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A review of rural and peri-urban sanitation infrastructure in South-East Asia and the Western Pacific: Highlighting regional inequalities and limited data

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ABSTRACT

Rural and peri-urban communities in developing countries rely on sanitation systems which are often unsafely managed. One of the major barriers to assess safely managed sanitation is a lack of data about the existing sanitation infrastructure and levels of containment safety. The aim was to review rural and peri-urban on-site sanitation studies in order to understand different infrastructure types, associated management practices and any impacts on human health. The scope was limited to South-East Asia and Western Pacific regions in order to better identify regional inequalities. Among the 155 reviewed articles, 73 studies (47%) linked sanitation infrastructure to poor human health. Nearly all articles reported latrine ownership (n = 149, 96%) while sanitation infrastructure types were covered less frequently (n = 104, 67%). In particular, there was a lack of published literature describing back-end characteristics (dimension and materials) (n = 12, 8%) and/or management practices (n = 4, 3%). This stems from a limited application of research methodologies that characterise sanitation infrastructure and faecal sludge management (containment, emptying and on-site treatment). Inequality between regions was prevalent with three quarters of the studies on latrine back-end infrastructure from Bangladesh and India in South-East Asia. A strategic research approach is needed to address the current knowledge gaps regarding sanitation infrastructure and safe faecal sludge management.

1. Introduction

Access to appropriate and safe sanitation is one of the key obstacles in achieving good human and environmental health. Globally, 4.2 billion people use unsafe sanitation facilities and 673 million people practice open defecation, highlighting the scale of the problem (UNICEF and WHO, 2020). Inadequate sanitation infrastructure poses major barriers to tackle sanitation challenges in developing countries in South-East Asia (DuChanois et al., 2019) and Western Pacific regions (Hadwen et al., 2015). Lack of access to adequate sanitation facilities is linked to increased rates of human diseases such as diarrhoea (Prüss-Ustün et al., 2014). According to the Global Burden of Disease Study, in 2017, 775,000 deaths were caused by diseases associated with inadequate sanitation (IHME, 2021). Inadequate sanitation is also a likely contributing factor in transmissions of highly infectious respiratory diseases like Severe Acute Respiratory Syndrome Coronavirus-2 (SARS-CoV-2) (Donde et al., 2020). In addition to human health consequences, inadequate sanitation facilities also contribute to a varied range of adverse environmental impacts such as groundwater pollution (Graham and Polizzotto, 2013) and declining coral reef health and coverage (Wear and Thurber, 2015).

Sustainable Development Goal (SDG) target 6.2, monitored by the Joint Monitoring Program (JMP) aims to ensure adequate and equitable sanitation for all (United Nations, 2018). The SDG sanitation ladder classifies sanitation by quality and access; the lowest level is open defecation, then unimproved, limited, basic, and safely managed is the highest (WHO and UNICEF, 2018). The indicator to achieve SDG target 6.2 is the proportion of population using safely managed sanitation which is defined as the use of improved facilities that are not shared with other households and where excreta are safely disposed of *in-situ* or transported and treated off-site (WHO and UNICEF, 2021). Although there has been an overall global increase in accessing safely managed sanitation facilities from 28% to 45% from the year 2000–2017, some individual regions underwent a reversal of past progress (UNICEF and WHO, 2019). The percentage coverage of basic sanitation in Pacific Island Countries in Western Pacific Region (excluding Australia and New

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Zealand) dropped from 38% to 30% from the year 2000–2017 (UNICEF and WHO, 2019). In contrast, South-East Asia achieved increased coverage (over 61%) of safely managed sanitation in 2017 (UNICEF and WHO, 2019). Estimates of safely managed sanitation coverage are somewhat contentious, due to differing assumptions around the safety of different types of latrine infrastructure. Also, data presented may not accurately represent the actual proportion of faecal sludge being safely contained (Bain et al., 2018; UNICEF and WHO, 2019). This can lead to an underestimation of the sanitation challenges for rural and peri-urban population and insufficient resources being allocated to achieve SDG 6 targets.

Inequality in attaining safely managed sanitation facilities is apparent not only between regions but also within countries. Uneven economic growth and distribution of resources between rural and urban communities have caused disparities in accessing limited or basic sanitation facilities (Ghosh and Cairncross, 2014; McClure et al., 2018). According to 2017 SDG household sanitation data, 93% of the 402 million people who practiced open defecation in South-East Asia and Western Pacific, were from rural communities (WHO and UNICEF, 2020). Hence, achieving SDG targets remains challenging due to the widening inequality within the sanitation sector.

On-site sanitation technologies are predominantly used by rural and peri-urban populations (Lourenço and Nunes, 2020). Two components of on-site sanitation are front-end and back-end. A latrine front-end refers to the part of the facility where the user deposits excreta (squats or sits) and the back-end refers to the containment facility where the human excreta is stored, treated, or disposed (Tilley et al., 2014). Septic tanks, pit latrines, and soak away pits are the most common types of latrine back-ends (Strande and Brdjanovic, 2014). Faecal sludge quantification and characterisation are crucial and difficult to assess due to the varied sanitation infrastructures practiced (Strande and Brdjanovic, 2014).

Globally, it is estimated that 48% of generated wastewater is discharged to the environment without any treatment (Jones et al., 2021), relates to the targets for SDG 6.3, which is focused on halving the proportion of untreated wastewater by 2030 (United Nations, 2018). Thus, to design safe faecal sludge management to meet the SDG targets, it is essential to understand the structural configuration of sanitation facilities. Also, the affordability of safely managed sanitation facilities and emptying services remains a challenge for low-income communities (Hutton and Varughese, 2016). As a result, unwillingness to upgrade sanitation infrastructure and reluctance in emptying latrine back-ends when filled are highly prevalent, which leads to unsafe faecal sludge containment (Tummers et al., 2016). In addition, lower resilience and adaptability of on-site sanitation increases the technological susceptibility to extreme weather events such as frequent flooding and drought (Howard et al., 2016). With increased climate variability in South-East Asia (Li, 2020) and Western Pacific (Sheng-jie and Dong-liang, 2018) regions and there are a number of sanitation-related challenges unique to these regions. Therefore, an in-depth investigation of on-site sanitation infrastructure and assessment of emptying and disposal practices are needed to tackle these complex challenges.

The factors discussed above – a lack of data on safe containment, inequality of sanitation infrastructure between and within countries, a commitment to achieve SDG sanitation targets – hence point to a clear need to better understand the sanitation infrastructure used by rural and peri-urban populations in South-East Asia and Western Pacific regions. Peri-urban communities are included in the scope of this study due to the similarity with rural communities with respect to the sanitation infrastructure in use and associated challenges (Angoua et al., 2018). The current review aims to deliver an assessment of on-site sanitation facilities in these two regions. The specific aims are: 1) to explore the impact of sanitation on human health within the specified regions; 2) to categorise the depth of rural and peri-urban sanitation infrastructure research (front-end and back-end types); 3) to identify knowledge gaps in rural and peri-urban on-site faecal sludge management practices (containment and any emptying or on-site treatment); and 4) to reveal any regional inequalities in sanitation-related research.

2. Methods

The geographic scope of the review is the rural and peri-urban population in South-East Asia and Western Pacific regions, based on the World Health Organization (WHO) regional grouping (WHO, 2021). WHO regional definition of South-East Asia varies from common geographical perspective and incorporates the following countries from central Asia: Bangladesh, India, Nepal, and Bhutan. The regions were chosen due to their comparable sanitation-related elements and associated challenges, in contrast to other regions such as Americas and Africa. Within the selected regions, South-East Asia has a larger total population (2 billion) compared to Western Pacific region (1.8 billion) (World Bank, 2021b). A low, medium and high score (<0.8) through the Human Development Index (HDI) published by the United Nations Development Program (UNDP) were employed for the selection of the countries within these regions (UNDP, 2019). This study selected 30 countries across South-East Asia and Western Pacific regions based on the 2019 HDI rankings (Fig. 1 and Table S1). Malaysia was also included despite having a very high HDI ranking (World Bank, 2021a). The rural population in Malaysia accounts for 23.4% of the total population and widening income inequality between rural and urban populations poses challenges that fit well with the concerns of the present study (Shahar et al., 2019; Tey et al., 2019).

The search for published full-text peer-reviewed academic journals in English was conducted using the Web of Science ™ database (Web of Science™, 2021). Grey literature was excluded from this review due to complexities around the validation of data quality and to ensure a manageable quantity of material to review. The search by topic was performed for each selected country and region using selected keywords: “Name of Country” AND Sanitation OR Latrine* OR Toilet*. The search was not limited by the publication year and articles returned spanned the period 1954–2020. The primary selection criterion was to include studies that conducted original field-based research by sampling (household surveys and/or interviews) from rural and peri-urban communities. For the studies that included both urban and rural/peri-urban settings, only data collected from rural and peri-urban areas was included in the review. Also, the studies that simply classified sanitation infrastructure as only improved or unimproved based on WHO/UNICEF JMP definitions were excluded due to insufficient information on the type of sanitation systems in use (WHO and UNICEF, 2018). Studies that only conducted secondary analyses on publicly available data such as country-based Demographic Health Surveillance (DHS) and census data, were excluded. Publications with unavailable full-text articles were also excluded, even if selected based on abstract screening.

A total of 3522 journal articles were identified from the initial screening (Step 1) (Fig. 2). After individual screening of title and elimination of duplicates in preliminary screening selection (Step 2), a total of 1584 relevant articles were selected for secondary (abstract) screening (Step 3). The screening of title and abstracts were based on the relevance of the selection criteria explained above. A total of 1014 articles satisfied the eligibility criteria for full-article review. Of these, 155 articles met the selection criteria for inclusion in the literature review (Step 4).

The following approach was employed to synthesise knowledge of on-site household sanitation facilities in South-East Asia and Western Pacific regions, by country. Firstly, the frequency of journal articles that reported negative human health outcomes (symptomatic effects, faecal-oral diseases, and parasitic infections) associated with sanitation practices was recorded. However, in studies which linked sanitation infrastructure with nutritional health outcomes (stunting and wasting), only latrine infrastructure-related information was extracted. Nutritional outcomes were not included in this review due to confounding risk factors such as hygiene practices, household income, and education

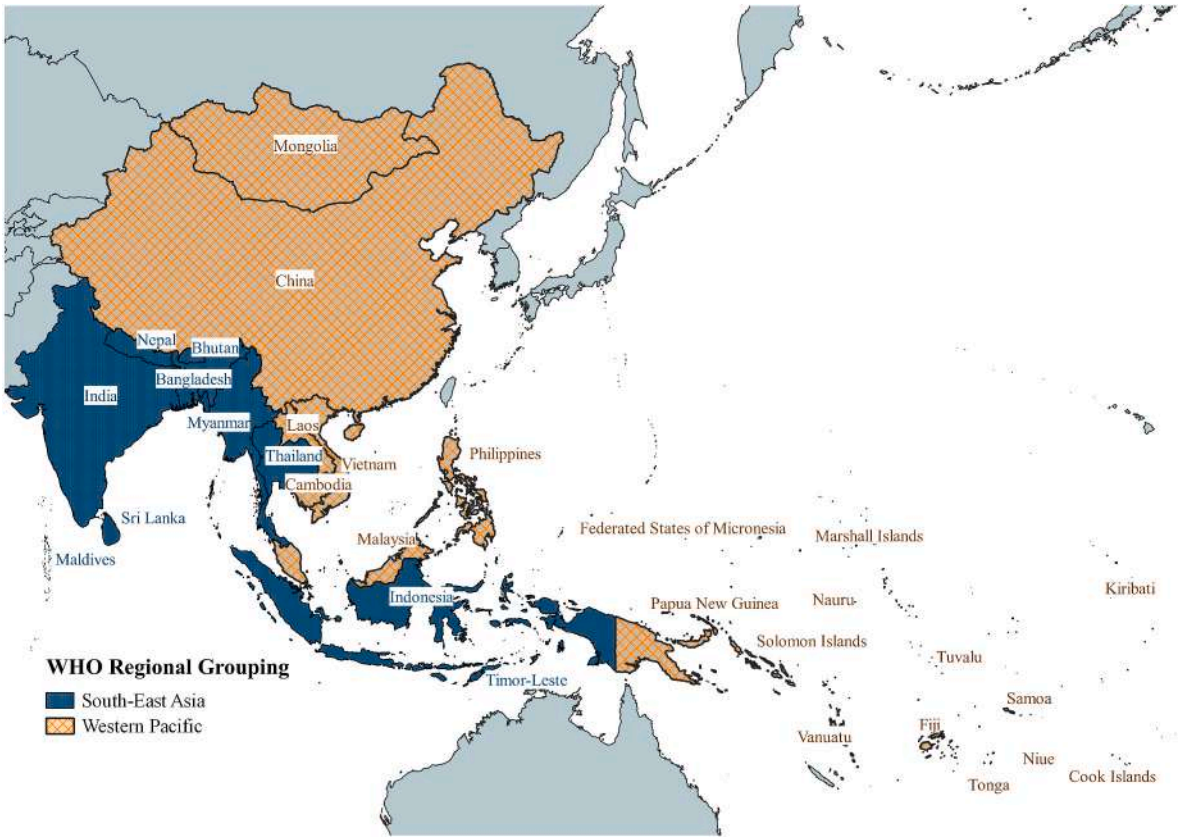

Fig. 1. Countries within South-East Asia and Western Pacific regions selected for the literature review based on WHO regional grouping definition (WHO, 2021). South-East Asia is denoted in blue and Western Pacific in orange. Only countries included are labelled. This map was developed using QGIS version 3.16-Hanover. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

level (Akombi et al., 2017; Vilcins et al., 2018). Secondly, sanitation data was classified into three major categories: latrine access, cleanliness, and infrastructure components (front-end and back-end) (Fig. 3).

3. Results

3.1. Geographic distribution of studies

The initial search (Step 1) returned 3522 articles with studies from South-East Asia (n = 2429, 69%), surpassing those from Western Pacific region (n = 1093, 31%) (Fig. 2). Following preliminary screening by title (Step 2, n = 1584), studies from South-East Asia (n = 1003, 63%) again exceeded those from Western Pacific (n = 581, 37%). Similarly, articles about South-East Asia (n = 667, 66%) were predominant during the secondary screening by abstract (Step 3). However, in the final selection of studies following full-text review (Step 4, n = 155), studies from South-East Asia (n = 74, 48%) and Western Pacific (n = 80, 52%) had nearly equal proportions. Of these, one article (Vannavong et al., 2017) included countries from both regions (Thailand and Lao PDR), and two studies from South-East Asia spanned multiple countries, namely Bangladesh and India (Baker et al., 2016) and Bangladesh and Indonesia (Nelson et al., 2014) (Table S2).

3.2. Sanitation and human health impact

Rural and peri-urban sanitation facilities in South-East Asia and Western Pacific regions were associated with negative human health outcomes in 73 (47%) out of 155 studies reviewed (Table 1). The reported sanitation criteria involved latrine access, cleanliness, and latrine infrastructure. The association of negative human health impact and sanitation components among the reviewed studies were established by using multiple research methodologies such as cross-sectional and longitudinal studies. Sanitation and human health-related studies were more numerous for Western Pacific (n = 50, 68%) compared to South-East Asia (n = 23, 32%). Despite having lower population (World Bank, 2021b), Western Pacific contributed to a higher number of disease-related studies, likely reflecting higher disease prevalence in this region. From South-East Asia, the largest number of studies were from Bangladesh (n = 10, 43%), while for Western Pacific, the most represented countries were Malaysia (n = 13, 26%) and Cambodia (n = 10, 20%), followed by Vietnam (n = 9, 18%).

Negative human health impacts were categorised as symptomatic (respiratory illness and diarrhoea), bacterial (typhoid and cholera) and viral (hepatitis), and parasitic infections. Parasitic infections (n = 51, 70%) were the most frequently reported public health impact related to sanitation. Of these, soil-transmitted helminths (n = 35, 69%) were more commonly reported infective organism across both regions. Of these, *Ascaris lumbricoides* (n = 23, 66%), hookworm (n = 23, 66%), whipworm (n = 17, 49%) and *Strongyloides stercoralis* (n = 9, 26%) were mentioned in the reviewed studies, indicating the role of inadequate sanitation in the transmission pathways of parasitic infections. The second most common reported human health impact was diarrhoea (n = 14, 19%), with five studies from Bangladesh making up 35% of the studies reporting diarrhoea. Moreover, four studies from Bangladesh and India associated respiratory illness (n = 4, 5%) with poor sanitation infrastructure and lack of latrine access and cleanliness. Inadequate sanitation infrastructure was associated with three faecal-oral diseases with serious outbreak potential: cholera (n = 3, 4%), Typhoid fever (n = 2, 3%) and hepatitis (n = 2, 3%) (Table 1), linking sanitation to human health.

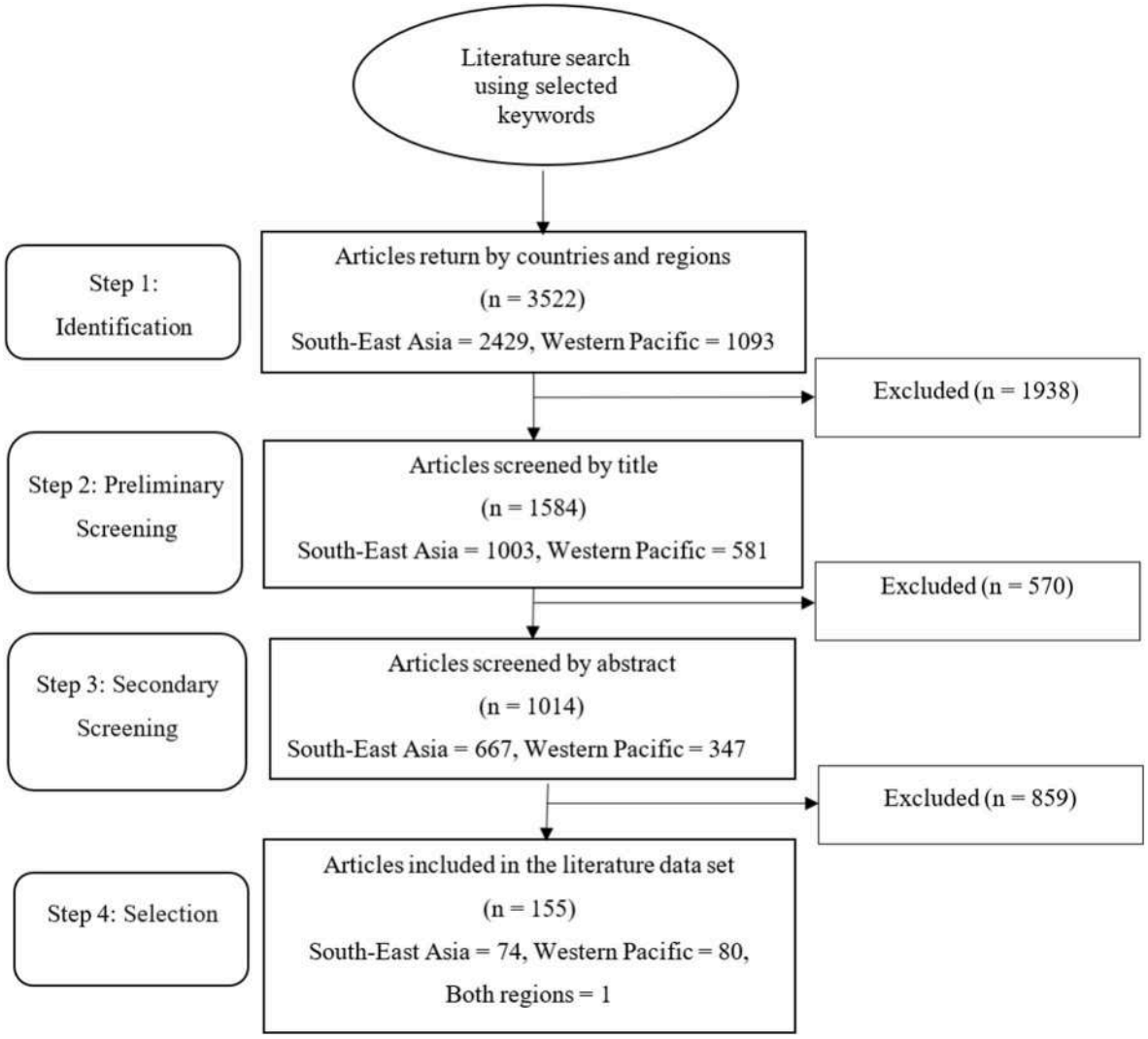

Fig. 2. Approach to the identification of eligible publications on rural and peri-urban household sanitation infrastructure and management practices in South-East Asia and Western Pacific regions.

3.3. Sanitation access and latrine cleanliness

Latrine access was reported in nearly all of the studies reviewed (n = 149, 96%) with a near even split between studies from South-East Asia (n = 72, 48%) and Western Pacific (n = 76, 51%) (Table S2). This indicates substantial research focus on overall sanitation access in existing literature. However, only 31 (21%) studies explicitly referred to the specific access type (private, shared, and public). Also, the higher number of studies from South-East Asian countries possibly reflects better research infrastructure in that region compared to Western Pacific. Also, among the reporting of latrine access type, private and shared (n = 20, 65%) latrine access was higher in number than reporting of public sanitation facilities (n = 3, 10%). Studies from Bangladesh were most consistent in their reporting of latrine access types: private (n = 10) and shared (n = 8). Access to public facilities is only stated in three studies from Cambodia (En and Gan, 2011), China (Li et al., 2015), and Mongolia (Uddin et al., 2014). Overall, fewer studies (n = 12) specified latrine cleanliness status. With the exception of Fiji (n = 1), all studies reporting on latrine condition were from South-East Asia (n = 11), depicting less emphasis on reporting of front-end maintenance within Western Pacific region. Studies reporting open defecation practices (n = 39) were larger in number in Western Pacific (n = 25, 64%) than South-East Asia (n = 14, 36%), possibly due to the increased open defecation practices in the region.

3.4. On-site sanitation infrastructure components

On-site sanitation infrastructure can be divided into front-end and back-end components (Fig. 3). From the latrine infrastructure studies (n = 104, 67%), about half included both front-end and back-end information (n = 48, 46%), the remaining studies reported either front-end (n = 26, 25%) or back-end (n = 30, 29%) details (Table S3). The studies reporting sanitation infrastructure were more frequently conducted in South-East Asia (n = 59, 57%) compared to Western Pacific region (n = 45, 43%). The majority of South-East Asian research was concentrated in two countries, Bangladesh (n = 22), and India (n = 15). For Western Pacific, Vietnam (n = 15) was the most frequently studied location. This highlights the imbalance in the quality and depth of sanitation research between the two regions and within the regions.

3.5. Front-end infrastructure

Front-end infrastructure was classified into four categories: presence of slab, toilet type (pedestal toilet, squat plate, or hole), flush mechanism (cistern flush, pour flush and dry toilet), and water seal. Among 74 articles reporting front-end characteristics (Table S4), studies from South-

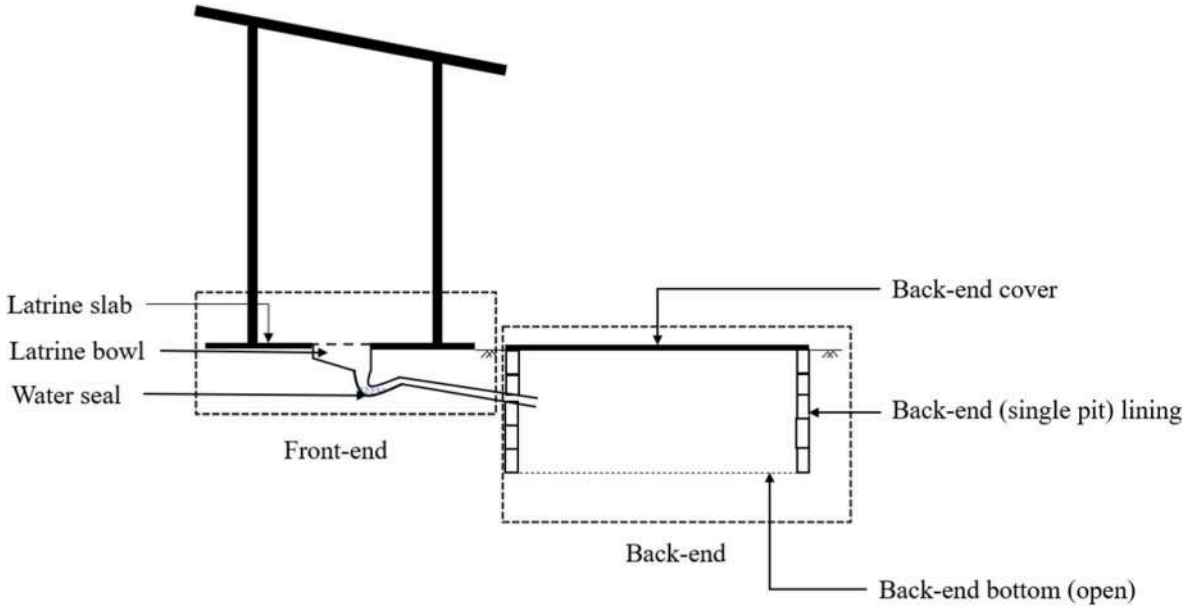

Fig. 3. A schematic of a typical rural latrine indicating the front-end (squat plate with pour flush) and back-end (off-set pit) division of structural components (dashed boxes).

East Asia (n = 45, 61%) again outnumbered those from the Western Pacific (n = 30, 41%). Looking at the presence of latrine slab, the majority of studies reported concrete slab (n = 21, 28%). Overall, a lower number of studies (n = 9, 12%) reported toilet type (pedestal toilet, squat, and hole). However, among them, squat plate type toilet (n = 9) was found to be the most common, followed by pedestal (n = 2) and hole (n = 2) toilet types. The studies revealed pour flush system was the most widespread latrine type in rural and peri-urban households across both regions, potentially due to the lower maintenance in rural and peri-urban context. In total 46 (62%) articles studied flush mechanism, of these, 37 (80%) cited pour flush latrine. Overall, the presence of functional water seal in latrines was reported in 17 studies (23%) with a majority of articles from South-East Asia (n = 15) and Bangladesh accounted for 12 studies.

3.6. Sanitation back-end types

Sanitation back-end types play a critical role in safe faecal sludge containment and treatment. A total of 79 of 104 articles, reporting latrine hardware, assessed different types of latrine back-ends across the two regions (Table 2). Single pit latrine (n = 67, 85%) was the most extensively studied back-end type in rural and peri-urban communities, likely due to the lower cost and easier construction process. Septic tank (n = 20, 25%) was the second most common type of back-end system in the reviewed literature. Current review combined both septic and aqua privy into a single category as only one study categorised the back-end as aqua-privy (Rashid and Pandit, 2020). None of the studies specified if the sampled septic tanks were built according to any international or country-specific standards. Therefore, it is assumed that the septic system described in the studies encompasses both standard and non-standard systems.

3.7. Sanitation back-end infrastructure

In addressing back-end characteristics, 12 out of 79 articles provided detailed information on the material, shape, and size of back-ends (Table 3) with two-thirds of studies based in South-East Asia (n = 8) and one-third in Western Pacific region (n = 4). Of these, seven articles studied pit latrines, followed by four studies reported composting latrines and only two reported on septic tanks.

The reported construction material and shape of back-end systems were consistent across multiple countries irrespective of sanitation technology in-use (Table 3). A majority of studies reported circular-shaped pit and composting latrine back-ends with concrete lining, potentially due to ease is construction method. The diameter for circular back-ends from reviewed studies ranges from 0.75 to 1.25 m across five countries: Bangladesh, India, Cambodia, Lao PDR, and Vietnam. The pit latrine depth ranged from 0.41 to 3.05 m, with the most frequently reported depth range between 0.9 and 1.5 m (Balasubramanya et al., 2016; Dey et al., 2016; Hussain et al., 2017; Naser et al., 2019). Only two studies reported deep pit latrines with 2 m and 3.05 m depth (Balasubramanya et al., 2016; Daniels et al., 2016). The shallowest latrines (0.41 m) were reported in Vietnam. Similarity in pit latrine configuration was observed in Bangladesh, Lao PDR, and Vietnam with concrete lining using three layers of pre-cast rings (depth of 0.3 m each) with permeable or open base. However, the number of concrete rings (from <3 to >3) varied in a study from India. The reviewed literature demonstrated similar infrastructure set-up between pit and compost type back-ends. The two studies from Bangladesh and one from Vietnam reported compost latrines (single and double) with three ring concrete lining (Table 3). Among the reviewed studies reporting septic system, other than construction material (concrete), none of the studies reported any further details or commented on the performance of the sampled latrine back-ends.

3.8. Sanitation back-end management and emptying practices

In addition to infrastructure, safe back-end management practices (including emptying) play a key role in achieving safely managed sanitation criteria. Only four studies out of 79 latrine back-end studies gave details on back-end management practices, even though this is essential to determine if the faecal sludge is safely managed or not (Table 4). Among them, the majority of studies were from Bangladesh (n = 3) and only one study from Cambodia. This follows the trend of South-East Asian dominance in the literature reporting sanitation hardware.

Balasubramanya et al. (2016) investigated 30 pit latrines from rural and peri-urban communities spread across five subdistricts in Bangladesh and estimated the average pit capacity at 733 L per household, considering a range of 30–50 L filling rates per person per year (Table 4). The average single pit filling frequency was estimated to be

Table 1
Association of negative human health impacts and diseases with sanitation in rural and peri-urban areas in South-East Asia and Western Pacific regions.

Region	Country	Human health impact					Reference
		Symptomatic		Bacterial and viral		Parasitic infection	
		Respiratory illness	Diarrhoea	Cholera and Typhoid	Hepatitis (A and E)		
South-East Asia	Bangladesh (n = 10)	4 (40%)	5 (50%)		1 (10%)	2 (20%)	(Ashraf et al., 2020; Baker et al., 2016; Dey et al., 2019; Ercumen et al., 2019; George et al., 2015; Huda et al., 2012; Islam et al., 2018; Labrique et al., 2013; Luby et al., 2018; Weaver et al., 2017)
South-East Asia	India (n = 2)	1	2			1	(Baker et al., 2016; Reese et al., 2019)
South-East Asia	Myanmar (n = 3)		1 (33%)			2 (67%)	(Gong et al., 2019a; Han et al., 2019; Maung et al., 1992)
South-East Asia	Nepal (n = 1)					1	Shrestha et al. (2018)
South-East Asia	Sri Lanka (n = 3)				1 (33%)	2 (67%)	(Ariyarathna and Abeysena, 2020; Galgamuwa et al., 2016; Perera et al., 2012)
South-East Asia	Thailand (n = 1)					1	Suntaravitun and Dokmaikaw (2018)
South-East Asia	Timor-Leste (n = 3)					3	(Campbell et al., 2016; Nery et al., 2019a, 2019b)
Western Pacific	Cambodia (n = 10)		2 (20%)			8 (80%)	(En and Gan, 2011; Forrer et al., 2016, 2018, 2019; Hunter et al., 2013; Khieu et al., 2013, 2014a, 2014b, 2014c; Moore et al., 2016)
Western Pacific	China (n = 4)					4	(Deng et al., 2020; Gong et al., 2019b; Openshaw et al., 2018; Xiao et al., 2015)
Western Pacific	Fiji (n = 2)			2			(Jenkins et al., 2019; Prasad et al., 2018)
Western Pacific	Kiribati (n = 1)		1				Psutka et al. (2013)
Western Pacific	Lao PDR (n = 6)					6	(Chard et al., 2020; Forrer et al., 2015; Kounnavong et al., 2011; Sayasone et al., 2007, 2011; Vonghachack et al., 2017)
Western Pacific	Malaysia (n = 13)		1 (8%)			12 (92%)	(Ahmed et al., 2011; Al-Delaimy et al., 2014; Brandon-Mong et al., 2017; Choy et al., 2014; Knight et al., 1992; Mohd-Shaharuddin et al., 2018; Nasr et al., 2013, 2020; Ngui et al., 2011, 2015; Nisha et al., 2020; Rajoo et al., 2017; Wong et al., 2016)
Western Pacific	Philippines (n = 3)		1 (25%)	1 (25%)		1 (25%)	(Baltazar et al., 1988; De Guzman et al., 2015; Labana et al., 2018)
Western Pacific	Solomon Islands (n = 2)		1	1			(Burnett et al., 2016; Harrington et al., 2015)
Western Pacific	Vietnam (n = 9)		1 (11%)	1 (11%)		7 (78%)	(Duc et al., 2011; Fuhrimann et al., 2016; Khandekar et al., 2006; Nguyen et al., 2017; Pham-Duc et al., 2014; Phuc et al., 2013; Trang et al., 2007; Verle et al., 2003; Yajima et al., 2009)
	n = 73[a,b]	4 (5%)	14 (19%)	5 (7%)	2 (3%)	51 (70%)	

[a] Sum of countries does not add up to the total number of studies reviewed as some studies were conducted in multiple countries.
[b] Sum of multiple categories does not add up to the total number of studies reviewed as some studies reported multiple categories.

3.68 years for a four-person household size, based on midpoint sludge accumulation rate of 40 L per person per year. However, only 20% (n = 6) of total surveyed households (n = 30) reported emptying their pit latrines that were four or more years old (Balasubramanya et al., 2016). This indicates that pits were either being used beyond safe filling capacity or degradation *in-situ* was reducing sludge volumes. The only other study to report pit capacities was also in Bangladesh and estimated an overall 0.832 m^3 (832 L) capacity for double pit latrines, with each pit having 0.416 m^3 (416 L) capacity (Hussain et al., 2017). Among the 18 households, only three households (17%) emptied their pits. They employed manual self-emptying method for the decomposed faecal sludge as it was easier to handle due to the reduced volume, less moisture, and odour (Hussain et al., 2017). Another study by Balasubramanya et al. (2017) interviewed 1091 households in rural areas of Bangladesh. Among 1080 households with latrine access, only 216 (20%) had emptied their back-ends, with 190 households (88%) hiring professionals to carry out the task. This implies that the remaining 12% of households either practiced self-emptying, dug a new pit, or abandoned the latrine. The reported average cost of back-end emptying was 322 taka (≈ USD 4). Among the households that emptied the pits (n = 216), 200 households (93%) confirmed disposing of the untreated emptied sludge to the nearby area by digging wide and shallow trenches (Balasubramanya et al., 2017). After the sludge infiltrates the soil, the faecal sludge is covered with soil, raising obvious questions about the safety of this disposal method. Of the households (n = 864) that were yet to empty their pits, 795 households (92%) expressed the intention of paying professionals to empty the pit and disposing of the untreated sludge on-site in trenches. Also, only a small fraction (6%) of them planned to dig new pit and there was no data on the remainder 2% of the households. Therefore, all studies reported disposal of untreated human excreta on-site, signifying potential risks to human health and environment.

4. Discussion

4.1. Summary of findings

In South-East Asia and Western Pacific region inadequate rural and peri-urban sanitation was found to be associated with viral, bacterial, and parasitic infections (Table 1). Latrine infrastructure studies were less frequent compared to studies reporting latrine access types (private, shared or no access) (Table S2). This indicates the current bias in the

Table 2
Sanitation back-end types in rural and peri-urban areas in South-East Asia and Western Pacific regions.

Country	Back-end types									Reference
	Septic tank[a]	Pit latrine		Compost[b]	Anaerobic digester	Soak pit[c]	Drum/ bucket	Hanging	Not defined[d]	
		Single	Double[e]							
Bangladesh (n = 17)	1 (6%)	13 (81%)	2 (12%)	2 (13%)			1 (6%)	4 (25%)		(Akter et al., 2014; Baker et al., 2016; Balasubramanya et al., 2016, 2017; Dey et al., 2016; Ercumen et al., 2019; George et al., 2015; Huda et al., 2012, 2018, 2019; Hussain et al., 2017; Islam et al., 2018, 2020; Labrique et al., 2013; Naser et al., 2019; Shimi et al., 2010; Uddin et al., 2013)
India (n = 13)	6 (46%)	10 (77%)	3 (23%)	3 (23%)	1 (8%)	2 (15%)				(Baker et al., 2016; Barnard et al., 2013; Bhallamudi et al., 2019; Daniels et al., 2016; Hylton et al., 2020; Jewitt et al., 2018; Kundu et al., 2018; O'Reilly et al., 2015; Padhi et al., 2015; Rashid and Pandit, 2020; Reese et al., 2019; Sinha et al., 2017; Yogananth and Bhatnagar, 2018)
Indonesia (n = 6)	3 (50%)	5 (83%)						1 (17%)		(Alisjahbana et al., 2019; Davis et al., 2018; Kurscheid et al., 2018; Rah et al., 2020; Sasongko et al., 2019; Torlesse et al., 2016)
Myanmar (n = 1)		1								Gong et al. (2019a)
Nepal (n = 7)	1 (14%)	7		1 (14%)			1 (14%)	1 (14%)		(Adhikari et al., 2017; Banks et al., 2019; Bhandari et al., 2019; McGinnis et al., 2019; Renzaho et al., 2018; Shrestha et al., 2018, 2020)
Sri Lanka (n = 1)		1								Galgamuwa et al. (2016)
Timor-Leste (n = 1)		1							1	Campbell et al. (2016)
Cambodia (n = 4)	1 (25%)	3 (75%)								(En and Gan, 2011; Harper et al., 2020; Janmohamed et al., 2016; Sinclair and Gerba, 2011)
China (n = 2)	1	2	1	1	1	1	1			(Gong et al., 2019b; Li et al., 2015)
Fiji (n = 2)	1	2								(Jenkins et al., 2019; Prasad et al., 2018)
Lao PDR (n = 2)		2								(Chard et al., 2020; Hiscox et al., 2016)
Malaysia (n = 2)		2								(Ngui et al., 2015; Wong et al., 2014)
Mongolia (n = 3)		3		2 (67%)			1 (33%)			(Barnes et al., 2020; Uddin et al., 2014, 2016)
Papua New Guinea (n = 1)		1								Olita'a et al. (2014)
Philippines (n = 2)	1	1		1						(De Guzman et al., 2015; Erismann et al., 2017)
Solomon Islands (n = 2)	1	2					1			(Fleming et al., 2019; Harrington et al., 2015)
Vanuatu (n = 1)		1								Morrison et al. (2020)
Vietnam (n = 13)	4 (31%)	11 (85%)	3 (23%)	4 (31%)	2 (15%)			4 (31%)	1 (8%)	(Duc et al., 2011; Fuhrimann et al., 2016; Grady et al., 2018; Jensen et al., 2008; Khandekar et al., 2006; Le et al., 2012; Minh et al., 2013; Pham-Duc et al., 2014; Rheinlander et al., 2010; Torondel et al., 2016; Trang et al., 2007; Verle et al., 2003; Yajima et al., 2009)
n = 79[f,g]	20 (25%)	67 (85%)	9 (11%)	14 (18%)	4 (5%)	3 (4%)	5 (6%)	10 (13%)	2 (3%)	

[a] Includes standard and non-standard septic tank (not constructed as per engineering standard) and aqua privy.
[b] Composting and ecological sanitation.
[c] Cesspit and soak pit.
[d] No information provided on the type of back-ends.
[e] If explicitly reported as double pits, if the information was absent, they were considered to be single pit latrine.
[f] Sum includes count of studies with multiple countries.
[g] Sum includes count of studies with multiple categories.

sanitation sector which focuses more on latrine ownership and patterns of use rather than the technical infrastructure components. Among the studies that described latrine infrastructure, front-end and back-end components shared similar proportion of studies. However, research on the latrine front-end was more rigorous in depicting physical characteristics compared to reports on back-end of latrines. Among the studies covering sanitation back-end types, very few researchers reported on the structural characteristics (dimensions and lining materials) (Table 3) and maintenance practices (emptying frequency, and emptied sludge disposal) (Table 4). Also, the type of information reported, did not allow for assessment of treatment safety. This highlights the scarcity of quality research on the topic within these two regions.

Table 3
Sanitation back-end infrastructure characteristics in rural and peri-urban areas.

Country	Latrines sampled	Back-end type	Construction material	Shape	Average cross-sectional size	Depth	Reference
Bangladesh	30	Single pit	Concrete	Circular	0.83 m	0.91–3.05 m	Balasubramanya et al. (2016)
Bangladesh	68	Single pit with 0.5 m sand layer around and the bottom	Concrete	Circular	0.75 m	0.9 m	Naser et al. (2019)
Bangladesh	18	Double pit	Concrete	Circular	0.8 m	0.9 m[a]	Hussain et al. (2017)
Bangladesh	20	Compost with single pit	Concrete	Circular			Uddin et al. (2013)
Bangladesh	70	Compost with double pit	Concrete	Circular		1.5 m	Dey et al. (2016)
India	447[b]	Single/double pit	Concrete	Circular			Barnard et al. (2013)
India	94[b]	Single/double pit		Circular		<1 m 1–2 m	Daniels et al. (2016)
India	15	Compost with single pit	Concrete Brick	Circular Square	1.25 m 0.84 m (Length) × 0.84 m (Width)		Hylton et al. (2020)
Cambodia	8	Septic tank	Concrete	Circular			Sinclair and Gerba (2011)
Lao PDR	205	Single pit	Concrete	Circular	0.8 m		Hiscox et al. (2016)
Vietnam	22	Single/double pit and compost				0.41–0.96 m	Torondel et al. (2016)
Vietnam	20[b]	Septic tank	Concrete				Le et al. (2012)

[a] Average depth.
[b] Number of households surveyed.

Table 4
Rural and peri-urban sanitation back-end management and emptying practices.

Country	Sample size	Respondents	Back-end type	Back-end volume (average)	Back-end age	Back-end emptying frequency	Latrine back-end emptying practices % of surveyed households					Reference
							Pay professional	Dig new pit	Self-empty	Undecided	Stop using toilet	
Bangladesh	30	Household latrines	Single pit	733 L[a]	4 years[b]	Once in 4 years[b]	20%					Balasubramanya et al. (2016)
Bangladesh	216	Households emptied their back-ends	Single pit		4 years[b]	Once in 4 years	88%					Balasubramanya et al. (2017)
	864	Households yet to empty their back-ends	Single pit				92%	6%				
Bangladesh	18	Household latrines	Double pit	Total 832 L (0.832 m^3)	3–18+ months				17%			Hussain et al. (2017)
Cambodia	3715	Households	Single pit		6–12 months		35%	24%	21%	16%	2%	Harper et al. (2020)

[a] Estimated value. The information was calculated by the data provided by BRAC (Bangladesh Rehabilitation Assistance Committee, a non-governmental organisation operating in Bangladesh).
[b] Data provided by BRAC and confirmed by interviewing households.

Such research is needed to tackle the sanitation challenges of rural and peri-urban communities. In addition, despite the initial equal distribution of studies between the two regions, Western Pacific had lower number of in-depth on-site sanitation back-end studies compared to the studies from South-East Asia. This uneven distribution of quality research within these regions highlights the challenges that need to overcome in achieving global SDG targets 6.2 and 6.3.

4.2. Impact of sanitation on human health

4.2.1. Soil-transmitted helminths (STH)

From a human health perspective across 73 studies (47%), this review found that parasitic infection caused by soil-transmitted helminths (STH) (n = 35, 48%) is the most commonly reported health impact relating to inadequate sanitation in rural and peri-urban populations in South-East Asia and Western Pacific regions (Table 1). The association of poor sanitation infrastructure with STH infections was also highlighted by Riaz et al. (2020), indicating the necessity of infrastructure assessment in reducing illness caused by STHs. The prevalence of non-washable latrine floors can facilitate STHs transmission and lead to higher risks of infection for the users. For example, a study from Vietnam (Torondel et al., 2016), reported the existence of brick latrine floors in peri-urban area and these types of non-washable latrine floors could be related to the higher instance of STH transmission reported in Vietnam. Also, 17 studies from this review cited the absence of latrine slabs across multiple countries (Table S4) which highlights the challenges in limiting human-faecal contact and increased risks of STH infections. On the other hand, there has been evidence of the contribution of adequate latrine back-end in reducing STH infections in rural populations. For instance, a study from Bangladesh (Ercumen et al., 2019) reported that upgrade to double pits of sanitation back-end reduced whipworm infections by 29% and hookworms infections by 24%. However, there was no reduction of *Ascaris* infections in this study, which was explained by slower decay rate of *Ascaris* eggs compared to other STHs. It has also been pointed out by other researchers that environmental conditions (temperature and moisture) can govern the prevalence of STHs transmission in certain

regions (Brooker et al., 2006; Brooker and Michael, 2000). The tropical climate of most South-East Asian and Western Pacific countries exacerbates health concerns linked to STH infections.

4.2.2. Diarrhoea

Lack of latrine access and inadequate sanitation infrastructure were associated with higher rates of diarrhoea in 14 studies reviewed (Table 1). The majority of sanitation-related studies related to diarrhoea occurred in Bangladesh (n = 5, 35%). Diarrhoea has been one of the leading causes of child morbidity and mortality in Bangladesh (Das et al., 2019) and hence it has been the focus on significant research efforts led by the International Centre for Diarrhoeal Diseases Research (icddrb, 2019). This might account for the greater attention and overall higher number of studies from Bangladesh reporting diarrhoea and sanitation infrastructure. According to WHO (2014) report, adequate on-site sanitation infrastructure can reduce diarrhoeal morbidity by 16% (WHO, 2014). This is consistent with one of the reviewed studies from Cambodia (En and Gan, 2011), which demonstrated the impact of using pour flush pit latrines on reduced diarrheal episodes among school children compared to open pit latrines. The study results showed that children using open pit latrines had higher odds of experiencing diarrhoea (OR 2.33, 95% CI 1.67–5). This reinforces the importance of adequate sanitation infrastructure in reducing diarrhoea. Clasen et al. (2010) reported similar findings, highlighting the effectiveness of sanitation infrastructure interventions in reducing the risks of diarrhoea. Proposed sanitation interventions included the construction of water-sealed double pit latrines and septic tanks, highlighting the importance of improving sanitation back-end infrastructure in reducing diarrhoeal morbidity. Other review articles (Fewtrell et al., 2005; Freeman et al., 2017; Li et al., 2016) also drew attention to the significance of sanitation infrastructure in reducing diarrhoeal morbidity. Therefore, there is a need to evaluate on-site sanitation technologies to ensure faecal sludge containment or treatment safety and enhance wellbeing of rural and peri-urban communities in South-East Asian and Western Pacific regions.

4.3. Current sanitation practices

4.3.1. Sanitation front-end structural characteristics

Among the reviewed studies (n = 155), 74 studies (47%) reported on latrine front-end characteristics. Pour flush latrines are the most common type of latrine front-end in rural and peri-urban settings for South-East Asia and Western pacific regions (Table S4). Likely because pour flush latrines are simpler and cheaper to build and generally requires less water compared to cistern flush toilets (Tilley et al., 2014). Considering rural and peri-urban settings, affordability and ease in construction can be a determining factor for this specific type of front-end choice. In addition, a higher number of studies reporting pour flush toilets across both regions may indicate their prevalence due to cultural preference.

4.3.2. Sanitation back-end structural characteristics

Latrine back-end physical characteristics play a key role in establishing safe faecal sludge management and limiting associated human risks and environmental contamination. The limited number of studies conducting in-depth latrine back-end infrastructure assessment (n = 12, 8%), suggests a deficit in quantifiable information to evaluate containment or treatment safety and efficiency. The dimensions and construction practices showed consistency among the reviewed studies (Table 3). The usual construction practice for pit and compost latrine was to use segmented concrete ring linings. The concrete lining segments are described as rings and used to increase durability by preventing soil collapse. Sanitation back-ends' overall dimensions (diameter and depth) play key role in determining the faecal sludge accumulation. The average diameter of pit latrines was 0.8 m among the studies from Bangladesh (Balasubramanya et al., 2016; Hussain et al., 2017; Naser et al., 2019) and Lao PDR (Hiscox et al., 2016). The back-end depth ranged from 0.41 to 3.05 m for multiple countries. However, most studies reported shallower pits (<1 m). Pits with smaller diameter and shallow depth can be dug by hand comfortably without collapsing the surrounding soil. A study conducted in Tanzania also showed similar findings with the most common pit depth among the 22 sampled pit latrines found to be 1 m (Mrimi et al., 2020). In addition to ease in installation, sanitation back-end depth can be influenced by environmental factors such as groundwater table and soil characteristics. For example, one of the reviewed studies from Bangladesh recommended an average appropriate pit depth of 1.5 m, considering relatively high groundwater level in Bangladesh (Hussain et al., 2017).

4.3.3. Sanitation back-end filling

Back-end filling process can be influenced by many site-specific factors. A study by Hussain et al. (2017) sampled 18 double pit latrines in a rural community of Bangladesh. Among the households, nine (50%) were using the first pit (household size ≤6 people), six (33%) changed to the second pit (household size 7–10 people) and three (17%) emptied the composted faecal sludge from the first pit (household size ≥11 people), demonstrating the link between household size and pit filling rates. Another study from Bangladesh by Balasubramanya et al. (2016) identified faecal sludge quality (% of moisture content), filling rates per person and excessive rainfall as driving factors of back-end filling frequency and increased demand for emptying. Also, depending on the soil types of each geographic location faecal sludge infiltration through soil may vary. Hence, the notion of using universal faecal sludge filling rate will incur inaccurate estimation. More region-specific research on faecal sludge and soil characterisation and consideration on climate variables (temperature, frequency of flooding and drought) are essential while assessing the safety of on-site containment and treatment systems.

4.3.4. Sanitation back-end emptying practices

The current literature review shows very low frequency of studies reporting sanitation back-end maintenance and emptying practices (n = 4, 3%) despite being one of the critical components in faecal sludge management (Table 4). Of the four studies, three were from Bangladesh, suggesting a lack of in-depth investigations of the operational aspects of sanitation back-ends in other countries within South-East Asia and Western Pacific regions.

A number of choices for back-end emptying practices have been observed in the reviewed literature for rural and peri-urban communities. The key factors that influenced the choice of practice amongst surveyed latrine owners are household income level, accessibility to local emptying services, and associated cost. Hussain et al. (2017) highlighted the advantages of using alternating double pit latrine as it enabled the studied population to swap the pits once filled. The studied households employed self-emptying practice as the decomposed faecal sludge volume was reduced, providing a financial advantage to the users. However, self-emptying practice is likely to be associated with unsafe faecal sludge handling, leading to adverse human health impacts (Strande and Brdjanovic, 2014). A study from Cambodia performed by Harper et al. (2020), described the intention of potential back-end emptying practices by interviewing households (n = 3715) as either desirable (paying professionals and digging new pit) or undesirable (self-empty, undecided and stop using toilet). More than half of the respondents (59%) reported having the intention of performing desirable pit emptying practices. However, 16% of the respondents reported being undecided regarding the back-end emptying practices, indicating potential unsafe faecal sludge management practice. Harper et al. (2020) also postulated that the intended back-end emptying practices recorded from the household surveys may not truly represent the actual emptying practices that will be executed. Households may have a tendency to report more favourable practices, whereas in reality they would employ the most affordable option. This adds to the challenges of understanding

the sludge management practices employed by rural and peri-urban communities.

4.3.5. Disposal practices of emptied faecal sludge

Adequate emptying, and safe disposal of faecal sludge play key role in meeting the safely managed sanitation criteria. Limited information on the fate of emptied faecal sludge in peer-reviewed studies represents significant concerns about unsafe faecal sludge management with increased risks of negative human health impact. Balasubramanya et al. (2017) reported that usual practice in rural areas in Bangladesh is to dispose untreated human waste in the nearby area by digging wide and shallow trenches and covering the sludge with soil. This would likely lead to subsurface nutrient and pathogen leaching, facilitating increased contaminant loading in groundwater and the surrounding waterbody, and may hence pose potential threats of disease outbreaks and environmental contaminations. A similar type of study performed in rural and peri-urban areas of Ghana (Appiah-Effah et al., 2014) showed that 61% of the surveyed households (n = 270) did not empty their back-ends though they were full due to high cost or lack of the back-end emptying services available within the community. As the latrine back-ends were full, they switched to either communal latrines or practiced open defecation. Similar to Balasubramanya et al. (2016), Appiah-Effah et al. (2014) also reported manual pit emptying was widespread among the studied population, leading to potential public health risks associated with unsafe faecal sludge handling. The lack of localised treatment facilities and associated services in developing countries potentially contribute to the prevalence of widespread unsafe faecal sludge management practices. These challenges are also highlighted by Koottatep et al. (2021), who studied 20 cities across Thailand. Their study showed that 70% of the faecal sludge generated from on-site sanitation systems are not safely managed. Similar to Balasubramanya et al. (2016), Koottatep et al. (2021) described the common practice is to discharge the untreated faecal sludge to the environment. From a global perspective, a study by Mara and Evans (2018) also identified lower or no coverage of adequate faecal sludge emptying and treatment facilities as major barriers in achieving safely managed sanitation. This signifies the necessity of understanding the back-end structures and volumes of faecal sludge present in order to design proper faecal sludge treatment facilities for rural and peri-urban communities to meet the safely managed sanitation target.

4.4. Challenges associated with on-site sanitation infrastructure and faecal sludge management

4.4.1. Geographic inequalities

The current literature review found no regional bias initially among the overall studies reviewed. The reviewed literature shared a nearly equal distribution of studies from South-East Asia (n = 74, 48%) and Western Pacific (n = 80, 52%). However, studies reporting negative human health outcome (n = 73) were more numerous in the Western Pacific (n = 50, 68%) compared to South-East Asia (n = 23, 32%) (Table 1). Also, 25 studies from Western Pacific reported open defecation practices, compared to 14 studies from South-East Asia (Table S2). According to Sustainable Development Goal (SDG) data, open defecation practices in Western Pacific has been increased from 13% to 14% between year 2000–2017, despite overall decline in global data (UNICEF and WHO, 2019). This clearly shows the challenge in achieving safely managed sanitation in Western Pacific. In contrast, South-East Asia was predominant among the studies conducting in-depth sanitation front-end (n = 45) (Table S4) and back-end (n = 8) (Tables 3 and 4) analyses compared to Western Pacific region. This indicates a regional bias in the production of quality research in the sanitation sector despite the vulnerability of Western Pacific to adverse public health impacts related to inadequate sanitation. From a country level perspective, an absence of sanitation-related studies for certain countries was observed, namely Cook Islands, Kiribati, Marshall Islands, Federated States of Micronesia, Nauru, Niue, Samoa, Tonga, Tuvalu, and Maldives. This suggests study bias for more populous countries and lack of spatial distribution of studies. The unavailability of sanitation data and research work for those countries indicates the presence of knowledge barriers to the achievement of global sanitation targets. The lack of sanitation studies in smaller Pacific Island Countries within Western Pacific region has been also identified by MacDonald et al. (2017). Their analysis showed that the research work within this region is mostly driven by the population density, suggesting countries with larger population produce more academic research works. This finding points to the need to distribute scientific resources to ensure sufficient attention is given to adequate sanitation and faecal sludge management practices across all nations.

4.4.2. Less focus on sanitation back-end infrastructure

The current review demonstrated a widening gap in the available information on rural and peri-urban sanitation infrastructure in South-East Asia and Western Pacific regions. The sanitation reporting type was dominated by latrine ownership and sharing status. Of the 155 studies included in this review, 149 (96%) reported latrine access data, compared to 104 (67%) of studies which focused on latrine infrastructure. Among the studies reporting latrine hardware, there was a lack of quality data on latrine back-ends infrastructure settings (n = 12) and management practices (n = 4) compared to front-end (n = 73). A plausible explanation can be the outcome of Millennium Development Goal (MDG), which was mostly concerned with latrine ownership and increased coverage of improved latrine front-end to prevent human contact with excreta during latrine use (United Nations, 2015; Weststrate et al., 2019). Current review identified latrine back-ends are less frequently studied, despite the fact that safe faecal sludge management is a key focus in sanitation sector (Strande and Brdjanovic, 2014). Therefore, there is a need to have an integrated overarching approach covering the overall sanitation system (front-end, back-end and faecal sludge management and emptying practices) to achieve the global SDG targets of safely managed sanitation. Also, the current JMP definition of improved sanitation facility encompasses different types of latrine back-ends such as pit, septic tank, or piped sewage system into one category (Baum et al., 2013; Gunawardana and Galagedara, 2013; WHO, 2013). As latrine back-end type plays a critical role in faecal sludge *in-situ* containment and treatment, it is essential to disaggregate the various technologies to have better assessment.

4.4.3. Lack of technological diversities

Addressing on-site back-end infrastructure, a lack of technological diversity has been observed. The most extensively reported on-site sanitation system in South-East Asia and Western Pacific was single pit latrine (Table 2). The current review demonstrated a lack of research conducted on safer sanitation systems with *in-situ* treatment, such as septic tanks, anaerobic digesters producing biogas, or compost latrines. This indicates a scarcity of quality research investigating their treatment efficiency and field implementation. Another review article by Graham and Polizzotto (2013) also showed the widespread use of pit latrines in other regions like Africa, South America, and Eastern Mediterranean regions. The high prevalence of simple pit latrines in rural and peri-urban settings across the globe is due to their lower-cost and ease of installation (Nakagiri et al., 2015). Also, household income and availability of government subsidies can increase the coverage of accessing safer sanitation systems. For example, one of the reviewed studies from China (Li et al., 2015) depicted the higher prevalence of engineered *in-situ* sanitation systems (89.8%) among 705 out of 790 respondents with higher income and households receiving financial incentives. Therefore, the household income level and availability of financial incentives from private and government organisations can be contributing factors in achieving higher coverage of safer on-site sanitation systems. Provision of financial incentives associated with alternative sanitation technologies by integrating both treatment and reuse of faecal sludge

may provide advantages to contribute to overcome the challenges. Andersson et al. (2018) also discussed the need for paradigm shift in sanitation sector by reusing resources like nitrogen and, phosphorus from faecal sludge. However, thorough safety assessments along with implementation and maintenance plans for the technologies still need to be undertaken to ensure sustainability in rural and peri-urban communities.

4.4.4. Environmental parameters and faecal sludge assessment

In addition to sanitation back-end infrastructure, evaluating the performance of on-site sanitation systems requires the reporting of environmental factors such as soil characteristics and groundwater table, as well as faecal sludge characterisation. The current review demonstrated a lack of information on both while reporting on-site sanitation system. For example, Naser et al. (2019) showed that using a sand barrier around the sampled pit latrines resulted in 27% reduction in *E. coli* loading in groundwater, hence highlighting the importance of assessing the soil properties. However, the extent of subsurface latrine leaching needs to be explored to understand overall environmental impact. A review article by Graham and Polizzotto (2013) showed that the lateral travel distance of microbial (virus and bacteria) and chemical (nitrate, nitrite, and chloride) contaminants sourced from latrines ranged from 5 to 50 m. The variable migration patterns were linked to site-specific soil characteristics and contaminant types. This implies the need for consideration of environmental parameters (soil type) and faecal sludge quantification to understand the contaminant migration patterns while designing sanitation infrastructure and safety assessment methods.

Also, microbial density within the latrine back-end and their interaction with the surrounding environment can vary greatly. For instance, Torondel et al. (2016) compared the microbial characteristics of the faecal sludge extracted from pit latrines from Vietnam and Tanzania. They determined that the bacterial distribution and composition of sludge were highly influenced by the influx of microbes from the surrounding environment with varying soil types and height of groundwater table surface. Multiple studies (Dzwairo, 2018; Pujari et al., 2012) pointed out that the flow rate and direction of nutrients sourced from sanitation infrastructure differ according to soil characteristics and their hydrogeological parameters. A study from Zimbabwe highlighted the importance of soil characterisation prior to on-site sanitation implementation (Dzwairo et al., 2006). A lateral travel distance of microbial contaminants up to 25 m were observed. Their study suggested shallower depth of latrine back-ends with lining may limit the contaminant transmission. Ravenscroft and associates (2017) performed a field investigation by constructing sample pit latrines (n = 4) and monitoring the pathway of faecal coliforms extending from latrine leach zones. Their study stated that the migration of faecal coliforms is influenced by the depth of unsaturated soil layer underneath the latrine back-ends. Therefore, factors such as geographic location, groundwater table, soil and faecal sludge characteristics, nutrient and microbial migration patterns need to be considered in determining the feasibility of on-site sanitation infrastructure and improving overall treatment effectiveness.

4.4.5. Research methodology development

This current review demonstrated a lack of reporting on the characteristics of latrine back-end infrastructures. Sanitation-related studies are generally based on observation and household interviews. Obtaining information on the visible latrine front-end is simpler compared to latrine back-end, which is often buried underground or not easily accessible. A thorough field assessment of latrine back-end involves the extraction of the infrastructure and sampling the deposited faecal sludge, which requires specialist skills and is also resource-intensive. This explains the dearth of information within peer-reviewed literature. Hence, there is a need to develop appropriate research methodologies and equipment (such as laser measures or hand-held on-site sampling equipment) to conduct field assessment. This will enable researchers to evaluate the characteristics of latrine back-ends and make better safety assessments of sanitation facilities in place. As there are no standardised methods for back-end infrastructure assessments, the findings of various studies are difficult to compare. Velkushanova et al. (2021) published, for the first time, standard method for the laboratory analysis of faecal sludge collected from on-site sanitation systems. They highlighted the challenges associated with faecal sludge testing as the current methodologies differ widely and are highly specific to the study location. Therefore, there is a need to establish a globally recognised standard method for latrine back-end infrastructure assessment which can be employed by sanitation researchers.

Another limitation within the existing literature is the inconsistencies in sanitation terminologies with no clear definitions for various sanitation back-end infrastructure such as soak-pits or cesspits. Furthermore, study-design methods and data were not always comparable as some reviewed studies only surveyed household members, while other studies only performed field investigations of latrine back-ends (Tables 3 and 4). One plausible explanation for the heterogeneity in sanitation terminologies can be the fact that sanitation sector incorporates researchers from wider range of disciplines such as public health, environmental science, and engineering. This heterogeneity makes the available data difficult to compare and could lead future researchers to misinterpret the findings of studies.

4.5. Limitations of the review

The key focus of this review was to analyse the academic research components addressing rural and peri-urban sanitation infrastructure in South-East Asia and Western Pacific region and highlight the existing knowledge gaps. Therefore, grey literature such as government and non-governmental organisation reports were excluded in this review. There is likely a wide range of information in non-peer reviewed literature which could provide more insight into sanitation infrastructure and local scale faecal sludge management practices within these regions. However, the exclusion of grey literature from the scope was also due to the complexities around the evaluation of data source, quality, and reliability.

The literature search was only limited to English language studies. The authors do not know the extent and quality of literature on the subject in other languages, and this remains a blind spot for this study. Also, the inability to access certain journal publications may have limited the extent of the reviewed literature within these two regions. In particular, it has been noted that six studies with relevance to sanitation infrastructure sourced from China mentioned in a review study by Li et al. (2016) were inaccessible. This limitation may have led to an incomplete representation of the overall sanitation situation in rural and peri-urban communities in China.

In some cases, reported sanitation technology required some interpretation. For example, two studies from India (Bhallamudi et al., 2019; Jewitt et al., 2018) reported the latrine back-end type as soak pit, whereas a Chinese study (Li et al., 2015) stated cesspit as the back-end system. These types of back-ends are combined in a single category in this study (Table 2). Where there is a variation in terminology, clear footnotes in the relevant tables have been provided highlighting those assumptions. Finally, varying sanitation terminologies may have led to misinterpretation of some reviewed articles.

4.6. Future recommendations

There is a scarcity of reporting in peer-reviewed literature detailing on-site sanitation infrastructure and assessing safe faecal sludge containment and on-site treatment in South-East Asia and Western Pacific regions. The Shit Flow Diagram approach has been employed for many urban areas in past decade (Sustainable Sanitation Alliance, 2022). This visual tool is useful to understand overall flow of faecal sludge within a specified location and determines the proportion of safe

and unsafe faecal sludge management practices. However, Shit Flow Diagram has not been applied to rural areas due to the lack of data. Therefore, more in-depth field investigation is needed on faecal sludge containment and on-site treatment are needed for rural communities to develop site specific Shit Flow Diagrams.

The lack of information can also be linked to the complexities around standardised data collection methodologies and field sampling of latrine back-ends. Development of hand-held field assessment tools and sampling equipment will support sanitation researchers and practitioners by reducing the level of difficulty in field sampling and data collection. Additionally, integrated study designs including both household surveys and site-specific field assessment of sanitation back-ends and consideration of socio-environmental factors (end users, faecal sludge characteristics, soil properties, hydrogeology, and climate conditions) will allow the researchers to perform thorough assessment on the system effectiveness and identify associated health and environmental risks.

The current review also identified lack of research focuses on this subject, especially in less populous countries. A substantial number of reviewed studies were from Bangladesh, which were directly linked to the dedicated water, sanitation, and hygiene research centre, International Centre for Diarrhoeal Disease Research, Bangladesh. Therefore, the establishment of sanitation-focused research initiatives are recommended across South-East Asia and Western Pacific, with special attention paid to the less populous nations and less researched countries. These types of initiatives will assist researchers to fill the existing knowledge gap by performing more quality research related to sanitation back-end infrastructure and their emptying and disposal practices. The increased research focus will facilitate better understanding of the safety of on-site sanitation systems and will eventually support the relevant agencies in mitigating the challenges associated with safe containment and treatment of faecal sludge and reduce associated health and environmental risks.

Also, there remains heterogeneity in sanitation terminologies and study designs. This creates difficulties for sanitation-related study design to incorporate diversified research across regions. This signifies the importance of future research work to have an integrated research approach with common sanitation infrastructure methodologies and terminologies overarching multiple disciplines to mitigate the knowledge gap in better reporting of on-site sanitation systems and their safety in containment or treatment.

5. Conclusion

This literature review provides for the first time an overview of the current understanding of rural and peri-urban sanitation infrastructure in South-East Asia and Western Pacific regions and its impact on human health. The first aim was to explore the impact of sanitation on human health within the specified regions and this review identified rural and peri-urban sanitation facilities in South-East Asia and Western Pacific regions were associated with negative human health outcomes in 73 (47%) out of 155 studies reviewed. Most commonly reported negative health impacts were soil transmitted helminths and diarrhoea. The second aim was to categorise the depth of rural and peri-urban sanitation infrastructure research (front-end and back-end types) and this review found there were fewer sanitation infrastructure-related studies (n = 104, 67%) compared to ownership status (n = 149, 96%) across both regions. The third aim was to identify knowledge gaps in rural and peri-urban on-site faecal sludge management practices (containment and any emptying or on-site treatment). Despite being a key factor in safe faecal sludge management, this review found latrine back-end structural characteristics (size, shape, and material type) (n = 12, 8%) and maintenance practices (faecal sludge extraction and on-site disposal) (n = 4, 3%) were less frequently reported components. Given the lack of information published on rural and peri-urban sanitation, there is a persistent need for standardised research methodology development by incorporating in-depth investigation of latrine back-end, emptying and on-site disposal. The fourth and final aim was to reveal any regional inequalities in sanitation-related research. This review identified that the Western Pacific region had the least number of studies reporting back-end infrastructure and management practices compared to the studies from South-East Asia. In particular, the majority of the studies from South-East Asia were from Bangladesh and India. This highlights the need for a more co-ordinated distribution of research resources. Addressing the identified research gaps will enable those in the sanitation sector to more effectively understand the efficiency and safety of on-site sanitation systems in rural and peri-urban communities.

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Author's contribution

Nabeela Nasim: Conceptualisation, study methodology design, execution of literature search, reviewing all literature, drafting manuscript, editing manuscript for final submission. Prof. Abbas El-Zein: Conceptualisation, critically revising the written work, editing manuscript for final submission. Dr. Jacqueline Thomas: Conceptualisation, study methodology design, reviewing key literature, critically revising the written work, editing manuscript for final submission.

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Declaration of competing interest

The authors declare no potential conflict of interest.

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Appendix A. Supplementary data

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References

Adhikari, D., Khatri, R.B., Paudel, Y.R., Poudyal, A.K., 2017. Factors associated with underweight among under-five children in eastern Nepal: community-based cross-sectional study. Front. Public Health 5.

Ahmed, A., Al-Mekhlafi, H.M., Choy, S.H., Ithoi, I., Al-Adhroey, A.H., Abdulsalam, A.M., Surin, J., 2011. The Burden of Moderate-To-Heavy Soil-Transmitted Helminth Infections Among Rural Malaysian Aborigines: an Urgent Need for an Integrated Control Programme, vol. 4. Parasites and Vectors.

Akombi, B.J., Agho, K.E., Hall, J.J., Wali, N., Renzaho, A., Merom, D., 2017. Stunting, wasting and underweight in Sub-Saharan Africa: a systematic review. Int. J. Environ. Res. Publ. Health 14 (8), 863.

Akter, T., Ali, A.R., Dey, N.C., 2014. Transition overtime in household latrine use in rural Bangladesh: a longitudinal cohort study. BMC Publ. Health 14 (1), 721.

Al-Delaimy, A.K., Al-Mekhlafi, H.M., Nasr, N.A., Sady, H., Atroosh, W.M., Nashiry, M., Anuar, T.S., Moktar, N., Lim, Y.A.L., Mahmud, R., 2014. Epidemiology of intestinal polyparasitism among Orang Asli school children in rural Malaysia. PLoS Neglected Trop. Dis. 8 (8).

Alisjahbana, B., Rivami, D.S., Octavia, L., Susilawati, N., Pangaribuan, M., Alisjahbana, A., Diana, A., 2019. Intrauterine growth retardation (IUGR) as determinant and environment as modulator of infant mortality and morbidity: the Tanjungsari cohort study in Indonesia. Asia Pac. J. Clin. Nutr. 28, S17–S31.

Andersson, K., Otoo, M., Nolasco, M., 2018. Innovative sanitation approaches could address multiple development challenges. Water Sci. Technol. 77 (4), 855–858.

Angoua, E.L.E., Dongo, K., Templeton, M.R., Zinsstag, J., Bonfoh, B., 2018. Barriers to access improved water and sanitation in poor peri-urban settlements of Abidjan, Côte d'Ivoire. PLoS One 13 (8), e0202928.

Appiah-Effah, E., Nyarko, K.B., Gyasi, S.F., Awuah, E., 2014. Faecal sludge management in low income areas: a case study of three districts in the Ashanti region of Ghana. J. Water, Sanit. Hyg. Dev. 4 (2), 189–199.

Ariyarathna, N., Abeysena, C., 2020. Risk factors for viral hepatitis A infection in Gampaha district, Sri Lanka: an unmatched case control study. BMC Publ. Health 20 (1).

Ashraf, S., Islam, M., Unicomb, L., Rahman, M., Winch, P.J., Arnold, B.F., Benjamin-Chung, J., Ram, P.K., Colford, J.M., Luby, S.P., 2020. Effect of improved water quality, sanitation, hygiene and nutrition interventions on respiratory illness in young children in rural Bangladesh: a multi-arm cluster-randomized controlled trial. Am. J. Trop. Med. Hyg. 102 (5), 1124–1130.

Bain, R., Johnston, R., Mitis, F., Chatterley, C., Slaymaker, T., 2018. Establishing sustainable development goal baselines for household drinking water, sanitation and hygiene services. Water 10 (12), 1711.

Baker, K.K., O'Reilly, C.E., Levine, M.M., Kotloff, K.L., Nataro, J.P., Ayers, T.L., Farag, T. H., Nasrin, D., Blackwelder, W.C., Wu, Y.K., Alonso, P.L., Breiman, R.F., Omore, R., Faruque, A.S.G., Das, S.K., Ahmed, S., Saha, D., Sow, S.O., Sur, D., Zaidi, A.K.M., Quadri, F., Mintz, E.D., 2016. Sanitation and hygiene-specific risk factors for moderate-to-severe diarrhea in young children in the global enteric multicenter study, 2007-2011: case-control study. PLoS Med. 13 (5).

Balasubramanya, S., Evans, B., Ahmed, R., Habib, A., Asad, N., Rahman, M., Hasan, M., Dey, D., Camargo-Valero, M., Rao, K.C., 2017. Take it away: the need for designing fecal sludge disposal services for single-pit latrines. J. Water, Sanit. Hyg. Dev. 7 (1), 121–128.

Balasubramanya, S., Evans, B., Ahmed, R., Habib, A., Asad, N., Vuong, L., Rahman, M., Hasan, M., Dey, D., Camargo-Valero, M., 2016. Pump it up: making single-pit emptying safer in rural Bangladesh. J. Water, Sanit. Hyg. Dev. 6 (3), 456–464.

Baltazar, J., Briscoe, J., Mesola, V., Moe, C., Solon, F., Vanderslice, J., Young, B., 1988. Can the case control method be used to assess the impact of water-supply and sanitation on diarrhea - a study in the Philippines. Bull. World Health Organ. 66 (5), 627–635.

Banks, L.M., White, S., Biran, A., Wilbur, J., Neupane, S., Neupane, S., Sharma, A., Kuper, H., 2019. Are current approaches for measuring access to clean water and sanitation inclusive of people with disabilities? comparison of individual- and household-level access between people with and without disabilities in the Tanahun district of Nepal. PLoS One 14, 10.

Barnard, S., Routray, P., Majorin, F., Peletz, R., Boisson, S., Sinha, A., Clasen, T., 2013. Impact of Indian total sanitation campaign on latrine coverage and use: a cross-sectional study in Orissa three years following programme implementation. PLoS One 8 (8).

Barnes, A.N., Baasandavga, U., Davaasuren, A., Gonchigoo, B., Gray, G.C., 2020. Knowledge and practices surrounding zoonotic disease among Mongolian herding households. Pastor. Res. Policy Pract. 10 (1).

Baum, R., Luh, J., Bartram, J., 2013. Sanitation: a global estimate of sewerage connections without treatment and the resulting impact on MDG progress. Environ. Sci. Technol. 47 (4), 1994–2000.

Bhallamudi, S.M., Kaviyarasan, R., Abilarasu, A., Philip, L., 2019. Nexus between sanitation and groundwater quality: case study from a hard rock region in India. J. Water, Sanit. Hyg. Dev. 9 (4), 703–713.

Bhandari, P., Bak, J., Lee, K.S., Chon, Y., Bhattachan, A., Rimal, P., Shrestha, B.R., Bhandari, B., Moon, J.O., Wu, N., Chu, W.S., Song, C.K., Lee, C.S., Mogasale, V., Ahn, S.H., 2019. Assessment of socio-demographic factors, mother and child health status, water, sanitation, and hygienic conditions existing in a hilly rural village of Nepal. Int. J. Environ. Res. Publ. Health 16, 20.

Brandon-Mong, G.J., Abdullah, N.A., Shukor, N., Jaturas, N., Richard, R.L., Choo, J.C., Majid, M.A.A., Mahboob, T., Tan, T.C., Sawangjaroen, N., Nissapatorn, V., 2017. Soil-transmitted helminths in Malaysia landscape: an aborigines study. Trop. Biomed. 34 (2), 363–374.

Brooker, S., Clements, A.C., Bundy, D.A., 2006. Global epidemiology, ecology and control of soil-transmitted helminth infections. Adv. Parasitol. 62, 221–261.

Brooker, S., Michael, E., 2000. The potential of geographical information systems and remote sensing in the epidemiology and control of human helminth infections. Adv. Parasitol. 47, 245–288.

Burnett, E., Dalipanda, T., Ogaoga, D., Gaiofa, J., Jilini, G., Halpin, A., Dietz, V., Date, K., Mintz, E., Hyde, T., Wannemuehler, K., Yen, C., 2016. Knowledge, attitudes, and practices regarding diarrhea and cholera following an oral cholera vaccination campaign in the Solomon Islands. PLoS Neglected Trop. Dis. 10 (8).

Campbell, S.J., Nery, S.V., D'Este, C.A., Gray, D.J., McCarthy, J.S., Traub, R.J., Andrews, R.M., Llewellyn, S., Vallely, A.J., Williams, G.M., Amaral, S., Clements, A. C.A., 2016. Water, sanitation and hygiene related risk factors for soil-transmitted helminth and Giardia Duodenalis infections in rural communities in Timor-Leste. Int. J. Parasitol. 46 (12), 771–779.

Chard, A.N., Levy, K., Baker, K.K., Tsaiid, K., Chang, H.H., Thongpaseuth, V., Sistrunk, J. R., Freeman, M.C., 2020. Environmental and spatial determinants of enteric pathogen infection in rural Lao People's Democratic Republic: a cross-sectional study. PLoS Neglected Trop. Dis. 14 (4).

Choy, S.H., Al-Mekhlafi, H.M., Mahdy, M.A.K., Nasr, N.N., Sulaiman, M., Lim, Y.A.L., Surin, J., 2014. Prevalence and associated risk factors of Giardia infection among indigenous communities in rural Malaysia. Sci. Rep. 4.

Clasen, T.F., Bostoen, K., Schmidt, W.P., Boisson, S., Fung, I.C.H., Jenkins, M.W., Scott, B., Sugden, S., Cairncross, S., 2010. Interventions to improve disposal of human excreta for preventing diarrhoea. Cochrane Database Syst. Rev. 6.

Daniels, M.E., Smith, W.A., Schmidt, W.P., Clasen, T., Jenkins, M.W., 2016. Modeling Cryptosporidium and Giardia in ground and surface water sources in rural India: associations with latrines, livestock, damaged wells, and rainfall patterns. Environ. Sci. Technol. 50 (14), 7498–7507.

Das, S., Chandra, H., Saha, U.R., 2019. District level estimates and mapping of prevalence of diarrhoea among under-five children in Bangladesh by combining survey and census data. PLoS One 14 (2), e0211062.

Davis, J., Macintyre, A., Odagiri, M., Suriastini, W., Cordova, A., Huggett, C., Agius, P.A., Faiqoh, Budiyani, A.E., Quillet, C., Cronin, A.A., Diah, N.M., Triwahyunto, A., Luchters, S., Kennedy, E., 2018. Menstrual hygiene management and school absenteeism among adolescent students in Indonesia: evidence from a cross-sectional school-based survey. Trop. Med. Int. Health 23 (12), 1350–1363.

De Guzman, A., de los Reyes, V.C., Sucaldito, M.N., Tayag, E., 2015. Availability of safe drinking-water: the answer to cholera outbreak? Nabua, Camarines Sur, Philippines. West. Pac. Surveill. Response 6 (3), 12–16, 2012.

Deng, Y., Zhang, S.X., Ning, C.Q., Zhou, Y.K., Teng, X.J., Wu, X.P., Chu, Y.H., Yu, Y.F., Chen, J.X., Tian, L.G., Wang, W., 2020. Molecular epidemiology and risk factors of Blastocystis sp. infections among general populations in Yunnan province, southwestern China. Risk Manag. Healthc. Pol. 13, 1791–1801.

Dey, N.C., Parvez, M., Islam, M.R., Mistry, S.K., Levine, D.I., 2019. Effectiveness of a community-based water, sanitation, and hygiene (WASH) intervention in reduction of diarrhoea among under-five children: evidence from a repeated cross-sectional study (2007–2015) in rural Bangladesh. Int. J. Hyg Environ. Health 222 (8), 1098–1108.

Dey, N.C., Ridwanul Haque, A., Kabir, B., Ubaid, S.F., 2016. Fecal indicator and Ascaris removal from double pit latrine content. J. Water Health 14 (6), 972–979.

Donde, O.O., Atoni, E., Muia, A.W., Yillia, P.T., 2020. COVID-19 Pandemic: Water, Sanitation and Hygiene (WASH) as a Critical Control Measure Remains a Major Challenge in Low-Income Countries. Water Research, 116793.

Duc, P.P., Nguyen-Viet, H., Hattendorf, J., Zinsstag, J., Cam, P.D., Odermatt, P., 2011. Risk Factors for Entamoeba Histolytica Infection in an Agricultural Community in Hanam Province, Vietnam', Parasites and Vectors, vol. 4.

DuChanois, R.M., Liddle, E.S., Fenner, R.A., Jeuland, M., Evans, B., Cumming, O., Zaman, R.U., Mujica-Pereira, A.V., Ross, I., Gribble, M.O., 2019. Factors associated with water service continuity for the rural populations of Bangladesh, Pakistan, Ethiopia, and Mozambique. Environ. Sci. Technol. 53 (8), 4355–4363.

Dzwairo, B., 2018. Multi-date trends in groundwater pollution from pit latrines. J. Water, Sanit. Hyg. Dev. 8 (4), 607–621.

Dzwairo, B., Hoko, Z., Love, D., Guzha, E., 2006. Assessment of the impacts of pit latrines on groundwater quality in rural areas: a case study from Marondera district, Zimbabwe. Phys. Chem. Earth, Parts A/B/C 31 (15–16), 779–788.

En, W.L., Gan, G.L., 2011. Factors associated with use of improved water sources and sanitation among rural primary schoolchildren in pursat province, Cambodia. Southeast Asian J. Trop. Med. Publ. Health 42 (4), 1022–1031.

Ercumen, A., Benjamin-Chung, J., Arnold, B.F., Lin, A., Hubbard, A.E., Stewart, C., Rahman, Z., Parvez, S.M., Unicomb, L., Rahman, M., Haque, R., Colford, J.M., Luby, S.P., 2019. Effects of water, sanitation, handwashing and nutritional interventions on soil-transmitted helminth infections in young children: a cluster-randomized controlled trial in rural Bangladesh. PLoS Neglected Trop. Dis. 13 (5).

Erismann, S., Knoblauch, A.M., Diagbouga, S., Odermatt, P., Gerold, J., Shrestha, A., Tarnagda, G., Savadogo, B., Schindler, C., Utzinger, J., Cisse, G., 2017. Prevalence and risk factors of undernutrition among schoolchildren in the Plateau Central and Centre-Ouest regions of Burkina Faso. Infect. Dis. Poverty 6.

Fewtrell, L., Kaufmann, R.B., Kay, D., Enanoria, W., Haller, L., Colford Jr., J.M., 2005. Water, sanitation, and hygiene interventions to reduce diarrhoea in less developed countries: a systematic review and meta-analysis. Lancet Infect. Dis. 5 (1), 42–52.

Fleming, L., Anthonj, C., Thakkar, M.B., Tikoisuva, W.M., Manga, M., Howard, G., Shields, K.F., Kelly, E., Overmars, M., Bartram, J., 2019. Urban and rural sanitation in the Solomon Islands: how resilient are these to extreme weather events? Sci. Total Environ. 683, 331–340.

Forrer, A., Khieu, V., Schar, F., Vounatsou, P., Chammartin, F., Marti, H., Muth, S., Odermatt, P., 2018. Strongyloides Stercoralis and Hookworm Co-infection: Spatial Distribution and Determinants in Preah Vihear Province, Cambodia, vol. 11. Parasites and Vectors.

Forrer, A., Khieu, V., Schindler, C., Schar, F., Marti, H., Char, M.C., Muth, S., Odermatt, P., 2016. Ivermectin treatment and sanitation effectively reduce Strongyloides stercoralis infection risk in rural communities in Cambodia. PLoS Neglected Trop. Dis. 10 (8).

Forrer, A., Khieu, V., Vounatsou, P., Sithithaworn, P., Ruantip, S., Huy, R., Muth, S., Odermatt, P., 2019. Strongyloides stercoralis: spatial distribution of a highly prevalent and ubiquitous soil-transmitted helminth in Cambodia. PLoS Neglected Trop. Dis. 13 (6).

Forrer, A., Vounatsou, P., Sayasone, S., Vonghachack, Y., Bouakhasith, D., Utzinger, J., Akkhavong, K., Odermatt, P., 2015. Risk profiling of hookworm infection and intensity in southern Lao People's Democratic Republic using bayesian models. PLoS Neglected Trop. Dis. 9 (3).

Freeman, M.C., Garn, J.V., Sclar, G.D., Boisson, S., Medlicott, K., Alexander, K.T., Penakalapati, G., Anderson, D., Mahtani, A.G., Grimes, J.E., 2017. The impact of sanitation on infectious disease and nutritional status: a systematic review and meta-analysis. Int. J. Hyg Environ. Health 220 (6), 928–949.

Fuhrimann, S., Winkler, M.S., Pham-Duc, P., Do-Trung, D., Schindler, C., Utzinger, J., Cisse, G., 2016. Intestinal Parasite Infections and Associated Risk Factors in Communities Exposed to Wastewater in Urban and Peri-Urban Transition Zones in Hanoi, Vietnam', Parasites and Vectors, vol. 9.

Galgamuwa, L., Iddawela, D., Dharmaratne, S.D., 2016. Factors associated with the prevalence of Ascaris lumbricoides infection among preschool children in a plantation community, Kandy district, Sri Lanka. South. Asian J. Trop. Med. Publ. Health 47 (6), 1143–1152.

George, C.M., Ahmed, S., Talukder, K.A., Azmi, I.J., Perin, J., Sack, R.B., Sack, D.A., Stine, O.C., Oldja, L., Shahnaij, M., Chakraborty, S., Parvin, T., Bhuyian, S.I., Bouwer, E., Zhang, X.T., Hasan, T.N., Luna, S.J., Akter, F., Faruque, A.S.G., 2015. Shigella infections in household contacts of pediatric shigellosis patients in rural Bangladesh. Emerg. Infect. Dis. 21 (11), 2006–2013.

Ghosh, A., Cairncross, S., 2014. The uneven progress of sanitation in India. J. Water, Sanit. Hyg. Dev. 4 (1), 15–22.

Gong, B.Y., Liu, X.H., Wu, Y.C., Xu, N., Xu, M., Yang, F.K., Tong, L., Zhou, K.X., Cao, J.P., Liu, A.Q., Shen, Y.J., 2019a. Prevalence and subtype distribution of Blastocystis in ethnic minority groups on both sides of the China-Myanmar border, and assessment of risk factors. Parasite 26.

Gong, B.Y., Yang, Y.M., Liu, X.H., Cao, J.P., Xu, M., Xu, N., Yang, F.K., Wu, F.W., Li, B.F., Liu, A.Q., Shen, Y.J., 2019b. First survey of Enterocytozoon bieneusi and dominant genotype Peru6 among ethnic minority groups in southwestern China's Yunnan province and assessment of risk factors. PLoS Neglected Trop. Dis. 13 (5).

Grady, C.A., Nguyen, K.V., Nguyen, T.V., Blatchley, E.R., 2018. Who is being left behind? an analysis of improved drinking water and basic sanitation access in the Vietnamese Mekong Delta. J. Water, Sanit. Hyg. Dev. 8 (3), 508–519.

Graham, J.P., Polizzotto, M.L., 2013. Pit latrines and their impacts on groundwater quality: a systematic review. Environ. Health Perspect. 121 (5), 521–530.

Gunawardana, I., Galagedara, L., 2013. A new approach to measure sanitation performance. J. Water, Sanit. Hyg. Dev. 3 (2), 269–282.

Hadwen, W.L., Powell, B., MacDonald, M.C., Elliott, M., Chan, T., Gernjak, W., Aalbersberg, W.G., 2015. Putting WASH in the water cycle: climate change, water resources and the future of water, sanitation and hygiene challenges in Pacific Island countries. J. Water, Sanit. Hyg. Dev. 5 (2), 183–191.

Han, K.T., Wai, K.T., Aye, K.H., Kyaw, K.W., Maung, W.P., Oo, T., 2019. Emerging neglected helminthiasis and determinants of multiple helminth infections in flood-prone township in Myanmar. Trop. Med. Health 47.

Harper, J., Bielefeldt, A., Javernick-Will, A., Veasna, T., Nicoletti, C., 2020. Context and intentions: practical associations for fecal sludge management in rural low-income Cambodia. J. Water, Sanit. Hyg. Dev. 10 (2), 191–201.

Harrington, H., Bradbury, R., Taeka, J., Asugeni, J., Asugeni, V., Igeni, T., Gwala, J., Newton, L., Anuabae, C.E.F., Kilivisi, F.L., Esau, D., Flores, A., Ribeyro, E., Liku, D., Muse, A., Asugeni, L., Talana, J., Shield, J., MacLaren, D.J., Massey, P.D., Muller, R., Speare, R., 2015. Prevalence of soil-transmitted helminths in remote villages in East Kwaio, Solomon Islands. West. Pacific Surveill. Res. 6 (3), 51–58.

Hiscox, A., Hirooka, R., Vongphayloth, K., Hill, N., Lindsay, S.W., Grandadam, M., Brey, P.T., 2016. Armigeres subalbatus colonization of damaged pit latrines: a nuisance and potential health risk to residents of resettlement villages in Laos. Med. Vet. Entomol. 30 (1), 95–100.

Howard, G., Calow, R., Macdonald, A., Bartram, J., 2016. Climate change and water and sanitation: likely impacts and emerging trends for action. Annu. Rev. Environ. Resour. 41, 253–276.

Huda, T.M.N., Schmidt, W.P., Pickering, A.J., Mahmud, Z.H., Islam, M.S., Rahman, M.S., Luby, S.P., Biran, A., 2018. A cross sectional study of the association between sanitation type and fecal contamination of the household environment in rural Bangladesh. Am. J. Trop. Med. Hyg. 98 (4), 967–976.

Huda, T.M.N., Schmidt, W.P., Pickering, A.J., Unicomb, L., Mahmud, Z.H., Luby, S.P., Biran, A., 2019. Effect of neighborhood sanitation coverage on fecal contamination of the household environment in rural Bangladesh. Am. J. Trop. Med. Hyg. 100 (3), 717–726.

Huda, T.M.N., Unicomb, L., Johnston, R.B., Halder, A.K., Sharker, M.A.Y., Luby, S.P., 2012. Interim evaluation of a large scale sanitation, hygiene and water improvement programme on childhood diarrhea and respiratory disease in rural Bangladesh. Soc. Sci. Med. 75 (4), 604–611.

Hunter, P.R., Risebro, H., Yen, M., Lefebvre, H., Lo, C., Hartemann, P., Longuet, C., Jaquenoud, F., 2013. Water source and diarrhoeal disease risk in children under 5 years old in Cambodia: a prospective diary based study. BMC Publ. Health 13.

Hussain, F., Clasen, T., Akter, S., Bawel, V., Luby, S.P., Leontsini, E., Unicomb, L., Barua, M.K., Thomas, B., Winch, P.J., 2017. Advantages and limitations for users of double pit pour-flush latrines: a qualitative study in rural Bangladesh. BMC Publ. Health 17.

Hutton, G., Varughese, M., 2016. The Costs of Meeting the 2030 Sustainable Development Goal Targets on Drinking Water, Sanitation, and Hygiene. http://hdl.handle.net/10986/23681. accessed. 04.04.2021.

Hylton, E., Noad, L., Templeton, M.R., Sule, M.N., 2020. The Rate of Vermi-Compost Accumulation within "Tiger Toilets" in India. Environmental Technology.

icddrb, 2019. Annual Report 2019. https://www.icddrb.org/dmdocuments/AR2019_17Jun2020.pdf. (Accessed 15 May 2021).

IHME, 2021. Global burden of disease study 2017 (GBD 2017) Results. http://ghdx.healthdata.org/gbd-results-tool accessed 01.04.2021.

Islam, M., Ercumen, A., Ashraf, S., Rahman, M., Shoab, A.K., Luby, S.P., Unicomb, L., 2018. Unsafe disposal of feces of children< 3 years among households with latrine access in rural Bangladesh: association with household characteristics, fly presence and child diarrhea. PLoS One 13 (4), e0195218.

Islam, M., Rahman, M., Unicomb, L., Kafi, M.A.H., Rahman, M., Alam, M., Sen, D., Islam, S., Pickering, A.J., Hubbard, A.E., 2020. Child defecation and feces management practices in rural Bangladesh: associations with fecal contamination, observed hand cleanliness and child diarrhea. PLoS One 15 (7).

Janmohamed, A., Karakochuk, C.D., McLean, J., Green, T.J., 2016. Improved sanitation facilities are associated with higher body mass index and higher hemoglobin concentration among rural Cambodian women in the first trimester of pregnancy. Am. J. Trop. Med. Hyg. 95 (5), 1211–1215.

Jenkins, A.P., Jupiter, S.D., Jenney, A., Rosa, V., Naucukidi, A., Prasad, N., Vosaki, G., Mulholland, K., Strugnell, R., Kama, M., Crump, J.A., Horwitz, P., 2019. Environmental foundations of typhoid fever in the Fijian residential setting. Int. J. Environ. Res. Publ. Health 16, 13.

Jensen, P.K.M., Phuc, P.D., Knudsen, L.G., Dalsgaard, A., Konradsen, F., 2008. Hygiene versus fertiliser: the use of human excreta in agriculture - a Vietnamese example. Int. J. Hyg Environ. Health 211 (3–4), 432–439.

Jewitt, S., Mahanta, A., Gaur, K., 2018. Sanitation sustainability, seasonality and stacking: improved facilities for how long, where and whom? Geogr. J. 184 (3), 255–268.

Jones, E.R., van Vliet, M.T., Qadir, M., Bierkens, M.F., 2021. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. Earth Syst. Sci. Data 13 (2), 237–254.

Khandekar, R., Thanah, T.T.K., Thi, P.D., 2006. Impact of face washing and environmental improvement on reduction of active trachoma in Vietnam - a public health intervention study. Ophthalmic Epidemiol. 13 (1), 43–52.

Khieu, V., Hattendorf, J., Schar, F., Marti, H., Char, M.C., Muth, S., Odermatt, P., 2014a. Strongyloides stercoralis infection and re-infection in a cohort of children in Cambodia. Parasitol. Int. 63 (5), 708–712.

Khieu, V., Schar, F., Forrer, A., Hattendorf, J., Marti, H., Duong, S., Vounatsou, P., Muth, S., Odermatt, P., 2014b. High prevalence and spatial distribution of Strongyloides stercoralis in rural Cambodia. PLoS Neglected Trop. Dis. 8 (6).

Khieu, V., Schar, F., Marti, H., Bless, P.J., Char, M.C., Muth, S., Odermatt, P., 2014c. Prevalence and Risk Factors of Strongyloides Stercoralis in Takeo Province, Cambodia, vol. 7. Parasites and Vectors.

Khieu, V., Schar, F., Marti, H., Sayasone, S., Duong, S., Muth, S., Odermatt, P., 2013. Diagnosis, treatment and risk factors of Strongyloides stercoralis in schoolchildren in Cambodia. PLoS Neglected Trop. Dis. 7 (2).

Knight, S.M., Toodayan, W., Caique, W.C., Kyi, W., Barnes, A., Desmarchelier, P., 1992. Risk-factors for the transmission of diarrhea in children - a case-control study in rural Malaysia. Int. J. Epidemiol. 21 (4), 812–818.

Koottatep, T., Taweesan, A., Kanabkaew, T., Polprasert, C., 2021. Inconvenient truth: unsafely managed fecal sludge after achieving MDG for decades in Thailand. J. Water, Sanit. Hyg. Dev. 11 (6), 1062–1070.

Kounnavong, S., Vonglokham, M., Houamboun, K., Odermatt, P., Boupha, B., 2011. Soil-transmitted helminth infections and risk factors in preschool children in southern rural Lao People's Democratic Republic. Trans. R. Soc. Trop. Med. Hyg. 105 (3), 160–166.

Kundu, A., Smith, W.A., Harvey, D., Wuertz, S., 2018. Drinking water safety: role of hand hygiene, sanitation facility, and water system in semi-urban areas of India. Am. J. Trop. Med. Hyg. 99 (4), 889–898.

Kurscheid, J., Bendrups, D., Susilo, J., Williams, C., Amaral, S., Laksono, B., Stewart, D. E., Gray, D.J., 2018. Shadow puppets and neglected diseases: evaluating a health promotion performance in rural Indonesia. Int. J. Environ. Res. Publ. Health 15 (9).

Labana, R.V., Dungca, J.Z., Nissapatorn, V., 2018. Community-based Surveillance of Cryptosporidium in the Indigenous Community of Boliwong, Philippines: from April to December 2017', Epidemiology and Health, vol. 40.

Labrique, A.B., Zaman, K., Hossain, Z., Saha, P., Yunus, M., Hossain, A., Ticehurst, J., Kmush, B., Nelson, K.E., 2013. An exploratory case control study of risk factors for hepatitis E in rural Bangladesh. PLoS One 8 (5).

Le, T.T.X., Hoat, L.N., Rheinlander, T., Dalsgaard, A., Konradsen, F., 2012. Sanitation behavior among schoolchildren in a multi-ethnic area of northern rural Vietnam. BMC Publ. Health 12.

Li, H.X., Yao, W., Dong, G.Q., Wang, L., Luo, Q., Wang, S., Xiong, C.L., Zhang, Q., 2016. Water and sanitation interventions to control diarrheal disease in rural China. J. Water, Sanit. Hyg. Dev. 6 (4), 640–649.

Li, X.-X., 2020. Heat wave trends in Southeast Asia during 1979–2018: the impact of humidity. Sci. Total Environ. 721, 137664.

Li, X.L., Hu, Q.H., Miao, Y.Q., Chen, W.J., Yuan, C.H., 2015. Household access to sanitation facilities in rural China. J. Water, Sanit. Hyg. Dev. 5 (3), 465–473.

Lourenço, N., Nunes, L., 2020. Review of dry and wet decentralized sanitation technologies for rural areas: applicability, challenges and opportunities. Environ. Manag. 65 (5), 642–664.

Luby, S.P., Rahman, M., Arnold, B.F., Unicomb, L., Ashraf, S., Winch, P.J., Stewart, C.P., Begum, F., Hussain, F., Benjamin-Chung, J., Leontsini, E., Naser, A.M., Parvez, S.M., Hubbard, A.E., Lin, A., Nizame, F.A., Jannat, K., Ercumen, A., Ram, P.K., Das, K.K., Abedin, J., Clasen, T.F., Dewey, K.G., Fernald, L.C., Null, C., Ahmed, T., Colford, J. M., 2018. Effects of water quality, sanitation, handwashing, and nutritional interventions on diarrhoea and child growth in rural Bangladesh: a cluster randomised controlled trial. Lancet Global Health 6 (3), E302–E315.

MacDonald, M.C., Chan, T., Elliott, M., Kearton, A., Shields, K.F., Barrington, D.J., Souter, R.T., Powell, B.R., Bartram, J., Hadwen, W.L., 2017. Temporal and thematic trends in water, sanitation and hygiene (WaSH) research in Pacific Island countries: a systematic review. J. Water, Sanit. Hyg. Dev. 7 (3), 352–368.

Mara, D., Evans, B., 2018. The sanitation and hygiene targets of the sustainable development goals: scope and challenges. J. Water, Sanit. Hyg. Dev. 8 (1), 1–16.

Maung, K., Khin, M., Wai, N.N., Hman, N.W., Myint, T.T., Butler, T., 1992. Risk-factors for the development of persistent diarrhea and malnutrition in Burmese children. Int. J. Epidemiol. 21 (5), 1021–1029.
McClure, K., Jun, J.K., Johnson, C.R., Fischer, P.R., Lu, L., Vy, S., Knutson, D., 2018. Disparities in rural-vs-urban achievement of millennium development goals in Cambodia: implications for current and future child health. Paediatr. Int. Child Health 38 (4), 235–243.
McGinnis, S., Marini, D., Amatya, P., Murphy, H.M., 2019. Bacterial contamination on latrine surfaces in community and household latrines in Kathmandu, Nepal. Int. J. Environ. Res. Publ. Health 16 (2).
Minh, H.V., Hung, N.V., Thanh, N.H., Yang, J.C., 2013. Assessing willingness to pay for improved sanitation in rural Vietnam. Environ. Health Prev. Med. 18 (4), 275–284.
Mohd-Shaharuddin, N., Lim, Y.A.L., Hassan, N.A., Nathan, S., Ngui, R., 2018. Soil-transmitted helminthiasis among indigenous communities in Malaysia: is this the endless malady with no solution? Trop. Biomed. 35 (1), 168–180.
Moore, C.E., Elwin, K., Phot, N., Seng, C., Mao, S., Suy, K., Kumar, V., Nader, J., Bousfield, R., Perera, S., Bailey, J.W., Beeching, N.J., Day, N.P.J., Parry, C.M., Chalmers, R.M., 2016. Molecular characterization of Cryptosporidium species and Giardia duodenalis from symptomatic Cambodian children. PLoS Neglected Trop. Dis. 10 (7).
Morrison, A.L., Lewthwaite, H., Houghton, L.A., Nasak, D.S.J., Sharples, K.J., Brown, P., Crump, J.A., Jack, S.J., 2020. Child undernutrition in households with microbiologically safer drinking water and 'improved water. In: Tanna, Vanuatu' (Ed.), J. Water Health 18 (3), 416–429.
Mrimi, E.C., Matwewe, F.J., Kellner, C.C., Thomas, J.M., 2020. Safe resource recovery from faecal sludge: evidence from an innovative treatment system in rural Tanzania. Environ. Sci. J. Integr. Environ. Res.: Water Res. Technol. 6 (6), 1737–1748.
Nakagiri, A., Niwagaba, C.B., Nyenje, P.M., Kulabako, R.N., Tumuhairwe, J.B., Kansiime, F., 2015. Are pit latrines in urban areas of Sub-Saharan Africa performing? a review of usage, filling, insects and odour nuisances. BMC Publ. Health 16 (1), 1–16.
Naser, A., Doza, S., Rahman, M., Ahmed, K.M., Gazi, M.S., Alam, G.R., Karim, M.R., Khan, G.K., Uddin, M.N., Mahmud, M.I., Ercumen, A., Rosenbaum, J., Annis, J., Luby, S.P., Unicomb, L., Clasen, T.F., 2019. Sand barriers around latrine pits reduce fecal bacterial leaching into shallow groundwater: a randomized controlled trial in coastal Bangladesh. Environ. Sci. Technol. 53 (4), 2105–2113.
Nasr, N.A., Al-Mekhlafi, H.M., Ahmed, A., Roslan, M.A., Bulgiba, A., 2013. Towards an Effective Control Programme of Soil-Transmitted Helminth Infections Among Orang Asli in Rural Malaysia. Part 1: Prevalence and Associated Key Factors, vol. 6. Parasites and Vectors.
Nasr, N.A., Al-Mekhlafi, H.M., Lim, Y.A.L., Elyana, F.N., Sady, H., Atroosh, W.M., Dawaki, S., Al-Delaimy, A.K., Al-Areeqi, M.A., Wehaish, A.A., Anuar, T.S., Mahmud, R., 2020. A holistic approach is needed to control the perpetual burden of soil-transmitted helminth infections among indigenous schoolchildren in Malaysia. Pathog. Glob. Health 114 (3), 145–159.
Nelson, K.B., Karver, J., Kullman, C., Graham, J.P., 2014. User perceptions of shared sanitation among rural households in Indonesia and Bangladesh. PLoS One 9 (8), e103886.
Nery, S.V., Clarke, N.E., Richardson, A., Traub, R., McCarthy, J.S., Gray, D.J., Vallely, A. J., Williams, G.M., Andrews, R.M., Campbell, S.J., Clements, A.C.A., 2019a. Risk factors for infection with soil-transmitted helminths during an integrated community level water, sanitation, and hygiene and deworming intervention in Timor-Leste. Int. J. Parasitol. 49 (5), 389–396.
Nery, S.V., Traub, R.J., McCarthy, J.S., Clarke, N.E., Amaral, S., Llewellyn, S., Weking, E., Richardson, A., Campbell, S.J., Gray, D.J., Vallely, A.J., Williams, G.M., Andrews, R. M., Clements, A.C.A., 2019b. WASH for WORMS: a cluster-randomized controlled trial of the impact of a community integrated water, sanitation, and hygiene and deworming intervention on soil-transmitted helminth infections. Am. J. Trop. Med. Hyg. 100 (3), 750–761.
Ngui, R., Aziz, S., Chua, K.H., Aidil, R.M., Lee, S.C., Tan, T.K., Sani, M.M., Arine, A.F., Rohela, M., Lim, Y.A.L., 2015. Patterns and risk factors of soil-transmitted helminthiasis among Orang Ashi subgroups in Peninsular Malaysia. Am. J. Trop. Med. Hyg. 93 (2), 361–370.
Ngui, R., Ishak, S., Chuen, C.S., Mahmud, R., Lim, Y.A.L., 2011. Prevalence and risk factors of intestinal parasitism in rural and remote west Malaysia. PLoS Neglected Trop. Dis. 5 (3).
Nguyen, T.V., Pham, Q.D., Do, Q.K., Diep, T.T., Phan, H.C., Ho, T.V., Do, H.T., Phan, L.T., Tran, H.N., 2017. Cholera returns to southern Vietnam in an outbreak associated with consuming unsafe water through iced tea: a matched case-control study. PLoS Neglected Trop. Dis. 11 (4).
Nisha, M., Aiman, M., Asyhira, N., Syafiq, H., Atiqah, N., Kumarasamy, V., Tan, M.P., Davamani, F., 2020. Risk factors associated with soil transmitted helminth (STH) infection in two indigenous communities in Malaysia. Trop. Biomed. 37 (2), 379–388.
O'Reilly, K., Louis, E., Thomas, E., Sinha, A., 2015. Combining sensor monitoring and ethnography to evaluate household latrine usage in rural India. J. Water, Sanit. Hyg. Dev. 5 (3), 426–438.
Olita'a, D., Vince, J., Ripa, P., Tefuarani, N., 2014. Risk factors for malnutrition in children at Port Moresby general hospital, Papua New Guinea: a case-control study. J. Trop. Pediatr. 60 (6), 442–448.
Openshaw, J.J., Medina, A., Felt, S.A., Li, T.Y., Huan, Z., Rozelle, S., Luby, S.P., 2018. Prevalence and risk factors for Taenia solium cysticercosis in school-aged children: a school based study in western Sichuan, People's Republic of China. PLoS Neglected Trop. Dis. 12 (5).
Padhi, B.K., Baker, K.K., Dutta, A., Cumming, O., Freeman, M.C., Satpathy, R., Das, B.S., Panigrahi, P., 2015. Risk of adverse pregnancy outcomes among women practicing poor sanitation in rural India: a population-based prospective cohort study. PLoS Med. 12 (7).
Perera, P.J., Disanayake, D., Fernando, M.P., Warnakulasoority, T.D., Ranathunga, N., 2012. Knowledge and practices related to helminth infections among mothers living in a suburban area of Sri Lanka. Southeast Asian J. Trop. Med. Publ. Health 43 (6), 1326–1332.
Pham-Duc, P., Nguyen-Viet, H., Hattendorf, J., Cam, P.D., Zurbrugg, C., Zinsstag, J., Odermatt, P., 2014. Diarrhoeal diseases among adult population in an agricultural community Hanam province, Vietnam, with high wastewater and excreta re-use. BMC Publ. Health 14.
Phuc, P.D., Hung, N.V., Hattendorf, J., Zinsstag, J., Cam, P.D., Zurbrugg, C., Odermatt, P., 2013. Ascaris lumbricoides and Trichuris trichiura infections associated with wastewater and human excreta use in agriculture in Vietnam. Parasitol. Int. 62 (2), 172–180.
Prasad, N., Jenkins, A.P., Naucukidi, L., Rosa, V., Sahu-Khan, A., Kama, M., Jenkins, K. M., Jenney, A.W.J., Jack, S.J., Saha, D., Horwitz, P., Jupiter, S.D., Strugnell, R.A., Mulholland, E.K., Crump, J.A., 2018. Epidemiology and risk factors for typhoid fever in central division, Fiji, 2014-2017: a case-control study. PLoS Neglected Trop. Dis. 12 (6).
Prüss-Ustün, A., Bartram, J., Clasen, T., Colford Jr., J.M., Cumming, O., Curtis, V., Bonjour, S., Dangour, A.D., De France, J., Fewtrell, L., 2014. Burden of disease from inadequate water, sanitation and hygiene in low-and middle-income settings: a retrospective analysis of data from 145 countries. Trop. Med. Int. Health 19 (8), 894–905.
Psutka, R., Priest, P., Davies, T., Rakunuea, T., Iddings, S., Reiffer, A., 2013. Assessing the demographic, behavioural and environmental characteristics and the potential effectiveness of a household water filter in the Republic of Kiribati. J. Water, Sanit. Hyg. Dev. 3 (4), 530–540.
Pujari, P.R., Padmakar, C., Labhasetwar, P.K., Mahore, P., Ganguly, A., 2012. Assessment of the impact of on-site sanitation systems on groundwater pollution in two diverse geological settings - a case study from India. Environ. Monit. Assess. 184 (1), 251–263.
Rah, J.H., Sukotjo, S., Badgaiyan, N., Cronin, A.A., Torlesse, H., 2020. Improved sanitation is associated with reduced child stunting amongst Indonesian children under 3 years of age. Matern. Child Nutr. 16.
Rajoo, Y., Ambu, S., Lim, Y.A.L., Rajoo, K., Tey, S.C., Lu, C.W., Ngui, R., 2017. Neglected intestinal parasites, malnutrition and associated key factors: a population based cross-sectional study among indigenous communities in Sarawak, Malaysia. PLoS One 12 (1).
Rashid, M., Pandit, D., 2020. An Assessment of Service Level of Household Toilet Attributes Based on the Users' Satisfaction in Rural Bihar. Environment Development and Sustainability, India.
Ravenscroft, P., Mahmud, Z.H., Islam, M.S., Hossain, A., Zahid, A., Saha, G.C., Ali, A., Islam, K., Cairncross, S., Clemens, J.D., Islam, M.S., 2017. The public health significance of latrines discharging to groundwater used for drinking. Water Res. 124, 192–201.
Reese, H., Routray, P., Torondel, B., Sinharoy, S.S., Mishra, S., Freeman, M.C., Chang, H. H., Clasen, T., 2019. Assessing longer-term effectiveness of a combined household-level piped water and sanitation intervention on child diarrhoea, acute respiratory infection, soil-transmitted helminth infection and nutritional status: a matched cohort study in rural Odisha, India. Int. J. Epidemiol. 48 (6), 1757–1767.
Renzaho, A., Chitekwe, S., Chen, W., Rijal, S., Dhakal, T., Chikazaza, I.R., Dahal, P., 2018. Impact of a multidimensional child cash grant programme on water, sanitation and hygiene in Nepal. J. Water, Sanit. Hyg. Dev. 8 (3), 520–532.
Rheinlander, T., Samuelsen, H., Dalsgaard, A., Konradsen, F., 2010. Hygiene and sanitation among ethnic minorities in Northern Vietnam: does government promotion match community priorities? Soc. Sci. Med. 71 (5), 994–1001.
Riaz, M., Aslam, N., Zainab, R., Aziz-Ur-Rehman, Rasool, G., Ullah, M.I., Daniyal, M., Akram, M., 2020. Prevalence, risk factors, challenges, and the currently available diagnostic tools for the determination of helminths infections in human. Eur. J. Inflamm. 18.
Sasongko, E.P.S., Ariyanto, E.F., Indraswari, N., Rachmi, C.N., Alisjahbana, A., 2019. Determinants of adolescent shortness in tanjungsari, west Java, Indonesia. Asia Pac. J. Clin. Nutr. 28, S43–S50.
Sayasone, S., Mak, T.K., Vanmany, M., Rasphone, O., Vounatsou, P., Utzinger, J., Akkhavong, K., Odermatt, P., 2011. Helminth and intestinal protozoa infections, multiparasitism and risk factors in Champasack province, Lao People's Democratic Republic. PLoS Neglected Trop. Dis. 5 (4).
Sayasone, S., Odermatt, P., Phoumindr, N., Vongsaravane, X., Sensombath, V., Phetsouvanh, R., Choulamany, X., Strobel, M., 2007. Epidemiology of opisthorchis viverrini in a rural district of southern Lao PDR. Trans. R. Soc. Trop. Med. Hyg. 101 (1), 40–47.
Shahar, S., Lau, H., Puteh, S.E.W., Amara, S., Razak, N.A., 2019. Health, Access and Nutritional Issues Among Low-Income Population in Malaysia: Introductory Note. Springer.
Sheng-jie, S., Dong-liang, L., 2018. Variability in the Western Pacific subtropical high and its relationship with sea temperature variation considering the background of climate warming over the past 60 years. J. Trop. Meteorol. 24 (4).
Shimi, A.C., Parvin, G.A., Biswas, C., Shaw, R., 2010. Impact and adaptation to flood', disaster prevention and management. Int. J.
Shrestha, A., Schindler, C., Odermatt, P., Gerold, J., Erismann, S., Sharma, S., Koju, R., Utzinger, J., Cisse, G., 2018. Intestinal Parasite Infections and Associated Risk Factors Among Schoolchildren in Dolakha and Ramechhap Districts, Nepal: a Cross-Sectional Study, vol. 11. Parasites and Vectors.
Shrestha, A., Six, J., Dahal, D., Marks, S., Meierhofer, R., 2020. Association of nutrition, water, sanitation and hygiene practices with children's nutritional status, intestinal

parasitic infections and diarrhoea in rural Nepal: a cross-sectional study. BMC Publ. Health 20 (1).
Sinclair, R.G., Gerba, C.P., 2011. Microbial contamination in kitchens and bathrooms of rural Cambodian village households. Lett. Appl. Microbiol. 52 (2), 144–149.
Sinha, A., Nagel, C.L., Schmidt, W.P., Torondel, B., Boisson, S., Routray, P., Clasen, T.F., 2017. Assessing patterns and determinants of latrine use in rural settings: a longitudinal study in Odisha, India. Int. J. Hyg Environ. Health 220 (5), 906–915.
Strande, L., Brdjanovic, D., 2014. Faecal Sludge Management: Systems Approach for Implementation and Operation. IWA publishing.
Suntaravitun, P., Dokmaikaw, A., 2018. Prevalence of intestinal parasites and associated risk factors for infection among rural communities of Chachoengsao province, Thailand. Kor. J. Parasitol. 56 (1), 33–39.
Sustainable Sanitation Alliance, 2022. The SFD Approach; Urban Sanitation and the SFD Approach. https://sfd.susana.org/about/the-sfd. (Accessed 8 March 2022).
Tey, N.P., Lai, S.L., Ng, S.T., Goh, K.L., Osman, A.F., 2019. Income inequality across states in Malaysia. Planning Malaysia 17, 10.
Tilley, E., Ulrich, L., Lüthi, C., Reymond, P., Schertenleib, R., Zurbrügg, C., 2014. Compendium of Sanitation Systems and Technologies. Eawag.
Torlesse, H., Cronin, A.A., Sebayang, S.K., Nandy, R., 2016. Determinants of stunting in Indonesian children: evidence from a cross-sectional survey indicate a prominent role for the water, sanitation and hygiene sector in stunting reduction. BMC Publ. Health 16.
Torondel, B., Ensink, J.H.J., Gundogdu, O., Ijaz, U.Z., Parkhill, J., Abdelahi, F., Nguyen, V.A., Sudgen, S., Gibson, W., Walker, A.W., Quince, C., 2016. Assessment of the influence of intrinsic environmental and geographical factors on the bacterial ecology of pit latrines. Microb. Biotechnol. 9 (2), 209–223.
Trang, D.T., Molbak, K., Cam, P.D., Dalsgaard, A., 2007. Helminth infections among people using wastewater and human excreta in peri-urban agriculture and aquaculture in Hanoi, Vietnam. Trop. Med. Int. Health 12, 82–90.
Tummers, L., Teo, S., Brunetto, Y., Palumbo, R., 2016. Contextualizing co-production of health care: a systematic literature review. Int. J. Public Sect. Manag.
Uddin, S.M., Ronteltap, M., van Lier, J.B., 2013. Assessment of urine diverting dehydrating toilets as a flood-resilient and affordable sanitation technology in the context of Bangladesh. J. Water, Sanit. Hyg. Dev. 3 (2), 87–95.
Uddin, S.M.N., Li, Z.F., Gaillard, J.C., Tedoff, P.F., Mang, H.P., Lapegue, J., Huba, E.M., Kummel, O., Rheinstein, E., 2014. Exposure to WASH-borne hazards: a scoping study on peri-urban Ger areas in Ulaanbaatar, Mongolia. Habitat Int. 44, 403–411.
Uddin, S.M.N., Tempel, A., Adamowski, J.F., Lapegue, J., Li, Z.F., Mang, H.P., 2016. Exploring alternative sources of funding for deploying sustainable sanitation technologies and services in Mongolia. Int. J. Water Resour. Dev. 32 (6), 881–894.
UNDP, 2019. Human Development Report 2019. http://hdr.undp.org/en/content/human-development-report-2019. accessed. 23.11.2020.
UNICEF and WHO, 2019. Progress on Household Drinking Water, Sanitation and Hygiene 2000-2017: Special Focus on Inequalities. https://washdata.org/sites/default/files/documents/reports/2019-07/jmp-2019-wash-households.pdf. accessed. 23.11.2020.
UNICEF and WHO, 2020. State of the World's Sanitation: an Urgent Call to Transform Sanitation for Better Health, Environments, Economies and Societies. https://washdata.org/sites/default/files/2020-11/UNICEF-WHO-state-of-the-worlds-sanitation-2020.pdf. accessed. 23.11.2020.
United Nations, 2015. The Millennium Development Goals Report 2015. https://www.un.org/millenniumgoals/2015_MDG_Report/pdf/MDG%202015%20rev%20(July%201).pdf. accessed. 14.05.2021.
United Nations, 2018. Sustainable Development Goal 6: Synthesis Report 2018 on Water and Sanitation. http://www.unglobalcompact.org/docs/publications/SDG6_SR2018.pdf. accessed. 24.11.2020.
Vannavong, N., Seidu, R., Stenstrom, T.A., Dada, N., Overgaard, H.J., 2017. Effects of Socio-Demographic Characteristics and Household Water Management on Aedes aegypti Production in Suburban and Rural Villages in Laos and Thailand, vol. 10. Parasites and Vectors.
Velkushanova, K., Brdjanovic, D., Koottatep, T., Strande, L., Buckley, C., Ronteltap, M., 2021. Methods for Faecal Sludge Analysis. IWA publishing.
Verle, P., Kongs, A., De, N.V., Thieu, N.Q., Depraetere, K., Kim, H.T., Dorny, P., 2003. Prevalence of intestinal parasitic infections in northern Vietnam. Trop. Med. Int. Health 8 (10), 961–964.
Vilcins, D., Sly, P.D., Jagals, P., 2018. Environmental risk factors associated with child stunting: a systematic review of the literature. Ann. Global Health 84 (4), 551.
Vonghachack, Y., Odermatt, P., Taisayyavong, K., Phounsavath, S., Akkhavong, K., Sayasone, S., 2017. Transmission of Opisthorchis viverrini, Schistosoma mekongi and soil-transmitted helminthes on the Mekong Islands, southern Lao PDR. Infect. Dis. Poverty 6.
Wear, S.L., Thurber, R.V., 2015. Sewage pollution: mitigation is key for coral reef stewardship. Ann. N. Y. Acad. Sci. 1355.
Weaver, A.M., Khatun-e-Jannat, K., Cercone, E., Krytus, K., Sohel, B.M., Ahmed, M., Rahman, M., Azziz-Baumgartner, E., Yu, J., Fry, A.M., 2017. Household-level risk factors for secondary influenza-like illness in a rural area of Bangladesh. Trop. Med. Int. Health 22 (2), 187–195.
Web of Science™ 2021 https://clarivate.com/webofsciencegroup/solutions/web-of-science-core-collection/.
Weststrate, J., Dijkstra, G., Eshuis, J., Gianoli, A., Rusca, M., 2019. The sustainable development goal on water and sanitation: learning from the millennium development goals. Soc. Indicat. Res. 143 (2), 795–810.
WHO, 2013. Progress on sanitation and drinking-water - 2013 update. https://apps.who.int/iris/bitstream/handle/10665/81245/9789241505390_eng.pdf?sequence=1 accessed. 14.05.2021.
WHO, 2014. Preventing diarrhoea through better water, sanitation and hygiene: exposures and impacts in low- and middle-income countries. http://apps.who.int/iris/bitstream/handle/10665/150112/9789241564823_eng.pdf?sequence=1 accessed. 14.05.2021.
WHO, 2021. Definition of regional groupings. https://www.who.int/about/who-we-are/regional-offices accessed 27.03.2021.
WHO and UNICEF, 2018. JMP methodology: 2017 update and SDG baselines. https://washdata.org/sites/default/files/documents/reports/2018-04/JMP-2017-update-methodology.pdf accessed. 27.03.2021.
WHO and UNICEF, 2021. Progress on household drinking water, sanitation and hygiene 2000-2020: five years into the SDGs. https://washdata.org/sites/default/files/2022-01/jmp-2021-wash-households_3.pdf. (Accessed 1 February 2022).
WHO and UNICEF, 2020. WHO/UNICEF JMP global database. https://washdata.org/data/household#!/. (Accessed 1 April 2021).
Wong, H.J., Moy, F.M., Nair, S., 2014. Risk factors of malnutrition among preschool children in Terengganu, Malaysia: a case control study. BMC Publ. Health 14.
Wong, W.K., Foo, P.C., Roze, M.N.M., Pim, C.D., Subramaniam, P., Lim, B.H., 2016. Helminthic infection and nutritional studies among orang asli children in sekolah kebangsaan pos legap, perak. Can. J. Infect Dis. Med. Microbiol. 2016.
World Bank, 2021a. Rural Population (% of Total Population) - Malaysia. https://data.worldbank.org/indicator/SP.RUR.TOTL.ZS?locations=MY. (Accessed 26 March 2021).
World Bank, 2021b. World Development Indicators. https://databank.worldbank.org/source/world-development-indicators/preview/on. (Accessed 18 June 2021).
Xiao, P.L., Zhou, Y.B., Chen, Y., Yang, Y., Shi, Y., Gao, J.C., Yihuo, W.L., Song, X.X., Jiang, Q.W., 2015. Prevalence and Risk Factors of Ascaris lumbricoides (Linnaeus, 1758), Trichuris Trichiura (Linnaeus, 1771) and HBV Infections in Southwestern China: a Community-Based Cross Sectional Study, vol. 8. Parasites and Vectors.
Yajima, A., Pascal, J., Dung, D.T., Thach, D.T.C., Dai, T.C., Didier, O., Antonio, M., 2009. High latrine coverage is not reducing the prevalence of soil-transmitted helminthiasis in Hoa Binh province, Vietnam. Trans. R. Soc. Trop. Med. Hyg. 103 (3), 237–241.
Yogananth, N., Bhatnagar, T., 2018. Prevalence of open defecation among households with toilets and associated factors in rural south India: an analytical cross-sectional study. Trans. R. Soc. Trop. Med. Hyg. 112 (7), 349–360.

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Assessment of passive human exposure to tobacco smoke by environmental and biological monitoring in different public places in Wuhan, central China

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ABSTRACT

Passive exposure to tobacco smoke is a global public health problem, while there are few data on public place monitoring and general population exposure assessment in central China. This study aimed to examine the levels of airborne nicotine ($n = 256$) in ten kinds of different public places in Wuhan, central China, and assess short-term and long-term smoke exposure in 340 non-smokers aged 18–67 who worked in these public places using tobacco biomarkers [i.e., cotinine and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL), respectively]. The highest median concentration of airborne nicotine (17.0 μg/m^3) was observed in internet cafes, approximately 304-fold of the lowest value found in nurseries (55.9 ng/m^3). Among the other studied public places, restaurants had the highest median concentrations (ng/m^3) of airborne nicotine (3,120), followed by subway stations (810), hotels (624), government officess (286), middle schools (269), health institutions (268), public institutions (190), and primary schools (140). Urinary cotinine and NNAL were found in almost all the participants, and the highest concentrations were found in non-smokers from the internet cafes [specific gravity (SG)-corrected urinary median concentrations: 23.1 ng/mL, geometric mean (GM): 24.1 ng/mL, range: 0.62–1679 ng/mL] for cotinine and 104 pg/mL (GM: 97.6 pg/mL, range: 32.3–236 pg/mL) for NNAL, respectively]. Urinary cotinine concentrations in male non-smokers (median: 2.02 ng/mL) were significantly higher than those in female non-smokers (1.44) ($P < 0.01$). Participants aged 18–27 were detected with the highest urinary cotinine and NNAL concentrations. Urinary cotinine and NNAL concentrations were significantly correlated with daily and monthly working hours, respectively. Besides, a positive correlation was observed between log-transformed urinary concentrations of cotinine and NNAL ($r = 0.32$, $P < 0.001$). This is the first time to report matched data on airborne nicotine and urinary cotinine/NNAL among employees in different public places. This study demonstrated ubiquitous exposure to environmental tobacco smoke in the studied public places.

1. Introduction

Tobacco smoking, including active smoking and passive exposure to environmental tobacco smoke (ETS), is one of the major contributors to the burden of disease worldwide, resulting in 12% of all deaths among adults aged ≥30 (WHO, 2012). Tobacco smoking is also one of the leading risk factors for deaths and disability-adjusted life-years in China (Zhou et al., 2019), and approximately 16.4% of all deaths (1.4 million) were attributed to tobacco use in 2010 (Yang et al., 2015). China is the largest tobacco producer and consumer in the world (Yang et al., 2015), producing nearly 2.4 trillion cigarettes in 2010 (National Bureau of Statistics of China, 2010). In China, 52.9% of adult men (288 million) and 2.4% of adult women (12.6 million) smoked, with 72.4% of non-smoking adults (740 million) were exposed to second-hand smoke in public places (including ninety percent of restaurants, more than half of government buildings, and more than one third of schools, public transports, and health care facilities) in the 2010 Global Adults Tobacco Survey (CDC, 2011).

ETS, also known as “second-hand smoke”, occurs in homes, workplaces, and public places (Law and Hackshaw, 1996). There is no safe

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level of exposure to ETS. Prenatal, infancy, and childhood exposure to ETS have been reported to be associated with sudden infant death syndrome (Schoendorf and Kiely, 1992), adverse respiratory outcomes (Zhuge et al., 2020), the development of childhood asthma (Simons et al., 2014), inflammatory bowel disease (Mahid et al., 2007), passive early childhood temperament behaviors (Merianos et al., 2021), sleep problems in children (Yolton et al., 2010), and so on. To adults, ETS exposure can increase the risk of lung cancer (Anderson et al., 2001; Vineis et al., 2005), non-cancer respiratory diseases (Vineis et al., 2005), pregnancy failure (Benedict et al., 2011), cardiovascular diseases (Messner and Bernhard, 2014), and type 2 diabetes mellitus (Huang et al., 2020).

Biomarkers specific to ETS exposure are nicotine (the main alkaloid of tobacco) and its metabolites, and metabolites of 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK) (Avila-Tang et al., 2013), a kind of nitrosamine and strong carcinogen, formed by the reaction of nicotine or pseudo-oxynicotine and nitrite in tobacco during the curing process (Hecht, 1998). Nicotine and cotinine have been reported to be ubiquitous in indoor air and dust (Agbenyikey et al., 2011; Caman et al., 2013; Chinthakindi and Kannan, 2021; Chinthakindi et al., 2022). Urinary cotinine (a metabolite of nicotine) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL; a metabolite of NNK) are specific biomarkers widely used in assessment of short-term (Benowitz, 1999; Avila-Tang et al., 2013) and long-term (Avila-Tang et al., 2013) ETS exposure, respectively. Currently, the majority of studies on second-hand smoke exposure in public places in China are conducted through questionare survey without biomonitoring. Several studies have measured nicotine concentrations in air of public places, such as Beijing, Sichuan, Jiangxi, Henan (Stillman et al., 2007), Tianjin (Li et al., 2014), and Qingdao (Liu et al., 2019); moreover, these studies only included seven types of public places at most. A study reported concentrations of airborne nicotine in public places and urinary cotinine of staffs there in Dalian, China in 2016 (Yang et al., 2016); however, only hospital, restaurant, and government offices were selected as the monitoring sites, and concentrations of urinary NNAL were not measured. Data on matched nicotine in air and NNAL&cotinine in urine of staffs from various public places are available in developed countries such as Korea (Park et al., 2016), nevertheless, China has no such data.

Wuhan municipal government issued a rule on prohibiting smoking in public places in January 1995, and revised it in 1998 and 2005 (PGWC, 2005), respectively. In 2005, China ratified the WHO Framework Convention on Tobacco Control, calling for effective legislation to create a 100% smoke-free environment in all indoor public places, indoor workplaces, public vehicles, and other public workplaces (FCTC, 2007). Beijing enacted the strictest smoking control law in China in 2015, making it 100% smoke-free in all indoor public places and workplaces (Yang et al., 2015), and other cities have followed suit. Since July 2018, Wuhan has carried out all kinds of special control smoking campaigns in public places, including government offices, hospitals, schools, hotels, restaurants, cinemas, internet cafes, and public transports, to boost the healthy Wuhan Campaign and civilized Military Games in November 2019. This study evaluated the effectiveness of tobacco control campaign in Wuhan by investigating ETS exposure in public places in August and September 2019, and provided a reference for the effectiveness of Wuhan regulations on smoking control ratified by Wuhan Municipal People's Congress (WMPC, 2019), which came into force in January 2020.

This study was designed: (1) to determine the levels of airborne nicotine in public places (government offices, health institutions, public institutions, nurseries, primary schools, middle schools, subway stations, restaurants, hotels, and internet cafes) in Wuhan; (2) to assess the short-term and long-term ETS exposure in non-smokers who worked in these public places using cotinine and NNAL, respectively, characterizing their sex-, and age-related variations; and (3) to explore the relationship between urinary concentrations of cotinine and NNAL and selected determinants such as working hours.

2. Materials and methods

2.1. Chemicals

Nicotine (CAS: 54-11-5; 99.42%), nicotine-d4 (CAS: 350818-69-8; 98.96%), cotinine (CAS: 486-56-6; 99.49%), and cotinine-d3 (CAS: 110952-70-0; 98.48%) were all purchased from Cerilliant (Sigma-Aldrich Corp. St. Louis, MO, USA). 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanol (NNAL) (CAS: 76014-81-8; 95.00%) and NNAL-d5 (CAS: 1794885-45-2; 98.00%) were obtained from Toronto Research Chemical (North York, Ontario, Canada). Acetonitrile, methanol, and water (OPTIMA LC-MS grade) were obtained from Fisher Chemical (Thermo Fisher Scientific, Inc. Waltham, MA, USA).

2.2. Study design

Four administrative districts were randomly selected for sampling in Wuhan during August and September 2019, and the following ten types of public places were selected as monitoring sites in each district: government offices, health institutions, public institutions, nurseries, primary schools, middle schools, subway stations, restaurants, hotels, and internet cafes. Among the ten types of microenvironments in the four studied districts, one site of nurseries, primary schools, and middle schools was randomly selected for monitoring, respectively; two sites were randomly selected for other seven types of microenvironments. A total of 68 monitoring sites were selected.

In each of the monitoring sites, four points were sampled for airborne nicotine measurement, except for the subway stations (only two sampling points). A total of 256 sampling points were monitored using passive samplers. Specifically, lobbies, meeting rooms, stairwells, and male toilets in government offices, health institutions, and public institutions were monitored for five days; offices, meeting rooms, stairwells, and male toilets in nurseries, primary schools, and middle schools were monitored for five days; entrances and male toilets in subway stations were monitored for seven days; halls, private rooms, waiting lounge, and male toilets in restaurants were monitored for seven days; lobbies, hallways, stairwells, and male toilets in hotels and internet cafes were monitored for seven days. Sampling was performed during Monday morning and Friday/Sunday afternoon (for five days and seven days, respectively). The exact time of each sampling was recorded. Informed consent were obtained from managers of all the sampling sites.

In addition, a total of 340 non-smokers who worked in the monitoring sites were selected as participants, with 5 individuals from each site. Informed consent was obtained from all the participants and urine samples were collected at the end of work time in a week. Age, sex, daily working hours, and monthly working hours of each employee were recorded. All the urine samples were stored at −80 °C until analysis. This study was approved by the ethics committee of Wuhan Centers for Disease Control and Prevention.

2.3. Measurement of airborne nicotine

Airborne nicotine at each monitoring site was collected using a passive sampler, a polystyrene sampling cassette. The structure includes a filter membrane treated with sodium bisulfate that can enrich environmental nicotine, a protective filter membrane allowing air to pass through it at a speed of 24 mL/min, of which the aperture size is suitable for nicotine to pass through, and a clamp for fixation. The sampler was hanged on something at 1–2 m from the floor (e.g., beams, nails, plants, or lights) and at least 1 m away from the window or ventilation, making it difficult to be accessed and as concealed as possible. Places where air does not circulate, such as corners and curtains were not chosen. After monitoring, the sampler was stored in a sealed plastic cup until pretreatment. Nicotine was extracted from the filters as follows: 100 μL of isotope-labeled internal standard (nicotine-d4; 25 μg/mL) was added to the filter in a 5 mL of centrifuge tube, then 2 mL of 50% methanol

Table 1
Air nicotine concentrations in various public places in Wuhan, China.

Monitoring sites	No.	Days	Nicotine (ng/m^3)				
			Mean ± SD	GM	Median	Range	DF (%)
Internet cafes	32	7	24.4[a] ±20.9[a]	14.4[a]	17.0[a]	1.06[a]–80.7[a]	100
Restaurants	32	7	3.60[a] ±3.04[a]	2.56[a]	3.12[a]	0.20[a]–10.7[a]	100
Subway stations	16	7	1.59[a] ±2.15[a]	747	810	0.11[a]–7.96[a]	100
Hotels	32	7	2.57[a] ±6.87[a]	665	624	0.05[a]–37.9[a]	100
Government offices	32	5	454 ± 598	289	286	<MDL–3.16[a]	93.8
Middle schools	16	5	424 ± 483	238	269	<MDL–1.73[a]	93.8
Public institutions	32	5	376 ± 449	244	190	0.07[a]–2.03[a]	100
Health institutions	32	7	384 ± 526	226	268	0.06[a]–2.89[a]	100
Primary schools	16	5	180 ± 126	137	140	<MDL–409	81.2
Nurseries	16	5	154 ± 242	79.6	55.9	<MDL–821	43.8
F							51.5
P							0.000

[a] μg/m^3; GM: geometric mean; DF: detection frequency.

aqueous solution were added and the sample was ultrasonic extracted for 30 min. The extracts were filtered by 0.22 μm of pore size membrane filter, and the filtrate was detected using an AB SCIEX 4500 electrospray triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) in positive ionization mode. The method lower limit of quantification (LLOQ) of nicotine were 10 ng per a filter membrane [signal/noise (S/N): 14.4; coefficient of variation (CV): 2.2%].

2.4. Measurement of urinary cotinine and NNAL

Liquid chromatograph-mass spectrometer (LC-MS) grade water was used as the blank samples. An aliquot of 50 μL of isotope-labeled internal standard (cotinine-d3; 50 ng/mL) was added to 1.5 mL centrifuge tube containing 50 μL urine samples. Each sample was added with 200 μL of 5% ammonia methanol solution and shaken for 15 min, and then centrifuged for 3 min at 12,000 *g*. The supernatant was collected and the analytes were detected using an AB SCIEX 5500 electrospray triple quadrupole mass spectrometer (Applied Biosystems, Foster City, CA, USA) in positive ionization mode. The method LLOQ of cotinine were 0.5 ng/mL (S/N: 12; CV: 4.0%).

An aliquot of 50 μL of isotope-labeled internal standard (NNAL-d5; 5 ng/mL) was then added to the urine sample (1 mL of urine sample or blank in a 5 mL centrifuge tube). After vortex, 50 μL of β-glucuronidase (20 mg/mL; pH = 5) was added into each sample, and the samples were water bathed at 37 °C overnight. Then, the sample was added with 100 μL of 50% K_2CO_3 aqueous solution (w/v) and 2 mL of toluene: n-butyl alcohol extract (7:3), shaken for 5 min, and then centrifuged for 3 min at 2500 *g*. The supernatant was collected and evaporated gently with nitrogen; the residue was dissolved with 100 μL of acetonitrile and then centrifuged for 3 min at 2500 *g* to remove any suspended solids for further analysis. The analytes were detected using an AB SCIEX 5500 electrospray triple quadrupole mass spectrometer in positive ionization mode. The method LLOQ of NNAL were 5 pg/mL (S/N: 10.0; CV: 2.5%).

2.5. Quality control and assurance

For quality control, a field blank and a duplicate were included in each batch of ten samples. The recoveries of nicotine, cotinine and NNAL spiked into each samples ranged from 85% to 115%. Background values of any target analytes in the blank samples (if found) were subtracted.

2.6. Estimated daily intake

The estimated daily intake (EDI; μg/kg-bw/day) of airborne nicotine in public places for employees was calculated based on the formula of air inhalation exposure (Wan et al., 2016): $EDI = C \times AIR \times \frac{IEF}{BW}$, where *C* is the median concentration of airborne nicotine (μg/m^3), *AIR* represents the air inhalation rate (15.7 m^3/day for adults), *IEF* is the working time exposure fraction (the fraction of time spent at work, 0.42), and *BW* is the adults bodyweight (60 kg). We hypothesized that airborne nicotine is absorbed with 100% efficiency to systemic blood circulation.

2.7. Statistical analysis

Data analysis was carried out using SPSS 20.0 (SPSS Inc, Chicago, USA). Mean, geometric mean (GM), median, and range were calculated. Concentrations below the LLOQ were substituted with a value equal to LLOQ divided by the square root of 2 for calculating GMs. Normal distribution of the residuals was tested using Shapiro–Wilk test and the homoscedasticity of the variance was checked by Levene's Test. The data was log-transformed for statistical analyses if the measured concentrations were not normally distributed. If the data still did not follow a normal distribution after log-transformation or with heterogeneity of variance, a nonparametric test (Mann–Whitney *U* or Kruskal–Wallis *H*) was performed. Statistical significance was considered at $P < 0.05$.

3. Results and discussion

3.1. Air nicotine concentrations and exposure assessment

The concentrations of airborne nicotine in the ten types of public places in Wuhan are listed in Tables 1 and 2. The highest nicotine values were observed in internet cafes and the lowest were found in nurseries, of which the GM concentrations were 14.4 μg/m^3 and 79.6 ng/m^3, respectively, and the former was approximately 181-fold of the latter (Table 1). Among the other public places, the GM concentrations of airborne nicotine were found in descending order as restaurants (2.56 μg/m^3), subway stations (747 ng/m^3), hotels (665), government offices (289), middle schools (238), public institutions (244), health institutions (226), and primary schools (137). Significant difference ($P < 0.01$) was observed in log-transformed air nicotine concentrations among the ten types of monitoring sites (Table 1). The airborne nicotine concentrations in this study and their ranking in the public places were similar or consistent with those in Barcelona, Spain 20 years ago (Jané et al., 2002), which had a reduction of environmental nicotine levels over 90% after fourteen years of tobacco control law in Spain (Córdoba-García, 2020). Compared with highly comparable research results in other parts of China, such as Qingdao, the nicotine concentration in other places is higher than that in Qingdao, except for internet cafes, which have half the nicotine concentration (Liu et al., 2019).

Except for nurseries and internet cafes, where the highest levels of nicotine were found in meeting rooms and hallways, respectively, the highest levels in other types of public places were found in male toilets (Table 2). And the nicotine level in male toilets of nurseries was next only to the nicotine level in meeting rooms. The difference between the highest and lowest concentrations of nicotine varied between 2.5 and

Table 2
Air nicotine concentrations in specific sampling points of the monitoring sites in public places in Wuhan, China.

Monitoring sites	Monitoring locations	No.	Nicotine (ng/m^3)						
			Mean ± SD	GM	25th	Median	75th	Range	DF (%)
Internet cafes	lobbies	8	30.3[a] ±21.0[a]	21.7[a]	13.1[a]	29.2[a]	48.4[a]	2.86[a]–60.3[a]	100
	hallways	8	36.7[a] ±25. 5[a]	26.0[a]	15.6[a]	36.2[a]	55.1[a]	2.79[a]–80.7[a]	100
	stairwells	8	8.14[a] ±10.2[a]	4.44[a]	2.24[a]	2.76[a]	14.9[a]	1.06[a]–29.7[a]	100
	male toilets	8	22.6[a] ±14.9[a]	17.4[a]	9.44[a]	20.2[a]	34.3[a]	3.69[a]–47.4[a]	100
F									4.93
P									0.007
Restaurants	halls	8	2.92[a] ±2.25[a]	2.00[a]	873	2.77[a]	4.68[a]	0.32[a]–6.70[a]	100
	private rooms	8	3.79[a] ±4.23[a]	1.25[a]	651	1.25[a]	8.26[a]	0.20[a]–10.7[a]	100
	waiting lounge	8	1.87[a] ±1.19[a]	1.72[a]	746	1.73[a]	3.17[a]	0.36[a]–3.23[a]	100
	male toilets	8	5.81[a] ±2.64[a]	6.08[a]	3.93[a]	6.08[a]	8.01[a]	1.08[a]–9.13[a]	100
F									2.21
P									0.11
Subway stations	entrances	8	513 ± 457	382	207	363	757	0.13[a]–1.50[a]	100
	male toilets	8	2.67[a] ±2.66[a]	1.46[a]	798	1.77[a]	4.35[a]	0.11[a]–7.96[a]	100
t									−2.39
P									0.032
Hotels	lobbies	8	798 ± 673	470	180	732	1.23[a]	0.05[a]–2.09[a]	100
	hallways	8	579 ± 527	379	148	487	884	0.08[a]–1.63[a]	100
	stairwells	8	501 ± 506	350	167	358	538	0.10[a]–1.69[a]	100
	male toilets	8	8.38[a] ±12.5[a]	3.14[a]	697	4.25[a]	10.3[a]	0.30[a]–37.9[a]	100
F									5.46
P									0.004
Government offices	lobbies	8	327 ± 309	196	55.2	228	690.4	<MDL–792	75.0
	meeting rooms	8	268 ± 103	249	163	257	347	134–430	100
	stairwells	8	343 ± 210	287	182	350	415	86.0–769	100
	male toilets	8	0.88[a] ±1.07[a]	496	230	356	1.56[a]	0.12[a]–3.16[a]	100
F									1.53
P									0.23
Middle schools	offices	4	167 ± 184	11	47.0	97.3	361	<MDL–439	75.0
	meeting rooms	4	218 ± 179	164	72.4	177	405	66.0–453	100
	stairwells	4	238 ± 171	188	86.5	214	414	64.4–460	100
	male toilets	4	1.07[a] ±0.57[a]	934	514	1.08[a]	1.62[a]	0.39[a]–1.73[a]	100
F									4.71
P									0.02
Public institutions	lobbies	8	243 ± 140	203	113	239	369	67.3–452	100
	meeting rooms	8	207 ± 237	139	74.9	98.4	304	67.3–743	100
	stairwells	8	250 ± 195	208	129	178	281	115–705	100
	male toilets	8	845 ± 671	599	274	655	1.44[a]	0.13[a]–2.03[a]	100
F									5.05
P									0.006
Health institutions	lobbies	8	236 ± 144	195	237	94.4	314	68.1–503	100
	meeting rooms	8	191 ± 160	141	69.5	107	325	58.4–481	100
	stairwells	8	280 ± 207	209	101	231	456	56.6–630	100
	male toilets	8	797 ± 932	456	182	470	1.16[a]	0.08[a]–2.89[a]	100
F									2.43
P									0.09
Primary schools	offices	4	250 ± 130	214	121	269	359	74.5–387	100
	meeting rooms	4	150 ± 42.3	146	117	140	193	110–210	100
	stairwells	4	47.6 ± 13.4	46.4	40.9	40.9	61.0	<MDL–67.7	25.0
	male toilets	4	273 ± 134	246	144	278	397	127–409	100
F									9.56
P									0.002
Nurseries	offices	4	48.6 ± 15.3	47.1	40.9	40.9	63.9	<MDL–71.6	25.0
	meeting rooms	4	269 ± 371	134	46.3	107	654	<MDL–821	75.0
	stairwells	4	65.8 ± 49.8	55.7	40.9	40.9	116	<MDL–140	25.0
	male toilets	4	233 ± 317	115	40.9	94.3	563	<MDL–702	50.0
F									1.06
P									0.40

[a] μg/m^3; GM: geometric mean; DF: detection frequency.

9.1 times in the sampling points across the ten types of monitoring sites. Among all the sampling points, median nicotine concentrations were the highest in the hallways of internet cafes (36.2 μg/m^3) and lowest in the stairwells of primary schools (47.6 ng/m^3). Air nicotine concentrations in different sampling points were compared, and significant differences among them were observed in public institutions, primary schools, middle schools, subway stations, hotels, and internet cafes among the ten types of sites ($P < 0.05$) (Table 2).

The EDIs of nicotine (μg/kg-bw/day) through inhalation for employees in public places in Wuhan, in descending order, were found as: internet cafes (1.87), restaurants (0.34), subway stations (0.09), hotels (0.07), government offices (0.03), middle schools (0.03), health institutions (0.03), public institutions (0.02), primary schools (0.02), and nurseries (0.01). The value of EDI for employees in the internet cafes was twice more than the upper limit of the suggested acute reference dose of nicotine (0.1–0.8 μg/kg-body weight/day) (EFSA. European Food Safety Authority, 2009), and the value in restaurants exceeded the lower limit. The EDIs of nicotine in this study were higher than those exposed through ingestion of indoor dust for adults in five other provinces in China (Hebei: 7.709 ng/kg-bw/day, Henan: 7.625, Shandong: 2.576, Shanxi: 6.767, and Sichuan: 1.256) (Chinthakindi et al., 2022) and ten Asian and western countries (Colombia: 0.062 ng/kg-bw/day, Greece:

Table 3
Urinary cotinine concentration of non-smokers in various monitoring sites.

		No.	Cotinine(ng/mL)						Cotinine (SG-corrected, ng/mL)				
			GM	25th	Median	75th	Range	DF (%)	GM	25th	Median	75th	Range
Monitoring sites	Internet cafes	40	23.6	7.84	22.3	50.8	0.59–1 764	100	24.1	8.54	23.1	55.0	0.62–1679
	Restaurants	40	6.67	2.15	3.24	11.6	0.52–1772	100	7.00	2.32	3.33	14.9	0.50–1611
	Subway stations	40	2.92	1.29	2.46	5.17	<MDL–361	95.0	3.06	1.26	2.48	5.97	<MDL–328
	Middle schools	20	2.94	0.57	1.60	7.19	<MDL–179	85.0	3.06	0.63	1.56	8.15	<MDL–199
	Government offices	40	1.47	0.67	1.14	1.89	<MDL–207	85.0	1.53	0.72	1.25	1.88	<MDL–259
	Hotels	40	2.68	0.74	1.18	10.1	<MDL–1762	85.0	2.73	0.73	1.16	11.3	<MDL–1678
	Health institutions	40	1.69	0.64	1.04	2.45	<MDL–301	90.0	1.76	0.67	1.10	2.42	<MDL–335
	Public institutions	40	1.24	0.35	0.88	2.18	<MDL–1220	72.5	1.27	0.41	1.01	2.08	<MDL–1355
	Primary schools	20	1.08	0.35	0.80	2.24	<MDL–15.1	35.0	1.12	0.42	0.83	2.40	<MDL–14.4
	Nurseries	20	0.60	0.35	0.35	1.09	<MDL–3.64	70.0	0.59	0.33	0.38	1.17	<MDL–3.46
	H							100					102
	P							0.000					0.000
Sex	Male	167	3.48	0.83	2.00	8.17	<MDL–1762	93.0	3.58	0.82	2.02	9.03	<MDL–1678
	Female	173	2.16	0.65	1.29	4.14	<MDL–1772	80.5	2.25	0.69	1.44	4.52	<MDL–1679
	Z							−4.89					−4.81
	P							0.000					0.000
Age	18–27	58	8.44	1.02	8.94	39.1	<MDL–1333	87.9	8.70	1.07	9.69	43.5	<MDL–1269
	28–37	119	2.56	0.71	1.35	4.21	<MDL–1772	85.7	2.66	0.75	1.43	4.68	<MDL–1611
	38–47	81	2.07	0.59	1.63	4.69	<MDL–1764	80.2	2.14	0.62	1.63	4.74	<MDL–1679
	48–57	76	1.69	0.63	1.42	3.18	<MDL–207	84.2	1.74	0.68	1.43	3.29	<MDL–259
	58–67	6	3.61	1.08	2.43	18.7	0.83–39.0	100	3.51	1.20	2.21	17.0	0.92–35.4
	H							17.9					17.6
	P							0.001					0.001
Overall		340	2.74	0.73	1.62	5.29	<MDL–1772	86.6	2.83	0.75	1.69	5.71	<MDL–1679

GM: geometric mean; DF: detection frequency.

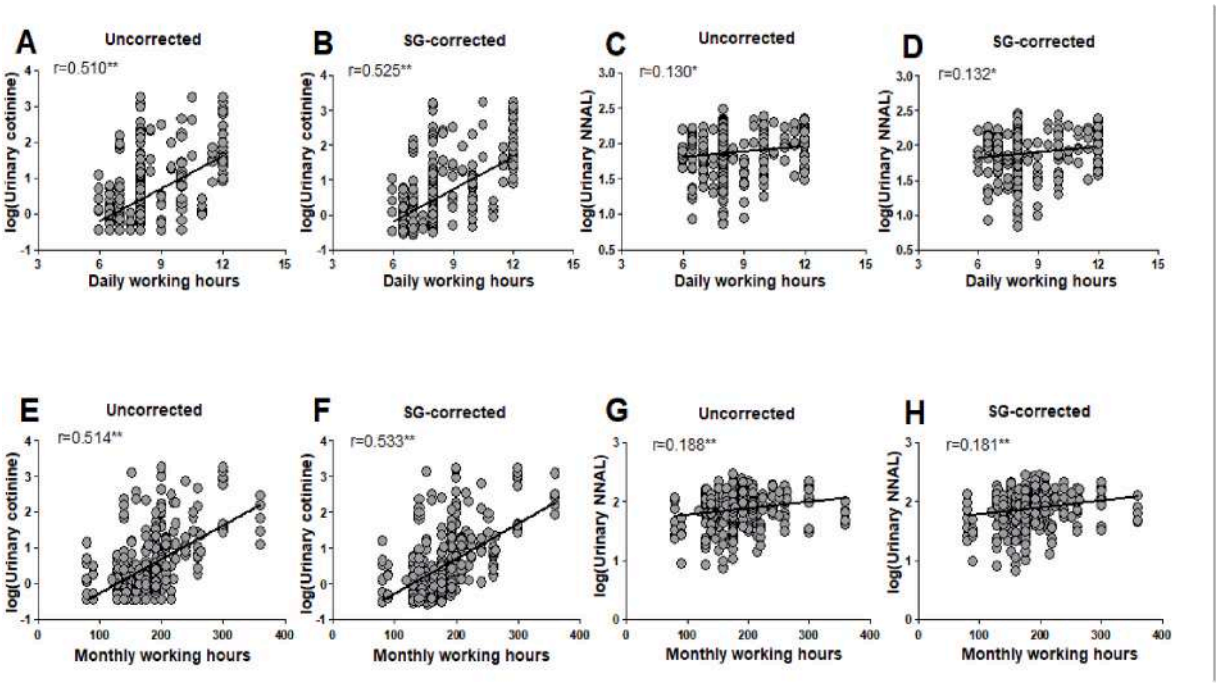

Fig. 1. Correlation of daily working hours with log-transformed(urinary cotinine) (uncorrected) (A), log(urinary cotinine) (SG-corrected) (B), log(urinary NNAL) (uncorrected) (C), log(urinary NNAL) (SG-corrected) (D), Correlation of monthly working hours with log(urinary cotinine) (uncorrected) (E), log(urinary cotinine) (SG-corrected) (F), log(urinary NNAL) (uncorrected) (G), log(urinary NNAL) (SG-corrected) (H). *Significant at $p < 0.05$, **Significant at $p < 0.001$.

2.406, India: 2.212, Japan: 0.027, Kuwait: 0.090, Pakistan: 0.603, Romania: 3.219, Saudi Arabia: 0.352, South Korea: 0.603, and Vietnam: 0.075) (Chinthakindi and Kannan, 2021). This might suggest that nicotine in the air was a major exposure source compared to that in indoor dust. Another explanation may be that nicotine exposure was higher in public places than in households for nonsmokers (Jané et al., 2002).

3.2. Urinary cotinine concentrations

Urinary cotinine concentrations of non-smokers in different monitoring sites are listed in Table 3. Cotinine was found in all the non-smokers in internet cafes and restaurants, and in other monitoring sites the detection frequencies ranged from 70.0% to 95.0%, except the primary schools with the lowest detection frequency of 35.0%. Among the ten types of monitoring sites, the highest concentrations of urinary cotinine (SG-corrected) were found in the internet cafes (median: 23.1

Table 4
Urinary NNAL concentration of non-smokers in various monitoring sites.

		No.	NNAL(pg/mL)				NNAL(SG-adjusted, pg/mL)		
			GM	Median	Range	DF (%)	GM	Median	Range
Monitoring sites	Internet cafes	40	95.6	96.5	29.9–224	100	97.6	104	32.3–236
	Hotels	40	91.5	95.9	20.6–217	100	93.4	98.7	18.8–210
	Restaurants	40	92.1	91.1	31.5–208	100	96.7	91.5	33.2–277
	Public institutions	40	66.7	87.6	8.59–299	100	68.7	87.3	8.18–284
	Health institutions	40	78.3	82.0	14.3–210	100	81.3	84.5	13.0–196
	Subway stations	20	73.4	83.6	9.65–179	100	76.8	82.1	11.3–170
	Government offices	40	69.7	72.8	16.4–222	100	72.6	77.2	16.4–211
	Nurseries	20	65.5	65.5	18.3–240	100	68.3	68.3	20.4–266
	Primary schools	20	45.7	56.1	<MDL–132	95.0	45.1	54.1	<MDL–125
	Middle schools	20	37.0	36.1	8.85–108	100	38.6	39.2	9.83–103
	H				43.9				42.0
	P				0.000				0.00
Sex	Male	167	76.8	82.6	15.5–224	100	78.9	81.0	14.1–277
	Female	173	70.2	79.6	<MDL–299	95.6	73.1	84.6	<MDL–284
	Z				−1.43				−1.32
	P				0.15				0.19
Age	18–27	58	86.9	101	21.9–240	100	89.6	105	20.8–266
	28–37	119	68.3	79.6	7.50–217	100	71.0	81.6	8.18–277
	38–47	81	71.8	77.3	<MDL–222	98.8	74.4	78.6	<MDL–236
	48–57	76	74.4	79.0	8.85–299	100	76.4	81.8	9.83–284
	58–67	6	64.4	67.5	41.1–95.5	100	62.6	67.0	37.3–86.8
	H				5.88				6.04
	P				0.21				0.20

GM: geometric mean; DF: detection frequency.

ng/mL), followed by restaurants (median: 3.33 ng/mL), subway stations (2.48), middle schools (1.56), government offices (1.25), hotels (1.16), health institutions (1.10), public institutions (1.01), primary schools (0.83), and nurseries (0.38). The difference among the various monitoring sites was statistically significant ($P < 0.01$) (Table 3).

Differences related with sex, age, and working hours were also analyzed. The median concentration of SG-corrected urinary cotinine for male non-smokers (2.02 ng/mL) working in the ten types of public places was 1.40-fold that of the female (1.44 ng/mL). Significant difference ($P < 0.01$) was observed in urinary cotinine concentrations between males and females (Table 3). We categorized the employees into five groups at the same interval of age according to the age range of all the employees: 18–27 years, 28–37 years, 38–47 years, 48–57 years, and 58–67 years. Employees aged 18–27 were detected with the highest SG-corrected urinary cotinine concentration (GM: 8.70 ng/mL), and were almost 5-fold that of the lowest group (48–57 years; GM: 1.74 ng/mL) (Table 3). The urinary cotinine concentrations (SG-corrected and uncorrected) relative to daily working hours and monthly working hours are depicted by the scatter plots in Fig. 1. Statistically significant correlations between urinary cotinine and working hours (daily and monthly) were observed (e.g., for both daily and monthly working hours: SG-corrected, r = 0.53; $P < 0.001$) (Fig. 1).

Our results indicated that urinary cotinine was detected (>0.5 ng/mL) in more than 90.0% of male non-smokers and 80.0% of female non-smokers in public places in Wuhan. The concentrations of cotinine in urine samples of non-smokers in this study (median, 1.62 ng/mL) were similar to the reported values of non-smoking adults (median, 1.66 ng/mL) in Israel (Berman et al., 2018), but higher than those in Canada (median: < 1.1 ng/mL) according to Canadian Health Measures Survey Cycle 4 (2014–2015) (Canada. Government of Canada, 2017) and Korea (median: 1.00 ng/mL) in 2012–2017 after expanding smoking restrictions in public places (Choi et al., 2017).

3.3. Urinary NNAL concentrations

The urinary NNAL concentrations are listed in Table 4. Urinary NNAL was detectable in all the non-smokers in the various monitoring sites (except for 95.0% of those in primary schools). Among the ten types of monitoring sites, the highest concentrations of urinary NNAL (SG-corrected; pg/mL) of non-smokers who worked in the monitoring sites were found in internet cafes (median: 104) followed by hotels (median: 98.7), restaurants (91.5), public institutions (87.3), health institutions (84.5), subway stations (82.1), government offices (77.2), nurseries (68.3), primary schools (54.1), and middle schools (39.2) (Table 4).

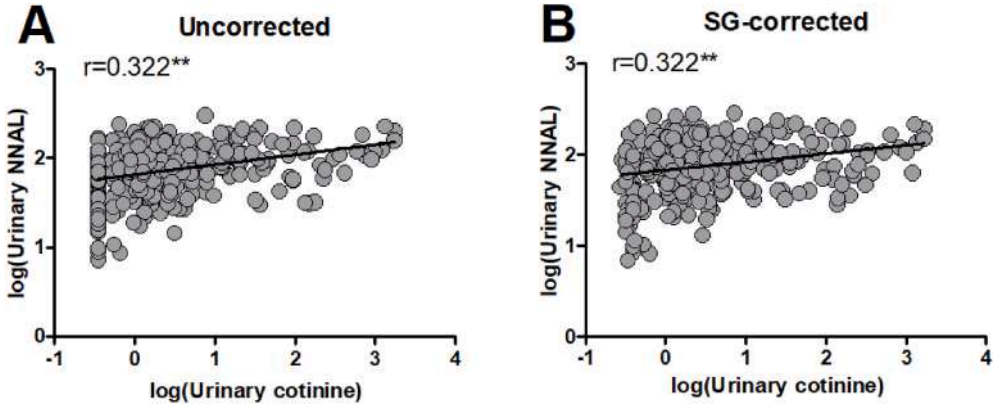

Fig. 2. Correlation of log(urinary cotinine) with log(urinary NNAL) (uncorrected) (A), log(urinary NNAL) (SG-corrected) (B). **Significant at $p < 0.001$.

Significant difference ($P < 0.001$) was observed in urinary NNAL concentrations among non-smokers who worked in the ten types of monitoring sites (Table 4).

Different from the urinary cotinine, there was no significant difference in urinary NNAL concentrations between male (median: 81.0 pg/mL) and female (84.6) non-smokers working in the ten types of public places in Wuhan ($P = 0.19$). In accordance with the urinary cotinine, employees aged 18–27 were detected with the highest urinary NNAL concentrations (SG-corrected median: 105 pg/mL). However, no significant difference ($P = 0.20$) was observed in urinary NNAL concentrations among the five age groups. The urinary NNAL concentration (SG-corrected and uncorrected) relative to daily working hours and monthly working hours are depicted by the scatter plots in Fig. 1C, D, G, H. Slightly but statistically significant correlations between urinary NNAL and working hours (daily and monthly) were observed (e.g., for daily working hours: SG-corrected, $r = 0.13$; $P < 0.05$; and for monthly working hours: SG-corrected, $r = 0.18$; $P < 0.001$) (Fig. 1).

In addition, urinary cotinine (log-transformed) and urinary NNAL (log-transformed) (Fig. 2) were positively correlated with each other but the coefficient was relatively low (e.g., SG-corrected, $r = 0.32$; $P < 0.001$), which was consistent with other relevant reports (Michael Meger Irmtrud Meger-Kossien Kirsten Riedel Gerhard, 2000; Chen et al., 2016; Chao et al., 2018).

The present study has several limitations. First, smoking status was based on self-report and did not distinguish between long-term non-smokers and those who quit smoking recently. Besides, we only monitored airborne nicotine concentrations in public places in Wuhan in a single season, and nicotine concentrations in other seasons should be analyzed in the future to characterize seasonal variations (Arku et al., 2015). Moreover, when calculating EDIs of airborne nicotine for adults, nicotine intake elsewhere by the employees (after work; such as in homes, vehicles, and so on), was not taken into account, thus, the 24-h EDIs of nicotine should be higher.

4. Conclusions

In this study, ETS exposure was ubiquitous in all the different public places monitored and concentrations of urinary cotinine and NNAL were detected among non-smokers who worked in these sites. The highest airborne nicotine concentrations were observed in internet cafes, and the lowest values were found in nurseries. Urinary cotinine and NNAL were found most of the non-smokers in the monitoring sites, and the highest concentrations of them were both found in non-smokers in internet cafes. This is the first time to demonstrate that urinary cotinine concentrations in male non-smokers were significantly higher than those in female non-smokers while such difference was not found in urinary NNAL. Employees aged 18–27 were detected with the highest urinary cotinine and NNAL concentrations compared with other age groups. Urinary cotinine and NNAL concentrations were significantly correlated with daily and monthly working hours. Besides, a positive correlation was observed between log-transformed urinary concentrations of cotinine and NNAL. In conclusion, these findings reveal the high-risk exposure areas and age groups of ETS in public places in Wuhan, and underscore the importance of adopting evidence-based strategies to protect people from SHS exposure, including educational interventions warning among vulnerable populations, smoke-free internet cafes and restaurants rules, and promotion of smoke-free laws.

Declaration of competing interest

None.

Acknowledgments

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References

Agbenyikey, W., Wellington, E., Gyapong, J., Travers, M.J., Breysse, P.N., McCarty, K.M., et al., 2011. Secondhand tobacco smoke exposure in selected public places (PM2.5 and air nicotine) and non-smoking employees (hair nicotine) in Ghana. Tobac. Control 20, 107–111.

Anderson, K.E., Carmella, S.G., Ye, M., Bliss, R.L., Le, C., Murphy, L., et al., 2001. Metabolites of a tobacco-specific lung carcinogen in nonsmoking women exposed to environmental tobacco smoke. J. Natl. Cancer Inst. 93, 378–381.

Arku, R.E., Adamkiewicz, G., Vallarino, J., Spengler, J.D., Levy, D.E., 2015. Seasonal variability in environmental tobacco smoke exposure in public housing developments. Indoor Air 25, 13–20.

Avila-Tang, E., Al-Delaimy, W.K., Ashley, D.L., Benowitz, N., Bernert, J.T., Kim, S., et al., 2013. Assessing secondhand smoke using biological markers. Tobac. Control 22, 164–171.

Benedict, M.D., Missmer, S.A., Vahratian, A., Berry, K.F., Vitonis, A.F., Cramer, D.W., et al., 2011. Secondhand tobacco smoke exposure is associated with increased risk of failed implantation and reduced IVF success. Hum. Reprod. 26, 2525–2531.

Benowitz, N.L., 1999. Biomarkers of environmental tobacco smoke exposure. Environ. Health Perspect. 107 (Suppl. 2), 349–355.

Berman, T., Barnett-Itzhaki, Z., Mery, N., Keinan-Boker, L., Shimony, T., Goldsmith, R., et al., 2018. Exposure to environmental tobacco smoke in non - smoking adults in Israel: results of the second Israel biomonitoring survey. Isr. J. Health Pol. Res. 7, 33.

Caman, O.K., I.Erguder, B., Ozcebe, H., Bilir, N., 2013. Urinary cotinine and breath carbon monoxide levels among bar and restaurant employees in ankara. Nicotine Tob. Res. 15, 1446–1452.

Canada. Government of Canada, 2017. Fourth report on human biomonitoring of environmental chemicals in Canada (accessed Nov 25, 2021). https://www.canada.ca/en/health-canada/services/environmental-workplace-health/reports-publications/environmental-contaminants/fourth-report-human-biomonitoring-environmental-chemicals-canada.html.

CDC, 2011. Chinese Center for Disease Control and Prevention. Global Adult Tobacco Survey (GATS) China 2010 Country Report. China Sanxia Press, Beijing.

Chao, M.R., Cooke, M.S., Kuo, C.Y., Pan, C.H., Liu, H.H., Yang, H.J., et al., 2018. Children are particularly vulnerable to environmental tobacco smoke exposure: evidence from biomarkers of tobacco-specific nitrosamines, and oxidative stress. Environ. Int. 120, 238–245.

Chen, C.Y., Jhou, Y.T., Lee, H.L., Lin, Y.W., 2016. Simultaneous, rapid, and sensitive quantification of 8-hydroxy-2'-deoxyguanosine and cotinine in human urine by on-line solid-phase extraction LC-MS/MS: correlation with tobacco exposure biomarkers NNAL. Anal. Bioanal. Chem. 408, 6295–6306.

Chinthakindi, S., Kannan, K., 2021. Primary aromatic amines in indoor dust from 10 countries and associated human exposure. Environ. Int. 157, 106840.

Chinthakindi, S., Zhu, Q., Liao, C., Kannan, K., 2022. Profiles of primary aromatic amines, nicotine, and cotinine in indoor dust and associated human exposure in China. Sci. Total Environ. 806, 151395.

Choi, W., Kim, S., Baek, Y.-W., Choi, K., Lee, K., Kim, S., et al., 2017. Exposure to environmental chemicals among Korean adults-updates from the second Korean National Environmental Health Survey (2012–2014). Int. J. Hyg Environ. Health 220, 29–35.

Córdoba-García, R., 2020. Fourteen years of tobacco control law in Spain. Current situation and proposals. Atención Primaria 52, 563–569.

EFSA. European Food Safety Authority, 2009. Potential risks for public health due to the presence of nicotine in wild mushrooms. EFSA J. https://efsa.onlinelibrary.wiley.com/doi/pdf/10.2903/j.efsa.2009.286r.

FCTC, 2007. Framework convention on tobacco control. Guidelines for Implementation of Article 8. https://www.who.int/fctc/guidelines/adopted/article_8/en/. (Accessed 18 November 2021).

Hecht, S.S., 1998. Biochemistry, biology, and carcinogenicity of tobacco-specific N-nitrosamines. Chem. Res. Toxicol. 11, 559–603.

Huang, C., Chen, G., Zhang, M., Lu, Y., Hua, Y., Hu, Y., et al., 2020. Association between environmental tobacco smoke exposure and risk of type 2 diabetes mellitus in Chinese female never smokers: a population-based cohort study. J. Diabetes 12, 339–346.

Jané, M., Nebot, M., Rojano, X., Artazcoz, L., Sunyer, J., Fernández, E., et al., 2002. Exposure to environmental tobacco smoke in public places in Barcelona, Spain. Tobac. Control 11, 83–84.

Law, M.R., Hackshaw, A.K., 1996. Environmental tobacco smoke. Br. Med. Bull. 52, 22–34.

Li, W., Jiang, G.H., Zheng, W.L., Yang, Y., Pan, Y., 2014. Hygienic survey about indoor PM2.5 and air nicotine in main public places in Tianjin. J. Environ. Health 31, 787–789.

Liu, H., Qi, F., Li, S.P., Li, Q.R., Jia, X.R., Qu, Y.B., 2019. The level of PM2.5 and nicotine monitoring and analysis of indoor environmental tobacco smoke pollution in some public places in Qingdao in 2016. Chin. J. Health Educ. 35, 1085–1088.

Mahid, S.S., Minor, K.S., Stromberg, A.J., Galandiuk, S., 2007. Active and passive smoking in childhood is related to the development of inflammatory bowel disease. Inflamm. Bowel Dis. 13, 431–438.

Merianos, A.L., Nabors, L.A., Fiser, K.A., Mahabee-Gittens, E.M., 2021. Exposure to tobacco smoke and temperament among U.S. Children 0-5 Years old. J. Pediatr. Psychol. 46, 454–464.
Messner, B., Bernhard, D., 2014. Smoking and cardiovascular disease: mechanisms of endothelial dysfunction and early atherogenesis. Arterioscler. Thromb. Vasc. Biol. 34, 509–515.
Michael Meger Irmtrud Meger-Kossien Kirsten Riedel Gerhard, S., 2000. Biomonitoring of environmental tobacco smoke (ETS)-related exposure to 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK). Biomarkers 5, 33–45.
National Bureau of Statistics of China. https://data.stats.gov.cn/easyquer.y.htm?cn=C01&zb=A0E0H&sj=2020.
Park, E.Y., Yun, E.H., Lim, M.K., Lee, D., Yang, W., Jeong, B.Y., et al., 2016. Consequences of incomplete smoke-free legislation in the Republic of Korea: results from environmental and biochemical monitoring: community based study. Cancer Res. Treat. 48 (1), 376–383.
PGWC, 2005. People's Government of Wuhan City. Regulations of Wuhan prohibiting smoking in public places. http://www.pkulaw.cn/fulltext_form.aspx?Gid=16845618 2005.
Schoendorf, K.C., Kiely, J.L., 1992. Relationship of sudden infant death syndrome to maternal smoking during and after pregnancy. Pediatrics 90, 905–908.
Simons, E., To, T., Moineddin, R., Stieb, D., Dell, S.D., 2014. Maternal second-hand smoke exposure in pregnancy is associated with childhood asthma development. J. Allergy Clin. Immunol. Pract. 2, 201–207.
Stillman, F., Navas-Acien, A., Ma, J., Ma, S.J., Avila-Tang, E., Breysse, P., et al., 2007. Second-hand tobacco smoke in public places in urban and rural China. Tobac. Control 16, 229–234.
Vineis, P., Airoldi, L., Veglia, F., Olgiati, L., Pastorelli, R., Autrup, H., et al., 2005. Environmental tobacco smoke and risk of respiratory cancer and chronic obstructive pulmonary disease in former smokers and never smokers in the EPIC prospective study. Br. Med. J. 330, 277.
Wan, Y., Xue, J., Kannan, K., 2016. Benzothiazoles in indoor air from Albany, New York, USA, and its implications for inhalation exposure. J. Hazard Mater. 311, 37–42.
WHO, 2012. WHO Global Report: Mortality Attributable to Tobacco. https://www.who.int/publications/i/item/9789241564434. (Accessed 28 October 2021).
WMPC, 2019. Wuhan Municipal People's Congress. Wuhan regulations on smoking control. http://www.whrd.gov.cn/mobile/detail-16729.html. (Accessed 27 October 2021).
Yang, G., Wang, Y., Wu, Y., Yang, J., Wan, X., 2015. The road to effective tobacco control in China. Lancet 385, 1019–1028.
Yang, L.J., Yu, X.C., Kang, Z.Y., Zhu, L., 2016. The exposure of tobacco smoke environment in several public places in downtown of Dalian. J. Prev. Med. Inf. 32, 533–538.
Yolton, K., Xu, Y., Khoury, J., Succop, P., Lanphear, B., Beebe, D.W., et al., 2010. Associations between secondhand smoke exposure and sleep patterns in children. Pediatrics 125, e261–e268.
Zhou, M., Wang, H., Zeng, X., Yin, P., Zhu, J., Chen, W., et al., 2019. Mortality, morbidity, and risk factors in China and its provinces, 1990-2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 394, 1145–1158.
Zhuge, Y., Qian, H., Zheng, X., Huang, C., Zhang, Y., Li, B., et al., 2020. Effects of parental smoking and indoor tobacco smoke exposure on respiratory outcomes in children. Sci. Rep. 10, 4311.

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Assessment of the association of exposure to polycyclic aromatic hydrocarbons, oxidative stress, and inflammation: A cross-sectional study in Augsburg, Germany

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ABSTRACT

Background: Exposure to polycyclic aromatic hydrocarbons (PAHs) has been linked to acute and chronic health effects through the suggested pathways of oxidative stress and inflammation. However, evidence is still limited. We aimed to investigate jointly the relationship of PAHs, oxidative stress, and inflammation.

Methods: We measured 13 biomarkers of PAH exposure (n = 6: hydroxylated polycyclic aromatic hydrocarbons, [OH-PAHs]), oxidative stress (n = 6: malondialdehyde (MDA); 8-hydroxy-2′-deoxyguanosine (8-OHdG); and 4 representatives of the compound class of $F_{2\alpha}$-isoprostanes) in urine, and inflammation (n = 1: high-sensitivity C-reactive protein, [hs-CRP]) in serum from 400 participants at the second follow-up (2013/2014) of the German KORA survey S4. Multiple linear regression models were applied to investigate the interplay between biomarkers.

Results: Concentrations of biomarkers varied according to sex, age, smoking status, season, and a history of obesity, diabetes, or chronic kidney disease. All OH-PAHs were significantly and positively associated with oxidative stress biomarkers. An interquartile range (IQR) increase in sum OH-PAHs was associated with a 13.3% (95% CI: 9.9%, 16.9%) increase in MDA, a 6.5% (95% CI: 3.5%, 9.6%) increase in 8-OHdG, and an 8.4% (95% CI: 6.6%, 11.3%) increase in sum $F_{2\alpha}$-isoprostanes. Associations were more pronounced between OH-PAHs and $F_{2\alpha}$-isoprostanes but also between OH-PAHs and 8-OHdG for participants with potential underlying systemic inflammation (hs-CRP $\geq$ 3 mg/L). We observed no association between OH-PAHs and hs-CRP levels. While 8-OHdG was significantly positively associated with hs-CRP (13.7% [95% CI: 2.2%, 26.5%] per IQR increase in 8-OHdG), $F_{2\alpha}$-isoprostanes and MDA indicated only a positive or null association, respectively.

Conclusion: The results of this cross-sectional study suggest, at a population level, that exposure to PAHs is associated with oxidative stress even in a low exposure setting. Oxidative stress markers, but not PAHs, were associated with inflammation. Individual risk factors were important contributors to these processes and should be considered in future studies. Further longitudinal studies are necessary to investigate the causal chain of the associations.

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1. Introduction

Polycyclic aromatic hydrocarbons (PAHs) are non-polar, semi-volatile, organic pollutants composed of several aromatic rings (Keyte et al., 2013). They are ubiquitous and hazardous pollutants which are generated during incomplete combustion of organic materials. Traffic emissions, domestic combustion, and industry practices are suggested to be the main anthropogenic sources of PAHs in urban areas (Kamal et al., 2015).

A large proportion of anthropogenically and naturally generated PAHs is occurring and transported in the air, and here – depending on physico-chemical parameters – bound to particles or in the gas phase (Kraus et al., 2011; Li et al., 2021; Liu et al., 2019). Worldwide, strong efforts are performed to control and decrease the ambient PAH-concentrations by creating limiting regulations and applying new technologies for anthropogenic processes such as heating, industrial processes and traffic. This is on the one hand reflected by relatively low PAH concentrations in our study region as previously reported (Li et al., 2018). On the other hand, depending on the current meteorological conditions (season in general but also specific events), PAHs levels can be significantly increased, either nationwide or only at specific sites (Fuchte et al., 2022). Additionally, it is expected that climate change has an impact on PAH-levels (Garrido et al., 2014) which is not yet fully understood. PAHs are one of the major groups of ambient pollutants that cause severe health effects and have been investigated for decades. Lipophilic PAHs can be absorbed via dermal, respiratory, or ingestion routes (Andersen et al., 2018; VanRooij et al., 1993). Short-term exposure to PAHs can cause acute health effects such as eye and skin irritation, headache, nausea, and vomiting. They can also induce inflammatory processes (Al-Delaimy et al., 2014). Long-term exposure to PAHs can lead to chronic health issues, such as chronic obstructive pulmonary disease, diabetes, and cardiovascular diseases (Alshaarawy et al., 2016; Cao et al., 2020; Yang et al., 2017). PAHs were also related to oxidative stress, genotoxicity, and carcinogenicity in both in vitro and in vivo studies (Danielsen et al., 2011; Kumar et al., 2020; Lan et al., 2004; Lu et al., 2016; McCarrick et al., 2019).

Individuals are always exposed to complex mixtures of low molecular weight, medium molecular weight and high molecular weight PAHs, and with current analytical methods it is impossible to comprehensively reflect the ways of metabolic transformation and excretion of all PAHs after their absorption in the organism. The use of biomarkers allows the assessment of the individual, internal PAH burden, and the determination of monohydroxylated PAHs (OH-PAHs) in urine have been previously used for this purpose (Aquilina et al., 2010; Ifegwu and Anyakora, 2016; Mesquita et al., 2014; Urbancova et al., 2016). In this study, we determined 1-OH-pyrene, the main urinary metabolite of pyrene, and five urinary isomeric OH-phenanthrenes originating from phenanthrene. Pyrene and phenanthrene are medium molecular weight PAHs, which are both abundant in typical environmental PAH-mixtures together with further, especially higher molecular weight PAHs. In contrast to those higher molecular weight PAHs, the OH-PAH metabolites of pyrene and phenanthrene can be reliably determined in low volume urine samples, and their concentrations – especially 1-hydroxypyrene concentrations - can be used for estimating the individual exposure to PAHs.

Oxidative stress is an important pathway linking exposure to ambient pollution and acute and chronic diseases (Peters et al., 2021). The induction of a disease process begins with the generation of oxidative stress in the organism. Once the pollutants are absorbed, the formation of reactive oxygen species (ROS) such as peroxides, superoxides, hydroxyl radicals, and singlet oxygen can be initiated (Apel and Hirt, 2004; Tao et al., 2003). These species can attack and modify adjacent macromolecules such as proteins, DNA, and lipids in vivo (Risom et al., 2005). Humans have protective mechanisms against ROS, such as antioxidants, or the activation of specific enzymatic processes to remove ROS species and maintain the oxidative stress balance. If this subtle balance is disturbed (Droge, 2002), oxidative stress and the resulting attack on macromolecules can lead to acute and chronic diseases of the respiratory, cardiovascular, or immunological systems (McCord, 1993; Michael et al., 2013; Miller, 2020; Taverne et al., 2013).

Due to the high reactivity of ROS species, direct quantification is critical. Some by-products or end products of oxidative stress that are excreted through faeces or urine can be quantified. We selected the established biomarkers malondialdehyde (MDA), 8-hydroxy-2′-deoxyguanosine (8-OHdG), and the compound class of $F_{2\alpha}$-isoprostanes. MDA, 8-OHdG, and $F_{2\alpha}$-isoprostanes reflect the amount of damaged double bonds of polyunsaturated fatty acids (PUFAs) (Ayala et al., 2014; Yoon et al., 2012), damaged DNA (Evans et al., 2010; Valavanidis et al., 2009), and damaged membrane phospholipids (Galano et al., 2017; Milne et al., 2008; Morrow and Roberts, 1996), respectively. Using a combination of oxidative stress biomarkers increases reliability when assessing individual oxidative stress levels (Zhu et al., 2021).

Inflammation is another possible pathway for air pollution-initiated health effects. Previous epidemiological studies have reported that long-term exposure to ambient pollutants is associated with increased serum levels of C-reactive protein (CRP), a well-known marker for inflammation (Everett et al., 2010; Hennig et al., 2014; Ostro et al., 2014; Pilz et al., 2018). Toxicological studies have led to similar observations—exposure to air pollution induces inflammatory responses such as increased CRP concentrations in human blood (Chuang et al., 2007). Systemic inflammation is induced by ambient air pollution via the production of cytokines such as tumor necrosis factor-α and interleukin-8 (Pope et al., 2016). Previous studies also indicated that PAH exposure is positively associated with oxidative stress and inflammation (Clark et al., 2012; Everett et al., 2010; Farzan et al., 2016; Ferguson et al., 2017; Gerlofs-Nijland et al., 2009; Lu et al., 2016; Vattanasit et al., 2014).

Although many studies have investigated the associations between OH-PAHs and oxidative stress or between OH-PAHs and inflammation or OH-PAHs and lifestyle factors and health characteristics, only two epidemiological studies have jointly considered the interplay, however both being limited by small and/or highly selected populations (Ferguson et al., 2017; Zhang et al., 2020). To fill this gap, we conducted this cross-sectional study among the general adult population and examined if (1) the concentration of OH-PAHs, oxidative stress markers, and high-sensitivity CRP (hs-CRP) varied among subgroups; (2) OH-PAHs were associated with oxidative stress, including potential effect modification by underlying systemic inflammation because large amount of ROS could be generated during the inflammatory process and disturb the balance (Fialkow et al., 2007); (3) OH-PAHs were associated with hs-CRP and (4) the selected oxidative stress markers were similarly associated with hs-CRP.

2. Methods

2.1. Study population

We included a selected subgroup of 400 subjects at different stages of impaired glucose metabolism without prior cardiovascular disease who participated in 2013/2014 in the second follow-up (FF4) of the baseline KORA (Cooperative Health Research in the region of Augsburg) S4 study (1999–2001, N = 4261) (Bamberg et al., 2017). Participants were invited to the study center in Augsburg, where they answered a computer-assisted personal interview and completed a self-administered questionnaire. All individuals were physically examined, and urine and blood samples were collected. The general KORA study design, sampling method, and data collection have been described in detail by Holle et al. (2005). All participants provided written informed consent to participate in the study which was approved by the ethics committee of the Bavarian Medical Association.

2.2. Urinary biomarker measurements

For each participant, a spot urine sample was collected. For further processing, the samples were stored at −80 °C in a central storage unit. All urinary biomarkers were analysed in our lab in 2015/16 using previously established liquid chromatography (LC)- based methods. OH-PAHs (1-OH-Phe, 1-hydroxyphenanthrene; 2-OH-Phe, 2-hydroxyphenanthrene; 3-OH-Phe, 3-hydroxyphenanthrene; 4-OH-Phe, 4-hydroxyphenanthrene; 9-OH-Phe, 9-hydroxyphenanthrene; 1-OH-Pyr, 1-hydroxypyrene) were measured on an Ultimate 3000 HPLC system with an RF 2000 fluorescence detector (Thermo Scientific, Dreieich, Germany) (Lintelmann et al., 2018). MDA, 8-OHdG, and $F_{2\alpha}$-isoprostanes (2,3-dinor-8-iso-$PGF_{2\alpha}$, 8-iso-15(R)-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, and ±5-$iPF_{2\alpha}$) were determined with LC-mass spectrometry, using a triple quadrupole mass spectrometer API-4000 (AB Sciex, Darmstadt, Germany) equipped with an electrospray ion source (ESI) (Wu et al., 2017). The sum concentrations of OH-PAHs (1-OH-Phe, 2-OH-Phe, and 3-OH-Phe) (Hou et al., 2019; Lu et al., 2016) and the sum concentration of $F_{2\alpha}$ -isoprostanes (Montuschi et al., 2004) were calculated. OH-PAHs, biomarkers of oxidative stress, and creatinine concentrations which were below the limit of detection (LOD) were set to half of the concentration of the LOD ($N_{1\text{–OH-Phe}} = 8$, $N_{2\text{–OH-Phe}} = 19$, $N_{3\text{–OH-Phe}} = 10$, $N_{4\text{–OH-phe}} = 152$, $N_{9\text{–OH-Phe}} = 260$, $N_{1\text{–OH-Pyr}} = 14$, $N_{MDA} = 0$, $N_{8\text{-OHdG}} = 0$, $N_{2,3\text{-dinor-8-iso-PGF2}\alpha} = 2$, $N_{8\text{-iso-15(R)-PGF2}\alpha} = 4$, $N_{8\text{-iso-PGF2}\alpha} = 6$, $N_{\pm5\text{-iPF2}\alpha} = 1$, $N_{creatinine} = 0$). Since more than 20% of 4-OH-Phe concentrations and 9-OH-Phe concentrations were below the LOD, we did not consider 4-OH-Phe and 9-OH-Phe in later data analysis. Creatinine was quantified on an HP 1100 LC system with an ultraviolet detector (Agilent, St. Clara, CA, USA) (Wu et al., 2017). The concentrations of urinary biomarkers were normalised to creatinine concentrations.

2.3. Individual characteristics and clinical parameters

Fasting venous blood samples were collected during the study participants' visits and stored at 4 °C until further processing. Hs-CRP concentrations in serum samples were assayed by latex-enhanced immunonephelometry on a BN II platform (Siemens Healthcare Diagnostics Product GmbH, Marburg, Germany) with an intra-assay coefficient variation of 2.13% (Pilz et al., 2018). Serum creatinine concentrations were determined using an automated Jaffé method (Technicon, SMAC autoanalyzer; Tarrytown, New York, USA) (Aumann et al., 2015). As a marker for kidney disease, we calculated the estimated glomerular filtration rate (eGFR) (mL/min per 1.73 m^2) using the 2009 CKD-EPI creatinine equation (Levey et al., 2009). Body mass index (BMI) was calculated as the weight divided by height (squared). Type 2 diabetes was validated either by an oral glucose tolerance test, a previous diagnosis, or a current intake of glucose-lowering agents. Pre-diabetes was defined as impaired fasting glucose and/or impaired glucose tolerance. Hypertension was defined as blood pressure >140/90 mmHg or treatment of known hypertension (WHO, 1999).

2.4. Ambient air pollution

Long-term ambient air pollution exposure was estimated using land use regression models for all KORA participants' residential addresses (Wolf et al., 2017). Ambient concentrations of particulate matter smaller than 2.5 μm in aerodynamic diameter ($PM_{2.5}$) and nitrogen dioxide (NO_2) were measured at 20 and 40 sites, respectively, between March 2014 and April 2015, and temporally adjusted for discontinuous site measurements. The annual average concentrations were then modelled using linear regression incorporating predictors such as traffic, land use, population, and building density. Details of the estimation can be found in our previous publication (Wolf et al., 2017).

2.5. Statistical analysis

Spearman correlation coefficients were calculated to explore the relationships of urinary biomarkers, individual characteristics, and clinical parameters.

We performed Kruskal-Wallis tests to compare biomarker concentrations across the subgroups. Obesity was defined as BMI ≥30 kg/m^2, potential underlying inflammation was defined by the hs-CRP concentration ≥3 mg/L, and potential renal impairment as eGFR <90 mL/min/1.73 m^2 (Inker et al., 2012). Seasons were defined as: spring: March to May; summer: June to August; autumn: September to November; winter: December to February.

We used multiple linear regression models to investigate the associations between i) OH-PAHs and oxidative stress, ii) OH-PAHs and hs-CRP, and iii) oxidative stress and hs-CRP. The normalised concentrations of urinary biomarkers and the concentration of hs-CRP were log-transformed to approximate normal distribution of the residuals and to stabilise the variance. In the base model, we included age, sex, smoking, and season as potential confounders, as suggested in a previous study (Yang et al., 2015). The time trend was included to adjust for potential fluctuations during the study period. The smoothing parameter for the trend was chosen by optimising the generalised cross-validation criteria (Wood, 2006). In an extended model, we additionally adjusted for obesity, diabetes, and potential renal impairment. We further included an interaction term to investigate the potential effect modification by underlying systemic inflammation. To evaluate the robustness of our results, we altered our base model adjustment by (1) removing season or (2) additionally adjusting for annual mean concentrations of ambient $PM_{2.5}$ and NO_2 exposure (Spearman correlation 0.71). All statistical analyses were performed using the R Statistical Software (version 3.5.1, R Foundation for Statistical Computing, Vienna, Austria). A two-sided P value < 0.05 was considered to be statistically significant. All effect estimates are presented as percent change of the geometric mean of the biomarkers with corresponding 95% confidence intervals for an interquartile range increase in exposure concentration.

3. Results

3.1. Study population

From the original group of 400 participants, 18 subjects were excluded due to missing values in the main outcomes (OH-PAHs: N = 7; MDA: N = 8; 8-OHdG: N = 9; $F_{2\alpha}$-isoprostanes: N = 9; creatinine: N = 6; extremely low creatinine concentration: N = 1; hs-CRP: N = 4). The mean age of the participants was 56 years (Table 1). Overall, 220 (57%) participants were male, 78 (20%) were smokers, and 163 (43%) reported a smoking history. The geometric mean concentration of hs-CRP was 1.31 mg/L. In total, 80 participants (21%) showed potential underlying inflammation (hs-CRP ≥ 3 mg/L). Overall, 53 participants were diagnosed with diabetes (14%), and 156 participants were diagnosed with pre-diabetes (41%).

The geometric means of the sum OH-PAHs concentration and the concentrations of 1-OH-Phe, 2-OH-Phe, 3-OH-Phe, and 1-OH-Pyr were 0.24, 0.11, 0.05, 0.06, and 0.16 ng/mg creatinine, respectively. The geometric means of the sum of $F_{2\alpha}$-isoprostanes concentration and the concentration of 2,3-dinor-8-iso-$PGF_{2\alpha}$ 8-iso-15(R)-$PGF_{2\alpha}$, 8-iso-$PGF_{2\alpha}$, and ±5-$iPF_{2\alpha}$ were 3.56, 1.71, 0.44, 0.22, and 1.06 ng/mg creatinine, respectively. The geometric mean concentrations of MDA, 8-OHdG, and creatinine in the study participants were 33.77 ng/mg creatinine, 2.94 ng/mg creatinine and 1.08 mg/mL, respectively. The annual means of individual ambient exposure of $PM_{2.5}$ and NO_2 were 11.72 μg/m^3 and 13.62 μg/m^3, respectively. Supplemental Fig. 1 shows the Spearman correlation coefficients between all pairwise combinations of biomarkers and clinical parameters. Strong correlations were observed within the $F_{2\alpha}$-isoprostane group (0.54–0.90) and the OH-PAH group (0.78–0.93). Therefore, we limited parts of the subsequent analyses to

Table 1
Descriptive statistics of the study population (N = 400).

Characteristics	Mean ± SD or Total N (%)	Missing N
Personal Characteristics		
Age (years)	56 ± 9.2	
<55	173 (45%)	
≥55, <65	126 (33%)	
≥65	83 (22%)	
Sex (male)	220 (57%)	
Socio-economic & lifestyle characteristics		
BMI (kg/m^2)	28.1 ± 4.8	
Obese (BMI ≥30 kg/m^2)	116 (30.4%)	
Smoking Status		
Non-smoker	141 (37%)	
Ex-smoker	163 (43%)	
Smoker	78 (20%)	
Clinical Characteristics		
hs-CRP (mg/L) (N = 382)	2.39 ± 3.36	4
Potential underlying inflammation (hs-CRP ≥ 3 mg/L)	80 (21%)	4
eGFR (mL/min/1.73 m^2)	89.9 ± 9.3	7
Potential renal impairment (eGFR <90 mL/min/1.73 m^2)	179 (47%)	7
Diabetes Status		
No Diabetes	173 (45%)	
Pre-diabetes	156 (41%)	
Diabetes	53 (14%)	
Hypertension Status		
Hypertension	130 (34%)	
Characteristics of the day of examination		
Season		
Spring (Mar–May)	129 (34%)	
Summer (Jun–Aug)	98 (26%)	
Autumn (Sep–Nov)	71 (18%)	
Winter (Dec–Feb)	84 (22%)	
Urinary biomarkers (ng/mg Creatinine)		
OH-PAHs	0.34 ± 0.39	7
1-OH-Phe	0.16 ± 0.18	7
2-OH-Phe	0.08 ± 0.12	7
3-OH-Phe	0.10 ± 0.14	7
1-OH-Pyr	0.30 ± 0.39	7
MDA	41.76 ± 38.69	8
8-OHdG	3.35 ± 1.93	9
$F_{2\alpha}$-isoprostanes	4.11 ± 2.92	9
2,3-dinor-8-iso-$PGF_{2\alpha}$	2.08 ± 1.63	7
8-iso-15(R)-$PGF_{2\alpha}$	0.52 ± 0.1	7
8-iso-$PGF_{2\alpha}$	0.27 ± 0.23	9
±5-$iPF_{2\alpha}$	1.24 ± 0.99	7
Creatinine (mg/mL)	1.34 ± 0.81	6
Annual mean of individual ambient exposure (μg/m^3)		
$PM_{2.5}$	11.72 ± 1.01	
NO_2	13.62 ± 4.35	

SD, standard deviation; OH-PAHs, sum OH-PAHs concentration; 1-OH-Phe, 1-hydroxyphenanthrene; 2-OH-Phe, 2-hydroxyphenanthrene; 3-OH-Phe, 1-hydroxyphenanthrene; 1-OH-Pyr, 1-hydroxypyrene; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; $F_{2\alpha}$-isoprostanes, sum $F_{2\alpha}$-isoprostanes concentration; BMI, Body Mass Index; hs-CRP, high sensitivity C-Reactive Protein; eGFR, Estimated Glomerular Filtration Rate from Serum Creatinine and Cystatin C; $PM_{2.5}$, particulate matter smaller than 2.5 μm in aerodynamic diameter; NO_2, nitrogen dioxide.

the sum of OH-PAH concentrations and the sum of $F_{2\alpha}$-isoprostanes concentrations as indicators to reduce the number of tests.

3.2. Distribution of biomarker levels across different subgroups

Table 2 shows the median concentrations of biomarkers in the selected subgroups. MDA concentrations were significantly higher in participants with higher age (≥55 years), potential underlying inflammation, potential renal impairment, or in participants who had their examinations in spring/winter. 8-OHdG concentrations were significantly higher in older participants (age ≥55 years). Concentrations of $F_{2\alpha}$-isoprostanes were significantly higher in female participants or participants who visited the study center in autumn/winter. Hs-CRP concentrations were significantly higher in older participants (≥65 years), potential renal impairment, diabetes, obesity, or hypertension.

3.3. Association between OH-PAHs and biomarkers of oxidative stress

All OH-PAHs were significantly positively associated with oxidative stress biomarkers (Fig. 1). For participants with a potential underlying state of inflammation (hs-CRP ≥ 3 mg/L), we observed stronger associations between OH-PAHs and $F_{2\alpha}$-isoprostanes and between OH-PAHs and 8-OHdG, whereas no differences were observed for MDA (Fig. 2). Additional adjustment for obesity, diabetes, and potential renal impairment did not considerably change the estimates.

3.4. Association between OH-PAHs and hs-CRP

We did not observe an association between the sum of OH-PAHs or single OH-PAHs and hs-CRP for both model adjustment sets, although the estimates tended to be slightly higher in the extended covariate model (Fig. 3).

3.5. Association between biomarkers of oxidative stress and hs-CRP

The effect estimates of the three biomarkers of oxidative stress indicated different patterns of association with hs-CRP (Fig. 4). In the base covariate model, MDA was not associated with hs-CRP, $F_{2\alpha}$-isoprostanes indicated a positive association, and 8-OHdG was significantly positively associated. When additionally adjusting for obesity, diabetes, and potential renal impairment, the positive association between $F_{2\alpha}$-isoprostanes and hs-CRP turned significant.

3.6. Robustness of multiple linear regression models

All effect estimates remained robust when excluding season from the regression models (Supplemental Tables 1–3) or when additionally adjusting for annual mean concentrations of ambient $PM_{2.5}$ and NO_2.

4. Discussion

4.1. Summary

In this cross-sectional study of 400 residents of the Augsburg region (Germany) conducted in 2013/2014, we determined biomarkers of PAH exposure, oxidative stress, and inflammation to investigate the interplay between these three groups of biomarkers. (1) The concentrations of OH-PAHs were comparably lower in our study than in other studies. For example, a study carried out among 300 participants from 7 Asian countries reported that the mean concentrations of 2-OH-Phe, 3-OH-Phe, and 1-OH-Pyr ranged between 0.072–0.58 ng/mL, 0.101–0.714 ng/mL, and 0.167–0.667 ng/mL, respectively (Guo et al., 2013) while in our study, the mean concentrations were 0.09, 0.11, and 0.35 ng/ml, respectively (before normalisation by urinary creatinine). The concentrations of the biomarkers of oxidative stress and inflammation were significantly higher in older (MDA, 8-OHdG, and hs-CRP) and obese (hs-CRP) participants, participants with potential underlying inflammation (MDA) and potential renal impairment (MDA, 8-OHdG and hs-CRP), as well as in participants with diagnosed diseases like type 2 diabetes (hs-CRP) or hypertension (hs-CRP), or for participants who had their clinical visit in spring/winter (MDA) or autumn/winter ($F_{2\alpha}$-isoprostanes) compared to their respective counterparts. (2) Positive associations were found between OH-PAHs and biomarkers of oxidative stress, and were more pronounced in participants with potential underlying inflammation. (3) However, no association was observed between OH-PAH and hs-CRP concentrations. (4) Among the three oxidative stress markers, only 8-OHdG was significantly positively associated with hs-CRP, whereas $F_{2\alpha}$-isoprostanes only indicated a positive association, and

Table 2
Kruskal-Wallis Test of OH-PAHs, oxidative stress, and inflammation biomarkers for different subsets of participants.

	N	OH-PAHs (ng/mg Creatinine)		MDA (ng/mg Creatinine)		8-OHdG (ng/mg Creatinine)		$F_{2\alpha}$-isoprostanes (ng/mg Creatinine)		hs-CRP (mg/L)	
		Median	p	Median	p	Median	p	Median	p	Median	p
Age											
Age <55	173	0.20	0.11	26.57	<0.01	2.66	<0.01	3.68	0.64	1.13	0.04
Age ≥55, <65	126	0.22		32.77		2.98		3.41		1.12	
Age ≥65	83	0.27		37.26		3.49		3.60		1.48	
Sex											
Female	162	0.21	0.16	29.47	0.19	3.20	0.17	3.77	0.01	1.37	0.08
Male	220	0.24		31.95		2.91		3.39		1.12	
Obesity											
No (BMI <30 kg/m^2)	266	0.24	0.01	30.80	0.69	3.10	0.12	3.61	0.57	0.97	<0.01
Yes (BMI ≥30 kg/m^2)	116	0.20		29.40		2.81		3.62		2.27	
Smoking Status											
Non-smoker	141	0.20	<0.01	32.85	0.20	3.25	0.50	3.77	0.08	1.13	0.50
Ex-smoker	163	0.21		29.38		2.96		3.41		1.15	
Smoker	78	0.30		29.54		2.79		3.64		1.51	
Potential underlying systemic inflammation											
No (hs-CRP < 3 mg/L)	302	0.21	0.04	29.74	0.03	2.96	0.08	3.58	0.55	–	–
Yes (hs-CRP ≥ 3 mg/L)	80	0.26		33.42		3.21		3.67		–	
Potential renal impairment											
No (eGFR ≥90 mL/min/1.73 m^2)	199	0.22	0.72	28.45	0.01	2.90	0.04	3.74	0.08	1.10	0.02
Yes (eGFR <90 mL/min/1.73 m^2)	179	0.23		33.32		3.18		3.45		1.38	
Type 2 Diabetes											
No	173	0.21	0.46	28.86	0.09	2.92	0.19	3.63	0.58	0.93	<0.01
Pre	156	0.22		31.24		3.06		3.51		1.62	
Yes	53	0.26		34.81		3.18		3.74		1.15	
Hypertension											
No	253	0.21	0.97	29.55	0.30	2.94	0.08	3.68	0.29	1.11	<0.01
Yes	139	0.23		32.18		3.18		3.41		1.41	
Season											
Spring (Mar–May)	129	0.20	<0.01	34.77	<0.01	3.09	0.24	3.68	<0.01	1.11	0.77
Summer (Jun–Aug)	98	0.19		24.35		2.79		2.73		1.15	
Autumn (Sep–Nov)	71	0.21		29.48		3.09		4.03		1.30	
Winter (Dec–Feb)	84	0.33		32.88		3.23		4.01		1.17	

hs-CRP, high sensitivity C-Reactive Protein; eGFR, Estimated Glomerular Filtration Rate from Serum Creatinine and Cystatin C, OH-PAHs, sum OH-PAHs concentration; 1-OH-Phe, 1-hydroxyphenanthrene; 2-OH-Phe, 2-hydroxyphenanthrene; 3-OH-Phe, 1-hydroxyphenanthrene; 1-OH-Pyr, 1-hydroxypyrene; MDA, malondialdehyde; 8-OHdG, 8-hydroxy-2′-deoxyguanosine; $F_{2\alpha}$-isoprostanes, sum $F_{2\alpha}$-isoprostanes concentration; BMI, Body Mass Index; potential renal impairment, chronic kidney disease.

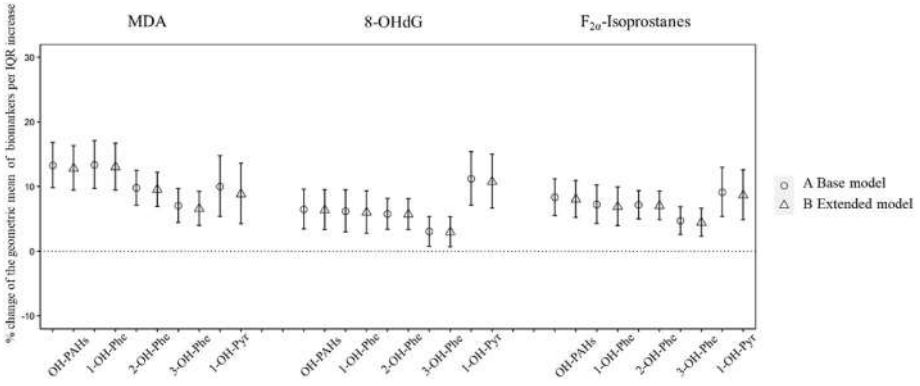

Fig. 1. Percent change in biomarkers of oxidative stress (95% CI) in association with an interquartile range increase in internal exposure biomarkers adjusted for age, sex, smoking, trend, and season (A Base model) and additionally adjusted for obesity, diabetes and potential renal impairment (B Extended model).

MDA was not associated at all. When additionally adjusting for obesity, diabetes, and potential renal impairment, the positive association between $F_{2\alpha}$-isoprostanes and hs-CRP turned significant.

4.2. Biomarkers varied across different subgroups

Sex: We observed higher concentrations of $F_{2\alpha}$-isoprostanes in women than in men. A similar study from the U.S. examining 65 participants (19 male, 46 female; 38.6 ± 11.1 years old) also found higher concentration of $F_{2\alpha}$-isoprostanes in females (Ma et al., 2017). Such different concentration levels in males and females may be caused by a decrease in oestrogen levels, which reduces the antioxidative capability in the postmenopausal period (Zaja-Milatovic et al., 2009), or due to the different ratios in content of lean body mass and bone mineral in male and female participants (Ma et al., 2017).

Age: Many studies have reported an association between aging and ROS, suggesting that free radicals play an important role in aging (Finkel and Holbrook, 2000; Guyton et al., 1998; Harman, 1956). In the present study, the biomarkers of ROS damage, MDA and 8-OHdG, showed higher concentrations in the older groups (between 55 and 65 years and

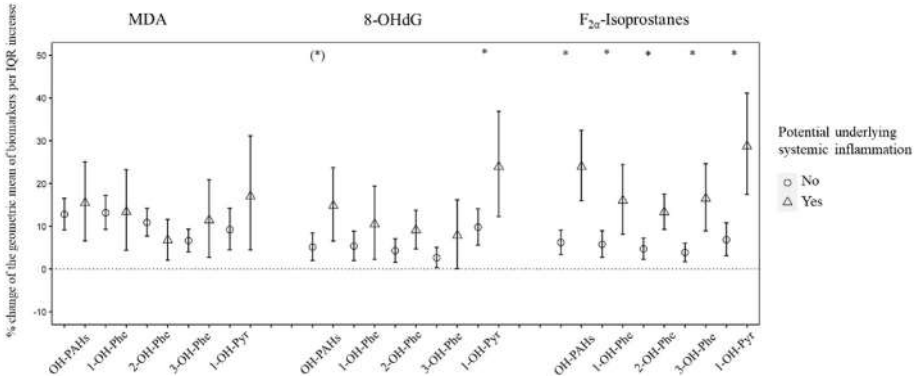

Fig. 2. Effect modification by potential underlying systemic inflammation (hs-CRP ≥ 3 mg/L vs. hs-CRP < 3 mg/L) of the association between OH-PAHs and oxidative stress biomarkers adjusted for age, sex, smoking, trend, and season (Base model). (* p-value of interaction <0.05, (*) p-value of interaction <0.1).

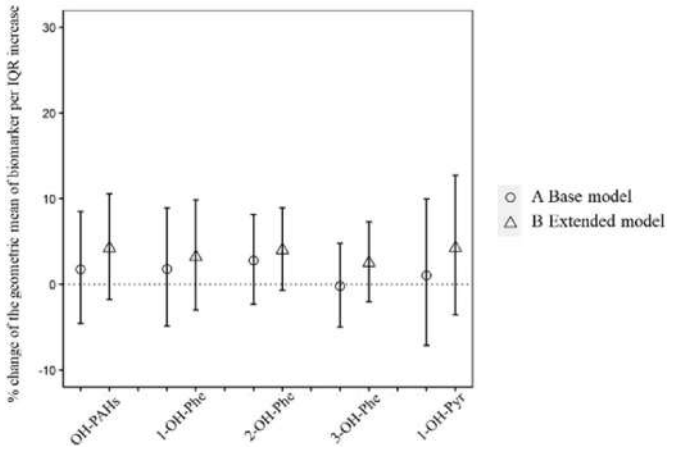

Fig. 3. Percent change in hs-CRP (95% CI) in association with an interquartile range increase in OH-PAHs adjusted for age, sex, smoking, trend, and season (A Base model) and additionally adjusted for obesity, diabetes and potential renal impairment (B Extended model).

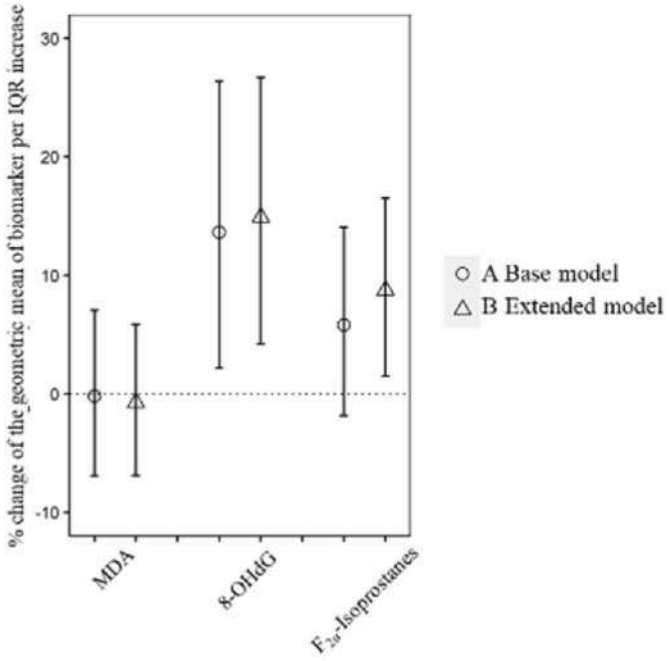

Fig. 4. Percent change in hs-CRP (95% CI) in association with an interquartile range increase in oxidative stress biomarkers adjusted for age, sex, smoking, trend, and season (A Base model) and additionally adjusted for obesity, diabetes and potential renal impairment (B Extended model).

older than 65 years) which is consistent with the theory that organisms age because they accumulate oxidative damage generated by ROS.

Smoking: Cigarette smoke contains large amounts of PAHs (Vu et al., 2015), and the group of smokers showed the highest level of PAH metabolites in urine. Similarly, a cohort study among 288 non-smokers and 100 smokers found highly significant differences and dose-response relationships with regard to cigarettes smoked per day for 2- OH-Phe, 3-OH-Phe, 4-OH-Phe, and 1-OH-Pyr (Heudorf and Angerer, 2001). In addition, a cross-sectional study among 4092 participants in China found significant correlations between urinary OH-PAH levels and cigarette smoking (Cao et al., 2020a).

Obesity and chronic diseases: Participants with obesity, potential renal impairment, diabetes, and hypertension showed higher concentrations of hs-CRP, indicating an underlying inflammatory state in these participants. Two studies compared patients at different stages of chronic kidney disease (CKD) with a control group and found higher oxidative stress levels and inflammation in patients with CKD (Karamouzis et al., 2008; Oberg et al., 2004). Accordingly, we observed higher concentrations of MDA, 8-OHdG, and hs-CRP in participants with potential renal impairment, as well as an indication for $F_{2\alpha}$-isoprostanes. Two studies among a cohort of North Indians and a cohort of African Americans found that higher concentrations of hs-CRP were associated with diabetes or, to a lesser degree, insulin resistance (Effoe et al., 2015; Mahajan et al., 2009). A study from Egypt, including 80 participants, reported that hypertension may increase the level of hs-CRP (Abd El Aziz et al., 2019), which matches our observations.

Season: We found significantly higher OH-PAH levels in urine samples collected during winter. We assume that a large part of the internal PAH burden is caused by exposure to PAH-polluted ambient air. Several studies have monitored atmospheric PAHs in Europe and China in recent years and reported similar seasonal variations due to different source contributions between autumn-winter and spring-summer (Albuquerque et al., 2016; Dvorská et al., 2011; Liu et al., 2014; Schnelle-Kreis et al., 2007). Moreover, Li et al. suggested that biomass burning for domestic heating during the heating season (October to March) was the major contributor to atmospheric PAHs in the Augsburg region (Germany) between 2014 and 2015 (Li et al., 2018). This observation was consistent with our results of seasonal variations in OH-PAHs, indicating that ambient PAHs might be an important source of PAH intake. In addition, the levels of oxidative stress markers (MDA and $F_{2\alpha}$-isoprostanes) were significantly higher in samples taken in spring/winter or autumn/winter, which may also be related to increased ambient PAHs pollution. Both short- and long-term studies suggest that higher concentrations of OH-PAHs and increased levels of oxidative

stress biomarkers can be detected after exposure to ambient pollutants (Bortey-Sam et al., 2017; Li et al., 2012; Lu et al., 2016; Moller and Loft, 2010; Motorykin et al., 2015; Suzuki et al., 1995).

4.3. Associations between OH-PAHs and oxidative stress and the role of inflammation

Two smaller cohort studies investigated the association between selected oxidative stress markers (MDA, 8-OHdG, and $F_{2\alpha}$-isoprostanes) and OH-PAHs in Japan and the United States (Bortey-Sam et al., 2017; Ferguson et al., 2017). Bortey-Sam et al. found significantly positive correlations between the sum of OH-PAHs, 2-OH-naphthalene, 2-3-OH-fluorenes, and MDA, and a positive correlation between 4-OH-Phe and 8-OHdG in urine samples collected from 202 residents of Kumasi, Japan. Ferguson et al. investigated urine samples of 200 pregnant women in the United States and reported that some PAH metabolites were consistently positively associated with urinary oxidative stress markers (8-OHdG and 8-isoprostane) (Ferguson et al., 2017). These findings were confirmed by our study, in which all examined oxidative stress markers showed positive associations with OH-PAHs. It should be mentioned that Ferguson et al. used specific gravity-corrected urinary concentrations in their study. Recent investigations showed that both, creatinine and specific gravity can be used as tools for normalisation or correction and no significant differences considering the results are expected in generally healthy individuals (Sallsten and Barregard, 2021). Although Kuiper et al. recently recommended the specific gravity method (Kuiper et al., 2021), at the time our samples were analysed (2015/16), most other studies were also based on creatinine normalised values.

In our study, the associations between OH-PAHs, 8-OHdG and $F_{2\alpha}$-isoprostanes were more pronounced in participants with potential underlying systemic inflammation, whereas no difference was observed for MDA. This finding indicates that oxidative stress is deteriorated, or is promoted by systemic inflammation. In a review article, Biswas et al. pointed out that inflammation processes can produce reactive species, and an inflammatory status may exaggerate the generation of reactive species (Biswas, 2016). Similarly, other underlying long-term risk factor profiles associated with obesity, diabetes, or CKD, may be responsible for increased levels of oxidative stress, as suggested by our data.

4.4. Associations between OH-PAHs and hs-CRP

A cohort study by Clark et al. using data of 3219 participants aged 20 years and older from the U.S National Health and Nutrition Examination Survey (NHANES) 2001–2004 investigated the relationship between OH-PAHs and inflammatory markers (homocysteine, fibrinogen, white blood cell count), but found no significant differences between high and low levels (75th vs. 25th percentiles) of all PAH metabolites in non-smoking participants (Clark et al., 2012). The study by Ferguson et al. observed positive associations between urinary OH-PAH concentrations and hs-CRP, but not with inflammatory cytokines (IL-1β, IL-6, IL-10, and TNF-α). In this analysis, we observed only slight indications for a positive association between OH-PAHs and hs-CRP levels. Clark et al. did not specify whether they used spot urine or 24 h urine, thus the comparability is limited.

In our previous KORA FF4 study across the full sample of 2252 participants, we observed significant positive associations between long-term exposure to ambient air pollution and hs-CRP (Pilz et al., 2018). In summary, our findings in this relatively low-exposure setting point to only weak associations between OH-PAHs and inflammation.

4.5. Association between oxidative stress and hs-CRP varied for different biomarkers

It has been shown that ROS play an important role in the signalling of inflammatory responses (Peters et al., 2021). In this analysis, 8-OHdG was significantly positively associated with hs-CRP, while MDA showed no association. When additionally adjusting for obesity, diabetes and potential renal impairment, the positive association between $F_{2\alpha}$-isoprostanes and hs-CRP turned significant. Although MDA, $F_{2\alpha}$-isoprostanes, and 8-OHdG are all indicators of ROS levels, they are generated from different pathways. While MDA (Ayala et al., 2014; Chen et al., 2011) and $F_{2\alpha}$-isoprostanes (Galano et al., 2017; Milne et al., 2008; Morrow and Roberts, 1996) are generated from non-enzymatic and free radical-mediated oxidation, 8-OHdG is formed enzymatically during DNA impairment and repair (Evans et al., 2010; Valavanidis et al., 2009). Different phases of oxidative stress from tolerance, adaptation, inflammation, and cell death were described by Peters et al. as a continuum (Peters et al., 2021). Therefore, MDA and $F_{2\alpha}$-isoprostanes can be generated through all phases of the continuum, while 8-OHdG is only generated in the later phases, for example, inflammation and cell death when ROS exceed antioxidation (Asanka Sanjeewa et al., 2021; Janovits et al., 2021; Luan et al., 2022; Trettin et al., 2014). Moreover, MDA is highly reactive and very polar. It is generated in the early phase of the exposure but gets cleared very fast (Siu and Draper, 1982; Traverso et al., 2004) whereas $F_{2\alpha}$-isoprostanes are relatively stable (Milne et al., 2008). This might be one explanation why we observed no association between hs-CRP and MDA but an indication for $F_{2\alpha}$-isoprostanes.

4.6. Strengths and limitations

One strength of this study is the selection of various biomarkers of oxidative stress, which were measured in our laboratory using established high-performance analytical methods. Each marker or each group of markers reflects characteristic damage to cellular macromolecules, namely the double bonds of PUFA, DNA, and membrane phospholipids. This allows the investigation of their interplay with respect to the internal PAH burden, which was also determined in our lab, along with the inflammation marker hs-CRP. Another strength of our study is the high number and diversity of individual participants' data available for the sub-cohort of the KORA study. The combination of lab-generated data with comprehensive information of the participants allowed us to perform more comprehensive investigations and better control of confounders than in previous studies.

There were several limitations to this study. First, our study was a cross-sectional study, and each participant was sampled only at a single time point from 2013 to 2014. Second, as a selected subset of participants was included in this analysis, our findings might not be representative for the general population. Third, OH-PAHs and biomarkers of oxidative stress were analysed from urine samples, while hs-CRP was analysed from serum samples. Metabolites in urine are considered as end products (Ayala et al., 2014; Evans et al., 2010; Morrow and Roberts, 1996) whereas metabolites in serum can function and participate in metabolic processes (Gewurz et al., 1982). However, this could also be considered an opportunity to observe and interpret the data within a larger frame. Fourth, the half-lives of the biomarkers might differ which could be one of the reasons why we did not observe an association between OH-PAHs and hs-CRP (Li et al., 2012; Pepys and Hirschfield, 2003). Finally, only spot urine samples were collected and analysed. However, we applied creatinine normalisation, an effective normalisation method to minimise the differences between the concentrations of OH-PAHs in spot, first-morning, and 24-h urine samples (Li et al., 2010).

5. Conclusion

In this cross-sectional study, we observed associations between exposure to PAHs, oxidative stress, and inflammation, even in a low exposure setting. We found positive associations between OH-PAHs and oxidative stress that were more pronounced in participants with an underlying inflammatory state. Additionally, hs-CRP was positively associated with increased markers of oxidative stress but not directly

with PAHs. Individual risk factors were important contributors to these processes and should be considered as potential confounders in future studies. Further longitudinal studies are necessary to investigate the causal chain of the associations.

Declaration of interest

All authors declare that no conflict of interest exists.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113993.

References

Abd El Aziz, W., Abou Elnour, E., Mena, M., El-brol, R., 2019. A comparative analysis of the level of high-sensitivity C-reactive protein in individuals with and without hypertension. Menoufia Medical Journal 32, 187–193.

Al-Delaimy, W.K., Larsen, C.W., Pezzoli, K., 2014. Differences in health symptoms among residents living near illegal dump sites in Los Laureles Canyon, Tijuana, Mexico: a cross sectional survey. Int. J. Environ. Res. Publ. Health 11, 9532–9552.

Albuquerque, M., Coutinho, M., Borrego, C., 2016. Long-term monitoring and seasonal analysis of polycyclic aromatic hydrocarbons (PAHs) measured over a decade in the ambient air of Porto. Portugal. Sci Total Environ 543, 439–448.

Alshaarawy, O., Elbaz, H.A., Andrew, M.E., 2016. The association of urinary polycyclic aromatic hydrocarbon biomarkers and cardiovascular disease in the US population. Environ. Int. 89–90, 174–178.

Andersen, M.H.G., Saber, A.T., Pedersen, J.E., Pedersen, P.B., Clausen, P.A., Lohr, M., Kermanizadeh, A., Loft, S., Ebbehoj, N.E., Hansen, A.M., Kalevi Koponen, I., Norskov, E.C., Vogel, U., Moller, P., 2018. Assessment of polycyclic aromatic hydrocarbon exposure, lung function, systemic inflammation, and genotoxicity in peripheral blood mononuclear cells from firefighters before and after a work shift. Environ. Mol. Mutagen. 59, 539–548.

Apel, K., Hirt, H., 2004. Reactive oxygen species: metabolism, oxidative stress, and signal transduction. Annu. Rev. Plant Biol. 55, 373–399.

Aquilina, N.J., Delgado-Saborit, J.M., Meddings, C., Baker, S., Harrison, R.M., Jacob 3rd, P., Wilson, M., Yu, L., Duan, M., Benowitz, N.L., 2010. Environmental and biological monitoring of exposures to PAHs and ETS in the general population. Environ. Int. 36, 763–771.

Asanka Sanjeewa, K.K., Herath, K.H.I.N.M., Yang, H.W., Choi, C.S., Jeon, Y.J., 2021. Anti-inflammatory mechanisms of fucoidans to treat inflammatory diseases: a review. Mar. Drugs 19.

Aumann, N., Baumeister, S.E., Rettig, R., Lieb, W., Werner, A., Doring, A., Peters, A., Koenig, W., Hannemann, A., Wallaschofski, H., Nauck, M., Stracke, S., Volzke, H., Meisinger, C., 2015. Regional Variation of Chronic Kidney Disease in Germany: Results from Two Population-Based Surveys, vol. 40. Kidney Blood Press Res, pp. 231–243.

Ayala, A., Munoz, M.F., Arguelles, S., 2014. Lipid peroxidation: production, metabolism, and signaling mechanisms of malondialdehyde and 4-hydroxy-2-nonenal. Oxid. Med. Cell. Longev. 2014, 360438.

Bamberg, F., Hetterich, H., Rospleszcz, S., Lorbeer, R., Auweter, S.D., Schlett, C.L., Schafnitzel, A., Bayerl, C., Schindler, A., Saam, T., Müller-Peltzer, K., Sommer, W., Zitzelsberger, T., Machann, J., Ingrisch, M., Selder, S., Rathmann, W., Heier, M., Linkohr, B., Meisinger, C., Weber, C., Ertl-Wagner, B., Massberg, S., Reiser, M.F., Peters, A., 2017. Subclinical disease burden as assessed by whole-body MRI in subjects with prediabetes, subjects with diabetes, and normal control subjects from the general population: the KORA-MRI study. Diabetes 66, 158–169.

Biswas, S.K., 2016. Does the interdependence between oxidative stress and inflammation explain the antioxidant paradox? Oxid. Med. Cell. Longev. 2016, 5698931.

Bortey-Sam, N., Ikenaka, Y., Akoto, O., Nakayama, S.M.M., Asante, K.A., Baidoo, E., Obirikorang, C., Saengtienchai, A., Isoda, N., Nimako, C., Mizukawa, H., Ishizuka, M., 2017. Oxidative stress and respiratory symptoms due to human exposure to polycyclic aromatic hydrocarbons (PAHs) in Kumasi, Ghana. Environ. Pollut. 228, 311–320.

Cao, L., Wang, D., Zhu, C., Wang, B., Cen, X., Chen, A., Zhou, H., Ye, Z., Tan, Q., Nie, X., Feng, X., Xie, Y., Yuan, J., Chen, W., 2020. Polycyclic aromatic hydrocarbon exposure and atherosclerotic cardiovascular disease risk in urban adults: the mediating role of oxidatively damaged DNA. Environ. Pollut. 265, 114860.

Chen, J.L., Huang, Y.J., Pan, C.H., Hu, C.W., Chao, M.R., 2011. Determination of urinary malondialdehyde by isotope dilution LC-MS/MS with automated solid-phase extraction: a cautionary note on derivatization optimization. Free Radic. Biol. Med. 51, 1823–1829.

Chuang, K.J., Chan, C.C., Su, T.C., Lee, C.T., Tang, C.S., 2007. The effect of urban air pollution on inflammation, oxidative stress, coagulation, and autonomic dysfunction in young adults. Am. J. Respir. Crit. Care Med. 176, 370–376.

Clark 3rd, J.D., Serdar, B., Lee, D.J., Arheart, K., Wilkinson, J.D., Fleming, L.E., 2012. Exposure to polycyclic aromatic hydrocarbons and serum inflammatory markers of cardiovascular disease. Environ. Res. 117, 132–137.

Danielsen, P.H., Moller, P., Jensen, K.A., Sharma, A.K., Wallin, H., Bossi, R., Autrup, H., Molhave, L., Ravanat, J.L., Briede, J.J., de Kok, T.M., Loft, S., 2011. Oxidative stress, DNA damage, and inflammation induced by ambient air and wood smoke particulate matter in human A549 and THP-1 cell lines. Chem. Res. Toxicol. 24, 168–184.

Droge, W., 2002. Free radicals in the physiological control of cell function. Physiol. Rev. 82, 47–95.

Dvorská, A., Lammel, G., Klánová, J., 2011. Use of diagnostic ratios for studying source apportionment and reactivity of ambient polycyclic aromatic hydrocarbons over Central Europe. Atmos. Environ. 45, 420–427.

Effoe, V.S., Correa, A., Chen, H., Lacy, M.E., Bertoni, A.G., 2015. High-sensitivity C-reactive protein is associated with incident type 2 diabetes among African Americans: the Jackson heart study. Diabetes Care 38, 1694–1700.

Evans, M.D., Saparbaev, M., Cooke, M.S., 2010. DNA repair and the origins of urinary oxidized 2'-deoxyribonucleosides. Mutagenesis 25, 433–442.

Everett, C.J., King, D.E., Player, M.S., Matheson, E.M., Post, R.E., Mainous 3rd, A.G., 2010. Association of urinary polycyclic aromatic hydrocarbons and serum C-reactive protein. Environ. Res. 110, 79–82.

Farzan, S.F., Chen, Y., Trachtman, H., Trasande, L., 2016. Urinary polycyclic aromatic hydrocarbons and measures of oxidative stress, inflammation and renal function in adolescents: NHANES 2003-2008. Environ. Res. 144, 149–157.

Ferguson, K.K., McElrath, T.F., Pace, G.G., Weller, D., Zeng, L., Pennathur, S., Cantonwine, D.E., Meeker, J.D., 2017. Urinary polycyclic aromatic hydrocarbon metabolite associations with biomarkers of inflammation, angiogenesis, and oxidative stress in pregnant women. Environ. Sci. Technol. 51, 4652–4660.

Fialkow, L., Wang, Y., Downey, G.P., 2007. Reactive oxygen and nitrogen species as signaling molecules regulating neutrophil function. Free Radic. Biol. Med. 42, 153–164.

Finkel, T., Holbrook, N.J., 2000. Oxidants, oxidative stress and the biology of ageing. Nature 408, 239–247.

Fuchte, H.E., Paas, B., Auer, F., Bayer, V.J., Achten, C., Schäffer, A., Smith, K.E.C., 2022. Identification of sites with elevated PM levels along an urban cycle path using a mobile platform and the analysis of 48 particle bound PAH. Atmos. Environ. 271, 118912.

Galano, J.M., Lee, Y.Y., Oger, C., Vigor, C., Vercauteren, J., Durand, T., Giera, M., Lee, J. C., 2017. Isoprostanes, neuroprostanes and phytoprostanes: an overview of 25years of research in chemistry and biology. Prog. Lipid Res. 68, 83–108.

Garrido, A., Jiménez-Guerrero, P., Ratola, N., 2014. Levels, trends and health concerns of atmospheric PAHs in Europe. Atmos. Environ. 99, 474–484.

Gerlofs-Nijland, M.E., Rummelhard, M., Boere, A.J., Leseman, D.L., Duffin, R., Schins, R. P., Borm, P.J., Sillanpaa, M., Salonen, R.O., Cassee, F.R., 2009. Particle induced toxicity in relation to transition metal and polycyclic aromatic hydrocarbon contents. Environ. Sci. Technol. 43, 4729–4736.

Guyton, K.Z., Gorospe, M., Wang, X., Mock, Y.D., Kokkonen, G.C., Liu, Y., Roth, G.S., Holbrook, N.J., 1998. Age-related changes in activation of mitogen-activated protein kinase cascades by oxidative stress. J. Invest. Dermatol. Symp. Proc. 3, 23–27.

Harman, D., 1956. Aging: a theory based on free radical and radiation chemistry. J. Gerontol. 11, 298–300.

Hennig, F., Fuks, K., Moebus, S., Weinmayr, G., Memmesheimer, M., Jakobs, H., Brocker-Preuss, M., Fuhrer-Sakel, D., Mohlenkamp, S., Erbel, R., Jockel, K.H., Hoffmann, B., Heinz Nixdorf Recall Study Investigative, G, 2014. Association between source-specific particulate matter air pollution and hs-CRP: local traffic and industrial emissions. Environ. Health Perspect. 122, 703–710.

Holle, R., Happich, M., Lowel, H., Wichmann, H.E., Group, M.K.S., 2005. KORA–a research platform for population based health research. Gesundheitswesen 67 (Suppl. 1), S19–S25.

Hou, J., Yin, W., Li, P., Huang, Y., Wan, Y., Hu, C., Xu, T., Cheng, J., Wang, L., Yu, Z., Yuan, J., 2019. Effect of exposure to phthalates on association of polycyclic aromatic hydrocarbons with 8-hydroxy-2'-deoxyguanosine. Sci. Total Environ. 691, 378–392.

Ifegwu, O.C., Anyakora, C., 2016. Chapter six - polycyclic aromatic hydrocarbons: Part II, urine markers. In: Makowski, G.S. (Ed.), Advances in Clinical Chemistry. Elsevier, pp. 159–183.

Inker, L.A., Schmid, C.H., Tighiouart, H., Eckfeldt, J.H., Feldman, H.I., Greene, T., Kusek, J.W., Manzi, J., Van Lente, F., Zhang, Y.L., Coresh, J., Levey, A.S., Investigators, C.-E., 2012. Estimating glomerular filtration rate from serum creatinine and cystatin C. N. Engl. J. Med. 367, 20–29.

Janovits, P.M., Leiguez, E., Portas, V., Teixeira, C., 2021. A metalloproteinase induces an inflammatory response in preadipocytes with the activation of cox signalling pathways and participation of endogenous phospholipases a2. Biomolecules 11.

Kamal, A., Qamar, K., Gulfraz, M., Anwar, M.A., Malik, R.N., 2015. PAH exposure and oxidative stress indicators of human cohorts exposed to traffic pollution in Lahore city (Pakistan). Chemosphere 120, 59–67.

Karamouzis, I., Sarafidis, P.A., Karamouzis, M., Iliadis, S., Haidich, A.B., Sioulis, A., Triantos, A., Vavatsi-Christaki, N., Grekas, D.M., 2008. Increase in oxidative stress but not in antioxidant capacity with advancing stages of chronic kidney disease. Am. J. Nephrol. 28, 397–404.
Keyte, I.J., Harrison, R.M., Lammel, G., 2013. Chemical reactivity and long-range transport potential of polycyclic aromatic hydrocarbons–a review. Chem. Soc. Rev. 42, 9333–9391.
Kraus, U., Breitner, S., Schnelle-Kreis, J., Cyrys, J., Lanki, T., Rückerl, R., Schneider, A., Brüske, I., Gu, J., Devlin, R., Wichmann, H.E., Zimmermann, R., Peters, A., 2011. Particle-associated organic compounds and symptoms in myocardial infarction survivors. Inhal. Toxicol. 23, 431–447.
Kuiper, J.R., O'Brien, K.M., Ferguson, K.K., Buckley, J.P., 2021. Urinary specific gravity measures in the U.S. population: implications for the adjustment of non-persistent chemical urinary biomarker data. Environ. Int. 156, 106656.
Kumar, A., Sankar, T.K., Sethi, S.S., Ambade, B., 2020. Characteristics, toxicity, source identification and seasonal variation of atmospheric polycyclic aromatic hydrocarbons over East India. Environ. Sci. Pollut. Res. Int. 27, 678–690.
Lan, Q., Mumford, J.L., Shen, M., Demarini, D.M., Bonner, M.R., He, X., Yeager, M., Welch, R., Chanock, S., Tian, L., Chapman, R.S., Zheng, T., Keohavong, P., Caporaso, N., Rothman, N., 2004. Oxidative damage-related genes AKR1C3 and OGG1 modulate risks for lung cancer due to exposure to PAH-rich coal combustion emissions. Carcinogenesis 25, 2177–2181.
Levey, A.S., Stevens, L.A., Schmid, C.H., Zhang, Y.L., Castro 3rd, A.F., Feldman, H.I., Kusek, J.W., Eggers, P., Van Lente, F., Greene, T., Coresh, J., Ckd, E.P.I., 2009. A new equation to estimate glomerular filtration rate. Ann. Intern. Med. 150, 604–612.
Li, F., Gu, J., Xin, J., Schnelle-Kreis, J., Wang, Y., Liu, Z., Shen, R., Michalke, B., Abbaszade, G., Zimmermann, R., 2021. Characteristics of chemical profile, sources and PAH toxicity of PM(2.5) in beijing in autumn-winter transit season with regard to domestic heating, pollution control measures and meteorology. Chemosphere 276, 130143.
Li, Z., Romanoff, L.C., Lewin, M.D., Porter, E.N., Trinidad, D.A., Needham, L.L., Patterson Jr., D.G., Sjodin, A., 2010. Variability of urinary concentrations of polycyclic aromatichydrocarbon metabolite in general population and comparison of spot, first-morning, and24-h void sampling. J. Expo Sci. Environ. Epidemiol. 20, 526–535.
Li, F., Schnelle-Kreis, J., Cyrys, J., Wolf, K., Karg, E., Gu, J., Orasche, J., Abbaszade, G., Peters, A., Zimmermann, R., 2018. Spatial and temporal variation of sources contributing to quasi-ultrafine particulate matter PM0.36 in Augsburg, Germany. Sci. Total Environ. 631–632, 191–200.
Li, Z., Romanoff, L., Bartell, S., Pittman, E.N., Trinidad, D.A., McClean, M., Webster, T.F., Sjodin, A., 2012. Excretion profiles and half-lives of ten urinary polycyclic aromatic hydrocarbon metabolites after dietary exposure. Chem. Res. Toxicol. 25, 1452–1461.
Lintelmann, J., Wu, X., Kuhn, E., Ritter, S., Schmidt, C., Zimmermann, R., 2018. Detection of monohydroxylated polycyclic aromatic hydrocarbons in urine and particulate matter using LC separations coupled with integrated SPE and fluorescence detection or coupled with high-resolution time-of-flight mass spectrometry. Biomed. Chromatogr. : BMC (Biomed. Chromatogr.) 32, e4183.
Liu, D., Xu, Y., Chaemfa, C., Tian, C., Li, J., Luo, C., Zhang, G., 2014. Concentrations, seasonal variations, and outflow of atmospheric polycyclic aromatic hydrocarbons (PAHs) at Ningbo site, Eastern China. Atmos. Pollut. Res. 5, 203–209.
Liu, X., Schnelle-Kreis, J., Schloter-Hai, B., Ma, L., Tai, P., Cao, X., Yu, C., Adam, T., Zimmermann, R., 2019. Analysis of PAHs associated with PM10 and PM2.5 from different districts in Nanjing. Aerosol Air Qual. Res. 19, 2294–2307.
Lu, S.Y., Li, Y.X., Zhang, J.Q., Zhang, T., Liu, G.H., Huang, M.Z., Li, X., Ruan, J.J., Kannan, K., Qiu, R.L., 2016. Associations between polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress in people living near e-waste recycling facilities in China. Environ. Int. 94, 161–169.
Luan, M., Wang, H., Wang, J., Zhang, X., Zhao, F., Liu, Z., Meng, Q., 2022. Advances in anti-inflammatory activity, mechanism and therapeutic application of ursolic acid. Mini-Rev. Med. Chem. 22, 422–436.
Mahajan, A., Tabassum, R., Chavali, S., Dwivedi, O.P., Bharadwaj, M., Tandon, N., Bharadwaj, D., 2009. High-sensitivity C-reactive protein levels and type 2 diabetes in urban North Indians. J. Clin. Endocrinol. Metab. 94, 2123–2127.
McCarrick, S., Cunha, V., Zapletal, O., Vondracek, J., Dreij, K., 2019. In vitro and in vivo genotoxicity of oxygenated polycyclic aromatic hydrocarbons. Environ. Pollut. 246, 678–687.
McCord, J.M., 1993. Human disease, free radicals, and the oxidant/antioxidant balance. Clin. Biochem. 26, 351–357.
Mesquita, S.R., van Drooge, B.L., Reche, C., Guimaraes, L., Grimalt, J.O., Barata, C., Pina, B., 2014. Toxic assessment of urban atmospheric particle-bound PAHs: relevance of composition and particle size in Barcelona (Spain). Environ. Pollut. 184, 555–562.
Michael, S., Montag, M., Dott, W., 2013. Pro-inflammatory effects and oxidative stress in lung macrophages and epithelial cells induced by ambient particulate matter. Environ. Pollut. 183, 19–29.
Miller, M.R., 2020. Oxidative stress and the cardiovascular effects of air pollution. Free Radic. Biol. Med. 151, 69–87.
Milne, G.L., Yin, H., Morrow, J.D., 2008. Human biochemistry of the isoprostane pathway. J. Biol. Chem. 283, 15533–15537.
Moller, P., Loft, S., 2010. Oxidative damage to DNA and lipids as biomarkers of exposure to air pollution. Environ. Health Perspect. 118, 1126–1136.
Montuschi, P., Barnes, P.J., Roberts 2nd, L.J., 2004. Isoprostanes: markers and mediators of oxidative stress. FASEB J 18, 1791–1800.
Morrow, J.D., Roberts 2nd, L.J., 1996. The isoprostanes. Current knowledge and directions for future research. Biochem. Pharmacol. 51, 1–9.
Motorykin, O., Santiago-Delgado, L., Rohlman, D., Schrlau, J.E., Harper, B., Harris, S., Harding, A., Kile, M.L., Massey Simonich, S.L., 2015. Metabolism and excretion rates of parent and hydroxy-PAHs in urine collected after consumption of traditionally smoked salmon for Native American volunteers. Sci. Total Environ. 514, 170–177.
Oberg, B.P., McMenamin, E., Lucas, F.L., McMonagle, E., Morrow, J., Ikizler, T.A., Himmelfarb, J., 2004. Increased prevalence of oxidant stress and inflammation in patients with moderate to severe chronic kidney disease. Kidney Int. 65, 1009–1016.
Ostro, B., Malig, B., Broadwin, R., Basu, R., Gold, E.B., Bromberger, J.T., Derby, C., Feinstein, S., Greendale, G.A., Jackson, E.A., Kravitz, H.M., Matthews, K.A., Sternfeld, B., Tomey, K., Green, R.R., Green, R., 2014. Chronic PM2.5 exposure and inflammation: determining sensitive subgroups in mid-life women. Environ. Res. 132, 168–175.
Pepys, M.B., Hirschfield, G.M., 2003. C-reactive protein: a critical update. J. Clin. Invest. 111, 1805–1812.
Peters, A., Nawrot, T.S., Baccarelli, A.A., 2021. Hallmarks of environmental insults. Cell 184, 1455–1468.
Pilz, V., Wolf, K., Breitner, S., Ruckerl, R., Koenig, W., Rathmann, W., Cyrys, J., Peters, A., Schneider, A., group, K.O.-S., 2018. C-reactive protein (CRP) and long-term air pollution with a focus on ultrafine particles. Int. J. Hyg Environ. Health 221, 510–518.
Pope 3rd, C.A., Bhatnagar, A., McCracken, J.P., Abplanalp, W., Conklin, D.J., O'Toole, T., 2016. Exposure to fine particulate air pollution is associated with endothelial injury and systemic inflammation. Circ. Res. 119, 1204–1214.
Risom, L., Moller, P., Loft, S., 2005. Oxidative stress-induced DNA damage by particulate air pollution. Mutat. Res. 592, 119–137.
Sallsten, G., Barregard, L., 2021. Variability of urinary creatinine in healthy individuals. Int. J. Environ. Res. Publ. Health 18, 3166.
Schnelle-Kreis, J., Sklorz, M., Orasche, J., Stolzel, M., Peters, A., Zimmermann, R., 2007. Semi volatile organic compounds in ambient PM2.5. Seasonal trends and daily resolved source contributions. Environ. Sci. Technol. 41, 3821–3828.
Siu, G.M., Draper, H.H., 1982. Metabolism of malonaldehyde in vivo and in vitro. Lipids 17, 349.
Suzuki, J., Inoue, Y., Suzuki, S., 1995. Changes in the urinary excretion level of 8-hydroxyguanine by exposure to reactive oxygen-generating substances. Free Radic. Biol. Med. 18, 431–436.
Tao, F., Gonzalez-Flecha, B., Kobzik, L., 2003. Reactive oxygen species in pulmonary inflammation by ambient particulates. Free Radic. Biol. Med. 35, 327–340.
Taverne, Y.J., Bogers, A.J., Duncker, D.J., Merkus, D., 2013. Reactive oxygen species and the cardiovascular system. Oxid. Med. Cell. Longev. 2013, 862423.
Traverso, N., Menini, S., Maineri, E.P., Patriarca, S., Odetti, P., Cottalasso, D., Marinari, U.M., Pronzato, M.A., 2004. Malondialdehyde, a lipoperoxidation-derived aldehyde, can bring about secondary oxidative damage to proteins. J. Gerontol. A Biol. Sci. Med. Sci. 59, B890–B895.
Trettin, A., Böhmer, A., Suchy, M.T., Probst, I., Staerk, U., Stichtenoth, D.O., Frölich, J.C., Tsikas, D., 2014. Effects of paracetamol on NOS, COX, and CYP activity and on oxidative stress in healthy male subjects, rat hepatocytes, and recombinant NOS. Oxid. Med. Cell. Longev. 2014.
Urbancova, K., Lankova, D., Rossner, P., Rossnerova, A., Svecova, V., Tomaniova, M., Veleminsky Jr., M., Sram, R.J., Hajslova, J., Pulkrabova, J., 2016. Evaluation of 11 polycyclic aromatic hydrocarbon metabolites in urine of Czech mothers and newborns. Sci. Total Environ. 577, 212–219.
Valavanidis, A., Vlachogianni, T., Fiotakis, C., 2009. 8-hydroxy-2' -deoxyguanosine (8-OHdG): a critical biomarker of oxidative stress and carcinogenesis. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 27, 120–139.
VanRooij, J.G., De Roos, J.H., Bodelier-Bade, M.M., Jongeneelen, F.J., 1993. Absorption of polycyclic aromatic hydrocarbons through human skin: differences between anatomical sites and individuals. J. Toxicol. Environ. Health 38, 355–368.
Vattanasit, U., Navasumrit, P., Khadka, M.B., Kanitwithayanun, J., Promvijit, J., Autrup, H., Ruchirawat, M., 2014. Oxidative DNA damage and inflammatory responses in cultured human cells and in humans exposed to traffic-related particles. Int. J. Hyg Environ. Health 217, 23–33.
Wolf, K., Cyrys, J., Harcinikova, T., Gu, J., Kusch, T., Hampel, R., Schneider, A., Peters, A., 2017. Land use regression modeling of ultrafine particles, ozone, nitrogen oxides and markers of particulate matter pollution in Augsburg, Germany. Sci. Total Environ. 579, 1531–1540.
Wood, S.N., 2006. Low-rank scale-invariant tensor product smooths for generalized additive mixed models. Biometrics 62, 1025–1036.
Wu, X., Lintelmann, J., Klingbeil, S., Li, J., Wang, H., Kuhn, E., Ritter, S., Zimmermann, R., 2017. Determination of air pollution-related biomarkers of exposure in urine of travellers between Germany and China using liquid chromatographic and liquid chromatographic-mass spectrometric methods: a pilot study. Biomarkers 22, 525–536.
Yang, L., Wang, W.C., Lung, S.C., Sun, Z., Chen, C., Chen, J.K., Zou, Q., Lin, Y.H., Lin, C. H., 2017. Polycyclic aromatic hydrocarbons are associated with increased risk of

chronic obstructive pulmonary disease during haze events in China. Sci. Total Environ. 574, 1649–1658.
Yang, Q., Qiu, X., Li, R., Ma, J., Li, K., Li, G., 2015. Polycyclic aromatic hydrocarbon (PAH) exposure and oxidative stress for a rural population from the North China Plain. Environ. Sci. Pollut. Res. Int. 22, 1760–1769.
Yoon, H.-S., Lee, K.-M., Lee, K.-H., Kim, S., Choi, K., Kang, D., 2012. Polycyclic aromatic hydrocarbon (1-OHPG and 2-naphthol) and oxidative stress (malondialdehyde) biomarkers in urine among Korean adults and children. Int. J. Hyg Environ. Health 215, 458–464.
Zhang, H., Han, Y., Qiu, X., Wang, Y., Li, W., Liu, J., Chen, X., Li, R., Xu, F., Chen, W., Yang, Q., Fang, Y., Fan, Y., Wang, J., Zhang, H., Zhu, T., 2020. Association of internal exposure to polycyclic aromatic hydrocarbons with inflammation and oxidative stress in prediabetic and healthy individuals. Chemosphere 253, 126748.
Zhu, H., Martinez-Moral, M.P., Kannan, K., 2021. Variability in urinary biomarkers of human exposure to polycyclic aromatic hydrocarbons and its association with oxidative stress. Environ. Int. 156, 106720.

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Chlorination for low-cost household water disinfection – A critical review and status in three Latin American countries

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ABSTRACT

Chlorination has historically provided microbiologically safe drinking water in public water supplies. Likewise, chlorine has also been introduced as a low-cost disinfection method in rural and marginalized communities, both at community and household level, as well as during emergencies. Although this practice is common and well established for use as a household water treatment technology in the Global South, several challenges in effective and efficient implementation still need to be addressed. Here, we explored these issues by a literature review and narrowed them to the status of three Latin American countries (Mexico, Colombia, and Brazil). Overall, it was found that although guidance on household-based chlorination includes information on health risks and hygiene, this may not create enough incentive for the user to adapt the method satisfactorily. Physicochemical quality of the water influences chlorination efficiency and it is found that variations in quality are rarely considered when recommending chlorine doses during implementation. These are far more often based on a few measurements of turbidity, thereby not considering dissolved organic matter, or seasonal and day-to-day variations. Other factors such as user preferences, chlorine product quality and availability also represent potential barriers to the sustainable use of chlorination. For chlorination to become a sustainable household water treatment, more focus should therefore be given to local conditions prior to the intervention, as well as support and maintenance of behavioural changes during and after the intervention.

1. Introduction

Access to sufficient, safe, acceptable, and affordable water is considered as a human right and is an integral part of the sustainable development goals formulated by the United Nations (WHO and UNICEF, 2017). Although there has been an increasing focus on realising this human right, it remains that 2.1 billion people did not have access to safely managed drinking water in 2020. Instead, they rely on water sources, which are prone to contamination and/or located far from the households (WHO and UNICEF, 2020; WHO, 2021). Consumption of unsafe drinking water can cause significant health problems and mortality from enteric infections, particularly among children (Nguyen et al., 2021). Some of the most prevalent causes of mortality due to diarrhoeal diseases are pathogens such as rotavirus, *Cryptosporidium*, and *Salmonella* (GBD, 2018). They are mainly transmitted through the faecal-oral route and are associated with unsafe drinking water, poor sanitation, and hygiene (Clasen et al., 2007).

Household water treatments (HWTs), where water is treated at the point of use, can be an important solution to help mitigate these problems, especially when centralised solutions are not feasible due to e.g. the high cost, maintenance requirement, or where the households are not located in clusters. Although many different technologies exist that have the potential to provide safe drinking water, they are challenged by

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Abbreviations

AC	activated carbon
DBPs	disinfection by-products
CD	chlorine demand
CDC	centre for diseases control
CBHI	community-based health insurance
CHWs	community health workers
COFEPRIS	Federal Commission for Protection against Health Risks
DRC	Democratic Republic of Congo
E. coli	*Escherichia coli*
FC	free Chlorine
FRC	free residual chlorine
HAAs	haloacetic acids
HH	households
HWT	household water treatment
HWTS	Household water treatment and storage
INEGI	Instituto Nacional de Estadística, Geografía e Informática (Mexico)
LRV	log reduction value
NGO	non-governmental organisation
NOM	natural organic matter
PSI	Population Service International
RAS	Reglamento Técnico para el Sector de Agua Potable y Saneamiento Básico (Colombia)
SS	safe storage
SWS	Safe Water System
TC	total coliforms
THMs	trihalomethanes
TOC	total organic carbon
TTC	thermotolerant coliforms
TTHM	total trihalomethanes
UNICEF	United Nations Children's Fund
USEPA	U.S. Environmental Protection Agency
WASEH	water, sanitation, and education for health
WASH	water sanitation and hygiene
WHO	World Health Organisation
WS	water supply

a general low effectiveness and compliance under real conditions in the field (WHO, 2011). This represents an important gap in research since little is known about the actual circumstances under which the methods are performed.

Chlorine has been used as a disinfectant since the early 1900s (USEPA, 1999), and in the Global South, it is the most common and cost-effective method at household level compared to filtration, solar disinfection, and combined flocculation/disinfection. Of these methods, only chlorination and combined flocculation/disinfection provides a residual disinfectant necessary to protect the water quality during storage in the households, where there is a risk of recontamination. Here, chlorination has the clear advantage over combined flocculation/disinfection in that it is cheaper and easier to perform (Clasen et al., 2007).

At the household level, chlorination can be easily achieved by adding a certain dosage of household bleach, sodium hypochlorite (NaOCl) solution, and is one of the most accessible methods, since NaOCl is produced and sold widely. An alternative to NaOCl is sodium dichloroisocyanurate (NaDCC) tablets ($NaCl_2(NOC)_3$), sodium dichloroisocyanurate, which have the advantages of being more stable during storage, safer, and more convenient (Clasen and Edmondson, 2006; Lantagne et al., 2011).

Chlorine when added to water in molecular or hypochlorite form undergoes hydrolysis to form aqueous molecular chlorine, hypochlorous acid and hypochlorite ion, which collectively are referred to as free chlorine (FC) (APHA et al., 2012). Both are antimicrobial agents, but HOCl is significantly more effective than OCl^- (Tvrdá and Benko, 2020). Free chlorine is effective against a range of bacteria and viruses associated with contaminated drinking water but it is less effective against protozoa, especially at the concentration range acceptable for drinking water (CDC, 2014a; Mohamed et al., 2015).

Although chlorination is a simple method for treating contaminated water, it faces several challenges for effective implementation, particularly in rural settings. The aim of this review is to deeply revise and critically analyse the scientific and grey literature on chlorination in practice to identify the main challenges and research gaps related to household chlorination in rural settings, the real scenarios for household chlorination, promotion and deployment, compliance, effectiveness and sustainability in the field. Special attention is put into the main barriers and enablers identified for adequate implementation in the Global South. For this, supported case studies from selected countries in Latin America, namely Mexico, Colombia, and Brazil have also been discussed.

2. Methodology

A literature review on household chlorination in rural settings in the Global South as well as supporting general technical information was performed. More than 3500 manuscripts were identified based on the search terms which were reviewed for inclusion of the relevant keywords and combinations of them by checking titles and abstracts. Peer reviewed literature was identified by searching electronic databases of Scopus, Web of Science, and Google Scholar using the keywords outlined in Table 1. Duplicate documents, review papers and publications in other languages than English, Spanish, and Portuguese were excluded. Research articles unrelated to chlorination in the Global South, to rural areas or purely based in lab studies were also excluded. Similar criteria were used for searching for grey literature. Additionally, some papers were found via reference chaining and information from the authors, who represent academics, researchers, and practitioners. Four hundred and eight articles were selected and the abstract (and method if necessary) were checked. The selected papers was reduced to 202 articles which were read in full length and studies were included in the final list if they were relevant to the topic.

3. Challenges with chlorination

3.1. Source water quality – dosing and storage

HWT systems must be robust in terms of their capability to treat water with variable microbial and physicochemical quality. When chlorine is added to water, it reacts with the organic material, metals, and other components, which render it unavailable for disinfection. The difference between the amount of added chlorine and the free residual

Table 1
Review search keywords, selecting the combination as ((‘chlorination method keyword’) AND (‘water treatment keyword) AND (‘setting keyword’).

Chlorination keyword	Water treatment keyword	Setting keyword
disinfect*	“drinking water” potable	rural
chlorin*	“water treat*" household	communit*
hypochlorite	HTW	“field trial”
“sodium dichloroisocyanurate"	“household water treatment” “Safe water system"	intervention
NaDCC bleach	SWS	“field implementation"
	“point-of-use” -POU	“developing countr*"
		LMIC
		“low and middle income country"

chlorine (FRC) after a given contact time is defined as the chlorine demand (CD). This CD depends on various parameters such as temperature, pH, turbidity, and chemical composition of the water and the CD of a given water source is therefore prone to temporal variations (WHO, 2019). This underlines the importance of a more thorough knowledge of the water quality on a larger time scale. In terms of chlorination interventions, few studies, as in Boisson et al. (2013) and Ercumen et al. (2015), have conducted pilot studies to determine the optimal chlorine dose based on the relationship between water quality and CD. In some cases, the measured water quality parameters are limited to a few measurements of e.g. *Escherichia coli* (*E. coli)* concentration (Mengistie et al., 2013) or FRC (Ercumen et al., 2015); but in most cases, no information is given of the water quality and the dosage is typically based on the general recommendations of turbidity (Mohamed et al., 2015).

Many studies find a correlation between the turbidity and the CD of the water (Levy et al., 2014; Mclaughlin et al., 2009; Mohamed et al., 2015) and the latest recommendations by Wilhelm et al. (2018) for dosing, as seen in Table 2, are based on testing of a large range of water sources with varying turbidity values. The CD exerted by the turbidity is due to the reaction of chlorine with organic and inorganic compounds in the water, but since some of the natural organic matter present in water is dissolved and it is not reflected in the turbidity. This is rarely taken into consideration when deciding the chlorine dosage in an intervention.

It is recognised by the WHO that chlorine dosing should be site-specific due to the varying CD, and therefore their recommendations are based on the maintenance of FRC concentrations as listed in Table 2. From a health perspective, the WHO further recommends that FRC should not exceed 5.0 mg/L at any time (WHO, 2011).

The FRC concentrations of 0.2–0.5 mg/L are recommended to protect the water from regrowth and recontamination during storage and usage, however bacterial regrowth has been observed at the recommended FRC levels within this range. Meierhofer et al. (2019) found that safe, chlorinated water from a water kiosk with a mean FRC of 0.39 mg/L was not sufficient to maintain drinking water quality after 24 h of storage, when filled into uncleaned jerry cans and used in households in Kenyan households. The uncleaned jerry cans contributed to the exerted CD and reduced the FRC to a mean concentration of 0.19 mg/L. This fact provoked contamination at storage level, *E. coli* was detected in 15.2% of the household stored water after 24 h of storage and a higher chlorine dosage would have been required to maintain the FRC level in the water. This emphasizes the importance of using clean water storage containers to prevent depletion of FRC during storage.

3.2. Pathogens, dose and contact time

The efficiency of chlorination depends on dosage and contact time. A 4-log inactivation of most waterborne pathogens have been shown at a dosage of a few mg/L and a 30–60 min contact time. However, several pathogens exhibit resistance to chlorination, influencing the required chlorine concentration and contact time necessary for sufficient inactivation (Clasen and Edmondson, 2006). Pathogens like the protist *Cryptosporidium parvum*, for instance, are highly resistant to conventional chlorination due to the production of resistant oocysts during their life cycle (Feng et al., 2021). At the household level, under less controlled conditions than in a water treatment plant, studies such as Mclaughlin et al. (2009) and Levy et al. (2014) have found the efficiency to be significantly reduced compared to laboratory testing, which is attributed to low user compliance, recontamination during storage, or high CD leading to insufficient disinfection and regrowth.

Although chlorination is supported by leading organisations such as UNICEF (Mohamed et al., 2015), the ineffectiveness against some protozoa means that chlorination products can only obtain a limited protection ('1-star' classification) as reflected in the Household Water Treatment Evaluation Scheme by the WHO (WHO, 2011). On this basis, WHO recommends that chlorination is only used in situations where the causative pathogens for disease are known or as part of a multi-barrier treatment approach (WHO, 2019). This is the case of chlorine used for emergency responses to cholera outbreaks (Patrick et al., 2013). The latter is outside the scope of this review.

Table 2
Recommendations for chlorine dosage and FRC for drinking water.

Applied dose/FRC	Concentration (mg/L)
Dosage at low turbidity (<10 NTU)	1.88 (Wilhelm et al., 2018)
Dosage at high turbidity (>10 NTU) (consumed within 8 h)	3.75 (Wilhelm et al., 2018)
FRC after 30 min	>0.50 (WHO, 2011)
FRC after 24 h	>0.20 (WHO, 2011)

3.3. Chlorine stability

Another issue that arises with chlorination is the quality of the disinfectant used (e.g. sodium hypochlorite solution, tablets, etc.). Lantagne et al. (2011) found that the stability of hypochlorite solutions from different production techniques (and different target concentrations and pH stabilization) were generally stable for up to 19 months unless exposed to direct sunlight, which reduces the concentration significantly. During normal use in households in Kenya, the stability of hypochlorite in 48 sampled bottles showed that 77% of the samples were within 20% of the initial concentration of 1.2% hypochlorite. About 17% were 50% of the target concentration while 4% were only 20% of the targeted concentration. The hypochlorite was on average manufactured 433 days before the sampling was conducted. It is evident that if the product quality is poor either due to poor initial quality (i.e. inaccurate concentration) or as a result of instability and degradation over time, there is a risk of underdosing, especially if households do not use the product before the expiration date, which Lantagne et al. (2011) recommended as a minimum 1-year for 1.25% hypochlorite solution, given that the pH is above 11.9 and it is stored below 35 °C.

3.4. Disinfection by-products

During chlorination there is a risk of the formation of a wide range of disinfection by-products (DBPs), which can be harmful to human health and thereby give rise to concern. The formation occurs when chlorine reacts with organic matter present in the water and the type and amount of DBPs formed depends on factors such as the water quality, contact time, temperature, and pH (USEPA, 1999). However, research has shown that under field conditions, the DBP formation does not exceed the WHO guideline values with health significance in drinking water (e.g. bromodichloromethane – 60 μg/L, bromoform – 100 μg/L, dibromochloremethane – 100 μg/L, chloroform – 300 μg/L, dichloroacetate −50 μg/L, dichloroacetonitrile – 20 μg/L, bromate – 10 μg/L) (WHO, 2017), even when using highly turbid water (up to 888 NTU) (Lantagne et al., 2008, 2010). Even so, the fear of DBP formation and the potential negative health effects may still pose an obstacle for a high user compliance. In centralised water supply (WS) systems, water quality monitoring is common to avoid such issue, but the presence of DBP precursors is not likely to be regularly tested in self-supplied communities, which may pose an additional risk to this disinfection method and might be considered in future research.

DBPs are present at sub-μg·L^{-1} or low-to-mid-μg· L^{-1} levels in drinking water (Xiao et al., 2020). At household level DBPs can be controlled by limiting their production which can be challenged by reducing the natural organic matter (NOM) before disinfection or adopting safer disinfection methods (e.g., ferrate(VI)). When DBPs are produced, their removal must be in place, typically by activated carbon (AC) adsorption, after disinfection although the volatile DBPs can be eliminated using other household water treatments (Deng, 2021).

According to a recent investigation on the efficiency of several HWTS – AC and membrane filtration, boiling, UV lamps, cold storage or a

combination of them – for the removal of DBPs in drinking water resulted on a significant removal of trihalomethanes (THMs) and haloacetic acids (HAAs) using all these HWTs except for storage. Their efficacy varied upon their purification mechanisms and depended on the chemical characteristic of the target DBPs (Xiao et al., 2020). It is also well recognised that pathogen removal should not be compromised in attempting to control DBPs, but also that more research is required on the trade-off between the chemical and microbiological risk during household treatment.

3.5. Evaluation of household chlorination

When dealing with chlorination, several indicators can be used to evaluate the outcome of an intervention. According to WHO and UNICEF (2012), these include the quality/presence of chlorine products in the household, FRC in the water, and improved water quality indicated by microbial indicators. FRC in the water can be an indicator of correct and consistent use as well as water safety and is therefore critical to monitor.

Some research has been done on the evaluation of drinking water treatment programs. The evaluation can be based on metrics such as the reported use, confirmed use, and effective use as illustrated in Fig. 1. Often the reported use is higher than the confirmed use and effective use and reported use is prone to being biased e.g. if users are affected by their participation in the intervention. Therefore, using self-reporting alone as an evaluation tool will typically overestimate the actual use of an HWT (Gallandat and Lantagne, 2018).

The lack of FRC in stored water does not necessarily mean that the HWT has not been performed by the user. It could also imply that the CD from raw water exceeded the applied dosage. This situation illustrates that different approaches are important to evaluate confirmed and effective use of the disinfectant. For instance, if there is no FRC, but a reduction in the pathogen threshold was found, this could indicate underdosing, an example of ineffective use. Incorrect use can be defined as either having too low or too high concentration of FRC. The latter will still provide safe drinking water, but it increases the risk of odour and taste issues.

4. Promotion of chlorination in rural settings in the Global South

In the 1990's, the Safe Water System (SWS) was developed by Centre for Disease Control and Prevention (CDC) and the Pan American Health Organisation, which combined chlorination with safe storage and behavioural change communication to improve the practices of safe storage, sanitation and hygiene (CDC, 2014b). These principles are being promoted worldwide by various non-governmental organisations (NGOs) such as Population Service International (PSI) and CARE and introduces chlorination both as liquid NaOCl (i.e. WaterGuard) and NaDCC tablets (i.e. Aquatabs).

Table 3 provides a summary of some intervention approaches and the key activities and outcomes related to these. The implementation strategies often combine collaboration with various stakeholders (e.g. governmental bodies, local leaders, and community health workers (CHWs)), training of local promoters, and education and information on topics such as water treatment, safe storage, hygiene, health, and causes of diarrhoea through personal interaction, community meetings and other activities in the communities as well as social marketing. These approaches built on creating acceptance as well as awareness of the products and health outcomes in communities, and in many cases result in an increased knowledge of the promoted products. Here, frequent household visits are often identified as a key factor in the uptake, where e.g. Wheeler and Agha (2013) observed that receiving household visits increased the likelihood of using and purchasing chlorine eight years after a WS and hygiene promotion campaign in Mozambique. However, these approaches do not always guarantee a high uptake of the treatment or consistent use as seen in Table 3.

The strategies vary in their financial approach, where some distribute a free supply of products during the study period, while others incorporate partly subsidized products or local companies and sales agent to ensure affordable products and distribution. The latter may be more sustainable in terms of long-term product supply in communities since it is not dependent on outside funding and ensures cost recovery. The drawback is that it may unintentionally exclude poorer parts of populations who are unable to afford the products even if they are available at a low cost. This trend was observed by Freeman et al. (2009), who found that the proportion of households who had detectable FRC in their stored water increased with increasing socioeconomic wealth and this was also associated with increasing awareness of chlorine products. The influence of cost was studied by Ritter et al. (2017) who showed higher purchase rates were associated with decreasing prices among rural Haitian households randomly assigned to five different prices of chlorine. Monthly household visits did not have a significant influence on the uptake at the two lowest prices nor at the highest price. At the low price, this indicates that some households do not need convincing to purchase, while others are unsusceptible to the promotional efforts. At the high price, household visits are not sufficient to overcome the price barrier. Similar price sensitivity was observed

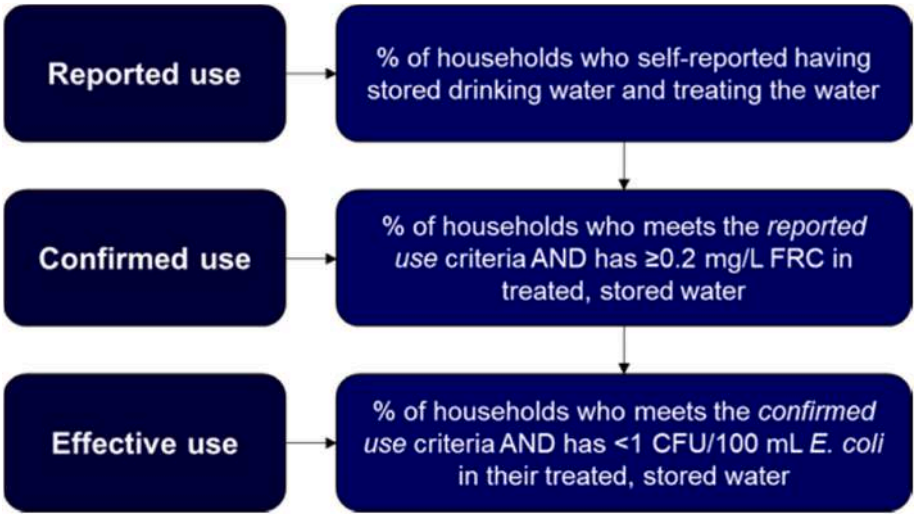

Fig. 1. Evaluation metrics for chlorination. Information from (Gallandat and Lantagne, 2018).

Table 3
Summary of strategies, activities, and outcomes for implementation of chlorination in rural communities. Sûr'Eau and Klorin are local brands of liquid NaOCl.

Country	Implementation strategy	Activities	Outcome
Rwanda (Chankova et al., 2012)	Implementation by PSI, the department for community-based health insurance (CBHI) scheme technical assistance at the Rwandan Ministry of Health and CBHI schemes in pilot districts. Training of 3,200 CHW, CBHI managers and committee members to promote and distribute Sûr'Eau to households in more than 1,100 villages. Training covered the technical aspects of household water treatment, management of Sûr'Eau stocks, and distribution and sales at the community level.	Regular small group out-reach sessions to provide education on safe water and sanitation, as well as to promote and sell Sûr'Eau at the community level. Promotion of safe water practices in general and Sûr'Eau in particular directly to households, including through interpersonal communication conducted during home visits and at community gatherings. Talks on safe water, hygiene, and sanitation, and promotion of Sûr'Eau to patients waiting to be seen at health centers.	After 18 months, the exposure to WASH (water sanitation and hygiene) messages was higher in the pilot districts compared to the control district. The knowledge of Sûr'Eau and its purpose increased by 10–35%-point among communities in pilot districts. In the pilot districts there was a decrease in the proportion of households that did not use any form of treatment of their drinking water, whereas this increased in the control district. At baseline, few households used any other treatment than boiling. After 18 months, 60% reported using HWTs every time they collected water, although this was not in total agreement with answers later in the questionnaires.
Madagascar (Ram et al., 2007)	Implementation by CARE Madagascar who trained community-based sales agent in four villages trained on causes and prevention of diarrhoea, proper use of the SWS, and techniques for changing health behaviours.	Sales agents conducted educational visits to neighbours to inform about the need for household water treatment and safe storage to prevent diarrhoea as well as to sell them Sûr'Eau and jerry cans with taps.	After 16 months, 99% of 242 surveyed households had heard of Sûr'Eau, primarily through CARE or the community-based sales agents. 95% of households reported using the product at least once, and of these 73% were currently using the product. 72% of current users were able to correctly state the dose, waiting time before drinking, and proper water storage procedure.
Malawi (Wood et al., 2012)	UNICEF, Ministry of Health and PSI promoted WaterGuard (liquid NaOCl) by distributing for free at antenatal clinics. Promotion of WaterGuard through media channels, i.e. radio announcements, signs, billboards, posters, and flyers. After the intervention, PSI continued its normal national distribution system and promotional activities for WaterGuard.	Distribution of free hygiene kits that included WaterGuard to 15,000 pregnant women attending health facilities. If enrolled in the program, the women were eligible to receive up to three free refills of WaterGuard at later visits. The women attending the health facility was educated by the health workers about health and hygiene, including water treatment and correct use of WaterGuard. Home visits by health surveillance assistants to reenforce educational messages.	Before the intervention, the awareness of WaterGuard was high. Three years after the intervention, the awareness of the need to treat household drinking water was high, where especially the influence of interpersonal communication with health workers and health surveillance assistants was reported as an important factor. After three years, participants varied between consistent year-round users, seasonal users, switching between different methods, and some no longer used WaterGuard.
Kenya (Makutsa et al., 2001)	Implementation of SWS by CARE in 12 rural villages by adopting community mobilisation and management structure of existing Water, Sanitation and Education for Health project. Support by Kenyan government officials, community management committees, and leaders of women's groups ensured. Agreement with a private company to produce 1% sodium hypochlorite (branded Klorin) and establishment of quality control system. Development of improved clay pots for safe storage. Subsidized and packaged with a free bottle of Klorin during the promotional period	Village health promoters held community meetings, schools, and village health training workshops to teach about SWS. Social marketing to encourage behavioural change, i.e. Klorin-themed posters, puppet shows, soccer tournaments, public product demonstrations, and Klorin quizzes with prizes. Village health promoters received T-shirts, water vessels, or Klorin bottles as incentives for meeting sales targets.	Before implementation, a survey showed that 90% used boiling as primary treatment but that most rarely treated their water even when acknowledging that contaminated drinking water causes diarrhoea. Six months after implementation, 33.5% of 173 surveyed households had detectable FRC and 18.5% were using the promoted clay pots.
Kenya (Freeman et al., 2009)	After receiving training on SWS by CARE Kenya, a local organisation, Safe Water and AIDS Project (SWAP) used a social entrepreneurship model of SWS dissemination. Unrelated to the SWAP activities, a number of self-help groups were selling PuR (combined flocculant-disinfectant), and	Training of SWAP group members on diarrhoea prevention and proper water treatment practices. SWAP group members were offered to purchase water treatment products (Klorin, PuR, and WaterGuard) at wholesale prices, sell them at retail prices to	After 2 years, 33% of 485 households in 6 rural villages and 2 peri-urban villages reported ever using Klorin, 9% using WaterGuard, and 10% using PuR. FRC attributed to Klorin was detected in 17.1% of stored water samples, while this was 2.5% for WaterGuard, and 0.8% for PuR.

(*continued on next page*)

Table 3 (*continued*)

Country	Implementation strategy	Activities	Outcome
	Klorin and promoted (but not selling) WaterGuard.	neighbours, and keep the difference as an incentive.	20% of the households reported repeat purchases of Klorin or WaterGuard, while 10% were still using their first supply. Only 5% reported having used more than 1 sachet of PuR and 1% had used more than 10 sachets.
India (Boisson et al., 2013)	Implemented by PSI by promotion and free distribution of NaDCC tablets. Training of interpersonal communicators.	Interpersonal communicators visited households fortnightly distributing free tablets, instructing on use, providing information on health and drinking water treatment, and engaging the households by using games and interactive pictures. Community-level activities including street plays, game shows, wall paintings, and distribution of information material	At baseline, 44% of households reported treating their drinking water by any form of treatment. Self-reported and confirmed use increased over the study period, with an overall 51% of household samples reported treated, and confirmed use was 32%. This varied between households, where 20% never had detectable FRC, and 76% had FRC on less than half of the visits.

among households in rural Kenya, where wealth was also a predictor of higher chlorine use, and health messages had little effect on households' decision to purchase chlorine (Blum et al., 2014).

5. Implementation of household-based chlorination

When chlorination is performed at the household level, NaDCC tablets or liquid NaOCl is often used. The commercially available chlorine-based products are typically of a high quality with only small deviations from the targeted concentration and are based on providing a fixed dosage of free chlorine. Common commercially available products such as WaterGuard comes in bottles, where e.g. the bottle cap is used as a measuring tool for a predesignated volume of water based on a target free chlorine concentration. The drawback with the fixed dosage-based products is that they require the household to have a certain size of container, which may not always be available. Therefore, there is a risk of incorrect dosing or discontinued use if the correct container size cannot be found (Kumwenda et al., 2014).

Field studies show that in some communities, local vendors buy concentrated NaOCl (10–15% free chlorine), which is diluted and re-sold in smaller plastic containers (Levy et al., 2014). This poses a risk of inaccurate dosing e.g. if the dilution is not performed correctly or the required dosage is not easily measured. Concentrations ranging from 0.9 to 15 mg/L in the treated drinking water were observed by Levy et al. (2014), when households used a locally diluted product in Ecuador. With the high concentrations of chlorine, the issues of increased risk of DBP formation and taste acceptability arise.

Chlorine solutions can also be produced locally i.e. by the electrolysis of brine using a simple electrolytical cell (Murray et al., 2020), dissolving calcium hypochlorite ($Ca(ClO)_2$), or injecting chlorine gas into a stream of deionized water (Lantagne et al., 2011). These products will have a lower quality in terms of the concentration of free chlorine (FC) and its stability compared to commercial products if the manufacturing and packaging are not of a high quality and thereby pose a risk of incorrect dosing if the target concentration is not met (Lantagne et al., 2011).

There can be a significant difference between the chlorination practices during drinking water treatment interventions for research purposes and the real-world conditions. According to Mclaughlin et al. (2009), the compliance rate is often higher in intervention studies compared to the actual situation. This can be caused by the oversight of investigators or the use of user surveys which may give biased results. In a rural community with no previous exposure to interventions or training, Mclaughlin et al. (2009) found that among nine households claiming to treat their water with chlorine, only two households reported always doing so during the 3-week study, while one household never chlorinated the water during the study. The remaining households reported chlorinating their water 14–77% of the time. There was no significant difference in the level of total chlorine between treated and untreated samples, but 50%, 58%, and 21% of the treated samples had detectable E. coli, enterococci, and phage respectively, compared to 86%, 82%, and 32% of untreated samples. Much higher self-reporting rates were found among 32 rural households in Zambia where about 42% of the households reported treated their water daily and 39% reported treating the water on every collection. However, this was partly contradicted by field observations i.e. unavailability of treated water upon visits (77%), reports of the available water being treated more than two days prior to the visit (50% of self-reported daily users) as well as inadequate FRC concentration of the drinking water present in the household (100%). In many cases, untreated water supplemented the treated water, and even among the self-reported users of chlorination, about half of the households reported drinking untreated water although they generally were aware of the associated risk to their health (Rosa et al., 2016).

One of the challenges with chlorination and the often-low observed effectiveness under field conditions is that, although confirmed and correct use can be validated, it does not factor in under which conditions chlorination is performed in the households. This is an area that has gained little attention and only a few studies have investigated how chlorination is practised, as summarized in Table 4. It is evident from these few studies, that applying the correct dose of chlorine can be problematic and across all three reports, no more than half of the respondents used the methods correctly. This proves, that even when users have an intent to treat, it may not always be enough to ensure safe drinking water. This is further complicated by varying source water quality, which would require the users to adjust the dosage. In the case of Malawi where 56% of respondents indicated either dosing correctly or overdosing, only 8% of water samples taken from storage containers in the households had an FRC concentration above 0.2 mg/L (Kumwenda et al., 2014). This discrepancy could be caused by non-treatment (over-reported use), high CD, or even recontamination during long storage time.

6. Efficiency, compliance, and sustainability of household chlorination

Chlorination of water at the point of use has been shown to reduce the risk of childhood diarrhoea (Boisson et al., 2013; Ercumen et al., 2015; Mengistie et al., 2013). Even so, in the past, field studies have shown that adopting and consistently using chlorination have been a challenge. If chlorination is practiced frequently, but the user does not consistently drink treated water (e.g. consumes untreated water outside the home) the compliance will still be low and thereby the health effects will also be low or absent (Murray et al., 2020; Patrick et al., 2013).

Looking at the short-term effect of interventions, case studies from Ethiopia have shown that two separate interventions with free supply of

Table 4
Practice and knowledge on correct use among rural households. HH: households. Sûr'Eau, Jif, and Clorox are local brands of liquid NaOCl.

Country	Project details	Product and correct use	Practice
Rwanda (Chankova et al., 2012)	2,402 HH studied over 18 months	Liquid Sûr'Eau: One bottle cap/20 L	About one third of respondents, who had used the method in past, could mention all 4 steps correctly; 1) Fill 1 bottle cap 2) Pour in 20 L jerry can 3) Close jerry can and shake well 4) Wait 30 min before drinking
Haiti (Patrick et al., 2013)	One-time survey of 433 HH 16 months after onset of cholera epidemic	- Aquatabs (available as 8.5,17, 33, and 67 mg): One 67 mg tablet/20 L - Jif or Clorox (5–15% active chlorine): 25 drops/ 20 L - PYAM tablets: two tablets (19 mg each)/20 L	Half of the Aquatabs users reported applying an acceptable dose, while 25% underdosed. 26% of Aquatabs users did not know how many tablets to dose and most thought that one tablet to 20 L water was correct, irrespective of tablet size. Liquid bleach users reported using the correct dose in 15.9% of the case. Almost 80% of the users were underdosing where the majority used five or less drops. All respondents using PYAM indicated underdosing
Malawi (Kumwenda et al., 2014)	One-time survey of 349 HH	WaterGuard: One bottle cap/10 L	330 HH reported to have used WaterGuard. Of these, 72% could explain how they treated the water, where 45% used the right dose, 44% underdosed and 11% overdosed. Of the WaterGuard users, 10% did not ensure mixing of the water after dosing.

WaterGuard had a high compliance during the 16-weeks study period. Here, on an overall average around 80% of households had FRC $\geq$0.2 mg/L during weekly or biweekly testing, which did not appear to decrease during the study period. Additionally, in both studies there was a significant improvement in the water quality and the proportion of water samples with detectable E. coli as well as the bacterial count (Mengistie et al., 2013; Solomon et al., 2020, 2021).

Similar results were reported by Rangel et al. (2003) in a study comparing a combined flocculation-disinfection product to regular diluted bleach and safe storage among rural households in Guatemala. Overall, water samples collected weekly during 4 weeks from households using bleach showed that 83% had FCR above 0.5 mg/L and 92% had less than 1 CFU/100 mL of *E. coli*, which demonstrates a high initial compliance. In contrast to this, a study from India showed a declining compliance to chlorine delivered free of charge, where the proportion of samples with <1 thermotolerant coliforms (TTC)/100 mL was about 32% after 1 week and declined to 7% after 4 months. This was attributed to inadequate chlorine dosing, low compliance due to a high resistance towards chlorination as well as in-home recontamination (Firth et al., 2010).

While some authors argue that the uptake of chlorine products is likely to be lower in the absence of free provision and intensive promotion (Ercumen et al., 2015), Garrett et al. (2008) found that in a combined water, sanitation, and education for health (WASEH) intervention in Kenya, where a low-cost chlorine product was promoted, FRC >0.1 mg/l was measured in 43% of stored water samples during weekly visits over 8 weeks. Although this intervention was limited in time, it indicates that free product distribution in not always the most essential motivator, but user acceptability may play a far more significant role when looking at the short-term compliance. Furthermore, comparison of short-term interventions can be complicated by the time of the year that they are conducted, i.e. during the rainy season, where the perceived risk of contamination is higher and therefore the incentive to treat water is higher (Wood et al., 2012).

For chlorination to be an effective and sustainable solution for securing safe drinking water, it is necessary to also look at the long-term compliance. Table 5 summarises the experiences from studies that lasted 10 months or longer. The results from these evaluations are varying significantly in their level of compliance, where the cases from India and Bangladesh are somewhat similar in their approach (frequency of visits, free distribution) but the compliance and disinfection efficiency was about twice as high in the study from Bangladesh. This suggests, that although frequent home visits and educational activities are important driving factors for a high and consistent uptake, other factors influence this as well and will vary across geographical regions. In the case of the Indian study, the intervention did not provide appropriately sized storage containers but reported that the typical container size in the households was 13 L, which is considerably smaller than the 20 L prescribed size for a 67 mg NaDCC tablet used in the study. Overdosing and consequently rejection of the treatment could be a contributing factor to the lower compliance.

Although chlorine on its own has been shown to reduce childhood diarrhoea (Crump et al., 2005), interventions that combine water treatment with other WASH (water, sanitation and hygiene) components have shown positive influence on the reduction of childhood diarrhoea. It is therefore interesting to look at these types of interventions to see if an increased focus on hygiene and sanitation has an impact on the compliance of water treatment. For comparison, Table 6 therefore summarises three studies where household chlorination has been introduced in communities in combination with other WASH components. In all studies, the compliance increased over time, but in the two studies that compared several treatment arms, the chlorine alone treatment arm interestingly had the highest compliance. Opryszko et al. (2010) argued that when multiple health messages and behaviours are promoted in combination, there is a risk that the messages will be diluted, and the inconvenient and time-consuming behaviours will be downgraded. However, the combined effects (even at lower treatment compliance) in terms of health improvements can still be greater compared to standalone chlorination.

7. Barriers and enablers for adequate implementation

The adequate implementation of chlorination in a household faces several barriers and enablers. These have different origins and can be divided into *user*, *chlorine product*, and *correct practice and storage* which are summarized in Table 7, Table 8, and Table 9 respectively.

Barriers that are directly related to the *user* include the user demand for HWTs. As seen in many field trials, there is often a lack of motivation to use the HWTs or a lack of understanding of the benefits, which leads to low user compliance and/or non-use. HWT is a preventative practice where the benefit, e.g. the aversion of diarrhoea incidences, is not obvious to the user immediately. Geremew et al. (2019) found that households in Ethiopia were more likely to use a chlorine product over time when they believed that it made their water safer to drink. This demands for better implementation strategies, including appropriate

Table 5
Examples of long-term effect of interventions or emergency responses focused on water treatment only. Sample size refers to the intervention participants (not including controls) at end line. HH: households. SS: safe storage. Gadyen Dlo is a local brand of liquid NaOCl.

Location	Sample size	Duration and frequency of visits/testing	Treatment	Effect on microbial quality	Compliance
Haiti (Lantagne and Clasen, 2013)	143 HH	10 months 1 follow up visit 10 months after an emergency response.	Free Aquatabs/Gadyen Dlo	46% of HH had *E. coli* <1 CFU/100 mL 41% of HH had *E. coli* <10 CFU/100 mL	81% of HH reported as current users. 90% of the treated samples had FCR above 0.2 mg/L
India (Boisson et al., 2013)	751 HH	12 months Biweekly educational visits Monthly testing visits	Free 67 mg NaDCC/bucket (about 13 L)	Overall mean 50 CFU/100 ml TTC (control 122 CFU/100 mL TTC). Reported use mean 24 CFU/100 mL TTC (control 138 CFU/100 mL). 37% of reported-use samples free of TTC (20% in control).	Confirmed use increased from 14% at baseline to 47% at end line. 30% of samples with detectable FRC exceeded the recommended 2.0 mg/L, where 11% was equal to or exceeding 5.0 mg/L.
Bangladesh (Ercumen et al., 2015)	537 HH	12 months Monthly promotion visits Monthly testing visits	Free 33 mg NaDCC/10 L + SS	74% of samples free from *E. coli* (11% in control). 9% of samples with *E. coli* >10 CFU/100 (61% in control). 2% of samples with *E. coli* >100 CFU/100 (21% in control).	91% of spot checks had stored water in the provided container. HH who had water stored during visits with FRC ≥0.2 mg/L varied between 76% and 90% during the study period.
Kenya (Parker et al., 2006)	51 health clinic clients	12 months Home visits after 2 weeks and 1 year	WaterGuard		71% of stored water samples had detectable FRC after 1 year
Guatemala (Rangel et al., 2003)	83 HH 87 HH w. SS	12 months Weekly visits 3 follow-up testing visits and monthly unannounced testing visits	Free chlorine bottles±SS	61% (+SS) and 51% (- SS) of samples on unannounced visits had <1 CFU/100 mL E. *coli*	On monthly unannounced visits, 44% (+SS) and 36% (- SS) of HH had FRC >0.1 mg/L
Haiti (Murray et al., 2020)	59 HH	13 months 4 follow-up testing visits	Free handheld electro chlorinator with target concentration of 2.5 mg/L chlorine in treated water	82% of samples free from *E. coli* 11% of samples with *E. coli* >10 CFU/100	Confirmed use ranged from 39% at two weeks to 13% at 13 months. 2% had confirmed use on every visit 77–91% reported drinking untreated water
Haiti (Harshfield et al., 2012)	201 HH	Up to 8 years after implementation 1 follow-up testing visit	Gadyen Dlo (NaOCl) + safe storage		75% of HH self-reported current use (10% of control), where 34% reported daily use (11% of controls) 56% confirmed use in the range 0.2–2.0 mg/L (10% of control).

HH: households, SS: safe sanitation, +SS: with safe sanitation, –SS: without safe sanitation.

behavioural intervention, including education and training in health and hygiene, to underline the benefits of treating the water both in terms of health outcome but also economic benefits e.g. higher school attendance, reduced number of missed workdays, and reduced expenditures on healthcare. However, even regular educational visits to households does not guarantee a high level of compliance (Boisson et al., 2013). Other user-related barriers include the user acceptability and preference. In terms of chlorination, one of the most frequently reported objections to chlorine is smell and taste. This is especially a problem when the users are not used to chlorine e.g. in the public WS. The user preference of technologies also plays an important part since some users will prefer methods that do not require regular purchases or offer a more convenient usage (Ercumen et al., 2015). Furthermore, in rural communities there may be cultural factors that cause a low adherence to the methods e.g. due to misconception/false rumours of chlorine toxicity or if water is associated with healing powers or being of a religious value to the people.

Barriers related to the *chlorine product* include the affordability and supply chain. Although chlorine products are generally cheap, they need to be replenished continuously and add an extra cost for households. Therefore, their willingness to pay after an intervention is implemented is very important, which is related to their awareness of the benefits of treating the water and the cost averted as a result thereof. However, in cases where chlorine is distributed free of charge, a low compliance can sometimes be observed, indicating that other factors contribute as well. Furthermore, it is seen that discontinued use after an intervention can be related to the lack of a supply chain in the communities, which can provide the chlorination products continuously. Prior to an intervention, it should therefore be investigated if the supply chain is sustainable. Although product quality is not reported as a concern by users, it is an important issue as discussed earlier. Therefore, product quality should be ensured through quality controls, especially when locally made products or diluted commercial products are used, where a poor quality may lead to ineffective treatment and false sense of security.

The lack of knowledge on *correct use and practices* is a significant barrier to the successful implementation of chlorination, where variations in available product concentrations and appropriate storage container size can lead to incorrect dosing. Underdosing will give a false sense of security, while overdosing may lead to rejection of the method due to taste issues. Although chlorination is seen as a simple method, identifying the correct practice can be a challenge for users as already discussed. Users who feel comfortable using the method have also been found to be more likely to continue to do so (Inungu et al., 2016). The successful implementation and adoption of HWT requires a significant behaviour change since consistent use requires an active choice to treat the water every time it Is collected and not just when the perceived risk of contamination is high. The behaviour change also means being aware of the risk associated with consuming untreated water and to stop that behaviour.

The common trait of many of these identified barriers is their common mitigation measures. Although most interventions are accompanied by information, education, and/or training to generate awareness of the importance of treating water, it has been shown that users prefer clearer messaging about the health benefits of safe water especially from

Table 6
Long-term effect of interventions or emergency responses combining water with other intervention types. Sample size refers to the intervention participants (not including controls) at end line. HH: households SS: safe storage. Clorin is a local brand of liquid NaOCl.

Location and intervention	Sample size	Duration and frequency of visits/ testing	Treatment	Compliance
Afghanistan (Opryszko et al., 2010) **a) Water b) Water, hygiene education, and improved water source**	288 HH Water 255 HH Combined	16 months Biweekly visits	FC (0.05% NaOCl) + SS	Self-reported use increased from overall 7%–72% in Water group and to 78% in Combined group. In both groups, the majority used Clorin as treatment (98% of Water group and 76% of Combined group).
Bangladesh (Parvez et al., 2018) **a) Water b) WASH c) WASH and nutrition**	698 HH Water 703 HH WASH 686 HH WASH + N	20 months Monthly visits	FC tablets + SS	Over 20 months, 84% of households in the Water group self-reported treating their water, while this was 78% for the WASH group, and 77% for the WASH + N group. Confirmed use was 76% for households in the Water group, 68% for WASH and 67 for WASH + N group.
Malawi (Loharikar et al., 2013) **Water and education (WASH) facilitated by antenatal care service**	232 pregnant women 386 relatives/ friends	3 years 1 follow-up survey after 1 year and after 3 years	Free WaterGuard (up to four free bottles) + FC stock during rainy season	Self-reported use increased from 44 to 69% of WaterGuard and 13–47% for chlorine stock solution among participants. Confirmed used increased from 9% to 54% among participants and 9%–43% among friends/ relatives

highly trusted sources of health advice such as nurses and health workers (Makutsa et al., 2001; Parker et al., 2006) rather than mass media (Freeman et al., 2009). Ongoing, positive interactions with health advisors (Wood et al., 2012) as well as high frequency of receiving instructions and/or home visits (Loharikar et al., 2013; Parker et al., 2006) have been observed to be some of the main driving factors for a high and sustainable compliance. This was also confirmed in an exploratory study about the challenges of tablet chlorine programs in emergencies. Interviews with emergency WASH professionals revealed that frequent distribution accompanied by education messages was key to a successful implementation. However, it was also found that if the individuals

Table 7
User dependent barriers and enablers to adequate implementation of chlorination based on field experience.

	Barriers	Enablers
Motivation	Lack of understanding of the importance of consistent use of treated water. (Patrick et al., 2013) Inability to acknowledge the link between health benefits and technology use. (Freeman et al., 2009; Roma et al., 2014) Perception of drinking water sources to be safe without treatment, i.e. due to natural treatment, protection, and clarity. (Freeman et al., 2009; Kumwenda et al., 2014; Lantagne and Clasen, 2013; Loharikar et al., 2013; Patrick et al., 2013).	Perceptions of health benefits generated by chlorine disinfection. (Roma et al., 2014). Perceived need for water treatment to prevent diarrhoea (Makutsa et al., 2001; Parker et al., 2006; Rosa et al., 2016; Wood et al., 2012). Desire to prevent sickness and protect their families. (Wood et al., 2012).
Acceptability	Dislike smell and/or taste even at low concentrations (Boisson et al., 2013; Freeman et al., 2009; Kumwenda et al., 2014; Mitro et al., 2019; Parker et al., 2006; Roma et al., 2014; Wood et al., 2012) Preference of other methods. (Kumwenda et al., 2014) Distorted perceptions of health benefits or problems caused by chlorine (i.e. infertility). (Makutsa et al., 2001; Mitro et al., 2019; Roma et al., 2014) Do not fully trust the method (Rosa et al., 2016)	Pre-existing experience with chlorine can reduce the sensitivity to taste and smell. (Mitro et al., 2019) Getting accustomed to the smell and taste, i.e. associating it with safety and good health. (Wood et al., 2012) Frequent distribution accompanied by education messages (Mitro et al., 2019) Interest in chemical disinfection (Makutsa et al., 2001)
Culture	Cultural perceptions of cause for contamination/disease. (Kumwenda et al., 2014) Cultural perceptions of chlorine products, i.e. causing diseases. (Roma et al., 2014) Water may be considered to have healing powers. (Roma et al., 2014) Low uptake of products, i.e. safe storage containers due to resistance towards non-traditional materials. (Ram et al., 2007)	Culturally appropriate educational methods (Parker et al., 2006).

conducting the training/education lack experience with the technical and/or behavioural aspects of water treatment, it will pose a barrier. It was also noted that the educational messages should be accompanied by implementors drinking water treated in the same way as they promote it. In some cases, it can also be relevant to include inputs from social scientists, who can assess the cultural and educational needs in a community (Mitro et al., 2019).

Although chlorination has been shown to be an effective approach to the disinfection of potable water, a significant limitation is that it is only effective under very specific conditions, and incorrect practices or discontinued use often lead to low efficiencies in the field. In the past, research has focused on either the quality of the water (i.e. in terms of presence of pathogens) and/or the presence of FRC concentration without considering the actual practices of chlorination. It is therefore uncertain if the reported failures are caused by user-dependent factors such as insufficient contact time and incorrect dosing, or if external factors such as high CD, poor quality of the product, or water/container recontamination are causing this.

While chlorination as an HWT only achieves a 1-star classification by the WHO, it can still be a highly effective solution to the disinfection of water containing bacterial or viral pathogens as it is easy to use and has a

Table 8
Chlorine product dependent barriers and enablers to adequate implementation of chlorination based on field experience.

	Barriers	Enablers
Cost	Lack of affordability (Freeman et al., 2009; Kumwenda et al., 2014; Loharikar et al., 2013; Opryszko et al., 2010; Parker et al., 2006; Patrick et al., 2013; Ram et al., 2007; Rosa et al., 2016). Use of less chlorine than directed on the bottle in order to make a bottle last longer (Mclaughlin et al., 2009). History of receiving free goods from NGOs, etc. (Makutsa et al., 2001)	Users see an economic value if it will save them money spent on water from vendors or fuel/wood for boiling. (Roma et al., 2014). Willingness to pay (Makutsa et al., 2001; Wood et al., 2012). Affordability, i.e. in comparison to other methods used by consumer (Makutsa et al., 2001; Parker et al., 2006). If perceived benefits outweigh the product's cost as long as users have the money to pay for it. (Wood et al., 2012). Extended free trial allows trying unfamiliar products without financial risk and should give sufficient time to experience health benefits. (Wood et al., 2012). Free access to products (Loharikar et al., 2013).
Availability of product	Unaware of the products or where to buy them. (Freeman et al., 2009; Loharikar et al., 2013). Lack of availability of products (Patrick et al., 2013; Rosa et al., 2016). Not aware of the presence of products in community (Freeman et al., 2009; Kumwenda et al., 2014). Product distribution challenged by poor road conditions and large distances between communities (Makutsa et al., 2001)	Knowledge of where to buy products. (Inungu et al., 2016; Loharikar et al., 2013). Ease in accessing the products (Makutsa et al., 2001; Wood et al., 2012).

Table 9
Current practice and storage dependent barriers and enablers to adequate implementation of chlorination based on field experience.

	Barriers	Enablers
Knowledge	Lack of understanding of correct use of products. (Mitro et al., 2019; Patrick et al., 2013). Confusion caused by use of multiple product types and products with different dosages (Mitro et al., 2019; Patrick et al., 2013). Extended storage time can contribute to the loss of FRC. (Patrick et al., 2013). One-time distribution without proper instruction. (Mitro et al., 2019)	Availability of products with different dosages may ensure that appropriate dosage is available for different container sizes (Mitro et al., 2019). Ease of using (Makutsa et al., 2001; Wood et al., 2012). Users feeling comfortable treating the water with product. (Inungu et al., 2016).
Behaviour	Lack of time to treat water (Murray et al., 2020; Roma et al., 2014). Forgetting to treat the water (Murray et al., 2020). Consumption of untreated water when outside of the home. (Murray et al., 2020; Patrick et al., 2013). Seasonal variation in compliance due to e.g. dry season when perceived risk of contamination is perceived as low (Wood et al., 2012).	Support of their family and the broader community can give incentive for behaviour change (Wood et al., 2012). Higher compliance when perceived risk of contamination is high, i.e. during outbreaks Patrick et al. (2013)

residual effect. This is especially true in emergency situations e.g. due to outbreaks of cholera, where *Vibrio cholerae* is effectively inactivated by chlorine. Similarly, chlorination can be an ineffective HWT, e.g. against *Cryptosporidium*. Unfortunately, few studies deal with this topic prior to implementing an HWT, which could also influence the effectiveness on health outcomes. Pre-intervention screening of the predominant pathogens in an area could prove to be an important decision-making tool when deciding on which HWT to implement.

8. Safe water and chlorination in Mexico, Colombia, and Brazil

In Latin America, there has been progress since 2000 in the coverage of drinking water service levels, as illustrated for Brazil, Colombia, and Mexico in Fig. 2. These countries are part of the GCRF-UKRI funded SAFEWATER research program focused on providing safe drinking water to rural communities in Brazil, Colombia, and Mexico (GCRF-UKRI, 2020). The service level, *safely managed water sources* is seen as a basic human right. These sources are improved sources, which have the potential to provide safe water due to their design/construction and additionally, the water should be available on premises and when needed. The other service levels include *basic* (less than 30 min round trip collection), *limited* (improved, but more than 30 min round trip collection), as well as *unimproved* (not protected against contamination) and raw *surface water* (WHO and UNICEF, 2017).

It is evident from Fig. 2, that rural households still lag behind their urban counterparts, where only rural households in Colombia are partly covered with safely managed drinking water sources. Rural areas pose a challenge since they are difficult to reach with on-premises safe drinking water. In Mexico, although more than 99% of the population had achieved at least basic drinking water services in 2017, both the rural and urban population are challenged by improved sources not being free from contamination (UNICEF and WHO, 2020). Although Fig. 2 does not show very significant differences between rural and urban areas of Mexico in the drinking water service level, there are many specific considerations that are not fully evidenced in the current data, and which would affect the actual conditions of the service: for example the type of basic service, the volume of water served, the continuity, etc.

Disaggregated data from Mexico shows that there was a difference in the coverage when differentiating between the poor and rich, where e.g. 88% of the poorest rural population had access to the basic service level compared to 99% of the richest rural population. This difference was even more pronounced in Colombia, where 58% and 93% of the poorest and richest respectively had access to at least basic service level in rural areas. No disaggregated data is available for Brazil (WHO and UNICEF, 2020).

In the following, the real scenario observed in rural communities of the three Latin American countries are described, where the focus is on household chlorination as an interim solution to provide safe drinking water in rural areas. There can be challenges to its implementation and compliance that differ on a regional scale and even chlorination of public water supplies can be a challenge in these areas as well as in the urban areas.

8.1. Chlorination in Mexico

In Mexico, chlorination is predominant in public water supplies, where the target FRC varies between 0.2 and 1.5 mg/L aligned with the Mexican drinking water regulations (Haro et al., 2012). However, it is insufficiently and intermittently applied, as confirmed by monthly evaluations of disinfection efficiency performed by the Federal Commission for Protection against Health Risks (COFEPRIS). The evaluations are carried out on sample parts of the public WS throughout the country

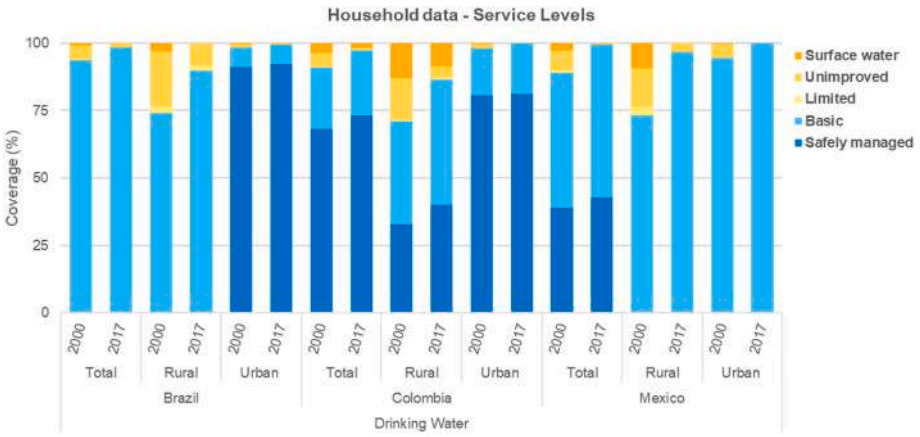

Fig. 2. Coverage (%) of drinking water resources at households in rural and urban areas of Brazil, Colombia and Mexico in years 2000 and 2017. Data from WHO and UNICEF (2017).

and showed that in 2016, only 47% of the sampled systems were efficient (i.e. more than 90% of the samples in a year meet the Mexican standard for FCR). In 22% of the systems, all the samples analysed were outside the Mexican standard in at least one of the monthly evaluations. In 4% of them, none of the samples from any evaluation met the standard (COFEPRIS, 2016). This poses a risk of deterioration of the water quality since most Mexican households store water either in tanks on rooftops or underground to ensure access to water, due to the generalized problem of intermittent WS. Only 45% of the country's population has a daily and continuous service of piped water. The rest of the population is subject to intermittent supply with variable frequencies (part of the day, every third day, once or twice a week) (INEGI, 2000).

Concerns regarding insufficient chlorination of public water supplies in Mexico are supported by several studies (Félix-Fuentes et al., 2007; Galdos-Balzategui et al., 2017; Rubino et al., 2019). In San Cristobal de Las Casas, Galdos-Balzategui et al. (2017) found that 32% of water samples from the public supply system had detectable *E. coli* in an annual monitoring of the bacteriological water quality, thereby confirming the irregularity in the disinfection process. In Guadalajara, Rubino et al. (2019) found that only 35% of 51 households had FRC meeting the required concentration and about 10% exceeded the FRC threshold. The FRC values fluctuated even from day to day in the same locations. Lower FRC concentrations were observed in water stored in households. Coliforms were observed in half of the tanks but in the study, only 8% of the households reported using the water for drinking and generally had a low confidence in the quality of the supplied water.

It should be noted that there are no national statistics for water quality monitoring of rural WS systems. COFEPRIS only provides surveillance to formal denomination systems, that have the necessary components to perform chlorination, which most rural systems do not have. In Mexico there are 49,440 locations with between 100 and 2,500 inhabitants, which are not part of the COFEPRIS evaluation system (CONAGUA, 2018). A study conducted by the Inter-American Development Bank and the NGO Cántaro Azul in 300 rural communities showed that only around 17% of piped WS systems met the FRC standards and more than 41% tested positive for the presence of *E. coli* (unpublished data). Navarro et al. (2007) found that in four studied rural communities, water samples from households at the water intake point and after passing through individual storage tanks only met the required FRC 59% and 49% of the time, respectively. Félix-Fuentes et al. (2007), studied the FRC level in three rural communities in the state of Sonora, and found that none of the water samples had detectable FRC in two of the communities, while 92% of the water samples from the third community were within norm.

A large part of Mexican rural communities, especially the most marginalized areas such as indigenous areas, are supplied with drinking water without any treatment. In these locations, not even chlorine is added to the community storage tank, since people reject chlorination (Soares, 2007). The general poor water quality of public water supplies means that many households must treat their own water to ensure potability (Vásquez et al., 2009). However, many choose to purchase bottled water instead, even in rural communities reaching up to half of the rural households (INEGI, 2018). Despite the need for water treatment, the research within HWT in rural Mexico is limited. In a study of a municipality in an indigenous region, boiling was the most reported treatment method among women respondents. The women reported the understanding of the relationship between water and health but, in fact, this could be associated with conditioning of a grant payment of the Prospera Government Program to women who meet the guidelines for hygiene behaviour, including boiling water for domestic use (Soares, 2007).

Today, to guarantee access to safe drinking water in Mexico, multiple strategies are needed. Governments need to develop public policies to improve the quality of the service of existing piped water systems and guarantee FRC values. Since the situation across the country in not likely to change in the short term, HWT is an important solution to increase access to safe water in rural and peri-urban communities. Although chlorination appears to be a common and effective treatment method in public WS, there is no relevant published scientific data on chlorine implementation in Mexican rural communities.

8.2. *Chlorination in Colombia*

The incorrect provision of drinking water and sanitation services in the rural sector and small municipalities is a common problem in Colombia (Quiroga et al., 2015). Although most Colombian rural communities have access to water, which is brought to homes through pipes, hoses, containers, or wells, only 42% of the rural population consumes safe water according to governmental sources (Colombia, 2018). There is only scarce information on the water quality in rural areas, which prevents knowing the real situation and consequently complicates adopting intervention strategies (Guzmán et al., 2015).

In urban areas in Colombia, chlorination is the most widely used disinfection method (RAS, 2010), but its application is not necessarily effective. In a study of chlorination in a pilot section of the drinking water distribution system in Cali, supplying approximately 30,000 inhabitants, it was found that the FRC was above the recommended 0.3 mg/L 80% of the time in the nine sampling points and never exceeded 1.0 mg/L. Lower concentrations of residual chlorine were observed when the residence time exceeded 24 h and at low water velocities in the pipes (Sánchez et al., 2010). It should be noted that the study did not mention the microbiological quality and thereby the potability of the

water.

Regarding rural areas, although sodium and calcium hypochlorite are available at health posts (RAS, 2010), the use of chlorination is challenged by low initial motivation and commitment of communities to maintain disinfection on a continuous and reliable basis, the lack of understanding of the importance of disinfection, and the scarce financial resources necessary for the proper operation and maintenance of the disinfection system (Quiroga et al., 2015).

At community level, Ávila de Navia et al. (2016) studied the water quality in the rural community El Charo, which is supplied by an aqueduct and the water treatment consists of a decanter tank, a multi-layer gravity filtration unit and disinfection with chlorine. The aqueduct of the rural community supplies water to 28 houses and a school. The water quality was analysed twice at several points, including the raw water and three delivery points (taps), namely one school, and a house with and without a storage tank. Coliforms and *Enterococcus* were present in the untreated water at concentrations up to 96 and 54 CFU/100 mL respectively, which was generally reduced during the treatment process. At the delivery points, only the household with a tank contained coliforms, but on the second sampling event, all delivery points had *Enterococcus*. All samples were free from *E. coli* (Ávila de Navia et al., 2016). The results indicate the need for improved process control to ensure that the water is completely disinfected as well as the importance of cleaning storage containers at the household level to prevent recontamination.

There is no published literature on the use of chlorination at household level in rural Colombia, but according to RAS (2010), laundry bleach (5.25% sodium hypochlorite) is used in some rural areas. Through interviews in the rural communities of Curití and El Carmelo in Antioquia, Colombia in the SAFEWATER project (GCRF-UKRI, 2020), it was found that although the communities were aware of the risks associated with untreated water and the willingness to implement treatments to improve the water quality, there was a resistance towards chlorination due to the alteration of taste of the water (unpublished data).

8.3. Chlorination in Brazil

In Brazil, chlorination is much more widespread in rural and isolated communities. Nationally, the most recommended chlorination practice for HWT is the use of 2.5% chlorine solution. The national Ministry of Health distribute, free of charge, bottles containing a 2.5% sodium hypochlorite solution and it is suggested to use 2 droplets (approximately 0.1 mL) for 1 L of water and a contact time of 30 min (Brazilian Ministry of Health, 2011) (Fig. 3a). Commercial and concentrated bleach, although it is not recommended, is also used for water treatment in some households. However, these methods depend on the user's proper dosage and storage. NaDCC tablets are also used for water disinfection in Brazil, where the recommended dosage is one tablet (2g) to treat 1000 L of water within 15 min.

The implementation of these chlorination methods in rural communities in Brazil is not consistent. In the Northern region, De Souza et al. (2016) showed that the chlorine treatment was performed by 0% and 20% of the households in a study of two communities. In another study of 97 households across 10 rural communities in the semi-arid region, 36% used chlorine tablets for drinking water disinfection, but chlorine was only detected in one sample (Peres et al., 2020). In the Northeast region, 24% of 66 studied households used chlorine prior to consumption (Xavier et al., 2011). The low adherence to this treatment was mostly reported due to: i) taste and odour caused by chlorine; ii) the lack of convenience to treat water every day; and iii) the belief that the water had good quality.

To increase the compliance and adequate dosage, different types of diffusion chlorinators have been used in Brazil. The Brazilian Agricultural Research Corporation developed a low-cost chlorinator (Fig. 3b), which can be installed by the user with PVC tubes between the water

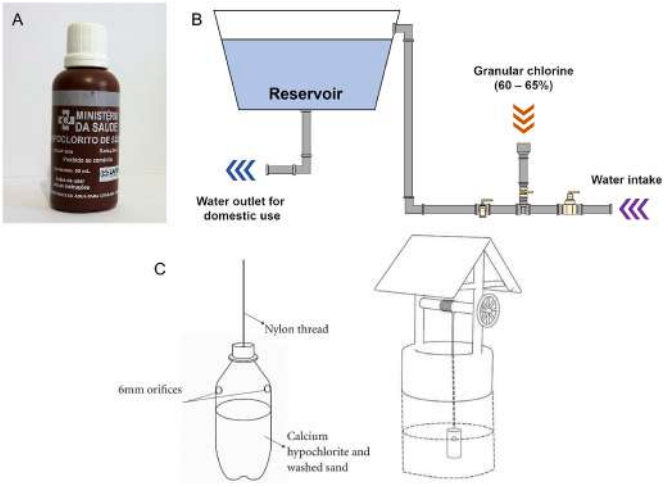

Fig. 3. A) Flask of 50 mL of 2.5% sodium hypochlorite distributed by the Brazilian Ministry of Health to population that are not supplied with treated water. B) Schematic of diffusion chlorinator developed by The Brazilian Agricultural Research Corporation, adapted from (Embrapa, 2015). C) Diffusion chlorinator made of plastic bottle fulfilled with sand and calcium hypochlorite, from (Ferreira et al., 2016).

intake inlet and the home reservoir (up to 1000 L) (Rodrigues, 2013). The Brazilian National Health Foundation developed a similar chlorinator suitable for reservoirs from 5000 L to 20000 L (Funasa, 2014). Both systems use granular calcium hypochlorite with 60–65% of active chlorine and consist of a point-of entry intervention, which reduces dependence on dosing at the point of use.

Ribeiro et al. (2018) evaluated the water quality from 20 rural schools in the Brazil's Northern region, prior and after the use of diffusion chlorinators. Prior to implementation, the bacteriological analysis from the water supplied showed that 100% and 70% were positive for TC and *E. coli*, respectively. After applying the chlorinator, only 25% of the schools presented TC and all of them were free from *E. coli*.

An older and simplified diffusion chlorinator, but still common in some Brazilian household wells, is made of a plastic bottle filled with sand and calcium hypochlorite (Fig. 3c). The chlorine can diffuse homogeneously into water by the two small holes at the top of the bottle, which is immersed in the well. It is reported that this system can ensure safe water for up to 30 days, depending on organic matter content in water.

This type of chlorinator was implemented in 11 wells in a rural community which were contaminated by TC (8 to >1,600 MPN/100 mL) and *E. coli* (4–50 MPN/100 mL). Ten days after the chlorinator implementation, no samples presented bacterial contamination. After 20 and 30 days, only one well was presenting TC counting, however the chlorine was not detectable. The users reported that in the first 3 days after implementation the taste and odour of chlorine was stronger, but after 10 days there was no more complaining. It is important to highlight that despite the efficiency observed in this study, the free chlorine concentration was always below the recommend by the Brazilian legislation (0.5 mg/L) (Guerra, 2006).

Another study in Brazil's Northern region used the same diffusion chlorinators in 20 wells contaminated with TC and *E. coli*. After 2 days of implementation only one sample was still contaminated. However, after 30 days 75% of the wells were presenting TC and 35% were presenting *E. coli* (Ferreira et al., 2016).

9. Concluding remarks

Chlorination has many advantages such as being very cost-effective, simple to use, and can be made widely available. This suggests that it

would be easy to achieve good results when implementing in households in rural communities, where the use is promoted by NGOs, local organisations, and governments.

In this review, we identified the main challenges of chlorination at household level, being the most relevant HWT the high user-dependence of this method to achieve high efficiency in disinfecting drinking water, the necessity of some technological approaches to apply the correct chlorination dose, the high risk of producing undesirable taste, smell and DBPs when the source of water has a certain organic matter content, and the chlorine dose is not accurately estimated by experts.

Despite that chlorine is the most effective, cheapest, easiest, most accessible and affordable household water treatment intervention, this paper also recognised the low compliance of chlorination as a big issue in rural settings and in the Global South in general. This is often attributed to the lack of motivation of the end-users, which points out at the need for research on user-behaviour changes in relation to water treatment and water quality awareness as well as the risks associated with consuming untreated water.

Social factors are often neglected in research done by scientists and engineers. For example, objection to the taste and smell of chlorine may even cause households to opt out drinking the disinfected water, as illustrated by the status of chlorination in Mexico. Even in public water supplies, the use of chlorine is challenged by insufficient dosing, as seen in Mexico, Colombia, and Brazil. Very few rigorous studies combine the social, scientific and engineering aspects to understand the challenges of real domestic scenarios, to investigate the limitations of existing technologies and to propose alternative approaches to face these issues. This illustrates the need to carry our more research in this area to change users' behaviour in the long term, improve compliance rates and reduce the high rates of users drop from chlorination programmes.

When the compliance concern is overcome, practical matters rise, as insufficient dosing found in Mexico, Colombia, and Brazil. This highlights the challenges associated with the technology and the need for capacity building when introducing an intervention. Here, we recommend that any public health solutions are accompanied by investigations aiming to avert taste and disinfection by-product issues at household, provide water quality assessment and pathogen screening, determine the potential need for multi-barrier approaches, as well as the design of user-friendly devices (e.g., inline chlorinators).

Microbiological contamination of water is a significant risk for rural communities in the Global South and is associated with deficiencies in water treatment and distribution. Intermittent supply, leaks, poor water quality and poor maintenance are common issues in rural areas. Barriers for an appropriate implementation of chlorination are responsible for low rates of success. The absence of risk assessment and the limitation for characterization, sampling and detection of biological contamination is a critical barrier. There is a lack of knowledge on the correct implementation of chlorination, which fails due to inadequate dosage, incorrect practice, and discontinued use causing low efficiencies in the households. Monitoring and evaluation of water sources in rural communities is a main barrier. There is general understanding of the benefits of chlorination in the community but there are not measures that warn the users about biological contamination of the water. Empowering the rural communities in water treatment knowledge and skills, via behaviour changes that include technical training on water quality, cleaning habits and water purification, is a necessity to create awareness at user level. A challenge for effective and sustainable chlorination is the creation of capacities and capabilities at the community level and for local governmental bodies to provide permanent support for sustainability.

Analysing the general status of the chlorination practice in three Latin American countries shows that, although the use of chlorine is widely known and considered a classic disinfection method by health care bodies, its application in households is not necessarily effective. Additionally, monitoring of proper practice is lacking, as well as compliance actions from medium to long-term follow-up in all three countries. There is a need for more research and solutions to address the effective implementation of chlorination at household level, specifically addressing compliance.

This work highlights the challenges associated with the implementation of household-based water treatment interventions and the need for capacity building when introducing interventions in communities and households. It is important to address some factors e.g. by gaining more knowledge on how chlorination is actually practised (dosing and contact time) and the specific water quality (physiochemical and microbial) to customize the dose and avert taste issues by overdosing as well as identifying the potential need for a multi-barrier approach.

Author contributions

Conceptualization, A.M. Nielsen, P. Fernández-Ibáñez and J.A. Byrne; methodology, A.M. Nielsen; supervision, S. Golden, P. Fernández-Ibáñez and J.A. Byrne; writing—original draft preparation, A.M. Nielsen, L.P.S. Paz, L.A.T. Garcia, K.J.S. Silva, M.M. Hincapié Pérez, A. Galdos-Balzategui and F. Reygadas; Supervision; writing—review and editing, S. Golden, P. Fernández-Ibáñez and J.A. Byrne.

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Declaration of competing interest

The authors declare no conflict of interest.

References

APHA, 2012. In: Rice, E.W., Baird, R.B., Eaton, A.D., Clesceri, L.S. (Eds.), Standard Methods for the Examination of Water and Wastewater, twenty-second ed., American Public Health Association (APHA), American Water Works Association (AWWA) and Water Environment Federation (WEF) Washington, D.C., USA.

Ávila de Navia, S.L., Estupiñán-Torres, S.M., Díaz González, L., 2016. Bacteriological water quality of the Vereda El Charco, San Miguel de Sema, Boyacá, Colombia (Calidad bacteriológica del agua Vereda El Charco, San Miguel de Sema, Boyacá, Colombia). Nova 14 (25), 139–145.

Blum, A., Null, C., Hoffmann, V., 2014. Marketing household water treatment: willingness to pay results from an experiment in rural Kenya. Water, 6(7), 1873-1886.

Boisson, S., Stevenson, M., Shapiro, L., Kumar, V., Singh, L., Ward, D., Clasen, T., 2013. Effect of household-based drinking water chlorination on diarrhoea among children under five in Orissa, India: a double-blind randomised placebo-controlled trial. PLoS Med. 10 (8), e1001497.

Brazilian Ministry of Health, 2011. Care for water for human consumption (Cuidados com água para consumo humano). Brazilian Ministry of Health. https://bvsms.saude.gov.br/bvs/folder/cuidados_agua_consumo_humano_2011.pdf.

CDC, 2014a. Free Chlorine Testing. https://www.cdc.gov/safewater/chlorine-residual-testing.html. (Accessed 23 October 2019).

CDC, 2014b. Safe Water System. https://www.cdc.gov/safewater/index.html. (Accessed 19 May 2020).

Chankova, S., Hatt, L., Musange, S., 2012. A community-based approach to promote household water treatment in Rwanda. J. Water Health 10 (1), 116–129.

Clasen, T., Edmondson, P., 2006. Sodium dichloroisocyanurate (NaDCC) tablets as an alternative to sodium hypochlorite for the routine treatment of drinking water at the household level. Int. J. Hyg Environ. Health 209 (2), 173–181. https://doi.org/10.1016/j.ijheh.2005.11.004.

Clasen, T., Haller, L., Walker, D., Bartram, J., Cairncross, S., 2007. Cost-effectiveness of water quality interventions for preventing diarrheal disease in developing countries. PLoS Med. 5 (4), 599–608.

COFEPRIS, 2016. https://datos.gob.mx/busca/dataset/calidad-del-agua-de-uso-y-consumo-humano. (Accessed 13 June 2020).

Colombia, 2018. Basic Water and Sanitation Master Plan. Strategic Vision 2018 – 2030 (Plan Director Agua Y Saneamiento Básico. Visión Estratégica 2018 – 2030). Bogotá

D.C., Colombia: Viceministerio de Agua y Saneamiento Básico, Gobierno de Colombia, Minvivienda.
Conagua, 2018. Water statistics in Mexico (Estadisticas del Agua en México). http://sina.conagua.gob.mx/publicaciones/EAM_2018.pdf.
Crump, J., Otieno, P., Slutsker, L., Keswick, B., Rosen, D., Hoekstra, M., Vulule, J.M., Luby, S., 2005. Household based treatment of drinking water with flocculant-disinfectant for preventing diarrhoea in areas with turbid source water in rural western Kenya: cluster randomised controlled trial. BMJ 331, 7515, 478.
De Souza, R.S., De Menezes, L.G., Felizzola, J.F., Figueiredo, R.O., Sá, T.D., Guerra, F.A., 2016. Water and health in igarapé-açu, pará, Brazil. Saúde Soc. 25 (4), 1095–1107.
Deng, Y., 2021. Making waves: principles for the design of sustainable household water treatment. Water Res. 198, 117151.
Embrapa, 2015. Planting Water – Embrapa Chlorinator (Plantando Água – Clorador Embrapa). São Carlos: Embrapa Instrumentação. Brazilian Agricultural Research Corporation. https://www.iniciativaverde.org.br/biblioteca-nossas-publicacoes.php.
Ercumen, A., Naser, A., Unicomb, L., Arnold, B., Colford, J., Luby, S., 2015. Effects of source-versus household contamination of tubewell water on child diarrhea in Rural Bangladesh: a randomized controlled trial. PLoS One 10 (3), e0121907.
Félix-Fuentes, A., Campas-Baypoli, O.N., Aguilar-Apodaca, M.G., Meza-Montenegro, M. M., 2007. Microbiological quality of water for human consumption in three rural communities in southern Sonora (Mexico) (Calidad microbiológica del agua de consumo humano de tres comunidades rurales del sur de Sonora (México)). RESPYN 8 (3), 1–13.
Feng, C., Xu, Z., Li, Y., Zhu, N., Wang, Z., 2021. Research progress on the contamination status and control policy of *Giardia* and *Cryptosporidium* in drinking water. J. Water, Sanit. Hyg. Dev. 11 (6), 867–886.
Ferreira, D.C., Luz, S.L., Buss, D.F., 2016. Evaluation of simple diffusion chlorinators for decontamination of wells in a rural settlement in Amazonia, Brazil. Ciência Saúde Coletiva 21 (3), 767–776.
Firth, J., Balraj, V., Muliyil, J., Roy, S., Rani, L., Chandresekhar, R., Kang, G., 2010. Point-of-use interventions to decrease contamination of drinking water: a randomized, controlled pilot study on efficacy, effectiveness, and acceptability of closed containers, Moringa oleifera, and in-home chlorination in rural South India. Am. J. Trop. Med. Hyg. 82 (5), 759–765.
Freeman, M., Quick, R., Abbott, D., Ogutu, P., Rheingans, R., 2009. Increasing equity of access to point-of-use water treatment products through social marketing and entrepreneurship: a case study in western Kenya. J. Water Health 7 (3), 527–534.
Funasa, 2014. Water chlorination manual in small communities using the simplified chlorinator developed by Funasa (Manual de cloração de água em pequenas comunidades utilizando o clorador simplificado desenvolvido pela Funasa). Brazilian National Health Fundation, Brazil.
Galdos-Balzategui, A., Torre, J.C., Sánchez-Pérez, H., López, J.J., Dosal, A.T., Urbina, S. G., 2017. Quantitative microbial risk assessment of drinking water in San Cristóbal de Las Casas, Chiapas, Mexico (Evaluación cuantitativa del riesgo microbiológico por consumo de agua en San Cristóbal de Las Casas, Chiapas, México). Tecnol. y Cienc. del Agua 8 (1), 133–153.
Gallandat, K., Lantagne, D., 2018. Lessons learned from fifteen drinking water treatment program evaluations in Haiti. Loughborough University. Transformation towards sustainable and resilient WASH services. In: Proceedings of the 41st WEDC International Conference, 2895. WEDC, Loughborough University, United Kingdom.
Garrett, V., Ogutu, P., Mabonga, P., Ombeki, S., Mwaki, A., Aluoch, G., Phelan, M., Quick, R., 2008. Diarrhoea prevention in a high-risk rural Kenyan populationthrough point-of-use chlorination, safe water storage,sanitation, and rainwater harvesting. Epidemiol. Infect. 136 (11), 1463–1471.
GBD, 2018. Causes of Death Collaborators, 2018. Global, regional, and national age-sex-specific mortality for 282 causes of death in 195 countries and territories, 1980–2017: a systematic analysis for the Global Burden of Disease Study 2017. Lancet 392 (10159), 1736–1788.
Gcrf-Ukri, 2020. Safewater project. https://www.safewater-research.com/. (Accessed 24 April 2020).
Geremew, A., Mengistie, B., Mellor, J., Lantagne, D., Alemayehu, E., Sahilu, G., 2019. Consistent point-of-use water chlorination among households using unimproved water sources and treatment preference in Eastern Ethiopia. Int. J. Environ. Health Res. 29 (6), 686–701.
Guerra, C.H., 2006. Evaluation of the Efficiency of the Clorador Simplified for Diffusion in the Disinfection of the Water for Human Consumption in Country Properties in the Basin of the Ribeirão O Laje – Caratinga/MG. Master thesis, Centro universitário de Caratinga, Brazil.
Guzmán, B., Nava, G., Díaz, P., 2015. Quality of water for human consumption and its association with morbimortality in Colombia, 2008-2012 (La calidad del agua para consumo humano y su asociación con la morbimortalidad en Colombia, 2008-2012). Biomedica 35 (2), 177–190.
Haro, J., Nubes, G., Ortiz, J.R., 2012. Sanitary risks in bacteriological quality of water: an evaluation in ten states of the Mexican Republic (Riesgos sanitarios en calidad bacteriológica del agua: una evaluación en diez estados de la república mexicana. Reg. soc. 24 (3), 257–288.
Harshfield, E., Lantagne, D., Turbes, A., Null, C., 2012. Evaluating the sustained health impact of household chlorination of drinking water in rural Haiti. Am. J. Trop. Med. Hyg. 87 (5), 786–795.
INEGI, 2000. https://www.inegi.org.mx/app/buscador/default.html?q=SEG%C3%9AN+FRECUENCIA+DE+RECEPCI%C3%93N+DE+AGUA. (Accessed 13 June 2020).
INEGI, 2018. Cerca de la mitad de los hogares realizan algún tipo de seperación o classificatción d la basura: módulo de hogares y medio ambiente.
Inungu, J., Zinsou, C., Mustafa, Y., Younis, M., Singbo, N., 2016. Factors associated with the uptake of Sodium dichloroisocyanurate (NADCC) tablets as household water-treatment product among caregivers of children under five in Benin, West Africa. J. Health Hum. Serv. Adm. 39 (1), 122–141.
Kumwenda, S., Morse, T.K., Lungu, K.T., Mwendera, C., Kasambara, A., 2014. Household use of water guard for treating drinking water in Chikwawa district, Southern Malawi. Malawi. J. Appl. Sci. 1 (1), 2–9.
Lantagne, D., Clasen, T., 2013. Effective use of household water treatment and safe storage in response to the 2010 Haiti earthquake. Am. J. Trop. Med. Hyg. 89 (3), 426–433.
Lantagne, D.S., Blount, B.C., Cardinali, F., Quick, R., 2008. Disinfection by-product formation and mitigation strategies in point-of-use chlorination of turbid and non-turbid waters in western Kenya. J. Water Health 6 (1), 67–82.
Lantagne, D.S., Cardinali, F., Blount, B.C., 2010. Disinfection by-product formation and mitigation strategies in point-of-use chlorination with sodium dichloroisocyanurate in Tanzania. Am. J. Trop. Med. Hyg. 83 (1), 135–143. https://doi.org/10.4269/ajtmh.2010.09-0431.
Lantagne, D.S., Blanton, E., Kotlarz, N., Gezagehn, H., van Dusen, E., Berens, J., Jellison, K., 2011. Hypochlorite solution expiration and stability in household water treatment in developing countries. J. Environ. Eng. 137 (2), 131–136. https://doi.org/10.1061/(ASCE)EE.1943-7870.0000299.
Levy, K., Anderson, L., Robb, K., Cevallos, W., Trueba, G., Eisenberg, J., 2014. Household effectiveness vs. laboratory efficacy of point-of-use chlorination. Water Res. 54, 69–77.
Loharikar, A., Russo, E., Sheth, A., Menon, M., Kudzala, A., Tauzie, B., Masuku, H.D., Ayers, T., Hoekstra, R.M., Quick, R., 2013. Long-term impact of integration of household water treatment and hygiene promotion with antenatal services on maternal water treatment and hygiene practices in Malawi. Am. J. Trop. Med. Hyg. 88 (2), 267–274.
Makutsa, P., Nzaku, K., Ogutu, P., Barasa, P., Ombeki, S., Mwaki, A., Quick, R., 2001. Challenges in implementing a point-of-use water quality intervention in rural Kenya. Am. J. Publ. Health 91 (10).
Mclaughlin, L., Levy, K., Beck, N., Shin, G., Meschke, J., Eisenberg, J., 2009. An observational study on the effectiveness of point-of-use chlorination. J. Environ. Health 71 (8), 48–53.
Meierhofer, R., Wietlisbach, B., Matiko, C., 2019. Influence of container cleanliness, container disinfection with chlorine, and container handling on recontamination of water collected from a water kiosk in a Kenyan slum. J. Water Health 17 (2), 308–317. https://doi.org/10.2166/wh.2019.282.
Mengistie, B., Berhane, Y., Worku, A., 2013. Household water chlorination reduces incidence of diarrhea among under-five children in rural Ethiopia: a cluster randomized controlled trial. PLoS One 8 (10).
Mitro, B., Wolfe, M., Galeano, M., Sikder, M., Gallandat, K., Lantagne, D., 2019. Barriers and facilitators to chlorine tablet distribution and use in emergencies: a qualitative assessment. Water 11 (6).
Mohamed, H., Brown, J., Njee, R., Clasen, T., Malebo, H., Mbuligwe, S., 2015. Point-of-use chlorination of turbid water: results from a field study in Tanzania. J. Water Health 13 (2), 544–552.
Murray, A., Napotnik, J., Rayner, J., Mendoza, A., Mitro, B., Norville, J., Faith, S.H., Eleveld, A., Jellison, K.L., Lantagne, D., 2020. Evaluation of consistent use, barriers to use, and microbiological effectiveness of three prototype household water treatment technologies in Haiti, Kenya, and Nicaragua. Sci. Total Environ. 718, 134685.
Navarro, I., Jiménez, B., Maya, C., Lucario, E.S., 2007. Assessment of potential cancer risks from THMs in water supply at Mexican rural communities. In: International Symposium on New Directions in Urban Water Management. UNESCO IHP, Paris, France.
Nguyen, K.H., Operario, D.J., Nyathi, M.E., Hill, C.L., Smith, J.A., Guerrant, R.L., Samie, A., Dillingham, R.A., Bessong, P.O., Rogawski McQuade, E.T., 2021. Seasonality of drinking water sources and the impact of drinking water source on enteric infections among children in Limpopo, South Africa. Int. J. Hyg Environ. Health 231, 113640.
Opryszko, M., Majeed, S., Hansen, P., Myers, J., Baba, D., Thompson, R., Burnham, G., 2010. Water and hygiene interventions to reduce diarrhoea in rural Afghanistan: a randomized controlled study. J. Water Health 8 (4), 687–702.
Parker, A., Stephenson, R., Riley, P., Ombeki, S., Komolleh, C., Sibley, L., Quick, R., 2006. Sustained high levels of stored drinking water treatment and retention of hand-washing knowledge in rural Kenyan households following a clinic-based intervention. Epidemiol. Infect. 134 (5), 1029–1036.
Parvez, S.M., Azad, R., Rahman, M., Unicomb, L., Ram, P., Naser, A., Stewart, C.P., Jannat, K., Rahman, M.J., Leontsini, E., Winch, P.J., Luby, S., 2018. Achieving optimal technology and behavioral uptake of single and combined interventions of water, sanitation hygiene and nutrition, in an efficacy trial (WASH benefits) in rural Banglades. Trials 19, 358.
Patrick, M., Berendes, D., Murphy, J., Bertrand, F., Husain, F., Handzel, T., 2013. Access to safe water in rural artibonite, Haiti 16 Months after the onset of the cholera epidemic. Am. J. Trop. Med. Hyg. 89 (4), 647–653.
Peres, M.R., Ebdon, J., Purnell, S., Taylor, H., 2020. Potential microbial transmission pathways in rural communities using multiple alternative water sources in semi-arid Brazil. Int. J. Hyg Environ. Health 224, 113431.
Quiroga, E., García, M., Solarte, Y., 2015. Community participation: a strategy to achieve low-risk microbiological water and effective disinfection (Participación comunitaria: Una estrategia para lograr agua de bajo riesgo microbiológica y una desinfección efectiva).
Ram, P., Kelsey, E., Rasoatiana, M.R., Rakotomalala, O., Dunston, C., Quick, R., 2007. Bringing safe water to remote populations: an evaluation of a portable point-of-use intervention in rural Madagascar. Am. J. Publ. Health 97 (3), 398–400.

Rangel, J., Lopez, B., Mejia, M., Mendoza, C., Luby, S., 2003. A novel technology to improve drinking water quality: a microbiological evaluation of in-home flocculation and chlorination in rural Guatemala. J. Water Health 1 (1), 15–22.
RAS, 2010. Technical Regulation for the Drinking Water and Basic Sanitation Sector-RAS (Reglamento Técnico para el Sector de Agua Potable y Saneamiento Básico-RAS). República de Colombia. Ministerio de Vivienda, Ciudad y Territorio.
Ribeiro, M.R., de Abreu, L.C., Laporta, G.Z., 2018. Drinking water and rural schools in the Western Amazon: an environmental intervention study. PeerJ 15 (6), e4993.
Ritter, M., Camille, E., Velcine, C., Guillaume, R., Lantagne, D., 2017. Optimizing household chlorination marketing strategies: a randomized controlled trial on the effect of price and promotion on adoption in Haiti. Am. J. Trop. Med. Hyg. 97 (1), 271–280.
Rodrigues, M.E., 2013. Water treatment: Tablet chlorinator (Tratamento de água: clorador de Pastilha). Belo Horizonte: Emater-MG.
Roma, E., Bond, T., Jeffrey, P., 2014. Factors involved in sustained use of point-of-use water disinfection methods: a field study from Flores Island, Indonesia. J. Water Health 12 (3), 573–583.
Rosa, G., Kelly, P., Clasen, T., 2016. Consistency of use and effectiveness of household water treatment practices among urban and rural populations claiming to treat their drinking water at home: a case study in Zambia. Am. J. Trop. Med. Hyg. 94 (2), 445–455.
Rubino, F., Corona, Y., Pérez, J.G., Smith, C., 2019. Bacterial contamination of drinking water in Guadalajara, Mexico. Int. J. Environ. Res. Publ. Health 16 (1), 67.
Sánchez, L., Rodríguez, S., Escobar, J., Torres, P., 2010. Modeling of residual chlorine and disinfection by-products in a pilot sector of the drinking water distribution system in the city of Cali. Inycompe 12 (1), 127–138.
Soares, D., 2007. Access, supply and control of water in an indigenous Chamula community in Chiapas. An analysis from the perspective of gender, environment and development. Reg. soc. 19 (38), 25–50.
Solomon, E., Robele, S., Kloos, H., Mengistie, B., 2020. Effect of household water treatment with chlorine on diarrhea among children under the age of five years in rural areas of Dire Dawa, eastern Ethiopia: a cluster randomized controlled trial. Infect. Dis. Poverty 9 (1), 64.
Solomon, E., Robele, S., Kloos, H., Mengistie, B., 2021. Comparing the effect of independent and combined interventions of household chlorination and handwashing on diarrhea of under-fives in rural Dire Dawa, eastern Ethiopia: a cluster randomized controlled trial. Pan. Afr. Med. J. 40, 239.
Tvrdá, E., Benko, F., 2020. Chapter 1 - free radicals: what they are and what they do. In: Preedy, V.R. (Ed.), Pathology. Academic Press, ISBN 9780128159729, pp. 3–13.
USEPA, 1999. Alternative Disinfectants and Oxidants Guidance Manual. USEPA.
Vásquez, W.F., Mozumder, P., Hernández-Arce, J., Berrens, R.P., 2009. Willingness to pay for safe drinking water: evidence from Parral, Mexico. J. Environ. Manag. 90 (11), 3391–3400.
Wheeler, J., Agha, S., 2013. Use of Certeza point-of-use water treatment product in Mozambique. J. Water, Sanit. Hyg. Dev. 3 (3), 341–348.
WHO, 2011. Evaluating Household Water Treatment Options: Health-Based Targets and Microbiological Performance Specifications. WHO, Geneva.
WHO, 2017. Guidelines for Drinking-Water Quality: Fourth Edition Incorporating the First Addendum. World Health Organization, Geneva. Licence: CC BY-NC-SA 3.0 IGO.
WHO, 2019. Results of Round II of the WHO Household Water Treatment Evaluation Scheme. WHO, Geneva.
WHO, 2021. Progress on Household Drinking Water, Sanitation and Hygiene 2000-2020: Five Years into the SDGs. WHO, Geneva.
WHO and UNICEF, 2012. A Toolkit for Monitoring and Evaluating Household Water Treatment and Safe Storage Programmes. UNICEF and WHO, Geneva.
WHO and UNICEF, 2017. Progress on Drinking Water, Sanitation, and Hygiene: 2017 Update and SDG Baselines. UNICEF and WHO, Geneva.
WHO and UNICEF, 2020. https://washdata.org/data/household#!/dashboard/3002. (Accessed 21 April 2020).
Wilhelm, N., Kaufmann, A., Blanton, E., Lantagne, D., 2018. Sodium hypochlorite dosage for household and emergency water treatment: updated recommendations. J. Water Health 16 (1), 112–125. https://doi.org/10.2166/wh.2017.012.
Wood, S., Foster, J., Kols, A., 2012. Understanding why women adopt and sustain home water treatment: insights from the Malawi antenatal care program. Soc. Sci. Med. 75 (4), 634–642.
Xavier, R.P., Siqueira, L.P., Vital, F.A., Rocha, F.J., I, J., Calazans, G.M., 2011. Microbiological quality of drinking rainwater in the inland region of Pajeú, Pernambuco, Northeast Brazil. Rev. Inst. Med. Trop. Sao Paulo 53 (3), 121–124.
Xiao, R., Duan, Y., Chu, W., 2020. The effectiveness of household water treatment and safe storage in improving drinking water quality: a disinfection by-product (DBP) perspective. J. Water Supply Res. Technol. - Aqua 69 (8), 785–806.

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Joint association of polycyclic aromatic hydrocarbons and heavy metal exposure with pulmonary function in children and adolescents aged 6–19 years

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ABSTRACT

Studies have reported associations between polycyclic aromatic hydrocarbon (PAH) or heavy metal (HM) exposure and respiratory diseases. However, evidence of their joint associations with pulmonary function, especially in children and adolescents aged 6–19 years, is lacking. We utilized cross-sectional data from 1,734 children and adolescents aged 6–19 years collected in the National Health and Nutrition Examination Survey 2007–2012 and analysed mixed PAH and mixed HM exposures and their joint association with pulmonary function by applying weighted quantile sum (WQS) regression and Bayesian kernel machine regression (BKMR). Multivariate linear regressions were carried out to determine the relationships between individual urinary PAH metabolites or blood HM levels and pulmonary function indices. We found that mixed PAHs and HMs were negatively related to forced expiratory volume in 1 s (FEV1) in subjects aged 6–12 years (all p values < 0.05). We found synergistic associations of PAH and HM exposure on pulmonary function impairment, mainly in children; lead (Pb) was the most damaging. In the analysis of individual PAH metabolites or HMs, Pb exposure was negatively associated with FEV1 values in all subgroups (all p values < 0.05). Thus, our findings indicate that increased PAH or HM exposure is associated with impairments to pulmonary function and that this association is more pronounced in children.

1. Introduction

Coexposure to polycyclic aromatic hydrocarbons (PAHs) and heavy metals (HMs) is inevitable because they are ubiquitous and coexist in various environmental media, such as street dirt, tobacco smoke and airborne fine particles (Huang et al., 2013; Saeedi et al., 2012). These pollutants are generated from a variety of sources. Specifically, PAHs are mainly released due to the incomplete combustion of biomass or fossil fuel; emissions from vehicles, paint, and asphalt pavement; industrial emissions; and residential fuel combustion (Kim et al., 2013). HMs are naturally occurring substances that originate from metal mining; oil, diesel and coal combustion; and natural geochemical processes (Loredo et al., 2003). Humans are mainly exposed to these pollutants through inhalation, ingestion or skin contact. Many PAH metabolites and HMs have been found in various biological samples, including those from serum, urine, breast milk, placenta, and umbilical cord blood (Olszowski et al., 2016; Sun et al., 2017; Yu et al., 2011; Zhou et al., 2019). Among the contact routes, the inhalation of PAHs or HMs makes it extremely likely that such compounds will deeply penetrate the respiratory tract (Valavanidis et al., 2008). Therefore, long-term exposure to PAHs or HMs is related to respiratory diseases (Bortey-Sam et al., 2018; Zhou et al., 2016).

As an early index of respiratory health status, pulmonary function is used to predict the long-term morbidity and mortality of many diseases, including nonrespiratory diseases (Schunemann et al., 2000). Epidemiological evidence indicates that impaired pulmonary function is related to exposure to PAHs, as demonstrated by several studies in Mexican schoolchildren (Barraza-Villarreal et al., 2014), Swedish young adults (Alhamdow et al., 2021), Chinese adults (Mu et al., 2019; Zhou et al., 2016), Canadian populations (Cakmak et al., 2017) and occupational populations (Shen et al., 2018). However, studies have not found any association between chronic pulmonary dysfunction and PAH exposure, as in Padula et al.'s study of exposure in asthmatic children (Padula et al., 2015). In contrast, extensive data have demonstrated that higher concentrations of HMs (mainly arsenic, cadmium or lead) in biological samples significantly predicted impaired pulmonary function among

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preschool children to 79-year-old adults (Madrigal et al., 2018; Yang et al., 2019; Zeng et al., 2017).

However, in the real world, no exposure occurs in isolation; humans are concurrently exposed to a wide range of environmental factors. Although the abovementioned epidemiological studies evaluated the effects of PAHs *or* HMs on the respiratory health of preadult populations, research on the joint effects of different classes of environmental chemicals remains scarce (Agier et al., 2019). While this has, to some extent, limited the risk of selective reporting (Siroux et al., 2016), further investigation is needed. Recently, the joint effects of PAHs and HMs on health, including thyroid hormone balance (Kim et al., 2021), cardiovascular endothelial inflammation (Zheng et al., 2019), semen quality (Shi et al., 2020) and children's growth and development (Jedrychowski et al., 2015; Zheng et al., 2019), have attracted increasing attention. However, their combined effect on pulmonary function in children and adolescents has rarely been examined. Children and adolescents compose one of the most susceptible age intervals in the general population because their respiratory systems have not yet fully developed. Notably, studies have shown that damage to pulmonary function before maturation is related to a higher incidence of respiratory, cardiovascular, and metabolic abnormalities; premature and all-cause death; and tendency to be hospitalized for chronic obstructive pulmonary disease in later life (Agusti et al., 2017; Mannino and Davis, 2006; Sin et al., 2005). Therefore, the combined effects of environmental hazards on pulmonary function prior to adulthood need to be determined.

Thus, in the present study, we used the National Health and Nutrition Examination Survey (NHANES) datasets from 2007 to 2012 to investigate the joint association of PAHs and HMs with pulmonary function indices by using the weighted quantile sum (WQS) and Bayesian kernel machine regression (BKMR) methods on data from children and adolescents aged 6–19 years.

2. Methods

2.1. Study participants

The NHANES was designed to survey the health and nutrition of a noninstitutionalized, representative U.S. civilian population with multicomponent and multistage data. The Research Ethics Review Board of the National Center for Health Statistics reviewed and approved the study protocol. Every selected participant signed written informed consent. Extensive individual data from interviews, examinations, laboratory tests, and nutritional status assessment in each cycle are provided on the NHANES website [https://www.cdc.gov/nchs/nhanes/].

This study was based on pooled data from 2007 to 2012, which comprised three consecutive cycles of the NHANES. Initially, 30,442 participants were included. Pregnant females were excluded, which reduced the sample size to 30,260 eligible subjects. Next, very young subjects (under 6 years) and adults (above 20 years) were excluded; thus, the sample size for analyses was reduced to 7,697. Then, adolescents with missing pulmonary function data were excluded (n = 1,543), as were individuals with missing PAH (n = 3,578) and HM exposure data (n = 742). We excluded individuals with extreme energy intake (n = 100), and extreme total energy intake outliers were defined as energy values above the 99th percentile or below the first percentile for sex (Neri et al., 2022). Therefore, the final analysis consisted of observations from 1,734 individuals (Fig. 1).

2.2. Urinary PAH metabolites

The detection of urinary PAH metabolites was based upon a well-

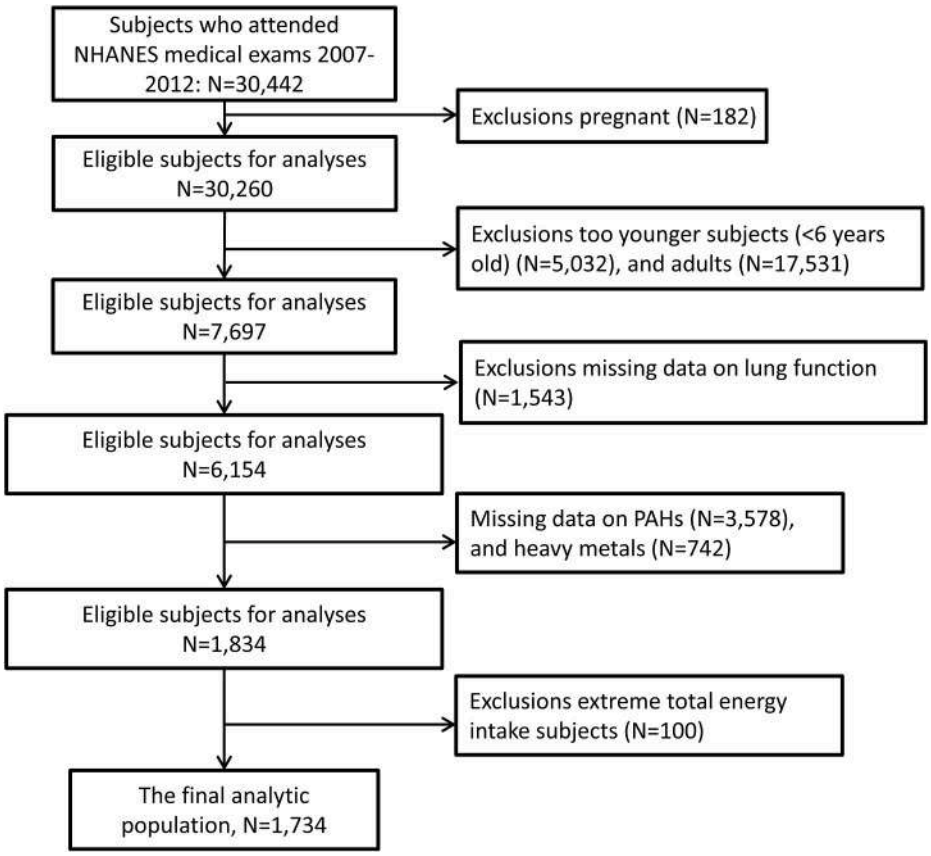

Fig. 1. Eligible participants and those included in the final analyses of the association between urinary PAH levels and/or blood HM levels and pulmonary function parameters in children and adolescents. NHANES, National Health and Nutrition Examination Survey; PAH, polycyclic aromatic hydrocarbon; HM, heavy metal.

established protocol, as described in previous studies (Alshaarawy et al., 2016;Xu et al., 2021). In brief, urine samples were gathered using identical collection cups; each sample was labelled, frozen below 20 °C and shipped to the National Center for Environmental Health for further testing. This detection method included a series of processes, including enzymatic hydrolysis of glucuronidated/sulfated monohydroxylated metabolites of PAHs (OH-PAHs) in urine, chemical extraction, derivatization, and final measurement with isotope dilution capillary gas chromatography tandem mass spectrometry (GC–MS/MS). The following metabolites of PAHs were analysed in this study: 1-naphthol, 2-naphthol, 3-hydroxyfluorene, 2-hydroxyfluorene, 1-hydroxyphenanthrene, 2-hydroxyphenanthrene, 3-hydroxyphenanthrene, 1-hydroxypyrene and 9-hydroxyfluorene. If the urinary PAH metabolite levels were below the limit of detection (LOD), these values were substituted with the LOD divided by the square root of 2.

2.3. Blood HM levels

Whole blood specimens were collected, frozen, stored, and delivered to the laboratory for testing on dry ice. Whole blood lead (Pb), cadmium (Cd), and total mercury (Hg) concentrations were measured using inductively coupled plasma–mass spectrometry. In this method, the pretreated samples were converted in nebulizers into aerosols, which are partially passed through the spray chamber and rapidly enter the central channel of the plasma, where the samples are atomized and ionized. The mass spectrometer rapidly detects ions with every mass-to-charge ratio, and the electrical signals are processed into digital signals that indicate the intensity of ions and ultimately the level of an element. The LODs were 0.25 μg/dL for Pb, 0.16 μg/L for Cd and 0.16 μg/L for Hg. If the measured value of HMs was less than the LOD, the value for that result was substituted with the LOD divided by the square root of two.

2.4. Pulmonary function

The NHANES spirometry protocol relied on standardized spirometric measurements and satisfied the recommendations of the American Thoracic Society (ATS) (Miller, Hankinson, Brusasco, Burgos, Casaburi, Coates, Crapo, Enright, van der Grinten, Gustafsson, Jensen, Johnson, MacIntyre, McKay, Navajas, Pedersen, Pellegrino, Viegi, Wanger and Force, 2005). A spirometer, which measures the amount of air a participant exhales and the rate at which the participant exhales the air, is used to perform the spirometry test. During the measurement, the subjects were instructed to exhale as forcefully as possible after taking in a full, deep breath. The result was then compared to standards established by NHANES III data, which were calculated based on an individual's age, height, sex, and race by virtue of the diagnostic thresholds for obstructive lung disease, as they differ for different body sizes and demographic subgroups. In our analysis, pulmonary function was evaluated by three metrics, namely, forced expiratory volume in 1 s (FEV1), forced vital capacity (FVC) and the FEV1: FVC ratio. Participant data were only included when participants provided at least three acceptable and repeatable values within eight measurements. FVC and FEV1 readings were ranked from A to F according to the ATS criteria. We only analysed data from subjects with FVC and FEV1 values of A or B in this study.

2.5. Covariates

We included the following variables as covariates in our analysis models due to their possible association with or influence on chemical exposure or pulmonary function: age (continuous; years); sex (male or female); race/ethnicity (non-Hispanic white, non-Hispanic black, Mexican-American, other Hispanic, or other/multiracial); body mass index (BMI); energy intake; serum cotinine levels; poverty-income ratio (PIR), urinary creatinine level (Hu et al., 2021; Madrigal et al., 2018), NHANES cycle and asthmatic status. BMI was calculated using weight in kilograms divided by height in metres squared. Serum cotinine, the metabolite of nicotine, indicates first- or second-hand exposure to cigarette smoke. Cotinine levels were determined with an isotope dilution high-performance liquid chromatography/atmospheric pressure chemical ionization tandem mass spectrometry method. Serum cotinine levels were classified as less than the LOD, from the LOD to 10 ng/mL, or more than 10 ng/mL in the analysis model. The PIR, which reflects the family socioeconomic level of participants, was calculated as the family income divided by the federal poverty threshold for that survey year. We classified the PIR as less than 1, greater than 1, or equal to 1 in our analysis model. The measurements of urinary PAH metabolites were adjusted with a new creatinine-involved method (O'Brien et al., 2016). The Roche/Hitachi Modular P Chemistry Analyser was employed to measure urinary creatinine concentrations. The covariate data were extracted from the NHANES website.

2.6. Statistical analysis

The descriptive statistics of demographic characteristics and pulmonary function indices of the participants are presented as the means ± standard deviations (for continuous variables) or frequencies and proportions (for categorical variables). The missing values of categorical variables were treated with the maximum value, and the missing values of continuous variables were imputed with the multivariate imputation chained equation (MICE) method (Marshall et al., 2009). Basic information of the children and adolescents aged 6–19 years was compared with Mann–Whitney *U* tests (continuous variables) or chi-squared tests (categorical variables). We employed fully adjusted multivariate linear regression models to investigate the relationship between each urinary PAH metabolite or each blood HM and pulmonary function indices. The urinary PAH metabolites, blood HM levels and pulmonary function indicators had naturally skewed distributions; thus, these variables were ln-transformed in the analysis models. Additionally, we adjusted for age, sex, race/ethnicity, BMI, PIR, serum cotinine category, NHANES cycle, and asthmatic status in all the models. WQS regression and BKMR were used to investigate whether 1) a mixture of urinary PAH metabolites, 2) a mixture of blood HM levels or 3) the overall PAH metabolites and HMs were associated with impaired pulmonary function. We employed a gWQS and bkmr R package to carry out the WQS and BKMR analysis as reported in previous studies (Duan et al., 2020; Xu et al., 2021). First, a bootstrap approach was applied to the data of the association of polycyclic aromatic hydrocarbons and heavy metals and pulmonary function to compute the empirical weights for each polycyclic aromatic hydrocarbon and heavy metal concentration. Second, the weighted index, namely, an index of the combined exposure, was estimated based on polycyclic aromatic hydrocarbons and heavy metal concentration quantiles by performing the weighted mean of the bootstrap weights and summed it. The data were randomly separated into two sets at a ratio of 40:60. One was the training set, which was utilized to measure the variable weights via a sum of 1,000 bootstrap samplings; the other was the validation set, which was used to determine the significance of mixed exposures through a survey-weighted Poisson regression. We used R version 3.5.0 to conduct all statistical analyses and applied a Holm–Bonferroni correction, where α was two-sided and a p value $<$ 0.05/12 was considered significant, for multiple comparisons of each chemical variable.

3. Results

The sociodemographic characteristics of the study participants are provided in Table 1. Our study contained a total of 1,734 subjects aged 6–19 years: 926 children and 808 adolescents. The average ages of the participants were 12.3 ± 3.9 years overall, 9.1 ± 1.9 years for the children and 16.0 ± 2.0 years for the adolescents. Among the participants, Mexican-Americans accounted for 25.1%, other Hispanic individuals accounted for 12.0%, non-Hispanic white individuals

Table 1
Characteristics of the study subjects, NHANES 2007 to 2012.

Variable	Overall (n = 1,734)	Children (6–12 years, n = 926)	Adolescents (13–19 years, n = 808)
Age (year)	12.3 ± 3.9	9.1 ± 1.9	16.0 ± 2.0
Sex (%)			
Males	51.2	51.1	48.9
Race/ethnicity (%)			
Mexican American	25.1	28.0	21.9
Other Hispanic	12.0	13.0	10.9
Non-Hispanic White	28.9	28.3	29.6
Non-Hispanic Black	24.6	22.7	26.7
Other Race-Including Multi-Racial	9.4	8.0	10.9
BMI (kg/m^2)	21.9 ± 6.3	19.4 ± 4.6	24.9 ± 6.7
PIR, %			
<1	30.5	33.1	27.5
≥1	61.7	59.4	64.4
Missing value	7.8	7.5	8.1
Cotinine, %			
<LOD	23.1	25.8	19.9
LOD-10	67.8	70.7	64.4
>10	6.9	0.1	14.7
Missing value	2.2	3.4	1.0
Energy intake (kcal/day)	2003.9 ± 766.2	1917.0 ± 658.8	2103.5 ± 862.9
FVC (mL)	3097.6 ± 1211.8	2246.6 ± 668.0	4073.0 ± 926.4
FEV1 (mL)	2654.7 ± 1031.4	1923.6 ± 557.8	3499.0 ± 775.1
FEV1/FVC	0.9 ± 0.1	0.9 ± 0.1	0.9 ± 0.1

Continuous variables were presented as mean values ± standard deviation, while categorical variables were showed as proportions.
NHANES, National Health and Nutrition Examination Survey; BMI, body mass index; PIR, poverty-income ratio; LOD, limit of detection; FVC, forced vital capacity; FEV1, forced expiratory volume at the 1 s.

Table 2
Result of WQS regression analysis showing the relationship between urinary PAHs and blood HM levels and FVC and FEV1 in children and adolescents.

	FVC		FEV1	
	Mean change (95% CI)	*p*	Mean change (95% CI)	*p*
PAHs + HMs				
Overall	−22.4 (−48.3, 3.5)	0.090	−26.8 (−49.2, −4.4)	0.019
Children	−37.8 (−56.1, −19.5)	5.7×10^{-5}	−38.2 (−54.1, −22.2)	3.1×10^{-6}
Adolescent	−47.1 (−81.6, −12.6)	0.008	−33.9 (−61.1, −6.7)	0.015
PAHs				
Overall	−2.8 (−20.7, 15.2)	0.763	−6.4 (−22.2, 9.4)	0.429
Children	−16.3 (−31.7, −1.0)	0.038	−1.9 (−3.2, −6.3)	0.004
Adolescent	−7.7 (−0.4, 21.6)	0.608	−18.4 (−43.3, 6.5)	0.149
HMs				
Overall	−25.8 (−46.9, −4.8)	0.016	−32.3 (−50.9, −13.7)	6.9×10^{-4}
Children	−28.5 (−42.9, −14.1)	1.2×10^{-4}	−3.0 (−4.3, −16.4)	1.6×10^{-5}
Adolescent	−43.4 (−71.0–15.8)	0.002	−27.6 (−51.0, −4.1)	0.022

WQS, weighted quantile sum; PAHs, polycyclic aromatic hydrocarbons; HMs, heavy metals; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; CI, confidence interval.
Age, sex, race/ethnicity, poverty-income ratio, body mass index, serum cotinine category, NHANES cycles, energy intake, and asthma status were adjusted in the model.

accounted for the largest proportion at 28.9%, non-Hispanic black individuals accounted for 24.6% and individuals of other races, including multiracial individuals, accounted for the lowest proportion at 9.4%. We found no significant difference in sex, race/ethnicity or PIR between the two groups. The average BMI was 19.4 ± 4.6 for children vs. 24.9 ± 6.7 for adolescents. The average values of FVC, FEV1 and FEV1/FVC for children vs. adolescents were 2246.6 ± 668.0 mL vs. 4073.0 ± 926.4 mL; 1923.6 ± 557.8 mL vs. 3499.0 ± 775.1 mL and 0.9 ± 0.1 vs. 0.9 ± 0.1, respectively. The FVC and FEV1 values were significantly lower in children than in adolescents (p values < 0.001).

The limits of detection and the values of urinary PAH metabolites and blood HM levels and their quantiles are presented in Supplemental Table 1. Except for 4-hydroxyphenanthrene, the detection rates of the chemical pollutants were greater than 80%. The chemical substance with the greatest median concentration was 2-napthol (at 3840.8 ng/L). The correlation results of PAHs and HMs are shown in Fig. S1.

Negative relationships between the joint mixtures (PAH metabolites and HM levels) and decreased FVC (mean change = −22.4, 95% CI: −48.3, 3.5, $p = 0.090$) and FEV1 (mean change = −26.8, 95% CI: −49.2, −4.4, $p = 0.019$) values were observed in the WQS regression among subjects aged 6–19 years (Table 2). An association between PAH and HM exposure and impaired pulmonary function was mainly found among children (Mean change $_{\text{for FVC}} = -37.8$, 95% CI: −56.1, −19.5, $p = 5.7 \times 10^{-5}$; Mean change $_{\text{for FEV1}} = -38.2$, 95% CI: −54.1, −22.2, $p = 3.1 \times 10^{-6}$). Our WQS model also indicated that increased mixed PAH exposure was related to decreased FVC (Mean change = −16.3, 95% CI: −31.7, −1.0, $p = 0.038$) and FEV1 (Mean change = −1.9, 95% CI: −3.2, −6.3, $p = 0.004$) values in children. In addition, significant negative relationships between mixed HM exposure and FVC and FEV1 values were observed among both children (Mean change $_{\text{for FVC}} = -28.5$, 95% CI: −42.9, −14.1, $p = 1.2 \times 10^{-4}$; Mean change $_{\text{for FEV1}} = -3.0$, 95% CI: −4.3, −16.4, $p = 1.6 \times 10^{-5}$) and adolescents (Mean change $_{\text{for FVC}} = -43.4$, 95% CI: −71.0, −15.8, $p = 0.002$; Mean change $_{\text{for FEV1}} = -27.6$, 95% CI: −51.0, −4.1, $p = 0.022$). The overall association between mixed HM exposure and pulmonary function impairment was greater than that of single components. Of the mixture of PAHs and HMs, Pb had the greatest contribution (Fig. 2), explaining 36.8% of the variance in FVC values and 38.1% of the variance in FEV1 values, followed by 2-napthol (explaining 24.0% of the variance in FVC values and 18.1% of the variance in FEV1 values). For children, Pb made the greatest contribution to pulmonary function impairment. Our BKMR analysis results were consistent with the result of WQS regression. We did not observe significant associations between the overall PAHs and HMs mixture and FVC and FEV1 values in adolescents or subjects aged 6–19 years by BKMR method (Fig. S2); however, it did show associations for PAHs and HMs mixture and FEV1 values in children (Fig. S3A.). Exposure-response functions and 95% credible bands for each metal in the mixture with all PAHs and HMs mixture in children fixed at their median are shown in Fig. S3B.

The results of multivariate linear regression models for each individual urinary PAH metabolite and HM are presented in Table 3 and Table 4. Total metals were negatively related to FVC values (Mean change = −90.4, 95% CI: −150.4, −30.5, $p = 0.003$). Additionally, Pb and total metals were inversely associated with FEV1 values (Mean change values: Pb = −72.2, 95% CI: −121.7, −22.7, $p = 0.004$; total metals = −90.8, 95% CI: −143.4, −38.2, $p = 0.001$). However, after age stratification, only Pb was positively related to the risk of pulmonary function impairment in children (Mean change $_{\text{for FVC}} = -78.7$, 95% CI: −127.8, −29.6, $p = 0.002$; Mean change $_{\text{for FEV1}} = -73.6$, 95% CI: −116.1, −31.0, $p = 0.001$) and in adolescents (Mean change $_{\text{for FEV1}} = -141.2$, 95% CI: −229.1, −53.2, $p = 0.002$). No correlations between any other chemicals (urinary PAH metabolites or blood HM levels) and FVC or FEV1 values were found. The associations between all individual chemicals (urinary PAH metabolites or blood HM levels) and FEV1/FVC values trended towards null, as shown in Supplemental Table 2. In addition, by adjusting different covariates for sensitivity analysis (Supplemental Tables 3 and 4), we found that our results were robust.

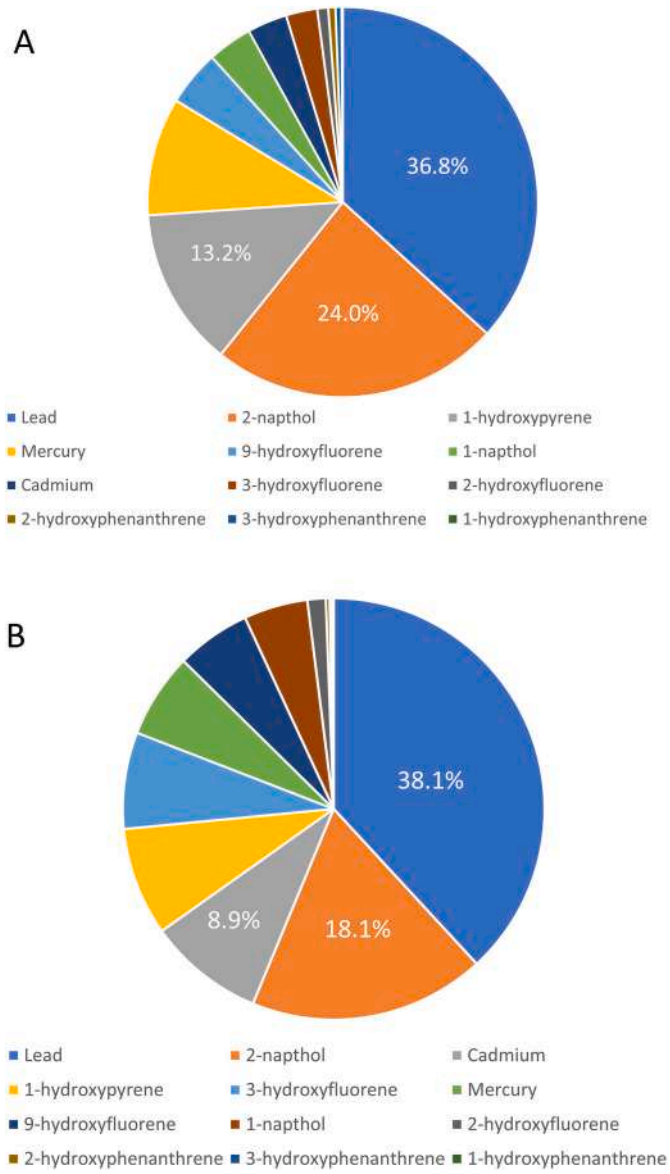

Fig. 2. The WQS regression model indicates weights for FVC (**A**) and FEV1 (**B**) values in participants aged 6–19 years. The following variables were adjusted for in the models: age, sex, race/ethnicity, PIR, BMI, serum cotinine category, NHANES cycle, and asthmatic status. WQS, weighted quantile sum; FVC, forced vital capacity; FEV1, forced expiratory volume in 1 s; PIR, poverty-income ratio; BMI, body mass index; NHANES, National Health and Nutrition Examination Survey.

4. Discussion

In our study, urinary PAH metabolites and blood HM levels were significantly related to pulmonary function impairment, mainly Pb, 1-hydroxypyrene, 2-naphthol, 3-hydroxyphenanthrene and Cd. In addition, exposure to PAHs and HMs had a synergistic association with the risk of pulmonary function impairment, predominantly in children.

PAH exposure has been reported to be associated with pulmonary function impairment in the general population over a large age span (Cakmak et al., 2017; Zhou et al., 2016). Additionally, studies have focused on specific age groups. As reported by Padula et al., ambient PAHs were associated with chronic pulmonary dysfunction, specifically in nonasthmatic U.S. children aged 9–18 years (Padula et al., 2015). Another pilot study conducted in Mexican schoolchildren aged 6–14 years found similar results, as urinary PAH levels were associated with pulmonary function as well as lower exhaled breath condensate (EBC) pH, a typical marker of airway inflammation (Barraza-Villarreal et al., 2014). In addition, two studies found that urinary PAH metabolites or $PM_{2.5}$-bound PAH exposure were related to decreased pulmonary function in Swedish young adults aged 22–25 years and in Chinese participants aged 40–60 years (Alhamdow et al., 2021; Mu et al., 2019). In addition to the findings in the general population, studies have focused on people with high PAH exposure levels, such as kitchen and coke oven workers; unsurprisingly, they found that such levels have adverse effects on pulmonary function (Kolena et al., 2020; Kolena et al., 2017). Thus, PAH exposure may result in human pulmonary function impairment; our study emphasized the susceptibility of children to PAH exposure relative to that of adolescents.

Studies have frequently suggested that exposure to HMs is related to decreased pulmonary function in smokers, adults and occupational workers (Hariri et al., 2018; Leem et al., 2015; Mannino et al., 2004; Verougstraete et al., 2004; Yang et al., 2019); however, the effect of HM exposure on young people has received increasing attention in recent years. A cross-sectional study conducted among preschool children of an electronic waste district in China found that blood Pb and Cd levels were negatively related to FEV1 values and that haemoglobin predicted pulmonary function (Zeng et al., 2017). Cakmak et al. found that Cd was associated with decreases in $FEF_{25–75}$, FEV1 and FVC (Cakmak et al., 2014). In another cross-sectional study based on NHANES 2011–2012 data, the blood and urinary levels of manganese (Mn) and the urinary levels of Pb in 1,234 children and youths were related to pulmonary function parameters (Madrigal et al., 2018). These findings are consistent with our conclusion, but we further emphasize the role of Pb in pulmonary function impairment by analysing the adverse effects of joint exposure to two kinds of chemicals.

Although the mechanism underlying pulmonary function impairment has not been fully elucidated, many studies have provided potential evidence concerning the action of PAHs or HMs. Exposure to PAHs or HMs causes oxidative damage by activating the cytochrome P450 enzyme family (Bolton et al., 2000; Valko et al., 2005). Thus, exposure to PAHs or HMs may increase reactive oxygen species, which can attack cellular macromolecules (mainly proteins, lipids and DNA) and ultimately damage the lung epithelium or tissue (Cao et al., 2020). A Taiwanese cross-sectional study on traffic conductors indicated that the joint effect of urinary PAH biomarkers and Cd levels may result in oxidative DNA damage (Huang et al., 2013). Moreover, a longitudinal study on coke oven workers in China found that PAH exposure was significantly associated with oxidative damage to DNA and lipids (Kuang et al., 2013). A persistent inflammatory response due to PAH or HM exposure may be another key mechanism underlying pulmonary function impairment. Epidemiological and animal studies have indicated that PAH or HM exposure may lead to persistent lung inflammation (Ma et al., 2020; Rokadia and Agarwal, 2013). When inflammation occurs, inflammatory cells secrete cytokines and growth factors, promoting airway hyperresponsiveness and structural changes, which influence pulmonary function (Chung, 2005). Other mechanisms of PAH or HM action have been examined, such as genetic polymorphisms (Joneidi et al., 2019; Ruchirawat et al., 2007) and epigenetic modifications (Bitto et al., 2014; Hew, Walker, Kohli, Garcia, Syed, McDonald-Hyman, Noth, Mann, Pratt, Balmes, Hammond, Eisen and Nadeau, 2015), both of which also play important roles in the pathogenesis of pulmonary function (House et al., 2015). For example, using two longitudinal cohort studies on occupational workers and general community residents, Wu et al. consistently discovered that rs1529672 in *RARB* altered and increased the impact of PAH exposure on the annual FEV1/FVC value (Wu et al., 2020). Mechanistic studies on the joint and synergistic associations of PAHs and HMs on pulmonary function are scarce, and this topic merits further exploration.

In our study, we found a significant age difference in the relationship between the joint association of PAH and HM exposure with pulmonary function impairment, which has only been hinted at in prior studies. The

Table 3
Results of multivariate regression model showing the relationship between urinary PAHs and blood HMs levels and FVC in children and adolescents.

	Overall			Children			Adolescents		
Chemicals	Mean change	95% CI	*p*	Mean change	95% CI	*p*	Mean change	95% CI	*p*
1-napthol	−3.6	−24.8, 17.5	0.737	4.9	−15.1, 24.9	0.633	9.9	−24.3, 43.9	0.573
2-napthol	−23.3	−46.9, 0.3	0.053	2.7	−19.8, 25.2	0.814	−33.1	−70.9, 4.8	0.087
3-hydroxyfluorene	−42.5	−78.9, −6.2	0.022	−18.9	−51.6, 13.9	0.258	−38.0	−98.7, 22.7	0.219
2-hydroxyfluorene	−31.9	−68.3, 4.7	0.087	−6.5	−42.8, 29.7	0.724	−26.2	−83.8, 31.4	0.373
3-hydroxyphenanthrene	−45.5	−87.7, −3.3	0.035	−32.2	−69.6, 5.2	0.091	−19.0	−90.5, 52.5	0.603
1-hydroxyphenanthrene	−53.9	−97.5, −10.2	0.016	−13.9	−53.6, 25.7	0.491	−52.2	−124.8, 20.4	0.159
2-hydroxyphenanthrene	−21.2	−61.1, 18.6	0.296	2.0	−32.8, 36.8	0.909	−42.4	−109.4, 24.7	0.215
1-hydroxypyrene	−50.2	−87.7, −12.6	0.009	−22.1	−56.5, 12.3	0.208	−22.0	−91.4, 47.4	0.534
9-hydroxyfluorene	−46.3	−82.9, −9.6	0.013	−18.8	−50.9, 13.3	0.250	−16.4	−77.6, 44.8	0.599
∑PAHs	−50.2	−89.8, −10.6	0.013	1.9	−33.6, 37.5	0.915	−45.5	−112.1, 21.1	0.180
Lead	−68.3	−124.7, −12.0	0.018	−78.7	−127.8, −29.6	0.002	−129.1	−227.2, −31.1	0.010
Cadmium	−71.2	−145.9, 3.5	0.062	−3.1	−101.6, 95.4	0.951	−55.4	−151.5, 40.7	0.258
Mercury	−24.5	−60.0, 11.0	0.175	−9.1	−42.3, 24.2	0.593	−27.8	−83.3, 27.7	0.326
∑Metals	−90.4	−150.4, −30.5	0.003	−80.1	−135.8, −24.3	0.005	−120.9	−216.6, −25.2	0.013

A Holm-Bonferroni correction was applied considering multiple comparisons of each chemical variable, p value$< (0.05/12 = 0.0041)$ was considered significant.
PAHs, polycyclic aromatic hydrocarbons; HMs, heavy metals; FVC, forced vital capacity; CI, confidence interval.
Age, sex, race/ethnicity, poverty-income ratio, body mass index, serum cotinine category, NHANES cycles, energy intake, and asthma status were adjusted in the model.

Table 4
Results of multivariate regression model showing the relationship between urinary PAHs and blood HMs levels and FEV1 in children and adolescents.

	Overall			Children			Adolescents		
Chemicals	Mean change	95% CI	*p*	Mean change	95% CI	*p*	Mean change	95% CI	*p*
1-napthol	1.4	−17.2, 20.0	0.880	3.9	−13.4, 21.3	0.657	16.6	−14.1, 47.2	0.289
2-napthol	−16.7	−37.5, 4.0	0.113	4.2	−15.3, 23.7	0.673	−22.2	−56.3, 11.8	0.200
3-hydroxyfluorene	−41.6	−73.5, −9.6	0.011	−23.7	−52.1, 4.6	0.101	−39.4	−93.9, 15.1	0.157
2-hydroxyfluorene	−25.1	−57.2, 6.9	0.124	−8.5	−40.0, 23.0	0.597	−17.6	−69.4, 34.1	0.504
3-hydroxyphenanthrene	−34.9	−71.9, 2.2	0.065	−23.3	−55.8, 9.2	0.159	−19.7	−84.0, 44.5	0.547
1-hydroxyphenanthrene	−44.1	−82.4, −5.8	0.024	−12.6	−47.0, 21.8	0.472	−48.5	−113.7, 16.8	0.145
2-hydroxyphenanthrene	−20.4	−55.3, 14.6	0.254	1.0	−29.2, 31.2	0.948	−30.4	−92.8, 31.9	0.339
1-hydroxypyrene	−47.7	−80.6, −14.7	0.005	−22.2	−52.1, 7.6	0.144	−27.0	−82.0, 28.0	0.336
9-hydroxyfluorene	−42.1	−74.3, −9.9	0.010	−16.0	−43.8, 11.9	0.260	−50.5	−107.2, 6.2	0.081
∑PAHs	−39.2	−74.0, −4.5	0.027	−1.4	−32.3, 29.4	0.928	−33.5	−93.4, 26.3	0.272
Lead	−72.2	−121.7, −22.7	0.004	−73.6	−116.1, −31.0	0.001	−141.2	−229.1, −53.2	0.002
Cadmium	−89.8	−155.3, −24.3	0.007	−26.6	−112.1, 58.9	0.541	−80.5	−166.7, 5.8	0.067
Mercury	−18.5	−49.6, 12.6	0.244	20.7	−49.5, 8.1	0.158	−5.8	−55.7, 44.1	0.819
∑Metals	−90.8	−143.4, −38.2	0.001	−88.4	−136.6, −40.2	<0.001	−114.2	−200.2, −28.2	0.009

A Holm-Bonferroni correction was applied considering multiple comparisons of each chemical variable, p value$< (0.05/12 = 0.0041)$ was considered significant.
PAHs, polycyclic aromatic hydrocarbons; HMs, heavy metals; FEV1, forced expiratory volume in 1 s; CI, confidence interval.
Age, sex, race/ethnicity, poverty-income ratio, body mass index, serum cotinine category, NHANES cycles, energy intake, and asthma status were adjusted in the model.

airway epithelium is more permeable to air pollutants in children than in adults, which makes the health risks of chemical exposure higher in children (Oliveira et al., 2019). Furthermore, children have a higher inhalation rate than adults, and more pollutants reach their lungs due to their body shape, physiology and daily activity level (Salvi, 2007). Accordingly, Oliveira et al. emphasized the increased impact of school indoor air quality on children's risk of exposure to particulate matter (PM) and PAHs (Oliveira et al., 2019); metals are a nonnegligible component of PM (Kim et al., 2015). In addition, recent data indicate that children in Asia or in urban and industrial areas experience higher environmental exposures, which may further aggravate cardiopulmonary diseases in children and increase their risk of developing cancer later in life (Oliveira et al., 2019). Therefore, to fully comprehend the health threat of PAH or HM exposure in children, many future studies evaluating both typical environmental exposure levels and concentrations in human samples are warranted. Furthermore, stringent implementation of pollution reduction and prevention strategies is needed.

This study has several strengths. First, it employed high-quality normalized data collection and sample processing from a nationally representative sample, which ensured the reliability and repeatability of the data. Second, we employed a mixed exposure model to analyse the associations of one class and the joint association of two classes of environmental exposures. To our knowledge, this study is the first to investigate the joint association of PAH and HM exposure with the risk of pulmonary function impairment. Third, we performed separate analyses stratified by age, enabling the investigation of the sensitivity of different groups to chemical pollutants. Furthermore, we implemented a recently verified creatinine adjustment method to correct for chemical concentrations. This method utilized covariate-adjusted standardization, which is suitable for evaluating the relationship between environmental chemical exposure and human health risks in aetiological studies (O'Brien et al., 2016).

Our study also had some limitations. First, its cross-sectional design prevented the establishment of causality between PAH or HM exposure and pulmonary function impairment. Second, although we adjusted for important confounders, it is likely that the effect of genes, females who were lactating, exercise, drinking, some diseases and other pollutants on pulmonary function may have biased the results; however, this information is unavailable. Third, the analysis of urinary PAH metabolite concentrations was carried out on a single spot urine sample, although Dobraca et al. suggested that exposure measurements from a single sample cannot accurately define the multiyear exposure timeframe of 1-naphthol (though it can for other PAH metabolites) (Dobraca et al., 2018). Finally, no data on the geographic location of subjects were provided; however, whether they lived in urban or rural areas, near traffic or in industrial settings would greatly influence the level of

pollutants in their bodies.

5. Conclusion

Joint exposure to PAHs and HMs is related to the risk of pulmonary function impairment; these associations were more prominent among children. Of the environmental pollutants, Pb had the greatest impact on health risks. This study highlighted the complexity of human health risks upon exposure to different kinds of chemicals. The mechanisms underlying their synergistic effects on background exposure to PAHs and HMs in children and adolescents aged 6–19 years need further elucidation.

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Declaration of competing interest

The authors declare that they have no competing interests.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114007.

References

Agier, L., Basagana, X., Maitre, L., Granum, B., Bird, P.K., Casas, M., Oftedal, B., Wright, J., Andrusaityte, S., de Castro, M., Cequier, E., Chatzi, L., Donaire-Gonzalez, D., Grazuleviciene, R., Haug, L.S., Sakhi, A.K., Leventakou, V., McEachan, R., Nieuwenhuijsen, M., Petraviciene, I., Robinson, O., Roumeliotaki, T., Sunyer, J., Tamayo-Uria, I., Thomsen, C., Urquiza, J., Valentin, A., Slama, R., Vrijheid, M., Siroux, V., 2019. Early-life exposome and lung function in children in Europe: an analysis of data from the longitudinal, population-based HELIX cohort. Lancet Planet. Health 3, e81–e92.

Agusti, A., Noell, G., Brugada, J., Faner, R., 2017. Lung function in early adulthood and health in later life: a transgenerational cohort analysis. Lancet Respir. Med. 5, 935–945.

Alhamdow, A., Zettergren, A., Kull, I., Hallberg, J., Andersson, N., Ekstrom, S., Berglund, M., Wheelock, C.E., Essig, Y.J., Krais, A.M., Georgelis, A., Lindh, C.H., Melen, E., Bergstrom, A., 2021. Low-level exposure to polycyclic aromatic hydrocarbons is associated with reduced lung function among Swedish young adults. Environ. Res. 197, 111169.

Alshaarawy, O., Elbaz, H.A., Andrew, M.E., 2016. The association of urinary polycyclic aromatic hydrocarbon biomarkers and cardiovascular disease in the US population. Environ. Int. 89–90, 174–178.

Barraza-Villarreal, A., Escamilla-Nunez, M.C., Schilmann, A., Hernandez-Cadena, L., Li, Z., Romanoff, L., Sjodin, A., Del Rio-Navarro, B.E., Diaz-Sanchez, D., Diaz-Barriga, F., Sly, P., Romieu, I., 2014. Lung function, airway inflammation, and polycyclic aromatic hydrocarbons exposure in mexican schoolchildren: a pilot study. J. Occup. Environ. Med. 56, 415–419.

Bitto, A., Pizzino, G., Irrera, N., Galfo, F., Squadrito, F., 2014. Epigenetic modifications due to heavy metals exposure in children living in polluted areas. Curr. Genom. 15, 464–468.

Bolton, J.L., Trush, M.A., Penning, T.M., Dryhurst, G., Monks, T.J., 2000. Role of quinones in toxicology. Chem. Res. Toxicol. 13, 135–160.

Bortey-Sam, N., Ikenaka, Y., Akoto, O., Nakayama, S.M.M., Asante, K.A., Baidoo, E., Obirikorang, C., Mizukawa, H., Ishizuka, M., 2018. Association between human exposure to heavy metals/metalloid and occurrences of respiratory diseases, lipid peroxidation and DNA damage in Kumasi, Ghana. Environ. Pollut. 235, 163–170.

Cakmak, S., Dales, R., Kauri, L.M., Mahmud, M., Van Ryswyk, K., Vanos, J., Liu, L., Kumarathasan, P., Thomson, E., Vincent, R., Weichenthal, S., 2014. Metal composition of fine particulate air pollution and acute changes in cardiorespiratory physiology. Environ. Pollut. 189, 208–214.

Cakmak, S., Hebbern, C., Cakmak, J.D., Dales, R.E., 2017. The influence of polycyclic aromatic hydrocarbons on lung function in a representative sample of the Canadian population. Environ. Pollut. 228, 1–7.

Cao, L.M., Mu, G., Chen, W.H., 2020. Polycyclic aromatic hydrocarbon: environmental sources, associations with altered lung function and potential mechanisms. Chin. Med. J. (Engl). 133, 1603–1605.

Chung, K.F., 2005. The role of airway smooth muscle in the pathogenesis of airway wall remodeling in chronic obstructive pulmonary disease. Proc. Am. Thorac. Soc. 2, 347–354. ; discussion 371-2.

Dobraca, D., Lum, R., Sjodin, A., Calafat, A.M., Laurent, C.A., Kushi, L.H., Windham, G. C., 2018. Urinary biomarkers of polycyclic aromatic hydrocarbons in pre- and peri-pubertal girls in Northern California: predictors of exposure and temporal variability. Environ. Res. 165, 46–54.

Duan, W., Xu, C., Liu, Q., Xu, J., Weng, Z., Zhang, X., Basnet, T.B., Dahal, M., Gu, A., 2020. Levels of a mixture of heavy metals in blood and urine and all-cause, cardiovascular disease and cancer mortality: a population-based cohort study. Environ. Pollut. 263, 114630.

Hariri, A., Mohamad Noor, N., Paiman, N.A., Ahmad Zaidi, A.M., Zainal Bakri, S.F., 2018. Heavy metals found in the breathing zone, toenails and lung function of welders working in an air-conditioned welding workplace. Int. J. Occup. Saf. Ergon. 24, 646–651.

Hew, K.M., Walker, A.I., Kohli, A., Garcia, M., Syed, A., McDonald-Hyman, C., Noth, E. M., Mann, J.K., Pratt, B., Balmes, J., Hammond, S.K., Eisen, E.A., Nadeau, K.C., 2015. Childhood exposure to ambient polycyclic aromatic hydrocarbons is linked to epigenetic modifications and impaired systemic immunity in T cells. Clin. Exp. Allergy 45, 238–248.

House, J.S., Li, H., DeGraff, L.M., Flake, G., Zeldin, D.C., London, S.J., 2015. Genetic variation in HTR4 and lung function: GWAS follow-up in mouse. Faseb. J. 29, 323–335.

Hu, P., Su, W., Vinturache, A., Gu, H., Cai, C., Lu, M., Ding, G., 2021. Urinary 3-phenoxybenzoic acid (3-PBA) concentration and pulmonary function in children: a National Health and Nutrition Examination Survey (NHANES) 2007-2012 analysis. Environ. Pollut. 270, 116178.

Huang, H.B., Chen, G.W., Wang, C.J., Lin, Y.Y., Liou, S.H., Lai, C.H., Wang, S.L., 2013. Exposure to heavy metals and polycyclic aromatic hydrocarbons and DNA damage in taiwanese traffic conductors. Cancer Epidemiol. Biomarkers Prev. 22, 102–108.

Jedrychowski, W.A., Perera, F.P., Majewska, R., Mrozek-Budzyn, D., Mroz, E., Roen, E.L., Sowa, A., Jacek, R., 2015. Depressed height gain of children associated with intrauterine exposure to polycyclic aromatic hydrocarbons (PAH) and heavy metals: the cohort prospective study. Environ. Res. 136, 141–147.

Joneidi, Z., Mortazavi, Y., Memari, F., Roointan, A., Chahardouli, B., Rostami, S., 2019. The impact of genetic variation on metabolism of heavy metals: genetic predisposition? Biomed. Pharmacother. 113, 108642.

Kim, K.H., Jahan, S.A., Kabir, E., Brown, R.J., 2013. A review of airborne polycyclic aromatic hydrocarbons (PAHs) and their human health effects. Environ. Int. 60, 71–80.

Kim, K.H., Kabir, E., Kabir, S., 2015. A review on the human health impact of airborne particulate matter. Environ. Int. 74, 136–143.

Kim, M.J., Kim, S., Choi, S., Lee, I., Moon, M.K., Choi, K., Park, Y.J., Cho, Y.H., Kwon, Y. M., Yoo, J., Cheon, G.J., Park, J., 2021. Association of exposure to polycyclic aromatic hydrocarbons and heavy metals with thyroid hormones in general adult population and potential mechanisms. Sci. Total Environ. 762, 144227.

Kolena, B., Petrovicova, I., Sidlovska, M., Hlisnikova, H., Bystricanova, L., Wimmerova, S., Trnovec, T., 2020. Occupational hazards and risks associated with phthalates among slovakian firefighters. Int. J. Environ. Res. Publ. Health 17.

Kolena, B., Petrovicova, I., Sidlovska, M., Pilka, T., Neuschlova, M., Valentova, I., Rybansky, L., Trnovec, T., 2017. Occupational phthalate exposure and health outcomes among hairdressing apprentices. Hum. Exp. Toxicol. 36, 1100–1112.

Kuang, D., Zhang, W., Deng, Q., Zhang, X., Huang, K., Guan, L., Hu, D., Wu, T., Guo, H., 2013. Dose-response relationships of polycyclic aromatic hydrocarbons exposure and oxidative damage to DNA and lipid in coke oven workers. Environ. Sci. Technol. 47, 7446–7456.

Leem, A.Y., Kim, S.K., Chang, J., Kang, Y.A., Kim, Y.S., Park, M.S., Kim, S.Y., Kim, E.Y., Chung, K.S., Jung, J.Y., 2015. Relationship between blood levels of heavy metals and lung function based on the Korean National Health and Nutrition Examination Survey IV-V. Int. J. Chronic Obstr. Pulm. Dis. 10, 1559–1570.

Loredo, J., Ordonez, A., Charlesworth, S., De Miguel, E., 2003. Influence of industry on the geochemical urban environment of Mieres (Spain) and associated health risk. Environ. Geochem. Health 25, 307–323.

Ma, H., Wang, H., Zhang, H., Guo, H., Zhang, W., Hu, F., Yao, Y., Wang, D., Li, C., Wang, J., 2020. Effects of phenanthrene on oxidative stress and inflammation in lung and liver of female rats. Environ. Toxicol. 35, 37–46.

Madrigal, J.M., Persky, V., Pappalardo, A., Argos, M., 2018. Association of heavy metals with measures of pulmonary function in children and youth: results from the National Health and Nutrition Examination Survey (NHANES). Environ. Int. 121, 871–878.

Mannino, D.M., Davis, K.J., 2006. Lung function decline and outcomes in an elderly population. Thorax 61, 472–477.

Mannino, D.M., Holguin, F., Greves, H.M., Savage-Brown, A., Stock, A.L., Jones, R.L., 2004. Urinary cadmium levels predict lower lung function in current and former smokers: data from the Third National Health and Nutrition Examination Survey. Thorax 59, 194–198.

Marshall, A., Altman, D.G., Holder, R.L., Royston, P., 2009. Combining estimates of interest in prognostic modelling studies after multiple imputation: current practice and guidelines. BMC Med. Res. Methodol. 9, 57.

Miller, M.R., Hankinson, J., Brusasco, V., Burgos, F., Casaburi, R., Coates, A., Crapo, R., Enright, P., van der Grinten, C.P., Gustafsson, P., Jensen, R., Johnson, D.C., MacIntyre, N., McKay, R., Navajas, D., Pedersen, O.F., Pellegrino, R., Viegi, G., Wanger, J., Force, A.E.T., 2005. Standardisation of spirometry. Eur. Respir. J. 26, 319–338.

Mu, G., Fan, L., Zhou, Y., Liu, Y., Ma, J., Yang, S., Wang, B., Xiao, L., Ye, Z., Shi, T., Yuan, J., Chen, W., 2019. Personal exposure to PM2.5-bound polycyclic aromatic hydrocarbons and lung function alteration: results of a panel study in China. Sci. Total Environ. 684, 458–465.

Neri, D., Martinez-Steele, E., Khandpur, N., Levy, R., 2022. Associations between ultra-processed foods consumption and indicators of adiposity in US adolescents: cross-

sectional analysis of the 2011-2016 national health and nutrition examination survey. J. Acad. Nutr. Diet.
O'Brien, K.M., Upson, K., Cook, N.R., Weinberg, C.R., 2016. Environmental chemicals in urine and blood: improving methods for creatinine and lipid adjustment. Environ. Health Perspect. 124, 220–227.
Oliveira, M., Slezakova, K., Delerue-Matos, C., Pereira, M.C., Morais, S., 2019. Children environmental exposure to particulate matter and polycyclic aromatic hydrocarbons and biomonitoring in school environments: a review on indoor and outdoor exposure levels, major sources and health impacts. Environ. Int. 124, 180–204.
Olszowski, T., Baranowska-Bosiacka, I., Rebacz-Maron, E., Gutowska, I., Jamiol, D., Prokopowicz, A., Goschorska, M., Chlubek, D., 2016. Cadmium concentration in mother's blood, milk, and newborn's blood and its correlation with fatty acids, anthropometric characteristics, and mother's smoking status. Biol. Trace Elem. Res. 174, 8–20.
Padula, A.M., Balmes, J.R., Eisen, E.A., Mann, J., Noth, E.M., Lurmann, F.W., Pratt, B., Tager, I.B., Nadeau, K., Hammond, S.K., 2015. Ambient polycyclic aromatic hydrocarbons and pulmonary function in children. J. Expo. Sci. Environ. Epidemiol. 25, 295–302.
Rokadia, H.K., Agarwal, S., 2013. Serum heavy metals and obstructive lung disease: results from the National Health and Nutrition Examination Survey. Chest 143, 388–397.
Ruchirawat, M., Settachan, D., Navasumrit, P., Tuntawiroon, J., Autrup, H., 2007. Assessment of potential cancer risk in children exposed to urban air pollution in Bangkok, Thailand. Toxicol. Lett. 168, 200–209.
Saeedi, M., Li, L.Y., Salmanzadeh, M., 2012. Heavy metals and polycyclic aromatic hydrocarbons: pollution and ecological risk assessment in street dust of Tehran. J. Hazard Mater. 227–228, 9–17.
Salvi, S., 2007. Health effects of ambient air pollution in children. Paediatr. Respir. Rev. 8, 275–280.
Schunemann, H.J., Dorn, J., Grant, B.J., Winkelstein Jr., W., Trevisan, M., 2000. Pulmonary function is a long-term predictor of mortality in the general population: 29-year follow-up of the Buffalo Health Study. Chest 118, 656–664.
Shen, M., Xing, J., Ji, Q., Li, Z., Wang, Y., Zhao, H., Wang, Q., Wang, T., Yu, L., Zhang, X., Sun, Y., Zhang, Z., Niu, Y., Wang, H., Chen, W., Dai, Y., Su, W., Duan, H., 2018. Declining pulmonary function in populations with long-term exposure to polycyclic aromatic hydrocarbons-enriched PM2.5. Environ. Sci. Technol. 52, 6610–6616.
Shi, Y., Gaurab, P., Wang, W., Yan, J., Sun, Z., Zhang, Y., Liu, J., Liu, X., Zheng, D., 2020. Interaction effect of polycyclic aromatic hydrocarbon metabolites and cadmium on semen quality in nonsmokers. Environ. Sci. Pollut. Res. Int. 27, 33990–33997.
Sin, D.D., Wu, L., Man, S.F., 2005. The relationship between reduced lung function and cardiovascular mortality: a population-based study and a systematic review of the literature. Chest 127, 1952–1959.
Siroux, V., Agier, L., Slama, R., 2016. The exposome concept: a challenge and a potential driver for environmental health research. Eur. Respir. Rev. 25, 124–129.
Sun, H., Hou, J., Zhou, Y., Yang, Y., Cheng, J., Xu, T., Xiao, L., Chen, W., Yuan, J., 2017. Dose-response relationship between urinary polycyclic aromatic hydrocarbons metabolites and urinary 8-hydroxy-2'-deoxyguanosine in a Chinese general population. Chemosphere 174, 506–514.
Valavanidis, A., Fiotakis, K., Vlachogianni, T., 2008. Airborne particulate matter and human health: toxicological assessment and importance of size and composition of particles for oxidative damage and carcinogenic mechanisms. J. Environ. Sci. Health C Environ. Carcinog. Ecotoxicol. Rev. 26, 339–362.
Valko, M., Morris, H., Cronin, M.T., 2005. Metals, toxicity and oxidative stress. Curr. Med. Chem. 12, 1161–1208.
Verougstraete, V., Mallants, A., Buchet, J.P., Swennen, B., Lison, D., 2004. Lung function changes in workers exposed to cobalt compounds: a 13-year follow-up. Am. J. Respir. Crit. Care Med. 170, 162–166.
Wu, X., Yang, S., Bai, Y., Li, G., Wei, W., He, M., Zhang, X., Wu, T., Chen, W., Guo, H., 2020. Interaction of RARB variant with polycyclic aromatic hydrocarbon exposure on annual lung function change. Am. J. Respir. Crit. Care Med. 201, 998–1002.
Xu, C., Liu, Q., Liang, J., Weng, Z., Xu, J., Jiang, Z., Gu, A., 2021a. Urinary biomarkers of polycyclic aromatic hydrocarbons and their associations with liver function in adolescents. Environ. Pollut. 278, 116842.
Xu, C., Xu, J., Zhang, X., Xu, S., Liu, Q., Weng, Z., Gu, A., 2021b. Serum nickel is associated with craniosynostosis risk: evidence from humans and mice. Environ. Int. 146, 106289.
Yang, G., Sun, T., Han, Y.Y., Rosser, F., Forno, E., Chen, W., Celedon, J.C., 2019. Serum cadmium and lead, current Wheeze, and lung function in a nationwide study of adults in the United States. J. Allergy Clin. Immunol. Pract. 7, 2653–2660 e3.
Yu, Y., Wang, X., Wang, B., Tao, S., Liu, W., Wang, X., Cao, J., Li, B., Lu, X., Wong, M.H., 2011. Polycyclic aromatic hydrocarbon residues in human milk, placenta, and umbilical cord blood in Beijing, China. Environ. Sci. Technol. 45, 10235–10242.
Zeng, X., Xu, X., Boezen, H.M., Vonk, J.M., Wu, W., Huo, X., 2017. Decreased lung function with mediation of blood parameters linked to e-waste lead and cadmium exposure in preschool children. Environ. Pollut. 230, 838–848.
Zheng, X., Huo, X., Zhang, Y., Wang, Q., Zhang, Y., Xu, X., 2019. Cardiovascular endothelial inflammation by chronic coexposure to lead (Pb) and polycyclic aromatic hydrocarbons from preschool children in an e-waste recycling area. Environ. Pollut. 246, 587–596.
Zhou, F., Yin, G., Gao, Y., Liu, D., Xie, J., Ouyang, L., Fan, Y., Yu, H., Zha, Z., Wang, K., Shao, L., Feng, C., Fan, G., 2019. Toxicity assessment due to prenatal and lactational exposure to lead, cadmium and mercury mixtures. Environ. Int. 133, 105192.
Zhou, Y., Sun, H., Xie, J., Song, Y., Liu, Y., Huang, X., Zhou, T., Rong, Y., Wu, T., Yuan, J., Chen, W., 2016. Urinary polycyclic aromatic hydrocarbon metabolites and altered lung function in Wuhan, China. Am. J. Respir. Crit. Care Med. 193, 835–846.

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Maternal and child biomonitoring strategies and levels of exposure in western Canada during the past seventeen years: The Alberta Biomonitoring Program: 2005–2021

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A R T I C L E I N F O

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A B S T R A C T

The Alberta Biomonitoring Program (ABP) was created in 2005 with the initial goal of establishing baseline levels of exposure to environmental chemicals in specific populations in the province of Alberta, Canada, and was later expanded to include multiple phases. The first two phases focused on evaluating exposure in pregnant women (Phase One, 2005) and children (Phase Two, 2004–2006) by analyzing residual serum specimens. Phase Three (2013–2016) employed active recruitment techniques to evaluate environmental exposures using a revised list of chemicals in paired serum pools from pregnant women and umbilical cord blood. These three phases of the program monitored a total of 226 chemicals in 285 pooled serum samples representing 31,529 individuals. Phase Four (2017–2020) of the ABP has taken a more targeted approach, focusing on the impact of the federal legalization of cannabis on the exposure of pregnant women in Alberta to cannabis, as well as tobacco and alcohol using residual prenatal screening serum specimens. Chemicals monitored in the first three phases include herbicides, neutral pesticides, metals, metalloids, and micronutrients, methylmercury, organochlorine pesticides, organophosphate pesticides, parabens, phthalate metabolites, perfluoroalkyl substances (PFAS), phenols, phytoestrogens, polybrominated compounds, polychlorinated biphenyls (PCBs), dioxins and furans, polycyclic aromatic hydrocarbons (PAHs), and tobacco biomarkers. Phase Four monitored six biomarkers of tobacco, alcohol, and cannabis. All serum samples were pooled. Mean concentrations and 95% confidence intervals (CIs) were calculated for the chemicals detected in ≥25% of the sample pools. cross the first three phases, the data from the ABP has provided baseline exposure levels for the chemicals in pregnant women, children, and newborns across the province. Comparison within and among the phases has highlighted differences in exposure levels with age,

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geography, seasonality, sample type, and time. The strategies employed throughout the program phases have been demonstrated to provide effective models for population biomonitoring.

Abbreviations

ABP	Alberta Biomonitoring Program
PFAS	perfluoroalkyl substances
CDC	Centers for Disease Control and Prevention
PBDE	polybrominated diphenyl ether
NHANES	National Health and Nutrition Examination Survey
CHMS	Canadian Health Measures Survey
POPs	persistent organic pollutants
AEPHIN	Alberta Environmental Public Health Information Network
ProvLab	Alberta Precision Laboratory – Public Health Laboratory
PAH	polycyclic aromatic hydrocarbon
ACFT	Alberta Centre for Toxicology
BPA	bisphenol A
LC	liquid chromatography
MSMS	tandem mass spectrometry
LLE	liquid-liquid extraction
GC	gas chromatography
ICP	inductively coupled plasma
HR	high resolution
EPA	Environmental Protection Agency
PCB	polychlorinated biphenyl
QC	quality control
LOD	limit of detection
CI	confidence interval
BE	biomonitoring equivalent
HBM	human biomonitoring
MIREC	Maternal-Infant Research on Environmental Chemicals

1. Introduction

Natural and anthropogenic chemicals of toxicological concern are ubiquitous in the environment. Humans are routinely exposed to such substances through various routes including inhalation, ingestion, or dermal absorption. The amount of a chemical absorbed by the body – the internal dose – is difficult to predict by modelling as there are multiple pathways of exposure and humans vary greatly in physiology, behaviour, and environment. The most accurate way of determining the internal dose is by measuring the chemical or a related biomarker in relevant biological samples such as blood, urine, nails or hair (Sexton et al., 2004). Biomonitoring has become an essential tool for identifying and assessing environmental exposures (Joas et al., 2012). The National Health and Nutrition Examination Survey (NHANES) in the United States (US) is one of the largest, longest-running surveys and the data is used extensively in studying environmental chemical exposure in the US (CDC, 2021). This program is run by the Centers for Disease Control and Prevention (CDC) and provides nationally representative cumulative exposure data that can be used to track exposures over time and monitor levels above known toxicity values. In addition to the US, several other countries have national (or international in the case of the European Biomonitoring Initiative (HBM4EU)) biomonitoring programs, utilizing the data for determining the effectiveness of public health policies; health risk assessments; and developing new health protection, promotion, and disease prevention programs (Gilles et al., 2021; Health Canada, 2019a; Schulz et al., 2007; Schoeters et al., 2017; Fillol et al., 2021; Perez-Gomez et al., 2013; Bocca et al., 2010; Černá et al., 2017; Lee et al., 2012). These national-level programs provide important data; however, little information is provided at regional or local levels or on sources of exposure (Fox and Latshaw, 2013). Health Canada's Canadian Health Measures Survey (CHMS) began collecting data in 2007 and samples at fifteen to sixteen sites across the country in each of their cycles with only two sites in the prairie region (Health Canada, 2010, 2013, 2015, 2017, 2019a), which would not provide a full picture of the variability throughout the province of Alberta. The Maternal-Infant Research on Environmental Chemicals (MIREC) study, which also began in 2007, examines the effects of environmental exposures on the health of pregnant women and their infants (MIREC, 2022). MIREC is a national-level cohort with samples collected from ten cities across Canada. The data cannot be easily extrapolated by region. Regional or local exposure data can be used to prioritize actions to target specific chemicals at a provincial, state, or local level and to further understand the variability across age, geography, and within specific populations (Fox and Latshaw, 2013; Valcke et al., 2020). However, regional programs suffer unique challenges compared to larger programs, such as inconsistent funding and fewer resources (Fox and Latshaw, 2013). Since 2009, the CDC has funded biomonitoring initiatives in individual states with the goal of increasing "states' capability and capacity to conduct targeted and population-based biomonitoring to assess human exposure to environmental chemicals in their communities" (CDC, 2019a). Health Canada has also begun combining the results from multiple cycles of the CHMS to examine exposure data at the provincial and regional levels (Valcke et al., 2020).

Alberta is a province in western Canada with a population of 4.4 million people (Government of Alberta, 2021) (Fig. 1). There is a significant oil and gas industry as well as agricultural, mining, and forestry activities in the province, which may result in characteristic chemical exposures in the population when compared to other parts of the country. The first provincial biomonitoring program in Canada was established in Alberta in 2005. The objective was to assess the regional, age-specific and temporal distribution of relevant environmental exposures in the province in a targeted and focussed manner (Gabos et al., 2008), and with a budget of only $1 million (CAD). The Alberta Biomonitoring Program (ABP) has focused on populations that traditionally may be more susceptible to environmental exposures, including pregnant women, children, and newborns. Maternal body burdens of some chemicals have been shown to have or be associated with deleterious effects on the mother and fetus including negative associations with neurodevelopment, fetal growth, behaviour, and obesity; low birth weight; maternal hypertension; and increased incidence of metabolic and cardiovascular disease in adulthood (Gibson et al., 2018; Govarts et al., 2012; Bergen, 2006; Nghiem et al., 2019; Forns et al., 2016; Waterfield et al., 2020; Berger et al., 2021; Bellinger, 2005). Most known environmental contaminants can cross the placenta to some extent (Aylward et al., 2014) and the disposition and effects of emerging chemicals of concern take many years to be defined. Children also cannot be considered to be "little adults" as they differ from adults not only in size, but also in physiology, behaviour and diet (Garbino--Pronczuk, 2005). Therefore, biomonitoring data from adults cannot be easily extrapolated to children.

Phase One of the ABP focused on exposures in pregnant women, including smoking biomarkers, organic contaminants, and trace metals, metalloids, and micronutrients in serum, while Phase Two examined similar exposures in children's serum. Phase Three expanded the suite of tested chemicals to include parabens and phthalate metabolites and

evaluated environmental exposures in maternal-newborn paired serum samples. Phase Four is a targeted study looking at the effect of the federal legalization of cannabis on exposure to cannabis, as well as alcohol and tobacco in pregnant women. All four phases of the ABP have used pooled samples. Pooling is a strategy that imparts multiple benefits to biomonitoring studies, including less blood required per study participant, higher detection rates, a simpler ethics approval process, the possibility of utilizing banked samples instead of active participant recruitment, and significant cost savings (Heffernan et al., 2013).

In addition to providing baseline levels of exposure to environmental chemicals in susceptible populations of Alberta and highlighting trends in exposure within the province by age, geography, and seasonality, the ABP has also provided a template for the development of a biomonitoring program in Saskatchewan. In 2011, the Alberta and Saskatchewan governments collaborated to develop a biomonitoring study for the province of Saskatchewan, based on a similar format as Phase One of the ABP (Government of Saskatchewan, 2019a). This study examined the same environmental chemicals analyzed in Phase Three of the ABP and provided baseline levels for northern Saskatchewan to compare within province and to other regions. Data from the ABP was used by Health Canada in risk assessments of boric acid (Health Canada, 2016) and zinc (Health Canada, 2019b) under their Chemical Management Plan.

The objectives of this paper are to review the scope and strategies used in the ABP for biomonitoring of pregnant women and children over the past seventeen years, to highlight findings and trends from the first three phases, and to provide information for other smaller jurisdictions to support the design of biomonitoring studies. The sample collection and recruitment methods used within each phase are presented as are the summary results and trends from Phases One, Two, and Three. A description of Phase Four, the current phase being carried out, is also provided.

2. Methods

2.1. Study population

Table 1 summarizes the study population for each phase of the ABP.

2.1.1. Phase One

Pregnant women aged 18 years or older who were receiving prenatal care in the province of Alberta at the time of sample collection and had a blood sample drawn in 2005 and sent to Alberta Precision Laboratory – Public Health Laboratory (previously known as Provincial Public Health Laboratory – Microbiology) (ProvLab) in Edmonton for routine prenatal screening were eligible for Phase One of the ABP.

2.1.2. Phase Two

Children aged two to thirteen years who resided in the southern region of Alberta and had a blood sample drawn prior to or post-elective surgery between 2004 and 2006 were eligible for Phase Two of the ABP.

2.1.3. Phase Three

Pregnant women aged 18 years or older who met the following criteria in the time span of 2013–2016 were eligible for Phase Three of the ABP:

Fig. 1. Map of Canada and the province of Alberta displaying the recruitment areas in Phases One, Two, Three and Four of the Alberta Biomonitoring Program. R1 through R9 represent the health regions in place in Alberta during the time of sample collection for Phases One and Two (2004–2006). R1: Chinook Regional Health Authority; R2: Palliser Health Region; R3: Calgary Health Region; R4: David Thompson Regional Health Authority; R5: East Central Health; R6: Capital Health; R7: Aspen Regional Health Authority; R8: Peace Country Health; R9: Northern Lights Health Region. Phase One Recruitment Area: Northern Alberta (R7, R8, R9); Central Alberta (R4, R5, R6); Southern Alberta (R1, R2, R3). Phase Two Recruitment Area: Southern Alberta (R1, R2, R3). Phase Three and Four Recruitment Area: Fort McMurray; Grande Prairie; Edmonton; Red Deer; Calgary; Lethbridge; Medicine Hat. (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

Table 1
Characteristics of the study populations in each phase of the Alberta Biomonitoring Program.

Characteristic	Phase One (2005)	Phase Two (2004–2006)	Phase Three (2013–2016)	Phase Four (2017–2020)
Population	Women in early pregnancy[a]	Children	Pregnant women (majority in third trimester) and newborns	Women in early pregnancy[a]
Age Stratification (years)	<25, 26–30, 31+	2–5, 6–13	18–25, 26–30, 31+	18–25, 26–30, 31+
Geographic Stratification	Provincial: northern, central, and southern Alberta	Southern Alberta	City of Calgary, Edmonton, Fort McMurray, Grande Prairie, Red Deer, Lethbridge, Medicine Hat	City of Calgary, Edmonton, Fort McMurray, Grande Prairie, Red Deer, Lethbridge, Medicine Hat
Total Pool Number	151	6	64 maternal/newborn pairs	622 (288 pre- and 312 post-legalization)
Total Sample Number	28,484	1,373	1,672 (836 maternal, 836 newborn cord)	7,200 (3,456 pre and 3,744 post-legalization)
Sample Collection	Leftover sample sequestration	Leftover sample sequestration	Prospective participant recruitment and collection	Leftover sample sequestration

[a] In Alberta, routine prenatal screening is recommended during the first trimester of pregnancy [37].

- delivering a newborn at a hospital with which there was an agreement to collect paired maternal and cord blood samples for the biomonitoring study;
- had lived in their current town/city for at least eight months of the pregnancy;
- had a singleton birth;
- were not banking or donating cord blood;
- were not currently a smoker or using products containing nicotine (self-reported).

2.1.4. Phase Four

Pregnant women aged 18 years or older who were residents of Calgary, Edmonton, Fort McMurray, Grande Prairie, Red Deer, Medicine Hat or Lethbridge, Alberta at the time of sample collection and had a blood sample drawn between October 17, 2017, and October 17, 2019, and sent to ProvLab for prenatal screening were eligible for Phase Four of the ABP. The post-legalization sampling date was extended to July 15, 2020, to ensure adequate samples were included from each of the seven regions.

2.2. Sample sequestration/participant recruitment and sample collection

2.2.1. Phase One

This phase of the ABP used the to-be-discarded serum samples leftover from routine prenatal communicable disease screening; therefore, no individual recruitment was performed. Aliquots of serum samples from 28,484 pregnant women from across Alberta were taken anonymously and randomly from a population of 44,584 eligible serum samples that were submitted to ProvLab between January 1st, 2005, and December 31st, 2005. These samples were obtained from pregnant women who resided in Alberta and sought prenatal care. All prenatal screening samples that included information on maternal age, geographic region, and had sufficient volume following routine screening were retained at ProvLab for this study. The following groups of samples were excluded:

- samples collected outside the province of Alberta;
- samples from pregnant women who tested positive for antibodies to HIV and/or syphilis serology and/or Hepatitis B surface antigen;
- duplicate samples from the same individual;
- samples collected from pregnant women with address information for one region of Alberta that was different from the region of the care provider who ordered the prenatal screening;
- samples with unknown maternal age.

All samples were frozen at −20 °C before selection for this study. These samples were then pooled as described in Section 2.4.1.

2.2.2. Phase Two

This phase of the ABP used archived serum samples from the Alberta Children's Hospital; therefore, no individual recruitment was carried out. These samples were obtained from children living in southern Alberta, aged 2–13 years, and presenting for elective surgeries at the Alberta Children's Hospital between 2004 and 2006. Only children with the American Society of Anesthetists (ASA) classification of I (normal, healthy patient) were eligible for the study. Samples from children with the following were excluded:

- chronic illnesses;
- autoimmune diseases;
- asthma;
- recurrent fractures;
- those undergoing diagnostic biopsies, various forms of endoscopy, or lipid modifying treatments;
- any child assigned an American Society of Anesthesiologists (ASA) score of II or more.

All samples were frozen in Nalgene cryogenic vials at −80 °C before selection for this study. 1,373 out of 1,845 eligible samples met the criteria and were used in the study. Samples were pooled as described in Section 2.4.2.

2.2.3. Phase Three

From 2013 until 2016, collection of serum samples took place at one of the obstetric units in seven cities in Alberta: Fort McMurray, Grande Prairie, Edmonton, Red Deer, Calgary, Medicine Hat, and Lethbridge. These were the seven largest population centers in Alberta in 2011. Study staff recruited potential participants at maternity clinics, physician offices, the assessment room on the labour and delivery unit, or over the phone to determine if the pregnant women were interested in and eligible for participation. If interested, the recruiter determined prior to labour and delivery if the woman met the eligibility criteria listed in Section 2.1. If eligible, the woman was informed with respect to the study and asked to read and sign a consent form and fill out a questionnaire (Supplementary Material S01 and S02) so that her serum sample and the cord blood serum sample could be pooled by age and geographic region. The participant was assigned a sample kit (Fig. S03) containing all the collection supplies required to draw the maternal and cord blood samples at delivery. Blood samples were collected using both glass and plastic vacutainer tubes without any additive or serum separator gel as some chemical classes could have been affected by the tube material (e.g., phthalate background from plastic tubes, metals leaching from glass tubes). The blood samples were spun down to obtain the serum. Pools prepared from serum collected in plastic tubes were analyzed for those chemical classes considered to not be affected by any constituent in plastic tubes or storage containers, but could be affected by the use of glass tubes or storage containers (i.e., metals, metalloids,

and micronutrients (Rodushkin et al., 2000) and PFAS (EPA, 2008)).

2.2.4. Phase Four

This phase of the ABP used serum leftover from routine prenatal screening; therefore, no individual recruitment was performed. Samples that were collected prior to federal legalization of cannabis (sample collection date range: November 6, 2017–October 16, 2018) and post cannabis legalization in Canada (sample collection date range: April 2, 2019–July 15, 2020) and received at ProvLab, having sufficient volume as well as age, geography, and collection date information were eligible for inclusion in this phase of the program. The following groups of samples were excluded: duplicates, low volume samples, and samples outside of the required age range or geographical area. A total of 3,456 samples collected pre-legalization and 3,744 samples collected post-legalization were placed into pools. The samples were pooled as described in Section 2.4.4.

2.3. Selection of environmental chemicals for monitoring

The list of chemical classes monitored in each phase of the ABP is provided in Table 2. A detailed list of each chemical and congener analyzed in Phases One, Two, and Three is provided in the Results section (Tables 6a-l) and Supplementary Material (Table S10a-l). The list of chemicals to be analyzed in Phase Four is provided in the Supplementary Material (Table S04).

2.3.1. Phase One

The targeted chemicals monitored in this phase were selected using expert advice on contaminants of concern in Alberta and by reviewing similar biomonitoring studies in other jurisdictions. Also considered were whether health effects were associated with exposure to these chemicals as well as the availability and costs of suitable analytical laboratory tests. Some of the chemical classes were only monitored in a subset of the population (southern Alberta pregnant women, aged 26–30 years) because it was uncertain if the study design would be sensitive enough to detect these in serum as they would typically be analyzed in urine (Table 2).

Table 2
Chemical classes monitored in each phase of the Alberta Biomonitoring Program.

	Phase One (2005)	Phase Two (2006)	Phase Three (2013–2016)	Phase Four (2017–2020)
Alcohol Biomarkers				✓
Cannabis Biomarkers				✓
Herbicides and Neutral Pesticides	✓	✓		
Metals, Metalloids and Micronutrients	✓	✓	✓	
Methylmercury	✓	✓	✓	
Organochlorine Pesticides	✓	✓	✓	
Organophosphate Pesticides	✓			
Parabens			✓	
Perfluoroalkyl Substances	✓	✓	✓	
Phenols	✓	✓	✓	
Phthalate Metabolites			✓	
Phytoestrogens	✓	✓	✓	
Polybrominated Compounds	✓	✓	✓	
Polychlorinated Biphenyls	✓	✓	✓	
Polychlorinated dibenzo-p-dioxins and polychlorinated dibenzofurans	✓	✓	✓	
Polycyclic Aromatic Hydrocarbons	✓			
Tobacco Biomarkers	✓	✓	✓	✓

2.3.2. Phase Two

The chemicals monitored in this phase were the same as those included in Phase One, except for organophosphate pesticides and PAHs, which were excluded in this phase because they were not detected above the limits of detection in any pooled sera in Phase One.

2.3.3. Phase Three

The chemicals monitored in this study were selected by reviewing data from the previous Alberta reports, large systematic biomonitoring studies such as Health Canada's CHMS and the CDC's National Biomonitoring Program in the United States, as well as through expert guidance. The targeted chemicals analyzed in this study are similar to those analyzed in Phase One with the exclusion of the organophosphate pesticides, PAHs, and herbicides and neutral pesticides as chemicals in these classes were not detected in more than 25% of the pooled samples in Phase One. Two new chemical classes, phthalate metabolites and parabens, were added to Phase Three based on their inclusion in other large biomonitoring studies and they were considered emerging chemicals of concern at that time.

2.3.4. Phase Four

The fourth phase of the ABP was designed to examine the effect of the legalization of cannabis by the federal government of Canada on the exposure of pregnant women in Alberta to cannabis as well as to tobacco and alcohol. In pregnant women using substances, it has been found that cannabis is often used in conjunction with alcohol and/or tobacco (Forray et al., 2015). Therefore, the chemicals monitored in this phase include serum biomarkers of tobacco, alcohol, and cannabis.

2.4. Sample size and pooling strategy

2.4.1. Phase One

Monte Carlo simulations were performed to determine the optimal number of pooled samples required within each age group and geographic region combination to have power to detect statistically significant differences across the strata. Fifteen pooled samples within each age/geographic stratum were determined to be necessary before decreasing gains would have occurred; however, by assuming that the distributional form was constant across all groups, the minimum number of pools per stratification could be reduced (Table 3). The final number of pools used ultimately depended on the number of available individual samples stored at ProvLab that met the inclusion criteria (44,584 samples). Within each geographic region and age group, 150–200 individual samples were pooled with a minimum of eight replicate pools for each age/geographic region combination (Table 3). 1 mL of serum from each sample was used to create the pools. The nine Health Regions of Alberta in 2005 (Fig. 1) were stratified into three general geographic regions representing northern, central, and southern Alberta. Three age classes were further defined within each geographic region, representing <25, 26–30, and 31+ years of age. In southern Alberta only, samples were also pooled by month of collection to investigate seasonal trends. Limited resources prevented investigations of seasonality in all geographic areas.

2.4.2. Phase Two

The number of children's samples available for Phase Two of the program was based on the number of eligible samples collected during elective surgeries in southern Alberta. As all samples came from children residing in one region, the samples were pooled according to two age categories (≤5 and 6–13 years) to investigate the effect of age upon exposure to the suite of environmental chemicals with the number of samples in each pool ranging from 196 to 240 (Table 4).

Table 3
Phase one pool numbers, pool volume, and samples per pool by age and geographic region.

Age Group (years)	Northern Alberta			Central Alberta			Southern Alberta		
	Number of Pools	Average Number of Samples per Pool	Pool Volume (mL)	Number of Pools	Average Number of Samples per Pool	Pool Volume (mL)	Number of Pools	Average Number of Samples per Pool	Pool Volume (mL)
<25	15	170	170	15	200	200	24	200	200
26–30	11	150	150	15	200	200	24	200	200
31+	8	150	150	15	200	200	24	200	200

Table 4
Phase two pool volumes and samples per pool by age group.

Pool Number	Age Group (years)	Number of Samples in Pool	Pool Volume (mL)
1	≤5	240	96.0
2	≤5	196	87.4
3	≤5	239	95.6
4	6–13	240	96.0
5	6–13	218	87.2
6	6–13	240	96.0

2.4.3. Phase Three

The original study design was to collect 180 maternal/cord blood sample pairs from each of the seven most populated regions in Alberta, including the city of Fort McMurray, Grande Prairie, Edmonton, Red Deer, Calgary, Medicine Hat, and Lethbridge. Sixty pairs were to be collected in each of three age groups (18–25, 26–30, and 31+ years). However, 180 sample pairs could not be collected at all sites due to some samples with low volume, difficulties with cord blood collection, and missed maternal and/or cord blood collections. Therefore, the number of samples per site was adjusted based on birth rates by geographic region and age category. Table 5 lists the number of pools and samples per pool by age group and geographic region in Phase Three. As there was only one pool per age group from Medicine Hat and from the 18–25-year-old group in Red Deer, no variance could be calculated for these stratifications. Pools were created separately using the serum collected in both the glass and plastic vacutainer tubes included in the sample collection kit. The pooling approach is outlined in Fig. S05.

2.4.4 Phase Four: To provide sufficient statistical power to determine differences in biomarker concentrations pre- and post-legalization of cannabis in Canada, 3,456 prenatal screening samples collected before and 3,744 samples collected after October 17, 2018, were sequestered and 12 samples were placed into each pool at ProvLab. The samples were obtained from Fort McMurray, Grande Prairie, Edmonton, Red Deer, Calgary, Medicine Hat, and Lethbridge across three age groups (18–25, 26–30, and 31+ years). The samples were pooled across the strata outlined in Fig. S06.

Table 5
Phase three pool numbers (number of samples per pool) by age and geographic region.

Region	Age 18–25 years	Age 26–30 years	Age 31+ years
Calgary	3 pairs[a] (12,12,20)	4 pairs (12,12,12,21)	5 pairs (12,12,12,12,20)
Edmonton	3 pairs (12,12,12)	4 pairs (12,12,12,20)	5 pairs (12,12,12,12,15)
Grande Prairie	2 pairs (12,20)	2 pairs (12,20)	2 pairs (12,20)
Medicine Hat	1 pair (12)	1 pair (12)	1 pair (12)
Red Deer	1 pair (12)	2 pairs (12,12)	2 pairs (12,12)
Fort McMurray	5 pairs (12,12,12,12,12)	5 pairs (12,12,12,12,12)	5 pairs (12,12,12,12,12)
Lethbridge	2 pairs (12,20)	4 pairs (12,12,12,12)	5 pairs (12,12,12,12,12)

[a] A pair is one maternal and one cord blood serum pool created from maternal and cord blood serum pairs of individual samples.

2.5. Ethical considerations

2.5.1. Phase One, Two, and Four

Ethics approval for Phase One of the ABP was obtained from the University of Alberta Research Ethics Office and the University of Calgary Conjoint Health Research Ethics Board (CHREB). An amendment to the original approvals for Phase One was obtained for the sequestration of the children's serum samples in Phase Two. Ethics approval for Phase Four was obtained from the University of Calgary's CHREB. For the prenatal samples in Phases One, Two, and Four, a waiver of consent was granted by CHREB (Phases One, Two, and Four) and the University of Alberta Research Ethics Office (Phases One and Two), consistent with the conditions under the Tri-Council Policy on Ethical Conduct for Research Involving Humans in place at the time of submission. These studies were considered to be surveillance and the residual samples were deemed medical waste that would otherwise have been discarded. As all samples were pooled (i.e., no individual samples were analyzed) no individual data could be retrieved from the results. Pooling of the samples was performed at ProvLab. Only the professional personnel of ProvLab who had handled the collection and testing of the samples had access to the demographic information of the individual samples to remove samples according to described criteria before random selection of samples by the various strata for pooling. The randomization process before pooling rendered anonymous participation as pooling prevented linkage to an individual.

2.5.2. Phase Three

Ethics approval for this study was granted by the University of Calgary CHREB and the University of Alberta Research Ethics Board. Informed consent was obtained from each participant. Participation was voluntary and participants were able to opt out at any time. Participant samples were assigned a code and the master code list was stored in a secure location at Alberta Centre for Toxicology (ACFT). When samples were sent to ProvLab for pooling, the only information provided to the professional personnel at ProvLab was the geographic region and age category for each sample. Samples were pooled according to geographic region and age category and each pool was assigned a new code. As samples in all phases were pooled, it is not possible to relate analysis results directly back to an individual participant.

2.6. Laboratory analytical methods

During the first three phases of the ABP, laboratory analyses were carried out at ACFT in Calgary, Alberta, and in private laboratories selected through competitive bid process [ALS Laboratory Group labs, Canada (Edmonton, Burlington, Waterloo), Sweden (ALS Scandinavia AB), and Prague (ALS Czech Republic)]. Phase Four analysis will be performed at ACFT. The laboratories were chosen based on quoted costs, demonstrated expertise in the analytical methods, and availability of modern instrumentation. Summaries of the analytical methods used in the environmental chemical analyses in Phases One and Two are provided in the first and second Alberta Health biomonitoring reports (Gabos et al., 2008, 2010). Summary method information for Phases

Three and Four is provided in the Supplementary Material (S07) with the laboratory that performed each analysis listed in parentheses.

2.7. Quality assurance

2.7.1. Phase One and Two

Seven control aliquots of bovine serum were prepared in Phase One: one aliquot was a baseline control (no manipulation), one aliquot replicated the automated handling of the real serum specimens prior to storage and use in the study, and five aliquots were manually aliquoted in the same manner as the real samples that were pooled. In Phase Two both bovine and water control aliquots were prepared to monitor potential contamination from the pipetting and pooling process, tubing, and vacutainer used for blood collection. Phase One used serum specimens originally collected in serum separator tubes (SSTs) while blood was collected in red top glass tubes with no additive in Phase Two. Neither of these tubes are the recommended collection tube for trace metals analysis; therefore, results that may have been affected are noted in Table 6a-l. Sample identification codes were assigned to each pool of samples and controls before testing so the subsequent chemical analyses were performed blind. Within each analysis batch, several quality control (QC) measures were implemented including method blanks, serum blanks, duplicates, QC samples spiked at multiple concentrations, and analysis of certified reference materials where available. ALS Laboratory Groups (Edmonton, Burlington, Prague, and Sweden) included standard reference materials in the organochlorine pesticide, PCBs, PFAS, PAH, PBDE, and methylmercury analysis. ACFT included certified materials in the metals, metalloids, and micronutrients analyses. No commercially available reference materials were included in the herbicides and neutral pesticides, organophosphate pesticides, phenols, phytoestrogens, or nicotine and cotinine analyses as none were readily available in serum to our knowledge. ALS Laboratories global quality management system met all requirements of International Standards ISO/IEC 17025:2017 and ISO 9001:2015. ALS Laboratory Group included quality control steps through sample preparation and analysis, inter-laboratory test programs, and regular internal audits. ACFT participated in the German External Quality Assessment Scheme for the metals, metalloids, and micronutrients analysis during these phases. Quality assurance reviews of the data were conducted to identify any disparities. Further details on the quality control measures for the analytical methods are provided in the Supplementary Material (S08).

2.7.2. Phase Three

All sample collection and storage materials were tested for background concentrations of a selected list of chemical classes analyzed prior to their use in the study. These classes were chosen based on the likelihood of their presence in the materials and included dioxins and furans, PBDEs, phthalates, PFAS, parabens, BPA, phenols, metals, metalloids, and micronutrients, and cotinine. Contributions of these chemicals from the collection and storage materials were considered negligible except where specifically acknowledged in Table 6a-l. QC pools were prepared at ACFT from volunteer serum using the same procedures and materials as those used to prepare the real sample pools. These QC pools were then spiked with representative compounds from the analysis suite. At least one QC pool was included with each batch. Each analysis batch included several QC measures such as method blanks, serum blanks, duplicates, QC samples spiked at multiple concentrations, and analysis of certified reference materials. ALS Laboratory Group Waterloo was accredited by the Canadian Association for Laboratory Accreditation (CALA) for the PFAS analysis. ALS Laboratory Group Burlington included standard reference materials in the PCBs, PBDEs, organochlorine pesticides, and dioxins and furans analyses. ACFT participated in the German External Quality Assessment Scheme for the metals, metalloids, and micronutrients analysis during this phase. Quality assurance reviews of the data were conducted to identify any disparities. Further details on quality control measures for the analytical methods (where available) are provided in the Supplementary Material (S07).

2.7.3. Phase Four

QC samples were prepared at two levels in blank bovine serum for the cannabis and tobacco biomarker analytical methods and in blank human serum for the analytical method to measure alcohol biomarkers. A set of QC samples was run at the beginning and end of each batch as well as after every twenty samples. In addition to these QC samples, each analysis batch also included several other QC measures such as method blanks, serum blanks, duplicates, and spiked samples. Further details on quality control measures for the analytical methods are provided in the Supplementary Material (S07).

2.8. Statistical analyses

2.8.1. Phase One and Two

The estimated mean concentrations were analyzed by regression methods in which age and geographic region (Phase One only) were considered as independent variables. Due to the pooled nature of the samples, arithmetic means were calculated (Heffernan et al., 2013). Since the pools were constructed with differing numbers of samples per pool, the data was differentially weighted for more accurate regression analysis according to the number of individual samples comprising the pool. These weights were verified to be accurate using Monte Carlo simulation. Weighted regression analyses were conducted using the Statistical Package for Social Science (SPSS) (Phase One: version 15; Phase Two: version 17). 95% confidence intervals (CIs) were derived separately for each estimate. When concentrations of chemicals analyzed at ALS Laboratories were below the limit of detection (LOD) for the analytical method, a value of LOD/2 was substituted. Substituting a value of LOD/2 is an appropriate method of estimation when the proportion of data that is below the limit of detection is low, and thereby the proportion of data that is given a substituted value, is low (Zeghnoun et al., 2007). For the chemical classes analyzed at ACFT, the actual values provided by the instrument were used if they were below the LOD or LOQ (limit of quantitation). When at least 25% of the pooled samples had detectable concentrations for any given chemical, a mean and confidence interval were calculated.

2.8.2. Phase Three

As in Phases One and Two, pools were constructed with differing numbers of samples, so data were differentially adjusted for accurate comparisons according to the number of individual samples used to create each pooled sample. Additionally, the size of the populations of the two largest cities in the study, Edmonton and Calgary, differ significantly from the populations of the smaller sites (Fort McMurray, Grande Prairie, Red Deer, Lethbridge, and Medicine Hat). Therefore, weights were determined for each age group within each site based on birth rates for each maternal age within a site. These weights were applied to the mean and variance calculations to adjust for differences in population size among sites. For comparisons within a site, the means and variances were only adjusted for the number of samples per pool. For comparisons across sites, the means and variances were adjusted for the number of samples per pool and weighted by the likelihood of a birth for a specific maternal age within a site. Descriptive statistics were only calculated when at least 25% of the pooled samples had detectable concentrations of a chemical. For chemicals analyzed at ALS Laboratories with at least 25%, but less than 100% of the pools having detectable concentrations, the value of LOD/2 was substituted as the analyte concentration for a pool with an analyte concentration lower than the LOD. For the analyses performed at ACFT (cotinine, phytoestrogens, and metals, metalloids, and micronutrients), actual instrument values below LOD were used in the statistical analysis. However, if more than 75% of the pooled samples did not have detectable concentrations of a chemical, descriptive statistics were not calculated. Details of the

Table 6a
Mean concentration, LOD, detection frequency and selected centiles of herbicides and neutral pesticides and metabolites measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One and Two of the Alberta Biomonitoring Program.

Herbicide, Neutral Pesticide, or Metabolite	Age Group (years), Sample Type[a]	Phase	Pool number (Total sample number)	LOD (ng/g)	%<LOD	Mean[b] (95% CI) (ng/g)	P5 (ng/g)	P25 (ng/g)	P50 (ng/g)	P75 (ng/g)	P95 (ng/g)
2,4-Dichlorophenoxyacetic acid (2,4-D)	≥18	One	151 (28,484)	0.1	79	–			–		
	2–13	Two	6 (1,373)	0.1	0	0.314 (0.221–0.407)	<LOD	<LOD	<LOD	0.190	1.20
trans-DCCA	≥18	One	151 (28,484)	0.03	83	–			–		
	2–13	Two	6 (1,373)	0.03	0	0.398 (0.193–0.603)	<LOD	<LOD	0.0407	0.170	1.40

[a] Phase Three only.
[b] Means for Phases One and Two are adjusted by number of samples within each pool.

calculations of the adjusted and weighted means are provided in the Supplementary Material (S09).

3. Results

A total of 188 chemicals were measured in the maternal serum pools in Phase One. A total of 154 chemicals were measured in children's serum in Phase Two. Of the 2,520 participants originally recruited across the seven sites for Phase Three, 1,125 eligible pairs of samples were obtained. 156 chemicals were analyzed in paired maternal and cord serum pools in Phase Three.

The mean concentrations of environmental chemicals measured and detected in serum pools in at least one of the first three phases of the ABP are listed in Table 6a–l along with the age group, program phase, number of pools, total number of samples, LOD, detection frequency, and selected estimated centiles. Means and centiles were not estimated for those chemicals with more than 25% of pools below the LOD (depicted in Table 6a-l as "-"). Those chemicals measured but not detected in more than 25% of pools in all phases in which they were measured are presented in the Supplementary Table S10a-l. The procedure for calculating the centiles requires a standard deviation, which was not calculated for chemicals with more than 25% of the pools below the LOD. The detection frequencies of the chemicals analyzed in each of the three phases ranged from 0 to 100%. Means, 95% CIs, and plots of chemical concentrations stratified by age and geography (Phase One only) are available in the Phase One and Two Alberta Biomonitoring Program Reports (Gabos et al., 2008, 2010).

Low detection rates for the majority of the herbicides and neutral pesticides and metabolites were observed in Phase Two, except for 2,4-D and trans-DCCA, which were both detected in all six children's serum pools. The mean concentrations of 2,4-D and trans-DCCA in the pooled children's serum were 3.14 (±0.93) $x10^2$ pg/g and 3.98 (±1.05) $x10^2$ pg/g, respectively.

Be, Pt, and U were not detected in any pools in any of the three phases. Other metals that were not detected in any pools include Cd (Phase Three cord pools), Cr (Phase Three), Tl (Phases One and Three), W (Phases Two and Three), and V (Phase Three). Low detection rates (≤17%) were observed for As (Phase One), Cd (Phase One and Phase Three maternal pools), Co (Phase Two), Pb, Ag (Phase Three cord pools), Tl (Phase Two), and W (Phase One). Mean concentrations for the micronutrients (B, Co, Cu, Fe, Mn, Mg, Mo, Ni, Se, Zn) ranged from 2.54 (±0.20) $x10^{-1}$ μg/L (Ni, Phase Three cord serum pools) to 1.64 (±0.02) $x10^4$ (Mg, Phase Three cord serum pools). Mean concentrations for the non-micronutrient metals and metalloids (Al, Sb, As, Ba, Be, Cd, Cs, Cr, Pb, Hg, Pt, Ag, Sr) ranged from 1.17 (±0.17) $x10^{-1}$ μg/L (Ag, Phase Two) to 3.06 (±0.07) $x10^1$ μg/L (Sr, Phase Three maternal serum pools).

Methylmercury was detected in all pools in Phases One and Two, while it was detected in 47% and 60% of the maternal and cord serum pools in Phase Three, respectively. Mean concentrations were similar across the three phases, ranging from 5.29 (±0.68) $x10^{-2}$ ng/g in the children's serum pools in Phase Two to 9.43 (±0.59) $x10^{-2}$ ng/g in the pregnant women's serum pools in Phase One.

Most of the organochlorine pesticides and metabolites analyzed across the three phases had low detection rates (<25%). The organochlorine pesticides detected in ≥72% of the serum pools were 4,4′-DDE (all three phases), HCB (Phases One and Three) and Mirex (Phases One and Three). Eight of the twenty-three organochlorine pesticides analyzed for in Phase Three had detection frequencies in maternal serum between 50% (heptachlor) and 100% (4,4′-DDE, HCB) and 27% (trans-nonachlor) and 100% (HCB) in cord serum samples. Mean wet weight concentrations within this class ranged from 2.52 (±0.39) $x10^{-3}$ pg/g (heptachlor, Phase Three maternal serum pools) to 6.71 (±0.59) $x10^{-1}$ pg/g (4,4′-DDE, Phase One) while lipid adjusted mean concentrations ranged from 5.18 (±0.76) $x10^{-1}$ pg/g lipid (heptachlor, Phase Three maternal serum pools) to 1.73 (±1.25) $x10^2$ pg/g lipid (4,4′-DDE, Phase Two).

Table 6b
Mean concentration, LOD, detection frequency and selected centiles of metals, metalloids, micronutrients, and methylmercury measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Metal, Metalloid, Micronutrient, or Methylmercury	Age Group (years), Sample Type[a]	Phase	Pool number (Total sample number)	LOD (μg/L)[b]	%<LOD	Mean[b,c] (95% CI) (μg/L)	P5 (μg/L)[b]	P25 (μg/L)[b]	P50 (μg/L)[b]	P75 (μg/L)[b]	P95 (μg/L)[b]
Aluminum (Al)	≥18	One	151 (28,484)	10	0	22.3 (21.4–23.2)[d]	<LOD	<LOD	<LOD	18.4	86.2
	2–13	Two	6 (1,373)	10	100	–			–		
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	1	67	<LOD	<LOD	<LOD	<LOD	<LOD	3.19
	≥18, cord				69	1.02 (<LOD–1.16)[e]	<LOD	<LOD	<LOD	<LOD	3.95
Antimony (Sb)	≥18	One	151 (28,484)	0.2	0	3.77 (3.61–3.93)[d]	<LOD	0.329	0.991	2.98	14.6
	2–13	Two	6 (1,373)	0.1	[f]						
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.25	0	0.684 (0.663–0.704)[e]	<LOD	0.273	0.480	0.847	1.91
	≥18, cord				0	0.833 (0.789–0.877)[e]	<LOD	<LOQ	0.411	0.917	2.90
Arsenic (As)	≥18	One	151 (28,484)	0.5	97	–			–		
	2–13	Two	6 (1,373)	0.2	0	0.272 (0.259–0.284)[g]	<LOD	<LOD	0.203	0.340	0.711
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.2	58	0.251 (0.219–0.283)[e]	<LOD	<LOD	<LOD	<LOD	0.970
	≥18, cord				66	0.232 (0.206–0.260)[e]	<LOD	<LOD	<LOD	<LOD	0.900
Barium (Ba)	≥18	One	151 (28,484)	0.2	0	8.51 (8.14–8.88)[d]	<LOQ	0.731	2.21	6.69	32.9
	2–13	Two	6 (1,373)	0.2	[f]						
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.5	0	1.64 (1.42–1.86)[e]	<LOD	<LOD	<LOD	1.17	6.32
	≥18, cord				3	1.26 (0.969–1.56)[e]	<LOD	<LOD	<LOD	0.638	4.55
Boron (B)	≥18	One	151 (28,484)	2	0	21.6 (20.9–22.3)[d]	<LOD	2.79	7.45	19.9	82.1
	2–13	Two	6 (1,373)	20[h]	0	31.0 (29.8–32.3)[g]	<LOD	<LOD	24.4	39.0	76.6
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	20	53	20.4 (<LOD–21.3)[e]	<LOD	<LOD	<LOD	24.0	65.8
	≥18, cord				75	<LOD	<LOD	<LOD	<LOD	<LOD	60.3
Cadmium (Cd)	≥18	One	151 (28,484)	0.2	99	–			–		
	2–13	Two	6 (1,373)	0.2	67	<LOD	<LOD	<LOD	<LOD	<LOD	0.592
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.05	98	–			–		
	≥18, cord				100	–			–		
Cesium (Cs)	≥18	One	151 (28,484)	0.2	0	0.480 (0.470–0.490)[d]	<LOD	<LOD	0.227	0.519	1.70
	2–13	Two	6 (1,373)	0.1	0	0.545 (0.496–0.594)	<LOD	0.125	0.276	0.606	1.88
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.05	0	0.357 (0.345–0.370)	0.0529	0.128	0.236	0.436	1.06
	≥18, cord			0.05	0	0.458 (0.439–0.476)	0.0523	0.139	0.275	0.543	1.45
Chromium (Cr)	≥18	One	151 (28,484)	0.2	0	1.51 (1.44–1.59)[d]	<LOD	<LOD	0.349	1.11	5.84
	2–13	Two	6 (1,373)	0.1	0	0.925 (0.812–1.04)[g]	<LOD	0.147	0.367	0.919	3.43
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.5	100	–			–		
	≥18, cord				100	–			–		
Cobalt (Co)	≥18	One	151 (28,484)	0.2	0	0.329 (0.284–0.375)[d]	<LOD	<LOD	<LOD	<LOD	1.07
	2–13	Two	6 (1,373)	0.2	83	–			–		
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.05	0	0.434 (0.425–0.443)	0.131	0.238	0.360	0.544	0.987
	≥18, cord				0	0.424 (0.414–0.433)	0.117	0.221	0.342	0.532	1.00
Copper (Cu)	≥18	One	151 (28,484)	0.2	0	1850 (1830–1870)[d]	423	862	1410	2320	4730
	2–13	Two	6 (1,373)	50	0	1690 (576–2800)	<LOD	<LOD	135	615	5440
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	2.5	0	2000 (1970–2030)	805	1290	1780	2470	3940
	≥18, cord				0	349 (339–358)	74.2	156	261	436	915
Iron (Fe)	≥18	One	151 (28,484)	10	0	1240 (1216–1265)[d]	91.5	285	626	1380	4280
	2–13	Two	6 (1,373)	50	0	1290 (1280–1310)	874	1080	1260	1460	1820
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	10	0	1740 (1610–1880)	61.0	243	633	1650	6570
	≥18, cord				0	4660 (4190–5130)	99.0	459	1340	3880	18000
Magnesium (Mg)	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	25	0	15900 (15800–16100)	8200	11700	14900	19100	27100
	≥18, cord				0	16400 (16200–16600)	7580	11400	15000	19900	29800
Manganese (Mn)	≥18	One	151 (28,484)	0.2	0	2.87 (2.61–3.13)[d]	<LOD	<LOD	0.369	1.45	10.3
	2–13	Two	6 (1,373)	0.2	[f]						
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.5	0	3.39 (2.96–3.81)[e]	<LOD	<LOD	0.790	2.50	13.1
	≥18, cord				0	3.86 (3.68–4.04)[e]	<LOD	0.997	2.10	4.42	12.9
Mercury (Hg)	≥18	One	151 (28,484)	0.2	28	0.251 (0.237–0.265)[d]	<LOD	<LOD	<LOD	<LOD	0.961
	2–13	Two	6 (1,373)	0.2	0	0.290 (0.229–0.351)	<LOD	<LOD	<LOD	0.219	1.12

(*continued on next page*)

Table 6b (*continued*)

Metal, Metalloid, Micronutrient, or Methylmercury	Age Group (years), Sample Type[a]	Phase	Pool number (Total sample number)	LOD (μg/L)[b]	%<LOD	Mean[b,c] (95% CI) (μg/L)	P5 (μg/L)[b]	P25 (μg/L)[b]	P50 (μg/L)[b]	P75 (μg/L)[b]	P95 (μg/L)[b]
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.1	28	0.155 (0.143–0.167)	<LOD	<LOD	<LOD	0.148	0.585
	≥18, cord				67	<LOD	<LOD	<LOD	<LOD	<LOD	0.365
Molybdenum (Mo)	≥18	One	151 (28,484)	0.2	0	1.38 (1.31–1.45)[d]	<LOD	<LOD	0.325	1.02	5.33
	2–13	Two	6 (1,373)	0.2	0	3.20 (2.87–3.54)	<LOD	0.622	1.45	3.39	11.5
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.5	0	1.16 (1.08–1.23)	<LOD	<LOD	<LOD	1.17	4.25
	≥18, cord				0	1.08 (1.05–1.11)	<LOD	0.504	0.827	1.36	2.76
Nickel (Ni)	≥18	One	151 (28,484)	0.2	0	0.881 (0.797–0.965)[d]	<LOD	<LOD	<LOD	0.428	3.14
	2–13	Two	6 (1,373)	0.5	0	1.24 (1.19–1.29)	<LOD	0.660	1.01	1.56	2.89
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.1	12	0.254 (0.234–0.274)[e]	<LOD	<LOD	<LOD	0.238	0.962
	≥18, cord				8	0.430 (0.377–0.483)[e]	<LOD	<LOD	0.102	0.321	1.66
Selenium (Se)	≥18	One	151 (28,484)	0.5	0	154 (151–157)[d]	13.0	38.6	82.2	175	520
	2–13	Two	6 (1,373)	50	0	117 (114–120)	<LOD	74.2	104	144	233
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	2.5	0	102 (99.0–104)	21.6	45.4	76.0	127	267
	≥18, cord				0	67.1 (66.1–68.2)	27.2	43.3	59.9	82.7	132
Silver (Ag)	≥18	One	151 (28,484)	0.2	20	0.266 (0.252–0.280)[d]	<LOD	<LOD	<LOD	<LOD	1.02
	2–13	Two	6 (1,373)	0.1	17	0.117 (<LOD–0.134)	<LOD	<LOD	<LOD	0.106	0.445
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.1	25	0.155 (0.128–0.182)	<LOD	<LOD	<LOD	<LOD	0.585
	≥18, cord				94	–			–		
Strontium (Sr)	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	1.0	0	30.6 (29.8–31.2)	8.06	15.5	24.4	38.4	73.7
	≥18, cord				0	27.6 (26.9–28.2)	6.89	13.5	21.6	34.6	68.0
Titanium (Ti)	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.1	0	0.253 (0.219–0.287)	<LOD	<LOD	<LOD	0.181	0.976
	≥18, cord				0	0.267 (0.235–0.298)	<LOD	<LOD	<LOD	0.203	1.03
Vanadium (V)	≥18	One	151 (28,484)	0.2	45	<LOD	<LOD	<LOD	<LOD	<LOD	0.554
	2–13	Two	6 (1,373)	0.2	0	0.313 (0.297–0.329)	<LOD	<LOD	0.222	0.388	0.868
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	1.0	100	–			–		
	≥18, cord				100	–			–		
Zinc (Zn)	≥18	One	151 (28,484)	5	0	1390 (1380–1410)[d]	296	621	1040	1740	3650
	2–13	Two	6 (1,373)	50	0	839 (821–856)	421	607	782	1010	1450
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	2.5	0	623 (612–634)	217	369	535	776	1320
	≥18, cord				0	1020 (1000–1040)	346	596	870	1270	2190
Methylmercury[i] (ng/g)	≥18	One	151 (28,484)	0.03	0	0.0943 (0.0884–0.100)	<LOD	<LOD	<LOD	0.0598	0.358
	2–13	Two	6 (1,373)	0.03	0	0.0529 (0.0461–0.0597)	<LOD	<LOD	<LOD	0.0515	0.198
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.04	53	0.0495 (<LOD–0.0702)	<LOD	<LOD	<LOD	<LOD	0.156
	≥18, cord				40	0.0592 (0.0508–0.0677)	<LOD	<LOD	<LOD	0.0413	0.228

[a] Phase Three only.

[b] Units are ng/g for methylmercury.

[c] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.

[d] Serum used in Phase One was collected in serum separator tubes (SSTs), which are not the recommended collection tubes for trace metals analysis. This should be considered when interpreting this result.

[e] Background contamination studies on blood collection and storage devices used in Phase Three suggested likely background contamination for this chemical. Results should be interpreted with caution.

[f] Statistics not performed due to suspected contamination of QC samples.

[g] Serum used in Phase Two was collected in red top glass tubes, which are not the recommended collection tubes for trace metals analysis. Background contamination studies on red top glass tubes (performed prior to Phase Three study) suggested possible background contamination for this chemical from the use of these tubes. This should be considered when interpreting this result.

[h] Tolerance range for accuracy and precision using this LOD may be as high as 35%.

[i] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class (Phase Three only) to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.

Of the six parabens analyzed in Phase Three, three had detection frequencies of ≤10% (benzyl, butyl, and isobutyl paraben) and three had detection frequencies ranging from 84 to 98% (ethyl, methyl, and propyl paraben). Mean concentrations ranged from 2.86 (±1.67) $x10^{-1}$ ng/mL (ethyl paraben, Phase Three cord serum pools) to 2.33 (±0.97) $x10^{1}$ ng/mL (methyl paraben, Phase Three cord serum pools).

Nine PFAS were analyzed in the Phase One and Two serum pools (eight were measured in the Phase Three serum pools). Six of the PFAS were detected in ≥63% of the serum pools in at least one of the phases: PFDA (Phase One), PFHxS, PFNA, PFOS, PFOA, PFUA (Phase Three maternal serum pools). Low detection frequencies (≤17%) were observed for PFDS, PFDA (Phase Two), PFDoA (Phases Two and Three), PFTA, and PFUA (Phase Two). Mean concentrations ranged from 7.92 (±2.38) $x10^{-2}$ ng/mL (PFUA, Phase One) to 9.85 (±1.87) ng/mL (PFOS, Phase Two).

Four chlorophenols and four alkylphenols were measured in Phases One and Two, while three of each were measured in Phase Three. 2,4/2,5-Dichlorophenol and o-phenylphenol had low detection frequencies in Phases One and Two (0–4%) while octylphenol, 2,4,5-, and 2,4,6-trichlorophenol had low detection rates across all three phases (0–4%). High detection rates (≥84%) were observed for BPA) (Phase One, Two and Phase Three maternal serum pools), nonylphenol (Phases One and Three) and pentachlorophenol (Phase Two). Mean concentrations of the phenols ranged from 2.60 (±0.92) $x10^{-1}$ ng/g (BPA, Phase One) to 3.00 (±1.25) $x10^{1}$ ng/mL (nonylphenol, Phase Three maternal serum pools).

Three of the fifteen phthalate metabolites measured in Phase Three were detected in 13% or fewer of the serum pools: MCHP, MiNP, and MOP. Ten metabolites were detected in ≥60% of the serum pools in which they were measured: MEHP, MiBP, MnBP, MECPP, MEHHP, MCPP, MBzP, MCOP, MEP, and MMP. Mean concentrations of the phthalate metabolites ranged from 4.44 (±0.42) $x10^{-1}$ ng/mL (MCPP, Phase Three cord serum pools) to 5.57 (±0.53) $x10^{1}$ ng/mL (MnBP, Phase Three cord serum pools).

Daidzein was detected in 100% of the pregnant women's serum pools in Phase One. Daidzein and genistein (Phase Two and Three only) were detected in ≥74% of the serum pools in which they were measured. Mean concentrations ranged from 4.23 (±0.81) $x10^{-1}$ ng/mL (daidzein, Phase Three cord serum pools) to 4.92 (±2.53) ng/mL (genistein, Phase Two).

Five of twelve PBDE congeners measured in the three phases were detected in ≤14% of the serum pools: BDE 66 (Phases One and Two), BDE 77, BDE 138 (Phases One and Two) [BDE 138/166 (Phase Three cord serum pools only)], BDE 183 (Phases One and Two), and BDE 209 (Phases One and Two). Ten congeners were detected in the serum pools in at least one phase at rates of ≥64%: BDE 28 (Phases One and Two) [BDE 28/33 (Phase Three)], BDE 47, BDE 66, BDE 85 (Phase Three), BDE 99, BDE 100, BDE 153, BDE 154, BDE 183 (Phase Three) and BDE 209 (Phase Three). Wet weight mean concentrations ranged from 3.52 (±1.75) $x10^{-1}$ pg/g (BDE 85, Phase Three cord serum pools) to 1.19 (±0.47) $x10^{2}$ pg/g (BDE 99, Phase One). Lipid adjusted mean concentrations fell within the range of 1.07 (±0.55) $x10^{2}$ pg/g lipid (BDE 138/166, Phase Three maternal serum pools) to 6.23 (±1.60) $x10^{4}$ pg/g lipid (BDE 47, Phase Two).

General trends in detection frequency for the PCB congeners across Phases One, Two, and Three are provided in Table 6j and Table S10i. For the congeners detected in 25% or more of the serum pools in Phase One, detection frequencies ranged from 30% (PCB 183) to 100% (PCB 180) [not including the low molecular weight congeners suspected of external contamination]. Six PCBs were detected in children's serum in Phase Two at frequencies ranging from 33 to 100%. Of the 37 congeners analyzed for in Phase Three, 35 were detected in maternal serum and 33 in cord serum. The Phase Three pools were analyzed in a different laboratory than the Phase One and Two pools, so the congeners/congener mixtures analyzed in Phase Three differ from those analyzed in the other two phases. Wet weight mean concentrations of the PCB congeners ranged across all three Phases from 8.29 (±1.21) $x10^{-2}$ pg/g (PCB 189, Phase Three cord serum pools) to 3.72 (±0.43) $x10^{1}$ pg/g (PCB 153/168, Phase Three maternal serum pools). Lipid adjusted concentrations ranged from 3.49 (±0.48) $x10^{1}$ pg/g lipid (PCB 189, Phase Three cord serum pools) to 9.02 (±1.87) $x10^{1}$ pg/g lipid (PCB 153/168, Phase Two).

Four (1,2,3,6,7,8-HxCDD; 1,2,3,7,8,9-HxCDD; 1,2,3,4,6,7,8-HpCDD; and 1,2,3,4,6,7,8,9-OCDD) of the seven dioxin congeners were detected in pregnant women's serum pools at rates of 30–99% in Phase One while five (2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; 1,2,3,4,6,7,8-HpCDF; and 1,2,3,4,6,7,8,9-OCDF) of the ten furan congeners were detected in 25–62% of the serum pools in Phase One. The same four dioxin congeners as detected in Phase One, and four furan congeners (2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF; 1,2,3,6,7,8-HxCDF; and 1,2,3,4,6,7,8-HpCDF) were detected in Phase Two at rates of 67–100% and 50–100%, respectively. 1,2,3,7,8-PeCDD; 1,2,3,6,7,8-HxCDD; 1,2,3,4,6,7,8-HpCDD; and 1,2,3,4,6,7,8,9-OCDD were detected in 48–98% of the maternal serum pools in Phase Three while 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8,9-OCDD were detected in 95–98% of the cord serum pools. Nine of the ten furan congeners were detected in both maternal and cord serum pools in Phase Three (only 1,2,3,4,7,8,9-HpCDF was detected in fewer than 25% of the maternal and cord serum pools in Phase Three) at rates of 35–87% (maternal) and 27–68% (cord). Low detection frequencies (≤17%) were observed across all three phases for 2,3,7,8-TCDD; 1,2,3,4,7,8-HxCDD; 2,3,7,8-TCDF; and 1,2,3,4,7,8,9-HpCDF. Low detection frequencies (≤25%) were also observed for 1,2,3,7,8-PeCDD; 1,2,3,7,8-PeCDF; 2,3,4,6,7,8-HxDCF; and 1,2,3,4,6,7,8,9-OCDF in Phases One and Two. Wet weight mean concentrations for the dioxins and furans ranged from 1.06 (±0.17) $x10^{-2}$ pg/g (1,2,3,4,7,8-HxCDF, Phase One) to 8.68 (±0.63) $x10^{-1}$ pg/g (1,2,3,4,6,7,8,9-OCDD, Phase Two). Lipid adjusted mean concentrations ranged from 1.85 (±0.29) pg/g lipid (1,2,3,4,7,8-HxCDF, Phase One) to 2.30 (±0.44) $x10^{2}$ pg/g lipid (1,2,3,4,6,7,8,9-OCDD, Phase Two).

Cotinine was measured in all three phases as a biomarker of tobacco exposure. Cotinine was detected in all serum pools in Phase One, while it was only detected in two of the six children's serum pools in Phase Two. Phase One included serum from pregnant women classified as both smokers and non-smokers while Phase Three only included pregnant women classified as non-smokers. Even though only non-smokers were included, the detection frequencies in Phase Three were high (73% and 75% for the maternal and cord serum pools, respectively). Mean detected concentrations ranged from 2.82 (±4.23) $x10^{-1}$ ng/mL (Phase Three maternal serum pools) to 2.72 (±0.18) $x10^{1}$ ng/mL (Phase One).

4. Discussion

The results for Phase One and Phase Two have been released in two separate reports by Alberta Health (Gabos et al., 2008, 2010) and visualized online on the AEPHIN website (Government of Alberta, 2020). The Phase Three report is currently under review by Alberta Health and analyses of the pooled serum for alcohol, tobacco, and cannabis biomarkers for Phase Four are ongoing.

Differences in exposure by age and geography were noted in Phase One, while differences in exposure between the children and southern Alberta pregnant women were observed when comparing Phases One and Two. These exposure differences are highlighted in the Phase One (Gabos et al., 2008) and Two (Gabos et al., 2010) reports and will not be discussed in detail here. The third phase of the ABP investigated differences in exposure between pregnant women and newborns by maternal age and by an even further division of geographic regions within the province. Serum was collected in Phase Three to allow comparison to the concentrations in the previous phases, although urine may be a better vehicle for the non-persistent chemicals due to their short half-lives in the body. The Phase Three data allowed for evaluation of temporal trends in exposure in pregnant women. Phase Four of the ABP is a targeted biomonitoring study rather than a broad exposure

Table 6c
Mean concentration, LOD, detection frequency and selected centiles of organochlorine pesticides measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Organochlorine Pesticide (wet weight)[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (ng/g)	%< LOD	Mean[c] (95% CI) (ng/g)	P5 (ng/g)	P25 (ng/g)	P50 (ng/g)	P75 (ng/g)	P95 (ng/g)
4,4′-dichlorodiphenyldichloroethylene (DDE)	≥18	One	151 (28,484)	0.1	4	0.671 (0.612–0.730)	<LOD	<LOD	<LOD	0.343	2.42
	2–13	Two	6 (1,373)	0.1	0	0.650 (0.266–1.07)	<LOD	<LOD	<LOD	0.239	2.10
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.016	0	0.569 (0.482–0.655)	<LOD	0.0340	0.114	0.382	2.18
	≥18, cord				2	0.193 (0.168–0.217)	<LOD	<LOD	0.0458	0.144	0.745
Dieldrin	≥18	One	151 (28,484)	0.1	100	–			–		
	2–13	Two	6 (1,373)	0.1	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.009	39	0.0191 (0.0174–0.0208)	<LOD	<LOD	<LOD	0.0171	0.0731
	≥18, cord				47	0.0111 (0.00999–0.0122)	<LOD	<LOD	<LOD	0.00938	0.0429
Heptachlor	≥18	One	151 (28,484)	0.1	93	–			–		
	2–13	Two	6 (1,373)	0.1	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.002	50	0.00252 (0.00213–0.00291)	<LOD	<LOD	<LOD	<LOD	0.00963
	≥18, cord				55	<LOD	<LOD	<LOD	<LOD	<LOD	0.00615
Heptachlor Epoxide	≥18	One	151 (28,484)	0.1	95	–			–		
	2–13	Two	6 (1,373)	0.1	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.002	16	0.0101 (0.00928–0.0110)	<LOD	<LOD	0.00362	0.00953	0.0383
	≥18, cord				36	0.00408 (0.00365–0.00452)	<LOD	<LOD	<LOD	0.00336	0.0158
Hexachlorobenzene (HCB)	≥18	One	151 (28,484)	0.1	28	0.158 (0.145–0.170)	<LOD	<LOD	<LOD	<LOD	0.578
	2–13	Two	6 (1,373)	0.1	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.001	0	0.0840 (0.0779–0.0901)	0.00346	0.0131	0.0329	0.0828	0.313
	≥18, cord				0	0.0539 (0.0413–0.0665)	<LOD	0.00183	0.00712	0.0277	0.195
Mirex	≥18	One	151 (28,484)	0.05	21	0.228 (0.206–0.250)	<LOD	<LOD	<LOD	0.108	0.805
	2–13	Two	6 (1,373)	0.05	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.003	19	0.0129 (0.0105–0.0152)	<LOD	<LOD	<LOD	0.00767	0.0482
	≥18, cord				26	0.00768 (0.00620–0.00916)	<LOD	<LOD	<LOD	0.00445	0.0286
Oxychlordane	≥18	One	151 (28,484)	0.05	100	–			–		
	2–13	Two	6 (1,373)	0.05	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.006	34	0.0114 (0.0103–0.0125)	<LOD	<LOD	<LOD	0.00987	0.0440
	≥18, cord				71	<LOD	<LOD	<LOD	<LOD	<LOD	0.0175
trans-Nonachlor	≥18	One	151 (28,484)	0.1	99	–			–		
	2–13	Two	6 (1,373)	0.1	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.015	37	0.0212 (0.0191–0.0233)	<LOD	<LOD	<LOD	0.0182	0.0817
	≥18, cord				73	<LOD	<LOD	<LOD	<LOD	<LOD	0.0401

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class (Phase Three only) to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.
[b] Phase Three only.
[c] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.

Table 6d
Mean concentration, LOD, detection frequency and selected centiles of parabens measured and detected in >25% of pooled blood serum samples in Phase Three of the Alberta Biomonitoring Program.

Paraben[a]	Age Group (years), Sample Type	Phase	Pool number (Total sample number)	LOD (ng/mL)	%<LOD	Mean[b] (95% CI) (ng/mL)	P5 (ng/mL)	P25 (ng/mL)	P50 (ng/mL)	P75 (ng/mL)	P95 (ng/mL)
Ethyl paraben	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.1	5	0.707 (0.596–0.818)	<LOD	<LOD	0.137	0.466	2.70
	≥18, cord				16	0.648 (0.478–0.819)	<LOD	<LOD	<LOD	0.308	2.29
Methyl paraben	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.318	10	6.39 (5.33–7.45)[c]	<LOD	0.338	1.17	4.06	24.2
	≥18, cord				3	30.9 (23.2–38.7) [c]	<LOD	0.954	3.80	15.1	110
Propyl paraben	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.1	2	2.47 (2.09–2.85)	<LOD	0.146	0.492	1.65	9.44
	≥18, cord				8	7.54 (5.38–9.70)	<LOD	0.195	0.811	3.37	26.1

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.
[b] Means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.
[c] Background contamination studies on blood collection and storage devices used in Phase Three suggested likely background contamination for this chemical. Results should be interpreted with caution.

analysis. The developing fetus is susceptible to exposure to cannabis, alcohol, and tobacco biomarkers with preventable negative associations reported in the literature such as low birth weight (Gabrhelik et al., 2021), impaired neurodevelopment (Cornelius and Day, 2009), adverse respiratory and cardiovascular outcomes (Banderali et al., 2015), and poor global developmental outcomes (Subramoney et al., 2018). The exposure levels determined in this phase may inform future public health policy with respect to these chemicals.

Phases One, Two, and Four took advantage of leftover serum specimens that otherwise would have been discarded while informed consent for sample collection at or near delivery was obtained upfront from pregnant women in Phase Three (Table 1). As samples in these studies were only available for pregnant women who sought prenatal care and for children presenting for elective surgeries, the samples are not representative of the total pregnant woman or child population in the province, which is a limitation of the study design. There are benefits and drawbacks to both sample collection methods. Leftover serum samples have the advantage of being ready for analysis once approvals for the study have been completed and the samples received at the analytical laboratory. A drawback to this type of sample is the lack of control over the sample collection or storage devices, which may limit the chemicals that can be reliably analyzed (Henriksen et al., 2020; CDC, 2018b). If leftover samples will be used, the medical devices and storage containers used for the original blood collection should be tested for background levels of the chemicals of interest. Susceptibility of the chemicals of interest to interference or background contamination should be investigated when deciding to use leftover samples. A good working relationship should be established with staff at the laboratory housing the samples. Actively recruiting participants provides an opportunity to collect more information from individuals in the form of a questionnaire, which allows for increased stratification of the chemical concentration data. Sample collection and storage devices can also be chosen to minimize potential contamination. However, prospective participant recruitment is resource intensive and time consuming. Collaboration across multiple disciplines was also required for Phase Three, which significantly added to the time required to carry out a biomonitoring study. Time constraints, budget, staff, and availability of collaborators must be carefully considered when deciding to utilize and active recruitment strategy. Communication among collaborators is crucial for timely sample collection. The European Human Biomonitoring Initiative (HBM4EU) website provides valuable resources in their online library for planning human biomonitoring studies such as protocols, questionnaires, and prioritization criteria for biomarkers (HBM4EU, n.d.).

Pooled serum samples were used in all four phases of the ABP. Pooled samples have the advantages of allowing more chemical agents to be tested with results representing an 'average' for the tested population as well as increased detection frequencies, reduced analytical cost, a less complicated ethics approval process, the possibility of using low-volume banked samples, and facilitating lower analytical method LODs (Heffernan et al., 2013). In the first three phases of the ABP, variations in the concentrations of environmental chemicals were detectable across geographic regions and age groups using pooled samples. The benefits of pooling have been recognized in other large, national biomonitoring programs, such as the Australia biomonitoring program, NHANES, and CHMS. The Australia program has used pooled samples collected since 2002 to evaluate exposure trends in environmental chemicals by age, gender, geography, and over time (Kärrman et al., 2006; Eriksson et al., 2017; Toms et al., 2018). NHANES began pooling serum samples in 2005 to achieve higher detection frequencies for the persistent chemicals PCBs, dioxins and furans, organochlorine pesticides and metabolites, and brominated flame retardants (Caudill, 2012). Means and variance measures for these chemical classes could not be reported previously because of low detection frequencies; however, pooling samples resulted in the detection and subsequent calculation of descriptive statistics for these chemicals. A Health Canada

Table 6e

Mean concentration, LOD, detection frequency and selected centiles of PFAS in pooled blood serum measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Perfluoroalkyl Substance[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (ng/mL)	%<LOD	Mean[c] (95% CI) (ng/mL)	P5 (ng/mL)	P25 (ng/mL)	P50 (ng/mL)	P75 (ng/mL)	P95 (ng/mL)
Perfluorodecanoic acid (PFDA)	≥18	One	151 (28,484)	0.02	32	0.0968 (0.0827–0.111)	<LOD	<LOD	<LOD	0.0351	0.312
	2–13	Two	6 (1,373)	0.5	83	–			–		
Perflurododecanoic acid (PFDoA)	≥18	One	151 (28,484)	0.05	71	0.112 (0.0844–0.140)	<LOD	<LOD	<LOD	<LOD	0.308
	2–13	Two	6 (1,373)	0.5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.076	100	–			–		
	≥18, cord				100	–			–		
Perfluorohexanesulfonic acid (PFHxS)	≥18	One	151 (28,484)	0.5	37	2.10 (1.77–2.44)	<LOD	<LOD	<LOD	0.714	6.60
	2–13	Two	6 (1,373)	0.5	0	9.07 (5.75–12.4)	<LOD	<LOD	1.30	4.91	33.2
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.04	0	0.600 (0.552–0.648)	<LOD	0.0822	0.216	0.566	2.27
	≥18, cord				0	0.340 (0.313–0.368)	<LOD	0.0470	0.123	0.322	1.29
Perfluorononanoic acid (PFNA)	≥18	One	151 (28,484)	0.05	1	0.470 (0.453–0.487)	<LOD	<LOD	<LOD	0.0622	0.554
	2–13	Two	6 (1,373)	0.5	0	1.00 (0.928–1.07)	<LOD	<LOD	0.594	1.18	3.18
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.04	0	0.336 (0.315–0.357)	<LOD	0.0637	0.150	0.353	1.21
	≥18, cord				0	0.180 (0.170–0.190)	<LOD	<LOD	0.0871	0.196	0.632
Perfluorooctanesulfonic acid (PFOS)	≥18	One	151 (28,484)	0.1	3	7.41 (6.91–7.91)	<LOD	0.356	1.27	4.50	27.9
	2–13	Two	6 (1,373)	0.1	0	9.85 (7.98–11.7)	0.183	0.883	2.64	7.89	38.1
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.187	0	1.95 (1.85–2.05)	<LOD	0.464	1.01	2.18	6.65
	≥18, cord				0	0.845 (0.810–0.880)	<LOD	0.259	0.510	1.00	2.66
Perfluorooctanoic acid (PFOA)	≥18	One	151 (28,484)	0.02	0	2.61 (2.55–2.67)	0.151	0.510	1.19	2.77	9.35
	2–13	Two	6 (1,373)	0.02	0	4.68 (4.26–5.11)	0.344	1.07	2.36	5.20	16.2
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.177	0	0.808 (0.744–0.872)	<LOD	<LOD	0.293	0.766	3.05
	≥18, cord				0	0.696 (0.633–0.758)	<LOD	<LOD	0.227	0.624	2.66
Perfluoroundecanoic acid (PFUA)	≥18	One	151 (28,484)	0.02	72	0.0792 (0.0554–0.103)	<LOD	<LOD	<LOD	<LOD	0.203
	2–13	Two	6 (1,373)	0.5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.04	6	0.0799 (0.0734–0.0864)	<LOD	<LOD	<LOD	0.0751	0.302
	≥18, cord				66	<LOD	<LOD	<LOD	<LOD	<LOD	0.0879

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class (Phase Three only) to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.

[b] Phase Three only.

[c] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.

Table 6f
Mean concentration, LOD, detection frequency and selected centiles of environmental phenols measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Phenol[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (ng/g)	%<LOD	Mean[c] (95% CI) (ng/g)	P5 (ng/g)	P25 (ng/g)	P50 (ng/g)	P75 (ng/g)	P95 (ng/g)
Bisphenol A (BPA)	≥18	One	151 (28,484)	0.01	0	0.260 (0.167–0.352)	<LOD	<LOD	0.0206	0.0942	0.836
	2–13	Two	6 (1,373)	0.01	0	3.56 (2.57–4.55)	0.0325	0.193	0.663	2.28	13.5
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.2[e]	16	0.731 (0.595–0.868)	<LOD	<LOD	<LOD	0.432	2.74
	≥18, cord				50	0.424 (0.255–0.593)	<LOD	<LOD	<LOD	<LOD	1.36
Nonylphenol	≥18	One	151 (28,484)	2.0	0	27.3 (20.4–34.3)	<LOD	<LOD	3.04	12.5	95.5
	2–13	Two	6 (1,373)	2.0	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.2[e]	0	30.0 (17.5–42.6)	<LOD	0.478	2.23	10.4	94.9
	≥18, cord				0	14.1 (<LOD–33.2)	<LOD	<LOD	0.325	2.07	29.8
Pentachlorophenol	≥18	One	151 (28,484)	0.25	[d]						
	2–13	Two	6 (1,373)	0.25	0	1.19 (1.02–1.35)	<LOD	<LOD	0.430	1.12	4.49
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.2	100	–			–		
	≥18, cord				100	–			–		

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class (Phase Three only) to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.
[b] Phase Three only.
[c] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.
[d] Statistics not performed due to suspected contamination of QC samples.
[e] Units are ng/mL for Phase 3 results.

report was released in 2020, in which CHMS plasma samples were pooled and analyzed for the same persistent chemicals as in the NHANES program (Health Canada, 2020). Caudill suggests a savings of $2.78 million US dollars if the NHANES 2005–2006 samples were pooled for the PCB analysis alone (Caudill, 2010). Approximating the cost of analysis of one sample for all the chemicals analyzed in Phase One as $3, 500, the cost of the analysis of the 28,484 individual samples would have been approximately $100 million, which is prohibitively expensive. Pooled samples have provided population level exposure data for the province of Alberta for over one hundred chemicals at a significantly reduced cost over individual sampling. There are limitations associated with pooled samples as well, including the inability to return results to individual patients; ability to only measure the arithmetic mean concentrations of the chemicals; and limited information on population variance, which can be mitigated through proper study design (Heffernan et al., 2013). When deciding whether to pool samples or analyze individual samples, the study objectives must be evaluated. For example, if the study requires linkage of exposure to health outcomes, pooling may not be a sufficient approach as individual results cannot be teased out of the data. However, if the goal is population surveillance, pooling is a good option for reducing operational costs, but still obtaining stratified exposure data.

Human biomonitoring data can be interpreted in terms of a population human health risk-based approach. Biomonitoring equivalents (BEs) are biomonitoring-based assessment values developed using pharmacokinetic data and are consistent with exposure-based guidance values such as reference doses (RfDs) or tolerable daily intakes (TDIs). These values are also sometimes called human biomonitoring (HBM) values. BEs and HBM values can be used as screening tools to determine how biomarker concentrations compare to levels consistent with risk-based population level exposure guidance values (Aylward et al., 2015). Biomarkers monitored in the ABP with available serum BEs or HBM values will be discussed in the sections below.

Current smokers were not excluded from the Phase One study. The mean cotinine levels detected in the pregnant women's serum from each age group and region were above the cut-off level of 10 ng/mL often cited for active smokers in individual serum samples (Hukkanen et al., 2005). Cotinine levels were highest in the youngest age group in the northern region of the province (Gabos et al., 2008). This followed the reported trend in maternal smoking in Alberta from 2005 to 2007 (Government of Alberta, 2009). Cotinine was below the LOD in Phase Two for the two children's age groups, suggesting low exposure at the time of sample collection (Gabos et al., 2010). In Phase Three, smokers or those using nicotine products were excluded from the study as the presence of samples from smokers in a pool with nonsmokers would skew the concentration of cotinine in the pool. The study was not designed to collect enough samples from smokers and non-smokers to have separate pools for comparison, as was carried out in the CHMS (Health Canada, 2010). In Phase Three, the objective was to determine the level of passive exposure to environmental tobacco smoke, similar to one of the objectives in NHANES (CDC, 2009). Maternal and newborn cotinine concentrations in Phase Three had large variances (i.e., the 95% CI was greater than the mean itself), suggesting that some women may have been using nicotine products, but did not report this on the questionnaire. The Spearman correlation between maternal and cord serum concentrations was high (0.78, unpublished data). A high Spearman correlation between maternal and cord serum concentrations of the environmental chemicals suggests maternal concentrations at the time of delivery may reliably predict fetal exposure later in gestation (Aylward et al., 2014). Phase Four included both smokers and non-smokers; therefore, a temporal trend can be evaluated when compared to Phase One levels.

Trends with age, geography, and seasonality were observed in the metals, metalloids, and micronutrients concentrations in Phase One. Pb was the only metal found to show concentration changes by season, with higher concentrations detected in all age groups in January than any

Table 6g
Mean concentration, LOD, detection frequency and selected centiles of phthalate metabolites measured and detected in >25% of pooled blood serum samples in Phase Three of the Alberta Biomonitoring Program.

Phthalate Metabolite[a]	Age Group (years), Sample Type	Phase	Pool number (Total sample number)	LOD (ng/mL)	%<LOD	Mean[b] (95% CI) (ng/mL)	P5 (ng/mL)	P25 (ng/mL)	P50 (ng/mL)	P75 (ng/mL)	P95 (ng/mL)
Mono (2-ethyl-5-carboxypentyl) phthalate (MECPP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.399	0	2.24 (1.78–2.70)	<LOD	<LOD	<LOD	1.25	8.27
	≥18, cord				0	2.56 (2.36–2.76)	<LOQ	<LOQ	0.953	2.46	9.63
Mono-(2-ethyl-5-hydroxyhexyl) phthalate (MEHHP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.399	15	0.514 (0.435–0.594)	<LOD	<LOD	<LOD	<LOD	1.96
	≥18, cord				3	0.658 (0.612–0.703)	<LOD	<LOD	<LOD	0.662	2.43
Mono-(2-ethyl-5-oxohexyl) phthalate (MEOHP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.39	65	<LOD	<LOD	<LOD	<LOD	<LOD	0.983
	≥18, cord				77	–				–	
Mono (2-ethylhexyl) phthalate (MEHP)[c]	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.4	0	21.9 (12.8–15.3)[d]	0.409	1.72	4.68	12.7	53.6
	≥18, cord				0	21.9 (19.4–24.3)[d]	0.425	2.02	6.00	17.8	84.6
Mono (3-carboxypropyl) phthalate (MCPP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.399	40	0.460 (0.418–0.501)	<LOD	<LOD	<LOD	0.408	1.76
	≥18, cord				34	0.444 (0.400–0.488)	<LOD	<LOD	<LOD	<LOD	1.71
Monobenzyl phthalate (MBzP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.399	3	1.80 (1.53–2.08)	<LOD	<LOD	<LOD	1.21	6.90
	≥18, cord				3	1.32 (1.12–1.53)	<LOD	<LOD	<LOD	0.885	5.06
Monocarboxyisooctyl phthalate (MCOP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.399	13	0.794 (0.723–0.864)	<LOD	<LOD	<LOD	0.715	3.03
	≥18, cord				8	0.924 (0.840–1.01)	<LOD	<LOD	<LOD	0.825	3.54
Monoethyl phthalate (MEP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.656	15	2.63 (2.33–2.94)	<LOD	<LOD	0.686	2.07	10.2
	≥18, cord				15	2.05 (1.88–2.22)	<LOD	<LOD	0.721	1.91	7.79
Monoisobutyl phthalate (MiBP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.399	0	41.9 (38.4–45.5)[d]	1.31	5.40	14.4	38.7	159
	≥18, cord				0	48.4 (43.5–53.3)[d]	1.09	4.96	14.2	40.9	187
Monoisodecyl phthalate (MiDP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.399	58	0.566 (0.496–0.637)	<LOD	<LOD	<LOD	0.428	2.19
	≥18, cord				52	0.568 (0.498–0.638)	<LOD	<LOD	<LOD	0.429	2.19
Monomethyl phthalate (MMP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.553	39	<LOD	<LOD	<LOD	<LOD	<LOD	2.10
	≥18, cord				39	<LOD	<LOD	<LOD	<LOD	<LOD	1.92
Mono-n-butyl phthalate (MnBP)	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.458	0	43.4 (39.4–47.4)[d]	1.16	5.02	13.9	38.5	166
	≥18, cord				0	55.7 (50.3–61.0)[d]	1.37	6.08	17.1	48.2	214

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.
[b] Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.
[c] One pair of Lethbridge 31+ year pools were removed from the MEHP analysis due to an extremely high concentrations of MEHP found in this pair of samples (~2,600x higher than the next highest concentration).
[d] Background contamination studies on blood collection and storage devices used in Phase Three suggested likely background contamination for this chemical. Results should be interpreted with caution.

other month and detectable levels in the ≤25 year group in July (Gabos et al., 2008). The reason for the difference in concentration by season is unknown. Trends in Phase One should be interpreted with caution as the serum in this phase was collected using SSTs, which may have contributed to background levels of metals, metalloids, and micronutrients. In Phase Two, As was detected in 100% of the children's serum pools, but was detected in <25% of the pregnant women's pooled serum samples. The LOD for As in Phase One was 0.5 μg/L while in Phase Two it was 0.2 μg/L, which may have contributed to the difference in detection rates between the two phases. In Phase Three, B, Cu, Hg, Se, Ag, and Sr were detected at higher concentrations in the maternal serum than the cord serum while Al, Sb, Cs, Fe, Mg, Ni and Zn had higher concentrations in the cord serum than in the maternal serum. Differences in metal, metalloid, and micronutrient concentration by geography were noted in Phase Three. However, the differences varied by metal/metalloid/micronutrient in that no one region had the overall highest or lowest concentration of the majority of the metals/metalloids/micronutrients (unpublished data). Maternal concentrations of As in Phase Three were higher than those in the Northern Saskatchewan Biomonitoring Study, while the concentration of Hg was higher in Saskatchewan pregnant women than Alberta pregnant women. Concentrations of mineral micronutrients were similar between both studies, with the exception of Zn, which was found to be lower in Alberta than in Saskatchewan (Government of Saskatchewan," 2019b). As food is the main source of exposure to these elements (CDC, 2007; World Health Organization, 2005; CDC, 2005), these differences may be due to variations in the levels of these elements found in food within each province. Spearman correlations between maternal and cord serum concentrations in Phase Three were high (≥0.70) for As, B, Hg, and Sr (unpublished data).

BEs have been derived for Mo in terms of both minimal nutrition requirements and potential toxicity in serum (Hays et al., 2016). The concentrations of Mo in all three phases are greater than the nutritionally derived BE of 0.5 μg/L. Mo is an essential trace element, and these data suggest the populations in each phase were exposed to sufficient levels of Mo in their diets. When compared to the toxicity based BEs, the mean values in all three phases were higher than the lowest BE in the range (0.9 μg/L), but significantly lower than the higher end of the range (27.9 μg/L). Zn is also an essential trace element so a recommended daily intake is required to prevent deficiency. The BE for Zn in serum/plasma based on a nutritional guidance value is 860 μg/L in women (Poddalgoda et al., 2019). Mean concentrations of zinc were higher than this BE in Phase One (1390 μg/L) and the maternal concentration in Phase Three was lower (623 μg/L). The toxicity based BEs ranged from 895 to 1281 μg/L. The collection materials were different between Phases One and Three, which should be considered when comparing the concentrations across phases and to the BEs. A BE of 9 μg/L has been derived for barium in plasma based on the U.S. EPA's RfD (Poddalgoda et al., 2017). This value is approximately 5.5x higher than the mean concentrations in Phase Three, but is similar to the mean concentration of 8.51 μg/L detected in Phase One. The authors suggest exercising caution when interpreting the BE for barium in plasma as it is not based on human clearance data and sampling, analytical and background level considerations must be taken into account as barium is known to leach from blood collection materials (Rodushkin and Ödman, 2001). The different sample collection tubes used in Phases One and Three may contribute to the observed difference. If a biomonitoring program will be ongoing over time, the supplies used should be carefully selected and tested for any background contamination prior to the beginning of the study and when any item is changed (Ward et al., 2018).

Methylmercury was analyzed in all three phases of the ABP. In Phase Three the mean concentrations in the maternal and cord serum pools were not significantly different from each other. A difference with age was not observed in Phase Three, which may be related to the lower detection frequency. Methylmercury was detected in four of the six maternal serum pools in the Northern Saskatchewan Biomonitoring

Table 6b
Mean concentration, LOD, detection frequency and selected centiles of phytoestrogens measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Phytoestrogen	Age Group (years), Sample Type[a]	Phase	Pool number (Total sample number)	LOD (ng/mL)	%<LOD	Mean[b] (95% CI) (ng/mL)	P5 (ng/mL)	P25 (ng/mL)	P50 (ng/mL)	P75 (ng/mL)	P95 (ng/mL)
Daidzein[c]	≥18	One	151 (28,484)	0.2	0	2.59 (2.37–2.81)	<LOD	<LOD	0.355	1.36	9.43
	2–13	Two	6 (1,373)	0.2	0	1.60 (0.861–2.34)	<LOD	<LOD	<LOD	0.743	5.62
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.2	26	0.863 (0.582–1.14)	<LOD	<LOD	<LOD	0.348	2.89
	≥18, cord				24	0.423 (0.342–0.504)	<LOD	<LOD	<LOD	0.243	1.57
Genistein	2–13	Two	6 (1,373)	0.2	0	4.92 (2.38–7.45)	<LOD	<LOD	0.502	2.12	16.8
	≥18, maternal	Three	64 pairs (836 maternal, 836 cord)	0.2	3	2.25 (1.82–2.67)	<LOD	<LOD	0.355	1.30	8.37
	≥18, cord				0	1.81 (1.57–2.05)	<LOD	<LOD	0.401	1.29	6.96

[a] Phase Three only.
[b] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.
[c] Two Edmonton 18–25 year old pools were removed from the daidzein data analysis (Phase Three only) due to non-quantifiable results from the analytical analysis.

Table 6i
Mean concentration, LOD, detection frequency and selected centiles of polybrominated compounds measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Polybrominated Compound (wet weight)[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (pg/g)	%<LOD	Mean[c] (95% CI) (pg/g)	P5 (pg/g)	P25 (pg/g)	P50 (pg/g)	P75 (pg/g)	P95 (pg/g)
BDE 28	≥18	One	151 (28,484)	0.410	5	9.24 (7.43–11.0)	<LOD	<LOD	0.546	2.72	27.3
	2–13	Two	6 (1,373)	0.273	0	4.51 (2.19–6.83)	<LOD	<LOD	0.461	1.95	15.5
BDE 28/33	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.051	0	3.00 (2.53–3.47)	<LOD	0.173	0.584	1.98	11.4
	≥18, cord				0	0.957 (0.842–1.07)	<LOD	0.0781	0.240	0.736	3.70
BDE 47	≥18	One	151 (28,484)	1.17	0	251 (216–287)	<LOD	4.56	20.6	93.2	816
	2–13	Two	6 (1,373)	0.780	0	242 (179–304)	2.54	14.5	48.4	162	925
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.039	0	73.1 (57.9–88.4)	0.430	2.88	10.8	40.3	269
	≥18, cord				0	18.3 (14.5–22.0)	0.108	0.721	2.70	10.1	67.3
BDE 66	≥18	One	151 (28,484)	1.17	92	–			–		
	2–13	Two	6 (1,373)	0.780	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.108	34	4.51 (2.76–6.25)	<LOD	<LOD	0.360	1.64	14.5
	≥18, cord				36	3.97 (2.57–5.37)	<LOD	<LOD	0.348	1.54	13.1
BDE 85	≥18	One	151 (28,484)	1.76	65	5.82 (3.22–8.42)	<LOD	<LOD	<LOD	<LOD	12.9
	2–13	Two	6 (1,373)	1.17	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.090	6	2.32 (1.15–3.56)	<LOD	<LOD	0.143	0.702	6.94
	≥18, cord				5	0.352 (0.177–0.527)	<LOD	<LOD	<LOD	0.108	1.06
BDE 99	≥18	One	151 (28,484)	1.76	0	122 (71.1–173)	<LOD	<LOD	3.39	20.6	277
	2–13	Two	6 (1,373)	1.17	0	74.0 (51.8–96.2)	<LOD	3.63	12.8	45.4	279
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.060	2	37.9 (20.2–55.6)	<LOD	0.522	2.51	12.1	116
	≥18, cord				0	5.15 (2.26–8.03)	<LOD	<LOD	0.284	1.44	14.9
BDE 100	≥18	One	151 (28,484)	1.17	0	62.8 (51.7–73.9)	<LOD	<LOD	4.11	19.9	191
	2–13	Two	6 (1,373)	0.780	0	62.2 (46.6–77.7)	<LOD	3.89	12.9	42.6	238
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.042	2	18.5 (14.7–22.3)	0.112	0.741	2.76	10.3	68.3
	≥18, cord				0	3.60 (2.82–4.38)	<LOD	0.135	0.511	1.94	13.2
BDE 138/166	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.197	52	0.618 (0.218–1.02)	<LOD	<LOD	<LOD	<LOD	1.71
	≥18, cord				84	–			–		
BDE 153	≥18	One	151 (28,484)	1.17	0	83.1 (74.4–91.7)	<LOD	2.23	9.18	37.8	290
	2–13	Two	6 (1,373)	0.780	0	80.3 (70.3–90.3)	3.27	12.4	31.3	79.0	299
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.159	0	43.0 (36.8–49.2)	0.503	2.78	9.12	29.9	165
	≥18, cord				0	6.82 (5.79–7.85)	<LOD	0.410	1.37	4.59	26.1
BDE 154	≥18	One	151 (28,484)	1.17	4	9.31 (5.57–13.0)	<LOD	<LOD	<LOD	1.62	21.4
	2–13	Two	6 (1,373)	0.780	0	6.08 (4.26–8.10)	<LOD	<LOD	1.04	3.72	23.2
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.101	2	3.32 (1.98–4.65)	<LOD	<LOD	0.254	1.17	10.6
	≥18, cord				15	0.422 (0.221–0.623)	<LOD	<LOD	<LOD	0.133	1.28
BDE 183	≥18	One	151 (28,484)	1.17	99	–			–		
	2–13	Two	6 (1,373)	0.780	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.073	0	1.24 (1.15–1.33)	<LOD	0.183	0.469	1.20	4.65
	≥18, cord				6	0.372 (0.322–0.421)	<LOD	<LOD	0.0839	0.269	1.43
BDE 209	≥18	One	151 (28,484)	1.17	99	–			–		
	2–13	Two	6 (1,373)	0.780	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.243	0	17.4 (16.1–18.7)[d]	0.661	2.56	6.56	16.8	65.2
	≥18, cord				0	5.02 (4.62–5.41)[d]	<LOD	0.711	1.84	4.79	18.9

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class (Phase Three only) to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.

[b] Phase Three only.

[c] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.

[d] Background contamination studies on blood collection and storage devices used in Phase Three suggested likely background contamination for this chemical. Results should be interpreted with caution.

Table 6j
Mean concentration, LOD, detection frequency and selected centiles of PCBs measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Polychlorinated Biphenyl (wet weight)[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (pg/g)	%<LOD	Mean[c] (95% CI) (pg/g)	P5 (pg/g)	P25 (pg/g)	P50 (pg/g)	P75 (pg/g)	P95 (pg/g)
PCB 28/20	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.087	0	3.68 (3.42–3.94)	0.159	0.592	1.47	3.67	13.6
	≥18, cord				0	1.46 (1.29–1.64)	<LOD	0.118	0.364	1.12	5.66
PCB 52	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.075	2	1.04 (0.795–1.29)	<LOD	<LOD	0.135	0.528	3.76
	≥18, cord				0	0.463 (0.372–0.554)	<LOD	<LOD	<LOD	0.266	1.72
PCB 61/70/74/76	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.080	0	7.38 (6.81–7.95)	0.271	1.06	2.74	7.08	27.7
	≥18, cord				0	2.47 (2.29–2.65)	0.102	0.385	0.968	2.44	9.20
PCB 66	≥18	One	151 (28,484)	5	[d]						
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.079	0	2.00 (1.74–2.27)	<LOD	0.142	0.455	1.45	7.73
	≥18, cord				0	0.743 (0.652–0.834)	<LOD	<LOD	0.183	0.565	2.87
PCB 77	≥18	One	151 (28,484)	5	96	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.085	66	<LOD	<LOD	<LOD	<LOD	<LOD	0.215
	≥18, cord				77	–			–		
PCB 83/99	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.077	0	9.59 (8.35–10.8)	0.136	0.710	2.24	7.08	37.0
	≥18, cord				0	2.64 (2.40–2.89)	<LOD	0.306	0.848	2.34	10.1
PCB 105	≥18	One	151 (28,484)	5	76	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.079	0	3.87 (3.21–4.54)	<LOD	0.196	0.688	2.41	14.6
	≥18, cord				0	1.07 (0.945–1.19)	<LOD	0.0919	0.278	0.840	4.13
PCB 109/86/97/125/87	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.078	23	0.746 (0.494–0.998)	<LOD	<LOD	<LOD	0.298	2.49
	≥18, cord				6	0.363 (0.239–0.486)	<LOD	<LOD	<LOD	0.144	1.21
PCB 113/90/101	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.068	3	1.97 (1.34–2.60)	<LOD	<LOD	0.189	0.816	6.66
	≥18, cord				0	0.657 (0.496–0.818)	<LOD	<LOD	0.0827	0.326	2.35
PCB 117/116/85/110/115	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.061	5	5.22 (3.11–7.34)	<LOD	0.0865	0.399	1.84	16.6
	≥18, cord				0	1.52 (1.04–1.99)	<LOD	<LOD	0.150	0.640	5.16
PCB 118	≥18	One	151 (28,484)	5	[d]						
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.074	0	14.3 (12.4–16.2)	0.192	1.02	3.26	10.4	55.3
	≥18, cord				0	4.18 (3.76–4.60)	0.0938	0.428	1.23	3.53	16.1
PCB 126	≥18	One	151 (28,484)	5	100	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.085	63	<LOD	<LOD	<LOD	<LOD	<LOD	0.284
	≥18, cord				77	–			–		
PCB 128/166	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.066	3	1.08 (0.897–1.26)	<LOD	<LOD	0.196	0.680	4.08
	≥18, cord				6	0.266 (0.230–0.302)	<LOD	<LOD	<LOD	0.191	1.02
PCB 138	≥18	One	151 (28,484)	5	[d]						
	2–13	Two	6 (1,373)	5	0	20.5 (17.3–23.6)	<LOD	<LOD	6.67	18.3	78.4
PCB 146	≥18	One	151 (28,484)	5	65	<LOD	<LOD	<LOD	<LOD	<LOD	15.1
	2–13	Two	6 (1,373)	5	100	–			–		
PCB 147/149	≥18	One	151 (28,484)	5	[d]						
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.074	2	0.746 (0.560–0.931)	<LOD	<LOD	0.0924	0.367	2.66
	≥18, cord				3	0.281 (0.200–0.362)	<LOD	<LOD	<LOD	0.125	0.973
PCB 151/135	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.068	2	0.773 (0.635–0.912)	<LOD	<LOD	0.132	0.469	2.91
	≥18, cord				10	0.222 (0.185–0.260)	<LOD	<LOD	<LOD	0.139	0.842
PCB 153/168	≥18	One	151 (28,484)	5	[d]						
	2–13	Two	6 (1,373)	5	0	34.3 (28.9–39.7)	<LOD	<LOD	11.0	30.4	132
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.059	0	37.2 (32.9–41.5)	0.641	3.16	9.60	29.1	144
	≥18, cord				0	8.68 (7.70–9.66)	0.158	0.766	2.30	6.90	33.6
PCB 156/157	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.066	0	5.03 (4.54–5.52)	0.120	0.539	1.53	4.32	19.3
	≥18, cord				0	1.33 (1.18–1.48)	<LOD	0.118	0.352	1.06	5.14
PCB 158	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.053	2	0.702 (0.587–0.816)	<LOD	<LOD	0.131	0.451	2.67

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Table 6j (*continued*)

Polychlorinated Biphenyl (wet weight)[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (pg/g)	%<LOD	Mean[c] (95% CI) (pg/g)	P5 (pg/g)	P25 (pg/g)	P50 (pg/g)	P75 (pg/g)	P95 (pg/g)
	≥18, cord				3	0.197 (0.170–0.224)	<LOD	<LOD	<LOD	0.141	0.759
PCB 167	≥18	One	151 (28,484)	5	99	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.051	0	1.20 (1.08–1.31)	<LOD	0.129	0.365	1.03	4.60
	≥18, cord				0	0.324 (0.296–0.352)	<LOD	<LOD	0.110	0.297	1.23
PCB 169	≥18	One	151 (28,484)	5	100	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.058	27	0.107 (0.0918–0.122)	<LOD	<LOD	<LOD	0.0749	0.411
	≥18, cord				73	<LOD	<LOD	<LOD	<LOD	<LOD	0.118
PCB 170	≥18	One	151 (28,484)	5	21	8.09 (7.35–8.84)	<LOD	<LOD	<LOD	<LOD	29.0
	2–13	Two	6 (1,373)	5	17	5.28 (<LOD–6.53)	<LOD	<LOD	<LOD	<LOD	20.3
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.060	0	9.20 (7.77–10.6)	0.0926	0.534	1.80	6.09	35.1
	≥18, cord				0	2.08 (1.76–2.41)	<LOD	0.121	0.409	1.38	7.96
PCB 172	≥18	One	151 (28,484)	5	99	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.058	2	1.22 (1.04–1.41)	<LOD	0.0744	0.248	0.828	4.68
	≥18, cord				8	0.236 (0.203–0.268)	<LOD	<LOD	<LOD	0.167	0.907
PCB 177	≥18	One	151 (28,484)	5	85	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.059	0	1.84 (1.61–2.08)	<LOD	0.141	0.442	1.38	7.13
	≥18, cord				0	0.426 (0.382–0.470)	<LOD	<LOD	0.122	0.355	1.64
PCB 178	≥18	One	151 (28,484)	5	90	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.058	2	1.66 (1.46–1.87)	<LOD	0.131	0.407	1.26	6.43
	≥18, cord				0	0.356 (0.315–0.397)	<LOD	<LOD	0.0926	0.280	1.38
PCB 180	≥18	One	151 (28,484)	5	0	26.8 (24.7–28.8)	<LOD	<LOD	<LOD	14.8	98.6
	2–13	Two	6 (1,373)	5	0	17.3 (14.6–20.0)	<LOD	<LOD	5.56	15.4	66.4
PCB 180/193	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.048	0	27.2 (22.3–32.0)	0.212	1.31	4.66	16.5	102
	≥18, cord				0	5.34 (4.40–6.28)	<LOD	0.263	0.929	3.28	20.2
PCB 183	≥18	One	151 (28,484)	5	70	<LOD	<LOD	<LOD	<LOD	<LOD	14.4
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.054	0	3.05 (2.64–3.46)	<LOD	0.215	0.688	2.20	11.8
	≥18, cord				2	0.593 (0.524–0.661)	<LOD	<LOD	0.154	0.466	2.29
PCB 187	≥18	One	151 (28,484)	5	17	11.8 (9.80–13.7)	<LOD	<LOD	<LOD	<LOD	36.5
	2–13	Two	6 (1,373)	5	33	5.05 (<LOD–6.64)	<LOD	<LOD	<LOD	<LOD	18.9
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.048	0	7.89 (6.85–8.94)	0.107	0.568	1.81	5.76	30.5
	≥18, cord				0	1.48 (1.31–1.65)	<LOD	0.130	0.391	1.18	5.73
PCB 189	≥18	One	151 (28,484)	5	100	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.042	2	0.422 (0.357–0.487)	<LOD	<LOD	0.0829	0.280	1.61
	≥18, cord				21	0.0829 (0.0707–0.0950)	<LOD	<LOD	<LOD	0.0568	0.318
PCB 194	≥18	One	151 (28,484)	5	86	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.045	0	4.35 (3.58–5.13)	<LOD	0.212	0.750	2.66	16.4
	≥18, cord				0	0.663 (0.566–0.761)	<LOD	<LOD	0.136	0.453	2.54
PCB 195	≥18	One	151 (28,484)	5	100	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.046	0	0.934 (0.763–1.10)	<LOD	<LOD	0.156	0.559	3.51
	≥18, cord				5	0.164 (0.139–0.190)	<LOD	<LOD	<LOD	0.110	0.628
PCB 196	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.048	2	1.45 (1.25–1.64)	<LOD	0.100	0.322	1.04	5.58
	≥18, cord				5	0.245 (0.214–0.275)	<LOD	<LOD	0.0586	0.183	0.945
PCB 198/199	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.047	0	4.36 (3.78–4.94)	0.0587	0.312	0.994	3.17	16.8
	≥18, cord				0	0.699 (0.618–0.780)	<LOD	0.0597	0.181	0.548	2.70
PCB 203	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.045	0	2.79 (2.36–3.22)	<LOD	0.165	0.554	1.86	10.7
	≥18, cord				2	0.421 (0.367–0.476)	<LOD	<LOD	0.0984	0.311	1.63

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Table 6j (*continued*)

Polychlorinated Biphenyl (wet weight)[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (pg/g)	%<LOD	Mean[c] (95% CI) (pg/g)	P5 (pg/g)	P25 (pg/g)	P50 (pg/g)	P75 (pg/g)	P95 (pg/g)
PCB 203/196	≥18	One	151 (28,484)	5	86	–	–	–	–		
	2–13	Two	6 (1,373)	5	67	<LOD	<LOD	<LOD	<LOD	<LOD	13.1
PCB 206	≥18	One	151 (28,484)	5	99	–	–	–	–		
	2–13	Two	6 (1,373)	5	100	–	–	–	–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.215	10	1.49 (1.35–1.62)	<LOD	<LOD	0.480	1.32	5.70
	≥18, cord				50	<LOD	<LOD	<LOD	<LOD	<LOD	0.780
PCB 209	≥18	One	151 (28,484)	5	99	–			–		
	2–13	Two	6 (1,373)	5	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.043	0	1.39 (1.24–1.53)	<LOD	0.138	0.400	1.16	5.36
	≥18, cord				5	0.203 (0.178–0.227)	<LOD	<LOD	0.0509	0.156	0.784

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class (Phase Three only) to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.
[b] Phase Three only.
[c] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.
[d] Statistics not performed due to suspected contamination of QC samples.

Study. The mean methylmercury concentration in maternal serum in Phase Three was lower than the concentrations detected in maternal serum pools in Saskatchewan (Government of Saskatchewan, 2019b). The Spearman correlation between maternal and cord serum mean methylmercury concentrations in Phase Three was high (0.84, unpublished data).

In Phase Three concentrations for the majority of the organochlorine pesticides were statistically higher in maternal than cord serum: 4,4'-DDE, dieldrin (wet weight only), heptachlor (wet weight only), heptachlor epoxide, HCB (wet weight only), Mirex (wet weight only), oxychlordane (wet weight only), and *trans*-nonachlor (wet weight only). Organochlorine pesticide concentrations increased with maternal age. Differences in concentrations were also noted by geographic region, with higher concentrations typically noted in Fort McMurray and Lethbridge and lower concentrations in Grande Prairie and Red Deer, although the differences were not always statistically significant from all other regions (unpublished data). Maternal concentrations of 4,4′-DDE and HCB in Phase Three were similar to those detected in Saskatchewan (Government of Saskatchewan, 2019b). 4,4′-DDE maternal concentrations in Phase Three were similar to the means calculated from the pooled NHANES data from 20 to 39 year old women in 2007–2009 while Phase Three maternal serum HCB concentrations were higher and oxychlordane and *trans*-nonachlor were lower than the NHANES data (CDC, 2018a). 4,4′-DDE and HCB were higher in Phase Three maternal serum than detected in pooled CHMS data from 20 to 39 year old people in 2014–2015 while concentrations of *trans*-nonachlor and oxychlordane were similar to the CHMS data from 20 to 39 year old people in 2012–2013 (Health Canada, 2020). 4,4′-DDE and *trans*-nonachlor maternal serum concentrations in Phase Three were higher than maternal data from Canadian women participating in the MIREC study between 2008 and 2011 while Phase Three oxychlordane concentrations were similar to the MIREC data (Fisher et al., 2016). Spearman correlation coefficients were calculated between maternal and cord serum concentrations and moderate to high correlations were noted for 4, 4′-DDE, Mirex, HCB, heptachlor, and *trans*-nonachlor (unpublished data). Most uses of DDT were phased out in the mid-1970s in Canada (Government of Canada, 2013) and all uses of Mirex have been banned in Canada since 1978 (United Nations, 2017), yet these persistent chemicals continue to be detected in the Alberta population.

BE values have been derived for DDT and its metabolites (Kirman et al., 2011). The lower BE concentration range derived using non-cancer endpoints ranged from 5,000 to 16,000 ng/g lipid for ∑DDT/DDE/DDD. The mean concentrations of 4,4′-DDE in all three phases were approximately 40 times lower than the low end of this BE range. This suggests low priority for risk assessment follow-up for the concentrations of 4,4′-DDE detected in the ABP. BE values have been derived for HCB by Aylward et al. using various exposure guidance values (Aylward et al., 2010). The concentrations of HCB detected in Phases One (27.9 ng/g lipid) and Three (17.9 ng/g lipid [maternal] and 21.9 ng/g lipid [cord]) were similar to or lower than the BE values estimated in Aylward et al. (25–340 ng/g lipid), suggesting a low priority for risk assessment follow-up. BEs were derived for the isomers and metabolites of chlordane in serum (Singh et al., 2019). The mean concentrations of these pesticides detected in Phase Three were 20x (*trans*-nonachlor) and 300x lower than the low end of the BE ranges derived by Singh et al. (2019).

All PFAS measured in Phase Two had higher mean concentrations in the children's serum pools compared to the pregnant women's serum pools from southern Alberta (Table 6e) (Gabos et al., 2010). This is a trend observed in many other studies and may be related to several factors including more hand to mouth activity in children than adults; proximity to the floor, which can result in more exposure to carpeted floors or house dust, and different pharmacokinetics in children compared to adults (Kato et al., 2009; Olsen et al., 2004). Maternal serum concentrations were greater than cord serum concentrations for PFHxS, PFOS, PFNA, and PFUA in Phase Three. There was not a

Table 6k

Mean concentration, LOD, detection frequency and selected centiles of dioxins and furans in pooled blood serum measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Polychlorinated di-benzo-p-dioxin or polychlorinated dibenzofuran (wet weight)[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (pg/g)	%<LOD	Mean[c] (95% CI) (pg/g)	P5 (pg/g)	P25 (pg/g)	P50 (pg/g)	P75 (pg/g)	P95 (pg/g)
1,2,3,7,8-Pentachlorodibenzodioxin (PeCDD)	≥18	One	151 (28,484)	0.01	91	–			–		
	2–13	Two	6 (1,373)	0.01	83	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.017	52	<LOD	<LOD	<LOD	<LOD	<LOD	0.0464
	≥18, cord				76	–			–		
1,2,3,6,7,8-HxCDD	≥18	One	151 (28,484)	0.01	7	0.0619 (0.0572–0.0666)	<LOD	<LOD	<LOD	0.0347	0.229
	2–13	Two	6 (1,373)	0.01	0	0.107 (0.0756–0.138)	<LOD	<LOD	0.0191	0.0666	0.404
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.028	32	0.0427 (0.0389–0.0466)	<LOD	<LOD	<LOD	0.0381	0.164
	≥18, cord				76	–			–		
1,2,3,7,8,9-HxCDD	≥18	One	151 (28,484)	0.01	70	0.0128 (0.0101–0.0156)	<LOD	<LOD	<LOD	<LOD	0.0369
	2–13	Two	6 (1,373)	0.01	33	0.0233 (<LOD–0.0371)	<LOD	<LOD	<LOD	<LOD	0.0774
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.029	76	–			–		
	≥18, cord				94	–			–		
1,2,3,4,6,7,8-Heptachlorodibenzodioxin (HpCDD)	≥18	One	151 (28,484)	0.01	2	0.129 (0.122–0.136)	<LOD	<LOD	0.0261	0.0871	0.494
	2–13	Two	6 (1,373)	0.01	0	0.132 (0.115–0.149)	<LOD	0.0194	0.0497	0.127	0.494
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.018	2	0.101 (0.0932–0.110)	<LOD	<LOD	0.0361	0.0952	0.384
	≥18, cord				5	0.0361 (0.0338–0.0384)	<LOD	<LOD	<LOD	0.0376	0.131
1,2,3,4,6,7,8,9-Octachlorodibenzodioxin (OCDD)	≥18	One	151 (28,484)	0.01	1	0.863 (0.820–0.906)	0.0114	0.0609	0.195	0.624	3.33
	2–13	Two	6 (1,373)	0.01	0	0.868 (0.805–0.932)	0.0935	0.254	0.510	1.02	2.78
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.023	2	0.672 (0.626–0.719)	0.030	0.111	0.274	0.676	2.48
	≥18, cord				2	0.148 (0.137–0.158)	<LOD	0.0231	0.0580	0.146	0.550
1,2,3,7,8-Pentachlorodibenzofuran (PeCDF)	≥18	One	151 (28,484)	0.01	84	–			–		
	2–13	Two	6 (1,373)	0.01	83	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.014	37	0.0148 (<LOD–0.0177)	<LOD	<LOD	<LOD	<LOD	0.0549
	≥18, cord				52	<LOD	<LOD	<LOD	<LOD	<LOD	0.0396
2,3,4,7,8-PeCDF	≥18	One	151 (28,484)	0.01	66	0.0118 (<LOD–0.0137)	<LOD	<LOD	<LOD	<LOD	0.0367
	2–13	Two	6 (1,373)	0.01	50	0.0275 (<LOD–0.0571)	<LOD	<LOD	<LOD	<LOD	0.0766
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.012	23	0.0203 (0.0183–0.0223)	<LOD	<LOD	<LOD	0.0173	0.0782
	≥18, cord				58	<LOD	<LOD	<LOD	<LOD	<LOD	0.0289
1,2,3,4,7,8-Hexachlorodibenzofuran (HxCDF)	≥18	One	151 (28,484)	0.01	67	0.0106 (<LOD–0.0124)	<LOD	<LOD	<LOD	<LOD	0.0331
	2–13	Two	6 (1,373)	0.01	0	0.0283 (0.0155–0.0412)	<LOD	<LOD	<LOD	0.0133	0.100
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.014	50	0.0145 (<LOD–0.0163)	<LOD	<LOD	<LOD	<LOD	0.0560
	≥18, cord				73	<LOD	<LOD	<LOD	<LOD	<LOD	0.0272
1,2,3,6,7,8-HxCDF	≥18	One	151 (28,484)	0.01	53	0.0124 (0.0108–0.0141)	<LOD	<LOD	<LOD	<LOD	0.0331
	2–13	Two	6 (1,373)	0.01	0	0.0250 (0.0183–0.0317)	<LOD	<LOD	<LOD	0.0164	0.0954
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.014	48	0.0161 (<LOD–0.0187)	<LOD	<LOD	<LOD	<LOD	0.0614
	≥18, cord				65	<LOD	<LOD	<LOD	<LOD	<LOD	0.0288
1,2,3,7,8,9-HxCDF	≥18	One	151 (28,484)	0.01	95	–			–		
	2–13	Two	6 (1,373)	0.01	83	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.019	53	<LOD	<LOD	<LOD	<LOD	<LOD	0.0614
	≥18, cord				47	<LOD	<LOD	<LOD	<LOD	<LOD	0.0494
2,3,4,6,7,8-HxCDF	≥18	One	151 (28,484)	0.01	93	–			–		
	2–13	Two	6 (1,373)	0.01	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.014	65	<LOD	<LOD	<LOD	<LOD	<LOD	0.0441
	≥18, cord				71	<LOD	<LOD	<LOD	<LOD	<LOD	0.0274
1,2,3,4,6,7,8-Heptachlorodibenzofuran (HpCDF)	≥18	One	151 (28,484)	0.01	38	0.0288 (0.0254–0.0322)	<LOD	<LOD	<LOD	0.0121	0.0977
	2–13	Two	6 (1,373)	0.01	0	0.0850 (0.0740–0.0960)	<LOD	0.0125	0.0321	0.0823	0.319
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.013	13	0.0267 (0.0226–0.0308)	<LOD	<LOD	<LOD	0.0178	0.102
	≥18, cord				32	<LOD	<LOD	<LOD	<LOD	<LOD	0.0470

(*continued on next page*)

Table 6k (*continued*)

Polychlorinated di-benzo-p-dioxin or polychlorinated dibenzofuran (wet weight)[a]	Age Group (years), Sample Type[b]	Phase	Pool number (Total sample number)	LOD (pg/g)	%<LOD	Mean[c] (95% CI) (pg/g)	P5 (pg/g)	P25 (pg/g)	P50 (pg/g)	P75 (pg/g)	P95 (pg/g)
1,2,3,4,6,7,8,9-Octachlorodibenzofuran (OCDF)	≥18	One	151 (28,484)	0.01	75	0.0110 (<LOD–0.0136)	<LOD	<LOD	<LOD	<LOD	0.0307
	2–13	Two	6 (1,373)	0.01	100	–			–		
	≥18, maternal	Three	62 pairs (812 maternal, 812 cord)	0.021	58	<LOD	<LOD	<LOD	<LOD	<LOD	0.0608
	≥18, cord				63	<LOD	<LOD	<LOD	<LOD	<LOD	0.0552

[a] Two pairs of Lethbridge 26–30 year pools were not analyzed for this chemical class (Phase Three only) to adjust for the difference in population among sites. The two pools not used in the analysis were received at a later date than the two pools that were used.

[b] Phase Three only.

[c] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.

consistent trend with maternal age among the PFAS congeners. Maternal serum PFOS concentrations were higher in Fort McMurray than in other regions, while PFUA concentrations were lower in Lethbridge than in other regions (unpublished data). Maternal serum concentrations of PFOA and PFNA in Phase Three were similar to or lower than those in the Saskatchewan (Government of Saskatchewan, 2019b), CHMS (individual females aged 20–39 years data, 2009–2011) (Health Canada, 2013), NHANES (females, 2013–2014 data) (CDC, 2019b), and MIREC (Fisher et al., 2016) studies. PFHxS, PFOS, and PFUA maternal serum concentrations in Phase Three were lower than those in the Saskatchewan, CHMS, NHANES, and MIREC studies listed above. The Spearman correlation coefficients observed for the PFAS in maternal and cord serum were highly correlated, except for PFUA, which had a lower detection rate than the other PFAS.

HBM I values for PFOS and PFOA have been established by the German Human Biomonitoring Commission at 5 ng/mL for PFOS and 2 ng/mL for PFOA (Apel et al., 2017). The HBM I value represents the concentration of a substance in human biological material at which and below which, according to the current knowledge and assessment by the HBM Commission, there is no risk of adverse health effects, and, consequently, no need for action (Apel et al., 2017). Concentrations of PFOS and PFOA in Phases One and Two were higher than these HBM values, but concentrations of PFAS have generally declined in recent years. The Phase Three PFOS and PFOA serum concentrations were below these HBM values, suggesting low priority for risk assessment.

BPA was detected at higher concentrations in children's serum in Phase Two when compared to the southern Alberta pregnant women's serum analyzed in Phase One. The mean BPA concentration in maternal serum in Phase Three was significantly higher than in cord serum, with higher concentrations detected in Grande Prairie than the other regions (unpublished data). An HBM value of 40 μg/L was derived for pentachlorophenol (Apel et al., 2017), which is approximately 33x higher than the mean concentration detected in Phase Two, suggesting a low priority for risk assessment follow-up of this compound. The mean maternal serum concentrations of nonylphenol in Phase Three increased with maternal age. Maternal serum concentrations were higher in Fort McMurray while cord serum concentrations were higher in Grande Prairie than in other regions (unpublished data). Maternal serum concentrations of BPA and nonylphenol were higher in Phase Three than in the Northern Saskatchewan Biomonitoring Study as the LODs were the same in each study, but BPA and nonylphenol were not detected in any serum pools in the Saskatchewan study; suggesting lower exposure to these compounds in northern Saskatchewan than Alberta (Government of Saskatchewan, 2019b).

Both primary and secondary phthalate metabolites were evaluated in Phase Three. Primary metabolites are more susceptible to artificially elevated levels due to external contamination with diester phthalates introduced during sample collection, storage, or analysis (Silva et al., 2005; Henriksen et al., 2020). Urine has been identified as the superior matrix for phthalate exposure biomonitoring, and it has been suggested in the literature that serum only be used for the evaluation of historical cohorts (Henriksen et al., 2020). MEHP, MnBP, and MEHHP were detected at higher mean concentrations in cord serum than maternal serum while MBzP and MEP were detected at higher concentrations in maternal serum than cord serum. For MCPP, MEHHP, MCOP, MEP and MMP, one or both of the younger age groups had higher mean concentrations than detected in the older age group(s). MiBP and MnBP had higher concentrations in the 26–30-year-old group than the 18–25-year-old group for both maternal and cord serum pools. MEHP (maternal pools only), MiBP, and MnBP had higher concentrations in Fort McMurray than in the other regions. These results should be interpreted with caution as these are primary metabolites. It was also noted that the secondary metabolites of DEHP (MECPP, MEHHP, and MEOHP) were higher in serum from Calgary and Edmonton, suggesting higher exposure to DEHP in these large cities than in the less populated regions (unpublished data). Maternal serum concentrations of MEHHP

Table 61
Mean concentration, LOD, detection frequency and selected centiles of tobacco biomarkers measured and detected in >25% of pooled blood serum samples in at least one phase of Phases One, Two, and Three of the Alberta Biomonitoring Program.

Tobacco Biomarker	Age Group (years), Sample Type[a]	Phase	Pool number (Total sample number)	LOD (ng/mL)	%<LOD	Mean[b] (95% CI) (ng/mL)	P5 (ng/mL)	P25 (ng/mL)	P50 (ng/mL)	P75 (ng/mL)	P95 (ng/mL)
Cotinine	≥18	One	151 (28,484)	5.0	0	27.2 (25.4–29.1)	<LOD	<LOD	<LOD	16.4	102
	2–13	Two	6 (1,373)	0.5	67	<LOD	<LOD	<LOD	<LOD	<LOD	1.60
	≥18, maternal	Three	63 pairs (824 maternal, 824 cord)[c]	0.025	27	0.282 (<LOD–0.705)	<LOD	<LOD	<LOD	0.0379	0.568
	≥18, cord				25	0.482 (<LOD–0.944)	<LOD	<LOD	<LOD	0.0901	1.15

[a] Phase Three only.
[b] Means for Phases One and Two are adjusted by number of samples within each pool; Phase Three means are adjusted for number of samples within each pool and weighted by likelihood of birth for maternal age within a geographic region.
[c] One pair of Fort McMurray 18–25 year old pools was removed from the cotinine data analysis (Phase Three only) due to a study participant in that pool confirming the use of a nicotine-containing product on her questionnaire.

were higher in Phase Three than in the Northern Saskatchewan Biomonitoring Study, while MBzP concentrations were similar between Phase Three and Saskatchewan, and MEP concentrations were lower in Phase Three than in the Saskatchewan study (Government of Saskatchewan, 2019b).

Phytoestrogens were measured in serum in all three phases of the ABP. Due to the similar structure of phytoestrogens to estrogen, much research has been done with varying outcomes to clarify the ability of phytoestrogens to cause endocrine disruption (Atkinson et al., 2005). Phytoestrogens have also been linked to adverse health effects on the immune system and thyroid function, and genotoxic effects (Schmitt et al., 2003; CDC, 2017). Daidzein was detected at higher concentrations in maternal serum than cord serum in Phase Three. In the maternal serum, daidzein and genistein concentrations were lower in the 26–30-year-old group than the other two age groups (unpublished data). Maternal serum concentrations of daidzein in Phase Three were similar to those in the Northern Saskatchewan Biomonitoring Study and concentrations of genistein in Saskatchewan were higher than those in the Phase Three maternal serum concentration (Government of Saskatchewan, 2019b).

Maternal serum concentrations of PBDEs were higher than cord serum concentrations in Phase Three. The maternal and cord serum concentrations were highly correlated, with the exceptions of BDE 183 and BDE 209. Maternal serum concentrations generally increased with maternal age, although the increase was not always statistically significant. The highest serum concentrations for most congeners were found in Red Deer and Lethbridge, while concentrations of BDE 209 were generally higher in Fort McMurray than in other sites (unpublished data). Maternal serum concentrations of all PBDE congeners detected in both Phase Three and the Northern Saskatchewan Biomonitoring Study were similar (Government of Saskatchewan, 2019b). Phase Three maternal serum concentrations of BDE 47 were similar to those detected in pooled CHMS data from 20 to 39-year-old people in 2012–2013 (Health Canada, 2020) and pooled NHANES data from 20 to 39-year-old non-Hispanic white women in 2009–2010 (CDC, 2018) and higher than detected in maternal serum from the participants in the MIREC study (Fisher et al., 2016). Maternal serum concentrations of BDE 85 in Phase Three were similar to those in the pooled NHANES data; BDE 99, 100, and 209 were slightly higher in Phase Three than in the pooled NHANES data; and BDE 153 was slightly lower in Phase Three than in the NHANES data (CDC, 2018). Maternal serum concentrations of BDE 99 and 100 in Phase Three were slightly higher than in the pooled CHMS data; BDE 153 concentrations were similar in Phase Three and the pooled CHMS data; and BDE 209 concentrations were lower in Phase Three than in the CHMS pooled data (Health Canada, 2020).

A BE of 520 ng/g lipid has been derived for BDE 99 (Krishnan et al., 2011). This value is 25–80x higher than the mean concentrations determined in the serum of pregnant women in Phases One and Three of the ABP, suggesting low priority for risk assessment of this compound.

Concentrations of PCBs increased with age for the highly detected congeners in Phase Three. For the less chlorinated congeners, concentrations were generally higher in Fort McMurray than the other regions, while concentrations of the more highly chlorinated congeners were generally higher in Red Deer. Concentrations were generally lowest in Grande Prairie (unpublished data). Maternal serum concentrations of PCB 105, 118, 168/153, 183, and 187 were similar in Phase Three and the Northern Saskatchewan Biomonitoring Study. PCB 147/149, 151/135, 206, and 209 concentrations were one to two orders of magnitude lower in maternal serum in Phase Three than the Saskatchewan study (Government of Saskatchewan, 2019a). Maternal serum concentrations in Phase Three were generally similar to or lower than the concentrations in the participants in the MIREC (Fisher et al., 2016), pooled CHMS data (20–39-year-old people in 2012–2013) (Health Canada, 2020), and pooled NHANES data (20–39-year-old non-Hispanic white women in 2009–2010) (CDC, 2018).

In Phase Three, maternal serum concentrations of the detected

dioxins and furans were higher than the cord serum concentrations. Concentrations of dioxins and furans in the older maternal age groups were generally higher or not significantly different than concentrations in the younger age groups (unpublished data). Maternal serum concentrations of 1,2,3,4,6,7,8-HpCDD and 1,2,3,4,6,7,8,9-OCDD in Phase Three were slightly higher than the concentrations in the Saskatchewan study (Government of Saskatchewan, 2019b). Maternal serum concentrations of the dioxins and furans were generally similar to the concentrations in 20–39-year-old pooled data from the 2014–2015 CHMS (Health Canada, 2020) and the pooled NHANES data from 2009 to 2010 (20–39-year-old non-Hispanic white females) (CDC, 2018a).

In some cases laboratory methods changed between phases. This resulted in increased sensitivity from Phase One to Phase Three for the organochlorine pesticides, most metals, PBDEs, PCBs, cotinine, nonylphenol, pentachlorophenol, PFHxS, and PFNA. Differences in detection frequencies between phases may be a result of these changes.

For the chemicals quantified in both Phase One and Phase Three, temporal trends in the environmental chemical concentrations within pregnant women were assessed. Arsenic was detected in fewer than 25% of the serum pools in Phase One, but it was detected in 42% of the maternal serum pools in Phase Three, likely because of the decreased detection limit in Phase Three. Al, Sb, and Cr were all detected at significantly higher concentrations in pregnant women in Phase One compared to Phase Three, which may be a result of SSTs being used to collect serum in Phase One as tubes with no additive were used in Phase Three. These three metals are known to be susceptible to background contamination and SSTs are not the recommended collection tubes for analysis of these metals (Mayo Clinic Laboratories, 2020a; 2020b; Filella et al., 2013). The concentrations of the micronutrients Cu, Fe, Co, and Mn all increased in pregnant women's serum from Phase One to Phase Three by 8%, 40%, 32% and 18%, respectively. The concentration of Hg and methylmercury in pregnant women's serum decreased from Phase One to Phase Three by 38% and 27%, respectively. Dieldrin, heptachlor (wet weight only), heptachlor epoxide, oxychlordane, and *trans*-nonachlor were detected in less than 25% of the serum pools in Phase One, but were detected in more than 25% of the serum pools in Phase Three, likely because of reduced LODs. Mean serum concentrations of DDE (wet weight only), HCB, and Mirex all decreased from Phase One to Phase Three by 15%, 47%, and 94%, respectively. Levels of the PFAS decreased in pregnant women's serum from Phase One to Phase Three. PFHxS, PFNA, PFOS, and PFOA decreased in concentration by 71%, 28%, 74, and 69%, respectively. The concentration of PFUA was similar between phases. The concentration of BPA was higher in maternal serum in Phase Three by more than 200% (BPA was only measured in southern Alberta pregnant women in Phase One so this concentration was only compared to the pregnant women in the southern regions in Phase Three, which includes Calgary, Red Deer and Medicine Hat). BPA has a short half-life in the body of only 5.3 h (Genuis et al., 2012), so the concentrations detected in serum are more dependent on time of sample collection and reflect recent rather than long-term exposure. The concentration of daidzein decreased by 67% from Phase One to Phase Three. The detection rate dropped from 100% to 74%, although the LOD remained the same. PBDE concentrations in maternal serum decreased from Phase One to Phase Three by 38% (BDE 153) to 68% (BDE 99). Three PBDEs were detected in more than 25% of serum pools in Phase Three that were detected in only 1–8% of serum pools in Phase One (BDE 66, 183, 209). Multiple congeners were detected in more than 25% of the serum pools in Phase Three that were detected in fewer than 25% in Phase One (PCB 105, 126, 156/157, 167, 169, 172, 177, 178, 189, 195, 206 and 209). Only PCB 170 increased in concentration from Phase One to Phase Three (38%) while PCB 183 and 187 decreased in concentration from Phase One to Phase Three (24% and 20%, respectively, based on lipid adjusted concentrations). Concentrations of 1,2,3,6,7,8-HxCDD; 1,2,3,4,6,7,8-HpCDD; and 1,2,3,4,6,7,8,9-OCDD decreased in maternal serum from Phase One to Phase Three by 31%, 22%, and 22%, respectively, based on wet weight concentrations. Concentrations of 2,3,4,7,8-PeCDF; 1,2,3,4,7,8-HxCDF (wet weight only); and 1,2,3,6,7,8-HxCDF (wet weight only) increased from Phase One to Phase Three by 72%, 37%, and 30%, respectively.

Concentrations of legacy and emerging chemicals were measured across the first three phases of the ABP, providing baseline levels for the province. Results from the ABP have highlighted chemicals that should be investigated in the future to further evaluate temporal trends, such as PFAS and PBDEs, which have shown declining levels from Phase One to Phase Three. Cotinine levels in Phase One showed that smoking is still a public health concern for pregnant women and levels in Phase Four will be compared to those in Phase One. Concentrations of the measured chemicals are generally similar or lower than those detected in other similar biomonitoring studies and the concentrations are not at levels of concern.

5. Conclusions

Since 2005, the ABP has provided complementary age and geography-stratified chemical exposure information on specific populations and has filled data gaps relevant to public health for the province of Alberta. The population is still being exposed to chemicals that have been banned in the province for decades, in addition to exposure to emerging chemicals, such as phthalates and parabens. The exposure of children and newborns differs to that of maternal exposure, highlighting the importance of evaluating levels in vulnerable populations. The ABP has adapted to changing exposures over time by adjusting analysis suites and has branched out to specifically investigate the effect of a new federal policy on cannabis. In addition to providing baseline level exposures, the data from the earlier phases has been used for health risk assessments by Health Canada (Health Canada, 2016, 2019b) and to generate new research hypotheses. This program has set up a framework in Alberta that can be used as a model for other regions.

Declaration of competing interest

None.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.113990.

References

Agency for Toxic Substances and Disease Registry, 2005. Toxicological Profile for Zinc. U.S. Department of Health and Human Services, Atlanta, GA, 20. https://www.atsdr.cdc.gov/toxprofiles/tp60.pdf. (Accessed 29 October 2020).

Agency for Toxic Substances and Disease Registry, 2007. Toxicological Profile for Arsenic. U.S. Department of Health and Human Services, Atlanta, GA, 20. http://www.atsdr.cdc.gov/toxprofiles/tp2.pdf. (Accessed 29 October 2020).

Apel, P., Angerer, J., Wilhelm, M., Kolossa-Gehring, M., 2017. New HBM values for emerging substances, inventory of reference and HBM values in force, and working principles of the German human biomonitoring commission. Int. J. Hyg Environ. Health 220 (2), 152–166. https://doi.org/10.1016/j.ijheh.2016.09.007.

Atkinson, C., Frankenfeld, C.L., Lampe, J.W., 2005. Gut bacterial metabolism of the soy isoflavone daidzein: exploring the relevance to human health. Exp. Biol. Med. 230 (3), 155–170. https://doi.org/10.1177/153537020523000302.

Aylward, L.L., Hays, S.M., Gagné, M., Nong, A., Krishnan, K., 2010. Biomonitoring equivalents for hexachlorobenzene. Regul. Toxicol. Pharmacol. 58 (1), 25–32. https://doi.org/10.1016/j.yrtph.2010.06.003.

Aylward, L.L., Hays, S.M., Kirman, C.R., Marchitti, S.A., Kenneke, J.F., English, C., Mattison, D.R., Becker, R.A., 2014. Relationships of chemical concentrations in maternal and cord blood: a review of available data. J. Toxicol. Environ.l Health - B: Crit. Rev. 17 (3), 175–203. https://doi.org/10.1080/10937404.2014.884956.

Aylward, L.L., Seiber, J.N., Hays, S.M., 2015. California biomonitoring data: comparison to NHANES and interpretation in a risk assessment context. Regul. Toxicol. Pharmacol. 73 (3), 875–884. https://doi.org/10.1016/j.yrtph.2015.10.002.

Banderali, G., Martelli, A., Landi, M., Moretti, F., Betti, F., Radaelli, G., Lassandro, C., Verduci, E., 2015. Short and long term health effects of parental tobacco smoking during pregnancy and lactation: a descriptive review. J. Transl. Med. 13 (1), 327. https://doi.org/10.1186/s12967-015-0690-y.

Bellinger, D.C., 2005. Teratogen update: lead and pregnancy. Birth Defects Res. Part A Clin. Mol. Teratol. 73 (6), 409–420. https://doi.org/10.1002/bdra.20127.

Bergen, H.T., 2006. Exposure to smoke during development: fetal programming of adult disease. Tob. Induc. Dis. 3 (August) https://doi.org/10.1186/1617-9625-3-2-5.

Berger, K., Hyland, C., Ames, J.L., Mora, A.M., Huen, K., Eskenazi, B., Holland, N., Harley, K.G., 2021. Prenatal exposure to mixtures of phthalates, parabens, and other phenols and obesity in five-year-olds in the CHAMACOS cohort. Int. J. Environ. Res. Publ. Health 18 (4). https://doi.org/10.3390/ijerph18041796.

Bocca, B., Mattei, D., Pino, A., Alimonti, A., 2010. Italian Network for human biomonitoring of metals: preliminary results from two regions. Ann. Inst. Super. Santa 46 (3), 259–265.

Caudill, S.P., 2010. Characterizing populations of individuals using pooled samples. J. Expo. Sci. Environ. Epidemiol. 20 (1), 29–37. http://10.0.4.14/jes.2008.72.

Caudill, S.P., 2012. Use of pooled samples from the national health and nutrition examination survey. Stat. Med. 31 (27), 3269–3277. https://doi.org/10.1002/sim.5341.

CDC, 2009. Fourth Report on Human Exposure to Environmental Chemicals. Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, GA.

CDC, 2018a. Fourth National Report on Human Exposure to Environmental Chemicals, Updated Tables, March 2018, Volume Two. Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, GA.

CDC, 2018b. Improving the Collection and Management of Human Samples Used for Measuring Environmental Chemicals and Nutrition Indicators Version 1.3 - March 2018. Centers for Disease Control and Prevetion, Deprtment of Health and Human Services, Atlanta, GA, 21. https://www.cdc.gov/biomonitoring/pdf/Human_Sample_Collection-508.pdf. (Accessed 16 March 2021).

CDC, 2019b. Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, February 2019, Volume One. Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta, GA.

CDC, 2021. Fourth National Report on Human Exposure to Environmental Chemicals Updated Tables, March 2021, Volume One. Centers for Disease Control and Prevention, Department of Health and Human Services, Atlanta,GA.

CDC (Centers for Disease Control and Prevention), 2017. Biomonitoring Summary Phytoestrogens. https://www.cdc.gov/biomonitoring/Phytoestrogens_BiomonitoringSummary.html. (Accessed 5 April 2022).

CDC (Centers for Disease Control and Prevention), 2019a. State biomonitoring programs. https://www.cdc.gov/biomonitoring/state_grants.html. (Accessed 3 August 2021).

Černá, M., Puklová, V., Hanzlíková, L., Sochorová, L., Kubínová, R., 2017. 25 Years of HBM in the Czech republic. Int. J. Hyg Environ. Health 220 (2), 3–5. Part A). https://doi.org/10.1016/j.ijheh.2016.08.004.

Cornelius, M.D., Day, N.L., 2009. Developmental consequences of prenatal tobacco exposure. Curr. Opin. Neurol. 22 (2), 121–125. https://doi.org/10.1097/WCO.0b013e328326f6dc.

EPA, 2008. Method 537. Determination of Selected Perfluorinated Alkyl Acids in Drinking Water by Solid Phase Extraction and Liquid Chromatography/Tandem Mass Spectrometry (LC/MS/MS). U.S. Environmental Protection Agency, Cincinnati, OH, 22. https://cfpub.epa.gov/si/si_public_file_download.cfm?p_download_id=525468&Lab=NERL. (Accessed 2 May 2022).

Eriksson, U., Mueller, J.F., Toms, L.-M.L., Hobson, P., Kärrman, A., 2017. Temporal trends of PFSAs, PFCAs and selected precursors in Australian serum from 2002 to 2013. Environ. Pollut. 220, 168–177. https://doi.org/10.1016/j.envpol.2016.09.036.

Filella, M., Belzile, N., Chen, Y.-W., 2013. Human exposure to antimony. IV. Contents in human blood. Crit. Rev. Environ. Sci. Technol. 43 (19), 2071–2105. https://doi.org/10.1080/10643389.2013.790741.

Fillol, C., Oleko, A., Saoudi, A., Zeghnoun, A., Balicco, A., Gane, J., Rambaud, L., Leblanc, A., Gaudreau, É., Marchand, P., Le Bizec, B., Bouchart, V., Le Gléau, F., Durand, G., Denys, S., 2021. Exposure of the French population to bisphenols, phthalates, parabens, glycol ethers, brominated flame retardants, and perfluorinated compounds in 2014–2016: results from the esteban study. Environ. Int. 147, 106340. https://doi.org/10.1016/j.envint.2020.106340.

Fisher, M., Arbuckle, T.E., Liang, C.L., LeBlanc, A., Gaudreau, É., Foster, W.G., Haines, D., Davis, K., Fraser, W.D., 2016. Concentrations of persistent organic pollutants in maternal and cord blood from the maternal-infant research on environmental chemicals (MIREC) cohort study. Environ. Health 15 (1), 59. https://doi.org/10.1186/s12940-016-0143-y.

Forns, J., Mandal, S., Iszatt, N., Polder, A., Thomsen, C., Lyche, J.L., Stigum, H., Vermeulen, R., Eggesbø, M., 2016. Novel application of statistical methods for analysis of multiple toxicants identifies DDT as a risk factor for early child behavioral problems. Environ. Res. 151, 91–100. https://doi.org/10.1016/j.envres.2016.07.014.

Forray, A., Merry, B., Lin, H., Ruger, J.P., Yonkers, K.A., 2015. Perinatal substance use: a prospective evaluation of abstinence and relapse. Drug Alcohol Depend. 150, 147–155. https://doi.org/10.1016/j.drugalcdep.2015.02.027.

Fox, M.A., Latshaw, M., 2013. State public health laboratory biomonitoring programs: implementation and early accomplishments. J. Environ. Health 75 (6), 90–94. https://ezproxy.lib.ucalgary.ca/login?url=https://search.ebscohost.com/login.aspx?direct=true&db=rzh&AN=84447290&site=ehost-live.

Gabos, S., Cheperdak, L., Kinniburgh, D., MacDonald, A., Lyon, M., Braakman, S., Lee, B., Hrudey, S., Le, X., Li, X.F., Mandal, R., Martin, J.W., Schopflocher, D., 2010. Alberta biomonitoring program chemicals in serum of children in southern Alberta 2004-2006 influence of age and comparison to pregnant women march 2010. Alberta health and wellness. Edmonton, AB 21. https://open.alberta.ca/dataset/c14199a8-e94a-4111-82fd-9f46c6382cd3/resource/eb93485b-78bd-4fb3-b96e-823ab31da1c4/download/chemical-biomonitoring-2010.pdf. (Accessed 16 February 2021).

Gabos, S., Zemanek, M., Cherperdak, L., Kinniburgh, D., Lee, B., Hrudey, S., Le, X., Li, X. F., Mandal, R., Martin, J.W., Schopflocher, D., 2008. Alberta Biomonitoring Program Chemicals in Serum of Pregnant Women in Alberta. Alberta Health and Wellness, Edmonton, AB, 21. https://open.alberta.ca/dataset/c5158a5c-b550-4ece-ab5e-86166c91738a/resource/424b3749-2f3a-4f71-9aaf-5a71be7038df/download/Chemical-Biomonitoring-2008.pdf. (Accessed 16 February 2021).

Gabrhelik, R., Mahic, M., Lund, I.O., Bramness, J., Selmer, R., Skovlund, E., Handal, M., Skurtveit, S., 2021. Cannabis use during pregnancy and risk of adverse birth outcomes: a longitudinal cohort study. Eur. Addiction Res. 27 (2), 131–141. https://doi.org/10.1159/000510821.

Garbino-Pronczuk, J., 2005. Children's Health and the Environment a Global Perspective. World Health Organization, Geneva, CH, 21. https://apps.who.int/iris/bitstream/handle/10665/43162/9241562927_eng.pdf?sequence=1&isAllowed=y. (Accessed 16 March 2021).

Genuis, S.J., Beesoon, S., Birkholz, D., Lobo, R.A., 2012. Human excretion of bisphenol A: blood, urine, and sweat (BUS) study. J. Environ. Public Health, 185731. https://doi.org/10.1155/2012/185731, 2012.

Gibson, E.A., Siegel, E.L., Eniola, F., Herbstman, J.B., Factor-Litvak, P., 2018. Effects of polybrominated diphenyl ethers on child cognitive, behavioral, and motor development. Int. J. Environ. Res. Publ. Health 15 (8). https://doi.org/10.3390/ijerph15081636.

Gilles, L., Govarts, E., Rambaud, L., Vogel, N., Castaño, A., Esteban López, M., Rodriguez Martin, L., Koppen, G., Remy, S., Vrijheid, M., Montazeri, P., Birks, L., Sepai, O., Stewart, L., Fiddicke, U., Loots, I., Knudsen, L.E., Kolossa-Gehring, M., Schoeters, G., 2021. HBM4EU combines and harmonises human biomonitoring data across the EU, building on existing capacity – the HBM4EU survey. Int. J. Hyg Environ. Health 237, 113809. https://doi.org/10.1016/j.ijheh.2021.113809.

Govarts, E., Nieuwenhuijsen, M., Schoeters, G., Ballester, F., Bloemen, K., de Boer, M., Chevrier, C., Eggesbø, M., Guxens, M., Krämer, U., Legler, J., Martínez, D., Palkovicova, L., Patelarou, E., Ranft, U., Rautio, A., Petersen, M.S., Slama, R., Stigum, H., Toft, G., Trnovec, T., Vandentorren, S., Weihe, P., Kuperus, N.W., Wilhelm, M., Wittsiepe, J., Bonde, J.P., 2012. Birth weight and prenatal exposure to polychlorinated biphenyls (PCBs) and dichlorodiphenyldichloroethylene (DDE): a meta-analysis within 12 European birth cohorts. Environ. Health Perspect. 120 (2), 162–170. https://doi.org/10.1289/ehp.1103767.

Government of Alberta, 2009. Alberta reproductive health pregnancies & births surveillance report 2009. Edmonton, AB 21. https://open.alberta.ca/dataset/06456e95-348e-403f-8c04-5eb79391d252/resource/77f0f8d6-1405-4b6b-a78c-6710c1dc7bd4/download/reproductive-health-2009.pdf. (Accessed 16 February 2021).

Government of Alberta, 2020. AEPHIN Alberta environmental public health information Network Alberta biomonitoring, 21. http://aephin.alberta.ca/biomonitoring/. (Accessed 16 February 2021).

Government of Alberta, 2021. Population statistics, 21. https://www.alberta.ca/population-statistics.aspx. (Accessed 8 September 2021).

Government of Canada, 2013. Toxic substances list: DDT, 21. https://www.canada.ca/en/environment-climate-change/services/management-toxic-substances/list-canadian-environmental-protection-act/dichlorodiphenyltrichloroethane.html. (Accessed 6 July 2021).

Government of Saskatchewan, 2019a. Biomonitoring in northern saskatchewan, 21. https://www.saskatchewan.ca/residents/environment-public-health-and-safety/biomonitoring-in-northern-saskatchewan. (Accessed 3 April 2021).

Government of Saskatchewan, 2019b. Northern Saskatchewan Prenatal Biomonitoring Study Technical Summary Report. Ministry of Health, Regina, SK, 22. https://pubsaskdev.blob.core.windows.net/pubsask-prod/112048/Northern-Saskatchewan-Prenatal-Biomonitoring-Study-Technical%252BSummary-Report.pdf. (Accessed 3 January 2022).

Hays, S.M., Macey, K., Poddalgoda, D., Lu, M., Nong, A., Aylward, L.L., 2016. Biomonitoring equivalents for molybdenum. Regul. Toxicol. Pharmacol. 77, 223–229. https://doi.org/10.1016/j.yrtph.2016.03.004.

HBM4EU, n.d. Online Library, 22. https://www.hbm4eu.eu/online-library/. (Accessed 26 April 2022).

Health Canada, 2010. Report on human biomonitoring of environmental chemicals in Canada results of the Canadian health measures survey cycle 1 (2007–2009). Ottawa, ON 20. https://www.canada.ca/content/dam/hc-sc/migration/hc-sc/ewh-semt/alt_formats/hecs-sesc/pdf/pubs/contaminants/chms-ecms/report-rapport-eng.pdf. (Accessed 29 October 2020).

Health Canada, 2013. Second report on human biomonitoring of environmental chemicals in Canada results of the Canadian health measures survey cycle 2 (2009–2011) April 2013. Ottawa, ON 20. http://ocrcnew.wpengine.com/wp-content/uploads/2013/05/2ndHumanBiomonitoringReport.pdf. (Accessed 29 October 2020).

Health Canada, 2015. Canadian health measures survey (CHMS) data user guide: cycle 3 july 2015. Ottawa, ON 21. https://usermanual.wiki/Pdf/CHMSUserGuideCycle3E.484319040/view. (Accessed 3 April 2021).
Health Canada, 2016. Draft screening assessment boric acid, its salts and its precursors. Ottawa, ON 22. http://www.ec.gc.ca/ese-ees/2A581398-E30E-4A57-925A-3F97A2EF4EB9/DSAR_Boric acid_EN.pdf. (Accessed 3 August 2022).
Health Canada, 2017. Fourth report on human biomonitoring of environmental chemicals in Canada results of the Canadian health measures survey cycle 4 (2014–2015) august 2017. Ottawa, ON. https://www.canada.ca/content/dam/hc-sc/documents/services/environmental-workplace-health/reports-publications/environmental-contaminants/fourth-report-human-biomonitoring-environmental-chemicals-canada/fourth-report-human-biomonitoring-environmental-chemicals-canada-eng.pdf. (Accessed 16 February 2021).
Health Canada, 2019a. Fifth report on human biomonitoring of environmental chemicals in Canada results of the Canadian health measures survey cycle 5 (2016–2017) november 2019. Ottawa, ON. https://www.canada.ca/content/dam/hc-sc/documents/services/environmental-workplace-health/reports-publications/environmental-contaminants/fifth-report-human-biomonitoring/pub1-eng.pdf. (Accessed 16 February 2021).
Health Canada, 2019b. Draft screening assessment zinc and its compounds. Ottawa, ON 22. https://www.canada.ca/content/dam/eccc/documents/pdf/pded/zinc/Draft-screening-assessment-zinc-compound.pdf. (Accessed 3 August 2022).
Health Canada, 2020. Report on human biomonitoring of environmental chemicals in pooled samples results of the Canadian health measures survey cycles 1 (2007–2009), 3 (2012–2013), 4 (2014–2015) and 5 (2016–2017) december 2020. Ottawa, ON 20. https://www.canada.ca/content/dam/hc-sc/images/services/environmental-workplace-health/reports-publications/environmental-contaminants/report-human-biomonitoring-environmental-chemicals-pooled-samples/27-20-2746-01-human-biomonitoring-final-en.pdf. (Accessed 21 December 2020).
Heffernan, A.L., Aylward, L.L., Toms, L.-M.L., Sly, P.D., Macleod, M., Mueller, J.F., 2013. Pooled biological specimens for human biomonitoring of environmental chemicals: opportunities and limitations. J. Expo. Sci. Environ. Epidemiol. 24 (November), 225. https://doi.org/10.1038/jes.2013.76.
Henriksen, L.S., Mathiesen, B.K., Assens, M., Krause, M., Skakkebæk, N.E., Juul, A., Andersson, A.-M., Hart, R.J., Newnham, J.P., Keelan, J.A., Pennell, C., Main, K.M., Frederiksen, H., 2020. Use of stored serum in the study of time trends and geographical differences in exposure of pregnant women to phthalates. Environ. Res. 184, 109231. https://doi.org/10.1016/j.envres.2020.109231.
Hukkanen, J., Jacob, P., Benowitz, N.L., 2005. Metabolism and disposition kinetics of nicotine. Pharmacol.l Rev. 57 (1), 79–115. LP. http://pharmrev.aspetjournals.org/content/57/1/79.abstract.
Joas, R., Casteleyn, L., Biot, P., MaKolossa-Gehring, M., Castano, A., Angerer, J., Schoeters, G., Sepai, O., Knudsen, L.E., Joas, A., Horvat, M., Bloemen, L., 2012. Harmonised human biomonitoring in europe: activities towards an EU HBM framework. Int. J. Hyg Environ. Health 215 (2), 172–175. https://doi.org/10.1016/j.ijheh.2011.08.010.
Kärrman, A., Mueller, J.F., van Bavel, B., Harden, F., Toms, L.-M.L., Lindström, G., 2006. Levels of 12 perfluorinated chemicals in pooled Australian serum, collected 2002−2003, in relation to age, gender, and region. Environ. Sci. Technol. 40 (12), 3742–3748. https://doi.org/10.1021/es060301u.
Kato, K., Calafat, A.M., Wong, L.-Y., Wanigatunga, A.A., Caudill, S.P., Needham, L.L., 2009. Polyfluoroalkyl compounds in pooled sera from children participating in the national health and nutrition examination survey 2001−2002. Environ. Sci. Technol. 43 (7), 2641–2647. https://doi.org/10.1021/es803156p.
Kirman, C.R., Aylward, L.L., Hays, S.M., Krishnan, K., Nong, A., 2011. Biomonitoring equivalents for DDT/DDE. Regul. Toxicol. Pharmacol. 60 (2), 172–180. https://doi.org/10.1016/j.yrtph.2011.03.012.
Krishnan, K., Adamou, T., Aylward, L.L., Hays, S.M., Kirman, C.R., Nong, A., 2011. Biomonitoring equivalents for 2,2′,4,4′,5-pentabromodiphenylether (PBDE-99). Reg. Toxicol. Pharmacol. 60 (2), 165–171. https://doi.org/10.1016/j.yrtph.2011.03.011.
Lee, J.W., Lee, C.K., Moon, C.S., Choi, I.J., Lee, K.J., Yi, S.-M., Jang, B.-K., Yoon, B.J., Kim, D.S., Peak, D., Sul, D., Oh, E., Im, H., Kang, H.S., Kim, J., Lee, J.-T., Kim, K., Park, K.L., Ahn, R., Park, S.H., Kim, S.C., Park, C.-H., Lee, J.H., 2012. Korea national survey for environmental pollutants in the human body 2008: heavy metals in the blood or urine of the Korean population. Int. J. Hyg Environ. Health 215 (4), 449–457. https://doi.org/10.1016/j.ijheh.2012.01.002.
Laboratories, Mayo Clinic, 2020a. Test ID: AL aluminum, serum, 20. https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/8373. (Accessed 30 October 2020).
Laboratories, Mayo Clinic, 2020b. Test ID: CRS chromium, serum, 20. https://www.mayocliniclabs.com/test-catalog/Clinical+and+Interpretive/8638. (Accessed 29 October 2020).
MIREC, 2022. Maternal-infant research on environmental chemicals what is MIREC, 22. https://www.mirec-canada.ca/en/. (Accessed 26 April 2022).
Nghiem, G.T.T., Nishijo, M., Pham, T.N., Ito, M., Pham, T.T., Tran, A.H., Nishimaru, H., Nishino, Y., Nishijo, H., 2019. Adverse effects of maternal dioxin exposure on fetal brain development before birth assessed by neonatal electroencephalography (EEG) leading to poor neurodevelopment; a 2-year follow-up study. Sci. Total Environ. 667 (June), 718–729. https://doi.org/10.1016/j.scitotenv.2019.02.395.
Olsen, G.W., Church, T.R., Hansen, K.J., Burris, J.M., Butenhoff, J.L., Mandel, J.H., Zobel, L.R., 2004. Quantitative evaluation of perfluorooctanesulfonate (PFOS) and other fluorochemicals in the serum of children. J. Child. Health 2 (1), 53–76. http://10.0.12.37/15417060490447378.
Perez-Gomez, B., Pastor-Barriuso, R., Cervantes-Amat, M., Esteban, M., Ruiz-Moraga, M., Aragones, N., Pollan, M., Navarro, C., Calvo, E., Roman, J., Lopez-Abente, G., Castano, A., 2013. BIOAMBIENT.ES study protocol: rationale and design of a cross-sectional human biomonitoring survey in Spain. Environ. Sci. Pollut. Res. 20 (2), 1193–1202. https://doi.org/10.1007/s11356-012-1320-3.
Poddalgoda, D., Macey, K., Assad, H., Krishnan, K., 2017. Development of biomonitoring equivalents for barium in urine and plasma for interpreting human biomonitoring data. Regul. Toxicol. Pharmacol. 86, 303–311. https://doi.org/10.1016/j.yrtph.2017.03.022.
Poddalgoda, D., Macey, K., Hancock, S., 2019. Derivation of biomonitoring equivalents (BE values) for zinc. Regul. Toxicol. Pharmacol. 106 (August), 178–186. https://doi.org/10.1016/j.yrtph.2019.04.018.
Rodushkin, I., Ödman, F., 2001. Assessment of the contamination from devices used for sampling and storage of whole blood and serum for element analysis. J. Trace Elem. Med. Biol. 15 (1), 40–45. https://doi.org/10.1016/S0946-672X(01)80025-0.
Rodushkin, I., Ödman, F., Olofsson, R., Axelsson, M.D., 2000. Determination of 60 elements in whole blood by sector field inductively coupled plasma mass spectrometry. J. Anal. At. Spectrom. 15 (8), 937–944. https://doi.org/10.1039/b003561k.
Schmitt, E., Metzler, M., Jonas, R., Dekant, W., Stopper, H., 2003. Genotoxic activity of four metabolites of the soy isoflavone daidzein. Mutat. Res. Genet. Toxicol. Environ. Mutagen 542 (1), 43–48. https://doi.org/10.1016/j.mrgentox.2003.08.003.
Schoeters, G., Govarts, E., Bruckers, L., Den Hond, E., Nelen, V., De Henauw, S., Sioen, I., Nawrot, T.S., Plusquin, M., Vriens, A., Covaci, A., Loots, I., Morrens, B., Coertjens, D., Van Larebeke, N., De Craemer, S., Croes, K., Lambrechts, N., Colles, A., Baeyens, W., 2017. Three cycles of human biomonitoring in flanders - time trends observed in the flemish environment and health study. Int. J. Hyg Environ. Health 220 (2), 36–45. https://doi.org/10.1016/j.ijheh.2016.11.006. , A).
Schulz, C., Conrad, A., Becker, K., Kolossa-Gehring, M., Seiwert, M., Seifert, B., 2007. Twenty years of the German environmental survey (GerES): human biomonitoring - temporal and spatial (west Germany/East Germany) differences in population exposure. Int. J. Hyg Environ. Health 210 (3–4), 271–297. https://doi.org/10.1016/j.ijheh.2007.01.034.
Sexton, K., Needham, L.L., Pirkle, J.L., 2004. Human biomonitoring of environmental chemicals. Am. Sci. 92 (1), 38–45. http://ezproxy.lib.ucalgary.ca/login?url=http://search.ebscohost.com/login.aspx?direct=true&db=a9h&AN=11701070&site=ehost-live.
Silva, M.J., Samandar, E., Preau Jr., J.L., Reidy, J.A., Needham, L.L., Calafat, A.M., 2005. Automated solid-phase extraction and quantitative analysis of 14 phthalate metabolites in human serum using isotope dilution-high-performance liquid chromatography-tandem mass spectrometry. J. Anal. Toxicol. 29 (8), 819–824. https://doi.org/10.1093/jat/29.8.819.
Singh, K., Nong, A., Feeley, M., Chan, H.M., 2019. Development of biomonitoring equivalents for chlordane and toxaphene with application to the general Canadian population. Regul. Toxicol. Pharmacol. 106 (August), 262–269. https://doi.org/10.1016/j.yrtph.2019.05.015.
Subramoney, S., Eastman, E., Adnams, C., Stein, D.J., Donald, K.A., 2018. The early developmental outcomes of prenatal alcohol exposure: a review. Front. Neurol. 9, 1108. https://doi.org/10.3389/fneur.2018.01108.
Toms, L.-M.L., Sjödin, A., Hobson, P., Harden, F.A., Aylward, L.L., Mueller, J.F., 2018. Temporal trends in serum polybrominated diphenyl ether concentrations in the Australian population, 2002–2013. Environ. Int. 121, 357–364. https://doi.org/10.1016/j.envint.2018.09.014.
United Nations, 2017. Mirex: supporting documentation provided by Canada. In: Rotterdam Convention on the Prior Informed Consent Procedure for Certain Hazardous Chemicals and Pesticides in International Trade Chemical Review Committee. United Nations, Rotterdam, NL, 22. file:///C:/Users/amacdona/AppData/Local/Temp/UNEP-FAO-RC-CRC.2-16-Add.1.En.pdf. (Accessed 1 July 2022).
Valcke, M., Karthikeyan, S., Walker, M., Gagné, M., Copes, R., St-Amand, A., 2020. Regional variations in human chemical exposures in Canada: a case study using biomonitoring data from the Canadian health measures survey for the provinces of quebec and ontario. Int. J. Hyg Environ. Health 225, 113451. https://doi.org/10.1016/j.ijheh.2020.113451.
Ward, C.D., Williams, R.J., Mullenix, K., Syhapanha, I., Jones, R.L., Caldwell, K., 2018. Trace metals screening process of devices used for the collection, analysis, and storage of biological specimens. At. Spectrosc. 39 (6), 219–228. https://doi.org/10.46770/AS.2018.06.001.
Waterfield, G., Rogers, M., Grandjean, P., Auffhammer, M., Sunding, D., 2020. Reducing exposure to high levels of perfluorinated compounds in drinking water improves reproductive outcomes: evidence from an intervention in Minnesota. Environ. Health 19 (1). https://doi.org/10.1186/s12940-020-00591-0.
World Health Organization, 2005. Mercury in drinking water, 20. https://www.who.int/water_sanitation_health/dwq/chemicals/mercuryfinal.pdf. (Accessed 30 October 2020).
Zeghnoun, A., Pascal, M., Fréry, N., Sarter, H., Falq, G., Focant, J.F., Eppe, G., 2007. Dealing with the non-detected and non-quantified data. The example of the serum dioxin data in the French dioxin and incinerators study. Organohalogen Compd. 69, 2288–2291. http://dioxin20xx.org/wp-content/uploads/pdfs/2007/07-261.pdf.

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Microbial contamination in surface water and potential health risks for peri-urban farmers of the Bengal delta

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ABSTRACT

Ensuring safe irrigation practices is vital to sustaining food production in water-scarce delta areas. Bangladesh and many other developing countries discharge untreated wastewater into their surrounding surface water bodies, serving as the primary irrigation source. This indirect irrigation of wastewater is believed to pose threats to the farmers, consumers and market vendors and may also affect crop and soil quality. To assess the risk, peri-urban farmers who use surrounding water bodies of Khulna city, Bangladesh, for crop irrigation were selected for the study. The microbial and heavy metal concentrations were measured in water samples collected from various locations over different seasons. For heavy metals As, Co, Ni, Cd, Cr, Cu and Pb, concentrations were below the detection limit, whereas Al, Fe, Mn, Ti and Zn were present but below the FAO recommendation limit for safe irrigation. The mean concentrations of microbial parameters were above the thresholds of WHO guidelines for crop irrigation intended for human consumption. Significant temporal variations in Faecal Coliform, *E. coli* and *Enterococcus* concentrations in the water samples were observed. The annual risk of infection for farmers was determined using the screening-level Quantitative Microbial Risk Assessment (QMRA). The results indicated that the annual probability of infection with pathogenic *E. coli* in different seasons ranges between 5×10^{-3} to 5×10^{-2}, above the WHO's acceptable threshold for annual risk of infection for safe water reuse in agriculture. During the farmers' survey, around 45% reported health-related issues and more than 26% reported suffering from water-borne diseases after getting in contact with polluted surface water. This illustrates the actuality of the risks in practice. To ensure safe irrigation, the health risks need to be reduced below the acceptable limits. Suggested technical measures include adequate treatment of wastewater before disposal into rivers and access to protective equipment for farmers. This should be complemented by raising awareness through education programs among farmers to reduce accidental ingestion.

1. Introduction

Global water scarcity is aggravated by the growing water demand caused by increasing populations and climate change (Mekonnen and Hoekstra, 2016). This issue is more clearly visible in the urbanizing delta such as the Bengal delta, which is also severely confronted with freshwater scarcity (Murshed and Kaluarachchi, 2018). In most situations, current water resource management practices in urban areas are linear and waste valuable resources such as water and nutrients. Though some countries have close to 100% coverage in collecting and treating urban wastewaters, only around 63% of the total wastewater generated globally is collected and 48% is discharged without treatment, which deteriorates the surface water quality (Jones et al., 2021; Kookana et al., 2020). Urban water reuse, in general, has been practiced globally to make this water reusable for irrigation and to mitigate the impact of freshwater scarcity on food production.

The use of wastewater for irrigation has gained attention during the last decade of the twentieth century because of the growing demands for irrigation and the raising concerns over the health effects to farmers and consumers (Jaramillo and Restrepo, 2017). For decades, farmers in Jordan and Israel have utilized wastewater for agricultural production due to the minimal local availability of water resources (Angelakis and Gikas, 2014; Carr et al., 2011). The examples in these countries

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List of abbreviations

CFU	Colony Forming Unit
DALYs	Disability Adjusted Life Years
FAO	Food and Agriculture Organization
FIB	Faecal Indicator Bacteria
GoB	Government of Bangladesh
MF	Membrane Filtration
QMRA	Quantitative Microbial Risk Assessment
WHO	World Health Organization
TC	Total coliform
FC	Faecal coliform
E. coli	*Escherichia coli*
Al	Aluminum
As	Arsenic
Cd	Cadmium
Cr	Chromium
Co	Cobalt
Cu	Copper
Fe	Iron
Pb	Lead
Mn	Manganese
Ni	Nickel
Ti	Titanium
Zn	Zinc

demonstrate good treated wastewater irrigation practices that also minimize the health risks. But these practices are not yet applied in many regions of the world. The use of untreated wastewater for irrigation can negatively impact human health as well as the quality of the environment (including soils) and crops. Wastewater commonly contains excreta-related pathogens (e.g. bacteria, viruses, protozoa and helminths) and toxic chemicals, such as heavy metals and micropollutants (e.g. pesticides, household chemicals, pharmaceutical residues) (Drechsel et al., 2010a; Gross et al., 2015; Jiménez and Asano, 2008; Mojid and Wyseure, 2013). In developing countries especially, untreated wastewater is discharged into the natural surface water streams which are major sources for irrigating crops. As a result, farmers and consumers are regularly exposed to unknown chemical and biological pollution.

To minimize the health risks due to the increasing unplanned and indirect wastewater irrigation practices, several risk assessments such as sanitary inspection, risk matrix, Quantitive Microbial Risk Assessment (QMRA) and risk mitigation frameworks such as Stockholm framework, sanitation safety planning, multiple-barrier approach have been drafted and used (WHO, 2006). These approaches are briefly discussed further in the manuscript. Sanitary inspection is an on-site visual evaluation tool, whereas the risk matrix approach provides a semi-quantitative and qualitative evaluation of the likelihood of a hazardous event. QMRA is a tool used for predicting the risk of infection or illness rates of humans exposed to pathogens, by using ingestion probability estimations and dose-response models based on a given population (Ferrer et al., 2012; Haas et al., 1999). QMRA is the formal WHO-approved quantitative risk assessment approach that integrates the scientific knowledge on the infectious effects of pathogens present in the water (WHO, 2016). The numerical outcomes of QMRA bring more specific insights useful for risk management as compared to other methods such as sanitary inspection and risk matrix (WHO, 2016). Though less sensitive, QMRA is less costly and less time-consuming than epidemiological studies and therefore, has become a preferred method for application (Ferrer et al., 2012). However, QMRA is a standardized model that is only applicable to a limited range of pathogens and is not yet developed to address the full range of pathogens actually present in wastewater, restricting its wider use for risk assessment (Hamilton and Haas, 2016). The Stockholm framework improved health-related guidelines and standards through a coherent system (WHO, 2006). Similarly, the multiple barrier approach is a risk mitigation framework that combines technical and non-technical strategies for risk mitigation and complements the sub-optimal wastewater treatment, which is seen as the best possible approach to reduce risks (Bos et al., 2010; Keraita et al., 2008; WHO, 2006). The multiple barrier approach stretches from wastewater generation to the consumption of irrigated crops (i.e. from farm to fork) and is vital for strategizing safe water reuse practices. This is crucial, especially for many urbanized deltas in developing and emerging economies where untreated urban wastewaters are regularly dumped into the rivers flowing to the sea. Meanwhile the very same water is also needed for irrigation to combat with the rising salinization.

Khulna: the third largest city of Bangladesh and has been taken as an example to assess the health risks (to later define risk mitigation) of the irrigation practices in urbanized deltas. The presence of elevated pathogen levels in surface water bodies due to anthropogenic activities has been reported in the coastal region of Bangladesh (Islam et al., 2017, 2018a, 2018b). Peri-urban agriculture in the delta area contributes to regional food production and surface water is the primary source of irrigation. Peri-urban farmers around the country have reported skin irritation, itchiness in the hands and legs while working with the surface water (Mojid et al., 2010). These effects are suspected to be related to untreated wastewater discharge in surface waters. Aside from skin contact, there is also a high probability that farmers and their family members have had contact with the wastewater pollutants through ingestion or aerosol inhalation (An et al., 2007). Several studies focused on assessing the health risks associated with river bathing or urban flooding; however, risk assessment related to indirect wastewater irrigation is scarce (Islam and Islam, 2020; Mark et al., 2018). Thus, there is a need to investigate the actual wastewater-related pollutant concentrations in surface waters and link these to actual risks for farmers as a base to design adequate risk mitigation measures. Faecal Indicator Bacteria (FIB) are widely used to understand the presence of pathogenic microorganisms in water (WHO, 2002). *E. coli*, faecal coliforms and faecal streptococci (with *enterococci* as subgroup) are commonly used as FIB (Islam et al., 2017). FIB could be useful to understand the microbial water quality and to formulate necessary risk mitigation strategies (Islam et al., 2017; Maimon et al., 2010; Teklehaimanot et al., 2014; Wu et al., 2011).

The first step in any set of measures is to mitigate risks due to direct or indirect use of wastewater for irrigation. This can first be approached by assessing the risks associated with pathogens, heavy metals and other (organic) chemical pollutants. In this study, the first steps of pollutant characterization were performed based on the local laboratory capacity. Therefore a set of selected microbial pathogens as indicators for domestic wastewater pollution and a suite of heavy metals as an indicator of industrial pollution is discussed in this study. The microbial contamination in surface water was evaluated and potential health risk for farmers was assessed assuming continuous exposure to pollutants in wastewater indirectly used for irrigation. Additionally, the farmers' risk perception towards the current irrigation practice was analyzed to address the required management strategies, including both technical and non-technical measures to reduce the risk of infection.

2. Methodology

2.1. Study area and sampling sites

Khulna City is positioned on the banks of rivers Rupsha and Bhairab,

with the tributary Mayur river as the primary source for irrigation for peri-urban farmers. Reliance on the Mayur river is significant, especially during the dry period (November–April) (Fig. 1). To evaluate the prevailing water quality, samples were collected from 20 sampling points localized in different surface water bodies in and around the city in winter (November to February), summer (March to June) and monsoon (July to October) seasons. Sampling points cover the various sources of irrigation, such as rivers, canals/drains, lakes and ponds (*supplementary materials: Table 1*). Canals and drains receive domestic wastewater directly from households and discharge to the surrounding rivers. Small lakes and urban ponds (too small to be made visible in Fig. 1) are used by a small part of the population for bathing, washing and fishing and generally do not connect with the rivers or canals, except in case of floods. Sampling points were selected with regards to the land use pattern of the city. For example, the eastern part of the city accommodates several small and medium-sized industries and thus, samples from the east were primarily selected for heavy metal analysis. Similarly, samples for microbial analysis were collected mainly from the western part, especially from the areas where farmers were extracting irrigation water. Sample collection for winter, summer and monsoon seasons occurred respectively in January, April and August 2018. 40 samples (20 for microbial and 20 for heavy metal analysis) were collected in each season.

2.2. Water quality assessment in laboratory and statistical analysis

Microbial assessment samples were collected in sterilized glass bottles to estimate the concentrations of Total coliform (TC), Faecal coliform (FC), *E. coli* and *Enterococcus* using the standard Membrane Filtration (MF) method number 9222 and 9230 as explained in literature (APHA/AWWA/WEF, 2012). Membrane filters (0.45 μm pore size, Sartorius RC White-sterile brand) were used to filter the samples that were used to inoculate agar plates in various dilution series. The plates were prepared from different agar media. After inoculation, petri dishes were incubated (35 °C for 24 h for TC, 44 °C for 24 h for FC, 44.5 °C for 24 h for *E. coli*, 35 °C for 48 h for *Enterococcus*). After incubation, the colonies formed were counted and back-calculated in colony-forming units per 100 ml (cfu/100 ml). Following the analysis, arithmetic mean was used to express the average number of microorganisms in water which was recommended in the literature (APHA/AWWA/WEF, 2012; Haas, 1996). Relevant and necessary chemical-physical water quality information was used based on the previous study carried out in the same sampling locations of the study area (Haldar et al., 2020).

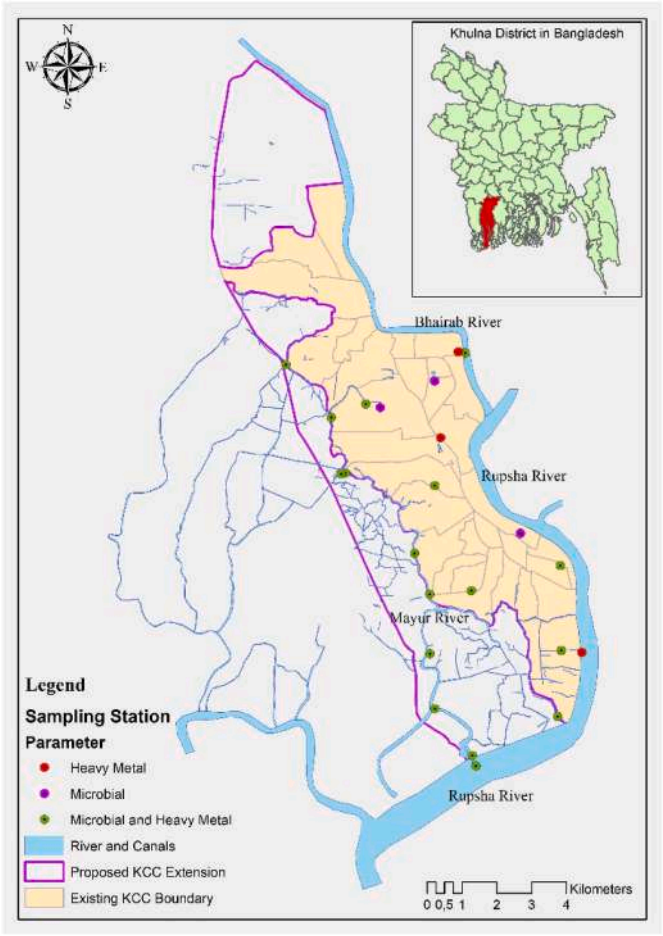

Fig. 1. Locations for collecting water samples.

The samples were collected in standard PPT bottles and transported to the laboratory to determine heavy metal contamination. First, the samples were filtered with filter paper (Whatman No. 41) and 1 ml HNO_3 (65%) per 100 ml was added to the samples to reduce the pH level for preservation. Second, the samples were homogenized and directly measured with the ICP-OES AVIO 500 machine from PerkinElmer. The presence of aluminum (Al), arsenic (As), cadmium (Cd), chromium (Cr), cobalt (Co), copper (Cu), iron (Fe), lead (Pb), manganese (Mn), nickel (Ni), titanium (Ti) and zinc (Zn) in the water samples were conducted following the standard method number 3120 (APHA/AWWA/WEF, 2012). The arithmetic mean was used to express the average heavy metal concentration in collected water samples. MS Excel and IBM SPSS 25.0 were used to perform necessary statistical analysis (e.g. descriptive statistics, ANOVA, correlation analysis) at 95% confidence interval and produce graphical illustrations such as graphs and scatter plots. ArcGIS 10.6 was used to generate maps based on the spatial information of the study area collected from the local municipal agencies.

2.3. Farmer's survey for perception analysis

A structured questionnaire was developed, pre-tested and deployed to understand health-related issues of the farmers who use surface water for irrigation. In total, 38 peri-urban farmers were surveyed in 2018 (Demographic information of the surveyed farmers can be found in *supplementary materials: Table 2*). The questionnaire included questions on crop health and yield, irrigation and fertilizer practices, perception towards water quality, experienced health-related problems, use of protective equipment during irrigation practices and risk perception. In addition, farmers were asked to rate their risk perception on various issues related to current irrigation practice on a scale of 1 (low-risk perception) to 5 (high-risk perception). Responses were recorded in an online-offline platform (Kobo Toolbox), including their GPS locations. Farmers were selected randomly among those whose farm was in the proximity of the Mayur river and had a higher chance of regularly exposing themselves to the water from indirect wastewater irrigation.

2.4. Quantitative Microbial Risk Assessment (QMRA)

In the early 1990s, QMRA was first proposed for water safety management (Regli et al., 1991; WHO, 2016). Since then, QMRA has been used to estimate risk levels for different water usage such as drinking water, recreational water, wastewater irrigation (WHO, 2016). In general, QMRA predicts risk based on exposure to one type of pathogen at a given time (Drechsel et al., 2010a; Haas et al., 1999). Based on the general characteristics, QMRA can have three different levels: screening, advanced and in-depth level and these levels include four steps for water-related risk assessment: hazard identification, exposure assessment, health effects assessment and risk characterization (Abrahams et al., 2004; Haas et al., 1999; WHO, 2016). Screening level QMRA provides a quick, low-cost overview of the level of risk, whereas advanced and in-depth level risk assessment offers more detailed and comprehensive information on risks but also requires higher cost and

time involvement (WHO, 2016).

The selection of appropriate levels and steps of QMRA depends on the overall aim of the risk assessment (WHO, 2016). This study aimed to highlight the concerns associated with the current irrigation practice and thus, an initial screening-level risk assessment was performed using a deterministic model with point estimates of pathogen concentrations. Theoretically superior and accurate to the deterministic model is the stochastic model, which accounts for the uncertainty over model elements; however, the model is complex and requires previously obtained knowledge on probability distributions and the use of Monte Carlo simulation (Hamilton and Stagnitti, 2008). Using a simple deterministic model, on the other hand, provide insights that could be useful in identifying the potential errors in complex stochastic models (Zwietering, 2009). As the necessary knowledge on variability and uncertainty over model inputs to quantify the risks was not available, this study oriented on determining the initial screening levels of risks, using single-point pathogen concentration estimates (WHO, 2016). This is the first step in risk assessment and can be followed (not done in this study) by a more quantitative assessment, eventually delivering risk results expressed in Disability Adjusted Life Years (DALYs). However, this requires much more detailed knowledge on probabilities of infection, illness and variability and needs to be accompanied by an uncertainty analysis based on Monte Carlo modeling techniques which was beyond the scope of this study.

2.4.1. Hazard identification

Hazard identification, the first step in QMRA, was performed to define the investigation's scope and purpose and formulate specific risk problems (WHO, 2016). The study area's local context and socio-cultural aspect were considered to select the particular pathogenic indicators and the relevant exposure pathways as done in literature (Ferrer et al., 2012). Pathogenic bacteria such as *E. coli* O157:H7, *Salmonella typhimurium*, *Shigella dysenteries* and *Vibrio cholerae* in water sources are associated with the major causes of diarrheal diseases and gastrointestinal infections worldwide (Momba et al., 2006; Teklehaimanot et al., 2014). In the study area, the presence of FIB in the surface water, especially *E. coli* and *Enterococcus*, is reported in previous studies (Islam et al., 2018b; Islam and Islam, 2020). Thus, in this study, the probability of infection is modeled assuming a fraction of the total counted *E. coli* being *E. coli* O157:H7. A ratio of 1:0.08 for *E. coli*:*E. coli* O157:H7 was used based on literature (Haas et al., 1999; Machdar et al., 2013) to assume the concentration of *E. coli* O157:H7 as this specific variant could not be detected in the local laboratory. The absence of research infrastructure in developing countries has been identified as a major challenge for an in-depth QMRA (Dias et al., 2019; Islam and Islam, 2020). This study focuses on the peri-urban farmers surrounding Khulna city who are indirectly using urban wastewater for irrigation and *E. coli* was selected as the microbial parameter to simulate the potential health risk.

2.4.2. Exposure assessment

In the exposure assessment, the frequency and magnitude of exposure to pathogens through different pathways were estimated (WHO, 2016). Exposure quantitatively indicates the pathogen's dose that a host ingests, inhales, or gets in contact with and is often identified as a route from the pathogen source (e.g. water) to the actual exposure event (e.g. accidental ingestion) (Haas et al., 1999). This study focused on the oral route of accidental ingestion by farmers while working in the field. Wastewater that enters the surface water body without any treatment typically contains remnants of human excreta. Similarly, animals grazing in the surrounding areas also excrete into the environment and the microbial pollutants in part reach surface water bodies through surface runoff. Farmers pump surface water to their agricultural fields and move around the field with bare feet. They come into contact with the surface water containing pathogens or may accidently ingest the polluted irrigated water (Fig. 2).

The exposure dose (cfu) per event was calculated using the following formula:

$$\text{Dose} = C \times q \quad \text{(i)}$$

where, C is the concentration of pathogens in the surface water (cfu/ml) and q is the volume of accidental irrigation water ingestion by farmers (ml).

Studies suggest that farmer's accidental ingestion of irrigation water range from 1 to 5 ml/event and for the simulation purpose a median value of 3 ml/event was assumed for single event per day spent in the field was (Moazeni et al., 2017; Symonds et al., 2014).

2.4.3. Health effect assessment and risk characterization

The health impact data for the identified hazards and the specific study population was assessed using a dose-response model in the health impact assessment (WHO, 2016). The dose-response model is a mathematical relationship between the dose of pathogen uptake by the receptor (i.e. farmer) through various routes (e.g. direct ingestion, inhalation or contact) and the probability of response (e.g. a form of infection, illness or death) (Haas et al., 1999). In this study, ingestion was assumed to be the main route because the study indicated that farmers work in the field without any protection which increases the chance of accidental ingestion (Mojid et al., 2010). In general, two types of models are being used to assess the dose-repose relation: the exponential model and the Beta-Poisson model (WHO, 2016). The exponential model assumes that the probability of infection can be shown as a function of ingested dose and Beta-Poisson is characterized by a median infectious dose and a slope parameter (Haas et al., 1999). In this study, for pathogenic microorganisms, the Beta-Poisson model is more appropriate and thus used due to the distribution of microbes in the environment and the interaction with the target population (Ferrer et al., 2012; Haas et al., 1999).

The probability of daily infection from a specific pathogenic microorganism was calculated using the following formula:

$$P_{i(d)} = 1 - \left[1 + Dose\frac{2^{\frac{1}{\alpha}} - 1}{N_{50}}\right]^{-\alpha} \quad \text{(ii)}$$

where, $P_{i(d)}$ is the daily probability of infection from specific pathogen *i*, N_{50} is the number of pathogens infecting 50% of the exposed population and α is the kinetic parameter (constant).

The annual probability of infection was calculated using the

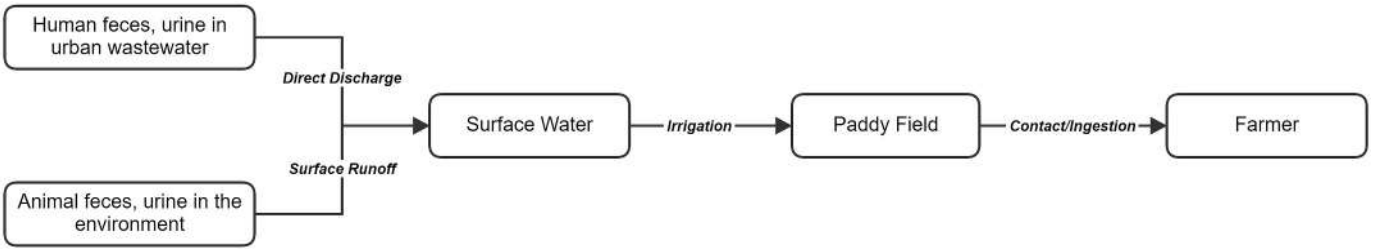

Fig. 2. The exposure route of accidental ingestion of wastewater.

following formula:

$$P_{i(A)} = 1\text{-}\ [1\text{-}P_{i(d)}]^{n} \quad \text{(iii)}$$

where, $P_{i(A)}$ is the annual probability of infection by ingesting pathogens and n is the exposed duration (days/year).

Literature indicates that farmers are exposed 50–80 days while irrigating fields, however, a default value of 75 days per year was used as exposure days for simulating the annual risk of infection (WHO, 2006). For seasonal risk of infection, the exposure days were determined based on the farmer's survey and other related information such as kinetic parameter α, a dose resulting in 50% infection, was also based on literature (Table 1). Reduction of pathogenic concentration ($\log_{10}$ removal) using technical and non-technical measures was simulated to formulate risk mitigation strategies to ensure the safe reuse of water in the study area.

3. Results and discussion

3.1. Microbial water quality

Laboratory analysis provided information on the concentration levels of TC, FC, *E. coli* and *Enterococcus* in the study area's surface water bodies (Fig. 3). The mean concentration of TC exceeded the local standards (<1000 cfu/100 ml) for inland surface water useable for irrigation for all sampling points around the year (GoB, 2002). The mean concentration of TC was the highest during the summer (1×10^6 cfu/100 ml) and lowest in the winter (8×10^5 cfu/100 ml). Similarly, the mean concentration of FC (in cfu/100 ml) was high during the summer and monsoon seasons ($4 \times 10^5$and 5×10^5 respectively) as compared to the winter (7×10^4). The mean *E. coli* concentration (in cfu/100 ml) was lower during the winter (4×10^4) than in summer and monsoon i.e., 3×10^5 and 4×10^5, respectively (*supplementary materials: Tables 3 and 4*). Several previous studies also indicated the elevated level of FC and *E. coli* during summer and monsoon in other areas of Bangladesh (Islam et al., 2011, 2017; Kostyla et al., 2015; Zabed et al., 2014). However, the concentration of enterococcus was lower during summer (7×10^3 cfu/100 ml) than in the monsoon season (2×10^4 cfu/100 ml). The presence of light accelerates *enterococcus*'s decay, which may have been linked with the lower concentration during summer compared to the monsoon season (Bordalo et al., 2002).

Correlation analysis indicates that water temperature had a significantly positive influence on the FC ($P < 0.01$) and *E. coli* ($P < 0.05$) concentrations. Similarly, a positive correlation between water temperature and TC was found but not statistically significant (*supplementary materials: Table 6*). The climatic data in the last two decades indicated that the region had an average maximum atmospheric temperature between 32 °C and 36 °C from April to October and the warm climate may have favored the growth of FC and *E. coli* resulting in higher concentrations (Barcina et al., 1986; Dey et al., 2017; Haque et al., 2019; Islam et al., 2017; Jang et al., 2017; Vermeulen and Hofstra, 2014). Similarly, ANOVA indicates the significant seasonal variation ($P < 0.05$) in FC, *E. coli* and *Enterococcus* concentrations except for TC. Heavy rainfall contributes to the higher dilution and excessive runoff during the monsoon season from nearby built-up areas where septic tanks, domestic animal sheds and wet markets are more common. The variation was highest during monsoon for all microbial indicators, which is most likely related to heterogeneous contributions of pollution sources and dilution by run-off waters. The presence of grazing cattle, wet markets, runoff from septic tanks and the dumping of untreated wastewater all most likely contribute to the high and varying concentrations of FIB in the surface waters also found by other authors (Ekklesia et al., 2015; Falardeau et al., 2017; Islam et al., 2018b; Jang and Liang, 2018; Myers and Kane, 2011; Ramos et al., 2006).

The standard deviation of pathogen concentrations indicates the very high concentration variability among sources, further validated using statistical analysis. ANOVA shows the significant ($P < 0.05$) spatial variation in TC, FC, *E. coli* concentrations among different sources in the study area (*supplementary materials: Table 5*). The overall mean concentration of TC for canal/drains was 2×10^6 cfu/100 ml, whereas for the river, the concentration was 8×10^5 cfu/100 ml and the concentration was highest during summer. The canals and drains occasionally receive effluents from the septic tanks via leakage or illegal dumping, whereas wastewater or runoff gets diluted with the river water and the tidal effect contributes to the movement of water, which may have an impact on the variability of the concentration over different sources.

FC and *E. coli* concentrations in all the sampling stations also exceeded the WHO guideline (≤1000 cfu/100 ml) for unrestricted use in agriculture, except for an urban pond owned by the local municipal authority. The pond is not open for regular activities and is occasionally treated with bleaching powder. The application of chlorinated lime or bleaching powder (calcium hypochlorite) can reduce (around 60%) the faecal contamination in water sources (Roy et al., 2016; Sirajul Islam et al., 2007). Two other urban ponds that were not under the municipal authority were used extensively by the local population for domestic activities, such as bathing and washing and had several folds higher TC and FC concentrations than the WHO threshold. Bathing in such microbially polluted waterbodies could lead to severe illness and increase infection chances, especially among children (Islam and Islam, 2020). Overall, the pathogen concentrations exceed the current national and international guidelines for using surface water for irrigation and daily activities, thus posing a health risk for the user groups.

3.2. Risk perception of farmers

Farmer survey indicated that most farmers (95%) have been using surface water sources, especially the Mayur river and nearby canals, as their primary source of irrigation for decades. Most of them (63%) understand their irrigation source regularly receives domestic wastewater from adjacent urban areas and mentioned the reliance on the existing sources due to lack of alternatives. Most farmers (84%) do not use any protective equipment during irrigation, thus enhancing the chance of accidental ingestion. Lack of protective equipment could lead to a higher risk of infection for farmers and their family members (Keraita et al., 2008; Mojid et al., 2010). In addition to accidental ingestion, peri-urban farmers also face other obstacles daily. More than 45% of the farmer

Table 1
Values used for QMRA simulation.

Parameter	Unit	*E. coli* O157:H7	Reference
Mean concentration (C)	cfu/ml	Winter: 2.8×10^3 Summer: 2.6×10^4 Monsoon: 3.3×10^4 Overall: 2×10^4	(Haas et al., 1999; Machdar et al., 2013) and this study
Kinetic parameter (α)	–	0.49	(Amha et al., 2015; Gibney et al., 2014; Haas et al., 1999, 2000)
Dose resulting 50% infection (N_{50})	–	5.96×10^5	
Volume of ingestion (q)	ml	1-5; Median: 3	(Moazeni et al., 2017; Symonds et al., 2014)
Exposed days (n)	days/year	50-80 (WHO default value 75)	(Moazeni et al., 2017; Symonds et al., 2014; WHO, 2006)
	days/season	22	This study

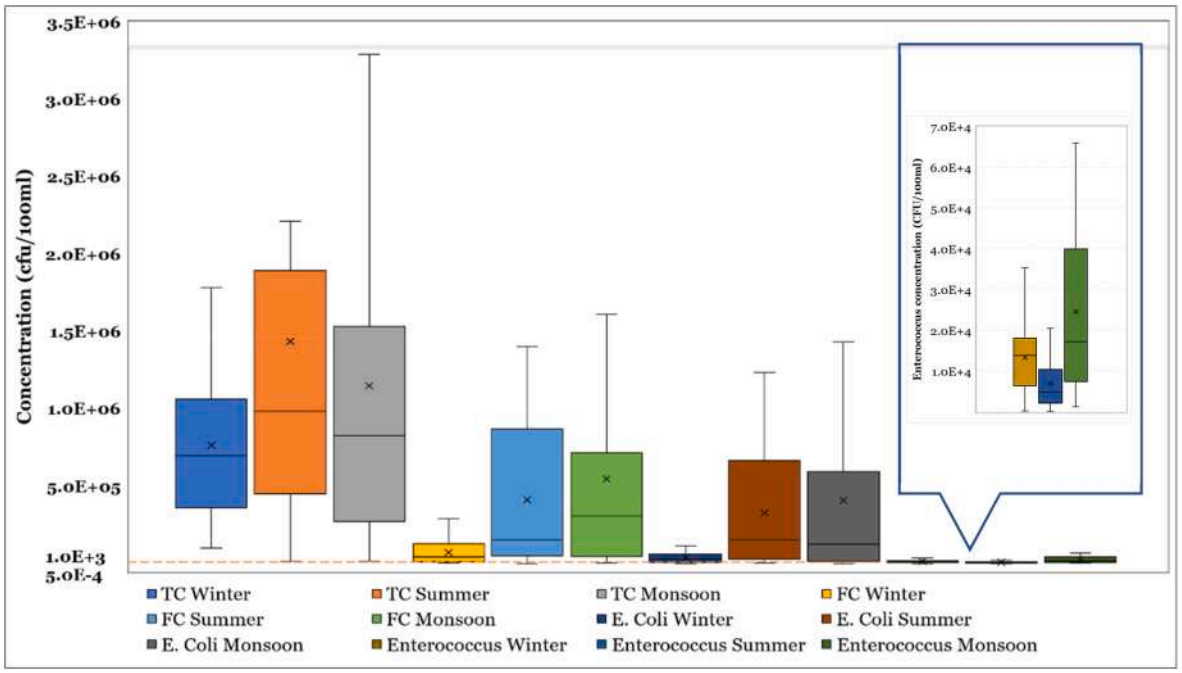

Fig. 3. Concentrations of TC, FC, *E. coli* and Enterococcus in the surface water (red dotted line indicates the allowable threshold for coliforms in WHO and local standards). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

reported odor, skin irritation, skin blistering and water-borne diseases like diarrhea after working in the field during irrigation which was also reported in previous studies (Haldar et al., 2021; Mojid et al., 2010). However, farmers' risk perception towards their current practice indicates that the peri-urban farmers rank health-related issues lower in the list compared to other issues (Fig. 4).

Farmers rank excessive presence of weeds and pests, which grow due to indirect wastewater irrigation in the field, as a top risk, followed by crop health. Their own health comes third in the list, followed by soil health and the local environment. Prioritizing farming-related issues over health issues is also observed in previous studies and farmers accepted those health risks considering the lack of available irrigation sources and potential economic gains of wastewater use (Adjaye-Gbewonyo, 2008; Drechsel et al., 2010a; Weldesilassie et al., 2011). Studies also indicated that experience in working with wastewater, education level, source of information, socio-economic condition influence the health risk perception among farmers (Drechsel et al., 2010a; Keraita et al., 2008; Obuobie et al., 2006; Weldesilassie et al., 2011). Similarly, in the study area, farmers who have been farming for more than 20 years did not perceive health risk as a major concern. Damage to the pump is the lowest on the list as the pumps are easily repairable and required materials are locally available. As excessive weed growth is common in the study area, farmers use chemical fertilizer to increase the crop yield and control weed growth and pest control in the field.

A very small number of farmers (16%) use a piece of cloth to cover their face during field activities, but that is not sufficient to protect them against the polluted surface water. The survey also revealed that the usability of the protective equipment, lack of information about the usefulness of protective equipment along with the high cost are the primary reasons for not using necessary protections during field work which is generally mentioned in other global studies (Lamnisos et al., 2013; Mayilla et al., 2016; Obuobie et al., 2006). Using necessary

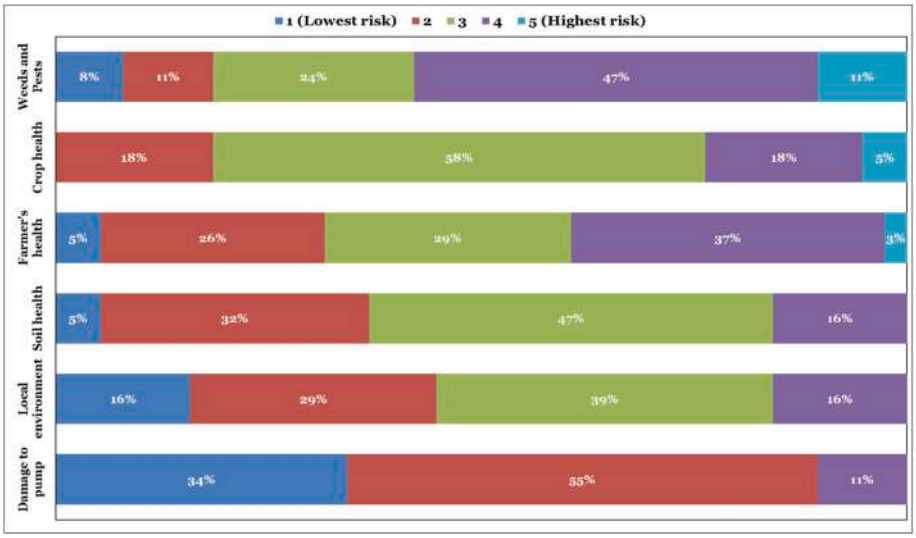

Fig. 4. Risk perception of farmers of their current irrigation practice.

protective equipment during farming activities is a low priority for their health due to their long-standing irrigation practices without any protection when the water used to be comparatively clean (Mayilla et al., 2016). Farmers also mentioned that they face difficulty in farming activities while wearing protective equipment such as boots or gloves, making it difficult to move and work in the muddy paddy field. However, this should not be a reason for failing to protect farmer's health as this equipment could easily be used for other farming activities such as vegetable or fruit farming. Farmers also mentioned taking basic medicines from local pharmacies and home remedies when they get sick after contacting polluted surface water.

3.3. Potential microbial health risks for farmers

The relation between the pathogen concentration and farmer's health risk due to accidental ingestion was simulated through the QMRA model and it indicates seasonal infection probabilities also risk of infection based on various irrigation water sources (Table 2). The daily probability of infection is higher in summer and monsoon (2×10^{-3}) compared to winter season (2×10^{-4}). The overall daily probability of infection for a single event is three orders of magnitude higher compared to the recommended limit of $<10^{-6}$ by WHO; similar to other studies from other parts of the world (Amha et al., 2015; Kouamé et al., 2017; Signor and Ashbolt, 2009; WHO, 2016). The infection probability also varies over the sources used for irrigation. The overall daily probability of infection is high for canal/drain (3×10^{-3}) followed by the river (8×10^{-4}) and lake/pond (5×10^{-5}) samples. This variation is understandable due to the variable *E. coli* concentrations across different sources; rivers and drains have a higher concentration than lakes and ponds.

Considering the 22 seasonal exposure days, the annual probability of infection in winter is the lowest (0.004), whereas the summer (0.04) and monsoon (0.05) have the highest probability (Fig. 5). However, the annual risk of infection is still much higher than the WHO guideline ($<10^{-4}$) for an acceptable risk limit (Amha et al., 2015; Signor and Ashbolt, 2009; WHO, 2016). Similar to values for the daily probability of infection based on sources, the annual risk of infection (considering WHO default 75 exposure days) is also high for river and canal/drain samples compared to the pond/lake samples. The overall annual risk of infection is highest (0.2) for canal/drain samples, followed by the river (0.06) and lake/pond (0.003) samples. Considering all samples, the overall annual risk of infection is 0.1 which is three orders of magnitude above the acceptable limit. The *E. coli* concentration was significantly different over sources, thus resulting in a higher annual risk of infection probability for canal/drain than lakes.

Farmers only rely on external irrigation during the dry period, i.e. the whole winter and parts of the summer season; thus, the calculated risks of infection for the monsoon season may not correspond to the practical situation of the farming practices of the past years. However, changes in the climatic variability in the Bengal delta will result in greater unpredictability of rainfall and droughts, which might force farmer's reliance on surface water throughout the year in the future (Gain et al., 2014; Kumar et al., 2020; Rahman et al., 2011). In addition to that, assuming the counted fractions of *E. coli* to be all *E. coli* O157:H7, one of the most infectious pathogenic E Coli variants, may result in overestimated values for infection probabilities has been indicated by others (WHO, 2016). However, additional simulations considering 0.01%, 0.05%, 0.1%, 0.5%, 1%, 2%, 5% and 10% of the initial concentrations being *E. coli* O157:H7 also resulted in daily and annual probability of infection above the WHO acceptable limit and the probability of infection start to decrease around 0.01% (*supplementary materials: Table 7*). Considering 10% of the counted *E. coli* concentrations to be this pathogenic variant, the overall annual risk of infection was 0.14, whereas for 0.1% of the annual risk of infection was 0.0002, which is still above the WHO acceptable limit.

Table 2
Daily probability of infection due to current practice.

Source	Winter	Summer	Monsoon	Overall
All Samples	2×10^{-4}	2×10^{-3}	2×10^{-3}	2×10^{-3}
River	2×10^{-4}	1×10^{-3}	1×10^{-3}	8×10^{-4}
Canal/Drain	4×10^{-4}	4×10^{-3}	5×10^{-3}	3×10^{-3}
Pond/Lake	1×10^{-5}	6×10^{-5}	7×10^{-5}	5×10^{-5}

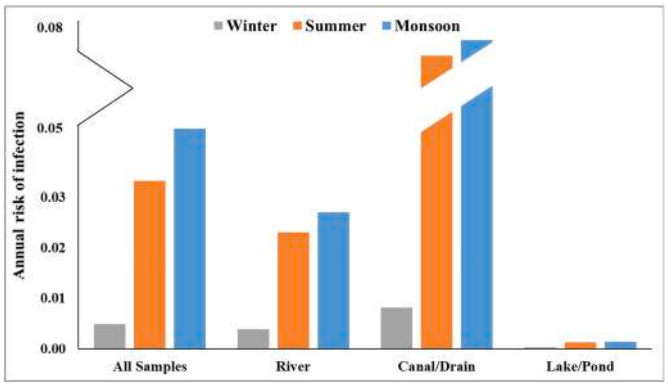

Fig. 5. Annual risk of infection over different sources for *E. coli*

The survey among the local farmers who has been using polluted surface water as irrigation water revealed that more than 26% of the farmers suffered from water-borne diseases after working in the field. We calculated an overall infection probability between 2 and 10% and only for pathogenic *E. coli*, so the actual observed infection risk from the survey is higher than this QMRA assessed value. This is logical since microbial indicators, such as *E. coli* and Salmonella (data not shown), were also present in these waters and others (viruses, protozoa, helminth eggs) can also be expected to be present; hence, an accumulative risk of multiple pathogens can be expected. Moreover, the actual infection rate in real-world situation may differ from the theoretical QMRA based risk assessment as infectivity varies between individuals based on the immune system, age and other health factors (WHO, 2016). The input model parameters of QMRA are often derived based on studies conducted in developed countries, raising the debate on the applicability of QMRA for developing countries (Mills et al., 2020). It is often generalized that people from developing countries have a stronger immune response system for water-related pathogens compared to their counterparts, though the opposite could also be easily reasoned. Thus, further investigation is necessary to estimate the actual risk in the context of the study area. The insights from this study on the seasonal probability of risk of infection were used to highlighting the current risk to take necessary strategies to mitigate the risks.

3.4. Risk mitigation for safe irrigation practice

The analysis has indicated that the concentrations of selected pathogenic microbial indicators in the surface water are exceeding the national and international guidelines for use, leading to an increased annual risk of infection. A multiple-barrier approach containing a series of technical and non-technical measures could reduce the current risk for the farmers (Drechsel et al., 2010b; Fuhrimann et al., 2016; Janeiro et al., 2020; Keraita et al., 2008). As the current irrigation sources receive a regular municipal discharge, a treatment system followed by necessary disinfection would remove $\log_{10}$ 3 of the prevailing concentrations of *E. coli* lowering it to the safe limits (Sperling et al., 2005). Implementation of technical strategies alone usually cannot reduce the health risk below the acceptable limit by only reducing the accidental ingestion volumes (*supplementary materials: Table 8*). Reducing pathogen concentration by treating wastewater before discharge as a technical strategy and reducing accidental ingestion to the minimum (1 ml/event)

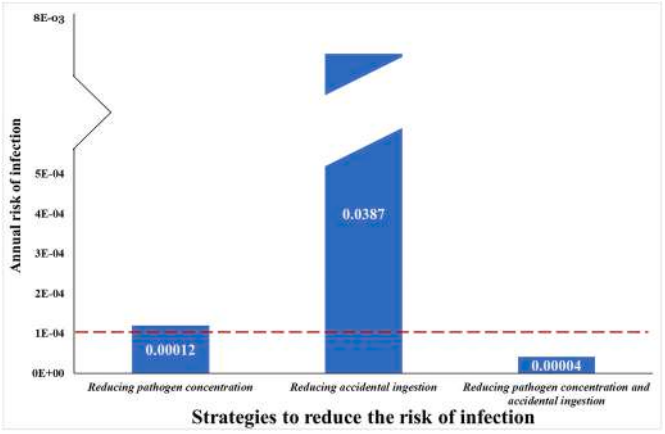

Fig. 6. Health risk after implementing technical and non-technical strategies (red dotted line indicates the acceptable health risk limit). (For interpretation of the references to colour in this figure legend, the reader is referred to the Web version of this article.)

using protective equipment and raising awareness and education programs as non-technical strategies, could significantly lower the health risk within the acceptable limit (Fig. 6). The authority should regularly monitor the water quality and enforce the necessary rules and regulations to prevent untreated discharge. The outflows from the septic tanks should be managed and de-sludged to prevent partially treated black water overflow into the surface water bodies as poorly managed system increases the chances of health risks (Foster et al., 2021). The sludge from the septic tanks could be further processed using appropriate technology suited to the local context (Drechsel et al., 2015; Fuhrimann et al., 2016; Hanjra et al., 2012; Tilley, 2014).

Farmers should be encouraged to use protective equipment, where possible, to reduce the incidents related to accidental ingestion. Only reducing the accidental ingestion to a minimum (1 ml/event) will be insufficient to reduce the health risk if the concentration remains high (*supplementary materials: Table 8*). Additionally, access to necessary health treatment (for severe illness), regular health awareness, an education program for farmers and their family members is crucial to reducing health risks (Utzinger et al., 2009). The agricultural extension agency could ensure access to protective equipment or education programs through government subsidies or grants, especially for economically marginalized farming groups. Combining technical and non-technical strategies would lead to reduced pathogen concentration in surface water sources and decreased chances of accidental ingestion, bringing the annual risk within the acceptable limit. Strategies should also include other stakeholder groups in the food chain i.e, market vendors and consumers as they also suffer from indirect wastewater irrigation (Barker et al., 2013; Ferrer et al., 2012). Awareness and information campaigns are necessary to prevent cross-contamination at the market level and increase safe storage and processing at the household level (Drechsel et al., 2010a; Fuhrimann et al., 2016; Tram et al., 2008). A strong monitoring and warning system for microbial contamination can help early detection take necessary measures to protect farmers' health (Fuhrimann et al., 2015; WHO, 2006).

3.5. Heavy and other metal contamination in surface water

Heavy metal analysis indicates that only Al, Fe, Mn, Ti and Zn were detected in the surface water and all, except Mn, had significant ($P < 0.05$) seasonal variations. However, all measured concentrations were below the FAO recommended limit for agricultural use (Table 3). The Mn concentration in surface water was near the FAO maximum allowable limit for safe irrigation (0.2 mg/L). Prevailing sources such as untreated dumping of wastewater could lead to the presence of manganese in the surface water (Metcalf & Eddy, 2013). The coastal districts of Bangladesh have manganese (Mn) concentrations beyond the national (BDS) and international (WHO) drinking water guidelines, which could also contribute to the Mn concertation in surface water (Rahman et al., 2021). Fe's concentration increases five-fold (from 0.26 mg/L to 1.37 mg/L) during monsoon compared to winter and Al concentration increases drastically (from 0.12 mg/L in winter to 1.41 mg/L in monsoon) due to the excessive runoff during that period (Bhardwaj et al., 2017; Measures et al., 2005).

The concentration of As, Co, Ni, Cd, Cr, Cu and Pb in the collected water samples was below the detection limits, which can be explained by the declining presence of traditional heavy mills and industries (jute, garments, cable) in the area (Rahman and Kabir, 2019) and prevalence of manufacturing SME's in categories like agro-processing, bakery, light engineering, timber and furniture. Several studies from the other parts of the country where heavy industrial zones (textiles, agro-chemical, dye, paint and ceramics) are dominant, the concentrations of heavy metals in water, soil and the crops (vegetables) were above the national and international standards (Ahmad and Goni, 2010; Ahmed et al., 2018, 2019). Two apparent reasons could cause a bit deviating situation in the study area i) a relatively low contribution of SMEs and other enterprises to water pollution or removing pollutants from the surface water resources. In addition to that, during the field survey, the excessive presence of water hyacinths - a fast-growing, free-flowing weed was observed in surface water bodies (*supplementary materials: Fig. 1*). Water hyacinth can absorb and remove heavy metals from wastewater through the roots (Ingole and Bhole, 2003; Muramoto and Oki, 1983; Rezania et al., 2015; Zheng et al., 2016). For example, studies show that water hyacinth removed almost 65% of Cr and Cu from wastewater simulated in a wetland-based system (Lissy and Madhu, 2011).

Table 3
Heavy metal concentration in the surface water of Khulna.

Parameters (mg/L)[a]	Season (N = 20) (Mean ± SD)			FAO Recommendation Limit (mg/L)	Detection Limit (mg/L)
	Winter	Summer	Monsoon		
Aluminium (Al)	0.12 ± 0.09	0.57 ± 1.09	1.41 ± 1.81	5	0.1
Iron (Fe)	0.26 ± 0.23	0.62 ± 1.14	1.37 ± 1.41	5	0.1
Manganese (Mn)	0.26 ± 0.37	0.18 ± 0.25	0.21 ± 0.25	0.2	0.01
Titanium (Ti)	0.05 ± 0	0.06 ± 0.04	0.09 ± 0.06	N/A	0.01
Zinc (Zn)	0.67 ± 1.23	0.1 ± 0.03	0.16 ± 0.27	2	0.1
Arsenic (As)				0.1	0.1
Cobalt (Co)				0.05	0.1
Nickel (Ni)				0.2	0.1
Cadmium (Cd)		Below the detection level		0.01	0.01
Chromium (Cr)				0.1	0.01
Copper (Cu)				0.2	0.01
Lead (Pb)				5	0.01

[a] Bold-italic parameter indicates the significant ($P < 0.05$) temporal variations.

Similarly, in artificial lake water Cu, Pb, Cd and Zn concentration decreased 24%, 26%, 50% and 57%, respectively, after 8 days of experiment with water hyacinths (Smolyakov, 2012). A similar process might have taken up a portion of heavy metals by the roots of water hyacinths from the surface water bodies, resulting in heavy metal concentrations below detection level. Another reason for the lower concentrations of heavy metals in surface water could be the deposition of heavy metal minerals in the riverbank soils and sediment, giving a delayed emission to the water phase due to sorption processes. Studies indicate that the riverbank soil can absorb heavy metals in large quantities of heavy metals even when repeatedly exposed to highly polluted mineral or effluent disposals (Chang et al., 1984; Kumar Kumar Sharma et al., 2007; Li et al., 2015; Yang et al., 2018). However, at some point, adsorption saturation would occur and higher emissions levels can then be expected. As the surface water bodies and riverbanks receive wastewater and mineral disposals for decades, the deposition of heavy metals in the riverbank soils and river sediments requires further investigation.

3.6. Limitations of the study and future research scope

This study has indicated a potential health risk related to current practice, but an in-depth level study would provide a more comprehensive understanding of the health risks, which would be useful in adopting required risk mitigation strategies. Future assessment considering the human enteric pathogens should include at least one virus, one bacteria, one protozoan, or even the presence of helminth eggs to understand the range of behaviors in pathogen groups to formulate specific risk mitigation strategies (WHO, 2016). Additionally, a study on plant uptake and deposition in the soil could provide further insights into the study area's heavy metal contamination. Currently, the surface water is deemed safe in terms of heavy metal contamination for agricultural use. However, increasing industrial activities may threaten the chemical health risk for farmers and consumers. Future studies should focus on quantifying the potential chemical risks to formulate risk mitigation strategies. The city is expected to have growing economic activities in the coming period, which may increase the presence of heavy metals in the surface water if not treated (ADB, 2020).

4. Conclusion

This study aimed to assess the risks related to indirect wastewater irrigation among peri-urban farmers based on a questionnaire survey among farmers and a determination of the microbial quality of surface water resources around the Bengal delta city of Khulna. In the survey, 26% of the farmers indicated water-borne-related health effects in the survey. Further, the results of the survey found that farmers rank excessive weed growth, nuisance of pest and crop health as the most important concerns, even above their own health. This seems to be related to their longstanding working experience with polluted surface water. The results found in this study for the city of Khulna indicate that surface water used for peri-urban agriculture has no significant concentrations of heavy metals, but does have very poor microbial quality. Further, when compared to national and international guidelines, the pathogen levels are several magnitudes too high. This pollution is linked to the direct discharge of domestic wastewater and the associated anthropogenic activities which excessively affecting surface water quality. Taking *E. coli* concentrations in surface water and the variations herein as the basis for a QMRA risk assessment, noteworthy health threats to farmers were identified (3–4 magnitudes too high compared to WHO limits), especially during the monsoon and summer seasons. Various measures were considered in mitigating these risks, including an education program for the farmers to protect their health and protective equipment for farmers while irrigating with polluted surface water. However, the most effective measure is the treatment of the urban water-reducing the pathogen levels in surface water within the recommended limit. Overall, the surface water quality needs to be improved by preventing the direct discharge of wastewater as well as applying adequate treatment. It is recommended that all stakeholder groups should be informed to ensure safe irrigation practices. This research showed possible health outcomes for farmers due to *E. coli* infections. An in-depth QMRA considering other microorganisms, such as bacteria, viruses, protozoa and helminth eggs, would provide a comprehensive image of the risks associated with indirect wastewater irrigation. Moreover, chemical pollution such as organic micropollutants, in addition to the heavy and other metals studied here, could further complete the picture of risks and treatment measures needed. Consumers and market vendors should also be considered in a complete risk assessment and strategies to reduce the risk of infection and chemical pollution. Implementation of technical and non-technical measures are needed to ensure safe water reuse for farming activities, which is crucial for sustaining agricultural production in this part of the Bengal delta.

CRediT authorship contribution statement

KH: Conceptualization, Methodology, Data Collection, Curation and Analysis, Writing the original draft. KKR, NH, DKD and HR: Conceptualization, Methodology, Supervision, Manuscript review & editing.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114002.

References

Abrahams, D., Den Broeder, L., Doyle, C., Fehr, R., Haigh, F., Mekel, O., Metcalfe, O., Pennington, A., Scott-Samuel, A., 2004. EPHIA-European Policy Health Impact Assessment: a Guide, International Health Impact Assessment Consortium. Brussels.

ADB, 2020. ADB approves $160 million loan to improve Sewerage system in Khulna, Bangladesh [WWW Document]. Bangladesh Khulna Sewerage Syst. Dev. Proj. URL. https://www.adb.org/news/adb-approves-160-million-loan-improve-sewerage-system-khulna-bangladesh, 9.29.20.

Adjaye-Gbewonyo, K., 2008. Farmers' perceptions of benefits and risks from wastewater irrigation in Accra, Ghana. Urban Agric. Mag. 27–28.

Ahmad, J.U., Goni, M.A., 2010. Heavy metal contamination in water, soil, and vegetables of the industrial areas in Dhaka, Bangladesh. Environ. Monit. Assess. 166, 347–357. https://doi.org/10.1007/s10661-009-1006-6.

Ahmed, M., Matsumoto, M., Kurosawa, K., 2018. Heavy metal contamination of irrigation water, soil, and vegetables in a multi-industry district of Bangladesh. Int. J. Environ. Res. 12, 531–542. https://doi.org/10.1007/s41742-018-0113-z.

Ahmed, M., Matsumoto, M., Ozaki, A., Van Thinh, N., Kurosawa, K., 2019. Heavy metal contamination of irrigation water, soil, and vegetables and the difference between dry and wet seasons near a multi-industry zone in Bangladesh. Water (Switzerland) 11. https://doi.org/10.3390/w11030583.

Amha, Y.M., Kumaraswamy, R., Ahmad, F., 2015. A probabilistic QMRA of Salmonella in direct agricultural reuse of treated municipal wastewater. Water Sci. Technol. 71, 1203–1211. https://doi.org/10.2166/wst.2015.093.

An, Y.J., Yoon, C.G., Jung, K.W., Ham, J.H., 2007. Estimating the microbial risk of E. coli in reclaimed wastewater irrigation on paddy field. Environ. Monit. Assess. 129, 53–60. https://doi.org/10.1007/s10661-006-9425-0.

Angelakis, A., Gikas, P., 2014. Water reuse: overview of current practices and trends in the world with emphasis on EU states. Water Util. J. 8, 67–78.

APHA/AWWA/WEF, 2012. Standard methods for the examination of water and wastewater. Stand. Methods 541. ISBN 9780875532356.
Barcina, I., Arana, I., Iriberri, J., Egea, L., 1986. Factors affecting the survival of E. coli in a river. Hydrobiologia 141, 249–253. https://doi.org/10.1007/BF00014218.
Barker, S.F., O'Toole, J., Sinclair, M.I., Leder, K., Malawaraarachchi, M., Hamilton, A.J., 2013. A probabilistic model of norovirus disease burden associated with greywater irrigation of home-produced lettuce in Melbourne, Australia. Water Res. 47, 1421–1432. https://doi.org/10.1016/j.watres.2012.12.012.
Bhardwaj, R., Gupta, A., Garg, J.K., 2017. Evaluation of heavy metal contamination using environmetrics and indexing approach for River Yamuna, Delhi stretch, India. Water Sci 31, 52–66. https://doi.org/10.1016/j.wsj.2017.02.002.
Bordalo, A.A., Onrassami, R., Dechsakulwatana, C., 2002. Survival of faecal indicator bacteria in tropical estuarine waters (Bangpakong River, Thailand). J. Appl. Microbiol. 93, 864–871. https://doi.org/10.1046/j.1365-2672.2002.01760.x.
Bos, R., Carr, R., Keraita, B., 2010. Assessing and mitigating wastewater-related health risks in low-income countries: an overview. In: Drechsel, P., Scott, C.A., Raschid-Sally, L., Redwood, Mark, Bahri, A. (Eds.), Wastewater Iriigation and Health: Assessing and Mitigating Risk in Low-Inc, 2010.
Carr, G., Potter, R.B., Nortcliff, S., 2011. Water reuse for irrigation in Jordan: perceptions of water quality among farmers. Agric. Water Manag. 98, 847–854.
Chang, A.C., Warneke, J.E., Page, A.L., Lund, L.J., 1984. Accumulation of heavy metals in Sewage sludge-treated soils. J. Environ. Qual. 13, 87–91. https://doi.org/10.2134/jeq1984.00472425001300010016x.
Dey, S., Uddin, M.S., Manchur, M.A., 2017. Physicochemical and bacteriological assessment of surface water quality of the karnaphuli river in Bangladesh. J. Pure Appl. Microbiol. 11, 1721–1728. https://doi.org/10.22207/JPAM.11.4.10.
Dias, E., Ebdon, J., Taylor, H., 2019. Estimating the concentration of viral pathogens and indicator organisms in the final effluent of wastewater treatment processes using stochastic modelling. Microb. Risk Anal. 11, 47–56. https://doi.org/10.1016/j.mran.2018.08.003.
Drechsel, P., Qadir, M., Wichelns, D. (Eds.), 2015. Wastewater Economic Asset in an Urbanizing World. Springer, Springer Netherlands, Dordrecht.
Drechsel, P., Scott, C.A., Raschid-Sally, L., Redwood, M., Bahri, A. (Eds.), 2010a. Wastewater Irrigation and Health, first ed. Earthscan, London.
Drechsel, P., Scott, C.A., Raschid-Sally, L., Redwood, M., Bahri, A., 2010b. Wastewater Irrigation and Health: Assessing and Mitigating Risk in Low-Income Countries. IWMI.
Ekklesia, E., Shanahan, P., Chua, L.H.C., Eikaas, H.S., 2015. Temporal variation of faecal indicator bacteria in tropical urban storm drains. Water Res. 68, 171–181. https://doi.org/10.1016/j.watres.2014.09.049.
Falardeau, J., Johnson, R.P., Pagotto, F., Wang, S., 2017. Occurrence, characterization, and potential predictors of verotoxigenic Escherichia coli, Listeria monocytogenes, and Salmonella in surface water used for produce irrigation in the Lower Mainland of British Columbia, Canada. PLoS One 12, 1–22. https://doi.org/10.1371/journal.pone.0185437.
Ferrer, A., Nguyen-Viet, H., Zinsstag, J., 2012. Quantification of diarrhea risk related to wastewater contact in Thailand. EcoHealth 9, 49–59. https://doi.org/10.1007/s10393-012-0746-x.
Foster, T., Falletta, J., Amin, N., Rahman, M., Liu, P., Raj, S., Mills, F., Petterson, S., Norman, G., Moe, C., Willetts, J., 2021. Modelling faecal pathogen flows and health risks in urban Bangladesh: implications for sanitation decision making. Int. J. Hyg Environ. Health 233, 113669. https://doi.org/10.1016/j.ijheh.2020.113669.
Fuhrimann, S., Pham-Duc, P., Cissé, G., Tram, N.T., Thu Ha, H., Dung, D.T., Ngoc, P., Nguyen-Viet, H., Anh Vuong, T., Utzinger, J., Schindler, C., Winkler, M.S., 2016. Microbial contamination along the main open wastewater and storm water channel of Hanoi, Vietnam, and potential health risks for urban farmers. Sci. Total Environ. 566 (567), 1014–1022. https://doi.org/10.1016/j.scitotenv.2016.05.080.
Fuhrimann, S., Stalder, M., Winkler, M.S., Niwagaba, C.B., Babu, M., Masaba, G., Kabatereine, N.B., Halage, A.A., Schneeberger, P.H.H., Utzinger, J., Cissé, G., 2015. Microbial and chemical contamination of water, sediment and soil in the Nakivubo wetland area in Kampala, Uganda. Environ. Monit. Assess. 187 https://doi.org/10.1007/s10661-015-4689-x.
Gain, A.K., Aryal, K.P., Sana, P., Uddin, M.N., 2014. Effect of river salinity on crop diversity: a case study of South west coastal region of Bangladesh. Nepal Agric. Res. J. 8, 29–37. https://doi.org/10.3126/narj.v8i0.11576.
Gibney, K.B., O'Toole, J., Sinclair, M., Leder, K., 2014. Disease burden of selected gastrointestinal pathogens in Australia, 2010. Int. J. Infect. Dis. 28, 176–185. https://doi.org/10.1016/j.ijid.2014.08.006.
GoB, 2002. The Environmental Conservation Rules. https://doi.org/10.1002/9781444319910.ch20. Bangladesh, 1997.
Gross, A., Maimon, A., Alfiya, Y., Friedler, E., 2015. Greywater Reuse. CRC Press, New York. https://doi.org/10.1201/b18217-1.
Haas, C.N., 1996. How to average microbial densities to characterize risk. Water Res. 30, 1036–1038. https://doi.org/10.1016/0043-1354(95)00228-6.
Haas, C.N., Rose, J.B., Gerba, C.P., 1999. Quantitative Microbial Risk Assessment. John Wiley & Sons.
Haas, C.N., Thayyar-Madabusi, A., Rose, J.B., Gerba, C.P., 2000. Development of a dose-response relationship for Escherichia Coli O157:H7. Int. J. Food Microbiol. 1748, 153–159.
Haldar, K., Kujawa-Roeleveld, K., Dey, P., Bosu, S., Datta, D.K., Rijnaarts, H.H.M., 2020. Spatio-temporal variations in chemical-physical water quality parameters influencing water reuse for irrigated agriculture in tropical urbanized deltas. Sci. Total Environ. 708, 134559 https://doi.org/10.1016/j.scitotenv.2019.134559.
Haldar, K., Kujawa-Roeleveld, K., Schoenmakers, M., Datta, D.K., Rijnaarts, H., Vos, J., 2021. Institutional challenges and stakeholder perception towards planned water reuse in peri-urban agriculture of the Bengal delta. J. Environ. Manag. 283 https://doi.org/10.1016/j.jenvman.2021.111974.
Hamilton, A.J., Stagnitti, F., 2008. Deterministic versus stochastic quantitative microbial risk assessment models for wastewater irrigation of food crops. Acta Hortic. 792, 333–339. https://doi.org/10.17660/ActaHortic.2008.792.38.
Hamilton, K.A., Haas, C.N., 2016. Critical review of mathematical approaches for quantitative microbial risk assessment (QMRA) of: Legionella in engineered water systems: research gaps and a new framework. Environ. Sci. Water Res. Technol. 2, 599–613. https://doi.org/10.1039/c6ew00023a.
Hanjra, M.A., Blackwell, J., Carr, G., Zhang, F., Jackson, T.M., 2012. Wastewater irrigation and environmental health: implications for water governance and public policy. Int. J. Hyg Environ. Health 215, 255–269. https://doi.org/10.1016/j.ijheh.2011.10.003.
Haque, M.A., Jewel, M.A.S., Sultana, M.P., 2019. Assessment of physicochemical and bacteriological parameters in surface water of Padma River, Bangladesh. Appl. Water Sci. 9, 1–8. https://doi.org/10.1007/s13201-018-0885-5.
Ingole, N.W., Bhole, A.G., 2003. Removal of heavy metals from aqueous solution by water hyacinth (Eichhornia crassipes). J. Water Supply Res. Technol. 52, 119–128.
Islam, M.A., Sakakibara, H., Karim, M.R., Sekine, M., Mahmud, Z.H., 2011. Bacteriological assessment of drinking water supply options in coastal areas of Bangladesh. J. Water Health 9, 415–428. https://doi.org/10.2166/wh.2011.114.
Islam, M.M.M., Hofstra, N., Islam, M.A., 2017. The impact of environmental variables on faecal indicator bacteria in the Betna river basin, Bangladesh. Environ. Process. 4, 319–332. https://doi.org/10.1007/s40710-017-0239-6.
Islam, M.M.M., Iqbal, M.S., Leemans, R., Hofstra, N., 2018a. Modelling the impact of future socio-economic and climate change scenarios on river microbial water quality. Int. J. Hyg Environ. Health 221, 283–292. https://doi.org/10.1016/j.ijheh.2017.11.006.
Islam, M.M.M., Islam, M.A., 2020. Quantifying public health risks from exposure to waterborne pathogens during river bathing as a basis for reduction of disease burden. J. Water Health 18, 292–305. https://doi.org/10.2166/wh.2020.045.
Islam, M.M.M., Sokolova, E., Hofstra, N., 2018b. Modelling of river faecal indicator bacteria dynamics as a basis for faecal contamination reduction. J. Hydrol. 563, 1000–1008. https://doi.org/10.1016/j.jhydrol.2018.06.077.
Janeiro, C.N., Arsénio, A.M., Brito, R.M.C.L., van Lier, J.B., 2020. Use of (partially) treated municipal wastewater in irrigated agriculture; potentials and constraints for sub-Saharan Africa. Phys. Chem. Earth 118–119. https://doi.org/10.1016/j.pce.2020.102906.
Jang, C.S., Liang, C.P., 2018. Characterizing health risks associated with recreational swimming at Taiwanese beaches by using quantitative microbial risk assessment. Water Sci. Technol. 77, 534–547. https://doi.org/10.2166/wst.2017.571.
Jang, J., Hur, H.G., Sadowsky, M.J., Byappanahalli, M.N., Yan, T., Ishii, S., 2017. Environmental Escherichia coli: ecology and public health implications—a review. J. Appl. Microbiol. 123, 570–581. https://doi.org/10.1111/jam.13468.
Jaramillo, M.F., Restrepo, I., 2017. Wastewater reuse in agriculture: a review about its limitations and benefits. Sustain. Times 9. https://doi.org/10.3390/su9101734.
Jiménez, B., Asano, T., 2008. Water Reuse: an International Survey of Current Practice, Issues and Needs. IWA Publishing, London.
Jones, E.R., van Vliet, M.T.H., Qadir, M., Bierkens, M.F.P., 2021. Country-level and gridded estimates of wastewater production, collection, treatment and reuse. Earth Syst. Sci. Data 13, 237–254. https://doi.org/10.5194/essd-13-237-2021.
Keraita, B., Drechsel, P., Konradsen, F., 2008. Perceptions of farmers on health risks and risk reduction measures in wastewater-irrigated urban vegetable farming in Ghana. J. Risk Res. 11, 1047–1061. https://doi.org/10.1080/13669870802380825.
Kookana, R.S., Drechsel, P., Jamwal, P., Vanderzalm, J., 2020. Urbanisation and emerging economies: issues and potential solutions for water and food security. Sci. Total Environ. 732, 139057 https://doi.org/10.1016/j.scitotenv.2020.139057.
Kostyla, C., Bain, R., Cronk, R., Bartram, J., 2015. Seasonal variation of fecal contamination in drinking water sources in developing countries: a systematic review. Sci. Total Environ. 514, 333–343. https://doi.org/10.1016/j.scitotenv.2015.01.018.
Kouamé, P.K., Nguyen-Viet, H., Dongo, K., Zurbrügg, C., Biémi, J., Bonfoh, B., 2017. Microbiological risk infection assessment using QMRA in agriculture systems in Côte d'Ivoire, West Africa. Environ. Monit. Assess. 189 https://doi.org/10.1007/s10661-017-6279-6.
Kumar Sharma, R., Agrawal, M., Marshall, F., 2007. Heavy metal contamination of soil and vegetables in suburban areas of Varanasi, India. Ecotoxicol. Environ. Saf. 66, 258–266. https://doi.org/10.1016/j.ecoenv.2005.11.007.
Kumar, U., Werners, S., Roy, S., Ashraf, S., Hoang, L.P., Datta, D.K., Ludwig, F., 2020. Role of information in farmers' response toweather and water related stresses in the lower Bengal Delta, Bangladesh. Sustainability 12. https://doi.org/10.3390/su12166598.
Lamnisos, D., Anastasiou, C., Grafias, P., Panayi, A., Larkou, A., Georgiou, E., Middleton, N., 2013. Awareness, attitudes towards wastewater reuse and perceptions of public health risks among the general public in Cyprus: demetris Lamnisos. Eur. J. Publ. Health 23, 151–152. https://doi.org/10.1093/eurpub/ckt123.015.
Li, N., Kang, Y., Pan, W., Zeng, L., Zhang, Q., Luo, J., 2015. Concentration and transportation of heavy metals in vegetables and risk assessment of human exposure to bioaccessible heavy metals in soil near a waste-incinerator site, South China. Sci. Total Environ. 521–522, 144–151. https://doi.org/10.1016/j.scitotenv.2015.03.081.
Lissy, M., Madhu, G., 2011. Removal of heavy metals from waste water using black teawaste. Arabian J. Sci. Eng. 1, 48–52. https://doi.org/10.1007/s13369-012-0264-8.

Machdar, E., van der Steen, N.P., Raschid-Sally, L., Lens, P.N.L., 2013. Application of Quantitative Microbial Risk Assessment to analyze the public health risk from poor drinking water quality in a low income area in Accra, Ghana. Sci. Total Environ. 449, 134–142. https://doi.org/10.1016/j.scitotenv.2013.01.048.

Maimon, A., Tal, A., Friedler, E., Gross, A., 2010. Safe on-site reuse of greywater for irrigation - a critical review of current guidelines. Environ. Sci. Technol. 44, 3213–3220. https://doi.org/10.1021/es902646g.

Mark, O., Jørgensen, C., Hammond, M., Khan, D., Tjener, R., Erichsen, A., Helwigh, B., 2018. A new methodology for modelling of health risk from urban flooding exemplified by cholera – case Dhaka, Bangladesh. J. Flood Risk Manag. 11, S28–S42. https://doi.org/10.1111/jfr3.12182.

Mayilla, W., Magayane, F., Konradsen, F., Keraita, B., Ngowi, H., 2016. Awareness of measures for reducing health risk of using low-quality irrigation water in Morogoro, Tanzania. Expo. Heal 8, 475–485. https://doi.org/10.1007/s12403-016-0207-9.

Measures, C.I., Brown, M.T., Vink, S., 2005. Dust deposition to the surface waters of the western and central North Pacific inferred from surface water dissolved aluminum concentrations. G-cubed 6. https://doi.org/10.1029/2005GC000922.

Mekonnen, M.M., Hoekstra, A.Y., 2016. Four billion people facing severe water scarcity. Sci. Adv. 2, e1500323.

Metcalf & Eddy, 2013. Wastewater Engineering: Treatment and Reuse, fifth ed. McGraw-Hill, Boston.

Mills, F., Willetts, J., Evans, B., Carrard, N., Kohlitz, J., 2020. Costs, climate and contamination: three drivers for Citywide sanitation investment decisions. Front. Environ. Sci. 8, 1–14. https://doi.org/10.3389/fenvs.2020.00130.

Moazeni, M., Nikaeen, M., Hadi, M., Moghim, S., Mouhebat, L., Hatamzadeh, M., Hassanzadeh, A., 2017. Estimation of health risks caused by exposure to enteroviruses from agricultural application of wastewater effluents. Water Res. 125, 104–113. https://doi.org/10.1016/j.watres.2017.08.028.

Mojid, M.A., Wyseure, G.C.L., 2013. Implications of municipal wastewater irrigation on soil health from a study in Bangladesh. Soil Use Manag. 29, 384–396. https://doi.org/10.1111/sum.12056.

Mojid, M.A., Wyseure, G.C.L., Biswas, S.K., Hossain, A.B.M.Z., 2010. Farmers' perceptions and knowledge in using wastewater for irrigation at twelve peri-urban areas and two sugar mill areas in Bangladesh. Agric. Water Manag. 98, 79–86. https://doi.org/10.1016/j.agwat.2010.07.015.

Momba, M.N.B., Osode, A.N., Sibewu, M., 2006. The impact of inadequate wastewater treatment on the receiving water bodies - case study: Buffalo City and Nkokonbe Municipalities of the Eastern Cape Province. WaterSA 32, 687–692. https://doi.org/10.4314/wsa.v32i5.47854.

Muramoto, S., Oki, Y., 1983. Removal of some heavy metals from polluted water by water hyacinth (Eichhornia crassipes). Bull. Environ. Contam. Toxicol. 30, 170–177. https://doi.org/10.1007/BF01610117.

Murshed, S.B., Kaluarachchi, J.J., 2018. Scarcity of fresh water resources in the Ganges Delta of Bangladesh. Water Secur 4–5, 8–18. https://doi.org/10.1016/j.wasec.2018.11.002.

Myers, L., Kane, J., 2011. The impact of summer cattle grazing on surface water quality in high elevation mountain Meadows. Water Qual. Expo. Heal. 3, 51–62. https://doi.org/10.1007/s12403-011-0043-x.

Obuobie, E., Keraita, B., Hope, L., Agodzo, S.K., 2006. Health risk perceptions of stakeholders in irrigated urban vegetable farming. Irrig. urban Veg. Prod. Ghana Charact. benefits risk Mitig. 116–135.

Rahman, M.A., Hashem, M.A., Rana, M.S., Islam, M.R., 2021. Manganese in potable water of nine districts, Bangladesh: human health risk. Environ. Sci. Pollut. Res. https://doi.org/10.1007/s11356-021-14016-z.

Rahman, M.H., Lund, T., Bryceson, I., 2011. Salinity impacts on agro-biodiversity in three coastal, rural villages of Bangladesh. Ocean Coast Manag. 54, 455–468. https://doi.org/10.1016/j.ocecoaman.2011.03.003.

Rahman, S.M.T., Kabir, A., 2019. Factors influencing location choice and cluster pattern of manufacturing small and medium enterprises in cities: evidence from Khulna City of Bangladesh. J. Glob. Entrep. Res. 9 https://doi.org/10.1186/s40497-019-0187-x.

Ramos, M.C., Quinton, J.N., Tyrrel, S.F., 2006. Effects of cattle manure on erosion rates and runoff water pollution by faecal coliforms. J. Environ. Manag. 78, 97–101. https://doi.org/10.1016/j.jenvman.2005.04.010.

Regli, S., Rose, J.B., Haas, C.N., Gerba, C.P., 1991. Modeling the risk from Giardia and viruses in drinking water. J. Am. Water Works Assoc. 83, 76–84. https://doi.org/10.1002/j.1551-8833.1991.tb07252.x.

Rezania, S., Ponraj, M., Talaiekhozani, A., Mohamad, S.E., Md Din, M.F., Taib, S.M., Sabbagh, F., Sairan, F.M., 2015. Perspectives of phytoremediation using water hyacinth for removal of heavy metals, organic and inorganic pollutants in wastewater. J. Environ. Manag. 163, 125–133. https://doi.org/10.1016/j.jenvman.2015.08.018.

Roy, P.K., Kumar, D., Ghosh, M., Majumder, A., 2016. Disinfection of water by various techniques – comparison based on experimental investigations. Desalination Water Treat. 57, 28141–28150. https://doi.org/10.1080/19443994.2016.1183522.

Signor, R.S., Ashbolt, N.J., 2009. Comparing probabilistic microbial risk assessments for drinking water against daily rather than annualised infection probability targets. J. Water Health 7, 535–543. https://doi.org/10.2166/wh.2009.101.

Sirajul Islam, M., Brooks, A., Kabir, M.S., Jahid, I.K., Shafiqul Islam, M., Goswami, D., Nair, G.B., Larson, C., Yukiko, W., Luby, S., 2007. Faecal contamination of drinking water sources of Dhaka city during the 2004 flood in Bangladesh and use of disinfectants for water treatment. J. Appl. Microbiol. 103, 80–87. https://doi.org/10.1111/j.1365-2672.2006.03234.x.

Smolyakov, B.S., 2012. Uptake of Zn, Cu, Pb, and Cd by water hyacinth in the initial stage of water system remediation. Appl. Geochem. 27, 1214–1219. https://doi.org/10.1016/j.apgeochem.2012.02.027.

Sperling, M. Von, Chernicharo, Lemos, De, C.A., 2005. Biological Wastewater Treatment in Warm Climate Regions. IWA Publ, pp. 1–856.

Symonds, E.M., Verbyla, M.E., Lukasik, J.O., Kafle, R.C., Breitbart, M., Mihelcic, J.R., 2014. A case study of enteric virus removal and insights into the associated risk of water reuse for two wastewater treatment pond systems in Bolivia. Water Res. 65, 257–270. https://doi.org/10.1016/j.watres.2014.07.032.

Teklehaimanot, G.Z., Coetzee, M.A.A., Momba, M.N.B., 2014. Faecal pollution loads in the wastewater effluents and receiving water bodies: a potential threat to the health of Sedibeng and Soshanguve communities, South Africa. Environ. Sci. Pollut. Res. 21, 9589–9603. https://doi.org/10.1007/s11356-014-2980-y.

Tilley, E., 2014. Compendium of Sanitation Systems and Technologies, second ed. Eawag.

Tram, N.T., Hoang, L.M.N., Cam, P.D., Chung, P.T., Fyfe, M.W., Isaac-Renton, J.L., Ong, C.S.L., 2008. Cyclospora spp. in herbs and water samples collected from markets and farms in Hanoi. Vietnam. Trop. Med. Int. Heal. 13, 1415–1420. https://doi.org/10.1111/j.1365-3156.2008.02158.x.

Utzinger, J., Raso, G., Brooker, S., De Savigny, D., Tanner, M., Ørnbjerg, N., Singer, B.H., N'Goran, E.K., 2009. Schistosomiasis and neglected tropical diseases: towards integrated and sustainable control and a word of caution. Parasitology 136, 1859–1874. https://doi.org/10.1017/S0031182009991600.

Vermeulen, L.C., Hofstra, N., 2014. Influence of climate variables on the concentration of Escherichia coli in the Rhine, Meuse, and drentse aa during 1985-2010. Reg. Environ. Change 14, 307–319. https://doi.org/10.1007/s10113-013-0492-9.

Weldesilassie, A.B., Boelee, E., Drechsel, P., Dabbert, S., 2011. Wastewater use in crop production in peri-urban areas of Addis Ababa: impacts on health in farm households. Environ. Dev. Econ. 16, 25–49. https://doi.org/10.1017/S1355770X1000029X.

WHO, 2016. Quantitative Microbial Risk Assessment: Application for Water Safety Management. WHO Press, Geneva.

WHO, 2006. Guidelines for the Safe Use of Wastewater, Excreta and Greywater. In: Watewater Use in Agriculture, ume 2. World Health, Geneva. https://doi.org/10.1007/s13398-014-0173-7.2.

WHO, 2002. Guidelines for Drinking-Water Quality, Second. World Health Organization, Geneva.

Wu, J., Long, S.C., Das, D., Dorner, S.M., 2011. Are microbial indicators and pathogens correlated? A statistical analysis of 40 years of research. J. Water Health 9, 265–278. https://doi.org/10.2166/wh.2011.117.

Yang, Q., Li, Z., Lu, X., Duan, Q., Huang, L., Bi, J., 2018. A review of soil heavy metal pollution from industrial and agricultural regions in China: pollution and risk assessment. Sci. Total Environ. 642, 690–700. https://doi.org/10.1016/j.scitotenv.2018.06.068.

Zabed, H., Suely, A., Faruq, G., Sahu, J.N., 2014. Water quality assessment of an unusual ritual well in Bangladesh and impact of mass bathing on this quality. Sci. Total Environ. 472, 363–369. https://doi.org/10.1016/j.scitotenv.2013.11.051.

Zheng, J.C., Liu, H.Q., Feng, H.M., Li, W.W., Lam, M.H.W., Lam, P.K.S., Yu, H.Q., 2016. Competitive sorption of heavy metals by water hyacinth roots. Environ. Pollut. 219, 837–845. https://doi.org/10.1016/j.envpol.2016.08.001.

Zwietering, M.H., 2009. Quantitative risk assessment: is more complex always better?. Simple is not stupid and complex is not always more correct. Int. J. Food Microbiol. 134, 57–62. https://doi.org/10.1016/j.ijfoodmicro.2008.12.025.

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Occurrence and genetic characteristics of multidrug-resistant *Escherichia coli* isolates co-harboring antimicrobial resistance genes and metal tolerance genes in aquatic ecosystems

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ABSTRACT

Multidrug-resistant (MDR) *Escherichia coli* isolates (n = 50) were recovered from aquatic ecosystems, which presented high counts of *E. coli* and metal values within the recommended range. These isolates showed different multidrug resistance profiles, highlighting the resistance to extended-spectrum cephalosporins, polymyxins, and fluoroquinolones. Several antimicrobial resistance genes (ARGs) were found, spotlighting the presence of at least one β-lactamase-encoding gene in each *E. coli* isolate. Substitutions in the quinolone resistance-determining regions and the two-component systems involving PhoP/PhoQ and PmrA/PmrB were also found. The metal tolerance gene *rcnA* (nickel and cobalt efflux pump) was the most prevalent. In this regard, 94% of *E. coli* isolates presented the co-occurrence of at least one ARG and metal tolerance gene. Furthermore, virulence genes and genetic diversity were found among MDR *E. coli* isolates. The emergence of potentially pathogenic isolates exhibiting multidrug resistance and metal tolerance emerged as a global health problem at the human-animal-environment interface.

1. Introduction

The evolution of antimicrobial resistance (AMR), a global public health crisis, is a multifactorial complex problem. In this regard, various strategies have been developed in recent years to slow down the progression of AMR (Hwang and Gums, 2016). The One Health approach has been applied to increase knowledge of AMR at the human-animal-environment interface, and in the environment, surveillance studies focusing on aquatic ecosystems have grown increasingly (McEwen and Collignon, 2018). The anthropogenic activities, mainly those related to urbanization and farming, have been responsible for introducing and spreading multidrug-resistant (MDR) strains to aquatic ecosystems (Dominguez et al., 2021).

The increased use of antimicrobials and metals in medicine for disease treatment and in livestock as feed supplements for animals raises concerns about the emergence of MDR and metal-tolerant strains, highlighting important pathogenic Gram-negative bacteria, such as diarrhoeagenic *Escherichia coli* (DEC) and extraintestinal pathogenic *E. coli* (ExPEC) (Klein et al., 2018). In hospitals, silver has been used in medical devices, topical creams, and as a disinfectant, while copper, in addition to being used to prevent infections, has been widely used in the chicken production industry for growth and therapy (Mijnendonckx et al., 2013; Arendsen et al., 2019; Bortoluzzi et al., 2020; Feng et al., 2020). Therefore, direct or indirect exposure of the environment to residues of antimicrobials and metals, as well as to bacteria adapted to these compounds is growing rapidly, which is worrying (Seiler and Berendonk, 2012).

In this regard, disposal of domestic sewage and hospital effluents stand out and contribute to the presence and spread of pathogenic strains, which are often also MDR. Furthermore, the presence of residues of antimicrobials and metals selects bacterial strains resistant to antimicrobials and tolerant to metals, respectively. The metals allow the co-selection of antimicrobial-resistant strains by mechanisms of co-resistance and cross-resistance (Baker-Austin et al., 2006; Tamhankar and Stålsby Lundborg, 2019). In metal-contaminated environments, the occurrence of antimicrobial resistance genes (ARG) and the co-selection

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and horizontal gene transfer (HGT) of plasmid-mediated ARGs have already been described (Yang et al., 2017; Imran et al., 2019).

The multidrug resistance phenotype of DEC and ExPEC has been associated mainly with co-existence of resistance genes to β-lactams [i.e., β-lactamase-encoding genes, highlighting the genes encoding extended-spectrum β-lactamase (ESBL) and plasmid-mediated AmpC β-lactamase (pAmpC)], fluoroquinolones [plasmid-mediated quinolone resistance (PMQR) genes], tetracyclines (*tet*-like), and folate pathway antagonists (FPA; *sul*-like) (Estaleva et al., 2021). These ARGs have been mainly detected on plasmids that co-harbored metal tolerance genes (MTGs), such as *silA* (*sil* operon; silver), *pcoA* (*pco* operon; copper), and *merA* (*mer* operon; mercury) (Hounmanou et al., 2021; Gaeta et al., 2022). Therefore, this study aimed to evaluate the co-occurrence of ARGs and metal tolerance genes in MDR and potentially pathogenic *E. coli* isolates recovered from pollution-affected aquatic ecosystems of southeastern Brazil.

2. Material and methods

2.1. Water sampling

Between 2018 and 2019, water samples were collected from 35 sampling sites of rivers, streams, and lakes in the state of São Paulo, Brazil (Fig. 1) by the Environmental Company of São Paulo State (CETESB). These points are part of the operation of the Inland Water Quality Monitoring Network in São Paulo State and have been used as supply for human consumption, primary and secondary contact recreation, irrigation, aquaculture, fishing activity, and marine navigation. Among the sampling sites, four have been used for recreational purposes with primary contact (MOGU-02351, GRDE-02271, QUEM-02700, and LVEN-02501), while three are classified as springs (TIET-02090, TIET-02050, and RPRE-02200) (Supplementary Table S1).

2.2. Bacterial isolation

One hundred milliliters of each water sample were filtered using a sterile membrane filter (0.45 μm pore size), which were placed on m-TEC ChromoSelect agar (Sigma-Aldrich, UK) and incubated for up to 24 h at 35 ± 2 °C. Finally, characteristic colonies of *E. coli* were randomly selected and stocked using BHI broth (Oxoid, UK) supplemented with 15% (v/v) glycerol at −80 °C. The extraction of genomic DNA was carried out using PureLink™ Genomic DNA Mini Kit (Thermo Fisher Scientific, USA) and the isolates were identified by sequencing of the *16S rRNA* gene.

2.3. Microbiological and physical-chemical analysis

Microbiological (*E. coli* counts) and physical-chemical [total dissolved solids (TDS), conductivity, biochemical oxygen demand (BOD), temperature, pH, turbidity, arsenic, cadmium, lead, copper, chrome, mercury, nickel, and zinc] analysis were carried out by CETESB. All aquatic ecosystems were evaluated for *E. coli* counts, while rivers and streams were analyzed for physical-chemical parameters. The microbiological and physical-chemical analyzes were performed following the technical norm L5.230 (CETESB, 2012) and CONAMA Resolutions 274 and 357 standards (CONAMA, 2000, 2005). The maximum values of the parameters are shown in Supplementary Table S2.

2.4. Antimicrobial susceptibility testing

The disk diffusion method was used to determine the antimicrobial resistance profile of *E. coli* isolates. Thirty-four antimicrobial agents were tested, including those belonging to the classes of β-lactams, FPA, fluoroquinolones, tetracyclines, aminoglycosides, phenicols, nitrofurans, and fosfomycins. The broth microdilution method was used to

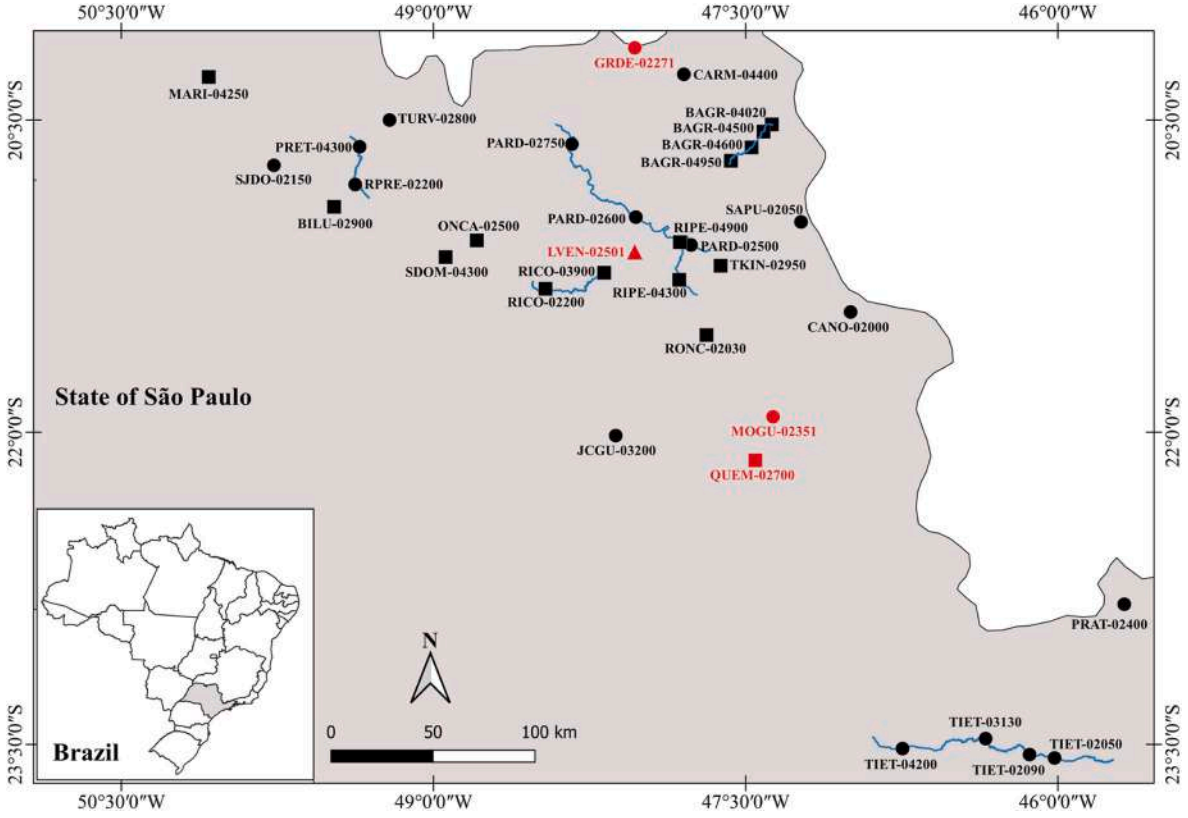

Fig. 1. Map showing the collection sites of the aquatic ecosystems used in this study. Black circles, squares and triangles represent rivers, streams, and lakes, respectively. The red color represents aquatic ecosystems used for recreational purposes. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

determine the minimal inhibitory concentration (MIC) for ceftazidime, ciprofloxacin, and colistin (CLSI, 2020). Multidrug resistance (i.e., resistant to ≥ 1 agent in ≥3 antimicrobial categories) was determined according to Magiorakos et al. (2012).

2.5. Detection of antimicrobial resistance genes (ARGs), plasmid replicon types, and integrons

Several ARGs associated with resistance to polymyxins (*mcr*), folate pathway inhibitors (*sul*), aminoglycosides [*aadA*, *aac*, *aph,* and *ant*], fluoroquinolones (*oqx*, *qnr*, and *qepA*), β-lactams (*bla*), fosfomycin (*fosA*), phenicols (*catA*, *floR*, and *cmlA*), and tetracyclines (*tet*) were detected by conventional polymerase chain reaction (PCR) (Supplementary Table S3). Incompatibility (Inc) groups and integrons were screened by conventional PCR (Supplementary Table S4).

2.6. Evaluation of substitutions in determinants of antimicrobial resistance

Amino acid substitutions in resistance determinants of polymyxins (MgrB, PhoP/PhoQ, and PmrA/PmrB) and fluoroquinolones (GyrA, ParC, and ParE) were determined using Sanger sequencing (Supplementary Table S5). The sequences were aligned using MEGA version X. *E. coli* K-12 substr. MG1655 (GenBank accession no. U00096) was used as the reference strain. The impact of the substitution of determinants of antimicrobial resistance was established using the Protein Variation Effect Analyzer (PROVEAN) software (http://provean.jcvi.org/index.php).

2.7. Detection of metal tolerance genes (MTGs)

Genes related to tolerance to copper (*pcoA*, *copA*), mercury (*merA*), silver (*silA*), chrome (*chrA*), arsenic (*arsB*), lead (*pbrA*), tellurite (*terF*), zinc (*zntB*), cadmium (*cadD*), cadmium/zinc (*czrC*), nickel/cobalt (*rcnA*), and nickel/cobalt/cadmium (*nccA*) were detected by conventional PCR (Supplementary Table S6).

2.8. Detection of virulence genes

Virulence genes related to DEC (*stx1*, *stx2*, *aap*, *aggR*, *AA probe*, *ipaH*, *aatA*, *aaiC*, *bfpA*, *est*, *elt ehxA*, and *eaeA*), ExPEC (*iutA*, *iucA*, *papA/papC*, *kpsMT II*, *sfa/foc*, and *afa/dra*), and other (*hlyF*, *iroN*, *gad*, *iss*, *ompT*, and *lpfA*) were searched by conventional PCR (Supplementary Table S7). *E. coli* isolates were classified as Enteroaggregative *E. coli* (EAEC), Shiga toxin-producing *E. coli* (STEC), and ExPEC following the criteria of Furlan and Stehling (2021) and Johnson et al. (2003).

2.9. Molecular typing

The phylogenetic groups were determined by Clermont *E. coli* phylotyping method (Clermont et al., 2013). The sequence types (STs) were determined using the multilocus sequence typing (MLST) of Achtman scheme (Wirth et al., 2006). The *fimH* allele was identified by *fimH* gene sequencing (Supplementary Table S8).

2.10. Conventional PCR and sequencing conditions

All conventional PCR used to detect genes related to antimicrobial resistance, metal tolerance, virulence, mobile genetic elements, and molecular typing were carried out using a reaction mixture of 25 μL containing 14.75 μL of PCR-grade water, 1 μL (1.25 U) of JumpStart™ Taq DNA Polymerase (Sigma-Aldrich, USA), and 2.5 μL (100 ng) of genomic DNA, 1.75 μL (25 mM) of $MgCl_2$, 1 μL (25 μM) of each primer (Supplementary Tables S3–S8), 2.5 μL of 10X PCR buffer without $MgCl_2$, and 0.5 μL (10 mmol L^{-1}) of deoxynucleotide solution mix. All amplicons were visualized in standard 1% agarose gel electrophoresis after staining with ethidium bromide. The amplicons were purified using the Illustra™ GFX™ PCR DNA and Gel Band Purification Kit (GE Healthcare, USA) and submitted to Sanger sequencing using BigDye™ Terminator v3.1 Cycle Sequencing Kit (Applied Biosystems, Lithuania). The sequences were analyzed using the Geneious Prime program (Biomatters Ltd., New Zealand) and compared to those available in the GenBank database (http://blast.ncbi.nlm.nih.gov/Blast.cgi).

3. Results

3.1. Data from aquatic ecosystems

Microbiological analysis revealed *E. coli* counts >600 CFU/100 mL in the great majority of aquatic ecosystems, highlighting the high counts in rivers. Among the physical-chemical parameters, the values ranged from 38.6 to 548 μS/cm for conductivity; 15–28.2 °C for temperature; 6.1 to 90 NTU for turbidity; < 50–327 mg/L for TDS; < 2–29 mg/L for BOD; 5.78 to 8.28 for pH; < 0.01 to 0.01 mg/L for arsenic; < 0.0008 to <0.001 mg/L to cadmium; < 0.007–0.12 mg/L for lead; < 0.004–0.065 mg/L for copper; < 0.01–0.39 mg/L for chrome; < 0.00001 to <0.0002 mg/L for mercury; < 0.003–0.03 mg/L to nickel; and 0.003 to 0.47 for zinc (Table 1; Supplementary Table S1).

In general, the values of turbidity, TDS, BOD, pH, arsenic, cadmium, lead, chrome, and nickel were higher in rivers, while conductivity, temperature, copper, and zinc were higher in streams (Table 1). Furthermore, BOD, lead and copper in some sampling sites were up to 5 × higher than the standard recommended (<5 for BOD, < 0.01 mg/L for lead, and <0.009 for copper), while the sampling site TIET-02090 showed pH (5.72) lower than the standard recommended (6–9) (Supplementary Tables S1 and S2).

3.2. E. coli isolates and multidrug resistance profiles

Two hundred *E. coli* isolates were obtained from aquatic ecosystems, of which 50 (25%) were MDR, and therefore were added to this study. Among the MDR *E. coli* isolates, all presented resistance to ampicillin, cefazolin, cefaclor, and cefuroxime. More than 70% of *E. coli* isolates presented resistance to trimethoprim-sulfamethoxazole, sulfonamides, trimethoprim, tetracycline, doxycycline, and streptomycin. In addition, resistance to colistin, amoxicillin-clavulanate, ampicillin-sulbactam, ceftazidime, cefotaxime, ceftriaxone, cefepime, aztreonam, gentamicin, tobramycin, nalidixic acid, ciprofloxacin, levofloxacin, norfloxacin, lomefloxacin, ofloxacin, and chloramphenicol (2%–16%) was also detected (Supplementary Table S1; Fig. S1).

3.3. Co-occurrence of ARGs and MTGs

Several ARGs were detected in this study, spotlighting the presence of at least one β-lactamase-encoding gene (bla_{TEM}, bla_{CMY}, bla_{SHV} and/or $bla_{CTX\text{-}M\text{-}8}$) in each MDR *E. coli* isolate. Other ARGs associated with resistance to fluoroquinolones (*qnrB* and *oqxB*), aminoglycosides [*aadA*, *aac(6′)-Ib*, and *ant(2″)-Ia*], tetracyclines [*tet(A)*, *tet(B)*, and *tet(C)*], phenicols (*floR* and *cmlA*), and FPA (*sul1*, *sul2* and *sul3*) were also found (GenBank accession no. OK001307-OK001307). The ARGs bla_{TEM} (n = 39), *aadA* (n = 30), *tet(A)* (n = 26), and *sul1* (n = 26) were the most prevalent (Table 2). Among the MTGs found, *rcnA* (nickel and cobalt efflux pump; n = 46) was the most prevalent, followed by *silA* (silver efflux pump; n = 29), *merA* (mercuric reductase; n = 3), and *pcoA* (copper efflux pump; n = 2) (GenBank accession no. OK001324-OK001327) (Table 2).

Among the MDR *E. coli* isolates, 47 (94%) showed the co-occurrence of at least one ARG and MTG, highlighting the association of β-lactamase-encoding genes and *silA*. The genes *floR*, *cmlA*, and *merA* were detected exclusively in rivers, while $bla_{CTX\text{-}M\text{-}8}$ and *ant(2″)-Ia* were found exclusively in streams (Fig. 2A and B). The genes bla_{SHV}, *qnrB*, *oqxB*, *tet(C)*, and *pcoA* were shared among rivers and streams. Besides, the genes

Table 1
Overview of microbiological and physical-chemical parameters of aquatic ecosystems.

Analysis	Parameters[a]	Rivers[b]		Streams[b]	
		Min	Max	Min	Max
Microbiological	*E. coli* (CFU/100 mL)	106	2,900,000	28	540,000
Physical-chemical	Conductivity (μS/cm)	46.1	544	38.6	548
	Temperature (°C)	15	27.9	19.4	28.2
	Turbidity (NTU)	6.12	90	6.1	60
	TDS (mg/L)	<50	327	<50	280
	BOD (mg/L)	<3	29	<2	18
	pH	5.78	8.28	6.36	8.12
	Arsenic (mg/L)	<0.01	0.01	<0.01	<0.01
	Cadmium (mg/L)	<0.007	<0.001	<0.0008	0.0001
	Lead (mg/L)	<0.007	0.12	<0.007	0.02
	Copper (mg/L)	<0.004	0.007	<0.004	0.065
	Chrome (mg/L)	<0.01	0.39	<0.01	0.05
	Mercury (mg/L)	<0.0001	<0.0002	<0.0001	<0.0002
	Nickel (mg/L)	<0.003	<0.03	<0.003	<0.02
	Zinc (mg/L)	<0.003	0.12	<0.004	0.47

[a] TDS, total dissolved solids; BOD, biochemical oxygen demand.
[b] Min, minimum values; Max, maximum values.

bla$_{TEM}$, *bla*$_{CMY}$, *aadA*, *aac(6′)-Ib*, *tet(A)*, *tet(B)*, *sul1*, *sul2*, *silA*, and *rcnA* were shared among all aquatic ecosystems (Fig. 2A and B). Furthermore, it was not possible to correlate the concentration of metals and the MRGs found since these genes were widely detected in aquatic environments with different concentrations of metals.

3.4. Detection of plasmid replicon types and integrons

Eleven Inc groups were detected among MDR *E. coli* isolates, highlighting IncF$_{repB}$ (24/50), ColE-like (19/50), and IncFIB (15/50) as the most prevalent. In addition, the *intI1* gene (class 1 integron-integrase gene) was found in eight MDR *E. coli* isolates (EW52, EW81, EW89, EW116, EW197, EW223, EW406, EW432, and EW494) (Supplementary Table S1).

3.5. Phylogenetic groups and virulence determinants

The commonest phylogenetic group was B1 (27/50), followed by A (14/50), C (5/50), E (3/50), and D (1/50). The phylogenetic groups A and B1 were distributed among rivers, streams, and lakes, while the phylogenetic groups C and E were distributed between rivers and streams (Table 2). Virulence genes related to DEC (*aap*, *AA probe*, and *stx2*), ExPEC (*iutA*, *kpsMT II*, *iucA*, *papA*, and *papC*), and other (*hlyF*, *iroN*, *gad*, *iss*, and *ompT*) were found in MDR *E. coli* isolates. The virulence genes *gad* (glutamate decarboxylase; $n = 40$) was the most prevalent (GenBank accession no. OK001328-OK001339) (Table 2). Of the 50 MDR *E. coli* isolates, 12%, 10% and 6%, were classified as ExPEC, EAEC, and STEC, respectively (Table 2). Only the *gad* gene was shared among all aquatic ecosystems, while ten virulence genes (*aap*, *AA probe*, *kpsMT II*, *iutA*, *iucA*, *papA*, *papC*, *iroN*, *iss*, and *ompT*) were shared among rivers and streams (Fig. 2C). EAEC and ExPEC isolates were detected in rivers and streams, while STEC isolates were found in streams and lakes. STEC isolates were assigned as B1, while EAEC and ExPEC isolates belonged to a diversity of phylogenetic groups (i.e., A, B1, C, D, and E). Three main aquatic ecosystems (Mogi-Guaçú, Pardo, and Bagres) were identified as potential disseminators of MDR EAEC and ExPEC isolates (Table 2; Supplementary Table S1).

3.6. Extended-spectrum cephalosporins (ESCs)-, ciprofloxacin-, and colistin-resistant E. coli isolates

Seventeen isolates showed resistance to ESCs, ciprofloxacin, and/or colistin, and were submitted to molecular typing and subtyping. ESCs-resistant *E. coli* isolates presented MICs between 16 and 32 mg/L to ceftazidime, harbored *bla*$_{CTX-M-8}$ and *bla*$_{CMY}$, and belonged to A-ST34-*H*972, B1-ST345-*H*31, A-ST398-*H*54, A-ST522-*H*40, A-ST1141-*H*25, B1-ST2521-*H*31, and B1-ST2359-*H*38. Curiously, four ESCs-resistant *E. coli* isolates (EW82, EW89, EW268, and EW126) were classified as EAEC and one (EW83) as STEC (Table 2; Supplementary Table S1). Among ciprofloxacin-resistant *E. coli* isolates, the MICs for ciprofloxacin ranged from 4 to 8 mg/L, and all of these isolates carried PMQR genes (*qnrB* and/or *oqxB*), except the EW442 isolate that showed substitutions in the quinolone resistance-determining regions (QRDR) of GyrA (S83S→L and 87D→N), ParC (80S→I). Those isolates belonged to B1-ST56-*H*54, D-ST106-*H*47, A-ST165-*H*54, A-ST398-*H*54, A-ST540-*H*54, A-ST744-*H*54, B1-ST7020-*H*31, and A-ST8583-NT (Table 3; Supplementary Table S1).

Six *E. coli* isolates exhibited resistance to colistin (MIC of 4 mg/L), which were assigned as D-ST106-*H*47, A-ST398-*H*54, A-ST744-*H*54, A-ST1408-*H*54, B1-ST1727-*H*31, and B1-ST2522-*H*38 (Table 4). None of *mcr*-like genes were found; however, substitutions in PhoP (44I→L), PhoQ (464E→D and 482A→T), PmrA (29S→G, 144G→S, 221E→K, and 222N→D), and PmrB (2H→R, 138S→N, 283D→G, and 358Y→N), were detected (Table 4). None of the substitutions have been predicted as deleterious by *in silico* analysis using PROVEAN. Furthermore, two isolates [EW236 (STEC) and EW423 (ExPEC)] showed resistance to ciprofloxacin and colistin (Table 3; Table 4). Surprisingly, an *E. coli* isolate (EW442) presented resistance to ceftazidime, ciprofloxacin, and colistin, as well as ARGs and resistance determinants for these antimicrobial agents (Table 2; Table 3; Table 4).

4. Discussion

This study reports the occurrence of MDR *E. coli* isolates co-harboring ARGs and MTGs, which also presented virulence genes related to DEC and ExPEC in aquatic ecosystems of Brazil. The environmental pollution by anthropogenic activities contributed powerfully to the occurrence and the dissemination of MDR pathogens to the environment (Pérez-Etayo et al., 2020). The aquatic ecosystems presented the microbiological parameter above the maximum values in the great majority of sampling sites analyzed, supporting the fecal contamination. Most of the genes found were shared between rivers and streams or exclusive to these aquatic environments. These findings may be related to the receiving of a higher pollution load when compared to lakes, which are mainly used for bathing. Accordingly, rivers and streams have the greatest potential for the spread of MDR DEC and ExPEC isolates. The presence of MDR pathogens in aquatic ecosystems has been increasingly reported worldwide, which causes a concerning scenario for public health (Gomi et al., 2017; Aijuka et al., 2018). In aquatic environments from Brazil, MDR and potentially pathogenic *E. coli* isolates have already been identified, corroborating with our

Table 2
Molecular characteristics of MDR *E. coli* isolates recovered from aquatic ecosystems.

Isolate[a,b]	PG[c]	Virulence gene[d]	Antimicrobial resistance gene	Metal tolerance gene[d]
EW82$^{a}_{RVR}$	A	*aap, AA probe, gad*	*bla*$_{CMY}$, *tet(A), tet(B), sul2*	*rcnA, silA*
EW414$^{c}_{RVR}$	E	*gad, iutA, iucA, kpsMT II*	*bla*$_{TEM}$, *sul2*	*rcnA, silA*
EW89$^{a}_{RVR}$	B1	*aap, AA probe, gad*	*bla*$_{CMY}$, *tet(A), tet(C), sul1*	*rcnA*
EW197$_{RVR}$	B1	*gad, iss, iroN, ompT, hlyF*	*bla*$_{TEM}$, *aadA, tet(B), sul1, sul2, floR*	*rcnA, merA, silA*
EW207$_{RVR}$	A	*gad*	*bla*$_{TEM}$, *tet(B), sul1, sul3*	*rcnA*
EW209$^{c}_{RVR}$	E	*gad, iutA, iucA, kpsMT II*	*bla*$_{TEM}$, *aadA, tet(B), sul1*	*rcnA*
EW434$_{RVR}$	A	*gad*	*bla*$_{TEM}$, *qnrB, aadA, aac (6′)-Ib, tet(B), tet(C), sul1*	*rcnA*
EW216$_{RVR}$	B1	*gad, ompT*	*bla*$_{TEM}$, *aadA, sul1*	*rcnA, silA*
EW268$^{a}_{RVR}$	A	*aap, AA probe, gad*	*bla*$_{CMY}$, *aadA, tet(B)*	*rcnA, silA*
EW223$_{RVR}$	B1	*gad, iss, iroN, ompT, hlyF*	*bla*$_{TEM}$, *aadA, tet(A), tet (B), sul1, sul3, cmlA*	*rcnA*
EW508$^{c}_{RVR}$	B1	*iss, ompT, hlyF, iutA, iucA, papA, papC*	*bla*$_{TEM}$, *aadA, tet(A), tet (B), sul1*	*rcnA, silA*
EW334$_{RVR}$	A	*gad*	*bla*$_{TEM}$, *qnrB, oqxB, tet (B)*	*rcnA, silA*
EW358$_{RVR}$	B1	*gad*	*bla*$_{TEM}$, *aadA, sul1*	-
EW374$_{RVR}$	B1	-	*bla*$_{TEM}$, *sul1*	*merA, silA*
EW384$_{RVR}$	B1	*gad*	*bla*$_{TEM}$, *aadA, sul1*	*rcnA*
EW420$_{RVR}$	B1	–	*bla*$_{TEM}$, *tet(A)*	-
EW476$_{RVR}$	B1	*gad*	*bla*$_{TEM}$, *aadA, sul3*	*rcnA, silA*
EW423$^{c}_{RVR}$	D	*iutA, iroN, papA, papC*	*bla*$_{TEM}$, *oqxB, tet(A), sul1, sul2*	*merA, rcnA, silA*
EW442$_{RVR}$	A	*gad*	*bla*$_{CMY}$, *aadA, tet(A), tet (B), sul1, floR*	*rcnA, pcoA*
EW396$_{RVR}$	B1	–	*bla*$_{TEM}$, *sul3, cmlA*	*rcnA*
EW454$_{RVR}$	A	*gad*	*bla*$_{TEM}$, *aadA, tet(A), sul1*	*rcnA, silA*
EW385$_{RVR}$	B1	*gad*	*bla*$_{TEM}$, *sul2*	-
EW460$_{RVR}$	A	–	*bla*$_{TEM}$, *aadA, sul1*	*rcnA, silA*
EW462$_{RVR}$	B1	–	*bla*$_{SHV}$, *aadA, sul2*	*rcnA, silA*
EW51$^{a}_{STR}$	C	*gad, aap, AA probe*	*bla*$_{TEM}$, *aadA, sul2*	*rcnA, silA*
EW52$^{b}_{STR}$	C	*gad, stx2*	*bla*$_{TEM}$, *aadA, tet(A), sul1*	*rcnA, silA*
EW500$_{STR}$	B1	*gad*	*bla*$_{TEM}$, *aadA, tet(A), sul1*	*rcnA*
EW81$_{STR}$	A	*gad, iss, iroN, ompT, hlyF*	*bla*$_{SHV}$, *aadA, tet(A)*	*rcnA*
EW205$_{STR}$	B1	*gad, iss, iroN, ompT*	*bla*$_{TEM}$, *tet(A), tet(B), sul1*	*rcnA, silA*
EW126$^{a}_{STR}$	B1	*gad, aap, AA probe*	*bla*$_{CTX-M-8}$, *tet(A), sul1*	*rcnA, silA*
EW128$^{c}_{STR}$	C	*gad, iutA, iucA, kpsMT II*	*bla*$_{TEM}$, *tet(A)*	*rcnA, silA*
EW494$_{STR}$	A	*gad*	*bla*$_{TEM}$, *aadA, tet(A), sul2*	*rcnA*
EW132$_{STR}$	C	*gad*	*bla*$_{TEM}$, *ant(2″)-Ia, tet (A), tet(C)*	*rcnA, silA*
EW488$_{STR}$	B1	*gad*	*bla*$_{TEM}$, *aadA, tet(A), tet (B), sul1*	*rcnA, silA*
EW173$_{STR}$	B1	*gad*	*bla*$_{SHV}$, *aac(6′)-lb, tet (A), tet(B)*	*rcnA*
EW213$_{STR}$	B1	*gad*	*bla*$_{TEM}$, *sul1*	*rcnA, silA*
EW504$_{STR}$	C	*gad, iutA*	*bla*$_{TEM}$, *aadA, tet(A), tet (B)*	*rcnA, silA, nccA*
EW506$_{STR}$	E	*iroN*	*bla*$_{TEM}$, *aadA, tet(A)*	*rcnA, silA*
EW236$^{b}_{STR}$	A	*gad, stx2*	*bla*$_{TEM}$, *qnrB, aadA, sul1*	*rcnA*
EW406$_{STR}$	A	–	*bla*$_{TEM}$, *tet(A), sul2*	*rcnA, silA*
EW464$_{STR}$	B1	*gad*	*bla*$_{TEM}$, *qnrB, oqxB, aadA, tet(A), sul1*	*rcnA, silA*
EW227$_{STR}$	B1	*gad*	*bla*$_{TEM}$, *aadA, tet(B), sul1, sul2*	*rcnA*

Table 2 (*continued*)

Isolate[a,b]	PG[c]	Virulence gene[d]	Antimicrobial resistance gene	Metal tolerance gene[d]
EW400$_{STR}$	B1	*gad*	*bla*$_{CMY}$, *qnrB, aac(6′)-Ib*	*rcnA, silA*
EW458$^{c}_{STR}$	B1	*gad, iutA, papA, papC*	*bla*$_{TEM}$, *aadA, tet(A), sul2*	*rcnA, pcoA*
EW496$_{STR}$	B1	*gad*	*bla*$_{TEM}$, *aadA, tet(B), sul2*	*rcnA*
EW502$_{STR}$	A	*gad*	*bla*$_{TEM}$, *qnrB, aadA, tet (A), sul2*	*rcnA, silA*
EW83$^{b}_{LK}$	B1	*gad, stx2*	*bla*$_{CMY}$, *sul3*	*rcnA, silA*
EW116 $_{LK}$	A	*gad*	*bla*$_{CMY}$, *tet(A), sul1, sul2*	*rcnA*
EW394 $_{LK}$	A	*gad*	*bla*$_{TEM}$, *aadA, tet(A), sul1*	*rcnA*
EW452$_{LK}$	B1	–	*bla*$_{TEM}$, *aadA, tet(B), sul3*	*rcnA, silA*

[a] Isolates recovered from rivers (RVR), streams (STR), and lakes (LK).
[b] Isolates classified as EAEC [(a)], STEC [(b)] and ExPEC [(c)].
[c] PG, phylogenetic group.
[d] (–), no genes found.

results and alerting for the presence and dissemination of these isolates in the environment (Furlan et al., 2020; Gomes et al., 2022).

In general, *E. coli* isolates exhibited resistance mainly to ampicillin, streptomycin, FPA, narrow-spectrum cephalosporins, and tetracyclines, and this multidrug resistance phenotype has been frequently reported at the human-animal-environment interface (Savin et al., 2020; Furlan and Stehling, 2021). In addition, some *E. coli* isolates exhibited resistance to critically important antimicrobials, such as colistin, ESC, and/or fluoroquinolones, which are historically linked to human and veterinary medicine, and more recently have been spreading in environmental sources (Codjoe and Donkor, 2017; Gharaibeh and Shatnawi, 2019; Furlan et al., 2020; Lopes et al., 2021). In this regard, several ARGs were detected, spotlighting genes encoding ESBL, pAmpC, and PMQR that are commonly described on plasmids, although they can also be integrated into the chromosome (Casella et al., 2018).

The resistance to ciprofloxacin and colistin can occur by the acquisition of PMQR genes and *mcr*-like, as well as by substitutions in the QRDR and two-component systems (TCS) involving PhoP/PhoQ and PmrA/PmrB, respectively (Hooper and Jacoby, 2015; El-Sayed Ahmed et al., 2020). These mechanisms were shared among MDR *E. coli* isolates, except the presence of *mcr*-like genes. A diversity of substitutions in chromosomal targets for acquired resistance to colistin were found, and in *E. coli* isolates susceptible and resistant to colistin were reported with substitutions in PhoP (44I→L), PmrA (29S→G), and PmrB (2H→R, 283D→G) (Luo et al., 2017; Sato et al., 2018), while substitutions in PmrA (144G→S) and PmrB (358Y→N) have already been described in colistin-resistant *E. coli* resistant from hospitals (Luo et al., 2017; Zakaria et al., 2021). Contrastingly, some isolates did not show *mcr*-like genes or deleterious substitutions in TCS involving PhoP/PhoQ and PmrA/PmrB, suggesting that other mechanisms may be involved in the colistin resistance. Besides, the phylogenetic distance to the reference strain used may also reflect on the substitutions found. Therefore, it is necessary to extend knowledge on phenotypic-genotypic correlations for colistin in various bacterial strains (Sato et al., 2018; Gogry et al., 2021).

Coexistence between ARGs and MTGs have been described on chromosome and plasmids of several bacterial species (Hobman and Crossman, 2015). In environmental sources, subinhibitory concentrations of antimicrobials and metals increase the HGT of ARGs and MTGs (Durão et al., 2018). However, due to the low concentrations of toxic forms of metals, the correlation between MTGs and metal concentrations does not seem to exist, reinforcing the co-existence of MTGs and ARGs as a process that is independent of selective pressure due to high concentrations of metals and corroborating with our findings (Gwin and Gunsch, 2018). The *rcnA* and *silA* were the most prevalent MTGs, although other MTGs were also found (e.g., *pcoA* and *merA*), suggesting

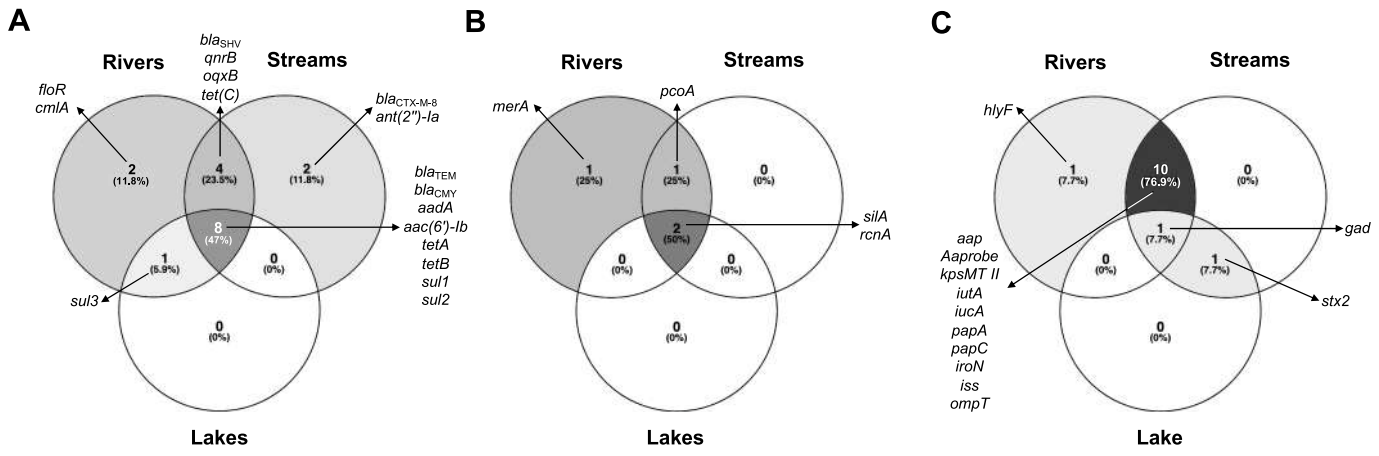

Fig. 2. Venn diagram of genes found according to aquatic ecosystems. A, antimicrobial resistance genes; B, metal tolerance genes; C, virulence genes.

Table 3
Molecular typing and substitutions in the QRDR of ciprofloxacin-resistant *E. coli* isolates.

Isolate	ST[a]	ST Cplx	*fimH*[b]	MIC CIP[c]	PMQR[d]	Amino acid substitution[e]		
				(mg/L)		GyrA	ParC	ParE
EW236	ST744	10	54	4^R	*qnrB*	WT	WT	WT
EW334	ST8583	10	NT	4^R	*qnrB, oqxB*	WT	WT	WT
EW400	ST7020	278	31	4^R	*qnrB*	WT	WT	WT
EW423	ST106	69	47	4^R	*oqxB*	WT	WT	WT
EW434	ST165	165	54	4^R	*qnrB*	WT	WT	WT
EW442	ST398	398	54	8^R	–	83S→L, 87D→N	80S→I	WT
EW464	ST56	155	54	4^R	*qnrB, oqxB*	WT	WT	WT
EW502	ST540	Singleton	54	4^R	*qnrB*	WT	WT	WT

[a] ST, sequence type.
[b] NT, non-typeable.
[c] MIC, minimum inhibitory concentration; CIP, ciprofloxacin.^{R}, resistance to ciprofloxacin.
[d] PMQR, plasmid-mediated quinolone resistance gene; $^{(-)}$, negative for PMQR.
[e] D, aspartic acid; I, isoleucine; L, leucine; N, asparagine; S, serine; WT, wild type.

Table 4
Molecular typing and substitutions in the TCS involving PhoP/PhoQ and PmrA/PmrB of colistin-resistant *E. coli* isolates.

Isolate	ST[a]	ST Cplx	*fimH*	MIC (mg/L)[b]	*mcr*-like[c]	Amino acid substitution[d]				
				Colistin		MgrB	PhoP	PhoQ	PmrA	PmrB
EW205	ST2522	Singleton	38	4^R	–	WT	44I→L	WT	29S→G, 144G→S	283D→G, 358Y→N
EW207	ST1408	Singleton	54	4^R	–	WT	WT	464E→D, 482A→T	29S→G, 222N→D	2H→R
EW236	ST744	10	54	4^R	–	WT	WT	WT	29S→G, 222N→D	WT
EW423	ST106	69	47	4^R	–	WT	44I→L	482A→T	29S→G	2H→R, 138S→N, 283D→G
EW442	ST398	398	54	4^R	–	WT	44I→L	482A→T	29S→G	2H→R, 138S→N, 283D→G
EW452	ST1727	446	31	4^R	–	WT	44I→L	WT	29S→G, 221E→K	283D→G, 358Y→N

[a] ST, sequence type.
[b] MIC, minimum inhibitory concentration;^{R}, resistance to colistin.
[c] $^{(-)}$, negative for *mcr*-like genes (*mcr-1* to *mcr-9*).
[d] A, alanine; D, apartic acid; E, glutamic acid; G, glycine; H, histidine; I, isoleucine; K, lysine; L, leucine; N, asparagine; R, arginine; S, serine; T, threonine; Y, tyrosine; WT, wild type.

a genetic linkage with plasmid-mediated ARGs (e.g., bla_{TEM} and bla_{CMY}) as described in aquatic environments (Randall et al., 2015; Furlan et al., 2020; Li et al., 2020; Hounmanou et al., 2021).

Molecular typing revealed a diversity of phylogenetic groups, STs and *fimH*-type, in which the association of phylogenetic group A and ST Cplx 10 was the most prevalent. *E. coli* A-ST Cplx 10 lineages, highlighting the high-risk clone ST744, exhibiting multidrug resistance phenotype associated with virulence genes have been reported in human and animal hosts, but infrequently in environmental sources (Pietsch et al., 2018). Surprisingly, the MDR *E. coli* isolate (EW126) was classified as EAEC, belonged to a rare ST2521 and co-harbored $bla_{CTX\text{-}M\text{-}8}$ and *silA*. In Southeastern Brazil, the same region of this study, MDR *E. coli* lineages A-ST744-*H*54 and B1-ST2522-*H*38 were isolated from wild fish in the Atlantic Coast (Sellera et al., 2018), and sheep in a farmhouse (Furlan et al., 2019), respectively, while B1-ST56-*H*54, B1-ST345-*H*31, A-ST34-*H*121, the latter differing in the *fimH*-type (*H*972), were described in soil samples (Furlan and Stehling, 2021), showing the spreading of these clones. Furthermore, some of the STs found in this study were not previously described in Brazil, nor in aquatic ecosystems around the world.

The emergence of potentially pathogenic bacteria exhibiting antimicrobial resistance and metal tolerance emerged as a global health

problem at the human-animal-environment interface. As metals are promising for the treatment of bacterial pathogens (Evans and Kavanagh, 2021), the appearance of MDR *E. coli* isolates co-carrying ARGs and MTGs calls attention since therapeutic failures can occur in the treatment of infections caused by bacteria with these characteristics. In addition, MDR and virulent *E. coli* isolates in aquatic ecosystems can migrate to surrounding environments and the food production chain, causing acquired and endogenous infections (Reid et al., 2020). On the other hand, these bacteria can colonize humans and animals, contributing to the HGT to the gut microbiota (Maeusli et al., 2020).

5. Conclusions

The occurrence of MDR and potentially pathogenic *E. coli* isolates, including those STs commonly reported in human and animal medicine and the high-risk clone ST744, in aquatic ecosystems represents a risk to global health, reinforcing the ability of these lineages to survive and spread in the environment. In addition, MDR *E. coli* isolates were recovered from aquatic ecosystems with high counts of *E. coli* but with metal values within the recommended range. Therefore, this study contributes to the monitoring of antimicrobial resistance worldwide, focusing on aquatic ecosystems of Brazil.

CRediT authorship contribution statement

João Pedro Rueda Furlan: Conceptualization, Resources, Investigation, Methodology, Software, Validation, Formal analysis, Data Curation, Writing — Original Draft, Writing — Review & Editing. Micaela Santana Ramos: Investigation, Methodology. Rafael da Silva Rosa: Investigation, Methodology. Eduardo Angelino Savazzi, Resources, Investigation. Eliana Guedes Stehling: Conceptualization, Funding acquisition, Supervision, Investigation, Project administration, Data Curation, Writing — Original Draft, Writing — Review & Editing; Visualization.

Declaration of competing interest

The authors declare no conflicts of interest.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.ijheh.2022.114003.

References

Aijuka, M., Santiago, A.E., Girón, V.E.R., Nataro, J.P., Buys, E.M., 2018. Enteroaggregative *Escherichia coli* is the predominant diarrheagenic *E. coli* pathotype among irrigation water and food sources in South Africa. Int. J. Food Microbiol. 278, 44–51. https://doi.org/10.1016/j.ijfoodmicro.2018.04.018.

Arendsen, L.P., Thakar, R., Sultan, A.H., 2019. The use of copper as an antimicrobial agent in health care, including obstetrics and gynecology. Clin. Microbiol. Ver. 32, e00125-18 https://doi.org/10.1128/CMR.00125-18.

Baker-Austin, C., Wright, M.S., Stepanauskas, R., McArthur, J.V., 2006. Co-selection of antibiotic and metal resistance. Trends Microbiol. 14, 176–182. https://doi.org/10.1016/j.tim.2006.02.006.

Bortoluzzi, C., Vieira, B.S., Applegate, T.J., 2020. Influence of dietary zinc, copper, and manganese on the intestinal health of broilers under *eimeria* challenge. Front. Vet. Sci. 7, 13. https://doi.org/10.3389/fvets.2020.00013.

Casella, T., Haenni, M., Madela, N.K., Andrade, L.K., Pradela, L.K., Andrade, L.N., Darini, A.L.D.C., Madec, J.Y., Nogueira, M.C.L., 2018. Extended-spectrum cephalosporin-resistant *Escherichia coli* isolated from chickens and chicken meat in Brazil is associated with rare and complex resistance plasmids and pandemic ST lineages. J. Antimicrob. Chemother. 73, 3293–3297. https://doi.org/10.1093/jac/dky335.

CETESB, 2012. Norma Técnica L5.230. *Escherichia coli* – Determination in Water Samples by Membrane Filtration Technique: Test Method [WWW Document]. URL, 01.02.22. https://cetesb.sp.gov.br/normas-tecnicas-cetesb/normas-tecnicas-vigentes/.

Clermont, O., Christenson, J.K., Denamur, E., Gordon, D.M., 2013. The Clermont *Escherichia coli* phylo-typing method revisited: improvement of specificity and detection of new phylo-groups. Environ. Microbiol. Rep. 5, 58–65. https://doi.org/10.1111/1758-2229.12019.

CLSI (Clinical and Laboratory Standards Institute), 2020. Performance Standards for Antimicrobial Susceptibility Testing. CLSI Supplement M100, 30th Edn. Clinical and Laboratory Standards Institute, Wayne, PA.

Codjoe, F.S., Donkor, E.S., 2017. Carbapenem resistance: a review. Med. Sci. 6, 1. https://doi.org/10.3390/medsci6010001.

CONAMA, 2000. Resolução 274, de 29 de novembro de 2000 [WWW Document]. URL, 01.02.22. http://www2.mma.gov.br/port/conama/legiabre.cfm?codlegi=272.

CONAMA, 2005. Resolução 357, de 17 de Março de 2005 [WWW Document]. URL, 01.02.22. http://www.mma.gov.br/port/conama/res/res05/res35705.pdf.

Durão, P., Balbontín, R., Gordo, I., 2018. Evolutionary mechanisms shaping the maintenance of antibiotic resistance. Trends Microbiol. 26, 677–691. https://doi.org/10.1016/j.tim.2018.01.005.

El-Sayed Ahmed, M.A.E., Zhong, L.L., Shen, C., Yang, Y., Doi, Y., Tian, G.B., 2020. Colistin and its role in the Era of antibiotic resistance: an extended review (2000-2019). Emerg. Microb. Infect. 9, 868–885. https://doi.org/10.1080/22221751.2020.1754133.

Estaleva, C.E.L., Zimba, T.F., Sekyere, J.O., Govinden, U., Chenia, H.Y., Simonsen, G.S., Haldorsen, B., Essack, S.Y., Sundsfjord, A., 2021. High prevalence of multidrug resistant ESBL- and plasmid mediated AmpC-producing clinical isolates of *Escherichia coli* at Maputo Central Hospital, Mozambique. BMC Infect. Dis. 21, 16. https://doi.org/10.1186/s12879-020-05696-y.

Evans, A., Kavanagh, K.A., 2021. Evaluation of metal-based antimicrobial compounds for the treatment of bacterial pathogens. J. Med. Microbiol. 70, 001363 https://doi.org/10.1099/jmm.0.001363.

Feng, C., Xie, B., Wuren, Q., Gao, M., 2020. Meta-analysis of the correlation between dietary copper supply and broiler performance. PLoS One 15, e0232876. https://doi.org/10.1371/journal.pone.0232876.

Furlan, J.P.R., Gallo, I.F.L., de Campos, A.C.L.P., Passaglia, J., Falcão, J.P., Navarro, A., Nakazato, G., Stehling, E.G., 2019. Molecular characterization of multidrug-resistant Shiga toxin-producing *Escherichia coli* harboring antimicrobial resistance genes obtained from a farmhouse. Pathog. Glob. Health 113, 268–274. https://doi.org/10.1080/20477724.2019.1693712.

Furlan, J.P.R., Savazzi, E.A., Stehling, E.G., 2020. Widespread high-risk clones of multidrug-resistant extended-spectrum β-lactamase-producing *Escherichia coli* B2-ST131 and F-ST648 in public aquatic environments. Int. J. Antimicrob. Agents 56, 106040. https://doi.org/10.1016/j.ijantimicag.2020.106040.

Furlan, J.P.R., Stehling, E.G., 2021. Multiple sequence types, virulence determinants and antimicrobial resistance genes in multidrug- and colistin-resistant *Escherichia coli* from agricultural and non-agricultural soils. Env. Poll. 288, 117804 https://doi.org/10.1016/j.envpol.2021.117804.

Gaeta, N.C., de Carvalho, D.U., Fontana, H., Sano, E., Moura, Q., Fuga, B., Munoz, P.M., Gregory, L., Lincopan, N., 2022. Genomic features of a multidrug-resistant and mercury-tolerant environmental *Escherichia coli* recovered after a mining dam disaster in South America. Sci. Total Environ. 823, 153590 https://doi.org/10.1016/j.scitotenv.2022.153590.

Gharaibeh, M.H., Shatnawi, S.Q., 2019. An overview of colistin resistance, mobilized colistin resistance genes dissemination, global responses, and the alternatives to colistin: a review. Vet. World 12, 1735–1746. https://doi.org/10.14202/vetworld.2019.1735-1746.

Gomi, R., Matsuda, T., Matsumura, Y., Yamamoto, M., Tanaka, M., Ichiyama, S., Yoneda, M., 2017. Whole-Genome analysis of antimicrobial-resistant and extraintestinal pathogenic *Escherichia coli* in river water. Appl. Environ. Microbiol. 83, e02703-e02716 https://doi.org/10.1128/AEM.02703-16.

Gogry, F.A., Siddiqui, M.T., Sultan, I., Haq, Q.M.R., 2021. Current update on intrinsic and acquired colistin resistance mechanisms in bacteria. Front. Med. 8, 1250. https://doi.org/10.3389/fmed.2021.677720.

Gomes, R.P., Oliveira, T.R., Gama, A.R., Vieira, J.D.G., Rocha, T.L., Carneiro, L.C., 2022. Gene resistance profile and multidrug-resistant bacteria isolated from a stream in midwestern Brazil. Environ. Nanotechnol. Monit. Manag. 18, 100688 https://doi.org/10.1016/j.enmm.2022.100688.

Gwin, C.A., Gunsch, C.K., 2018. Examining relationships between total silver concentration and Sil silver resistance genes in domestic wastewater treatment plants. J. Appl. Microbiol. 124, 1638–1646. https://doi.org/10.1111/jam.13731.

Hobman, J.L., Crossman, L.C., 2015. Bacterial antimicrobial metal ion resistance. J. Med. Microbiol. 64, 471–497. https://doi.org/10.1099/jmm.0.023036-0.

Hooper, D.C., Jacoby, G.A., 2015. Mechanisms of drug resistance: quinolone resistance. Ann. N. Y. Acad. Sci. 1354, 12–31. https://doi.org/10.1111/nyas.12830.

Hounmanou, Y.M.G., Bortolaia, V., Dang, S.T.T., Truong, D., Olsen, J.E., Dalsgaard, A., 2021. ESBL and AmpC β-lactamase encoding genes in *E. coli* from pig and pig farm

workers in vietnam and their association with mobile genetic elements. Front. Microbiol. 12, 629139 https://doi.org/10.3389/fmicb.2021.629139.
Hwang, A.Y., Gums, J.G., 2016. The emergence and evolution of antimicrobial resistance: impact on a global scale. Bioorg. Med. Chem. 24, 6440–6445. https://doi.org/10.1016/j.bmc.2016.04.027.
Imran, Md, Das, K.R., Naik, M.M., 2019. Co-selection of multi-antibiotic resistance in bacterial pathogens in metal and microplastic contaminated environments: an emerging health threat. Chemosphere 215, 846–857. https://doi.org/10.1016/j.chemosphere.2018.10.114.
Johnson, J.R., Murray, A.C., Gajewski, A., Sullivan, M., Snippes, P., Kuskowski, M.A., Smith, K.E., 2003. Isolation and molecular characterization of nalidixic acid-resistant extraintestinal pathogenic *Escherichia coli* from retail chicken products. Antimicrob. Agents Chemother. 47, 2161–2168. https://doi.org/10.1128/AAC.47.7.2161-2168.2003.
Klein, E.Y., Van Boeckel, T.P., Martinez, E.M., Pant, S., Gandra, S., Levin, S.A., Goossens, H., Laxminarayan, R., 2018. Global increase and geographic convergence in antibiotic consumption between 2000 and 2015. Proc. Natl. Acad. Sci. USA 115, E3463–E3470. https://doi.org/10.1073/pnas.1717295115.
Li, Y., Dai, X., Zeng, J., Gao, Y., Zhang, Z., Zhang, L., 2020. Characterization of the global distribution and diversified plasmid reservoirs of the colistin resistance gene *mcr-9*. Sci. Rep. 10, 811. https://doi.org/10.1038/s41598-020-65106-w.
Lopes, R., Furlan, J.P.R., Dos Santos, L.D.R., Gallo, I.F.L., Stehling, E.G., 2021. Colistin-resistant *mcr-1*-positive *Escherichia coli* ST131-H22 carrying $bla_{CTX\text{-}M\text{-}15}$ and *qnrB19* in agricultural soil. Front. Microbiol. 12, 659900 https://doi.org/10.3389/fmicb.2021.659900.
Luo, Q., Yu, W., Zhou, K., Guo, L., Shen, P., Lu, H., Huang, C., Xu, H., Xu, S., Xiao, Y., Li, L., 2017. Molecular epidemiology and colistin resistant mechanism of *mcr*-positive and *mcr*-negative clinical isolated *Escherichia coli*. Front. Microbiol. 8, 2262. https://doi.org/10.3389/fmicb.2017.02262.
Maeusli, M., Lee, B., Miller, S., Reyna, Z., Lu, P., Yan, J., Ulhaq, A., Skandalis, N., Spellberg, B., Luna, B., 2020. Horizontal gene transfer of antibiotic resistance from *Acinetobacter baylyi* to *Escherichia coli* on lettuce and subsequent antibiotic resistance transmission to the gut microbiome. mSphere 5, e00329-20. https://doi.org/10.1128/mSphere.00329-20.
Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin. Microbiol. Infect. 18, 268–281. https://doi.org/10.1111/j.1469-0691.2011.03570.x.
McEwen, S.A., Collignon, P.J., 2018. Antimicrobial resistance: a one health perspective. Microbiol. Spectr. 6, 2. https://doi.org/10.1128/microbiolspec.ARBA-0009-2017.
Mijnendonckx, K., Leys, N., Mahillon, J., Silver, S., Van Houdt, R., 2013. Antimicrobial silver: uses, toxicity and potential for resistance. Biometals 26, 609–621. https://doi.org/10.1007/s10534-013-9645-z.
Pérez-Etayo, L., González, D., Leiva, J., Vitas, A.I., 2020. Multidrug-resistant bacteria isolated from different aquatic environments in the north of Spain and south of France. Microorganisms 8, 1425. https://doi.org/10.3390/microorganisms8091425.
Pietsch, M., Irrgang, A., Roschanski, N., Brenner Michael, G., Hamprecht, A., Rieber, H., Käsbohrer, A., Schwarz, S., Rösler, U., Kreienbrock, L., Pfeifer, Y., Fuchs, S., Werner, G., RESET Study Group, 2018. Whole genome analyses of CMY-2-producing *Escherichia coli* isolates from humans, animals and food in Germany. BMC Genom. 19, 601. https://doi.org/10.1186/s12864-018-4976-3.
Randall, C.P., Gupta, A., Jackson, N., Busse, D., O'Neill, A.J., 2015. Silver resistance in Gram-negative bacteria: a dissection of endogenous and exogenous mechanisms. J. Antimicrob. Chemother. 70, 1037–1046. https://doi.org/10.1093/jac/dku523.
Reid, C.J., Blau, K., Jechalke, S., Smalla, K., Djordjevic, S.P., 2020. Whole genome sequencing of *Escherichia coli* from store-bought produce. Front. Microbiol. 10, 3050. https://doi.org/10.3389/fmicb.2019.03050.
Sato, T., Shiraishi, T., Hiyama, Y., Honda, H., Shinagawa, M., Usui, M., Kuronuma, K., Masumori, N., Takahashi, S., Tamura, Y., Yokota, S.I., 2018. Contribution of novel amino acid alterations in PmrA or PmrB to colistin resistance in *mcr*-negative *Escherichia coli* clinical isolates, including major multidrug-resistant lineages O25b:H4-ST131-H30Rx and non-x. Antimicrob. Agents Chemother. 62, e00864-18 https://doi.org/10.1128/AAC.00864-18.
Savin, M., Bierbaum, G., Hammerl, J.A., Heinemann, C., Parcina, M., Sib, E., Voigt, A., Kreyenschmidt, J., 2020. Antibiotic-resistant bacteria and antimicrobial residues in wastewater and process water from German pig slaughterhouses and their receiving municipal wastewater treatment plants. Sci. Total Environ. 727, 138788 https://doi.org/10.1016/j.scitotenv.2020.138788.
Seiler, C., Berendonk, T.U., 2012. Heavy metal driven co-selection of antibiotic resistance in soil and water bodies impacted by agriculture and aquaculture. Front. Microbiol. 3, 399. https://doi.org/10.3389/fmicb.2012.00399.
Sellera, F.P., Fernandes, M.R., Moura, Q., Carvalho, M.P.N., Lincopan, N., 2018. Extended-spectrum-β-lactamase (CTX-M)-producing *Escherichia coli* in wild fishes from a polluted area in the Atlantic Coast of South America. Mar. Pollut. Bull. 135, 183–186. https://doi.org/10.1016/j.marpolbul.2018.07.012.
Tamhankar, A.J., Stålsby Lundborg, C., 2019. Antimicrobials and antimicrobial resistance in the environment and its remediation: a global one health perspective. Int. J. Environ. Res. Publ. Health 16, 4614. https://doi.org/10.3390/ijerph16234614.
Wirth, T., Falush, D., Lan, R., Colles, F., Mensa, P., Wieler, L.H., Karch, H., Reeves, P.R., Maiden, M.C., Ochman, H., Achtman, M., 2006. Sex and virulence in *Escherichia coli*: an evolutionary perspective. Mol. Microbiol. 60, 1136–1151. https://doi.org/10.1111/j.1365-2958.2006.05172.x.
Yang, Y., Xu, C., Cao, X., Lin, H., Wang, J., 2017. Antibiotic resistance genes in surface water of eutrophic urban lakes are related to heavy metals, antibiotics, lake morphology and anthorpic impact. Ecotoxicology 26, 831–840. https://doi.org/10.1007/s10646-017-1814-3.
Zakaria, A.S., Edward, E.A., Mohamed, N.M., 2021. Genomic insights into a colistin-resistant uropathogenic *Escherichia coli* strain of O23:H4-ST641 lineage harboring *mcr-1.1* on a conjugative IncHI2 plasmid from Egypt. Microorganisms 9, 799. https://doi.org/10.3390/microorganisms9040799.

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Prevalence of ESKAPE pathogens in the environment: Antibiotic resistance status, community-acquired infection and risk to human health

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ABSTRACT

The ESKAPE (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.) pathogens are characterised by increased levels of resistance towards multiple classes of first line and last-resort antibiotics. Although these pathogens are frequently isolated from clinical environments and are implicated in a variety of life-threatening, hospital-associated infections; antibiotic resistant ESKAPE strains have been isolated from environmental reservoirs such as surface water, wastewater, food, and soil. Literature on the persistence and subsequent health risks posed by the ESKAPE isolates in extra-hospital settings is however, limited and the current review aims to elucidate the primary reservoirs of these pathogens in the environment, their antibiotic resistance profiles, and the link to community-acquired infections. Additionally, information on the current state of research regarding health-risk assessments linked to exposure of the ESKAPE pathogens in the natural environment, is outlined.

1. Introduction

Antimicrobial resistance (AMR) presents a major challenge towards achieving universal health care and impedes the accomplishment of several of the sustainable development goals (SDGs), including: (i) zero hunger, improved nutrition, food security, and sustainable agriculture [e.g., as populations grow, there is an increased demand for animal proteins, resulting in increased antibiotic consumption in the food-animal sector (SDG 2)]; (ii) good health and well-being (SDG 3); and, (iii) clean water and sanitation (SDG 6) (Interagency Coordination Group on Antimicrobial Resistance, 2019). To tackle the threat of AMR, in 2017, the World Health Organisation (WHO) developed a list of antibiotic resistant, global priority pathogens to aid the research and development of new and effective antibiotic treatments (World Health Organisation, 2017). Established according to multi-criteria analyses, the list was ranked into three priority tiers: medium, high, and critical (Tacconelli et al., 2018).

Within the "Priority 1: Critical" pathogen group on the WHO's priority pathogens list are the multidrug resistant (MDR; bacteria that are resistant to three or more classes of antibiotics) Gram-negative ESKAPE (Rice, 2008) pathogens; *Acinetobacter baumannii* (carbapenem-resistant), *Pseudomonas aeruginosa* (carbapenem-resistant), *Klebsiella pneumoniae* (third generation cephalosporin-resistant) and *Enterobacter* spp. (third generation cephalosporin-resistant). The "Priority 2: High" pathogen group contains the Gram-positive ESKAPE pathogens; *Enterococcus faecium* (vancomycin-resistant) and *Staphylococcus aureus* (methicillin-resistant, vancomycin intermediate and resistant). These pathogens contribute significantly to the burden of disease in developed and developing nations and are frequently isolated from clinical settings where they are associated with several life-threatening, hospital--acquired (HA) infections [e.g., bacteraemia, urinary tract infections (UTIs), pneumonia, meningitis, and wound infections, amongst others], particularly in intensive care units (ICUs) (Navidinia et al., 2017). Consequently, infections caused by the ESKAPE pathogens are a leading cause of mortality and morbidity worldwide.

To evade the effects of antibiotics, the mechanisms utilised by the ESKAPE pathogens may include target alteration (e.g., modification of topoisomerase enzymes or ribosomal subunits), decreased drug uptake [e.g., mutations in outer membrane (OM) proteins], production of

Abbreviations: ESBL, Extended-spectrum beta-lactamase.

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Abbreviations

ABR	Antibiotic resistance
AeMBR	Aerobic membrane bioreactor
AMR	Antimicrobial resistance
AnMBR	Anaerobic membrane bioreactor
ARB	Antibiotic resistant bacteria
CA	Community-acquired
CAP	Community-acquired pneumonia
ESKAPE	*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* spp.
HA	Hospital-acquired
HIV	Human immunodeficiency virus
ICU	Intensive care unit
KPC	*K. pneumoniae* carbapenemase
MBR	Membrane bioreactor
MDR	Multidrug resistance
MRSA	Methicillin-resistant *S. aureus*
OM	Outer membrane
PDR	Pan-drug resistant
QMRA	Quantitative microbial risk assessment
SDG	Sustainable development goal
SSTI	Skin-and-soft tissue infection
ST	Sequence-type
UTI	Urinary tract infection
VRE	Vancomycin-resistant enterococci
VRSA	Vancomycin-resistant *S. aureus*
WHO	World Health Organisation
WWTP	Wastewater treatment plant
XDR	Extensively drug resistant

biofilms or a protective exopolysaccharide matrix, production of degrading enzymes [e.g., beta(β)-lactamases], overexpression of efflux pumps or the adaption of alternative metabolic pathways (e.g., folic acid metabolism) (Gajdács et al., 2021). It is thus well established that the ESKAPE pathogens exhibit resistance towards several major classes of antibiotics including macrolides, tetracyclines, oxazolidinones, β-lactams, lipopeptides, β-lactam-β-lactamase inhibitor combinations, as well as last-line antibiotics such as glycopeptides, carbapenems and polymyxins (Zaman et al., 2017). Numerous studies have however, shown that varying degrees of antibiotic resistance (ABR), as well as heteroresistant phenotypes, exist in ESKAPE pathogen strains (El-Halfawy and Valvano, 2015; Jayol et al., 2015; Charretier et al., 2018; Wang et al., 2018; Uechi et al., 2019; Zhou et al., 2020a; Jo and Ko, 2021; Nurjadi et al., 2021). Moreover, while most research has focused on the identification, prevalence, and ABR classification of these pathogens in hospital/clinical environments, the ESKAPE strains have also been isolated from several extra-hospital reservoirs. In fact, common environmental reservoirs of the ESKAPE pathogens include soil, food (vegetables, fruit, and animal products), various water sources (surface runoff, drinking water, streams, dams, and rivers, amongst others), plants and sewage or municipal waste (Havenga et al., 2019; Klockgether and Tümmler, 2017; Pachori et al., 2019). As AMR is an ecological issue defined by complex interactions between a diverse range of microbial populations which affect the health of animals, humans, and the environment (e.g., ABR genes may be transferred from environmental reservoirs to pathogenic bacteria, affecting future interactions between these organisms and their surrounding environment), a multi-disciplinary approach is needed to better understand the emergence of various diseases associated with the ESKAPE pathogens (e.g., zoonotic, vector-borne, and chronic), conduct risk assessments, and implement control strategies for these pathogens in the environment (Collignon and McEwen, 2019). Therefore, as humans, food, and the environment have all been identified as drivers of AMR, the importance of a One Health approach has been highlighted as a major strategy in overcoming the threat of AMR (Interagency Coordination Group on Antimicrobial Resistance, 2019). This approach is crucial as human populations grow and expand to new geographic regions (Gudipati et al., 2020) and the One Health strategy thus aims to reduce the rise and spread of AMR at the human-animal-environment interface across the globe.

This review will thus focus on literature outlining the environmental niches of the ESKAPE pathogens, community-acquired (CA) infections associated with these pathogens, and the health risks associated with contact between humans and environmental reservoirs harbouring antibiotic resistant strains of the ESKAPE pathogens.

2. ESKAPE pathogens: antibiotic resistance profiles, environmental niches and community-acquired infection

The presence of the ESKAPE pathogens in the environment is likely due to contamination via sewage spills, hospital waste that has been discarded incorrectly, anthropogenic activities such as bathing and swimming, and the disposal of agricultural waste, amongst others (Amarasiri et al., 2020; Hrenovic et al., 2017; Nyenje et al., 2012). Additionally, several antibiotic resistant microorganisms (including the ESKAPE species) and antibiotic resistance genes (ARGs), have been detected in beach sand and intertidal beach water (Akanbi et al., 2017), industrial and municipal wastewater systems (Gwenzi, 2020), soils impacted by human activities and dumpsites (Hrenovic et al., 2017), vegetables (Ebomah and Okoh, 2020), raw or ready-to-eat foods (Lee, 2003; Verraes et al., 2013), irrigation water, groundwater, and surface water systems, including systems for drinking water (Fig. 1) (Ebomah and Okoh, 2020). In fact, it has been estimated that around 46.4% of bacteria obtained from hospitals, sewerage plants, and pharmaceutical factories are resistant to multiple antibiotics (Sukul and Spiteller, 2007; Baquero et al., 2008).

Additionally, a multitude of non-human, animal-specific antimicrobials are used in the animal husbandry sector as growth promoters, as a means of preventative healthcare (prophylaxis), and as a method of controlling disease caused by pathogenic microorganisms (Van et al., 2020). It is thus well-known that human, industrial, and agricultural activities are intensifying the environmental resistome (i.e., the total collection of all genes that may indirectly or directly confer ABR in the environment). However, limited information is available regarding the percentage of bacteria that exhibit AMR towards antimicrobial growth promoters. Moreover, the environmental resistome remains a largely underexplored area of research, and limited information is available on the preservation and transfer of resistance genes in various environmental niches and to and amongst bacteria, animals or humans (Pal et al., 2016).

Of particular concern is that most antimicrobials (73%) that are used in human healthcare, are also used in the food-animal industry, due to the increased global demand for animal protein (Van Boeckel et al., 2017). Moreover, it is becoming increasingly recognised that the extensive use of general antimicrobials (i.e., they may either be human-specific, or non-human animal-specific) in the food-animal production sector, may result in the development of resistance to commonly used antimicrobials in human healthcare (Landers et al., 2012; Aidara-Kane et al., 2018).

To date, certain countries that lack strict regulations, administer antibiotics for use as animal growth promoters for food production (Eagar et al., 2012; Woolhouse et al., 2015), while other regions,

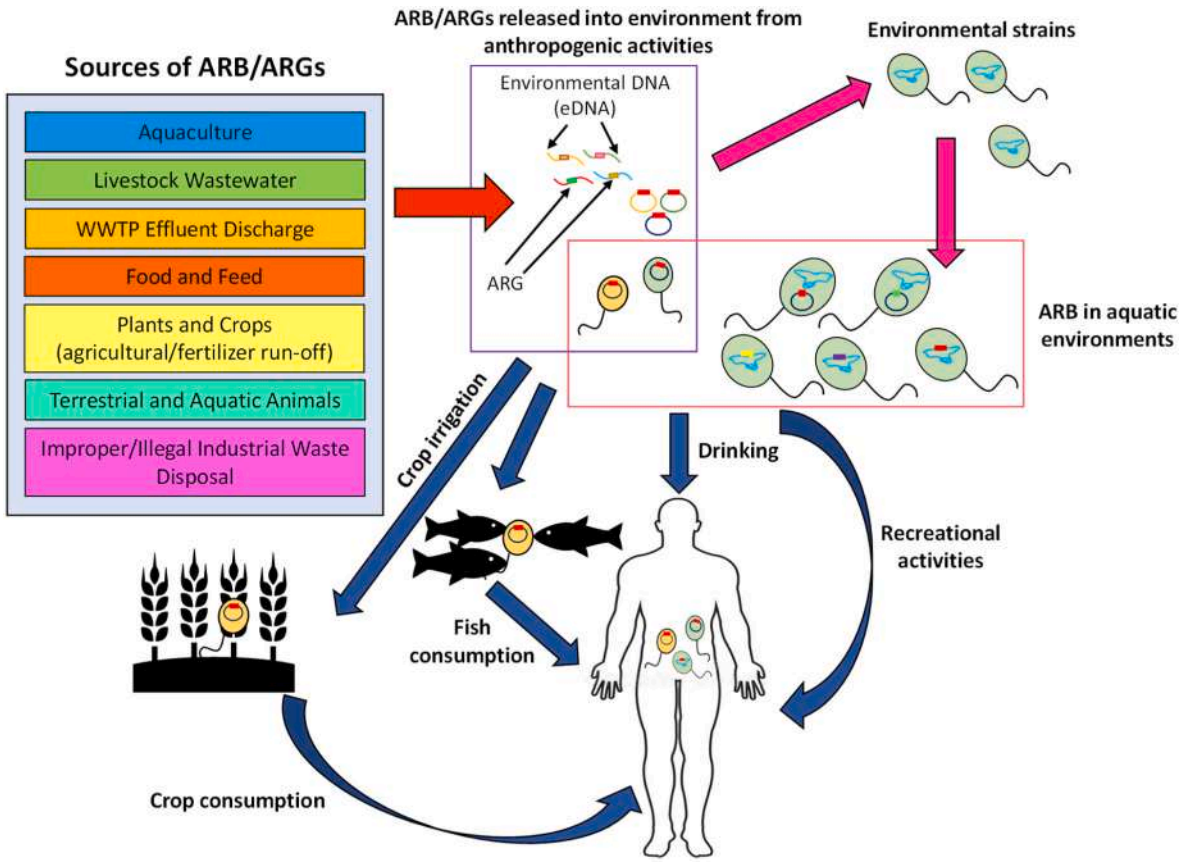

Fig. 1. Potential transmission routes of antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARGs) from the environment to humans (adapted from Amarasiri et al., 2020).

particularly those in the European Union, have banned the use of antimicrobials as growth promoters. Nonetheless, the use of these banned antimicrobials is still reserved for prophylactic (i.e., treatment of animals before clinical signs of disease appear, to prevent disease/infection occurrence) and metaphylactic (i.e., treatment of a group of animals which do not exhibit evidence of disease but remain in close proximity/contact to other animals which exhibit signs of infectious disease) purposes in these regions, and thus the ban of these substances has not led to a consistently observable decrease in antibiotic consumption (Woolhouse et al., 2015; Argudín et al., 2017; Founou et al., 2018).

In contrast, the control and use of antimicrobials in underdeveloped and developing countries remains largely unregulated (Alonso et al., 2017). South Africa is, however, an exception to this observation. More specifically, the "Antimicrobial Resistance National Strategy Framework for 2014–2024" was developed and implemented in South Africa, with one of the primary objectives of this framework being to promote appropriate antimicrobial use in human and animal health (Van et al., 2020). Similarly, the Vietnamese Ministry of Health devised a national AMR action plan in 2013 and was the first country in the Eastern region of the WHO to do this (Van Kinh et al., 2017), while Columbia has undertaken a surveillance initiative since 2003 (called the "Centro Internacional de Entrenamiento e Investigaciones Médicas"), which aims to track ABR amongst Gram-negative bacteria (Perez and Villegas, 2015).

Another important aspect to consider is whether antimicrobials used specifically in the food-animal industry (i.e., non-human antimicrobials) are effective at preventing cross ABR following pathogen transmission to humans. However, the information available on this topic predominantly focuses on *Campylobacter* and *Salmonella* infections, as well as indicator organisms such as enterococci and *Escherichia coli* (*E. coli*), which cause disease in humans (Phillips et al., 2004).

Originally, it was argued that the use of antibiotic growth promoters (e.g., avoparcin, bacitracin, tylosin, virginiamycin) as feed additives in animal husbandry, does not pose a threat to human health as they are not conventionally administered as human medication. However, indirect evidence has shown that the use of antibiotic growth promoters may expedite resistance to clinically relevant antibiotics that are used in human healthcare, via cross-resistance or co-selection (Chen et al., 2016; Gupta et al., 2021). For example, strains of glycopeptide-resistant *E. faecium* possessing the *vanA* gene were isolated by Klare et al. (1993) from WWTPs in small German towns, where no hospitals were located. This led to the assumption that the use of avoparcin (a glycopeptide animal-specific antibiotic) as a feed additive in the food-animal sector, led to a larger reservoir of glycopeptide-resistant *E. faecium*. It was then hypothesised that if glycopeptide-resistant *E. faecium* were present in meat products, these strains could potentially spread to non-hospitalised, healthy humans. This hypothesis has since been confirmed (Klare et al., 1995a, 1995b; Schouten et al., 1997) as the resistance determinants implicated in the co-selection of AMR may be linked to the same transposon or plasmid (Bager, 2000).

Therefore, the use of one specific antimicrobial agent, may select for resistance to other antimicrobials which are closely related, and evidence suggests that animal-specific antimicrobials may enhance cross-resistance, following the transmission of resistant pathogens to humans. However, no consensus currently exists to determine whether non-human animal-specific antimicrobials are in fact effective at preventing cross antibiotic-resistance after pathogen transmission to humans (Tang et al., 2017). Therefore, it is difficult to draw definitive conclusions regarding this topic.

Furthermore, in developed countries, where established water and wastewater treatment processes are available, food-borne rather than waterborne transmission routes are responsible for many reported cases of disease and infection. For example, transmission of ARGs or ARB may

occur by directly contaminating poultry or meat during the slaughter or processing stages, or indirectly from vegetables and fruit that have been grown and irrigated using contaminated manure or water (Hanning et al., 2009). In contrast, in developing nations, where water and sewage treatment may be sub-standard, there is a higher probability that water acts as an important route of transmission for resistant bacteria and/or ARGs from humans and animals (Aubertheau et al., 2017).

Thus, while nosocomial infections are generally acquired within a hospital or clinical setting while receiving healthcare (Sikora and Zahra, 2021), CA infections are contracted outside of hospital/clinical settings (without any previous health care encounter or exposure) and are diagnosed within 48 h of being admitted to a nosocomial environment (Kaplan et al., 2005). However, due to the variability of selective environmental pressures, notable differences may exist between hospital-associated and CA strains of the ESKAPE pathogens (Zetola et al., 2005) and research focusing on CA cases caused by the ESKAPE pathogens will be summarised.

It should, however, be noted, that while not included in the ESKAPE pathogens, *E. coli* has also been identified as a leading cause of both HA and CA infections. These may include sepsis, bloodstream infections, and/or UTIs. *Escherichia coli* is a common gut commensal of humans and animals, and similar to the formal ESKAPE members, can acquire resistance genes from other bacteria, particularly those in the *Enterobacterales* order, resulting in high rates of AMR being observed in this species. In fact, the first case of colistin resistance (the mobile colistin resistance gene, *mcr-1*) was observed in 2016, in *E. coli* isolates obtained from a pig farm in China (Australian Commission on Safety and Quality in Health Care, 2016; European Centre for Disease Prevention and Control, 2018; Cassini et al., 2019; De Oliveira et al., 2020).

Certain strains of *E. coli* are also highly pathogenic and may possess several virulence factors. These pathogenic strains have subsequently been divided into different pathotypes such as enteroinvasive, enterohemorrhagic, enteropathogenic, enterotoxigenic, diffusely adherent, and enteroaggregative *E. coli* (Enciso-Martínez et al., 2022). Bichon et al. (2018) then reported on a case of CA meningitis in a 67-year-old woman. The patient had a history of good health and was admitted to the emergency department of a hospital in France, following reports of altered consciousness and fever. Two days after being admitted, the patient experienced symptoms of a UTI, intermittent vomiting, and abdominal pain. Upon testing, *E. coli* expressing low AmpC β-lactamase levels was identified as the causative agent of infection. However, following antimicrobial treatment with acyclovir, ceftriaxone, and amoxicillin, the patient returned to relatively good health. The only recurring issue was a slight hearing impairment caused by chronic otitis, as well as inconsistent anosognosia (i.e., when it is hard to distinguish other health conditions/problems that a person may have). Thus, while not discussed in this review, AMR *E. coli* is one of the major pathogenic burdens challenging the health of humans and animals. It is thus vital for teams involved in surveillance, research, and development, as well as AMR policies, to consider and include this Gram-negative pathogen as a critical concern to public health (De Oliveira et al., 2020).

2.1. *Enterococcus faecium*

Two distinct subpopulations of *E. faecium* have been identified. The first subpopulation are commensals which occupy the gastrointestinal tract of animals and humans, which are generally not associated with clinical infections. The second subpopulation is comprised of hospital-associated lineages [e.g., vancomycin-resistant enterococci (VRE)], which commonly cause opportunistic infections and nosocomial outbreaks (Zhou et al., 2020b). The lineages in the second subpopulation are thus known for their role as the leading causative agents of HA infections (e.g., endocarditis, bacteraemia, post-surgery wounds and UTIs) due to the intrinsic resistance of these organisms to a wide range of broad- and narrow-spectrum antibiotics, as well as their tolerance towards various stresses (e.g., disinfectants, desiccation, starvation) (Chilambi et al., 2020; Zhou et al., 2020b).

Amongst the most effective antimicrobial drugs for the treatment of infections caused by enterococci are penicillins, such as ampicillin and penicillin. However, isolates of the *E. faecium* hospital-associated subpopulation are generally characterised by ampicillin resistance. Moreover, several other traits including virulence genes, ARGs, and enhanced colonisation and biofilm formation, ensure the persistence of *E. faecium* in clinical settings (Zhou et al., 2020b). Enterococci are also inherently resistant to a multitude of antibiotics including anti-staphylococcal penicillins, cephalosporins, low concentrations of clindamycin, and trimethoprim (in vivo) (Murray, 2000), while several *E. faecium* strains have acquired resistance to other antibiotic classes, such as aminoglycosides and β-lactams. For example, high-level gentamicin-resistant and vancomycin-resistant *E. faecium* isolates have emerged as pathogens of concern and present a major challenge for the treatment of patients infected with these strains. In fact, vancomycin-resistant strains of *E. faecium* have been identified as the primary MDR *Enterococcus* spp. in clinical settings (Emaneini et al., 2008; Lee et al., 2019).

Moreover, enterococci may be found in environmental reservoirs such as surface water, plants, soil, vegetables, fermented food products, and are sometimes even used in human probiotics (Mannu et al., 2003). The presence of enterococci in environmental waters is also frequently used as an indicator of faecal pollution (Zhou et al., 2020b). A recent study conducted by Dos Santos et al. (2021) isolated *E. faecium* from soil (from a riverbank, recreation club, zoo, and farm) and water (from a zoo, recreation club, lagoon, rivers, and beaches) samples in nine different cities of the south-eastern region of Brazil (Table 1). Following isolation, the authors aimed to characterise the antimicrobial resistance profiles of the isolates. Overall, 40 *E. faecium* isolates were obtained, of which 100% ($n = 40$) were resistant to all the tested fluoroquinolone antibiotics and exhibited resistance to four (or more) antimicrobial agents. All the isolates were thus classified as MDR however, none of the isolates exhibited resistance towards teicoplanin or ampicillin. The MDR *E. faecium* isolates were then screened for the presence of various ARGs [e.g., *tetK*, *tetL*, *tetM*, *tetO*, *aac(6′)-Ie-aph(2″)-Ia*, *ant(4′)-Ia*, *ant(6′)-Ia*, *aph(3′)-IIIa*, *aph(2″)-Ib*, *aph(2″)-Id*, *aph(2″)-Ic*, *ermA*, *ermB*, *ermC*, *mefAE*, *vanA*, *vanB*, *vanC1*, *vanC2/3*, *cfr*, and *optrA*], and while a great diversity of genes were detected, overall, five (12.5%) of the isolates possessed three or more ARGs. It is thus clear that soil and water samples can act as reservoirs of MDR *E. faecium* and that these environmental sources may contribute to the dissemination of *E. faecium* and clinically relevant ARGs to other environments (Dos Santos et al., 2021).

Monteiro and Santos (2020) then conducted a study in Portugal, where wastewater and environmental samples were collected at various stages (conventional biological secondary treatment, as well as disinfection via ultra-violet radiation) of treatment to determine whether antibiotic resistant organisms were present in wastewater treatment plants (WWTPs; Table 1). In total, 186 enterococci isolates were obtained (158 isolates from the WWTPs and 28 isolates from the receiving waters of the WWTPs), of which 98 isolates (53%) were identified as *E. faecium*. Results of the antibiotic assays then indicated that the enterococci isolates (all the detected species) exhibited the highest resistance to linezolid (41%), tetracycline (40%) and ciprofloxacin (34%), while the lowest levels of ABR were recorded for gentamicin (1%). Of importance was the detection of VRE in the water samples, as well as the *vanA* and *vanB* genes in all the VRE isolates, as VRE are important nosocomial pathogens. Results of this study also showed that although WWTPs were effective at reducing the level of ABR in the water, 72% of the treated/disinfected water samples, including reclaimed water, contained antibiotic resistant enterococci, while VRE were detected in 6% of these samples. It has thus been highlighted that the use of reclaimed water containing MDR enterococci and VRE for activities such as garden irrigation, crop production, and street cleaning, largely increases the associated potential human health risks (Monteiro and Santos, 2020).

The emergence of VRE has necessitated the use of modified and novel

Table 1
Summary of studies focusing on the prevalence and antibiotic resistance profiles of environmental ESKAPE isolates.

ESKAPE Pathogen	Environmental Reservoir of Isolation	Number of Positive Isolates	Antibiotic Resistance Profile (Percentage of resistant isolates)	Antibiotic Resistance Genes Detected (Percentage of isolates possessing the genes)	Reference
E. faecium	Pastoralist cattle in the interface areas of the Kafue basin (Zambia)	$n = 29$	Resistance to gentamicin (96.6%), cotrimoxazole (89.7%), penicillin (79.3%), erythromycin (72.4%), amoxicillin (65.5%), nitrofurantoin (65.5%), ampicillin (51.7%), and tetracycline (51.7%)		Mubita et al. (2008)
	Wastewater treatment plant (WWTP) water and the receiving waters from the WWTPs	$n = 98$	[a] Resistance to linezolid (41%), tetracycline (40%), ciprofloxacin (34%), ampicillin (16%), chloramphenicol (6%), vancomycin (6%), and gentamicin (1%)	*vanA* (2%) and *vanB* (2%)	Monteiro and Santos (2020)
	Soil (riverbank, recreation club, zoo, and farm) and water (zoo, recreation club, lagoon, rivers, and beaches)	$n = 40$	Resistance to all fluoroquinolones tested (100%), erythromycin (92.5%), rifampin (80%), nitrofurantoin (65%), linezolid (35%), fosfomycin (27.5%), tetracycline (25%), doxycycline (25%), vancomycin (17.5%), minocycline (15%), and chloramphenicol (10%)	*ermB* (25%), *tetL* (22.5%), *tetM* (22.5%), *ant(6′)-Ia* (10%), *mefAE* (7.5%), *ermC* (5%), *vanC1* (5%), *aac (6′)-Ie-aph(2″)-Ia* (2.5%), *ant(4′)-Ia* (2.5%), and *aph(3′)-IIIa* (2.5%)	Dos Santos et al. (2021)
	Excavated ponds and masonry tanks in a fish farming environment	$n = 11$	Varying degrees of resistance to ciprofloxacin, oxytetracycline, erythromycin, and tetracycline	*ace* (18.1%), *agg* (18.1%), and *gelE* (72.3%)	Araújo et al. (2021)
S. aureus	Public marine beaches	$n = 10$	Resistance to methicillin (60%), erythromycin (40%), kanamycin (30%), trimethoprim-sulfamethoxazole (30%), tetracycline (30%), and chloramphenicol (10%)	*ccrB* (50%), *ermA* (50%), *tetK* (20%), and *tetM* (20%)	Soge et al. (2009)
	Ready-to-eat foods from roadside cafeterias	$n = 8$			Nyenje et al. (2012)
S. aureus	Beach sand and intertidal beach water	$n = 30$	Resistance to penicillin (96.7%), rifampicin (80%), clindamycin (80%), oxacillin (73.3%), and erythromycin (70%)	*mecA* (22.7%), *femA* (53.3%), *rpoB* (45.8%), *blaZ* (55.2%), *ermB* (71.4%), and *tetM* (72.7%)	Akanbi et al. (2017)
	Effluent of treated wastewater and receiving surface waters	$n = 80$	Resistance to ampicillin (96.3%), cefoxitin (97.5%), penicillin (97.5%), oxacillin (98.8%), lincomycin (100%), cefazolin (72.5%), azithromycin (66.3%), amoxicillin-clavulanic acid (52.5%), erythromycin (40%), and vancomycin (33.8%).	*aac(6′)/aph(2″)* (56.3%), *ermC* (62.5%), *msrA* (22.5%), *blaZ* (70%), and *tetK* (70%)	Ramessar and Olaniran (2019)
K. pneumoniae	Streams, lakes, and the Baltic Sea	$n = 62$			Podschun et al. (2001)
	Water and sediment	$n = 55$	Resistance to neomycin (50.9%), streptomycin (7.3%), kanamycin (9.1%), gentamicin (1.8%), amoxicillin-clavulanic acid (7.3%), nalidixic acid (3.6%), tetracycline (9.1%), chloramphenicol (1.8%), and trimethoprim-sulfamethoxazole (9.1%)		Barati et al. (2016)
	Surface and drinking waters	Not specified	Resistance to ampicillin (100%)		Dobrijević et al. (2017)
	Water from the Krapina River (Croatia)	$n = 4$	All resistant to ampicillin, amoxicillin-clavulanic acid, piperacillin-tazobactam, all cephalosporins, all carbapenems, trimethoprim-sulfamethoxazole, ciprofloxacin, gentamicin, and amikacin; Susceptible to colistin	bla_{SHV-1} (100%), *aac(3′)-II* (100%), *aac (6′)-Ib* (100%), *and aph(3′)-Ia* (100%)	Jelić et al. (2019)
A. baumannii	Water from the Seine River (France)	$n = 1$	Susceptible to tobramycin, amikacin, netilmicin, kanamycin, and gentamicin; Resistant to quinolones, fluoroquinolones, chloramphenicol, tetracycline, and tigecycline	bla_{OXA-23}	Girlich et al. (2010)
	Technol/dumpsite	$n = 3$	All resistant to carbapenems, fluoroquinolones, carbapenems, penicillins/β-lactamase inhibitors, and aminoglycosides	bla_{OXA-72} (66.7%) and bla_{OXA-23} (33.3%)	Hrenovic et al. (2017)
	Stream	$n = 1$	Resistant to cefepime, gentamicin, and cefotaxime; Susceptible to amikacin, ceftazidime, ciprofloxacin, imipenem, levofloxacin, tetracycline, piperacillin-tazobactam, and colistin		Havenga et al. (2019)
P. aeruginosa	Surface water of the Woluwe River (Brussels, Belgium)	$n = 65$	One strain, (isolated from the section of the river with the most pollution), exhibited resistance to several antibiotics including β-lactams, fluoroquinolones, and aminoglycosides		Pirnay et al. (2005)
	Ready-to-eat foods from roadside cafeterias	$n = 46$			Nyenje et al. (2012)

(*continued on next page*)

Table 1 (*continued*)

ESKAPE Pathogen	Environmental Reservoir of Isolation	Number of Positive Isolates	Antibiotic Resistance Profile (Percentage of resistant isolates)	Antibiotic Resistance Genes Detected (Percentage of isolates possessing the genes)	Reference
P. aeruginosa	Wastewater (hospital, urban, treated, and untreated), sludge, and river water from upstream and downstream of a WWTP	$n = 238$	Wild-type (76.5%), resistant (16.4%), and multidrug resistant (7.1%)		Slekovec et al. (2012)
	Soil and water	$n = 40$			Martins et al. (2014)
	Sub-surface seawaters along the Indian Peninsula	$n = 32$	High susceptibility to quinolones, polymyxins, and aminoglycosides; Moderately susceptible to penicillins; Resistance to tetracycline families and cefepime		Nair et al. (2015)
	Wastewater received and discharged into the Puck Bay (Baltic Sea, Northern Poland)	$n = 27$	Resistance to cefepime (18.5%), ceftazidime (18.5%), ticarcillin (29.6%), ticarcillin-clavulanic acid (22.2%), and aztreonam (70.4%)		Luczkiewicz et al. (2015)
Enterobacter spp.	Water from the Narmada River (India)	$n = 57$	Varying degrees of resistance to ceftriaxone, cefpodoxime, cefepime, ceftazidime, cefuroxime, cefotaxime, ceftazidime-clavulanic acid, cefoxitin, aztreonam, and chloramphenicol antibiotics	bla_{TEM} (42%) and bla_{SHV} (44%)	Sharma et al. (2008)

[a] Referring to all enterococci isolated in the study, not specifically *E. faecium*.

therapeutic agents (e.g., tigecycline, linezolid, and daptomycin) (Mendes et al., 2016; Zaheer et al., 2020). In fact, daptomycin is amongst the most common therapeutic agents used to treat VRE infections (Kamboj et al., 2011). While its mode of action remains unclear, it is known that daptomycin is a cyclic lipopeptide which acts in a bactericidal manner to bind to phosphatidylglycerol (PEG; a phospholipid required for the successful activity of daptomycin) in bacterial cell membranes. This subsequently leads to the disruption of the cell membrane and critical processes that occur in the cell envelope, resulting in cell death (Prater et al., 2019).

Although the transmission of daptomycin-resistant enterococci has been reported in nosocomial environments, limited information is available regarding the prevalence of environmental daptomycin-resistant *E. faecium* isolates, as the majority of global enterococcal isolates (>99.8%) are susceptible to this antibiotic (Lellek et al., 2015; Bender et al., 2018). However, Zhang et al. (2010), Diarra et al. (2010), and Furtula et al. (2013) isolated daptomycin resistant enterococci isolates from conventional beef sources, faecal and caecal samples, and poultry farm and surrounding surface- and groundwater, respectively.

Prater et al. (2019) then observed that *E. faecium* is capable of alternating its resistance to daptomycin, based on its surrounding environmental pressures. For example, under planktonic conditions, cellular pathways responsible for increasing cell surface charge (by upregulating *yvcRS* of *mrpF* and *dltABCD*), caused decreased daptomycin binding to the cell. In contrast, environments which favoured communities with a complex structure (including biofilms), developed repulsion and diversion strategies, by inducing mutations in *oatA* and *divIVA,* respectively. Moreover, the importance of modifying the cell membrane was highlighted, as both environments studied converged on *cls* (cardiolipin synthase) mutations. *Enterococcus faecium* is therefore capable of changing its level of daptomycin resistance by evolving its cellular trajectories, based on environmental surroundings and biochemical pathways. Moreover, continued exposure to daptomycin may result in a polymorphic population of *E. faecium* which appear phenotypically resistant, thereby highlighting the recalcitrant nature of this species (Prater et al., 2019).

Although VRE are rarely reported as causative agents of CA infection, Raja et al. (2005) reported an interesting case of CA VRE infection in Malaysia (Table 2). In this case, a 38-year-old man applied herbal leaves as a remedy for his right cheek, which had reportedly been swollen and sore due to an open burn wound. Following application of the leaves, the pain and redness of the area increased. Laboratory analysis of pus samples obtained from the infected cheek identified the source of the infection as *E. faecium*, which was further identified as resistant towards clindamycin, erythromycin, ampicillin, teicoplanin and vancomycin. Upon analysis of the results, it was hypothesised that the *E. faecium* had contaminated the leaves of the herb applied, which caused the onset of infection in the patient via the open burn wound (Raja et al., 2005).

2.2. *Staphylococcus aureus*

Staphylococcus aureus possesses intrinsic resistance mechanisms [efflux systems, OM permeability, and the production of large quantities of β-lactamase], or may acquire ARGs (Guo et al., 2020). For instance, aminoglycoside resistance in strains of *S. aureus* is caused by a decreased permeability of the OM, which reduces drug uptake, while strains of penicillin-resistant *S. aureus* destroy the antibacterial activity of penicillin by expressing a β-lactamase (mediated by the *blaZ* gene) that hydrolyses the crucial β-lactam bond present in this compound (Guo et al., 2020). In fact, the resistance rate of human *S. aureus* isolates to penicillin is currently greater than 90%, resulting in the inefficiency of this antibiotic to treat infections caused by this pathogen (McGuinness et al., 2017; Walsh and Wencewicz, 2020). *Staphylococcus aureus* acquired resistance mechanisms may then fall into one of four categories, namely (i) mutations that induce resistance, (ii) acquired resistance genes, (iii) biofilm-mediated resistance, or (iv) persister cells (Guo et al., 2020). Acquired resistance to several antimicrobials, such as methicillin, vancomycin [a last-resort treatment option for severe infections caused by methicillin-resistant *S. aureus* (MRSA)] and other, newer antimicrobial agents (e.g., daptomycin and linezolid), has been reported (Gould et al., 2012; Rodvold and McConeghy, 2014).

In particular, MRSA and emerging vancomycin-resistant *S. aureus* (VRSA) strains are capable of causing devastating health outcomes in the absence of appropriate treatment and containment options and are thus considered agents of extreme importance. This is concerning, as vancomycin is considered a common last-resort treatment option for MRSA infections and with the emergence of VRSA in 2002 (Michigan, USA), less treatment options are available for diseases caused by MRSA strains. Additionally, VRSA isolates commonly exhibit MDR status against a broad range of antimicrobials (Purrello et al., 2016; Khan et al., 2018; Cong et al., 2020), limiting the treatment options for VRSA infections.

Vancomycin is a glycopeptide which exerts a bactericidal effect by targeting the peptidoglycan layer of the bacterial cell wall, in susceptible bacterial isolates. It forms hydrogen bonds with the D-Ala-D-Ala residues in the N-acetylglucosamine-N-acetylmuramic acid-Pentapeptide, which makes up part of the peptidoglycan layer. The resultant drug

Table 2
Case reports of community-acquired infections caused by the ESKAPE pathogens.

Causative ESKAPE Pathogen	Disease Diagnosis	Risk Factors/Exposure Route	Antibiotic Resistance/Susceptibility of the Isolate	Reference
E. faecium	Cheek infection/burn wound	It was hypothesised that the *E. faecium* had contaminated the herbal leaves which were applied as a remedy to the wound	Resistant to clindamycin, erythromycin, ampicillin, teicoplanin, and vancomycin	Raja et al. (2005)
S. aureus	Skin and soft-tissue infections, and invasive infections		[a] Methicillin resistant isolates – resistance to clindamycin (2–6%), and erythromycin (94–97%); Methicillin-susceptible isolates – resistance to clindamycin (3–11%), and erythromycin (44–50%)	Kaplan et al. (2005)
	Pneumonia	Underlying diseases, influenza, or risk factors for methicillin resistant *S. aureus*	All ($n = 11$) resistant to oxacillin and erythromycin; All susceptible to linezolid, rifampin, trimethoprim-sulfamethoxazole, and vancomycin; Susceptible to clindamycin (91%), and vancomycin (55%)	Hageman et al. (2006)
	Necrotising pneumonia and deep venous thrombosis	Healthy medical record with no underlying comorbidities, thus the cause was unknown	Sensitive to vancomycin	Lu et al. (2022)
K. pneumoniae	Liver abscess	No underlying comorbidities, thus the cause was unknown	Resistant to cefazolin, ceftriaxone, cefotaxime, and cefepime; Susceptible to trimethoprim-sulfamethoxazole, gentamicin, ciprofloxacin, imipenem, and ertapenem	Su et al. (2008)
	Urinary tract infection	Travel to Asia, The Middle East or Africa in the six weeks prior to the study (or in the last 6 weeks to 24 months), recent use of β-lactams or fluoroquinolones, recreational freshwater swimming during the past year, and diabetes mellitus		Søraas et al. (2013)
K. pneumoniae	Urinary tract infection	Being a nursing home resident	Of the 83 isolates, resistance to cefotaxime (96.4%), cefazolin (85.5%), amoxicillin-clavulanic acid (80.7%), ciprofloxacin (72.3%), trimethoprim-sulfamethoxazole (72.3%), gentamicin (42.2%), nitrofurantoin (37.3%), piperacillin-tazobactam (32.5%), and fosfomycin (22.9%) was observed; 86.7% of the isolates were MDR	Boix-Palop et al. (2017)
	Invasive infection	Patient was HIV positive, had diabetes mellitus and chronic alcoholism. No history of recent hospital admissions or travel abroad, thus the cause was unknown	Excellent susceptibility to ampicillin	Rodrigues et al. (2020)
A. baumannii	Pneumonia	No underlying comorbidities but was a smoker and alcoholic, thus the cause was unknown	Susceptible to piperacillin-tazobactam, cefepime, ceftazidime, amikacin, imipenem, ciprofloxacin, tobramycin, gentamicin, levofloxacin, ampicillin-sulbactam, sulfamethoxazole, and cefoperazone-sulbactam	Xu et al. (2020)
	Pyogenic liver abscess	Exposure to people at work combined with hepatitis B infection, frequent alcohol consumption and potential poor nutrition	Resistant to ciprofloxacin, amikacin, meropenem, levofloxacin, and piperacillin-tazobactam	Chukwurah et al. (2021)
P. aeruginosa	Pneumonia with septicaemia	No underlying comorbidities but was a smoker, thus the cause was unknown	Susceptible to and treated using meropenem, ciprofloxacin, clindamycin, and norepinephrine	Imai et al. (2016)
	Pneumonia	Use of alternative water sources (e.g., greywater, rainwater, and borehole water) during a drought period in South Africa		John et al. (2017)
	Pneumonia and progression to septic shock	Poor living conditions	Developed resistance to carbapenems over the course of treatment	Wang et al. (2019)
Enterobacter spp.	Bloodstream infection	Malignancy, ulcers of the upper gastrointestinal tract, central venous catheterization, and mechanical ventilation	All isolates (100%) susceptible to imipenem and meropenem; the 26 MDR isolates (43.3%) exhibited resistance to all other β-lactam antibiotics (except cefepime), susceptibility to piperacillin-tazobactam (38.5%; $n = 26$), amikacin (53.8%; $n = 26$), and levofloxacin (53.8%; $n = 26$); the 34 non-MDR isolates were susceptible to third generation cephalosporins, aztreonam and piperacillin-tazobactam (94.1%–100%; $n = 34$), gentamicin (85.3%; $n = 34$), amikacin (94.1%; $n = 34$), and quinolones (85.3%–94.1%; $n = 34$)	Liu et al. (2004)
	Round pneumonia	History of liver cirrhosis and alcohol consumption, but the cause was unknown	Sensitive to levofloxacin; susceptible to amikacin, cefepime, ciprofloxacin, gentamicin, imipenem-cilastatin, meropenem, tetracycline, tigecycline, tobramycin, and trimethoprim-sulfamethoxazole; Intermediately susceptible to piperacillin-tazobactam, ertapenem; Resistant to cefuroxime, ceftriaxone, cefotaxime, ceftazidime, cefazolin, ampicillin, ampicillin-sulbactam, and amoxicillin-clavulanate	Jiménez-Castillo et al. (2021)

[a] Results obtained each year for a three-year period.

complex inhibits the transglycosylation and transpeptidation phases of peptidoglycan production, resulting in the effective antimicrobial activity of vancomycin (Baëtz et al., 2021). However, the presence of *van* gene clusters in pathogens (which may be transferred between species via horizontal gene transfer), may also provide resistance to this antibiotic. In fact, at least eleven *van* gene clusters have been identified (VanN, VanL, VanG, VanE, VanM, VanI, VanC, VanD, VanF, VanB, and VanA) to date, which confer vancomycin resistance in various species (e.g., *E. faecium*, *E. faecalis*, *Clostridium difficile*, and *S. aureus*, amongst others; Table 1) (Boyd et al., 2008; Xu et al., 2010; Lebreton et al., 2011; Cong et al., 2020). It is thus essential to implement frequent surveillance and control strategies for HA infections and vancomycin resistance, to curb and prevent the further spread and emergence of VRSA strains (Wu et al., 2021).

Like *E. faecium*, *S. aureus* is a commensal bacterium that is asymptomatically distributed on various parts of the human body (e.g., mucous membranes, skin, and skin glands) (Lakhundi and Zhang, 2018). However, it has also been identified as the primary causative agent of bacterial skin-and-soft tissue infections (SSTIs) and has been implicated in the onset of several other invasive infections such as complex pneumonia, musculoskeletal infections, and endocarditis in immunosuppressed individuals (McNeil, 2014). This Gram-positive ESKAPE bacterium is also widely distributed in environmental reservoirs such as sewage, untreated water, and raw milk (Nyenje et al., 2012).

As previous studies have linked the presence and dissemination of *S. aureus* in marine waters to recreational human activities and bather density on beaches, Akanbi et al. (2017) determined the prevalence and antibiotic sensitivity profiles of *S. aureus* isolates obtained from beach sand and intertidal beach water collected in the Eastern Cape Province of South Africa (Table 1). A total of 249 samples (water and sand) were collected from 10 beaches between April 2015 and April 2016. From these samples, 245 presumptive isolates were screened using PCR analysis with 12.3% ($n = 30/245$) of the isolates positively identified as *S. aureus*. Moreover, antibiotic resistance profiling indicated that more than 50% of the *S. aureus* isolates exhibited phenotypic resistance to methicillin. Results of this study thus indicate that sand and water collected from beaches in the Eastern Cape Province of South Africa contain antibiotic resistant *S. aureus* strains, which may be transmitted and possibly pose a health risk to humans and animals.

Moreover, the resistance or susceptibility of microorganisms to various antibiotics is complex and can vary in different regions. There is thus a need to identify and classify ABR patterns acco rding to geographical location (Vola et al., 2013; Ni et al., 2015; Wang et al., 2015). Although the fraction of invasive infections caused by hospital-acquired MRSA (HA-MRSA) and community-acquired MRSA (CA-MRSA) has recently declined in the United States and several European countries (Wyllie et al., 2011; Dantes et al., 2013; European Centre for Disease Prevention and Control, 2018), CA cases of *S. aureus* infection, primarily caused by MRSA, have been increasing at a rapid rate in developing countries (e.g., Taiwan, Philippines, Sri Lanka, Vietnam, amongst others).

Community-acquired infections commonly arise due to crowded living conditions, poor hygiene, lack of access to sufficient healthcare, and from being exposed to asymptomatic carriers of pathogenic microorganisms (Loewen et al., 2017). Furthermore, in hospital and community settings of developing countries, it is common to follow incorrect treatment approaches (e.g., when the incorrect antibiotic is prescribed or the prescription/treatment time is too long/short), contributing to increased AMR amongst pathogenic microorganisms (Skov et al., 2012; Hosaka et al., 2022). Thus, while the issue of antibiotic resistant *S. aureus* remains a global concern, it is likely that CA *S. aureus* infections are less common/decreasing in developed regions, as these areas may have less crowded living conditions, better hygiene practices, access to high-quality healthcare facilities and experienced medical professionals, increased ABR surveillance, correct clinical use of antimicrobials, and stricter access to antibiotics. However, due to reports of a lack of ABR data from developing regions, it is unclear whether the total antibiotic consumption in developing regions definitively contributes to increased rates of CA infections (Sakeena et al., 2018; Chokshi et al., 2019; Castro et al., 2020; Van et al., 2020).

Ramessar and Olaniran (2019) then aimed to determine the virulence gene profiles and antibiograms of MRSA isolates recovered from the effluent of treated wastewater and receiving surface waters in Durban, KwaZulu-Natal (South Africa; Table 1). A total of 80 MRSA isolates were obtained from the sampling sites, with ABR observed towards ampicillin (96.25%), cefoxitin and penicillin (97.50%), oxacillin (98.75%) and lincomycin (100%). Additionally, several isolates exhibited resistance to cefazolin (72.50%), azithromycin (66.25%), amoxicillin-clavulanic acid (52.50%), erythromycin (40%) and vancomycin (33.75%). The detection of MDR and virulent strains of MRSA in the receiving waters of treated effluent emphasises the need for the implementation of effective wastewater treatment options to prevent the spread of MDR bacteria to other environmental reservoirs (Ramessar and Olaniran, 2019).

As previously mentioned, *S. aureus* is well-known for its role as a natural commensal of the human body, particularly on the surface of the skin. The skin consequently acts as an efficient barrier to prevent infection with *S. aureus*. It is thus thought that the onset of CA *S. aureus* infection is linked to risk factors such as exposure to an asymptomatic carrier of the species, living in crowded environments, and poor hygiene (Loewen et al., 2017). However, Lu et al. (2022) recently reported on a case of sepsis, necrotising pneumonia, and deep venous thrombosis in an infant, associated with a CA-MRSA isolate. In 2017, the female infant (one month and 5 days old at the time), with a healthy medical history, was admitted to the emergency department due to a persistent fever. Upon investigation, MRSA was identified as the causative agent of the infection however, where the exposure to the pathogen occurred is still unknown. Subsequently, vancomycin was administered and combined with multiple anticoagulant therapy, which led to the eventual recovery of the patient. Nonetheless, at the three-year check-up, the patient still exhibited deep venous thrombosis and requires ongoing check-ups. This CA case is a rare report, as it is not common for healthy infants to simultaneously exhibit sepsis, necrotising pneumonia and deep venous thrombosis caused by CA MRSA (Lu et al., 2022).

2.3. *Klebsiella pneumoniae*

Several human infections, including UTIs, respiratory tract infections and bloodstream infections are caused by *K. pneumoniae*, which most commonly occur in immunocompromised or hospitalised patients. Regularly used treatment options for *K. pneumoniae* associated infections include β-lactams (penicillins and carbapenems), β-lactamase inhibitor (piperacillin-tazobactam), polymyxins (colistin or polymyxin B), or cephalosporins (cefepime) (Girometti et al., 2014; Martin and Bachman, 2018). Interestingly, *K. pneumoniae* carbapenemase (KPC) enzymes and several other AMR determinants, have contributed to several outbreaks caused by this species (Paczosa and Mecsas, 2016). For example, research has shown that strains of *K. pneumoniae* possess intrinsic resistance to penicillin due to the presence of the chromosomally encoded SHV-1 (sulfhydryl reagent variable) penicillinase (Holt et al., 2015; Wand et al., 2015). Similarly, KPCs are capable of effectively hydrolysing all monobactams, cephalosporins, carbapenems and β-lactam inhibitors (Akova et al., 2012), making treatment with these antimicrobials particularly difficult due to their low effectivity rate.

In addition to the utilisation of enzymes, *K. pneumoniae* employs numerous other resistance mechanisms to evade the effects of antimicrobials. For instance, to evade the effects of fluoroquinolones, *K. pneumoniae* induces mutations in the antibiotic's target gene [e.g., mutations in the quinolone-determining area of two *K. pneumoniae* genes (*parC* – codes for topoisomerase IV; *gyrA* – codes for gyrase)]. Similarly, several studies have observed resistance to quinolones due to the

presence of the *qnrB* and *qnrS* genes. This pathogen may also utilise MDR efflux pumps (e.g., via the *tetA* gene which encodes an efflux pump) or modify enzymes and proteins which protect the antibiotic's cellular target by pumping out toxic substances (Wang et al., 2020).

Klebsiella pneumoniae naturally inhabits the gastrointestinal tract and nasopharynx of healthy animals and humans but is also commonly isolated from environmental reservoirs such as water, soil, and plants (Wang et al., 2020). Thus, despite its broad environmental distribution, *K. pneumoniae* has emerged as an opportunistic pathogen that is commonly associated with one third of all Gram-negative hospital--linked infections, yet also causes a substantial number of CA infections (e.g., meningitis, pneumonia, pyogenic liver abscess) (Holt et al., 2015; Martin and Bachman, 2018). For example, Jelić et al. (2019) isolated four *K. pneumoniae* isolates from water of the Krapina River in Croatia (Table 1). All isolates were found to produce KPC-2 and were genetically closely related, belonging to sequence-type 258 (ST258), as identified using pulsed-field gel electrophoresis and multi-locus sequence typing (Pasteur scheme). The isolates all exhibited uniform, MDR antimicrobial resistance profiles, but were susceptible to colistin. Additionally, the bla_{KPC-2}, bla_{SHV-1}, *aac(3′)-II*, *aac(6′)-Ib*, and *aph(3′)-Ia* genes were detected in all the isolates. These observations are concerning as the isolates were thus confirmed to share similar features with clinical *K. pneumoniae* isolates from the nearby hospital due to their carbapenem resistance, MDR status, and affiliation with ST258. The isolates were thus hypothesised to have originated in a clinical setting and it was assumed that they were subsequently discharged into the river via untreated hospital wastewater (Jelić et al., 2019).

Ebomah and Okoh (2020) then assessed several sampling sites in the Amathole, Chris Hani, and Sarah Baartman District Municipalities, in the Eastern Cape Province of South Africa for the presence of carbapenemase-producing *Klebsiella* spp. (CPK; Table 1). Two-hundred and ninety-one ($n = 291$) *Klebsiella* spp. isolates were obtained from the 243 samples collected (hospital effluents, surface water, WWTP final effluents, soil, vegetables, and irrigation water), of which *K. pneumoniae* comprised 63% ($n = 182$) of the isolates obtained. Following AMR profiling, the highest percentage of resistance observed in the *K. pneumoniae* isolates was to imipenem (51%), followed by ertapenem (35%), doripenem (35%), and meropenem (34%). All the *Klebsiella* spp. isolates that showed resistance to the tested carbapenems were then screened for the $bla_{OXA-48-like}$, bla_{KPC}, and bla_{NDM-1} carbapenem-resistance genes, which were detected at different frequencies in the various *Klebsiella* strains. It was subsequently speculated that the isolation of CPK isolates from the environmental sources in this study may be due to improperly treated WWTP final effluent being discharged into receiving aquatic environments (Ebomah and Okoh, 2020).

Since the 1980s, hypervirulent *K. pneumoniae* strains have been responsible for the onset of severe CA infections. Commonly observed CA infections caused by this species include pneumonia, endophthalmitis (infection of the eye), pyogenic liver abscess, meningitis, or brain abscess, which are often followed by bacteraemia (Siu et al., 2012; Russo and Marr, 2019). Correspondingly, Rodrigues et al. (2020) obtained four hypermucoviscous *K. pneumoniae* isolates from a patient with a known history of diabetes mellitus and chronic alcoholism, and who was human immunodeficiency virus (HIV) positive (Table 2). The patient presented with an invasive CA infection however, the exact cause was unknown. Analysis of the four *K. pneumoniae* isolates obtained, then indicated that all of them were fully susceptible to all the antibiotic classes tested (βeta-lactams, aminoglycosides, fluoroquinolones, and macrolides), including ampicillin.

2.4. *Acinetobacter baumannii*

Acinetobacter baumannii is predominantly associated with nosocomial infections (such as pneumonia, bacteraemia, meningitis, UTIs, gastrointestinal infections and SSTIs) and primarily affects immunocompromised individuals suffering from a variety of underlying diseases. The significant ability of *A. baumannii* to survive for extended periods of time within clinical settings, dynamically reorganise and rapidly evolve its genome, as well as acquire ARGs under selective pressure from the environment, has resulted in the association of this pathogen with increased mortality and morbidity rates, as well as extended hospital stays (Levy-Blitchtein et al., 2018).

In general, *A. baumannii* is intrinsically resistant to glycopeptides, macrolides, streptogramins and lincosamides. Therefore, as carbapenems and polymyxins (colistin or polymyxin B), are typically used to treat infections caused by Gram-negative pathogens, they have been effective in treating regular and MDR *A. baumannii* infections. However, due to the frequent application of carbapenems in recent years, resistance towards this antibiotic class is increasing in strains of *A. baumannii* (Meletis, 2016). Multidrug resistant *A. baumannii* are thus generally resistant to three antibiotic classes, namely aminoglycosides, fluoroquinolones, and β-lactam antibiotics (all cephalosporins and penicillins) (Eichenberger and Thaden, 2019). Conversely, extensively drug resistant (XDR; resistant to at least one antimicrobial agent in all but two or less of the antimicrobial categories) strains of *A. baumannii* are classified as resistant to the previously mentioned three (aminoglycosides, fluoroquinolones, and β-lactams) or more antimicrobial classes, while pan-drug resistant (PDR; susceptible/sensitive to one or two potential antimicrobial agents, or resistant to all currently available antibacterial agents) strains are those that exhibit additional resistance to tigecycline and polymyxins, including colistin (Karakonstantis et al., 2020; Mulani et al., 2019). Multidrug resistant, XDR and PDR strains of *A. baumannii* utilise a broad range of ABR mechanisms such as multidrug efflux systems, enzymes that modify aminoglycosides, β-lactamase enzymes, and alterations to cell permeability, amongst others (Kyriakidis et al., 2021), to evade the effects of antimicrobials.

Acinetobacter baumannii can survive in environments characterised by either dry or moist conditions and can remain viable under these conditions for several weeks, and even months. Consequently, while *A. baumannii* has been isolated from natural environments (e.g., water, soil, vegetables, and animal sources), albeit rarely, moist tissues (e.g., mucous membranes, open wounds) and on the human skin, it is the primary opportunistic pathogen detected and isolated in ICUs (Eveillard et al., 2013). Subsequently, *A. baumannii* has been linked to the onset of nosocomial infections, with an increasing resistance to carbapenems being observed, causing a significant reduction in available treatment options for infections caused by this species. Since the initial identification of a carbapenem-hydrolysing class D β-lactamase (OXA-23) in a clinical *A. baumannii* isolate, in Scotland in 1995, the bla_{OXA-23} gene has since been observed in several clinical *A. baumannii* isolates worldwide (Bonnet et al., 2002).

Girlich et al. (2010) then reported on the successful isolation of an *A. baumannii* isolate possessing the bla_{OXA-23} gene, from water of the Seine River (Table 1). The isolate, classified as *A. baumannii* B9, was found to be susceptible to tobramycin, amikacin, netilmicin, kanamycin, and gentamicin, but resistant to quinolones, fluoroquinolones, chloramphenicol, tetracycline, and tigecycline. Hrenovic et al. (2017) reported on the successful isolation of three carbapenem-resistant *A. baumannii* isolates from technosol (i.e., a soil site that has been modified by human-made materials) rich in heavy metals and petroleum hydrocarbons at an illegal Croatian dumpsite (Table 1). This dumpsite was used for the disposal of hazardous industrial waste from 1955 to 1990 and has since become an illegal dumpsite. Upon analysis of the *A. baumannii* isolates, it was observed that they shared many features with previously identified clinical isolates. All three isolates were found to exhibit carbapenem resistance, with two of the three isolates carrying the bla_{OXA-72} gene and the third carrying the bla_{OXA-23} gene. Additionally, all isolates exhibited resistance to fluoroquinolones, carbapenems, penicillins/β-lactamase inhibitors and aminoglycosides, with sensitivity towards gentamicin, tobramycin and colistin observed. Isolates 1 and 2 remained susceptible to trimethoprim-sulfamethoxazole, minocycline and colistin, while isolate 3 remained susceptible to colistin only.

Multidrug resistant status was thus awarded to isolates 1 and 2, while isolate 3 was classified as XDR. It was thus hypothesised that the most likely source of the MDR and XDR *A. baumannii* isolates in the technosol was due to the illegal disposal of hospital waste at this site (Hrenovic et al., 2017).

Although *A. baumannii* is primarily isolated from hospital environments, this species has also been implicated in the onset of CA outbreaks, albeit very rarely. In a recent study, Chukwurah et al. (2021) reported on a CA case of pyogenic liver abscess caused by *A. baumannii* (Table 2). In this study, the male patient had a weakened immune system due to the combined effects of a hepatitis B infection, frequent alcohol consumption and potential poor nutrition. These factors were hypothesised to have initiated the spread of the bacteria from his gut to the liver, leading to eventual abscess formation. Following analysis of pus samples, obtained from the abscess, the patient was found to be infected with an *A. baumannii* isolate that exhibited resistance towards ciprofloxacin, amikacin, meropenem, levofloxacin and piperacillin-tazobactam. In addition, rare cases of CA bacteraemia and pneumonia caused by *Acinetobacter* spp., have been reported (Chukwurah et al., 2021). For example, Xu et al. (2020) report on a severe case of CA infection caused by *A. baumannii*. In this case study, a 66-year-old male experienced acute fever for 24 h, an intermittent cough (with pale, bloody sputum), a heavy chest accompanied by chest pain, and shortness of breath while at rest (Table 2). Consequently, he was admitted to the emergency department of a hospital in Wenzhou, China, and subsequently diagnosed with severe CA pneumonia (CAP), acute injury to the kidneys, respiratory failure, and septic shock. Samples of the patients' blood and sputum were then sent for next-generation sequencing, which identified the presence of several nucleotide sequences belonging to *A. baumannii* in the sputum sample. This observation was confirmed using subsequent sputum culture, and prompted the diagnosis and treatment of *A. baumannii* instigated CAP.

2.5. Pseudomonas aeruginosa

Pseudomonas aeruginosa has been associated with several life-threatening infections such as septicaemia and endocarditis, pneumonia, cystitis, UTIs, as well as surgical wound infections (Pachori et al., 2019), with treatment becoming increasingly difficult due its MDR status (Goli et al., 2016). Cephalosporins (cefepime, ceftazidime or cefoperazone), monobactams (aztreonam), carbapenems (meropenem or doripenem), fluoroquinolones (ciprofloxacin), aminoglycosides (tobramycin, amikacin, or gentamicin), antipseudomonal penicillins, combined with a β-lactamase inhibitor (piperacillin-tazobactam and ticarcillin-clavulanate) and polymyxins (colistin or polymyxin B), are commonly used to treat *P. aeruginosa* infections (Ibrahim et al., 2020). However, *P. aeruginosa* makes use of several mechanisms to evade the effects of antipseudomonal antimicrobials, including the expression of porins, efflux pumps [e.g., the multidrug efflux pumps mexAB-oprM, mexCD-oprJ, mexEF-oprN and mexXY-(oprA)], mutations in the target sites of antibiotics, activity of enzymes that can inactivate drugs (e.g., β-lactamases, aminoglycoside-modifying enzymes), or biofilm formation. As a result, this opportunistic pathogen is resistant to several antibiotics within the aforementioned classes including chloramphenicol, colistin, rifampin, trimethoprim-sulfamethoxazole, tetracycline, and several β-lactams (Goli et al., 2016; Rossolini and Mantengoli, 2005).

Pseudomonas aeruginosa thrives in aquatic and soil environments, while typically colonising the surfaces of plants, humans, and animals (Klockgether and Tümmler, 2017; Pachori et al., 2019). The ability of *P. aeruginosa* to survive in environments characterised by minimal nutrients and varying physical conditions, as well as its resistance to certain medical disinfection methods, has enabled the persistence of this organism in both hospital and community settings (Lister et al., 2009; Mena and Gerba, 2009). Nair et al. (2015) then conducted a comparative study where environmental (obtained from sub-surface water samples along the Indian Peninsula) and clinical *P. aeruginosa* isolates were compared based on their metabolic, genotypic, and phenotypic characteristics. In total, 32 (~4.1%) of the marine isolates were classified as *P. aeruginosa*. Upon analysis of the phenotypic characterisation results, the patterns of antibiotic susceptibility of the *P. aeruginosa* isolates varied depending on the family of antibiotics being tested (Table 1). All isolates showed increased susceptibility to the tested polymyxin, quinolone and aminoglycoside antibiotic groups, moderate susceptibility to penicillin antibiotics, and resistance to the tetracycline families and cefepime. Interestingly, the environmental isolates were found to exhibit similar or higher resistance profiles than the clinical isolates (except for gentamicin and polymyxin). Nonetheless, the overall isolation frequency for *P. aeruginosa* was low for the coastal waters, and this was hypothesised to be due to factors such as local competition [e.g., with pollutants and allochthonous (i.e., originating a distance from its original position) bacteria] and external stimuli.

Imai et al. (2016) then reported on a case of CA pneumonia with septicaemia in a healthy 53-year-old woman, for which the exposure/causative scenario was not known (Table 2). The patient had no previous history of medical ailments or recent overseas travel but admitted to being an active smoker for 30 years prior to the infection. Upon clinical investigation, the patient was diagnosed with septic shock due to lobar pneumonia, with the causative pathogen identified as *P. aeruginosa*. John et al. (2017) reported on an outbreak of CA *P. aeruginosa* pneumonia, which coincided with a severe drought period in the Western Cape of South Africa and was associated with the increased use of alternative water sources (e.g., greywater, rainwater, and borehole water) by community members. The authors consequently hypothesised that contaminated water could have been a possible exposure scenario, leading to the CA outbreak (Table 2). This is concerning as individuals residing in underdeveloped and developing countries which lack adequate sanitation and healthcare may thus be at higher risk of contracting life-threatening infections which in many cases, are untreatable (Prüss-Ustün et al., 2019).

2.6. Enterobacter spp.

Infections with *Enterobacter* spp. may result in meningitis, pneumonia, cerebral abscess, wound infections, septicaemia, UTIs, and intestinal/abdominal cavity infections (Davin-Regli et al., 2019; Mezzatesta et al., 2012). Subsequently, as most infections are nosocomial, the majority of *Enterobacter* spp. isolates exhibit extensive resistance to quinolones, penicillins, and third generation cephalosporins, due to the treatment of infected patients with these antibiotic classes. However, certain antibiotics including carbapenems (e.g., meropenem, ertapenem, doripenem) and fourth generation cephalosporins (e.g., cefepime, cefpirome), remain effective and are attractive options for the treatment of *Enterobacter* spp. infections (Son et al., 2018). Moreover, the aminoglycosides, especially amikacin, have shown promising activity in treating over 95% of *Enterobacter* spp. infections (Davin-Regli et al., 2019). Nonetheless, the expression of various enzymes, such as carbapenemases and extended-spectrum beta-lactamases (ESBLs), including KPC, OXA, VIM and metallo-β-lactamase-1 (encoded by variants of the bla_{NDM}, bla_{OXA}, bla_{KPC}, bla_{VIM}, $bla_{CTX\text{-}M}$, bla_{IMP}, and bla_{TEM} genes), implies that *Enterobacter* spp. are intrinsically resistant to amoxicillin, ampicillin, cefoxitin and first-generation cephalosporins (Yuan et al., 2019).

Enterobacter spp. are associated with several environmental reservoirs such as water, soil, plants (where they act as endophytes and are sometimes considered phytopathogens for various species of plants) and are considered natural commensals of the human and animal gastrointestinal tract (Davin-Regli et al., 2019). While various ESKAPE strains have been detected in environmental waters, contamination of food by enteric pathogens such as *Enterobacter* spp. may also occur if sewage or contaminated water is used to fertilise farm soils, or if crops are irrigated using untreated water sources (Lee, 2003; Verraes et al., 2013).

Sharma et al. (2008) investigated the prevalence of MDR, and

ESBL-producing strains of *Enterobacter* spp. isolated from points along the Narmada River (Table 1). In total, 57 *Enterobacter* isolates were obtained, of which 73.7% ($n = 42$) were identified as ESBL producers. Additionally, all isolates were susceptible to imipenem, while varying degrees of resistance to ceftriaxone, cefpodoxime, cefepime, ceftazidime, cefuroxime, cefotaxime, ceftazidime-clavulanic acid, cefoxitin, aztreonam and chloramphenicol antibiotics was observed. The bla_{TEM} and bla_{SHV} genes were then detected in 42% and 44% of the isolates, respectively. Jiménez-Castillo et al. (2021) then reported on a case of round pneumonia (i.e., when round or oval densities are visible via chest x-ray) in a 64-year-old male who had a history of liver cirrhosis and alcohol consumption. Upon analysis of sputum taken from the patient, *E. hormaechei* was identified as the causative agent and was found to be sensitive to levofloxacin. Forty-eight hours after admission to the emergency room, respiratory distress syndrome developed in the patient, and he was admitted to the ICU. However, the disease worsened, and the patient died of septic shock (Table 2).

It is however important to note that the risk linked to CA ESKAPE infections may not be limited to a single ESKAPE pathogen, but that the risk of polymicrobial infections also exists. For example, Liu et al. (2004) reported that sixty patients in a medical centre of Northern Taiwan, had bloodstream infections caused by *E. cloacae* (Table 2). Of the sixty infected patients, fourteen (5.4%) were polymicrobial infections: four with *E. coli*, seven with *K. pneumoniae*, one with *S. marcescens*, and one with *A. baumannii*. Additionally, out of the fourteen polymicrobial infections, six of these cases were CA, with several of the causative *E. cloacae* isolates exhibiting MDR status. It is thus clear that risk of CA infection may be attributed to more than one ESKAPE species at any particular time, and that the causative agent(s) may possess MDR status.

3. Quantitative microbial risk assessment of environmental ESKAPE pathogens

The potential health risks posed by the ESKAPE pathogens in environmental reservoirs have not been extensively investigated, as most research has focused on the detection and ABR classification of these organisms in hospital environments. To assess the health risk of human exposure to the ESKAPE pathogens in the environment, epidemiological studies may be used however, these tend to be expensive and time-consuming (Abia et al., 2016). Quantitative microbial risk assessment (QMRA) has thus been employed as an essential, complimentary tool to epidemiological studies, and to better understand the implications and risks that exposure to environmental sources may have on human health (Soller et al., 2015).

Quantitative microbial risk assessment is a widely recognised tool that has been used for more than two decades to estimate the pathogen-associated risk to populations or communities (Owens et al., 2020; Pecson et al., 2017). To perform QMRA, four stages of analysis need to be conducted, namely (i) hazard identification, (ii) exposure assessment, (iii) dose-response modelling, and (iv) risk characterisation (Owens et al., 2020; Zhang et al., 2019). Through the combination of these stages, a prediction-based analysis model can be generated, which

Table 3
Dose response models available for the risk assessment of the ESKAPE pathogens.

Organism	Dose-response model			QMRA application	
	Parameters	Background	Reference	Background	Reference
E. faecium	Exponential $k = 177$	Model: Human Exposure: Immersion in sea water contaminated with faecal streptococci Response: Gastrointestinal illness	Kay et al. (1994)	Exposure: Ingestion of sea water Response: Gastrointestinal illness Exposure: Ingestion of grey/groundwater Response: Infection	Stone et al. (2008) Ottoson and Stenström (2003) Goh et al. (2015)
S. aureus	Exponential $k = 7.64 \times 10^{-8}$	Model: Human Exposure: Subcutaneous application on the forearm Response: Infection	Rose and Haas (1999)	Exposure: Dermal contact via washing hands with contaminated greywater Response: Infection	Busgang et al. (2018)
	Beta-Poisson $\alpha = 0.3999$ $N_{50} = 26\ 660$	Model: Human Exposure: Auto-inoculation during food preparation Response: Nasal colonisation with MRSA	Schoen et al. (2020)	Exposure: Immersion in wastewater or greywater Response: Colonisation and Infection	Schoen et al. (2021)
K. pneumoniae	Exponential $k = 1.62 \times 10^{-6}$	Model: Rats Exposure: Transtracheal instillation into lungs Response: Lobar pneumonia	Domenico et al. (1982)	Exposure: Dermal contact to liquid particulates and accidental ingestion of particulates Response: Infection	Harb and Hong (2017)
A. baumannii	Exponential $k = 2.76 \times 10^{-7}$	Model: Mice Exposure: Intraperitoneal inoculation Response: Peritoneal sepsis	López-Rojas et al. (2011)	Exposure: Dermal contact to liquid particulates and accidental ingestion of particulates Response: Infection	Harb and Hong (2017)
P. aeruginosa	Beta-Poisson $\alpha = 1.9 \times 10^{-1}$ $N_{50} = 18\ 500$	Model: White rabbit Exposure: Contaminated contact lens Response: Corneal ulceration	Lawin-Brüssel et al. (1993)	Exposure: Cleaning contact lenses with potentially contaminated tap water Response: Corneal ulceration	Rasheduzzaman et al. (2019)
P. aeruginosa	Exponential $k = 1.05 \times 10^{-4}$	Model: Swiss webster mice (5 days old) Exposure: Injected in eyelids Response: Death	Hazlett et al. (1978)	Exposure: Dermal exposure to liquid particles (from reusing influent, effluent, and chlorinated effluent in agricultural irrigation) Response: Infection	Al-Jassim et al. (2015)
Enterobacter spp. (*E. sakazakii*)	Exponential $k = 1.0 \times 10^{-5}$ to 1.0×10^{-10}	Model: Infant Exposure: Ingestion of contaminated powdered infant formula Response: Infection	Iversen and Forsythe (2003) Paoli and Hartnett (2006)		

k - probability of the pathogen surviving the host's defence to initiate infection; N_{50} - median infective dosage; α - shape factor.

elucidates the potential health risks that correlate to specific pathogens based on exposure scenarios (e.g., linked to a certain activity or environment) (Owens et al., 2020).

Goh et al. (2015) then investigated the microbial risks associated with the ingestion of sea water containing *Salmonella* and *Enterococcus* from the Marina Reservoir (Singapore) and its four primary feeders (Table 3). Results of this study indicated that the microbial risk levels were below the recreational illness rate (0.8%) set out by the United States Environmental Protection Agency for fresh water. However, the risks calculated for the probability of exhibiting gastrointestinal illness were exceeded for *Enterococcus* in one of the four upstream catchments. Nonetheless, this catchment area was located a distance away from the area used for regular recreational activities, thus the authors hypothesised that the identified risk of infection was not of particular concern.

In an effort to sustain the increasing demand for water supplies, the re-use of various water sources has been endorsed by municipal water managers and international agencies (Sharvelle et al., 2017). Wastewater and greywater are thus increasingly re-used for various non-potable and potable purposes however, treatment of these waters is required before use to control the variety of infectious human pathogens that may be present (Sharvelle et al., 2017). Schoen et al. (2021) thus determined the annual risks of colonisation, bloodstream infection, skin infection, and the burden of disease linked to the exposure of healthy adults to antibiotic susceptible and resistant *S. aureus* (Table 3). The exposure scenario assessed was immersion in treated (using various techniques) greywater or wastewater, leading to subsequent nasal colonisation and infection with *S. aureus*. Results of this study indicated that as the level of treatment applied to the greywater or wastewater increased, the associated risk of infection decreased. It was then identified that the estimated risk of infection and burden of disease resulting from nasal colonisation was below the health risk benchmarks for risk-based, potable, or non-potable reuse systems, suggesting that there was no risk posed by nasal colonisation with susceptible or resistant *S. aureus* from treated greywater or wastewater. However, as this risk may increase to above the recommended limit if an individual is immersed in minimally treated wastewater or greywater, it has been recognised that strain-specific data for concentration and dose-response in wastewater is needed to fully validate QMRA (Schoen et al., 2021).

While risk assessment has not been frequently conducted for *K. pneumoniae* or *A. baumannii*, Harb and Hong (2017) examined the efficacy of two membrane bioreactor (MBR) systems (responsible for treating municipal wastewater) in removing potentially pathogenic bacteria from wastewater (Table 3). Quantitative microbial risk assessment was performed for *K. pneumoniae*, *A. baumannii* and *P. aeruginosa* to determine what exposure doses of these pathogens are imposed by aerobic MBRs (AeMBR) and anaerobic MBRs (AnMBR) on agricultural workers via dermal contact to liquid particulates during irrigation events and accidental ingestion of particulates during land filling/land applying dewatered activated sludge. Exposure doses from irrigation with AeMBR effluent for *A. baumannii* (6×10^{-3}), *P. aeruginosa* (3.2×10^{-1}) and *K. pneumoniae* (4.2×10^{-3}) were above the acceptable microbial risk level of 1×10^{-4} infections per person per year. The risks from AnMBR effluent were also exceeded for *P. aeruginosa* (6.3×10^{-2}) and *K. pneumoniae* (6.3×10^{-1}), while the risk limit for *A. baumannii* was below the benchmark (4.3×10^{-5}). The risk associated with exposure and ingestion of AeMBR-produced activated sludge was then also assessed for *K. pneumoniae* and *A. baumannii*, with the risk for *K. pneumoniae* exposure (4.9×10^{-3}) and ingestion (2.6×10^{-2}) and *A. baumannii* exposure (5.0×10^{-3}) and ingestion (2.6×10^{-2}) exceeding the benchmark limit. Based on these results, it was proposed that there is a high-risk level associated with the use of wastewater from AeMBRs and AnMBRs for irrigation purposes. Additionally, as the risks for *K. pneumoniae* and *A. baumannii* were exceeded for the exposure to and accidental ingestion of AeMBR sludge during land application activities and disposal, the need for correct treatment of activated sludge before disposal, was highlighted (Harb and Hong, 2017).

In contrast to the other ESKAPE pathogens, several studies have been conducted to determine the risk associated with *P. aeruginosa* in different water sources as this pathogen commonly inhabits moist environments such as soil and water (Klockgether and Tümmler, 2017; Pachori et al., 2019). *Pseudomonas aeruginosa* is typically associated with ocular infections and is not regularly described as an ingestion hazard (Mena and Gerba, 2009). Rodriguez-Alvarez et al. (2015) therefore used QMRA and an eye-exposure scenario to assess the risks associated with *E. coli*, *P. aeruginosa* and *Giardia* from various water sources obtained from a peri-urban area in Salta, Argentina (Table 3). The results were compared to the general 1×10^{-4} risk level. In most cases, the drinking regulations were achieved for *E. coli* however, the risk level associated with *P. aeruginosa,* and *Giardia* were exceeded for water obtained from all the studied sources (neighbourhood boreholes, an irrigation channel, a new and an old water treatment plant, as well as shallow wells). Results from this study thus indicate that water which undergoes treatment and is considered safe for consumption may still contain a significant level of pathogenic microorganisms that may potentially negatively impact the health of the consumer (Rodriguez-Alvarez et al., 2015).

Similar to *A. baumannii* and *K. pneumoniae*, limited information is available regarding risk assessment models for *Enterobacter* spp. Paoli and Hartnett (2006) however, devised a QMRA model to estimate the risk associated with *E. sakazakii* in powdered infant formula. Based on the limited number of QMRA studies evaluating the risk posed by the ESKAPE pathogens in environmental sources, it is thus clear that additional studies are required to determine the risk of CA ESKAPE infections. For example, many of the CA outbreaks reported in Table 2 for the ESKAPE pathogens are UTIs, pneumonia, or bloodstream infections. Therefore, ESKAPE-specific dose-response models should be established for these particular infection end-points, as they pose the greatest risk to communities. Additionally, if ESKAPE-specific dose-response models are not available, suitable surrogate models (similar infection end-point caused by another closely related bacterium) need to be identified and used to generate risk assessment data (Schoen et al., 2021).

Overall, the QMRA studies that have been discussed, assessed exposure scenarios that include recreational water use, accidental ingestion of seawater, immersion/exposure to greywater and wastewater, and the accidental exposure to treated wastewater or sludge during agricultural activities. However, these only represent a fraction of the possible real-world scenarios where individuals may be exposed to the ESKAPE pathogens. For example, many of the studies detected the ESKAPE pathogens in environmental reservoirs other than wastewater, such as soil, river water, irrigation water, ready-to-eat foods, and on vegetables (Table 1). Therefore, a wider range of exposure scenarios should and need to be evaluated in future research for both developed and developing countries (Busgang et al., 2018; Denissen et al., 2021; Reyneke et al., 2020).

In line with this, an important factor to consider when conducting risk assessments, is that the microbial exposure volumes and routes of exposure/transmission may be vastly different between low-income/developing countries and developed countries. For example, applicable exposure scenarios linked to the use of greywater differ between developed (e.g., toilet flushing and garden irrigation) versus developing countries (e.g., cleaning, washing laundry, garden work), while the way in which the activity is completed may also differ significantly [e.g., use of a high-pressure garden hose (developed) versus a watering can (developing) for garden irrigation] (Reyneke et al., 2020).

In fact, current research primarily focuses on microbial transmission via food and water in developing countries however, evidence has suggested that non-dietary ingestion pathways (e.g., ingesting soil or other contaminants via hand-to-mouth contact events) may play a role in children's microbial exposures in developing or lower income countries. Additionally, high concentrations of faecal bacteria have been observed in soils and on various domestic surfaces (such as a broom, bucket, bag, clothing, plate, and a tool handle, amongst others) in developing countries, suggesting that these environments may

subsequently influence human exposure to faecal contamination which may harbour a multitude of pathogens (Julian and Pickering, 2015; Mattioli et al., 2012).

Another limitation to the current state of QMRA research, is that the description of an individual's interactions with the environment may not always be accurate (Julian and Pickering, 2015). Additionally, the description of the same interaction might differ significantly between studies. For example, Busgang et al. (2018) determined the microbial risks associated with the reuse of greywater for garden irrigation using best-practice and worst-case scenarios for *Salmonella enterica*, *Shigella* spp., and *S. aureus*. In this study, an average ingestion volume of 100 mL was used for the "accidental drinking" scenario. However, in a similar study, Ottoson and Stenström (2003) investigated the risk associated with the accidental ingestion of treated greywater for several species, including enterococci, using an ingestion volume of 1 mL. These studies highlighted that the appropriate selection of exposure volumes (i.e., accurate description of exposure scenarios) is important as this variable may result in an over- or under-estimation of the posed microbial risk.

Another important factor to consider when conducting QMRA is the differences which may be observed between the severity of infections in adults and children, or healthy individuals and immunocompromised individuals, particularly if the infections are caused by polymicrobial communities. Additionally, the presence of ARGs in the ESKAPE pathogens hinders the accurate calculation of risk assessment results. In fact, ARGs act as novel environmental pollutants and several studies have shown that many ARGs and pathogens that are tolerant to disinfection, may survive in water distribution systems, treated water, and tap water (Hu et al., 2021). Future QMRA research and risk assessment models should thus incorporate the presence and effect of ARGs, as well as the degree to which the causative pathogen(s) under study may affect people of different ages, demographics, living conditions, and health statuses.

4. Conclusion

Enterococcus faecium, *S. aureus*, *K. pneumoniae*, *A. baumannii*, *P. aeruginosa* and *Enterobacter* spp. are well known for their role in the onset of a variety of life-threatening, clinical infections. As a result of the imprudent use of commercially available antimicrobials, very few antibiotics are effective against these pathogens, and this problem is exacerbated by their intrinsic and acquired resistance mechanisms. This is concerning as the issue of ABR could lead to prolonged illnesses, failure of treatment therapies, increased health care expenses, and even the risk of death if humans are infected with MDR/XDR/PDR strains of these pathogens (Tanwar et al., 2014).

Despite their prevalence in nosocomial settings, studies have reported on the isolation of the ESKAPE pathogens from various environmental reservoirs such as soil, dumpsites, beach sand, wastewater, food, fish farms, amongst others. Moreover, many of the environmental ESKAPE isolates obtained in such studies are found to exhibit increased levels of ABR and possess a variety of ARGs. This is likely because animals and humans have been identified as reservoirs which harbour antibiotic resistant organisms, including the ESKAPE pathogens, and subsequently may transfer ARGs or antibiotic resistant pathogens to other humans, animals or into the environment (Akanbi et al., 2017).

Furthermore, due to the detection of antibiotic resistant ESKAPE isolates in the environment and incidences of CA infection, it is crucial that stringent control and disinfection methods are implemented with regards to the disposal of clinical waste, wastewater, and agricultural waste, worldwide. Not only does the spread of ARB from person to person need to be prevented, but also between and within the human and agricultural sectors, as well as in the environment, with a particular focus on contaminated waters. A One Health approach will thus greatly aid in reducing the amount of ARB and ARGs that are released into the environment, as it will improve the awareness and understanding of ABR through effective education, training, and communication across various sectors. Additionally, it will prompt the implementation of various control measures to improve the quality of water and food, particularly in low- and middle-income countries (Aslam et al., 2021). In turn, this could reduce the human-associated health risks linked to contaminated environmental water reservoirs (Collignon and McEwen, 2019).

Furthermore, when calculating health risks, it is important to consider the approximate percentage of immunocompromised individuals living in the communities under study, as this will more accurately aid in estimating the risk associated with the use of environmental waters (or other sources that are being investigated) for potable and domestic activities (Denissen et al., 2021). Moreover, as most risk assessments focus on pathogen concentration only, models need to be developed which take into consideration the ABR mechanisms (e.g., ARGs) used by the causative agent. By doing this, a more accurate evaluation can be made regarding the impact of pathogens, particularly the ESKAPE pathogens, and antimicrobial resistance on human, animal, and environmental health (i.e., a One Health approach).

Author contributions

Conceptualisation: JD and WK. Compiled and edited the manuscript: JD, BR, MW, BH, TB, SK, and WK. All authors approved the submitted version of the article.

Declaration of competing interest

The authors declare that the review was compiled in the absence of any financial or commercial relations that could be construed as a possible conflict of interest.

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References

Abia, A.L.K., Ubomba-Jaswa, E., Genthe, B., Momba, M.N.B., 2016. Quantitative microbial risk assessment (QMRA) shows increased public health risk associated with exposure to river water under conditions of riverbed sediment resuspension. Sci. Total Environ. 566, 1143–1151.

Aidara-Kane, A., Angulo, F.J., Conly, J.M., Minato, Y., Silbergeld, E.K., McEwen, S.A., Collignon, P.J., 2018. World Health Organization (WHO) guidelines on use of medically important antimicrobials in food-producing animals. Antimicrob. Resist. Infect. Control 7 (1), 1–8.

Akanbi, O.E., Njom, H.A., Fri, J., Otigbu, A.C., Clarke, A.M., 2017. Antimicrobial susceptibility of *Staphylococcus aureus* isolated from recreational waters and beach sand in Eastern Cape Province of South Africa. Int. J. Environ. Res. 14, 1001.

Akova, M., Daikos, G.L., Tzouvelekis, L., Carmeli, Y., 2012. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. Clin. Microbiol. Infect. 18, 439–448.

Al-Jassim, N., Ansari, M.I., Harb, M., Hong, P.Y., 2015. Removal of bacterial contaminants and antibiotic resistance genes by conventional wastewater treatment processes in Saudi Arabia: is the treated wastewater safe to reuse for agricultural irrigation? Water Res. 73, 277–290.

Alonso, C.A., Zarazaga, M., Ben Sallem, R., Jouini, A., Ben Slama, K., Torres, C., 2017. Antibiotic resistance in *Escherichia coli* in husbandry animals: the African perspective. Lett. Appl. Microbiol. 64 (5), 318–334.

Amarasiri, M., Sano, D., Suzuki, S., 2020. Understanding human health risks caused by antibiotic resistant bacteria (ARB) and antibiotic resistance genes (ARG) in water environments: current knowledge and questions to be answered. Crit. Rev. Environ. Sci. Technol. 50, 2016–2059.

Araújo, A.J.G.D., Grassotti, T.T., Frazzon, A.P.G., 2021. Characterization of *Enterococcus* spp. isolated from a fish farming environment in southern Brazil. Braz. J. Biol. 81, 954–961.

Argudín, M.A., Deplano, A., Meghraoui, A., Dodémont, M., Heinrichs, A., Denis, O., Nonhoff, C., Roisin, S., 2017. Bacteria from animals as a pool of antimicrobial resistance genes. Antibiotics 6 (2), 12.

Aslam, B., Khurshid, M., Arshad, M.I., Muzammil, S., Rasool, M., Yasmeen, N., Shah, T., Chaudhry, T.H., Rasool, M.H., Shahid, A., Xueshan, X., 2021. Antibiotic resistance: one health one world outlook. Front. Cell. Infect. Microbiol. 11, 771510.

Aubertheau, E., Stalder, T., Mondamert, L., Ploy, M.C., Dagot, C., Labanowski, J., 2017. Impact of wastewater treatment plant discharge on the contamination of river

biofilms by pharmaceuticals and antibiotic resistance. Sci. Total Environ. 579, 1387–1398.
Australian Commission on Safety and Quality in Health Care (ACSQHC), 2016. AURA 2016: First Australian Report on Antimicrobial Use and Resistance in Human Health. ACSQHC, Sydney, ISBN 978-1-925224-42-9, 2016, Available: https://www.safetyandquality.gov.au/sites/default/files/migrated/AURA-2016-First-Australian-Report-on-Antimicrobial-use-and-resistance-in-human-health.pdf.
Baëtz, B., Boudrioua, A., Hartke, A., Giraud, C., 2021. Alternatives to fight vancomycin-resistant staphylococci and enterococci. Antibiotics 10 (9), 1116.
Bager, F., 2000. DANMAP 99—Consumption of Antimicrobial Agents and Occurrence of Antimicrobial Resistance in Bacteria from Food Animals, Food and Humans in Denmark. Statens Serum Institut, Danish Veterinary and Food administration, Danish Medicines Agency, Danish Veterinary Laboratory, Copenhagen, Denmark, 1600-2032.
Baquero, F., Martínez, J.L., Cantón, R., 2008. Antibiotics and antibiotic resistance in water environments. Curr. Opin. Biotechnol. 19 (3), 260–265.
Barati, A., Ghaderpour, A., Chew, L.L., Bong, C.W., Thong, K.L., Chong, V.C., Chai, L.C., 2016. Isolation and characterization of aquatic-borne *Klebsiella pneumoniae* from tropical estuaries in Malaysia. Int. J. Environ. Res. Publ. Health 13, 426.
Bender, J.K., Cattoir, V., Hegstad, K., Sadowy, E., Coque, T.M., Westh, H., Hammerum, A. M., Schaffer, K., Burns, K., Murchan, S., Novais, C., 2018. Update on prevalence and mechanisms of resistance to linezolid, tigecycline and daptomycin in enterococci in Europe: towards a common nomenclature. Drug Resist. Updates 40, 25–39.
Bichon, A., Aubry, C., Dubourg, G., Drouet, H., Lagier, J.C., Raoult, D., Parola, P., 2018. *Escherichia coli* spontaneous community-acquired meningitis in adults: a case report and literature review. Int. J. Infect. Dis. 67, 70–74.
Boix-Palop, L., Xercavins, M., Badía, C., Obradors, M., Riera, M., Freixas, N., Pérez, J., Rodríguez-Carballeira, M., Garau, J., Calbo, E., 2017. Emerging extended-spectrum β-lactamase-producing *Klebsiella pneumoniae* causing community-onset urinary tract infections: a case-control-control study. Int. J. Antimicrob. Agents 50, 197–202.
Bonnet, R., Marchandin, H., Chanal, C., Sirot, D., Labia, R., De Champs, C., Jumas-Bilak, E., Sirot, J., 2002. Chromosome-encoded class D β-lactamase OXA-23 in Proteus mirabilis. Antimicrob. Agents Chemother. 46, 2004–2006.
Boyd, D.A., Willey, B.M., Fawcett, D., Gillani, N., Mulvey, M.R., 2008. Molecular characterization of *Enterococcus faecalis* N06-0364 with low-level vancomycin resistance harboring a novel D-Ala-D-Ser gene cluster, *vanL*. Antimicrob. Agents Chemother. 52 (7), 2667–2672.
Busgang, A., Friedler, E., Gilboa, Y., Gross, A., 2018. Quantitative microbial risk analysis for various bacterial exposure scenarios involving greywater reuse for irrigation. Water 10, 413.
Cassini, A., Högberg, L.D., Plachouras, D., Quattrocchi, A., Hoxha, A., Simonsen, G.S., Colomb-Cotinat, M., Kretzschmar, M.E., Devleesschauwer, B., Cecchini, M., Ouakrim, D.A., 2019. Attributable deaths and disability-adjusted life-years caused by infections with antibiotic-resistant bacteria in the EU and the European Economic Area in 2015: a population-level modelling analysis. Lancet Infect. Dis. 19 (1), 56–66.
Castro, P.B.N.E., da Silva Rodrigues, D.A., Roeser, H.M.P., da Fonseca Santiago, A., de Cássia Franco Afonso, R.J., 2020. Antibiotic consumption in developing countries defies global commitments: an overview on Brazilian growth in consumption. Environ. Sci. Pollut. Res. 27 (17), 21013–21020.
Charretier, Y., Diene, S.M., Baud, D., Chatellier, S., Santiago-Allexant, E., van Belkum, A., Guigon, G., Schrenzel, J., 2018. Colistin heteroresistance and involvement of the PmrAB regulatory system in *Acinetobacter baumannii*. Antimicrob. Agents Chemother. 62 (9), e00788–e00818.
Chen, M.Y., Lira, F., Liang, H.Q., Wu, R.T., Duan, J.H., Liao, X.P., Martínez, J.L., Liu, Y. H., Sun, J., 2016. Multilevel selection of bcrABDR-mediated bacitracin resistance in *Enterococcus faecalis* from chicken farms. Sci. Rep. 6 (1), 1–7.
Chilambi, G.S., Nordstrom, H.R., Evans, D.R., Ferrolino, J.A., Hayden, R.T., Marón, G.M., Vo, A.N., Gilmore, M.S., Wolf, J., Rosch, J.W., Van Tyne, D., 2020. Evolution of vancomycin-resistant *Enterococcus faecium* during colonization and infection in immunocompromised pediatric patients. Proc. Natl. Acad. Sci. U.S.A. 117, 11703–11714.
Chokshi, A., Sifri, Z., Cennimo, D., Horng, H., 2019. Global contributors to antibiotic resistance. J. Global Infect. Dis. 11 (1), 36.
Chukwurah, S.N., Ushie, S.N., Ezenekwe, F.E., Ugwunze, E.O., Ufoaroh, C.U., Maduekwe, N.P., 2021. Community acquired pyogenic liver abscess caused by a nosocomial organism: a case report. Orient J. Med. 33, 117–122.
Collignon, P.J., McEwen, S.A., 2019. One health – its importance in helping to better control antimicrobial resistance. Trav. Med. Infect. Dis. 4, 22.
Cong, Y., Yang, S., Rao, X., 2020. Vancomycin resistant *Staphylococcus aureus* infections: a review of case updating and clinical features. J. Adv. Res. 21, 169–176.
Dantes, R., Mu, Y., Belflower, R., Aragon, D., Dumyati, G., Harrison, L.H., Lessa, F.C., Lynfield, R., Nadle, J., Petit, S., Ray, S.M., 2013. National burden of invasive methicillin-resistant *Staphylococcus aureus* infections, United States, 2011. JAMA Intern. Med. 173 (21), 1970–1978.
Davin-Regli, A., Lavigne, J.P., Pagès, J.M., 2019. *Enterobacter* spp.: update on taxonomy, clinical aspects, and emerging antimicrobial resistance. Clin. Microbiol. Rev. 32, e00002–e00019.
Denissen, J.K., Reyneke, B., Waso, M., Khan, S., Khan, W., 2021. Human pathogenic bacteria detected in rainwater: risk assessment and correlation to microbial source tracking markers and traditional indicators. Front. Microbiol. 12, 1027.
De Oliveira, D.M., Forde, B.M., Kidd, T.J., Harris, P.N., Schembri, M.A., Beatson, S.A., Paterson, D.L., Walker, M.J., 2020. Antimicrobial resistance in ESKAPE pathogens. Clin. Microbiol. Rev. 33 (3), e00181–e00219.
Diarra, M.S., Rempel, H., Champagne, J., Masson, L., Pritchard, J., Topp, E., 2010. Distribution of antimicrobial resistance and virulence genes in *Enterococcus* spp. and characterization of isolates from broiler chickens. Appl. Environ. Microbiol. 76 (24), 8033–8043.
Dobrijević, D., Trudić, A., Borčić, V., Bekut, M., 2017. Antibiotic susceptibility profile of *Klebsiella pneumoniae* strains isolated from drinking and surface water. Racionalna terapija 9, 1–13.
Domenico, P., Johanson Jr., W.G., Straus, D.C., 1982. Lobar pneumonia in rats produced by clinical isolates of *Klebsiella pneumoniae*. Infect. Immun. 37, 327–335.
Dos Santos, L.D.R., Furlan, J.P.R., Gallo, I.F.L., Ramos, M.S., Savazzi, E.A., Stehling, E.G., 2021. Occurrence of multidrug-resistant *Enterococcus faecium* isolated from environmental samples. Lett. Appl. Microbiol. 73, 237–246.
Eagar, H., Swan, G., Van Vuuren, M., 2012. A survey of antimicrobial usage in animals in South Africa with specific reference to food animals. J. S. Afr. Vet. Assoc. 83 (1), 1–8.
Ebomah, K.E., Okoh, A.I., 2020. Detection of carbapenem-resistance genes in *Klebsiella* species recovered from selected environmental niches in the Eastern Cape Province, South Africa. Antibiotics 9, 425.
Eichenberger, E.M., Thaden, J.T., 2019. Epidemiology and mechanisms of resistance of extensively drug resistant Gram-negative bacteria. Antibiotics 8, 37.
El-Halfawy, O.M., Valvano, M.A., 2015. Antimicrobial heteroresistance: an emerging field in need of clarity. Clin. Microbiol. Rev. 28 (1), 191–207.
Emaneini, M., Aligholi, M., Aminshahi, M., 2008. Characterization of glycopeptides, aminoglycosides and macrolide resistance among *Enterococcus faecalis* and *Enterococcus faecium* isolates from hospitals in Tehran. Pol. J. Microbiol. 57, 173–178.
Enciso-Martínez, Y., González-Aguilar, G.A., Martínez-Téllez, M.A., González-Pérez, C.J., Valencia-Rivera, D.E., Barrios-Villa, E., Ayala-Zavala, J.F., 2022. Relevance of tracking the diversity of *Escherichia coli* pathotypes to reinforce food safety. Int. J. Food Microbiol. 374, 109736.
European Centre for Disease Prevention and Control, 2018. Surveillance of Antimicrobial Resistance in Europe–Annual Report of the European Antimicrobial Resistance Surveillance Network (EARS-Net) 2017. ECDC, Stockholm, ISBN 978-92-9498-282-7, 2018, Available: https://www.ecdc.europa.eu/sites/default/files/documents/AMR%202017_Cover%2BInner-web_v3.pdf.
Eveillard, M., Kempf, M., Belmonte, O., Pailhoriès, H., Joly-Guillou, M.L., 2013. Reservoirs of *Acinetobacter baumannii* outside the hospital and potential involvement in emerging human community-acquired infections. Int. J. Infect. Dis. 17, e802–e805.
Founou, L.L., Amoako, D.G., Founou, R.C., Essack, S.Y., 2018. Antibiotic resistance in food animals in Africa: a systematic review and meta-analysis. Microb. Drug Resist. 24 (5), 648–665.
Furtula, V., Jackson, C.R., Farrell, E.G., Barrett, J.B., Hiott, L.M., Chambers, P.A., 2013. Antimicrobial resistance in *Enterococcus* spp. isolated from environmental samples in an area of intensive poultry production. Int. J. Environ. Res. Publ. Health 10 (3), 1020–1036.
Gajdács, M., Urbán, E., Stájer, A., Baráth, Z., 2021. Antimicrobial resistance in the context of the sustainable development goals: a brief review. Eur. J. Investig. Health Psychol. Educ. 11, 71–82.
Girlich, D., Poirel, L., Nordmann, P., 2010. First isolation of the bla OXA-23 carbapenemase gene from an environmental *Acinetobacter baumannii* isolate. Antimicrob. Agents Chemother. 54, 578–579.
Girometti, N., Lewis, R.E., Giannella, M., Ambretti, S., Bartoletti, M., Tedeschi, S., Tumietto, F., Cristini, F., Trapani, F., Gaibani, P., Viale, P., 2014. *Klebsiella pneumoniae* bloodstream infection: epidemiology and impact of inappropriate empirical therapy. Medicine 93, 298–309.
Goh, S.G., Fang, H.M., Chang, S.Y., Gin, K.Y., 2015. Quantitative microbial risk assessment of *Salmonella* and *Enterococcus* in marina reservoir and catchments. Water Pract. Technol. 10, 527–531.
Goli, H.R., Nahaei, M.R., Rezaee, M.A., Hasani, A., Kafil, H.S., Aghazadeh, M., 2016. Emergence of colistin resistant *Pseudomonas aeruginosa* at Tabriz hospitals, Iran. Iran. J. Microbiol. 8, 62–69.
Gould, I.M., David, M.Z., Esposito, S., Garau, J., Lina, G., Mazzei, T., Peters, G., 2012. New insights into meticillin-resistant *Staphylococcus aureus* (MRSA) pathogenesis, treatment and resistance. Int. J. Antimicrob. Agents 39, 96–104.
Gudipati, S., Zervos, M., Herc, E., 2020. Can the one health approach save us from the emergence and re-emergence of infectious pathogens in the era of climate change: implications for antimicrobial resistance? Antibiotics 9, 599.
Guo, Y., Song, G., Sun, M., Wang, J., Wang, Y., 2020. Prevalence and therapies of antibiotic-resistance in *Staphylococcus aureus*. Front. Cell. Infect. Microbiol. 10, 107.
Gupta, C.L., Blum, S.E., Kattusamy, K., Daniel, T., Druyan, S., Shapira, R., Krifucks, O., Zhu, Y.G., Zhou, X.Y., Su, J.Q., Cytryn, E., 2021. Longitudinal study on the effects of growth-promoting and therapeutic antibiotics on the dynamics of chicken cloacal and litter microbiomes and resistomes. Microbiome 9 (1), 1–19.
Gwenzi, W., 2020. The 'thanato-resistome'-The funeral industry as a potential reservoir of antibiotic resistance: early insights and perspectives. Sci. Total Environ. 749, 141120.
Hageman, J.C., Uyeki, T.M., Francis, J.S., Jernigan, D.B., Wheeler, J.G., Bridges, C.B., Barenkamp, S.J., Sievert, D.M., Srinivasan, A., Doherty, M.C., McDougal, L.K., 2006. Severe CA pneumonia due to *Staphylococcus aureus*, 2003–04 influenza season. Emerg. Infect. Dis. 12, 894–899.
Hanning, I.B., Nutt, J.D., Ricke, S.C., 2009. Salmonellosis outbreaks in the United States due to fresh produce: sources and potential intervention measures. Foodb. Pathog. Dis. 6, 635–648.
Harb, M., Hong, P.Y., 2017. Molecular-based detection of potentially pathogenic bacteria in membrane bioreactor (MBR) systems treating municipal wastewater: a case study. Environ. Sci. Pollut. Res. 24, 5370–5380.

Havenga, B., Ndlovu, T., Clements, T., Reyneke, B., Waso, M., Khan, W., 2019. Exploring the antimicrobial resistance profiles of WHO critical priority list bacterial strains. BMC Microbiol. 19, 303.
Hazlett, L.D., Rosen, D.D., Berk, R.S., 1978. Age-related susceptibility to *Pseudomonas aeruginosa* ocular infections in mice. Infect. Immun. 20, 25–29.
Holt, K.E., Wertheim, H., Zadoks, R.N., Baker, S., Whitehouse, C.A., Dance, D., Jenney, A., Connor, T.R., Hsu, L.Y., Severin, J., Brisse, S., 2015. Genomic analysis of diversity, population structure, virulence, and antimicrobial resistance in *Klebsiella pneumoniae*, an urgent threat to public health. Proc. Natl. Acad. Sci. U.S.A. 112, E3574–E3581.
Hrenovic, J., Durn, G., Music, M.S., Dekic, S., Troskot-Corbic, T., Skoric, D., 2017. Extensively and multi drug-resistant *Acinetobacter baumannii* recovered from technosol at a dump site in Croatia. Sci. Total Environ. 607–608, 1049–1055.
Hosaka, Y., Yahara, K., Clark, A., Kitagawa, H., Hisatsune, J., Sugai, M., Shibayama, K., Stelling, J., 2022. Surveillance of multi-drug resistance phenotypes in *Staphylococcus aureus* in Japan and correlation with whole-genome sequence findings. J. Hosp. Infect. 123, 34–42.
Hu, Y., Jiang, L., Sun, X., Wu, J., Ma, L., Zhou, Y., Lin, K., Luo, Y., Cui, C., 2021. Risk assessment of antibiotic resistance genes in the drinking water system. Sci. Total Environ. 800, 149650.
Ibrahim, D., Jabbour, J.F., Kanj, S.S., 2020. Current choices of antibiotic treatment for *Pseudomonas aeruginosa* infections. Curr. Opin. Infect. Dis. 33, 464–473.
Imai, Y., Iida, R., Nitta, M., Takasu, A., 2016. *Pseudomonas aeruginosa* community acquired pneumonia with septicemia in a previously healthy woman. Case Rep. Clin. Med. 5, 335–341.
Interagency Coordination Group on Antimicrobial Resistance, 2019. No Time to Wait: Securing the Future from Drug Resistant Infections. IACG Report to the Secretary General of the United Nations, 07.09.21. https://www.who.int/antimicrobial-resistance/interagency-coordination-group/IACG_final_report_EN.pdf.
Iversen, C., Forsythe, S., 2003. Risk profile of *Enterobacter sakazakii*, an emergent pathogen associated with infant milk formula. Trends Food Sci. Technol. 14, 443–454.
Jayol, A., Nordmann, P., Brink, A., Poirel, L., 2015. Heteroresistance to colistin in *Klebsiella pneumoniae* associated with alterations in the PhoPQ regulatory system. Antimicrob. Agents Chemother. 59 (5), 2780–2784.
Jelić, M., Hrenović, J., Dekić, S., Goić-Barišić, I., Andrašević, A.T., 2019. First evidence of KPC-producing ST258 *Klebsiella pneumoniae* in river water. J. Hosp. Infect. 103, 147–150.
Jiménez-Castillo, R.A., Aguilar-Rivera, L.R., Carrizales-Sepúlveda, E.F., Gómez-Quiroz, R.A., Llantada-López, A.R., González-Aguirre, J.E., Náñez-Terreros, H., Rendón-Ramírez, E.J., 2021. A case of round pneumonia due to *Enterobacter hormaechei*: the need for a standardized diagnosis and treatment approach in adults. Rev. Inst. Med. Trop. Sao Paulo 20, e3.
Jo, J., Ko, K.S., 2021. Tigecycline heteroresistance and resistance mechanism in clinical isolates of *Acinetobacter baumannii*. Microbiol. Spectr. 9, e01010–e01021.
John, T.J., Lalla, U., Taljaard, J.J., John, K.G., Slabbert, J., Koegelenberg, C.F.N., 2017. An outbreak of community-acquired *pseudomonas aeruginosa* pneumonia in a setting of high water stress. QJM: Int. J. Med. 110, 855–856.
Julian, T.R., Pickering, A.J., 2015. A pilot study on integrating videography and environmental microbial sampling to model fecal bacterial exposures in peri-urban Tanzania. PLoS One 10, 0136158.
Kamboj, M., Cohen, N., Gilhuley, K., Babady, N.E., Seo, S.K., Sepkowitz, K.A., 2011. Emergence of daptomycin-resistant VRE: experience of a single institution. Infect. Control Hosp. Epidemiol. 32 (4), 391–394.
Kaplan, S.L., Hulten, K.G., Gonzalez, B.E., Hammerman, W.A., Lamberth, L., Versalovic, J., Mason Jr., E.O., 2005. Three-year surveillance of CA *Staphylococcus aureus* infections in children. Clin. Infect. Dis. 40, 1785–1791.
Karakonstantis, S., Kritsotakis, E.I., Gikas, A., 2020. Pandrug-resistant Gram-negative bacteria: a systematic review of current epidemiology, prognosis and treatment options. J. Antimicrob. Chemother. 75, 271–282.
Kay, D., Jones, F., Wyer, M.D., Fleisher, J.M., Salmon, R.L., Godfree, A.F., Zelenauch-Jacquotte, A., Shore, R., 1994. Predicting likelihood of gastroenteritis from sea bathing: results from randomised exposure. Lancet 344, 905–909.
Khan, A., Wilson, B., Gould, I.M., 2018. Current and future treatment options for community-associated MRSA infection. Expet Opin. Pharmacother. 19 (5), 457–470.
Klare, I., Heier, H., Claus, H., Witte, W., 1993. Environmental strains of *Enterococcus faecium* with inducible high-level resistance to glycopeptides. FEMS Microbiol. Lett. 106 (1), 23–29.
Klare, I., Heier, H., Claus, H., Reissbrodt, R., Witte, W.V., 1995a. *vanA*-mediated high-level glycopeptide resistance in *Enterococcus faecium* from animal husbandry. FEMS Microbiol. Lett. 125 (2), 165–171.
Klare, I., Heier, H., Claus, H., Böhme, G., Marin, S., Seltmann, G., Hakenbeck, R., Antanassova, V., Witte, W., 1995b. *Enterococcus faecium* strains with *vanA*-mediated high-level glycopeptide resistance isolated from animal foodstuffs and fecal samples of humans in the community. Microb. Drug Resist. 1 (3), 265–272.
Klockgether, J., Tümmler, B., 2017. Recent advances in understanding *Pseudomonas aeruginosa* as a pathogen. F1000Research 6, 1216.
Kyriakidis, I., Vasileiou, E., Pana, Z.D., Tragiannidis, A., 2021. *Acinetobacter baumannii* antibiotic resistance mechanisms. Pathogens 10, 373.
Lakhundi, S., Zhang, K., 2018. Methicillin-resistant *Staphylococcus aureus*: molecular characterization, evolution, and epidemiology. Clin. Microbiol. Rev. 31, e00020–e00118.
Landers, T.F., Cohen, B., Wittum, T.E., Larson, E.L., 2012. A review of antibiotic use in food animals: perspective, policy, and potential. Publ. Health Rep. 127 (1), 4–22.
Lawin-Brüssel, C.A., Refojo, M.F., Leong, F.L., Hanninen, L., Kenyon, K.R., 1993. Effect of *Pseudomonas aeruginosa* concentration in experimental contact lens-related microbial keratitis. Cornea 12, 10–18.
Lebreton, F., Depardieu, F., Bourdon, N., Fines-Guyon, M., Berger, P., Camiade, S., Leclercq, R., Courvalin, P., Cattoir, V., 2011. D-Ala-d-Ser VanN-type transferable vancomycin resistance in *Enterococcus faecium*. Antimicrob. Agents Chemother. 55 (10), 4606–4612.
Lee, J.H., 2003. Methicillin (Oxacillin)-resistant *Staphylococcus aureus* strains isolated from major food animals and their potential transmission to humans. Appl. Environ. Microbiol. 69, 6489–6494.
Lee, T., Pang, S., Abraham, S., Coombs, G.W., 2019. Antimicrobial-resistant CC17 *Enterococcus faecium*: the past, the present and the future. J. Glob. Antimicrob. Resist. 16, 36–47.
Levy-Blitchtein, S., Roca, I., Plasencia-Rebata, S., Vicente-Taboada, W., Velásquez-Pomar, J., Muñoz, L., Moreno-Morales, J., Pons, M.J., Del Valle-Mendoza, J., Vila, J., 2018. Emergence and spread of carbapenem-resistant *Acinetobacter baumannii* international clones II and III in Lima, Peru. Emerg. Microb. Infect. 7, 1–9.
Lister, P.D., Wolter, D.J., Hanson, N.D., 2009. Antibacterial-resistant *Pseudomonas aeruginosa*: clinical impact and complex regulation of chromosomally encoded resistance mechanisms. Clin. Microbiol. Rev. 22, 582–610.
Liu, C.P., Wang, N.Y., Lee, C.M., Weng, L.C., Tseng, H.K., Liu, C.W., Chiang, C.S., Huang, F.Y., 2004. Nosocomial and CA *Enterobacter cloacae* bloodstream infection: risk factors for and prevalence of SHV-12 in multiresistant isolates in a medical centre. J. Hosp. Infect. 58, 63–77.
Lellek, H., Franke, G.C., Ruckert, C., Wolters, M., Wolschke, C., Christner, M., Büttner, H., Alawi, M., Kröger, N., Rohde, H., 2015. Emergence of daptomycin non-susceptibility in colonizing vancomycin-resistant *Enterococcus faecium* isolates during daptomycin therapy. International J. Med. Microbiol. 305 (8), 902–909.
Loewen, K., Schreiber, Y., Kirlew, M., Bocking, N., Kelly, L., 2017. Community-associated methicillin-resistant *Staphylococcus aureus* infection: literature review and clinical update. Can. Fam. Physician 63, 512–520.
López-Rojas, R., Domínguez-Herrera, J., McConnell, M.J., Docobo-Peréz, F., Smani, Y., Fernández-Reyes, M., Rivas, L., Pachón, J., 2011. Impaired virulence and in vivo fitness of colistin-resistant *Acinetobacter baumannii*. J. Infect. Dis. 203, 545–548.
Lu, M., Fu, M., Zhang, Y., Shen, T., Xie, H., Liu, D., 2022. Septicaemia with deep venous thrombosis and necrotising pneumonia caused by acute community-acquired methicillin-resistant Staphylococcus aureus in a*n infant with a three*-year follow-up: a case report. BMC Infect. Dis. 22, 1–6.
Luczkiewicz, A., Kotlarska, E., Artichowicz, W., Tarasewicz, K., Fudala-Ksiazek, S., 2015. Antimicrobial resistance of *Pseudomonas* spp. isolated from wastewater and wastewater-impacted marine coastal zone. Environ. Sci. Pollut. Res. 22, 19823–19834.
Mannu, L., Paba, A., Daga, E., Comunian, R., Zanetti, S., Duprè, I., Sechi, L.A., 2003. Comparison of the incidence of virulence determinants and antibiotic resistance between *Enterococcus faecium* strains of dairy, animal and clinical origin. Int. J. Food Microbiol. 88, 291–304.
Martins, V.V., Pitondo-Silva, A., de Melo Manço, L., Falcão, J.P., Freitas, S.D., da Silveira, W.D., Stehling, E.G., 2014. Pathogenic potential and genetic diversity of environmental and clinical isolates of *Pseudomonas aeruginosa*. Apmis 122, 92–100.
Martin, R.M., Bachman, M.A., 2018. Colonization, infection, and the accessory genome of *Klebsiella pneumoniae*. Front. Cell. Infect. Microbiol. 8.
Mattioli, M.C.M., Pickering, A.J., Gilsdorf, R., Davis, J., Boehm, A.B., 2012. Hands and water as vectors of diarrheal pathogens in Bagamoyo, Tanzania. Environ. Sci. Technol. 47, 355–363.
McGuinness, W.A., Malachowa, N., DeLeo, F.R., 2017. Focus: infectious diseases: vancomycin resistance in *Staphylococcus aureus*. Yale J. Biol. Med. 90, 269–281.
McNeil, J.C., 2014. *Staphylococcus aureus* - antimicrobial resistance and the immunocompromised child. Infect. Drug Resist. 7, 117–127.
Meletis, G., 2016. Carbapenem resistance: overview of the problem and future perspectives. Ther. Adv. Infect. Dis. 3, 15–21.
Mena, K.D., Gerba, C.P., 2009. Risk assessment of *Pseudomonas aeruginosa* in water. Rev. Environ. Contam. Toxicol. 201, 71–115.
Mendes, R.E., Castanheira, M., Farrell, D.J., Flamm, R.K., Sader, H.S., Jones, R.N., 2016. Longitudinal (2001–14) analysis of enterococci and VRE causing invasive infections in European and US hospitals, including a contemporary (2010–13) analysis of oritavancin in vitro potency. J. Antimicrob. Chemother. 71 (12), 3453–3458.
Mezzatesta, M.L., Gona, F., Stefani, S., 2012. *Enterobacter cloacae* complex: clinical impact and emerging antibiotic resistance. Future Microbiol. 7, 887–902.
Monteiro, S., Santos, R., 2020. Incidence of enterococci resistant to clinically relevant antibiotics in environmental waters and in reclaimed waters used for irrigation. J. Water Health 18, 911–924.
Mubita, C., Syakalima, M., Chisenga, C., Munyeme, M., Bwalya, M., Chifumpa, G., Mudenda Hang'ombe, B., Sinkala, P., Simuunza, M., Fukushi, H., Isogai, H., 2008. Antibiograms of faecal *Escherichia coli* and Enterococci species isolated from pastoralist cattle in the interface areas of the Kafue basin in Zambia-short communication. Vet. Arh. 78, 179–185.
Mulani, M.S., Kamble, E.E., Kumkar, S.N., Tawre, M.S., Pardesi, K.R., 2019. Emerging strategies to combat ESKAPE pathogens in the era of antimicrobial resistance: a review. Front. Microbiol. 10.
Murray, B.E., 2000. Vancomycin-resistant enterococcal infections. N. Engl. J. Med. 342, 710–721.
Nair, A.V., Joseph, N., Krishna, K., Sneha, K.G., Tom, N., Jangid, K., Nair, S., 2015. A comparative study of coastal and clinical isolates of *Pseudomonas aeruginosa*. Braz. J. Microbiol. 46, 725–734.

Navidinia, M., Goudarzi, M., Rameshe, S.M., Farajollahi, Z., Asl, P.E., Mounesi, M.R., 2017. Molecular characterization of resistance genes in MDR-ESKAPE pathogens. J. Pure Appl. Microbiol. 11, 779–792.
Ni, N., Nam, E.M., Hammersmith, K.M., Nagra, P.K., Azari, A.A., Leiby, B.E., Dai, Y., Cabrera, F.A., Ma, J.F., Lambert Jr., C.E., Honig, S.E., 2015. Seasonal, geographic, and antimicrobial resistance patterns in microbial keratitis: 4-year experience in eastern Pennsylvania. Cornea 34 (3), 296–302.
Nurjadi, D., Chanthalangsy, Q., Zizmann, E., Stuermer, V., Moll, M., Klein, S., Boutin, S., Heeg, K., Zanger, P., 2021. Phenotypic detection of hemin-inducible trimethoprim-sulfamethoxazole heteroresistance in *Staphylococcus aureus*. Microbiol. Spectr. 9 (2), e01510–e01521.
Nyenje, M.E., Odjadjare, C.E., Tanih, N.F., Green, E., Ndip, R.N., 2012. Foodborne pathogens recovered from ready-to-eat foods from roadside cafeterias and retail outlets in Alice, Eastern Cape Province, South Africa: public health implications. Int. J. Environ. Res. Publ. Health 9, 2608–2619.
Owens, C.E., Angles, M.L., Cox, P.T., Byleveld, P.M., Osborne, N.J., Rahman, M.B., 2020. Implementation of quantitative microbial risk assessment (QMRA) for public drinking water supplies: systematic review. Water Res. 174, 1–18.
Ottoson, J., Stenström, T.A., 2003. Faecal contamination of greywater and associated microbial risks. Water Res. 37, 645–655.
Pachori, P., Gothalwal, R., Gandhi, P., 2019. Emergence of antibiotic resistance *Pseudomonas aeruginosa* in intensive care unit; A critical review. Genes and Diseases 6, 109–119.
Paczosa, M.K., Mecsas, J., 2016. *Klebsiella pneumoniae*: going on the offense with a strong defense. Microbiol. Mol. Biol. Rev. 80, 629–661.
Pal, C., Bengtsson-Palme, J., Kristiansson, E., Larsson, D.J., 2016. The structure and diversity of human, animal and environmental resistomes. Microbiome 4 (1), 1–15.
Paoli, M.G., Hartnett, E., 2006. Overview of a risk assessment model for *Enterobacter sakazakii* in powdered infant formula. Available:. The Food and Agriculture Organization of the United Nations and the World Health Organisation, Rome, Italy, 18.04.22. https://www.who.int/foodsafety/publications/micro/RA_Overview.pdf.
Pecson, B.M., Triolo, S.C., Olivieri, S., Chen, E.C., Pisarenko, A.N., Yang, C.C., Olivieri, A., Haas, C.N., Trussell, R.S., Trussell, R.R., 2017. Reliability of pathogen control in direct potable reuse: performance evaluation and QMRA of a full-scale 1 MGD advanced treatment train. Water Res. 122, 258–268.
Perez, F., Villegas, M.V., 2015. The role of surveillance systems in confronting the global crisis of antibiotic-resistant bacteria. Curr. Opin. Infect. Dis. 28 (4), 375.
Phillips, I., Casewell, M., Cox, T., De Groot, B., Friis, C., Jones, R., Nightingale, C., Preston, R., Waddell, J., 2004. Does the use of antibiotics in food animals pose a risk to human health? A critical review of published data. J. Antimicrob. Chemother. 53 (1), 28–52.
Pirnay, J.P., Matthijs, S., Colak, H., Chablain, P., Bilocq, F., Van Eldere, J., De Vos, D., Zizi, M., Triest, L., Cornelis, P., 2005. Global *Pseudomonas aeruginosa* biodiversity as reflected in a Belgian river. Environ. Microbiol. 7, 969–980.
Podschun, R., Pietsch, S., Höller, C., Ullmann, U., 2001. Incidence of *Klebsiella* species in surface waters and their expression of virulence factors. Appl. Environ. Microbiol. 67, 3325–3327.
Prater, A.G., Mehta, H.H., Kosgei, A.J., Miller, W.R., Tran, T.T., Arias, C.A., Shamoo, Y., 2019. Environment shapes the accessible daptomycin resistance mechanisms in *Enterococcus faecium*. Antimicrob. Agents Chemother. 63 (10), e00790–e00819.
Prüss-Ustün, A., Wolf, J., Bartram, J., Clasen, T., Cumming, O., Freeman, M.C., Gordon, B., Hunter, P.R., Medlicott, K., Johnston, R., 2019. Burden of disease from inadequate water, sanitation and hygiene for selected adverse health outcomes: an updated analysis with a focus on low- and middle-income countries. Int. J. Hyg Environ. Health 222, 765–777.
Purrello, S.M., Garau, J., Giamarellos, E., Mazzei, T., Pea, F., Soriano, A., Stefani, S., 2016. Methicillin-resistant *Staphylococcus aureus* infections: a review of the currently available treatment options. J. Glob. Antimicrob. Resist. 7, 178–186.
Raja, N.S., Karunakaran, R., Ngeow, Y.F., Awang, R., 2005. Community-acquired vancomycin-resistant *Enterococcus faecium*: a case report from Malaysia. J. Med. Microbiol. 54, 901–903.
Ramessar, K., Olaniran, A.O., 2019. Antibiogram and molecular characterization of methicillin-resistant *Staphylococcus aureus* recovered from treated wastewater effluent and receiving surface water in Durban, South Africa. World J. Microbiol. Biotechnol. 35, 1–11.
Rasheduzzaman, M., Singh, R., Haas, C.N., Tolofari, D., Yassaghi, H., Hamilton, K.A., Yang, Z., Gurian, P.L., 2019. Reverse QMRA as a decision support tool: setting acceptable concentration limits for *Pseudomonas aeruginosa* and *Naegleria fowleri*. Water 11, 1–15.
Reyneke, B., Hamilton, K.A., Fernandez-Ibanez, P., Polo-López, M.I., Mcguigan, K.G., Khan, S., Khan, W., 2020. EMA-amplicon-based sequencing informs risk assessment analysis of water treatment systems. Sci. Total Environ. 743, 1–11.
Rice, L.B., 2008. Federal funding for the study of antimicrobial resistance in nosocomial pathogens: no ESKAPE. J. Infect. Dis. 197 (8), 1079–1081.
Rodrigues, C., d'Humières, C., Papin, G., Passet, V., Ruppé, E., Brisse, S., 2020. Community-acquired infection caused by the uncommon hypervirulent *Klebsiella pneumoniae* ST66-K2 lineage. Microb. Genom. 6, 1–5.
Rodriguez-Alvarez, M.S., Weir, M.H., Pope, J.M., Seghezzo, L., Rajal, V.B., Salusso, M.M., Morana, L.B., 2015. Development of a relative risk model for drinking water regulation and design recommendations for a peri urban region of Argentina. Int. J. Hyg Environ. Health 218, 627–638.
Rodvold, K.A., McConeghy, K.W., 2014. Methicillin-resistant *Staphylococcus aureus* therapy: past, present, and future. Clin. Infect. Dis. 58, S20–S27.
Rose, J.B., Haas, C.N., 1999. A risk assessment framework for the evaluation of skin infections and the potential impact of antibacterial soap washing. Am. J. Infect. Control 27, S26–S33.
Rossolini, G.M., Mantengoli, E., 2005. Treatment and control of severe infections caused by multiresistant *Pseudomonas aeruginosa*. Clin. Microbiol. Infect. 11, 17–32.
Russo, T.A., Marr, C.M., 2019. Hypervirulent *Klebsiella pneumoniae*. Clin. Microbiol. Rev. 32, e00001–e00019.
Sakeena, M.H.F., Bennett, A.A., McLachlan, A.J., 2018. Enhancing pharmacists' role in developing countries to overcome the challenge of antimicrobial resistance: a narrative review. Antimicrob. Resist. Infect. Control 7 (1), 1–11.
Schoen, M.E., Peckham, T.K., Shirai, J.H., Kissel, J.C., Thapaliya, D., Smith, T.C., Meschke, J.S., 2020. Risk of nasal colonization of methicillin-resistant *Staphylococcus aureus* during preparation of contaminated retail pork meat. Microb. Risk Anal. 16, 100136.
Schoen, M.E., Jahne, M.A., Garland, J., Ramirez, L., Lopatkin, A.J., Hamilton, K.A., 2021. Quantitative microbial risk assessment of antimicrobial resistant and susceptible *Staphylococcus aureus* in reclaimed wastewaters. Environ. Sci. Technol. 55, 15246–15255.
Schouten, M.A., Voss, A., Hoogkamp-Korstanje, J.A.A., 1997. VRE and meat. Lancet 349 (9060), 1258.
Sharma, A., Dour, P., Singh, T.N., 2008. The prevalence of extended-spectrum β-lactamase in environmental isolates of *Enterobacter*. Indian J. Pathol. Microbiol. 51, 130–136.
Sharvelle, S., Dozier, A., Arabi, M., Reichel, B., 2017. A geospatially-enabled web tool for urban water demand forecasting and assessment of alternative urban water management strategies. Environ. Model. Software 97, 213–228.
Sikora, A., Zahra, F., 2021. Nosocomial infections. In: StatPearls [Internet]. StatPearls Publishing.
Siu, L.K., Yeh, K.M., Lin, J.C., Fung, C.P., Chang, F.Y., 2012. *Klebsiella pneumoniae* liver abscess: a new invasive syndrome. Lancet Infect. Dis. 12, 881–887.
Skov, R., Christiansen, K., Dancer, S.J., Daum, R.S., Dryden, M., Huang, Y.C., Lowy, F.D., 2012. Update on the prevention and control of community-acquired meticillin-resistant *Staphylococcus aureus* (CA-MRSA). Int. J. Antimicrob. Agents 39 (3), 193–200.
Slekovec, C., Plantin, J., Cholley, P., Thouverez, M., Talon, D., Bertrand, X., Hocquet, D., 2012. Tracking down antibiotic-resistant *Pseudomonas aeruginosa* isolates in a wastewater network. PLoS One 7, e49300.
Soge, O.O., Meschke, J.S., No, D.B., Roberts, M.C., 2009. Characterization of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* spp. isolated from US West Coast public marine beaches. J. Antimicrob. Chemother. 64, 1148–1155.
Soller, J., Bartrand, T., Ravenscroft, J., Molina, M., Whelan, G., Schoen, M., Ashbolt, N., 2015. Estimated human health risks from recreational exposures to stormwater runoff containing animal faecal material. Environ. Model. Software 72, 21–32.
Son, S.K., Lee, N.R., Ko, J.H., Choi, J.K., Moon, S.Y., Joo, E.J., Peck, K.R., Park, D.A., 2018. Clinical effectiveness of carbapenems versus alternative antibiotics for treating ESBL-producing Enterobacteriaceae bacteraemia: a systematic review and meta-analysis. J. Antimicrob. Chemother. 73, 2631–2642.
Søraas, A., Sundsfjord, A., Sandven, I., Brunborg, C., Jenum, P.A., 2013. Risk factors for community-acquired urinary tract infections caused by ESBL-producing enterobacteriaceae–a case–control study in a low prevalence country. PLoS One 8, e69581.
Stone, D.L., Harding, A.K., Hope, B.K., Slaughter-Mason, S., 2008. Exposure assessment and risk of gastrointestinal illness among surfers. J. Toxicol. Environ. Health, Part A 71, 1603–1615.
Su, S.C., Siu, L.K., Ma, L., Yeh, K.M., Fung, C.P., Lin, J.C., Chang, F.Y., 2008. Community-acquired liver abscess caused by serotype K1 *Klebsiella pneumoniae* with CTX-M-15-type extended-spectrum β-lactamase. Antimicrob. Agents Chemother. 52, 804–805.
Sukul, P., Spiteller, M., 2007. Fluoroquinolone antibiotics in the environment. Rev. Environ. Contam. Toxicol. 191, 131–162.
Tacconelli, E., Carrara, E., Savoldi, A., Harbarth, S., Mendelson, M., Monnet, D.L., Pulcini, C., Kahlmeter, G., Kluytmans, J., Carmeli, Y., Ouellette, M., 2018. Discovery, research, and development of new antibiotics: the WHO priority list of antibiotic-resistant bacteria and tuberculosis. Lancet Infect. Dis. 18, 318–327.
Tang, K.L., Caffrey, N.P., Nóbrega, D.B., Cork, S.C., Ronksley, P.E., Barkema, H.W., Polachek, A.J., Ganshorn, H., Sharma, N., Kellner, J.D., Ghali, W.A., 2017. Restricting the use of antibiotics in food-producing animals and its associations with antibiotic resistance in food-producing animals and human beings: a systematic review and meta-analysis. Lancet Planet. Health 1 (8), e316–e327.
Tanwar, J., Das, S., Fatima, Z., Hameed, S., 2014. Multidrug resistance: an emerging crisis. Interdiscip. Perspect. Infect. Dis. 541340, 1–7.
Uechi, K., Tada, T., Shimada, K., Nakasone, I., Kirikae, T., Fujita, J., 2019. Emergence of a carbapenem-resistant and colistin-heteroresistant *Enterobacter cloacae* clinical isolate in Japan. J. Infect. Chemother. 25 (4), 285–288.
Van, T.T.H., Yidana, Z., Smooker, P.M., Coloe, P.J., 2020. Antibiotic use in food animals worldwide, with a focus on Africa: pluses and minuses. J. Glob. Antimicrob. Resist. 20, 170–177.
Van Boeckel, T.P., Glennon, E.E., Chen, D., Gilbert, M., Robinson, T.P., Grenfell, B.T., Levin, S.A., Bonhoeffer, S., Laxminarayan, R., 2017. Reducing antimicrobial use in food animals. Science 357 (6358), 1350–1352.
Van Kinh, N., Wertheim, H.F., Thwaites, G.E., Khue, L.N., Thai, C.H., Khoa, N.T., Trung, N.V., Crook, D., van Doorn, H.R., 2017. Developing an antimicrobial resistance reference laboratory and surveillance programme in Vietnam. Lancet Global Health 5 (12), e1186–e1187.
Verraes, C., Van Boxstael, S., Van Meervenne, E., Van Coillie, E., Butaye, P., Catry, B., de Schaetzen, M., Van Huffel, X., Imberechts, H., Dierick, K., Daube, G., Saegerman, C., De Block, J., Dewulf, J., Herman, L., 2013. Antimicrobial resistance in the food chain: a review. Int. J. Environ. Res. Publ. Health 10, 2643–2669.

Vola, M.E., Moriyama, A.S., Lisboa, R., Vola, M.M., Hirai, F.E., Bispo, P.J.M., Höfling-Lima, A.L., 2013. Prevalence and antibiotic susceptibility of methicillin-resistant *Staphylococcus aureus* in ocular infections. Arq. Bras. 76, 350–353.

Walsh, C., Wencewicz, T., 2020. Antibiotics: Challenges, Mechanisms, Opportunities. John Wiley & Sons, Inc., Hoboken, NJ, United States America.

Wand, M.E., Baker, K.S., Benthall, G., McGregor, H., McCowen, J.W., Deheer-Graham, A., Sutton, J.M., 2015. Characterization of pre-antibiotic era *Klebsiella pneumoniae* isolates with respect to antibiotic/disinfectant susceptibility and virulence in *Galleria mellonella*. Antimicrob. Agents Chemother. 59, 3966–3972.

Wang, N., Yang, Q., Tan, Y., Lin, L., Huang, Q., Wu, K., 2015. Bacterial spectrum and antibiotic resistance patterns of ocular infection: differences between external and intraocular diseases. J. Ophthalmol. 2015, 1–7.

Wang, J., He, J.T., Bai, Y., Wang, R., Cai, Y., 2018. Synergistic activity of colistin/fosfomycin combination against carbapenemase-producing *Klebsiella pneumoniae* in an in vitro pharmacokinetic/pharmacodynamic model. BioMed Res. Int. 2018, 1–10.

Wang, T., Hou, Y., Wang, R., 2019. A case report of community-acquired *Pseudomonas aeruginosa* pneumonia complicated with MODS in a previously healthy patient and related literature review. BMC Infect. Dis. 19, 1–6.

Wang, G., Zhao, G., Chao, X., Xie, L., Wang, H., 2020. The characteristic of virulence, biofilm and antibiotic resistance of *Klebsiella pneumoniae*. Int. J. Environ. Res. Publ. Health 17, 6278.

Woolhouse, M., Ward, M., Van Bunnik, B., Farrar, J., 2015. Antimicrobial resistance in humans, livestock and the wider environment. Philos. Trans. R. Soc. Lond. B Biol. Sci. 370 (1670), 20140083.

World Health Organisation (WHO), 2017. Global Priority List of Antibiotic-Resistant Bacteria to Guide Research, Discovery, and Development of New Antibiotics, 16.06.21. http://www.who.int/medicines/publications/WHO-PPL-Short_Summary_25 Feb-ET_NM_WHO.pdf.

Wu, Q., Sabokroo, N., Wang, Y., Hashemian, M., Karamollahi, S., Kouhsari, E., 2021. Systematic review and meta-analysis of the epidemiology of vancomycin-resistance *Staphylococcus aureus* isolates. Antimicrob. Resist. Infect. Control 10 (1), 1–13.

Wyllie, D.H., Walker, A.S., Miller, R., Moore, C., Williamson, S.R., Schlackow, I., Finney, J.M., O'Connor, L., Peto, T.E., Crook, D.W., 2011. Decline of meticillin-resistant Staphylococcus aureus in O*xfordshire hospitals* is strain-specific and preceded infection-control intensification. BMJ Open 1 (1), e000160.

Xu, X., Lin, D., Yan, G., Ye, X., Wu, S., Guo, Y., Zhu, D., Hu, F., Zhang, Y., Wang, F., Jacoby, G.A., 2010. *vanM*, a new glycopeptide resistance gene cluster found in *Enterococcus faecium*. Antimicrob. Agents Chemother. 54 (11), 4643–4647.

Xu, A., Zhu, H., Gao, B., Weng, H., Ding, Z., Li, M., Weng, X., He, G., 2020. Diagnosis of severe community-acquired pneumonia caused by *Acinetobacter baumannii* through next-generation sequencing: a case report. BMC Infect. Dis. 20, 1–7.

Yuan, S., Wu, G., Zheng, B., 2019. Complete genome sequence of an IMP-8, CTX-M-14, CTX-M-3 and QnrS1 co-producing *Enterobacter asburiae* isolate from a patient with wound infection. J. Glob. Antimicrob. Resist. 18, 52–54.

Zaheer, R., Cook, S.R., Barbieri, R., Goji, N., Cameron, A., Petkau, A., Polo, R.O., Tymensen, L., Stamm, C., Song, J., Hannon, S., 2020. Surveillance of *Enterococcus* spp. reveals distinct species and antimicrobial resistance diversity across a One-Health continuum. Sci. Rep. 10 (1), 1–16.

Zaman, S.B., Hussain, M.A., Nye, R., Mehta, V., Mamun, K.T., Hossain, N., 2017. A review on antibiotic resistance: alarm bells are ringing. Cureus 9, e1403.

Zetola, N., Francis, J.S., Nuermberger, E.L., Bishai, W., 2005. Community-acquired meticillin-resistant *Staphylococcus aureus*: an emerging threat. Lancet Infect. Dis. 5, 275–286.

Zhang, J., Wall, S.K., Xu, L., Ebner, P.D., 2010. Contamination rates and antimicrobial resistance in bacteria isolated from "grass-fed" labelled beef products. Foodb. Pathog. Dis. 7 (11), 1331–1336.

Zhang, Q., Gallard, J., Wu, B., Harwood, V.J., Sadowsky, M.J., Hamilton, K.A., Ahmed, W., 2019. Synergy between quantitative microbial source tracking (qMST) and quantitative microbial risk assessment (QMRA): a review and prospectus. Environ. Int. 130, 104703.

Zhou, Y., Yang, Y., Ding, L., Chen, C., Xu, X., Wang, M., 2020a. Vancomycin heteroresistance in *vanM*-type *Enterococcus faecium*. Microb. Drug Resist. 26 (7), 776–782.

Zhou, X., Willems, R.J., Friedrich, A.W., Rossen, J.W., Bathoorn, E., 2020b. *Enterococcus faecium*: from microbiological insights to practical recommendations for infection control and diagnostics. Antimicrob. Resist. Infect. Control 9, 1–13.