






A scoping review of human pathogens detected in untreated human wastewater and sludge

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ABSTRACT

Wastewater monitoring is an approach to identify the presence or abundance of pathogens within a population. The objective of this scoping review (ScR) was to identify and characterize research on human pathogens and antimicrobial resistance detected in untreated human wastewater and sludge. A search was conducted up to March 2023 and standard ScR methodology was followed. This ScR included 1,722 articles, of which 56.5% were published after the emergence of COVID-19. Viruses and bacteria were commonly investigated, while research on protozoa, helminths, and fungi was infrequent. Articles prior to 2019 were dominated by research on pathogens transmitted through fecal–oral or waterborne pathways, whereas more recent articles have explored the detection of pathogens transmitted through other pathways such as respiratory and vector-borne. There was variation in sampling, samples, and sample processing across studies. The current evidence suggests that wastewater monitoring could be applied to a range of pathogens as a public health tool to detect an emerging pathogen and understand the burden and spread of disease to inform decision-making. Further development and refinement of the methods to identify and interpret wastewater signals for different prioritized pathogens are needed to develop standards on when, why, and how to monitor effectively.

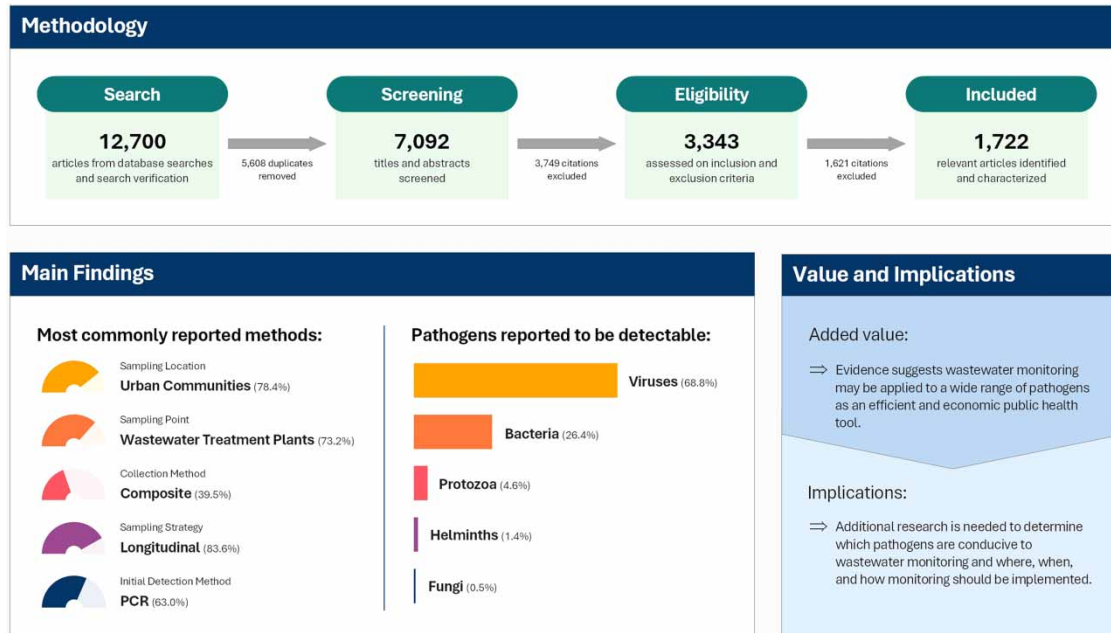
Key words: infectious disease, knowledge synthesis, pathogen, scoping review, wastewater

HIGHLIGHTS

- A wide range of pathogens can be detected in wastewater.
- 56.5% of studies were published since 2020.
- Viruses were the most commonly investigated pathogen type followed by bacteria.
- Earlier studies focused on pathogens transmitted through fecal–oral or waterborne pathways.
- Additional research is needed to determine which pathogens are conducive to wastewater monitoring and where, when, and how it should be implemented.

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GRAPHICAL ABSTRACT



INTRODUCTION

The recent coronavirus disease 2019 (COVID-19) pandemic demonstrated that the risk of the emergence and rapid spread of new human health threats can only be combatted by innovative public health tools including strengthened surveillance networks and the integration of rapidly evolving tools, such as wastewater monitoring, into traditional surveillance systems (Mao *et al.* 2020; Baker *et al.* 2022).

Wastewater monitoring or surveillance is an approach to identify the presence or abundance of pathogens, biological or chemical indicators of disease, drug and substance use patterns, and other signals within a population over time and may inform public health decision-making (Mao *et al.* 2020; Sims & Kasprzyk-Hordern 2020). For the surveillance of human diseases, a wastewater monitoring approach can provide a less biased and cost-effective measure of disease prevalence as wastewater data are not dependent on human behavior and health system capacity (Safford *et al.* 2022; Kilaru *et al.* 2023). Wastewater monitoring has historically been used to identify pathogens transmitted through water and fecal-oral routes (Kilaru *et al.* 2023). However, wastewater monitoring has gained momentum globally with innovative research and developments in surveillance for respiratory pathogens during the COVID-19 pandemic (Thagale *et al.* 2022; Boehm *et al.* 2023). The potential to use wastewater to monitor a wide range of pathogens remains unclear (Kilaru *et al.* 2023). Therefore, the aim of this scoping review (ScR) was to identify and characterize the current evidence on human pathogens and antimicrobial resistance detected in untreated human wastewater and sludge.

METHODS

Review type, protocol, and team

An ScR uses systematic methodology to identify and map relevant evidence on an existing or emerging topic (Arksey & O'Malley 2005; Levac *et al.* 2010; Tricco *et al.* 2016; Peters *et al.* 2020a). While similar in the structure and rigor of systematic reviews, their purpose is to identify and map the landscape of the literature and determine possible gaps in the literature (Arksey & O'Malley 2005; Levac *et al.* 2010; Tricco *et al.* 2016; Peters *et al.* 2020a).

To ensure transparency, reproducibility, and consistency during all stages of the ScR, a protocol was created prior to conducting the ScR (Supplementary 1). The protocol includes a list of definitions, inclusion/exclusion criteria, search strategy, title and abstract screening forms, and the data characterization form. A few small deviations were made to the original protocol and are outlined in Supplementary 1. This ScR adheres to the Joanna Briggs Institute methodology for ScRs (Peters *et al.*

2020b) and was prepared in accordance with the PRISMA extension for ScRs (PRISMA-ScR) (Tricco *et al.* 2018). A multi-disciplinary team with expertise in evidence synthesis, epidemiology, infectious diseases, wastewater, and public health created the protocol and executed the ScR.

Review question and eligibility criteria

The objective of this ScR was to identify and summarize relevant evidence that addresses the research question: what human pathogen(s) or antimicrobial resistant organisms/antimicrobial resistance genes have been detected through wastewater testing or surveillance of untreated, human wastewater or sludge? The following inclusion criteria were applied:

- (1) Publication date: All
- (2) Language: English and French
- (3) Study design: All
- (4) Country: All
- (5) Document type: Primary research (represents a study where the authors collected and analyzed their own data)
- (6) Pathogens: Human (known to infect and cause diseases in humans). Indicator organisms (bacteria and viruses that are used as surrogates to evaluate the presence of pathogens in water and to monitor water quality) were excluded, except if the study was looking at the presence of antimicrobial resistance genes.
- (7) Type of wastewater: Raw/untreated human wastewater or sludge

Search strategy and verification

A comprehensive search strategy was developed by a Health Canada research librarian in collaboration with the authors and applied in five bibliographic databases: Scopus, Embase, Medline, Global Health, and Europe PMC (Supplementary 1). The initial search was conducted on February 15–17, 2022 and was updated on March 21, 2023.

The reference lists of relevant reviews on the topic were hand-searched to the point of saturation to ensure that the database search captured all relevant primary research. During the search verification process of 18 reviews, an additional 304 articles were identified and added to the search results (Hovi *et al.* 2012; O'Brien & Xagorarakis 2019; Bhatt *et al.* 2020; Corpuz *et al.* 2020; Hassoun-Kheir *et al.* 2020; Michael-Kordatou *et al.* 2020; Ali *et al.* 2021; Mousazadeh *et al.* 2021; O'Keeffe 2021; Pruden *et al.* 2021; Saawarn & Hait 2021; Zaatout *et al.* 2021; Bonanno Ferraro *et al.* 2022; Chau *et al.* 2022; Dzinamarira *et al.* 2022; Huang *et al.* 2022; Shah *et al.* 2022; Kilaru *et al.* 2023). Due to the volume of literature on the topic identified through the database search and a lack of resources, a grey literature search was not conducted.

Review management

Search results were imported into the web-based systematic review software DistillerSR (Version 2023.6.2; Evidence Partners, 2023) and duplicates were removed. If an included preprint was subsequently published after our search date, then the preprint version was manually replaced with the published version in DistillerSR, and extractions were completed on the published version. All stages of the ScR were conducted within DistillerSR, including relevance screening and data characterization.

Relevance screening and data characterization

A relevance screening form was developed to determine if the article was relevant to the review using the inclusion and exclusion criteria. The full text of potentially relevant articles was then procured and reviewed using a data characterization form, which aimed to confirm article relevance and extract data on pertinent information from each study such as study design, wastewater sample, sampling frequency and location, method of detection, and pathogens identified. For antimicrobial resistance, we captured whether the article studied antimicrobial resistant organisms in wastewater and how it was tested. Both forms (50 relevance screening and 10 data characterization) were developed *a priori* and piloted by all reviewers for clarity and consistency. Modifications were implemented based on reviewer feedback, where necessary, prior to implementation. Two reviewers independently used these forms to screen for relevance and extract data. During both stages, reviewers resolved conflicts by consensus or a third reviewer. Both forms including all definitions and data extracted are outlined in detail in the protocol (Supplementary 1). Excluded studies and the reasons for exclusion are documented in Supplementary 2.

Data analysis and reporting

The completed dataset was exported into Microsoft Excel[®] for Microsoft 365 MSO (Version 2307 Build 16.0.16626.20198) for data cleaning, categorization, descriptive analysis, and narrative summarization. The complete dataset is available in Supplementary 3.

RESULTS

ScR descriptive statistics

Of the 7,092 unique citations screened for relevance, full articles were procured for 3,343 potentially relevant studies and 1,722 (English = 1,713, French = 9) were considered primary research relevant to the research question (Figure 1). Only two articles were excluded from the ScR on the basis of language: one Chinese and one Spanish.

Articles were published between 1929 and 2023 with 56.5% (972/1,722) published since 2020. The majority of the articles were primary peer-reviewed research articles (1,525/1,722) and originated from Europe (569/1,722), North America (480/1,722), and Asia (413/1,722) (Table 1). The articles utilized observational (1,468/1,722) or experimental (580/1,722) study designs to investigate human pathogens in wastewater, with the majority being longitudinal/surveillance (includes monitoring) study designs (1,299/1,722). Experimental studies included the evaluation of detection methods (277/1,722), evaluation of sampling/sample preparation methods (254/1,722), or controlled or challenge trials (49/1,722), in which the majority analyzed the fate of pathogens through wastewater treatment plants. A small number of relevant primary research also included a

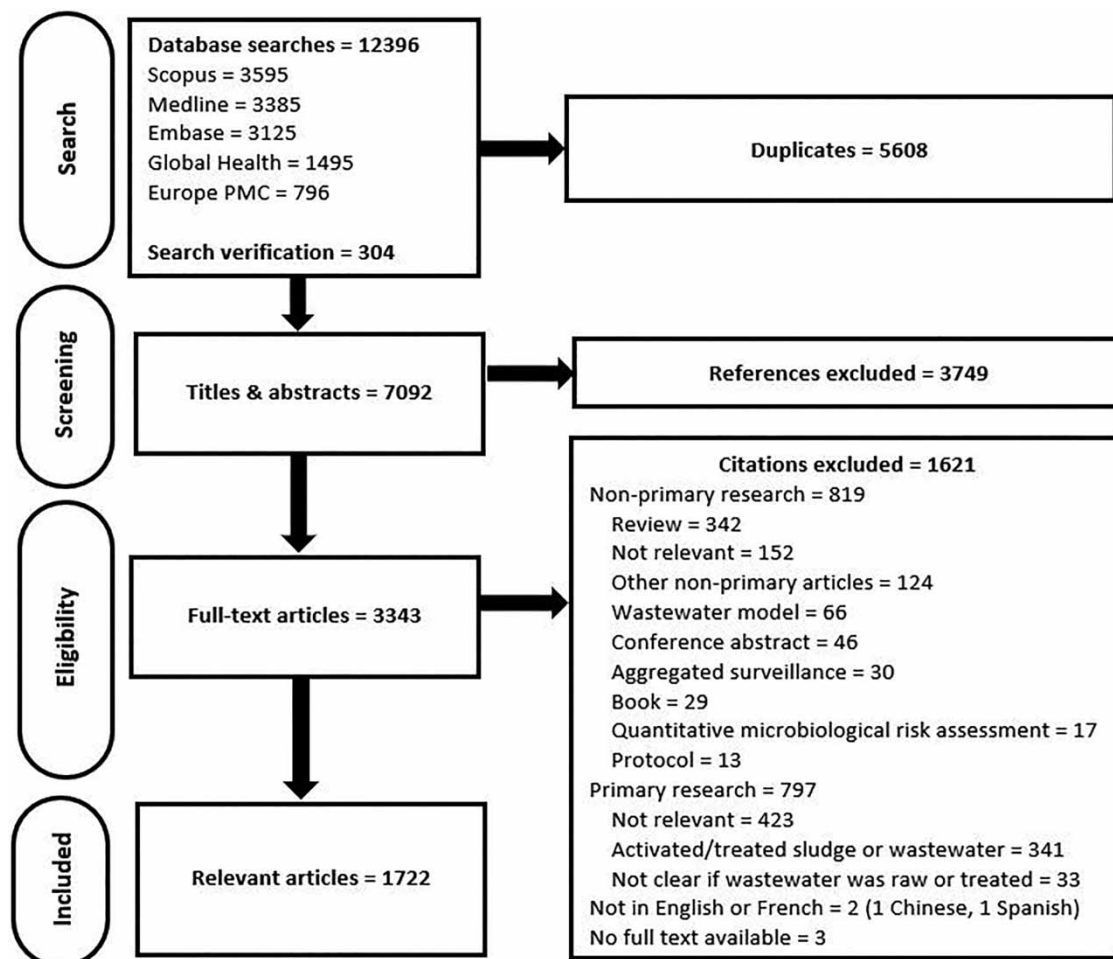


Figure 1 | PRISMA flow diagram of the article through the scoping review process.

Table 1 | General characteristics of 1,722 primary research publications on human pathogens found in untreated wastewater

Category	Count
Type of document	
Primary peer-reviewed research	1,525
Preprint	102
Short communications	79
Letter to the editor with data	10
Conference proceeding abstract with data	6
Continent ^a	
Europe	569
North America ^b	480
Asia	413
Africa	136
South America	113
Australia/Oceania	51
Date of publication	
< 1970	10
1970–1979	30
1980–1989	44
1990–1999	39
2000–2009	156
2010–2019	471
2020–2023	972
Study design ^a	
Observational	1,468
Longitudinal/surveillance (includes monitoring)	1,299
Cross-sectional	168
Case-control	1
Experimental	580
Controlled trial	45
Challenge trial	4
Evaluation of detection methods	277
Evaluation of sampling/sample preparation methods	254
Risk assessment/predictive models	103
Type of wastewater sampled ^a	
Untreated/raw wastewater	1,675
Untreated/raw/primary sludge	102

^aTotal number sums to >1,722 as some studies were conducted on more than one continent, had more than one type of detection or study design, or sampled more than one type of wastewater.

^bNorth America includes the United States, Canada, Mexico, Central America, and the Caribbean.

risk assessment or predictive model (103/1,722). Many papers encompassed multiple study designs, most often to validate the detection of a pathogen in wastewater under controlled conditions prior to conducting a field study. The pathogen or antimicrobial resistance organism concentration or signal strength was quantified in 61.6% (1,060/1,722) of the studies. Untreated/raw wastewater was sampled in 97.3% (1,675/1,722) of studies, and untreated/raw/primary sludge was sampled in 5.9% (102/1,722) of studies.

Wastewater sampling

The wastewater sampling details of the 1,722 included studies can be found in [Table 2](#) and are described briefly below. The sampling of wastewater occurred in multiple locations including urban communities (1,350/1,722), small rural communities (<10,000 people, 85/1,722), buildings or facilities (372/1,722), and airplane/ship/other transport vehicles (12/1,722). Of the building or facility sample locations, the majority were hospitals (225/372) and universities/colleges (88/372), followed by residential buildings (20/372), schools/daycares/nurseries (18/372), retirement/nursing homes (17/372), retail/shopping (14/372), workplace/offices/factories (13/371), airports/bus terminals (11/372), quarantine/isolation centers (10/372), worker accommodations (9/372), prisons (6/372), hotels/hostels (4/372), and homeless shelters (4/372). For the sampling point, 73.2% (1,261/1,722) of studies obtain their samples from a wastewater treatment plant. Other sampling points included facilities/institutes (372/1,722), sewers (215/1,722), pumping stations (67/1,722), and open drains/canals (66/1,722). Composite (681/1,722), grab (470/1,722), and passive (72/1,722) sampling were the main methods used to collect wastewater samples. The sampling strategies were longitudinal sampling (1,440/1,722) or one-time detection (235/1,722). The most commonly reported sampling frequencies were weekly (278/1,722), one-time (265/1,722), and monthly (222/1,722). The study period was most often 1–2 years (368/1,722) followed by 1–3 months (360/1,722). Polymerase chain reaction (PCR) (1,085/1,722) and culture/biochemical identification (500/1,722) were the most frequently used methods of initially detecting pathogens in wastewater. PCR was also the most commonly used secondary or confirmatory detection method (435/1,722), followed by gel electrophoresis (325/1,722) and Sanger sequencing (326/1,722).

Human pathogens

Pathogens detected in wastewater

The detection of human pathogens in untreated wastewater or sludge was described in 99.3% (1,709/1,722) of studies. Of these, pathogens were reported at the genus or species level (1,655/1,709), family level (85/1,709), and at a higher taxonomic rank (14/1,709). Across these studies, 551 pathogens were described at the genus or species level, belonging to 142 distinct microbial families. There were also 10 additional microbial families studied without further taxonomic ranking being provided. A full list of pathogens found in wastewater is available in Supplementary 4.

Viruses were the most commonly investigated pathogen type (68.8%; 1,176/1,709), followed by bacteria (26.4%; 451/1,709), protozoa (4.6%; 78/1,709), helminths (1.4%; 23/1,709), and fungi (0.5%; 9/1,709) ([Figure 2](#)). The top 10 pathogens in each pathogen type are shown in [Figure 2](#). Within each pathogen type, most of the research was conducted on fewer than 10 pathogens. One experimental study described the persistence of a prion protein in wastewater ([Maluquer de Motes et al. 2012](#)).

Overall, the top eight families studied (five viruses and three bacteria) over time depict a steady increase in wastewater research between the years 2000–2019 with a steep rise in 2020–2023 ([Figure 3](#)), mainly driven by SARS-CoV-2 (675/682, [Figure 2](#)) within the *Coronaviridae* family (682/1,709). The *Enterobacteriaceae* (325/1,709) family includes the frequently studied pathogens *Escherichia* sp., *Klebsiella* sp., *Salmonella* sp., *Enterobacter* sp., and *Citrobacter* sp., as shown in [Figure 2](#). *Picornaviridae* (303/1,709) includes the commonly studied pathogens such as non-polio enteroviruses, polioviruses, and Hepatovirus A (Hepatitis A virus). The *Caliciviridae* family (163/1,709) included only *Norovirus* sp. and Sapporo virus (Sapovirus). Pathogens in the family *Adenoviridae* (127/1,709) were almost exclusively Adenoviruses. The most common species investigated in the *Pseudomonadaceae* family (113/1,709), *Sedoreoviridae* family (93/1,709), and *Moraxellaceae* family (91/1,709) were *Pseudomonas* sp., Rotaviruses, and *Acinetobacter* sp., respectively.

Studies conducted prior to 2019, the year COVID-19 emerged, focused on pathogen families transmitted primarily through fecal–oral or waterborne pathways such as *Enterobacteriaceae*, *Picornaviridae*, and *Caliciviridae*, as shown in [Figure 3](#). Starting in the early 2000s, research studies started exploring the detection of pathogens that were primarily transmitted through other pathways such as respiratory, vector-borne, sexual, and blood-borne. Aside from SARS-CoV-2 ($n = 675$), the number of research studies investigating other respiratory pathogens was low and included SARS-CoV-1 ($n = 5$) and MERS-CoV ($n = 1$), influenza A & B ($n = 22$), respiratory syncytial virus (RSV) ($n = 9$), and measles virus ($n = 5$). These studies were primarily conducted in 2020–2023, with the exception of one study on influenza A conducted in 2011 and three studies on SARS-CoV-1 conducted in 2005. Over the last 3 years, sporadic publications on vector-borne pathogens in wastewater have been published on chikungunya ($n = 1$), dengue ($n = 2$), yellow fever ($n = 1$), zika ($n = 1$), tularensis ($n = 1$), and tick-borne relapsing fever ($n = 1$). Starting in the late 2000s, several pathogens causing sexually transmitted and blood-borne infections

Table 2 | Wastewater sampling details of 1,722 primary research publications on human pathogens found in untreated wastewater

Category	Count
Sampling location ^a	
Urban community	1,350
Buildings or facilities	372
Urban/rural community – not specified	140
Small rural community	85
Not reported	20
Airplane/ship/other transport vehicle	12
Sampling point ^a	
Wastewater treatment plant	1,261
Facility/institute	372
Sewer	215
Not reported	86
Pumping station	67
Open drains/canal	66
Sewage outlet/discharge	19
Septic tank/truck	17
Airplane/ship lavatory tank	12
Entry point to sewage lagoon or septic ponds	8
Storage tank/pit latrine	6
Sample collection method ^a	
Composite sample	681
Not reported	674
Grab sample	470
Passive sample	72
Other ^b	7
Sampling strategy ^a	
Longitudinal	1,440
One-time detection	235
Not applicable (e.g., experiment)	157
Sampling period	
< 1 month	263
1–3 months	365
4–6 months	257
7–11 months	317
1–2 years	376
3+ years	81
Not reported	63
Sampling frequency ^a	
Once ^c	265
Daily	99
2–3 times a week	154
Weekly	278

(Continued.)

Table 2 | Continued

Category	Count
Bi-weekly (every second week)	91
Twice a month	32
Monthly	222
Every two months	36
By season or quarter	23
Other ^d	65
Not reported	561
Initial detection method ^a	
PCR	1,085
Culture/biochemical identification	500
Immunoassay	57
Next-generation or whole genome sequencing	50
Microscopy	37
Spectrometry	29
Other DNA/RNA sequencing (e.g., Sanger)	10
Loop-mediated isothermal amplification (LAMP)	8
Not reported	8
Animal studies	5
Other ^e	5
Secondary/confirmatory detection method ^a	
PCR	435
Gel electrophoresis	326
Other DNA/RNA sequencing (e.g., Sanger)	325
Next-generation or whole genome sequencing	301
Immunoassay	94
Spectrometry	85
Culture/biochemical identification	73
Hybridization assay	28
Microscopy	16
Other ^f	7

^aTotal number sums to >1,722 as some studies were conducted in multiple locations, at different sampling points, over large time periods, using multiple collection methods, sampling strategies, and detection methods.

^bOther sampling methods included swabbing ($n = 5$), tongue depressor attached to a stick ($n = 1$), and a NanoCeram virus sampler filter ($n = 1$).

^cOne-time detection for the sampling frequency ($n = 265$) includes 30 experimental studies that took a single sample.

^dOther sampling frequencies included multiple times a week ($n = 41$), every 1–6 weeks ($n = 10$), multiple times a month ($n = 8$), every few months ($n = 4$), yearly ($n = 2$).

^eOther detection methods included the hybridization assay ($n = 3$), the optical sensor array ($n = 1$), and the electrochemical genosensor ($n = 1$).

^fOther secondary/confirmatory detection methods included loop-mediated isothermal amplification ($n = 4$) and animal studies ($n = 3$).

have also been investigated, including herpes simplex viruses ($n = 4$), *Neisseria gonorrhoeae* ($n = 1$), *Treponema pallidum* ($n = 1$), and human papillomaviruses (HPV) ($n = 13$).

Pathogens not detected in wastewater

There were 6.4% (111/1,722) of studies where pathogens being studied were not detected in wastewater or sludge. This included 120 pathogens belonging to 65 distinct microbial families (Supplementary 4). Of these pathogens, 81.1% (99/122) were detected in wastewater or sludge in other studies and 18.9% (23/122) of pathogens were not detected in individual studies when they were looked for, including hantaviruses, *Toxoplasma gondii*, and simian immunodeficiency virus (SIV).

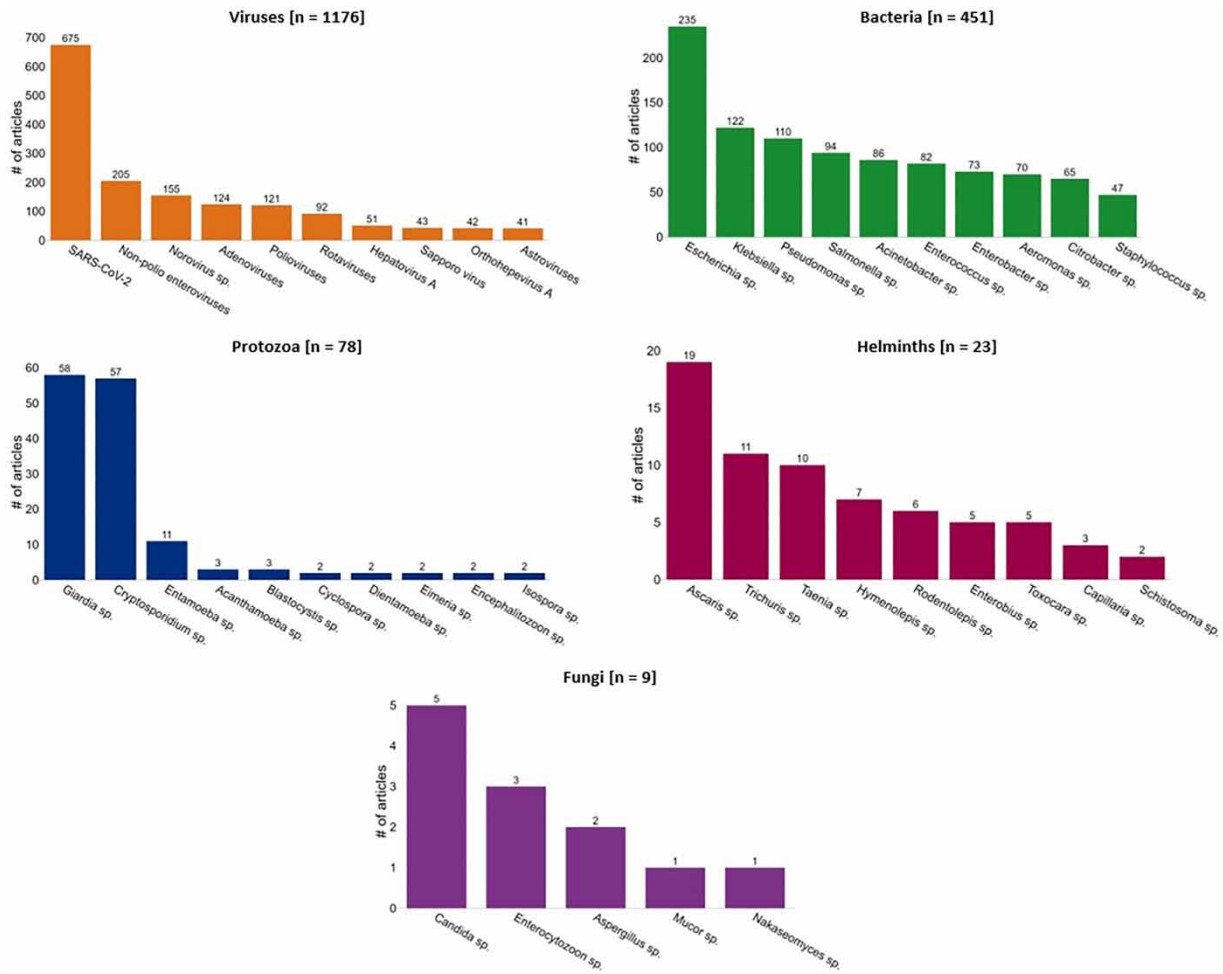


Figure 2 | Breakdown of the number of studies for the top 10 pathogens investigated within each pathogen category.

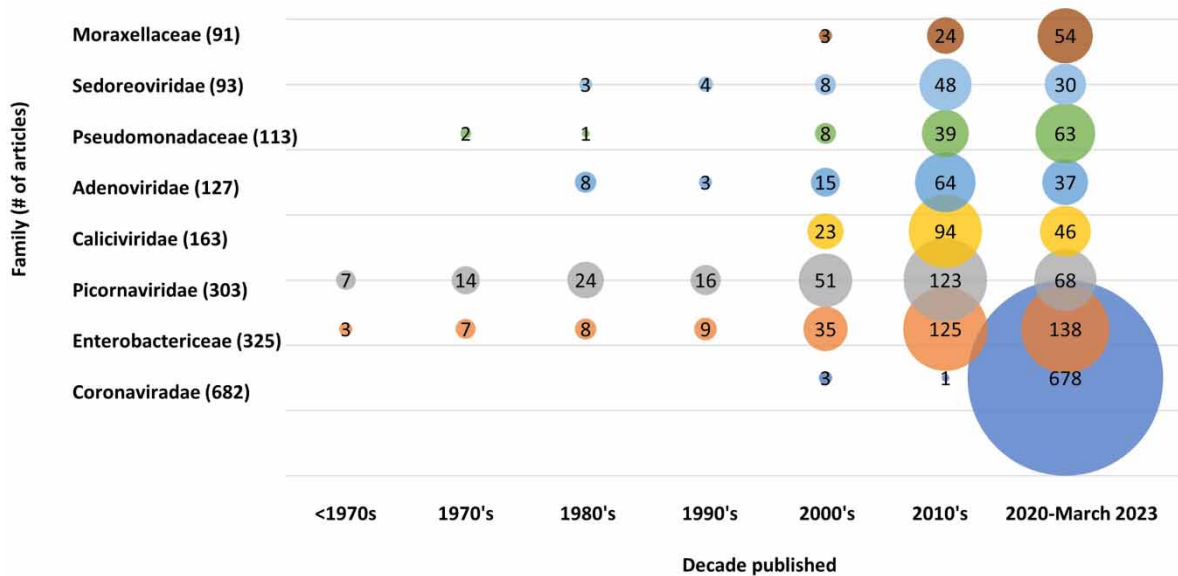


Figure 3 | Bubble chart of the top eight most frequently studied pathogen families over time.

Antimicrobial resistance

Wastewater detection of antimicrobial resistant organisms or genes was described in 20.6% (355/1,722) of studies. The majority of these studies were conducted in Europe (148/355) and Asia (119/355), followed by North America (45/355), Africa (34/355), Australia/Oceania (34/355), and South America (20/355). Antimicrobial susceptibility was tested phenotypically (109/355), genotypically (96/355), or using both methods (150/355). *Escherichia coli* (177/355) and *Klebsiella pneumoniae* (86/355) were the most commonly reported organisms in antimicrobial resistance studies.

Wastewater and population health

Researchers connected their wastewater findings with population health data in 43.0% (741/1,722) of studies. The majority of these studies correlated wastewater results with clinical surveillance or case data (710/741) for SARS-CoV-2 (471/710), Enteroviruses (63/710), antimicrobial resistant organisms (55/710), or viruses causing acute gastroenteritis (rotaviruses, noroviruses, adenoviruses, sapoviruses, and astroviruses) (49/710).

Public health action

Wastewater detection or monitoring of pathogens was reported to lead to public health action in 3.4% (58/1,722) of studies. This included decisions to commence (43/58) or cease (1/58) public health measures (e.g., additional surveillance, targeted testing, and isolation), implement public health messaging (8/58), and support decision-making (8/58). The majority of these studies were on SARS-CoV-2 (43/58) followed by poliovirus (7/58).

DISCUSSION

This ScR summarizes the characteristics of the global research on human pathogens and antimicrobial resistance targets found in untreated wastewater and sludge. The results demonstrate that a wide range of pathogens can be detected in wastewater. However, the usefulness of wastewater monitoring for different pathogens is dependent on both the burden of disease in a community, as well as if and how much the pathogen is excreted into wastewater during infection.

In this ScR, there are several notable topic areas that are not underpinned by many studies including certain pathogens, antimicrobial resistance, and some methods for wastewater monitoring. Most studies focused on viral and bacterial pathogens with minimal research on other pathogen types, protozoa, helminths and fungi; the latter are a growing international concern (Benedict *et al.* 2017; Nnadi & Carter 2021). Some priority fungal pathogens designated by the World Health Organization have been identified in wastewater studies including *Candida auris* and *Nakaseomyces glabratus* (Brumfield *et al.* 2022; World Health Organization 2022; Barber *et al.* 2023; Rossi *et al.* 2023). However, this ScR shows that more research is needed to determine if wastewater monitoring is an appropriate method for identifying emerging fungi of international concern. Further syntheses on the identified studies related to antimicrobial resistance may be warranted to understand how and why wastewater has been used to address this important global public health threat.

Tracking antimicrobial resistance burden and trends is an area for which wastewater monitoring could potentially be useful; however, this ScR identified little research on strategies or utility for such monitoring. In theory, monitoring antimicrobial resistance in wastewater could be beneficial for the purpose of identifying multidrug resistant pathogens currently circulating at the population level. Indeed, this is data that has historically been difficult to acquire, and it may provide insights into potential resistance acquisition and dissemination in the environment as sewage can contain a confluence of antimicrobials, human pathogens, resistance genes, and mobile genetic elements (Liguori *et al.* 2022).

Within the current literature, most of the studies on sampling, sample handling, and extrapolation of wastewater results to the burden of infection in a population were conducted on SARS-CoV-2. For other pathogens, there were few studies that examined the sensitivity of methodologies used to understand the emergence or fluctuations of a pathogen within a population. As the utility of wastewater monitoring is being considered for a broader range of pathogens, research to establish the best practices will need to be conducted. Wastewater samples were the most common for monitoring human pathogens compared to sludge samples. In studies examining both, sludge samples have been described as containing a broader diversity of human pathogens but may also have more inhibitors and other contaminants that change the sample handling and extrapolation of results. Thus, more research is needed into the assay sensitivity, sample processing, and result extrapolation trade-offs for different sample types and how this may influence pathogen detection within a community wastewater catchment (Bibby & Peccia 2013; Peccia *et al.* 2020). Optimal sampling frequencies may vary depending on the pathogen and characteristics of the outbreak or circulation of the pathogen in the population. Studies conducted on SARS-CoV-2 show that

differences in sampling frequencies influenced the characterization of incident COVID-19 cases at the population level (Chan *et al.* 2023). Daily data were described as the ‘gold standard’ for the trend analysis of SARS-CoV-2 and a minimum of 2–5 samples per week were necessary for capturing trends within the community for SARS-CoV-2 (Chan *et al.* 2023; Kuroita *et al.* 2023). For other pathogens, daily and twice weekly sampling approaches are less common in wastewater monitoring, and most studies reported weekly or monthly sampling. An evaluation of the optimal sampling strategy to capture community trends would need to be established for each pathogen and may vary depending on the epidemiologic situation (e.g., outbreak vs. seasonal activity) and the goals of the monitoring system.

This ScR identified that a limited number of studies were conducted in the Caribbean, Central America, Africa, and the Middle East. Wastewater monitoring may offer the greatest benefit in countries with limited clinical surveillance capacity, especially considering that wastewater has several advantages over sampling individuals and case counting, including its ability to be a cost-effective strategy for the detection and evaluation of the burden of infectious diseases in a population (Ali *et al.* 2022; Ngwira *et al.* 2022). Considerations for the application of wastewater monitoring in developing countries include an assessment of the wastewater infrastructure and the design of a sampling strategy to get representative samples (Pandey *et al.* 2021).

It is our intention that the information synthesized and knowledge gaps discussed in this ScR will be used to support evidence-informed decision-making and further research on this topic. Although we attempted to capture all relevant literature on this topic, it is possible that some research was not captured due to the complexity of the search, publication bias, absence of citation indexing, lack of citations by included papers, or the omission of an extensive grey literature search. Articles published in any language other than English and French were not characterized in this ScR due to translation resource constraints; however, only two studies were excluded on the basis of publication language.

CONCLUSION

While wastewater testing to monitor pathogens is not a new concept, the success of using wastewater to detect SARS-CoV-2 and estimate community burden during the pandemic has demonstrated the use of wastewater to inform public health responses and provided a unique opportunity to compare the traditional surveillance methods against wastewater monitoring data for a sustained period of time. Outside of SARS-CoV-2, a few studies on enteric pathogens also correlated wastewater signals to clinical surveillance data. These suggest that a multifaceted approach to surveillance that incorporates wastewater signals and other traditional epidemiological metrics (e.g., incidence, hospitalizations, and pharmaceutical sales) would be beneficial for both outbreak situations and routine monitoring of many pathogens, including but not limited to influenza, RSV, and antimicrobial resistance. Recent research on SARS-CoV-2 and mpox has shown that wastewater can be used to monitor the burden and spread of a pathogen in a population fairly quickly and economically, which has the potential to influence case-based surveillance strategies in the future (Sherchan *et al.* 2023; Tiwari *et al.* 2023). From a public health preparedness lens, additional research is needed to determine which pathogens are conducive to wastewater monitoring, and where, when, and how monitoring should be implemented.

ACKNOWLEDGEMENTS

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AUTHORS' CONTRIBUTION

T.C. served as the project lead, contributed to the design, performed as a reviewer, and wrote the manuscript. P.R. was the project lead, contributed to the design, performed as a reviewer, and edited the manuscript. K.Y. contributed to the design and manuscript, performed as a reviewer, and edited the manuscript. G.M. served as a reviewer, contributed to and edited the manuscript. A.B. performed as a reviewer and data cleanup, and edited the manuscript. K.P. performed as a reviewer and edited the manuscript. L.W. was responsible for the conceptualization, design and initiation of the project, and also edited the manuscript, supervisor. All authors approved the final version for publication.

DATA AVAILABILITY STATEMENT

All relevant data are included in the paper or its Supplementary Information (Supplementary Files 1–4: <https://doi.org/10.17605/OSF.IO/2Y6ZU>).

CONFLICT OF INTEREST

The authors declare there is no conflict.

REFERENCES

- Ali, W., Zhang, H., Wang, Z., Chang, C., Javed, A., Ali, K., Du, W., Niazi, N. K., Mao, K. & Yang, Z. 2021 Occurrence of various viruses and recent evidence of SARS-CoV-2 in wastewater systems. *Journal of Hazardous Materials* **414**, 125439. <https://doi.org/10.1016/j.jhazmat.2021.125439>.
- Ali, S., Gudina, E. K., Gize, A., Aliy, A., Adankie, B. T., Tsegaye, W., Hundie, G. B., Muleta, M. B., Chibssa, T. R., Belaineh, R., Negessu, D., Shegu, D., Froeschl, G. & Wieser, A. 2022 Community wastewater-based surveillance can be a cost-effective approach to track COVID-19 outbreak in low-resource settings: Feasibility assessment for Ethiopia context. *International Journal of Environmental Research and Public Health* **19** (14), 8515. doi:10.3390/ijerph19148515.
- Arksey, H. & O'Malley, L. 2005 Scoping studies: Towards a methodological framework. *International Journal of Social Research Methodology* **8** (1), 19–32. doi:10.1080/1364557032000119616.
- Baker, R. E., Mahmud, A. S., Miller, I. F., Rajeev, M., Rasambainarivo, F., Rice, B. L., Takahashi, S., Tatem, A. J., Wagner, C. E., Wang, L. F., Wesolowski, A. & Metcalf, C. J. E. 2022 Infectious disease in an era of global change. *Nature Reviews Microbiology* **20** (4), 193–205. doi:10.1038/s41579-021-00639-z.
- Barber, C., Crank, K., Papp, K., Innes, G. K., Schmitz, B. W., Chavez, J., Rossi, A. & Gerrity, D. 2023 Community-scale wastewater surveillance of *Candida auris* during an ongoing outbreak in Southern Nevada. *Environmental Science & Technology* **57** (4), 1755–1763. doi:10.1021/acs.est.2c07763.
- Benedict, K., Richardson, M., Vallabhaneni, S., Jackson, B. R. & Chiller, T. 2017 Emerging issues, challenges, and changing epidemiology of fungal disease outbreaks. *Lancet Infectious Diseases* **17** (12), e403–e411. doi:10.1016/s1473-3099(17)30443-7.
- Bhatt, A., Arora, P. & Prajapati, S. K. 2020 Occurrence, fates and potential treatment approaches for removal of viruses from wastewater: A review with emphasis on SARS-CoV-2. *Journal of Environmental Chemical Engineering* **8** (5), 104429. <https://doi.org/10.1016/j.jece.2020.104429>.
- Bibby, K. & Peccia, J. 2013 Identification of viral pathogen diversity in sewage sludge by metagenome analysis. *Environmental Science & Technology* **47** (4), 1945–1951. doi:10.1021/es305181x.
- Boehm, A. B., Hughes, B., Duong, D., Chan-Herur, V., Buchman, A., Wolfe, M. K. & White, B. J. 2023 Wastewater concentrations of human influenza, metapneumovirus, parainfluenza, respiratory syncytial virus, rhinovirus, and seasonal coronavirus nucleic-acids during the COVID-19 pandemic: A surveillance study. *The Lancet Microbe* **4** (5), e340–e348. [https://doi.org/10.1016/S2666-5247\(22\)00386-X](https://doi.org/10.1016/S2666-5247(22)00386-X).
- Bonanno Ferraro, G., Veneri, C., Mancini, P., Iaconelli, M., Suffredini, E., Bonadonna, L., Lucentini, L., Bowo-Ngandji, A., Kengne-Nde, C., Mbada, D. S., Mahamat, G., Tazokong, H. R., Ebogo-Belobo, J. T., Njouom, R., Kenmoe, S. & La Rosa, G. 2022 A state-of-the-art scoping review on SARS-CoV-2 in sewage focusing on the potential of wastewater surveillance for the monitoring of the COVID-19 pandemic. *Food and Environmental Virology* **14** (4), 315–354. doi:10.1007/s12560-021-09498-6.
- Brumfield, K. D., Leddy, M., Usmani, M., Cotruvo, J. A., Tien, C. T., Dorsey, S., Graubics, K., Fanelli, B., Zhou, I., Registe, N., Dadlani, M., Wimalarante, M., Jinasena, D., Abayagunawardena, R., Withanachchi, C., Huq, A., Jutla, A. & Colwell, R. R. 2022 Microbiome analysis for wastewater surveillance during COVID-19. *mBio* **13** (4), e00591–22. doi:10.1128/mbio.00591-22.
- Chan, E. M. G., Kennedy, L. C., Wolfe, M. K. & Boehm, A. B. 2023 Identifying trends in SARS-CoV-2 RNA in wastewater to infer changing COVID-19 incidence: Effect of sampling frequency. *PLoS Water* **2** (4), e0000088. doi:10.1371/journal.pwat.0000088.
- Chau, K. K., Barker, L., Budgell, E. P., Vihta, K. D., Sims, N., Kasprzyk-Hordern, B., Harriss, E., Crook, D. W., Read, D. S., Walker, A. S. & Stoesser, N. 2022 Systematic review of wastewater surveillance of antimicrobial resistance in human populations. *Environment International* **162**, 107171. <https://doi.org/10.1016/j.envint.2022.107171>.
- Corpuz, M. V. A., Buonerba, A., Vigliotta, G., Zarra, T., Ballesteros, F., Campiglia, P., Belgiorno, V., Korshin, G. & Naddeo, V. 2020 Viruses in wastewater: Occurrence, abundance and detection methods. *Science of the Total Environment* **745**, 140910. <https://doi.org/10.1016/j.scitotenv.2020.140910>.
- Dzinamarira, T., Murewanhema, G., Iradukunda, P. G., Madziva, R., Herrera, H., Cuadros, D. F., Tungwarara, N., Chitungo, I. & Musuka, G. 2022 Utilization of SARS-CoV-2 wastewater surveillance in Africa – A rapid review. *International Journal of Environmental Research and Public Health* **19** (2). doi:10.3390/ijerph19020969.
- Hassoun-Kheir, N., Stabholz, Y., Kreft, J. U., de la Cruz, R., Romalde, J. L., Nesme, J., Sørensen, S. J., Smets, B. F., Graham, D. & Paul, M. 2020 Comparison of antibiotic-resistant bacteria and antibiotic resistance genes abundance in hospital and community wastewater: A systematic review. *Science of the Total Environment* **743**, 140804. <https://doi.org/10.1016/j.scitotenv.2020.140804>.
- Hovi, T., Shulman, L. M., van der Avoort, H., Deshpande, J., Roivainen, M. & de Gourville, E. M. 2012 Role of environmental poliovirus surveillance in global polio eradication and beyond. *Epidemiology & Infection* **140** (1), 1–13. doi:10.1017/s095026881000316x.

- Huang, Y., Zhou, N., Zhang, S., Yi, Y., Han, Y., Liu, M., Han, Y., Shi, N., Yang, L., Wang, Q., Cui, T. & Jin, H. 2022 Norovirus detection in wastewater and its correlation with human gastroenteritis: A systematic review and meta-analysis. *Environmental Science and Pollution Research* **29** (16), 22829–22842. doi:10.1007/s11356-021-18202-x.
- Kilaru, P., Hill, D., Anderson, K., Collins, M. B., Green, H., Kmush, B. L. & Larsen, D. A. 2023 Wastewater surveillance for infectious disease: A systematic review. *American Journal of Epidemiology* **192** (2), 305–322. doi:10.1093/aje/kwac175.
- Kuroita, T., Yoshimura, A., Iwamoto, R., Ando, H., Okabe, S. & Kitajima, M. 2023 Quantitative analysis of SARS-CoV-2 RNA in wastewater and evaluation of sampling frequency during the downward period of a COVID-19 wave in Japan. *Science of the Total Environment* 166526. <https://doi.org/10.1016/j.scitotenv.2023.166526>.
- Levac, D., Colquhoun, H. & O'Brien, K. K. 2010 Scoping studies: Advancing the methodology. *Implementation Science* **5**, 69. doi:10.1186/1748-5908-5-69.
- Liguori, K., Keenum, I., Davis, B. C., Calarco, J., Milligan, E., Harwood, V. J. & Pruden, A. 2022 Antimicrobial resistance monitoring of water environments: A framework for standardized methods and quality control. *Environmental Science & Technology* **56** (13), 9149–9160. doi:10.1021/acs.est.1c08918.
- Maluquer de Motes, C., Espinosa, J. C., Esteban, A., Calvo, M., Girones, R. & Torres, J. M. 2012 Persistence of the bovine spongiform encephalopathy infectious agent in sewage. *Environmental Research* **117**, 1–7. <https://doi.org/10.1016/j.envres.2012.06.010>.
- Mao, K., Zhang, K., Du, W., Ali, W., Feng, X. & Zhang, H. 2020 The potential of wastewater-based epidemiology as surveillance and early warning of infectious disease outbreaks. *Current Opinion in Environmental Science & Health* **17**, 1–7. doi:10.1016/j.coesh.2020.04.006.
- Michael-Kordatou, I., Karaolia, P. & Fatta-Kassinos, D. 2020 Sewage analysis as a tool for the COVID-19 pandemic response and management: The urgent need for optimised protocols for SARS-CoV-2 detection and quantification. *Journal of Environmental Chemical Engineering* **8** (5), 104306. <https://doi.org/10.1016/j.jece.2020.104306>.
- Mousazadeh, M., Ashoori, R., Paital, B., Kabdaşlı, I., Frontistis, Z., Hashemi, M., Sandoval, M. A., Sherchan, S., Das, K. & Emamjomeh, M. M. 2021 Wastewater based epidemiology perspective as a faster protocol for detecting coronavirus RNA in human populations: A review with specific reference to SARS-CoV-2 virus. *Pathogens* **10** (8). doi:10.3390/pathogens10081008.
- Ngwira, L. G., Sharma, B., Shrestha, K. B., Dahal, S., Tuladhar, R., Manthalu, G., Chilima, B., Ganizani, A., Rigby, J., Kanjerwa, O., Barnes, K., Anscombe, C., Mfutso-Bengo, J., Feasey, N. & Mvundura, M. 2022 Cost of wastewater-based environmental surveillance for SARS-CoV-2: Evidence from pilot sites in Blantyre, Malawi and Kathmandu, Nepal. *PLoS Global Public Health* **2** (12), e0001377. doi:10.1371/journal.pgph.0001377.
- Nnadi, N. E. & Carter, D. A. 2021 Climate change and the emergence of fungal pathogens. *PLoS Pathogens* **17** (4), e1009503. doi:10.1371/journal.ppat.1009503.
- O'Brien, E. & Xagorarakis, I. 2019 A water-focused one-health approach for early detection and prevention of viral outbreaks. *One Health* **7**, 100094. <https://doi.org/10.1016/j.onehlt.2019.100094>.
- O'Keeffe, J. 2021 Wastewater-based epidemiology: Current uses and future opportunities as a public health surveillance tool. *Environmental Health Review* **64** (3), 44–52. doi:10.5864/d2021-015.
- Pandey, D., Verma, S., Verma, P., Mahanty, B., Dutta, K., Daverey, A. & Arunachalam, K. 2021 SARS-CoV-2 in wastewater: Challenges for developing countries. *International Journal of Hygiene & Environmental Health* **231**, 113634. doi:10.1016/j.ijheh.2020.113634.
- Peccia, J., Zulli, A., Brackney, D. E., Grubaugh, N. D., Kaplan, E. H., Casanovas-Massana, A., Ko, A. I., Malik, A. A., Wang, D., Wang, M., Warren, J. L., Weinberger, D. M., Arnold, W. & Omer, S. B. 2020 Measurement of SARS-CoV-2 RNA in wastewater tracks community infection dynamics. *Nature Biotechnology* **38** (10), 1164–1167. doi:10.1038/s41587-020-0684-z.
- Peters, M. D. J., Marnie, C., Tricco, A. C., Pollock, D., Munn, Z., Alexander, L., McInerney, P., Godfrey, C. M. & Khalil, H. 2020a Updated methodological guidance for the conduct of scoping reviews. *JBI Evidence Synthesis* **18** (10), 2119–2126. doi:10.11124/jbies-20-00167.
- Peters, M. D. J., Godfrey, C., McInerney, P., Munn, Z., Tricco, A. C., Khalil, H., 2020b Chapter 11: Scoping reviews (2020 version). In: *JBI Manual for Evidence Synthesis* (Aromataris, E. & Munn, Z., eds). <https://doi.org/10.46658/JBIMES-20-12>.
- Pruden, A., Vikesland, P. J., Davis, B. C. & de Roda Husman, A. M. 2021 Seizing the moment: Now is the time for integrated global surveillance of antimicrobial resistance in wastewater environments. *Current Opinion in Microbiology* **64**, 91–99. <https://doi.org/10.1016/j.mib.2021.09.013>.
- Rossi, A., Chavez, J., Iverson, T., Hergert, J., Oakeson, K., LaCross, N., Njoku, C., Gorzalski, A. & Gerrity, D. 2023 *Candida auris* discovery through community wastewater surveillance during healthcare outbreak, Nevada, USA, 2022. *Emerging Infectious Disease Journal* **29** (2), 422. doi:10.3201/eid2902.221523.
- Saawarn, B. & Hait, S. 2021 Occurrence, fate and removal of SARS-CoV-2 in wastewater: Current knowledge and future perspectives. *Journal of Environmental and Chemical Engineering* **9** (1), 104870. doi:10.1016/j.jece.2020.104870.
- Safford, H. R., Shapiro, K. & Bischel, H. N. 2022 Wastewater analysis can be a powerful public health tool – If it's done sensibly. *Proceedings of the National Academy of Sciences* **119** (6), e2119600119. doi:10.1073/pnas.2119600119.
- Shah, S., Gwee, S. X. W., Ng, J. Q. X., Lau, N., Koh, J. & Pang, J. 2022 Wastewater surveillance to infer COVID-19 transmission: A systematic review. *Science of the Total Environment* **804**, 150060. <https://doi.org/10.1016/j.scitotenv.2021.150060>.
- Sherchan, S. P., Solomon, T., Idris, O., Nwaubani, D. & Thakali, O. 2023 Wastewater surveillance of Mpox virus in Baltimore. *Science of the Total Environment* **891**, 164414. doi:10.1016/j.scitotenv.2023.164414.
- Sims, N. & Kasprzyk-Hordern, B. 2020 Future perspectives of wastewater-based epidemiology: Monitoring infectious disease spread and resistance to the community level. *Environment International* **139**, 105689. doi:10.1016/j.envint.2020.105689.

- Tiwari, A., Adhikari, S., Kaya, D., Islam, M. A., Malla, B., Sherchan, S. P., Al-Mustapha, A. I., Kumar, M., Aggarwal, S., Bhattacharya, P., Bibby, K., Halden, R. U., Bivins, A., Haramoto, E., Oikarinen, S., Heikinheimo, A. & Pitkänen, T. 2023 **Monkeypox outbreak: Wastewater and environmental surveillance perspective**. *Science of the Total Environment* **856** (Pt 2), 159166. doi:10.1016/j.scitotenv.2022.159166.
- Tthagale, M., Liphadzi, S., Bhagwan, J., Naidoo, V., Jonas, K., van Vuuren, L., Medema, G., Andrews, L., Been, F., Ferreira, M. L., Saatci, A. M., Alpaslan Kocamemi, B., Hassard, F., Singer, A. C., Bunce, J. T., Grimsley, J. M. S., Brown, M. & Jones, D. L. 2022 **Establishment of local wastewater-based surveillance programmes in response to the spread and infection of COVID-19 – Case studies from South Africa, the Netherlands, Turkey and England**. *Journal of Water and Health* **20** (2), 287–299. doi:10.2166/wh.2022.185.
- Tricco, A. C., Lillie, E., Zarin, W., O'Brien, K., Colquhoun, H., Kastner, M., Levac, D., Ng, C., Pearson Sharpe, J., Wilson, K., Kenny, M., Warren, R., Wilson, C., Stelfox, H. T. & Straus, S. E. 2016 **A scoping review on the conduct and reporting of scoping reviews**. *BMC Medical Research Methodology* **16** (1), 15. doi:10.1186/s12874-016-0116-4.
- Tricco, A. C., Lillie, E., Zarin, W., O'Brien, K. K., Colquhoun, H., Levac, D., Moher, D., Peters, M. D. J., Horsley, T., Weeks, L., Hempel, S., Akl, E. A., Chang, C., McCowan, J., Stewart, L., Hartling, L., Aldcroft, A., Wilson, M. G., Garrity, C., Lewin, S., Godfrey, C. M., Macdonald, M. T., Langlois, E. V., Soares-Weiser, K., Moriarty, J., Clifford, T., Tuncalp, O. & Straus, S. E. 2018 **PRISMA extension for Scoping Reviews (PRISMA-ScR): Checklist and explanation**. *Annals of Internal Medicine* **169** (7), 467–473. doi:10.7326/m18-0850.
- World Health Organization 2022 **WHO Fungal Priority Pathogens List to Guide Research, Development and Public Health Action**. Available from: <https://www.who.int/publications/i/item/9789240060241> (accessed 20 September 2023).
- Zaatout, N., Bouras, S. & Slimani, N. 2021 **Prevalence of extended-spectrum β -lactamase (ESBL)-producing Enterobacteriaceae in wastewater: A systematic review and meta-analysis**. *Journal of Water and Health* **19** (5), 705–723. doi:10.2166/wh.2021.112.

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