Science of the Total Environment 926 (2024) 171401

Contents lists available at ScienceDirect

Science of the Total Environment

journal homepage: www.elsevier.com/locate/scitotenv

Developing wastewater-based surveillance schemes for multiple pathogens: The WastPan project in Finland

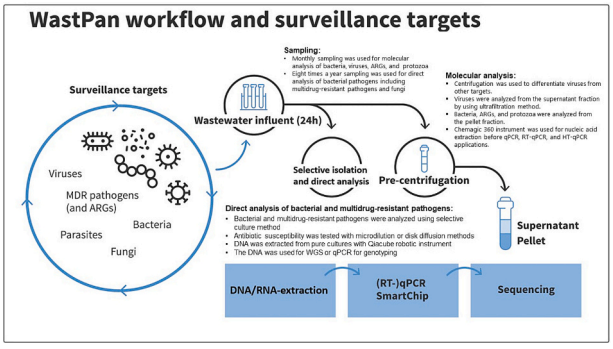
Ananda Tiwari [a,*], Kirsi-Maarit Lehto [b], Dafni K. Paspaliari [a,f], Ahmad I. Al-Mustapha [c,d], Anniina Sarekoski [a,c], Anna-Maria Hokajärvi [a], Annika Länsivaara [b], Rafiqul Hyder [b], Oskari Luomala [a], Anssi Lipponen [a,1], Sami Oikarinen [b], Annamari Heikinheimo [c,e], Tarja Pitkänen [a,c,*], WastPan Study Group

[a] Finnish Institute for Health and Welfare, Department of Health Security, Kuopio and Helsinki, Finland
[b] Tampere University, Faculty of Medicine and Health Technology, Tampere, Finland
[c] University of Helsinki, Faculty of Veterinary Medicine, Helsinki, Finland
[d] Department of Veterinary Public Health and Preventive Medicine, Faculty of Veterinary Medicine, University of Ibadan, Ibadan, Nigeria
[e] Finnish Food Authority, Seinäjoki, Finland
[f] ECDC Fellowship Programme, Public Health Microbiology path (EUPHEM), European Centre for Disease Prevention and Control (ECDC), Solna, Sweden.

HIGHLIGHTS

- Wastewater surveillance programme for multiple pathogens is explained.
- Wastewater surveillance sampling frequency and population coverage vary on pathogen.
- The N2 assay detected SARS-CoV-2 RNA in wastewater more often than the N1 assay.
- A single community wastewater sample can yield considerable public health data.

GRAPHICAL ABSTRACT



ARTICLE INFO

Editor: Warish Ahmed

Keywords:
Wastewater-based epidemiology
Multiple pathogens

ABSTRACT

Wastewater comprises multiple pathogens and offers a potential for wastewater-based surveillance (WBS) to track the prevalence of communicable diseases. The Finnish WastPan project aimed to establish wastewater-based pandemic preparedness for multiple pathogens (viruses, bacteria, parasites, fungi), including antimicrobial resistance (AMR). This article outlines WastPan's experiences in this project, including the criteria for target selection, sampling locations, frequency, analysis methods, and results communication. Target selection relied on

* Corresponding authors.
E-mail addresses: ananda.tiwari@thl.fi (A. Tiwari), kirsi-maarit.lehto@tuni.fi (K.-M. Lehto), ahmad.al-mustapha@helsinki.fi (A.I. Al-Mustapha), anniina.sarekoski@thl.fi, anniina.sarekoski@helsinki.fi (A. Sarekoski), anna-maria.hokajarvi@thl.fi (A.-M. Hokajärvi), annika.lansivaara@tuni.fi (A. Länsivaara), rafiqul.hyder@tuni.fi (R. Hyder), oskari.luomala@thl.fi (O. Luomala), anssi.lipponen@thl.fi (A. Lipponen), sami.oikarinen@tuni.fi (S. Oikarinen), annamari.heikinheimo@helsinki.fi, annamari.heikinheimo@ruokavirasto.fi (A. Heikinheimo), tarja.pitkanen@thl.fi, tarja.m.pitkanen@helsinki.fi (T. Pitkänen).
[1] Present address: University of Eastern Finland, Department of Medicine, Unit of Biomedicine, Kuopio, Finland.

https://doi.org/10.1016/j.scitotenv.2024.171401
Received 2 December 2023; Received in revised form 28 February 2024; Accepted 28 February 2024
Available online 10 March 2024

Pandemic preparedness
Antimicrobial resistance

epidemiological and microbiological evidence and practical feasibility. Within the WastPan framework, wastewater samples were collected between 2021 and 2023 from 10 wastewater treatment plants (WWTPs) covering 40 % of Finland's population. WWTP selection was validated for reported cases of Extended Spectrum Beta-lactamase-producing bacterial pathogens (*Escherichia coli* and *Klebsiella pneumoniae*) from the National Infectious Disease Register. The workflow included 24-h composite influent samples, with one fraction for culture-based analysis (bacteria and fungi) and the rest of the sample was reserved for molecular analysis (viruses, bacteria, antibiotic resistance genes, and parasites). The reproducibility of the monitoring workflow was assessed for SARS-CoV-2 through inter-laboratory comparisons using the N2 and N1 assays. Identical protocols were applied to same-day samples, yielding similar positivity trends in the two laboratories, but the N2 assay achieved a significantly higher detection rate (Laboratory 1: 91.5 %; Laboratory 2: 87.4 %) than the N1 assay (76.6 %) monitored only in Laboratory 2 (McNemar, $p < 0.001$ Lab 1, $= 0.006$ Lab 2). This result indicates that the selection of monitoring primers and assays may impact monitoring sensitivity in WBS. Overall, the current study recommends that the selection of sampling frequencies and population coverage of the monitoring should be based on pathogen-specific epidemiological characteristics. For example, pathogens that are stable over time may need less frequent annual sampling, while those that are occurring across regions may require reduced sample coverage. Here, WastPan successfully piloted WBS for monitoring multiple pathogens, highlighting the significance of one-litre community composite wastewater samples for assessing community health. The infrastructure established for COVID-19 WBS is valuable for monitoring various pathogens. The prioritization of the monitoring targets optimizes resource utilization. In the future legislative support in target selection, coverage determination, and sustained funding for WBS is recomended.

1. Introduction

Wastewater-based surveillance (WBS) is an emerging approach for monitoring many critical seasonal or emerging pathogens at the population level (Diamond et al., 2022; Farkas et al., 2020; Keshaviah et al., 2023; Kilaru et al., 2023). Municipal sewage comprises pathogens from the entire community served within a single sewershed, potentially released through various body fluids, including respiratory and nasal secretions, saliva, urine, faeces, and skin lesions, during the various stages of infection, i.e., symptomatic, asymptomatic, pre-symptomatic and post-symptomatic (Ahmed et al., 2022a; Bibby et al., 2021; Mao et al., 2020). WBS is a cost-effective approach for monitoring pathogens at the population level, as the cost of analysis of an aetiological agent from a wastewater sample that could provide information from a whole population is almost equivalent to the cost of analysis of the agent in a clinical sample (Kitajima et al., 2020; Mao et al., 2020; Tiwari et al., 2023a). A single composite sample from a community can have an enormous potential to inform health authorities about many diseases that are currently not reported or for which there is no information about circulation at the population level. Community-level WBS also has minimal individual privacy concerns and ethical challenges (Bowes et al., 2023; Lundy et al., 2021), and it is independent of the healthcare-seeking behaviours of individuals and their access to healthcare facilities.

WBS has tremendous flexibility in the selection of sampling sites, surveillance targets, sampling frequency, sample storage for future analysis, the selection of monitoring methods, data analysis, and data presentation based on local needs (Ahmed et al., 2020; Bibby et al., 2021; Farkas et al., 2020; Medema et al., 2020; Tiwari et al., 2023a). WBS has succeeded in guiding public health policy for at least two pathogens: poliovirus (Hovi et al., 2001; Levican et al., 2019; O'Reilly et al., 2020; Pöyry et al., 1988) and SARS-CoV-2 (Ahmed et al., 2020; Hokajärvi et al., 2021; Islam et al., 2023; Kitajima et al., 2020; Kumar et al., 2023; Maal-Bared et al., 2023; Medema et al., 2020). Since 2020, the use of WBS has accelerated globally, mainly during the coronavirus disease 2019 (COVID-19) pandemic (Ahmed et al., 2020; Hokajärvi et al., 2021; Islam et al., 2023; Kitajima et al., 2020; Kumar et al., 2023; Maal-Bared et al., 2023; Medema et al., 2020), as rapid, real-time monitoring and early warning tool (Bibby et al., 2021; Farkas et al., 2020). WBS has been done for many pathogens, such as influenza A virus (Ahmed et al., 2023a; Boehm et al., 2022; Boehm et al., 2023), enterovirus (Ahmed et al., 2023a; Faleye et al., 2021; Shrestha et al., 2024a), noroviruses (Hellmér et al., 2014; Prevost et al., 2015; Santiso-Bellón et al., 2020; Shrestha et al., 2024b), hepatitis A virus (Hellmér et al., 2014), hepatitis E virus (Iaconelli et al., 2020), rotavirus (Santiso-Bellón et al., 2020), adenovirus (Fong et al., 2010), dengue virus (Thakali et al., 2022), mpox (de Jonge et al., 2022; Tiwari et al., 2022a) and antimicrobial resistant (AMR) pathogens and related genes (Hendriksen et al., 2019; Karkman et al., 2020; Pärnänen et al., 2019) for evaluating their circulation at the population level. However, for many of these pathogens, the approach is in the early developmental stage and has mainly focused on determining their prevalence in wastewater and optimizing monitoring pipelines, such as sampling, concentration, nucleic acid extraction, and enumeration (Ahmed et al., 2023a; Ahmed et al., 2023b; Ahmed et al., 2023c; Kilaru et al., 2023; Markt et al., 2023).

The selection of targets for WBS is affected by local needs, microbiological evidence, and the availability of resources. Each community can have its own special needs for monitoring a target, and local health authorities and epidemiologists are the main end-users of WBS data. Therefore, fulfilling their demands, i.e., regarding the types of data they need and how they want to receive the data, can affect the selection of surveillance targets and presentation of data, respectively. Thus, identifying the priorities and needs of public health authorities can be useful when defining the targets for WBS. Targets with significant surveillance gaps in clinical surveillance and those that are useful for social interventions (such as announcing advisories or launching mass vaccination campaigns) might be the ones prioritized.

Finland has already used WBS for monitoring poliovirus since the 1960s (Hovi et al., 2001; Pöyry et al., 1988), illicit drug use since the 2010s (Kankaanpää et al., 2014; Kankaanpää et al., 2016) and coronavirus disease-2019 (COVID-19) almost from the beginning of the pandemic (March 2020) (Pitkänen & Gunnar, 2022). WBS has been an important management tool for determining spatial and temporal trends, confirming slowdowns of outbreaks, providing early warning of virus (re-)emergence, following the introduction of new variants, and estimating the size of the population infected with COVID-19 (Tiwari et al., 2022b). After the onset of the COVID-19 pandemic, a three-year project named "Wastewater-based Surveillance as Pandemic Preparedness Tool (WastPan)" was started in Finland (Pitkänen, 2023). Overall, WastPan aimed to integrate WBS of communicable diseases and AMR pathogens into national pandemic preparedness as an early warning tool. Specifically, it aimed to (a) establish a detection and quantification methodology for clinically relevant pathogens, including AMR pathogens and related genes, in community wastewater, (b) identify the existing temporal trends and geographical distribution of communicable pathogens, (c) examine the potential of studying the metagenome content of wastewater to reveal emerging trends in communicable disease prevalence and (d) develop a platform to support the open sharing of

environmental data to enable WBS (Pitkänen, 2023).

Herein, we share experiences and understanding gained during the WastPan project, with special consideration of the expansion of WBS beyond COVID-19 for monitoring multiple pathogens. This paper reports our experiences concerning: 1) the selection of the priority targets for monitoring; 2) the definition of sample location and sampling frequency; 3) the definition of sample logistics and the analytical laboratories that would perform the actual work of the surveillance programme; and 4) the communication of wastewater data to health authorities, the public and other stakeholders. This information can be useful for public authorities, as well as for the research community globally, who wish to develop and expand WBS for multiple pathogens and AMR.

2. Methodology

2.1. Selection of monitoring targets

WastPan used comprehensive criteria for selecting WBS targets (Table 1). It used a knowledge-based approach by consulting and discussing with epidemiologists from the Infectious Disease Control and Vaccinations Unit of the Finnish Institute for Health and Welfare (THL, 2023). This unit is responsible for the surveillance of pathogen outbreaks in Finland. WastPan assessed the surveillance gaps in the current system based on the opinions of epidemiologists so that WBS information could fill such gaps and provide an extra line of evidence about the current seasonal and emerging outbreaks of pathogens.

The subsequent criterion for target selection in WastPan involved microbiological evidence (Gentry et al., 2023). This implies choosing targets with adequate detectable background counts, preferably exhibiting seasonal and temporal variations. However, this criterion does not apply to emerging pathogens like *Candida auris*. WBS can infer seasonal and temporal variations in the occurrence of pathogens, thereby helping authorities in preparing timely interventions (Gentry et al., 2023). This is especially relevant for viral outbreaks with mild symptoms that follow epidemic patterns, for which clinical testing can be infrequent. WBS aids in comprehending their spatial and temporal trends, potentially bridging surveillance gaps for emerging pathogens without reliable monitoring systems.

Moreover, regarding AMR pathogens, we selected for WBS those that are clinically the most relevant globally (Elstrøm et al., 2019; Graber et al., 2021; Pinholt et al., 2019; Southon et al., 2020), and such pathogens may be acquired via travel (Tiwari et al., 2024). Many of our AMR targets have been included in the current public health surveillance system. However, the current public health surveillance is based on clinical cases, and as many AMR pathogens monitored in WBS are majorly part of the gut microbiota providing their information on both clinical and asymptomatic carriage in the community (Hendriksen et al., 2019; Karkman et al., 2020; Pärnänen et al., 2019). Therefore, WBS enables preparedness for this silent pandemic, as if an AMR pathogen increases in wastewater, there can be an increased risk of a lack of effective medication. Furthermore, WastPan used Oxford Nanopore metagenomics (Wu et al., 2022), and high-throughput qPCR (Karkman et al., 2016; Lai et al., 2021; Majlander et al., 2021) methods for screening potential emerging antibiotic resistance genes (ARGs) at the population level.

Table 1
Summary of criteria for selecting microbial targets for wastewater-based surveillance.

	Category	Description
Epidemiological evidence		
1	Inconsistent surveillance	Clinical cases frequently reported, but public health surveillance currently inadequate
2	Actionable	Surveillance of targets can guide public health actions for mitigating the disease burden, e.g., virus infections with mild symptoms are often not subject to clinical testing
Microbiological evidence		
3	Prevalence	Not ubiquitous in large numbers, but having some background level that is high enough to be monitored and whose changes can easily be detected
4	Seasonality	Having some level of seasonal variation
5	Source	Community acquired rather than through travelling, as the latter is difficult to predict, so it may require a high frequency of sampling
6	Reservoirs and routes	Targets for which knowledge of the reservoirs and sources is lacking (e.g., Enterohemorrhagic *E. coli*)
7	Early detection and warning	Potential for pandemic preparedness. Targets with pandemic potential are of particular interest. The threshold of WBS detection needs to be low for very early warning of the introduction of a pandemic agent

2.2. Definition of sampling sites and sampling frequency

A high sample coverage increases the approximation of the actual population size. However, increasing the sample coverage always comes with the trade-off of an increased demand for resources and practical challenges. Thus, the best option can be an informed and appropriate selection of sample coverage (with good geographical representation), which does not necessarily require the largest sample size. Such a selection can optimize WBS at a minimal financial cost with fewer practical challenges (Andrade, 2020). As part of WastPan, we sought to determine the appropriate sample coverage, i.e., the minimum number of WWTPs, but with a good representation of the whole nation. WWTPs for sample collection were selected after comprehensive consideration of the geographical distribution of WWTPs in all 21 Wellbeing Services Counties (i.e., administrative divisions based on hospital coverage area) of Finland, the population size, the proximity of farming areas, and the proximity of the international border.

WastPan collected wastewater samples from 10 WWTPs serving 44 Finnish municipalities, including densely populated areas and/or key gateways to the nation (international airports and seaports, Helsinki, Espoo, Turku, Oulu, Tampere, Kuopio, Rovaniemi). The selection also included Lappeenranta, a city on the eastern border with Russia, and small cities with notable animal farming and slaughterhouse activities (Seinäjoki, Pietarsaari), aiming for comprehensive geographical coverage across the country. These municipalities collectively represent 40 % of Finland's population (about 2.2 million out of the 5.5 million total population of Finland) (Table S1).

We evaluated the WBS for coverage and frequency for ESBL-producing *E. coli* and *K. pneumoniae* as model targets. We collected all clinical cases of these model targets reported to the National Infectious Diseases Register (NIDR) between January 2018 and August 2021. Based on the municipality of residence of the ESBL-producing *E. coli* and *K. pneumoniae* cases in the NIDR database, we compared the incidence of ESBL cases nationwide with the incidence for the service area of: a) 28 WWTPs that are included in the national illicit drug surveillance and were included in early SARS-CoV-2 WBS, b) the smaller-scale representative network of 10 WWTPs proposed for WastPan and c) the WWTP serving the capital region.

A challenge in calculating cases per service area arose because the service areas for certain WWTPs precisely aligned with entire municipal territories, while for others, the WWTP service areas encompassed only parts of municipalities (Tiwari et al., 2022b). To account for the lack of perfect overlap between the WWTP service areas and the boundaries of the municipalities as reported in the NIDR, we adjusted the case numbers per WWTP service area with the use of correction factors as described earlier in detail (Tiwari et al., 2022b). The correction factors describe the percentage of the total population living in the respective municipalities that are serviced by the WWTP. The monthly incidence rate in the service area of each WWTP was calculated with the following equation:

$$I = \frac{N.C}{P} \bullet 100,000 \quad (1)$$

where I = the incidence per 100,000 inhabitants or the serviced population, N = the monthly number of new cases notified in the NIDR, C = a correction factor defined based on the total population of the municipality and population serviced by the WWTP, with C = 1 when the entire population of the municipality is serviced by the WWTP, and P = the total serviced population.

In this paper, we compare the trend of ESBL-producing *E. coli* in wastewater monitoring during the WastPan study years with the clinical data trends from 2018 to 2021. We monitored ESBL-producing *E. coli* in wastewater eight times per year from February 2021 to February 2023 by collecting a total of seventeen 24-h composite influent samples from 10 WastPan WWTPs. ESBL-producing Gram-negative bacteria were selectively isolated using CHROMagarESBL (CHROMagar™, Paris, France) with a spread-plate technique. After incubation at 37 °C for 18–24 h, presumptive *E. coli* isolates were confirmed based on colony colour morphology, and some of them were confirmed with MALDI-TOF, as reported earlier (Tiwari et al., 2022c; Tiwari et al., 2023b).

2.3. Sampling, analysis and calculation of results

WastPan monitored 13 viruses (pathogenic and faecal indicators), six bacteria (pathogenic and faecal indicators), eight multidrug-resistant bacteria, clinically relevant antibiotic resistance genes (ARGs), two parasites, and one pathogenic fungus (Table 2). The reason for monitoring faecal indicator bacteria and viruses was to collect background information on the faecal microbiota, and such targets can be also used for normalization of the dilution of wastewater, as performed earlier (Langeveld et al., 2023).

The project was carried out by a governmental public health laboratory [Expert Microbiology Unit of the Finnish Institute for Health and Welfare (THL)], an environmental health research group [Tampere University (TAU)], and a zoonotic antimicrobial resistance research group [Faculty of Veterinary Medicine at the University of Helsinki and the Finnish Food Authority (UH)]. THL monitored faecal bacteria with selective culture-based methods and bacteria, parasites, and viruses with qPCR and RT-qPCR methods, depending on whether the nucleic acid template was either DNA or RNA, respectively (Hokajärvi et al., 2013; Kauppinen et al., 2019; Tiwari et al., 2018; Tiwari et al., 2022b), and ARGs with high-throughput qPCR (Karkman et al., 2016; Majlander et al., 2021). TAU monitored DNA viruses with qPCR methods and RNA viruses with RT-qPCR methods (Länsivaara et al., 2023a; Länsivaara et al., 2023b; Lehto et al., 2023). UH monitored clinically relevant multidrug-resistant pathogens with selective isolation (Heljanko et al., 2023; Heljanko et al., 2024; Tiwari et al., 2022c; Tiwari et al., 2023b), and ARGs with Oxford Nanopore-based metagenomics approach (Wu et al., 2022).

Table 2
Microbial targets analysed monthly in the WastPan project 2/2021–2/2023 from community wastewater in 10 Finnish cities with a national coverage of 40 % of the population (about 2.2 million out of the 5.5 million population).

Microbial group	Monitoring targets
Viruses	SARS-CoV-2, HCoV-OC43, HCoV-229E, Influenza A virus, Respiratory syncytial virus (pan assay), Human metapneumovirus, Adenovirus, Norovirus (GI and GII), Sapovirus, Enterovirus, Rhinovirus, Pepper mild mottle virus (PMMoV), Cross-assembly phage (CrAssphage)
Bacteria	*Campylobacter*[1], *Salmonella*[1], *Escherichia coli, Enterococcus, Pseudomonas aeruginosa, Clostridium difficile*[1]
Antimicrobial-resistant (AMR) pathogens[1]	*Escherichia coli* (ESBL, CP), *Staphylococcus aureus* (MR), *Enterococcus* species (VR), *Enterobacter* species (CP), *Klebsiella pneumoniae* (CP), *Acinetobacter baumannii* (CP), *Pseudomonas aeruginosa* (CP), *Citrobacter freundii* (CP)
Antibiotic resistance genes (ARGs)	Environmental DNA targeted with high-throughput qPCR and Oxford nanopore metagenomics
Parasites	*Giardia, Cryptosporidium*
Fungi[1]	*Candida auris*

[1] Sampled eight times/year, SARS-CoV-2 = severe acute respiratory syndrome coronavirus 2, HCoV = human coronavirus, ESBL = extended-spectrum beta-lactamases, CP = carbapenemase producing, MR = methicillin resistant, VR = vancomycin resistant, PMMoV monitoring was only started in December 2021.

The reproducibility of the monitoring workflow was confirmed by interlaboratory comparison tests for SARS-CoV-2 as a model organism in two laboratories with expertise in WBS of SARS-CoV-2, here named Laboratory 1 and Laboratory 2. Samples collected from all 10 WWTPs between 21 February 2021 and 11 December 2022 were employed for this inter-laboratory comparison (Table S2). The samples were kept at 4 °C as soon as they arrived at the laboratory and were analysed within 24–48 h, as previously described (Hokajärvi et al., 2021; Tiwari et al., 2022b). Both laboratories followed the same protocol for virus concentration in wastewater, as described earlier (Hokajärvi et al., 2021). Briefly, samples were centrifuged after removing the particulate debris, and the supernatant was concentrated using a Centricon® Plus-70 centrifugal ultrafilter. Sterile deionized water was used as a negative process control. Both laboratories identically extracted RNA by taking 300 μL of the concentrate and used a Chemagic Viral300 DNA/RNA extraction kit with the Chemagic-360D instrument (Perkin-Elmer, Germany) and PerkinElmer Chemagic Viral DNA/RNA 300 (Wallac Oy, Turku, Finland). The elution volume was 50 or 60 μL for both laboratories. Laboratory 1 used a COVID-19-positive swab sample from a clinically diagnosed patient as a positive control, and molecular-grade water was used as a negative control in both laboratories, as done earlier (Tiwari et al., 2022b). Laboratory 1 used plasmid standard from IDT (#10006625, 2019_nCoV_N Positive Control) and Laboratory 2 synthetic RNA (Codex DNA, CA, USA) with 10-fold dilution series (1–10,000 copies/μL) for relative quantification of SARS-CoV-2 gene copies.

RT-qPCR assays were performed using a QuantStudio 6 Flex real-time PCR system (Applied Biosystems, ThermoFisher Scientific) in Laboratory 1 and a QuantStudio 5 real-time PCR system (Applied Biosystems, ThermoFisher Scientific) in Laboratory 2, using TaqMan Fast Virus 1-Step Master Mix according to the manufacturer's protocol (Applied Biosystems by Thermo Fisher Scientific, Vilnius, Lithuania). Laboratory 1 only used the US CDC N2 assay, but Laboratory 2 used both US CDC N1 and N2 primer-probe sets (Table S3). For Laboratory 2, the reaction mixture for the TaqMan N1 and N2 assays included 6.25 μL of the TaqMan Fast Virus 1-step Master Mix, 200 nM forward primer, 200 nM reverse primer, 200 nM probe, and 5 μL template. Non-diluted and 10-fold-diluted fractions of the extracted nucleic acids of each wastewater sample were analysed in duplicate. Laboratory 1 used mengovirus as an internal process control for estimating the recovery efficiency, as described earlier (Tiwari et al., 2022b).

3. Results and discussion

3.1. Selection of monitoring targets

The WastPan project comprehensively selected priority targets and WWTPs providing wide national coverage for WBS of these pathogens in Finland and piloted the monitoring for two years. Its targets covered a wide range of pathogens causing respiratory diseases and gastrointestinal infections, multidrug-resistant pathogens, and several ARGs (Table 2). Comprehensive evaluation and prioritization of targets can be the best solution when resources are limited, and mainly when there are no clear legislative requirements and no social pressure due to pandemics. Many of the pathogens selected in the WastPan project had earlier been reported in Finnish wastewater (Kauppinen et al., 2019; Laine et al., 2011).

Target selection for WBS is influenced by local needs, microbiological evidence and resource availability. Each community can have its own unique reasons for monitoring specific targets. The selection of targets based on local health authority needs and priorities can enhance surveillance actions and address current surveillance gaps. Information derived from WBS of such selected targets needs to impact public health action. For example, many viruses cause flu-like symptoms, so knowing the circulation of pathogens in a community may help in timing the local vaccination campaigns and selecting appropriate vaccines. Respiratory disease is one of the major causes of morbidity and mortality in Finland (Statistics Finland, 2021). In many cases, these epidemics are silent due to mild symptoms, and infections rarely require clinical testing, as the epidemics are self-limiting.

WastPan results demonstrated that not only SARS-CoV-2 but also other respiratory viruses and AMR pathogens can be quantified for outbreak detection purposes from wastewater (Länsivaara et al., 2023b; Lehto et al., 2023; Tiwari et al., 2023a). Based on these promising results, Finland is currently planning to expand the national surveillance programme to include influenza A virus and respiratory syncytial virus (RSV) as new targets. WBS of various respiratory viruses such as SARS-COV-2, influenza A and RSV has been carried out also elsewhere (Ahmed et al., 2023d; Boehm et al., 2023). Boehm et al. reported various respiratory viruses (influenza A and B viruses, RSV A and B viruses, parainfluenza viruses, rhinoviruses, seasonal coronaviruses and metapneumovirus) in wastewater in California, USA (Boehm et al., 2023). The reported virus concentrations and detection rates were concordant with viral diseases in sentinel laboratories (Boehm et al., 2023). The surveillance infrastructure built during COVID-19 is useful and still available for surveillance, and for the development of WBS for multiple other pathogens in the future (Pruden et al., 2021). As there will certainly be resource limitations, it might be best to focus on priority pathogens with pandemic potential that cause severe infections. Recently, Gentry et al. discussed the importance of the prioritization of targets during WBS of community diseases based on epidemiological knowledge and microbiological evidence (Gentry et al., 2023).

The WastPan project categorized communicable disease agents into four priority groups based on expert evaluation of diseases, and transmission mode of the pathogens, and the estimated level of priority to generate epidemiological surveillance data from wastewater to support the other available health indicators (Fig. 1). Based upon subjective experience rather than empirical evidence, the surveillance goals and sampling frequency requirements vary depending on the pathogen groups (Keshaviah et al., 2021). For pandemic preparedness and absence verification, more frequent sampling may be needed in large cities and travel hubs (Williams et al., 2023). For long-term trend detection, less frequent sampling with broader population coverage may suffice (Williams et al., 2023). In Finland, targets from groups 2 and 3 are notably affected by international travelling (Fig. 1). Thus, conducting WBS for these targets at least during peak travel months (January and August) and tentatively also in May is proposed.

The proposed revised Urban Wastewater Treatment Directive (UWWTD) of the European Union, Article 17, has a provision for urban wastewater surveillance (EU Regulation 2020/741, 2022). Based on the recast proposal, Member States are required to establish a national system for cooperation and coordination between health authorities and wastewater treatment authorities. The intention of the national systems would be to identify essential public health parameters, including SARS-CoV-2 and its variants, poliovirus, influenza virus, emerging pathogens and any other relevant public health parameters, which are to be monitored at least in the inlets at wastewater treatment plants (EU Regulation 2020/741, 2022). Therefore, many of the pathogens piloted in WastPan may potentially continue as targets for WBS in Finland and elsewhere.

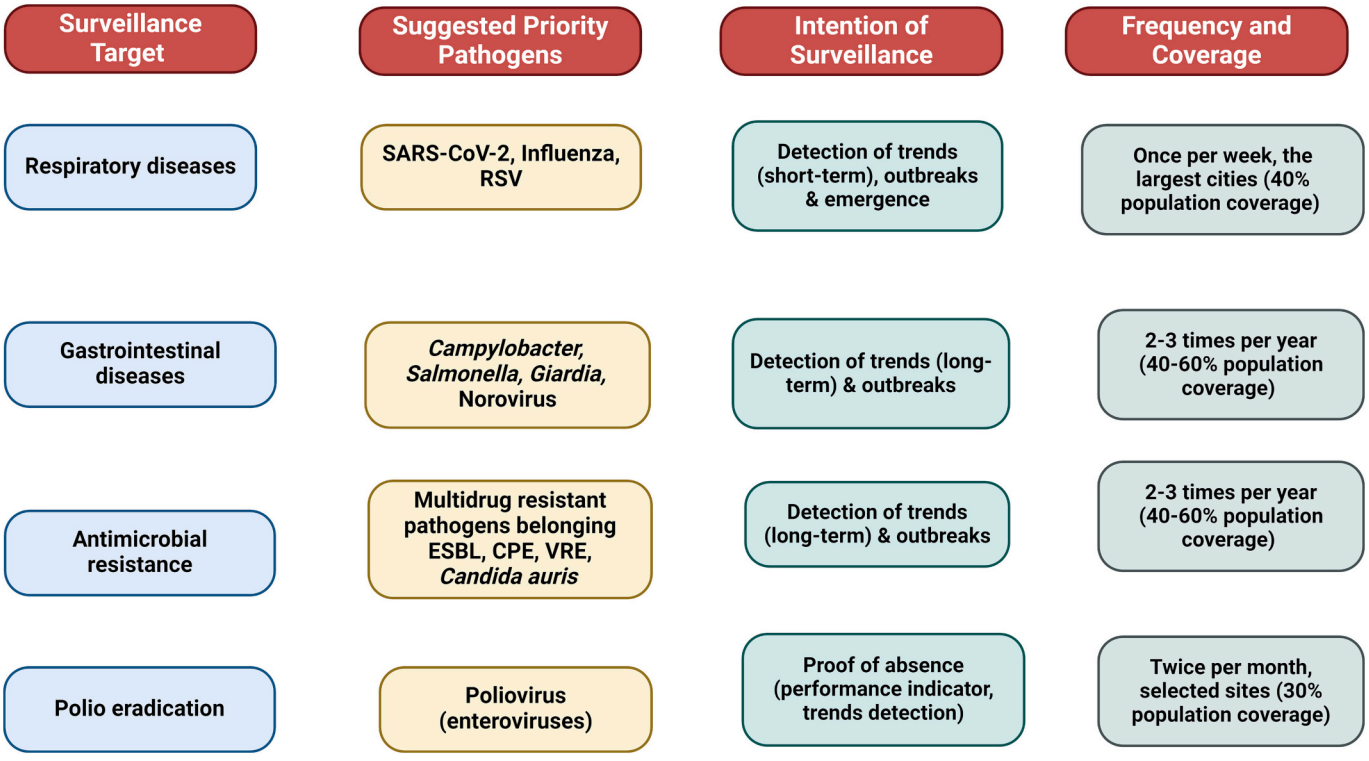


Fig. 1. Division of wastewater surveillance targets into four priority groups in the WastPan project and the proposal for suitable frequency and coverage of the sampling for national surveillance purposes. The proposal is based on the stakeholder views, including the national public health authority, about the surveillance needs and possibilities taking into account the practicalities such as logistics and presumed resource limitations. SARS-CoV-2 = Severe acute respiratory syndrome coronavirus 2, RSV = Respiratory syncytial virus, regarding multidrug resistant pathogens belonging to ESBL and CP were targeted *E. coli, K. pneumoniae, and Citrobacter freundii*, ESBL = Extended-spectrum beta-lactamases, CPE = Carbapenemase producing *Enterobacterieae*, VRE = Vancomycin-resistant enterococci.

3.2. Monitoring workflow and selection of methods

The 24-h composite samples collected from each WWTP (Table S1) were shipped in cold boxes to the respective laboratories for analysis of the targets. WastPan used a culture-based approach for selective isolation and enumeration of faecal bacterial pathogens, faecal indicator bacteria, fungal counts, and antibiotic-resistant bacteria, evaluating their antibiotic susceptibility, as well as for whole-genome sequencing (Heljanko et al., 2023; Heljanko et al., 2024; Tiwari et al., 2023a). Culture-based methods provide evidence of the prevalence of viable targets in wastewater samples that can be grown in a selective culture media. Culture-dependent methods are still the gold standard methods in clinical settings across the globe, and for counting colonies of faecal indicator microbes from water samples too (Tiwari et al., 2021). As a limitation, culture-dependent methods cannot monitor many environmental microbes. Culture-independent methods (PCR-based and metagenomics) overcome these culturing biases.

WastPan used molecular methods for analyzing viruses, some bacteria, ARG, and parasites. Regarding the laboratory workflow, water samples were pre-centrifuged, the supernatant was used for virus analysis, and the pellet fraction was used for the analysis of bacteria, ARG, and parasites. The qPCR/RT-qPCR methods are rapid and useful for understanding the spatial and temporal distribution of microbial targets and also for routine surveillance of targets in wastewater (Tiwari et al., 2022a). These methods can be more sensitive than culture-based methods, as they enable the measurement of both culturable and difficult-to-culture strains, inactivated microbes, and even free nucleic acids in wastewater (Pitkänen et al., 2013).

WastPan used sequencing-based methods to monitor the prevalence of different SARS-CoV-2 variants in wastewater. Monitoring of SARS-CoV-2 variants through sequencing requires a PCR amplification step, which increases the abundance of genes to a detectable level (Tiwari et al., 2023a). In general, sequencing and metagenomics methods are used to define phylogeny and for screening novel genes, variants, and species. The metagenomics approach further elucidates the functional potential of a microbial community and does not require previous information about the pathogens circulating in the community. However, sequence-based methods can have disadvantages due to possible biases introduced by PCR amplification, the selection of primer pairs, and also because the results are more likely to be affected by the most frequent species (Tiwari et al., 2023a). Moreover, both sequencing and metagenomics methods may be unable to detect many low-abundance organisms (Tiwari et al., 2023a).

3.3. Definition of sampling sites and sampling frequencies

The selection of WWTPs and sampling frequency was validated for reported ESBL *E. coli* and *K. pneumoniae* cases in the NIDR in Finland (Fig. 2). Indeed, it is essential to note that this validation was not extended to all pathogens, and the unique epidemiology of each pathogen may influence the choice of sampling sites and frequencies.

The NIDR-reported incidence in the sewershed areas of the 28 WWTPs, and the 10 WastPan WWTPs align well with the national trend in ESBL cases (Fig. 2). The 28 WWTPs were involved in illicit drug

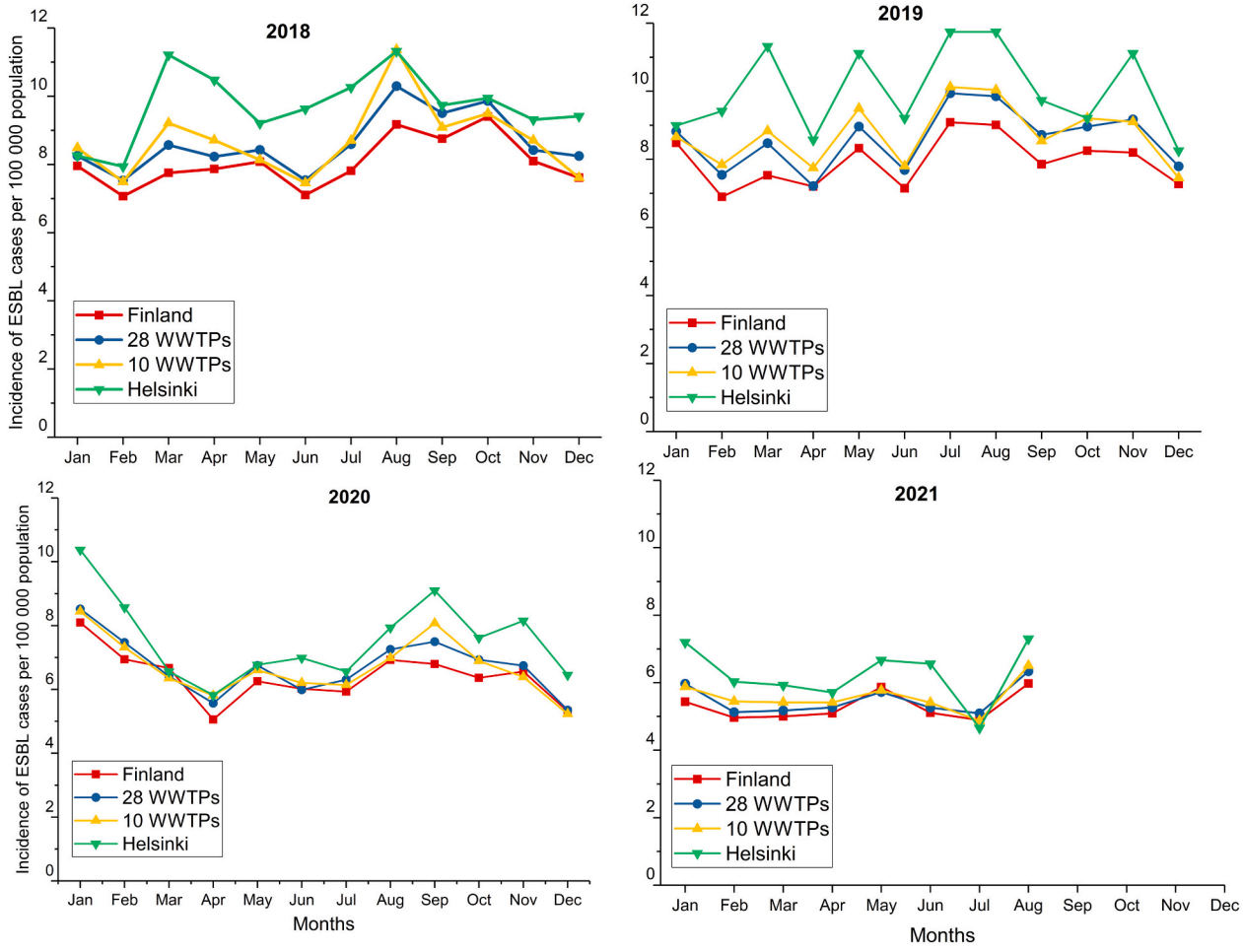


Fig. 2. The monthly incidence of NIDR-notified ESBL *E. coli* and *K. pneumoniae* cases per 100,000 population. Finland covers the entire nation, while 28 and 10 WWTPs and Helsinki represent the reported cases with their home address in the sewer catchment areas of the respective treatment plants.

monitoring (Kankaanpää et al., 2014; Kankaanpää et al., 2016), and early national SARS-CoV-2 surveillance (Tiwari et al., 2022b), sampling from all 28 WWTPs would provide a more comprehensive representation of nationwide trends than limiting it to the 10 WastPan WWTP areas. However, the cost and time savings of sampling only from these 10 WWTPs surpass this drawback. Focusing solely on Helsinki (Viikinmäki) WWTP would further compromise representativeness, but it could be a viable option for capturing trends, especially if other choices are unavailable. Monitoring Helsinki offers an early warning for imported infectious agents, considering its significance as a major gateway to the country, and the Viikinmäki WWTP being the largest in the Nordic countries (Viikinmäki, 2021). Consequently, WastPan identified the selection of 10 WWTPs including Helsinki, as the optimal and cost-effective approach for regular surveillance of AMR and infectious agents (Table S1).

During the four years covered in the analysis of NIDR data, ESBL *E. coli* cases surpassed *K. pneumoniae* cases in Finland (total reported cases = 17,201; 88.73 % *E. coli* and 11.27 % *K. pneumoniae*) (Fig. 2). The total number of reported cases was around 400–500 per month for the first two years (2018–2019) but dropped to around 300–400 cases during the COVID-19 pandemic (2020−2021). The reason behind the fewer ESBL cases during the COVID-19 period could be reduced testing and restrictions for international travel (Tiwari et al., 2024). Many earlier studies have reported international travel and hospitalization abroad in AMR hotspot regions are the main sources of ESBL and CPE in Finland and other Nordic countries (Elstrøm et al., 2019; Graber et al., 2021; Österblad et al., 2012; Pinholt et al., 2019; Southon et al., 2020; Tiwari et al., 2024). The monthly trends in ESBL cases and incidence within the catchment area of 10 WastPan WWTPs closely mirrored those in the area of the 28 WWTPs, the nationwide trends, and to a lesser extent, the catchment area of the Helsinki WWTP (Fig. 2).

The monthly fluctuation in ESBL cases in the NIDR clinical dataset was moderate, with no clear seasonal patterns being observed, except for a modest increase during the post-holiday months (August and January), likely linked to travel patterns. Therefore, we propose sampling in August and January due to the heightened incidence of ESBL *E. coli* during these months in Finland. For AMR pathogens, seasonal sample collection might suffice, given their slower pathogenicity changes compared to viruses, eliminating the need for monthly sampling in Finland. ESBL *E. coli* is proposed as an AMR indicator for WBS due to its high wastewater prevalence (WHO, 2015). Currently, clinical testing provides limited evidence of its population prevalence temporal and spatial variations.

In wastewater monitoring, out of the total of 168 samples analysed from 10 WWTPs during 17 sampling events, the minimum, median and maximum counts of ESBL *E. coli* were 12.1, 13.3 and 15.0 log10 CFU/24-h influent flow/100000 population, respectively. From Helsinki WWTP, all samples surpass the median value of ESBL *E. coli* CFU counts. At the WWTP located in Espoo, which treats about half of the wastewater produced in the capital area of Finland, 10 out of 16 samples surpassed the median value among the total of 168 samples analysed in this study. In Tampere, Oulu, and Rovaniemi, other larger cities had 8, 7, and 5 samples respectively out of 17 total samples analysed exceeded the median value out of 168 samples (Figs. S1 and S2). This suggests that significantly higher ESBL counts were found in major gateway cities to Finland, potentially imported from abroad. This aligns as previously mentioned, Nordic countries have largely imported AMR cases (Tiwari et al., 2024).

However, over both years, August stood out with a high count (15 out of 20 samples had >168 samples median value) of ESBL *E. coli* compared to other months. When combining all samples, the peak average ESBL *E. coli* CFU/100,000 population per 24 h occurred in May and July 2021, contrasting with August and September 2022 (Fig. 3). This contrasts with our earlier assumption that January could see higher counts due to increased international travel returning home. Nevertheless, the monthly fluctuations in ESBL *E. coli* counts within wastewater samples showed significant variability in each WWTP (Fig. S3).

Monitoring of ESBL *E. coli* in wastewater provides insights into both potential infections and the asymptomatic carriage of these bacteria within the sewershed population's microbiota. The WHO Tricycle Protocol recognizes ESBL *E. coli* as an excellent AMR indicator from a One Health perspective (WHO, 2021).

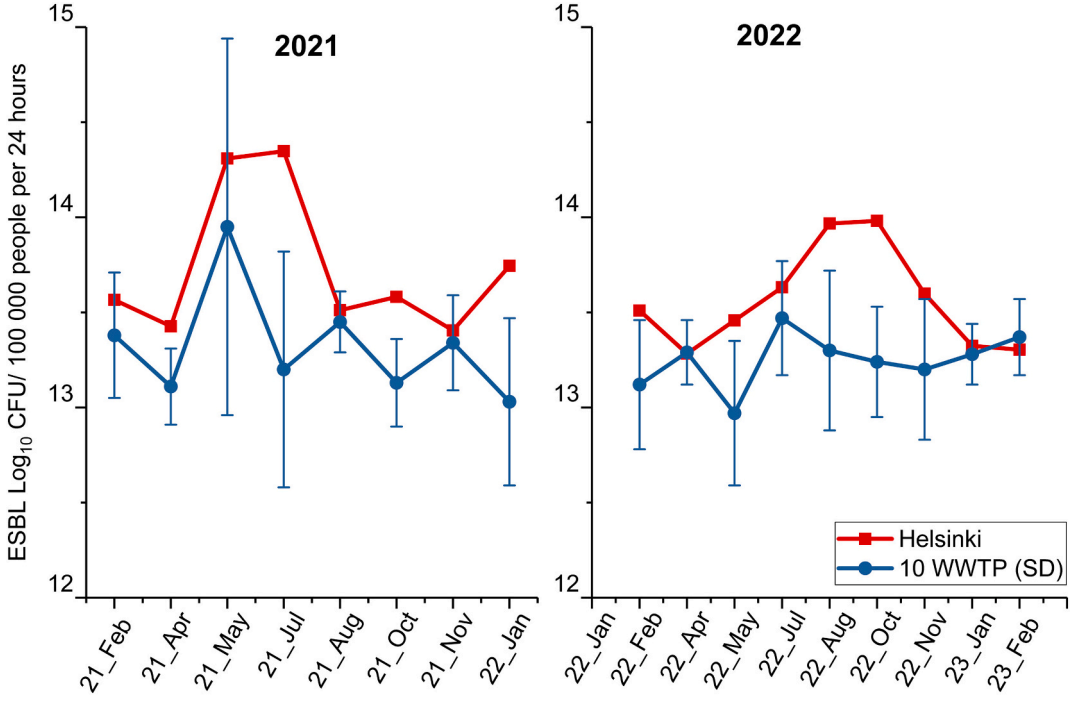


Fig. 3. The monthly variation in ESBL *E. coli* counts (Log_{10} CFU/day/100000 population) in wastewater samples during the WastPan study years (SD = ± standard deviation). Helsinki refers to one out of the 10 WastPan WWTPs, the Viikinmäki wastewater treatment plant, located at Helsinki.

3.4. Interlaboratory and gene assay comparisons

Reproducible methods are important in all laboratory analysis, including microbiological and WBS investigations. Herein, an inter-laboratory and inter-assay comparison study was conducted for SARS-CoV-2 RNA by considering the detection rate monitored in parallel in Laboratory 1 using the N2 assay and Laboratory 2 using the N1 and N2 assays. Details of the RT-qPCR reactions are presented in Table S4. The detection rate of SARS-CoV-2 between Laboratories 1 and 2 with the N2 assay (Table 3A) was concordant (McNemar test, $p = 0.146$), and detection in wastewater followed similar trends to COVID-19 clinical cases (Table S5). When comparing SARS-CoV-2 gene copies, we observed no relationship (Pearson correlation coefficient or Pearson's $r = -0.022$, linear regression coefficient, the goodness of fit: $R^2 = -0.0062$) (Fig. 4A). Such a poor relationship can be attributed to variations in the standard materials used for the relative quantification of genome copies. Lab 1 utilized IDT plasmid and Lab 2 employed Codex synthetic RNA as the standard material for quantifying SARS-CoV-2 RNA. While not confirmed, potential human-induced biases, possibly linked to staff turnover in Lab 2, cannot be dismissed, as the relatively higher estimation of gene copies was systematically observed in the samples analysed in a few months (Fig. 4A and B).

SARS-CoV-2 RNA was more frequently detected using the N2 assay (Laboratory 1: 91.5 %; Laboratory 2: 87.4 %) compared to the N1 assay (Lab 2 only, 76.6 %) (McNemar, $p < 0.001$ Lab 1, $p = 0.006$ Lab 2) (Tables 3B and 3C). When comparing SARS-CoV-2 RNA detection in wastewater using N1 and N2 assays with clinically reported cases, N2 assays detected the virus even when there were few clinical cases (Table S5). The relative quantification of SARS-CoV-2 RNA was higher with the N2 assay than with N1 assay in both laboratories (Fig. 4B and C). There was a strong relationship between SARS-CoV-2 gene copies monitored with N2 assay in Lab 1 and N1 assay in Lab 2 (Pearson's $r = 0.8358$, the goodness of fit: $R^2 = 0.6956$) (Fig. 4C).

Sensitivity of the monitoring methods used for WBS is important since the monitoring results can be used to provide an early warning of pandemic threats and enable the monitoring of low abundance targets (Länsivaara et al., 2023b; Tiwari et al., 2022b). Bustin et al. defined the analytical sensitivity of a method as its ability to detect minimum genome copies (GC) with reasonable certainty or a minimum number of GC that can be reliably detected with a given analytical system (Bustin et al., 2009). Variations in detection rates with the N1 and N2 assays within the same monitoring workflow can be attributed to various factors such as genomic variability, assay sensitivity and specificity, and mutation changes, and have been reported in many earlier studies (Gonzalez et al., 2020; Matsumura et al., 2021; Nalla et al., 2020).

3.5. Communication of results

While communication is an important component of WBS, its approach has not fully developed in the WBS, in comparison to the conventional clinical-based approach (Tiwari et al., 2022b). A communication dashboard informs authorities and stakeholders promptly about WBS results, through user-friendly data visualizations (Hill et al., 2023; Wettstone et al., 2023).

WastPan aimed to integrate and utilize all the information produced in the project in developing an open platform to enable future WBS initiatives, i.e., a pandemic preparedness platform. WastPan developed a dashboard for communicating WBS data to wide stakeholders, including communities, epidemiologists, healthcare providers, government agencies, and others (Pitkänen, 2023). The goal is to engage stakeholders and raise awareness about the usage of WBS (https://www.thl.fi/episeuranta/jatevesi/wastpan/en/).

During portal development, we initially mapped existing water supply network coverage and analysed available background data from WWTPs at sampling times. Our dashboard reports both microbial target loads and background wastewater characteristics, aiding public health professionals and relevant stakeholders in interpreting results. The dashboard offers insights into spatial and temporal variations in target concentrations, enabling stakeholders to assess pathogen prevalence and make informed decisions, such as regarding resource allocation and vaccine distribution.

WastPan has prepared an open data entry and transfer interface that can be used for monitoring and modelling epidemics and microbiological trends. The goal is to develop an automated comparison of wastewater-based findings with other surveillance databases, such as NIDR. The influenza A virus (Lehto et al., 2023) and RSV (Länsivaara et al., 2023a) were chosen as pilot microbes for the results website, whose reports contain observation matrices of the results, followed by treatment plant-specific graphs and, where possible, the corresponding NIDR cases. The map view is based on each microbe and is published as detected or not detected. All WastPan results are archived on the WastPan website as an electronic data source after publication and will be openly available. In addition, the results pages contain links to all WastPan publications for more detailed analysis information. The website has been published in both Finnish and English and is available from: https://www.thl.fi/episeuranta/jatevesi/wastpan/en/.

3.6. Challenges in multi-target WBS monitoring

WBS has not yet been fully developed, and its use has some considerable limitations and uncertainties. One of the early aims to verify the usefulness of WBS has been to establish a reliable relationship between wastewater data and clinical cases in sewershed communities, facilitating communication with epidemiologists and health authorities (Pang et al., 2022). However, the varying shedding rates of targets in different body fluids, such as urine, faeces, and oral secretions, and in different infection stages (Table S6) complicate the correlation between WBS data and clinical cases (Lowry et al., 2023). This variation is significant, even among different targets. Thus, when using WBS for multiple targets, detecting one target while not detecting another does not guarantee the absence of clinical cases in the sewershed for the undetected target. For example, a study by Lowry et al. (Lowry et al., 2023) demonstrated that the median concentration of influenza virus in stool samples from infected individuals is around 5–6 $\log_{10}$ copies per gram (Lowry et al., 2023). By comparison, Lee et al. (Lee et al., 2007) estimated the norovirus concentration in human stool samples to range from 8.5 to 10.5 $\log_{10}$ copies per gram, depending on factors such as age and diarrhoea stage (Lee et al., 2007). Therefore, to achieve equal detection rates, influenza would require a much larger number of clinical cases than norovirus in the sewershed if both have identical decay rates in wastewater distribution systems and identical responses to concentration stages and monitoring platforms. Furthermore, pathogens from a wide taxonomic range and differing in physiology and morphology may act differently during various sample processing and analysis pipelines (Ahmed et al., 2023a; Ahmed et al., 2023d). In the future, it is essential to consider WBS as an independent data source, complementing the information gained from other means of public health surveillance.

However, basic information about the pathogen excretion and stability in the wastewater systems is needed for each WBS target. For example, in the early times after the initiation of COVID-19 pandemic, we experimentally evaluated the persistence of SARS-CoV-2 RNA biomarkers in wastewater under cold and freezing storage conditions and

Table 3A
Comparing SARS-CoV-2 RNA detection in Laboratories 1 and 2 with N2 assay.

		Laboratory 1 (N2-assay)	
		Detected	Not detected
Laboratory 2 (N2-assay)	Detected	150 (86 %)	3 (2 %)
	Not detected	9 (5 %)	12 (7 %)

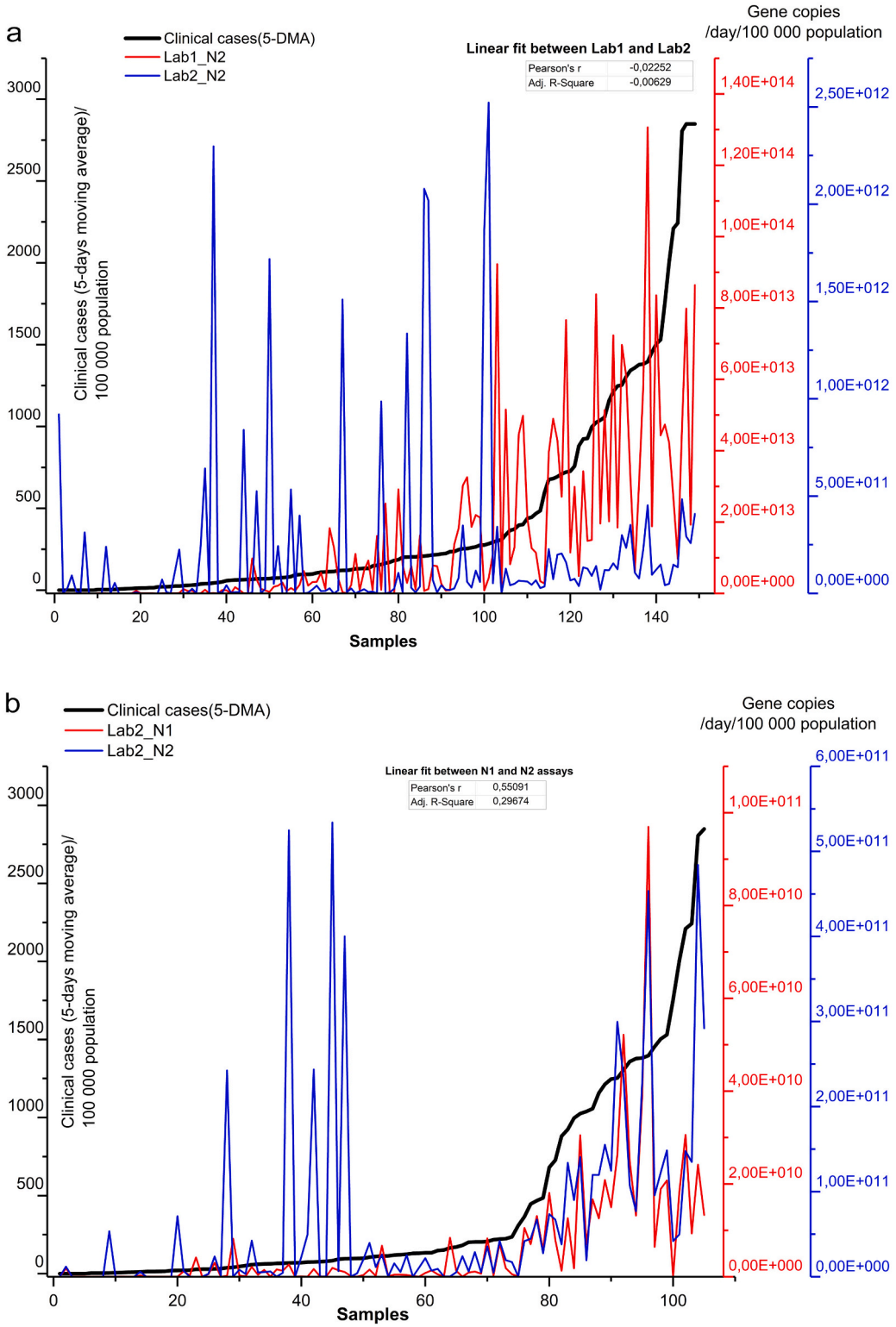


Fig. 4. Comparing SARS-CoV-2 RNA copies monitored via A) the N2 assay in Laboratories 1 and 2, B) N1 assay and N2 assay in Laboratory 2, and C) N2 assay in Laboratory 1 and N1 assay in Laboratory 2, illustrating their relationships with clinically reported COVID-19 cases on the respective sampling day. The reported clinical cases are presented as a five-day moving average. SARS-CoV-2 gene copies are normalized with the 24-h influent flow volume and adjusted per 100,000 population.

the prevalence of viral RNA in pellets or supernatant after centrifugation of samples (Hokajärvi et al., 2021). We detected a decline in viral particles over 28 days at 4 °C but not at −20 °C or − 75 °C. Further, Crank et al. analysed faecal routes as the primary source of SARS-CoV-2 in the sewage system, estimating that infected individuals release different amounts of SARS-CoV-2 via saliva, sputum, urine, and stool (Crank et al., 2022). They estimated that infected individuals contribute about 8.05 $\log_{10}$ SARS-CoV-2 GC via saliva, 7.92 $\log_{10}$ via sputum, 8.15 $\log_{10}$

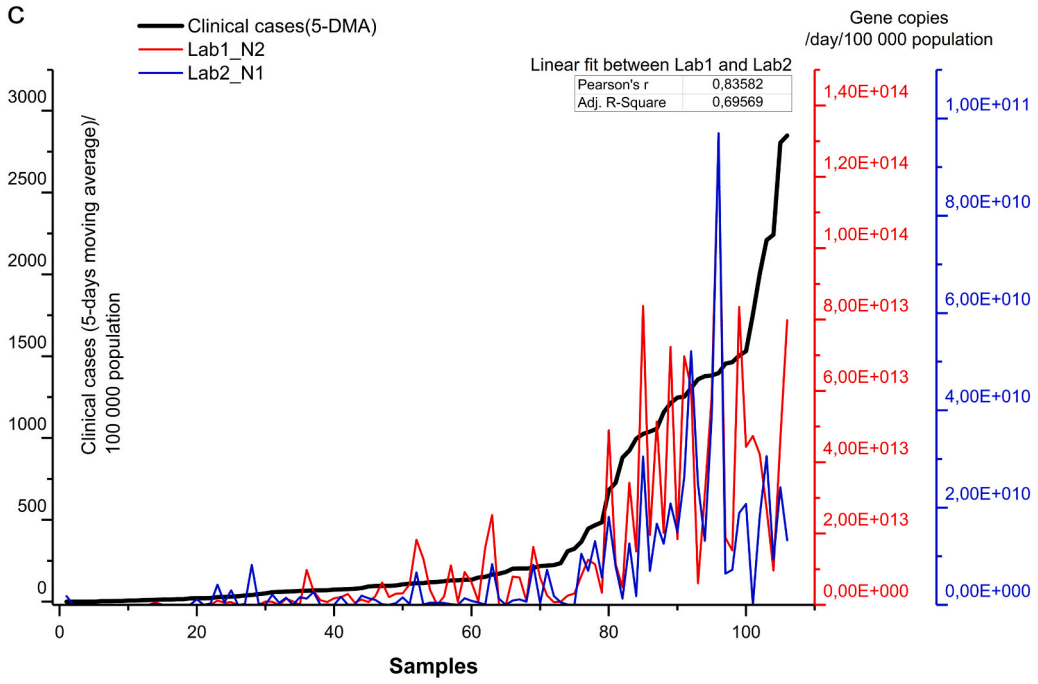


Fig. 4. (*continued*).

Table 3B
Comparing SARS-CoV-2 RNA detection: N2 assay in Laboratory 1 versus N1 assay in Laboratory 2.

		Laboratory 1 (N2-assay)	
		Detected	Not detected
Laboratory 2 (N1-assay)	Detected	95 (78 %)	0 (0 %)
	Not detected	19 (16 %)	10 (8 %)

Table 3C
Comparing SARS-CoV-2 RNA detection: N1 and N2 assays in Laboratory 2.

		Laboratory 2 (N2-assay)	
		Detected	Not detected
Laboratory 2 (N1-assay)	Detected	93 (66 %)	11 (8 %)
	Not detected	19 (14 %)	18 (13 %)

via urine, and about 10.55 $\log_{10}$ via stool in a day (Crank et al., 2022). The cumulative impact of these aspects on the target concentration in the sewage system can be substantial. However, limited longitudinal data on the shedding rates and stability of various respiratory viruses in human excretions complicates these assessments, as seen in previous studies (Lowry et al., 2023).

To effectively use wastewater monitoring for outbreak detection, long-term monitoring, the establishment of baseline levels and the definition of outbreak thresholds are essential. The definition of an outbreak threshold depends on the shedding rate, as high shedding rates can result in significant peaks with only a few clinical cases, while low shedding rates may require a larger number of cases to create noticeable peaks (Li et al., 2023). Furthermore, data on asymptomatic cases, vaccination effects on shedding, pathogen decay in the wastewater distribution system, and variations in the behaviour of different microbial targets are critical. The sensitivity of the monitoring method, the presence of PCR inhibitors in wastewater and dilution effects during weather events such as heavy rain or snowmelt should also be considered. For WBS of antimicrobial resistance (AMR) and its correlation with clinical data, additional complexities arise due to the diversity of bacterial species and strains, as well as the presence of resistance genes in mobile genetic elements (Elisabeth et al., 2021; Tiwari et al., 2022d). These resistance genes can be transferred between environmental and clinical bacteria and may originate from various sources, including asymptomatic carriers and normal human microbiota (Larsson & Flach, 2022).

Managing variations in the distance between different wastewater treatment plants and monitoring laboratories is a significant concern in WBS. Effective sample logistics, including proper sample transportation and storage, are crucial to ensure the reliability and integrity of the analytes (Ahmed et al., 2022b). Centralizing all analyses in a single laboratory ensures uniformity and consistent procedural steps, minimizing variations related to human factors, platforms, and monitoring pipelines. However, this approach introduces variation due to varying transportation distances for samples from different municipalities. The establishment of standardized protocols for sample collection, including timing, frequency, and location, is imperative to capture dynamic changes in targets in WBS.

The normalization procedures enhance the comparability of results across different sampling sites and times. Common normalization techniques involve based on factors such as wastewater flow rates or the abundance of faecal indicators (such as pepper mild mottle virus and CrAssphage) (Maal-Bared et al., 2023). These normalization methods contribute to more accurate and meaningful data interpretation, allowing for reliable comparisons and trend analyses (Maal-Bared et al., 2023). However, there is no universal solution regarding the best normalization method. WastPan has experience with the superiority of the flow normalization method over using Pepper mild mottle virus or CrAssphage (Tiwari et al., 2022b).

4. Conclusions

This paper clearly describes how the WastPan project in Finland selected priority microbial targets and extensive WWTP coverage for multi-pathogen monitoring, piloting the programme for two years. The WastPan project has demonstrated that a single composite sample from a community has a power to reveal information about diseases that are currently under clinical surveillance. WBS can offer insights into the

incidence, seasonality, locality, and reservoirs of multiple pathogens. WBS has considerable flexibility in the selection of sampling sites, surveillance targets, sampling frequency, sample storage for future analysis, the selection of monitoring methods, data analysis, and data presentation based on local needs. Comprehensive evaluation and prioritization of targets can be the best solution for using limited resources. The selection of a new target, establishment of the sampling strategy, practical arrangement of sample shipments, selection of a sensitive and specific monitoring assay, collection of baseline data, establishment of a communication and action plan, as well as the threshold for action, and integration of WBS in routine surveillance actions for public health represent a simplified pipeline for WBS of new pathogens. Thus, WBS provides an opportunity to monitor a wide range of pathogens, but the selection of pathogens for inclusion in routine surveillance should align with local epidemiological requirements. The infrastructure established during WBS for COVID-19 can serve as a valuable resource for monitoring a wide array of other pathogens, making it a cost-effective approach to gathering community-wide microbiological evidence from wastewater.

WastPan study group

The WastPan consortium members are the following: Tarja Pitkänen, Anssi Lipponen, Anna-Maria Hokajärvi, Anniina Sarekoski, Ananda Tiwari, and Aleksi Kolehmainen, from the Expert Microbiology Unit, Finnish Institute for Health and Welfare, Kuopio, Finland; Soile Blomqvist, Kati Räisänen and Carita Savolainen-Kopra from the Expert Microbiology Unit, Finnish Institute for Health and Welfare, Helsinki, Finland; Teemu Möttönen, Oskari Luomala and Aapo Juutinen from the Infectious Disease Control and Vaccinations Unit, Finnish Institute for Health and Welfare, Helsinki, Finland; Sami Oikarinen, Kirsi-Maarit Lehto, Annika Länsivaara, Rafiqul Hyder and Erja Janhonen from the Faculty of Medicine and Health Technology, Tampere University, Tampere, Finland; Annamari Heikinheimo, Viivi Heljanko, Venla Johansson, Paula Kurittu, Ananda Tiwari and Ahmad Al-Mustapha from the Department of Food Hygiene and Environmental Health, Faculty of Veterinary Medicine, University of Helsinki, Finland.

Funding

This study formed a part of the WastPan consortium project, funded by the Research Council of Finland (formerly the Academy of Finland) (grant numbers: 1339415, 1339416, 1339417), the Finnish Government, the Ministry of Agriculture and Forestry Finland, the Ministry of the Environment Finland, the Finnish Water Utilities Development Fund and the National Emergency Supply Agency Finland. Dafni Paspaliari was supported financially by the European Centre of Disease Prevention and Control.

Ethics approval

Not required.

CRediT authorship contribution statement

Ananda Tiwari: Writing – review & editing, Writing – original draft, Methodology, Investigation, Formal analysis, Data curation, Conceptualization. **Kirsi-Maarit Lehto:** Writing – review & editing, Project administration, Investigation, Data curation. **Dafni Paspaliari:** Writing – review & editing, Writing – original draft, Visualization, Formal analysis, Data curation. **Ahmad I. Al-Mustapha:** Writing – review & editing, Investigation. **Anniina Sarekoski:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Anna-Maria Hokajärvi:** Writing – review & editing, Methodology, Investigation, Data curation. **Annika Länsivaara:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Rafiqul Hyder:** Writing – review & editing, Investigation, Formal analysis, Data curation. **Oskari Luomala:** Writing- Review & editing, Methodology and Investigation. **Anssi Lipponen:** Writing – Review & editing, Investigation, Project administration, Methodology, Data curation. **Sami Oikarinen:** Writing – review & editing, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Formal analysis, Data curation. **Annamari Heikinheimo:** Writing – review & editing, Supervision, Resources, Project administration, Methodology, Funding acquisition. **Tarja Pitkanen:** Writing – review & editing, Writing – original draft, Validation, Supervision, Software, Resources, Project administration, Methodology, Investigation, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest. Kirsi-Maarit Lehto and Sami Oikarinen are the stakeholders of GreenSeq Ltd. Finland. All claims expressed in this article are solely those of the authors and do not necessarily represent those of their affiliated organizations or those of the publisher, the editors, or the reviewers. Dafni Paspaliari was a fellow of the ECDC Fellowship Programme, supported financially by the European Centre for Disease Prevention and Control. The views and opinions expressed herein do not state or reflect those of ECDC. ECDC is not responsible for the data and information collation and analysis and cannot be held liable for conclusions or opinions drawn.

Data availability

All the data produced in the project have been made publicly available in each different publication. All the data included in this paper have been made public as results and supplemental materials.

Acknowledgements

The authors would like to express special thanks to Ms. Mirka Korhonen, Ms. Marjo Tiittanen, Ms. Tiina Heiskanen, Ms. Tarja Rahkonen, Ms. Eveliina Nurmi, Ms. Annika Laaksonen and Ms. Kirsi Ristkari for their assistance in the laboratory, Ms. Kristiina Valkama for her help with sample transportation arrangements, and Ms. Suvi Hyökki for preparing the graphical abstract. We thank Professor Outi Lyytikainen for their feedback they provided on the criteria for Table 1. The personnel of the wastewater treatment facilities in all WastPan surveillance locations in Finland are gratefully acknowledged for their support and timely efforts in wastewater composite sampling and sample transportation arrangements for the work. We acknowledge university lecturer Mr. Roy Siddall, Helsinki University Language Centre, for English language revision.

Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.scitotenv.2024.171401.

References

Ahmed, W., Angel, N., Edson, J., Bibby, K., Bivins, A., O'Brien, J.W., Choi, P.M., Kitajima, M., Simpson, S.L., Li, J., Tscharke, B., Verhagen, R., Smith, W.J.M., Zaugg, J., Dierens, L., Hugenholtz, P., Thomas, K.V., Mueller, J.F., 2020. First confirmed detection of SARS-CoV-2 in untreated wastewater in Australia: A proof of concept for the wastewater survei- llance of COVID-19 in the community. Sci. Total Environ. 728, 138764 https://doi.org/10.1016/j.scitotenv.2020.138764.

Ahmed, W., Bivins, A., Payyappat, S., Cassidy, M., Harrison, N., Besley, C., 2022a. Distribution of human fecal marker genes and their association with pathogenic viruses in untreated wastewater determined using quantitative PCR. Water Res. 226, 119093 https://doi.org/10.1016/j.watres.2022.119093.

Ahmed, W., Simpson, S.L., Bertsch, P.M., Bibby, K., Bivins, A., Blackall, L.L., Bofill-Mas, S., Bosch, A., Brandão, J., Choi, P.M., Ciesielski, M., Donner, E., D'Souza, N., Farnleitner, A.H., Gerrity, D., Gonzalez, R., Griffith, J.F., Gyawali, P., Haas, C.N., Shanks, O.C., 2022b. Minimizing errors in RT-PCR detection and quantification of SARS-CoV-2 RNA for wastewater surveillance. Sci. Total Environ. 805, 149877 https://doi.org/10.1016/j.scitotenv.2021.149877.

Ahmed, W., Smith, W.J.M., Tiwari, A., Bivins, A., Simpson, S.L., 2023a. Unveiling indicator, enteric, and respiratory viruses in aircraft lavatory wastewater using adsorption-extraction and Nanotrap® microbiome A particles workflows. Sci. Total Environ. 2 (2), 165007 https://doi.org/10.1016/j.scitotenv.2023.165007.

Ahmed, W., Bivins, A., Korajkic, A., Metcalfe, S., Smith, W.J.M., Simpson, S.L., 2023b. Comparative analysis of adsorption-extraction (AE) and Nanotrap® magnetic virus particles (NMVP) workflows for the recovery of endogenous enveloped and non-enveloped viruses in wastewater. Sci. Total Environ. 859, 160072 https://doi.org/10.1016/j.scitotenv.2022.160072.

Ahmed, W., Smith, W.J.M., Sirikanchana, K., Kitajima, M., Bivins, A., Simpson, S.L., 2023c. Influence of membrane pore-size on the recovery of endogenous viruses from wastewater using an adsorption-extraction method. J. Virol. Methods 317, 114732. https://doi.org/10.1016/j.jviromet.2023.114732.

Ahmed, W., Bivins, A., Stephens, M., Metcalfe, S., Smith, W.J.M., Sirikanchana, K., Kitajima, M., Simpson, S.L., 2023d. Occurrence of multiple respiratory viruses in wastewater in Queensland, Australia: potential for community disease surveillance. Sci. Total Environ. 864, 161023 https://doi.org/10.1016/j.scitotenv.2022.161023.

Andrade, C., 2020. The limitations of online surveys. Indian J. Psychol. Med. 42 (6), 575–576. https://doi.org/10.1177/0253717620957496.

Bibby, K., Bivins, A., Wu, Z., North, D., 2021. Making waves: plausible lead time for waste- water based epidemiology as an early warning system for COVID-19. Water Res. 202 (January), 117438 https://doi.org/10.1016/j.watres.2021.117438.

Boehm, A.B., Hughes, B., Wolfe, M.K., White, B.J., Duong, D., Chan-Herur, V., 2022. Regional replacement of SARS-CoV-2 variant omicron BA.1 with BA.2 as observed through wastewater surveillance. Environ. Sci. Technol. Lett. 9 (6), 575–580. https://doi.org/10.1021/acs.estlett.2c00266.

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.

Bowes, D.A., Darling, A., Driver, E.M., Kaya, D., Maal-Bared, R., Lee, L.M., Goodman, K., Adhikari, S., Aggarwal, S., Bivins, A., Bohrerova, Z., Cohen, A., Duvallet, C., Elnimeiry, R.A., Hutchison, J.M., Kapoor, V., Keenum, I., Ling, F., Sills, D., Mansfeldt, C., 2023. Structured ethical review for wastewater-based testing in support of public health. Environ. Sci. Technol. 57 (35), 12969–12980. https://doi.org/10.1021/acs.est.3c04529.

Bustin, S.A., Benes, V., Garson, J.A., Hellemans, J., Huggett, J., Kubista, M., Mueller, R., Nolan, T., Pfaffl, M.W., Shipley, G.L., Vandesompele, J., Wittwer, C.T., 2009. The MIQE guidelines: minimum information for publication of quantitative real-time PCR experiments. Clin. Chem. 55 (4), 611–622. https://doi.org/10.1373/clinchem.2008.112797.

Crank, K., Chen, W., Bivins, A., Lowry, S., Bibby, K., 2022. Contribution of SARS-CoV-2 RNA shedding routes to RNA loads in wastewater. Sci. Total Environ. 806, 150376 https://doi.org/10.1016/j.scitotenv.2021.150376.

Diamond, M.B., Keshaviah, A., Bento, A.I., Conroy-Ben, O., Driver, E.M., Ensor, K.B., Halden, R.U., Hopkins, L.P., Kuhn, K.G., Moe, C.L., Rouchka, E.C., Smith, T., Stevenson, B.S., Susswein, Z., Vogel, J.R., Wolfe, M.K., Stadler, L.B., Scarpino, S.V., 2022. Wastewater surveillance of pathogens can inform public health responses. Nat. Med. 28 (10), 1992–1995. https://doi.org/10.1038/s41591-022-01940-x.

Elisabeth, M., Maneesh, P.-S., Katarina, S.F., Slobodan, Z., Michael, S., 2021. Antimicrobial resistance & migrants in Sweden: poor living conditions enforced by migration control policies as a risk factor for optimal public health management. Front. Public Health 9. https://doi.org/10.3389/fpubh.2021.642983.

Elstrøm, P., Astrup, E., Hegstad, K., Samuelsen, Ø., Enger, H., Kacelnik, O., 2019. The fight to keep resistance at bay, epidemiology of carbapenemase producing organisms (CPOs), vancomycin resistant enterococci (VRE) and methicillin resistant Staphylococcus aureus (MRSA) in Norway, 2006-2017. PLoS One 14 (2), e0211741. https://doi.org/10.1371/journal.pone.0211741.

EU Regulation 2020/741, 2022. Proposal for a directive of the European parlia- ment and of the council concerning urban wastewater treatment (recast). Off. J. Eur. Union 0345 (October), 1–68.

Faleye, T.O.C., Bowes, D.A., Driver, E.M., Adhikari, S., Adams, D., Varsani, A., Halden, R. U., Scotch, M., 2021. Wastewater-based epidemiology and long-read sequencing to identify enterovirus circulation in three municipalities in Maricopa County, Arizona, Southwest United States between June and October 2020. Viruses 13 (9), 1803. https://doi.org/10.3390/v13091803.

Farkas, K., Hillary, L.S., Malham, S.K., McDonald, J.E., Jones, D.L., 2020. Wastewater and public health: the potential of wastewater surveillance for monitoring COVID-19. Current Opinion in Environmental Science & Health 17, 14–20. https://doi.org/10.1016/j.coesh.2020.06.001.

Fong, T.-T., Phanikumar, M.S., Xagoraraki, I., Rose, J.B., 2010. Quantitative detection of human adenoviruses in wastewater and combined sewer overflows influencing a Michigan river. Appl. Environ. Microbiol. 76 (3), 715–723. https://doi.org/10.1128/AEM.01316-09.

Gentry, Z., Zhao, L., Faust, R.A., David, R.E., Norton, J., Xagoraraki, I., 2023. Wastewater surveillance beyond COVID-19: a ranking system for communicable disease testing in the tri-county Detroit area, Michigan, USA. Front. Public Health 11. https://doi.org/10.3389/fpubh.2023.1178515.

Gonzalez, R., Curtis, K., Bivins, A., Bibby, K., Weir, M.H., Yetka, K., Thompson, H., Keeling, D., Mitchell, J., Gonzalez, D., 2020. COVID-19 surveillance in southeastern Virginia using wastewater-based epidemiology. Water Res. 186, 116296 https://doi.org/10.1016/j.watres.2020.116296.

Graber, T.E., Mercier, É., Bhatnagar, K., Fuzzen, M., D'Aoust, P.M., Hoang, H.-D., Tian, X., Towhid, S.T., Plaza-Diaz, J., Eid, W., Alain, T., Butler, A., Goodridge, L., Servos, M., Delatolla, R., 2021. Near real-time determination of B.1.1.7 in proportion to total SARS-CoV-2 viral load in wastewater using an allele-specific primer extension PCR strategy. Water Res. 205, 117681 https://doi.org/10.1016/j.watres.2021.117681.

Heljanko, V., Johansson, V., Räisänen, K., Anttila, V.-J., Lyytikäinen, O., Jalava, J., Weijo, I., Lehtinen, J.-M., Lehto, K.-M., Lipponen, A., Oikarinen, S., Pitkänen, T., Heikinheimo, A., 2023. Genomic epidemiology of nosocomial carbapenemase-producing Citrobacter freundii in sewerage systems in the Helsinki metropolitan area, Finland. Front. Microbiol. 14 https://doi.org/10.3389/fmicb.2023.1165751.

Heljanko, V., Tyni, O., Johansson, V., Virtanen, J.-P., Räisänen, K., Lehto, K.-M., Lipponen, A., Oikarinen, S., Pitkänen, T., Al-Mustapha, A., Kurittu, P., Länsivaara, A., Hyder, R., Janhonen, E., Tiwari, A., Hokajärvi, A.-M., Kolehmainen, A., Möttönen, T., Luomala, O., Heikinheimo, A., 2024. Clinically relevant sequence types of carbapenemase-producing Escherichia coli and Klebsiella pneumoniae detected in Finnish wastewater in 2021–2022. Antimicrob. Resist. Infect. Control 13 (1), 14. https://doi.org/10.1186/s13756-024-01370-z.

Hellmér, M., Paxéus, N., Magnius, L., Enache, L., Arnholm, B., Johansson, A., Bergström, T., Norder, H., 2014. Detection of pathogenic viruses in sewage provided early warnings of hepatitis A virus and norovirus outbreaks. Appl. Environ. Microbiol. 80 (21), 6771–6781. https://doi.org/10.1128/AEM.01981-14.

Hendriksen, R.S., Munk, P., Njage, P., van Bunnik, B., McNally, L., Lukjancenko, O., Röder, T., Nieuwenhuijse, D., Pedersen, S.K., Kjeldgaard, J., Kaas, R.S., Clausen, P.T. L.C., Vogt, J.K., Leekitcharoenphon, P., van de Schans, M.G.M., Zuidema, T., de Roda Husman, A.M., Rasmussen, S., Petersen, B., Aarestrup, F.M., 2019. Global monitoring of antimicrobial resistance based on metagenomics analyses of urban sewage. Nat. Commun. 10 (1), 1124. https://doi.org/10.1038/s41467-019-08853-3.

Hill, D., Dunham, C., Larsen, D.A., Collins, M., 2023. Operationalizing an open-source dashboard for communicating results of wastewater-based surveillance. MethodsX 11, 102299. https://doi.org/10.1016/j.mex.2023.102299.

Hokajärvi, A.M., Pitkanïen, T., Siljanen, H.M.P., Nakari, U.M., Torvinen, E., Siitonen, A., Miettinen, I.T., 2013. Occurrence of thermotolerant Campylobacter spp. and adenoviruses in Finnish bathing waters and purified sewage effluents. J. Water Health 11 (1), 120–134. https://doi.org/10.2166/wh.2012.192.

Hokajärvi, A.M., Rytkönen, A., Tiwari, A., Kauppinen, A., Oikarinen, S., Lehto, K.M., Kankaanpää, A., Gunnar, T., Al-Hello, H., Blomqvist, S., Miettinen, I.T., Savolainen-Kopra, C., Pitkänen, T., 2021. The detection and stability of the SARS-CoV-2 RNA biomarkers in wastewater influent in Helsinki, Finland. Sci. Total Environ. 770, 145274 https://doi.org/10.1016/j.scitotenv.2021.145274.

Hovi, T., Stenvik, M., Partanen, H., Kangas, A., 2001. Poliovirus surveillance by examining sewage specimens. Quantitative recovery of virus after introduction into sewerage at remote upstream location. Epidemiol. Infect. 127 (01), 101–106. https://doi.org/10.1017/S0950268801005787.

Iaconelli, M., Bonanno Ferraro, G., Mancini, P., Suffredini, E., Veneri, C., Ciccaglione, A. R., Bruni, R., Della Libera, S., Bignami, F., Brambilla, M., De Medici, D., Brandtner, D., Schembri, P., D'Amato, S., La Rosa, G., 2020. Nine-year Nationwide environmental surveillance of hepatitis E virus in urban wastewaters in Italy (2011-2019). Int. J. Environ. Res. Public Health 17 (6), 2059. https://doi.org/10.3390/ijerph17062059.

Islam, M.A., Rahman, M.A., Jakariya, M., Bahadur, N.M., Hossen, F., Mukharjee, S.K., Hossain, M.S., Tasneem, A., Haque, M.A., Sera, F., Jahid, I.K., Ahmed, T., Hasan, M. N., Islam, M.T., Hossain, A., Amin, R., Tiwari, A., Didar-Ul-Alam, M., Dhama, K., Ahmed, F., 2023. A 30-day follow-up study on the prevalence of SARS-COV-2 genetic markers in wastewater from the residence of COVID-19 patient and comparison with clinical positivity. Sci. Total Environ. 858, 159350 https://doi.org/10.1016/j.scitotenv.2022.159350.

de Jonge, E.F., Peterse, C.M., Koelewijn, J.M., van der Drift, A.-M.R., van der Beek, R.F. H.J., Nagelkerke, E., Lodder, W.J., 2022. The detection of monkeypox virus DNA in waste- water samples in the Netherlands. Sci. Total Environ. 852 (August), 158265 https://doi.org/10.1016/j.scitotenv.2022.158265.

Kankaanpää, A., Ariniemi, K., Heinonen, M., Kuoppasalmi, K., Gunnar, T., 2014. Use of illicit stimulant drugs in Finland: A wastewater study in ten major cities. Sci. Total Environ. 487, 696–702. https://doi.org/10.1016/j.scitotenv.2013.11.095.

Kankaanpää, A., Ariniemi, K., Heinonen, M., Kuoppasalmi, K., Gunnar, T., 2016. Current trends in Finnish drug abuse: wastewater based epidemiology combined with other national indicators. Sci. Total Environ. 568, 864–874. https://doi.org/10.1016/j.scitotenv.2016.06.060.

Karkman, A., Johnson, T.A., Lyra, C., Stedtfeld, R.D., Tamminen, M., Tiedje, J.M., Virta, M., 2016. High-throughput quantification of antibiotic resistance genes from an urban waste- water treatment plant. FEMS Microbiol. Ecol. 92 (3) https://doi.org/10.1093/femsec/fiw014.

Karkman, A., Berglund, F., Flach, C.-F., Kristiansson, E., Larsson, D.G.J., 2020. Predicting clinical resistance prevalence using sewage metagenomic data. Communications Biology 3 (1), 711. https://doi.org/10.1038/s42003-020-01439-6.

Kauppinen, A., Pitkänen, T., Al-Hello, H., Maunula, L., Hokajärvi, A.-M., Rimhanen-Finne, R., Miettinen, I.T., 2019. Two drinking water outbreaks caused by wastewater intrusion including Sapovirus in Finland. Int. J. Environ. Res. Public Health 16 (22), 4376. https://doi.org/10.3390/ijerph16224376.

Keshaviah, A., Hu, X.C., Henry, M., 2021. Developing a flexible National Wastewater Surveillance System for COVID-19 and beyond. Environ. Health Perspect. 129 (4) https://doi.org/10.1289/EHP8572.

Keshaviah, A., Diamond, M.B., Wade, M.J., Scarpino, S.V., Ahmed, W., Amman, F., Aruna, O., Badilla-Aguilar, A., Bar-Or, I., Bergthaler, A., Bines, J.E., Bivins, A.W., Boehm, A.B., Brault, J.-M., Burnet, J.-B., Chapman, J.R., Chaudhuri, A., de Roda Husman, A.M., Delatolla, R., Zanoli Sato, M.I., 2023. Wastewater monitoring can anchor global disease surveillance systems. Lancet Glob. Health 11 (6), e976–e981. https://doi.org/10.1016/S2214-109X(23)00170-5.

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. Am. J. Epidemiol. 192 (2), 305–322. https://doi.org/10.1093/aje/kwac175.

Kitajima, M., Ahmed, W., Bibby, K., Carducci, A., Gerba, C.P., Hamilton, K.A., Haramoto, E., Rose, J.B., 2020. SARS-CoV-2 in wastewater: state of the knowledge and research needs. Sci. Total Environ. 739 (139076), 139076 https://doi.org/10.1016/j.scitotenv.2020.139076.

Kumar, M., Joshi, M., Jiang, G., Yamada, R., Honda, R., Srivastava, V., Mahlknecht, J., Barcelo, D., Chidambram, S., Khursheed, A., Graham, D.W., Goswami, R., Kuroda, K., Tiwari, A., Joshi, C., 2023. Response of wastewater-based epidemiology predictor for the second wave of COVID-19 in Ahmedabad, India: A long-term data perspective. Environ. Pollut. 337, 122471 https://doi.org/10.1016/j.envpol.2023.122471.

Lai, F.Y., Muziasari, W., Virta, M., Wiberg, K., Ahrens, L., 2021. Profiles of environmental antibiotic resistomes in the urban aquatic recipients of Sweden using high-throughput quantitative PCR analysis. Environ. Pollut. 287, 117651 https://doi.org/10.1016/j.envpol.2021.117651.

Laine, J., Huovinen, E., Virtanen, M.J., Snellman, M., Lumio, J., Ruutu, P., et al., 2011. An extensive gastroenteritis outbreak after drinking-water contamination by sewage effluent, Finland. Epidemiol. Infect. 139 (7), 1105–1113. https://doi.org/10.1017/S0950268810002141.

Langeveld, J., Schilperoort, R., Heijnen, L., Elsinga, G., Schapendonk, C.E.M., Fanoy, E., de Schepper, E.I.T., Koopmans, M.P.G., de Graaf, M., Medema, G., 2023. Normalisation of SARS-CoV-2 concentrations in wastewater: the use of flow, electrical conductivity and crAssphage. Sci. Total Environ. 865, 161196 https://doi.org/10.1016/j.scitotenv.2022.161196.

Länsivaara, A., Lehto, K.-M., Hyder, R., Janhonen, E., Lipponen, A., Heikinheimo, A., Pitkänen, T., Oikarinen, S., 2023a. Comparison of different PCR methods for the detection of SARS-CoV-2 RNA in wastewater based on the reported incidence of COVID-19 in Finland. MedRxiv Preprint, 23295183. https://doi.org/10.1101/2023.09.07.23295183.

Länsivaara, A., Lehto, K., Hyder, R., Luomala, O., 2023b. Wastewater-based surveillance of respiratory syncytial virus epidemic at the national level in, pp. 1–30. https://doi.org/10.1101/2023.09.04.23295011.

Larsson, D.G.J., Flach, C.-F., 2022. Antibiotic resistance in the environment. Nat. Rev. Microbiol. 20 (5), 257–269. https://doi.org/10.1038/s41579-021-00649-x.

Lee, N., Chan, M.C.W., Wong, B., Choi, K.W., Sin, W., Lui, G., Chan, P.K.S., Lai, R.W.M., Cockram, C.S., Sung, J.J.Y., Leung, W.K., 2007. Fecal viral concentration and diarrhea in norovirus gastroenteritis. Emerg. Infect. Dis. 13 (9), 1399–1401. https://doi.org/10.3201/eid1309.061535.

Lehto, K.-M., Hyder, R., Länsivaara, A., Luomala, O., Lipponen, A., Hokajärvi, A.-M., Heikinheimo, A., Pitkänen, T., Oikarinen, S., Group, W.S., 2023. Wastewater-based surveillance is an efficient monitoring tool for tracking influenza A virus in the community. MedRxiv 2008–2023.

Levican, J., Levican, A., Ampuero, M., Gaggero, A., 2019. JC polyomavirus circulation in one-year surveillance in wastewater in Santiago, Chile. Infection, Genetics and Evolution : Journal of Molecular Epidemiology and Evolutionary Genetics in Infectious Diseases 71 (January), 151–158. https://doi.org/10.1016/j.meegid.2019.03.017.

Li, Q., Lee, B.E., Gao, T., Qiu, Y., Ellehoj, E., Yu, J., Diggle, M., Tipples, G., Maal-Bared, R., Hinshaw, D., Sikora, C., Ashbolt, N.J., Talbot, J., Hrudey, S.E., Pang, X., 2023. Number of COVID-19 cases required in a population to detect SARS-CoV-2 RNA in wastewater in the province of Alberta, Canada: sensitivity assessment. J. Environ. Sci. 125, 843–850. https://doi.org/10.1016/j.jes.2022.04.047.

Lowry, S.A., Wolfe, M.K., Boehm, A.B., 2023. Respiratory virus concentrations in human excretions that contribute to wastewater: a systematic review and meta-analysis. J. Water Health 21 (6), 831–848. https://doi.org/10.2166/wh.2023.057.

Lundy, L., Fatta-Kassinos, D., Slobodnik, J., Karaolia, P., Cirka, L., Kreuzinger, N., Castiglioni, S., Bijlsma, L., Dulio, V., Deviller, G., Lai, F.Y., Alygizakis, N., Barneo, M., Baz-Lomba, J.A., Béen, F., Cíchová, M., Conde-Pérez, K., Covaci, A., Donner, E., Viklander, M., 2021. Making waves: collaboration in the time of SARS-CoV-2- rapid development of an international co-operation and wastewater surveillance database to support public health decision-making. Water Res. 199 (January), 117167 https://doi.org/10.1016/j.watres.2021.117167.

Maal-Bared, R., Qiu, Y., Li, Q., Gao, T., Hrudey, S.E., Bhavanam, S., Ruecker, N.J., Ellehoj, E., Lee, B.E., Pang, X., 2023. Does normalization of SARS-CoV-2 concentrations by pepper mild mottle virus improve correlations and lead time between wastewater surveillance and clinical data in Alberta (Canada): comparing twelve SARS-CoV-2 normalization approaches. Sci. Total Environ. 856, 158964 https://doi.org/10.1016/j.scitotenv.2022.158964.

Majlander, J., Anttila, V.-J., Nurmi, W., Seppälä, A., Tiedje, J., Muziasari, W., 2021. Routine wastewater-based monitoring of antibiotic resistance in two Finnish hospitals: focus on carbapenem resistance genes and genes associated with bacteria causing hospital-acquired infections. J. Hosp. Infect. 117, 157–164. https://doi.org/10.1016/j.jhin.2021.09.008.

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. https://doi.org/10.1016/j.coesh.2020.04.006.

Markt, R., Stillebacher, F., Nägele, F., Kammerer, A., Peer, N., Payr, M., Scheffknecht, C., Dria, S., Draxl-Weiskopf, S., Mayr, M., Rauch, W., Kreuzinger, N., Rainer, L., Bachner, F., Zuba, M., Ostermann, H., Lackner, N., Insam, H., Wagner, A.O., 2023. Expanding the pathogen panel in wastewater epidemiology to influenza and norovirus. Viruses 15 (2), 263. https://doi.org/10.3390/v15020263.

Matsumura, Y., Shimizu, T., Noguchi, T., Nakano, S., Yamamoto, M., Nagao, M., 2021. Comparison of 12 molecular detection assays for severe acute respiratory syndrome coronavirus 2 (SARS-CoV-2). J. Mol. Diagn. 23 (2), 164–170. https://doi.org/10.1016/j.jmoldx.2020.11.007.

Medema, G., Heijnen, L., Elsinga, G., Italiaander, R., Brouwer, A., 2020. Presence of SARS-Coronavirus-2 RNA in sewage and correlation with reported COVID-19 prevalence in the early stage of the epidemic in the Netherlands. Environ. Sci. Technol. Lett. 7 (7), 511–516. https://doi.org/10.1021/acs.estlett.0c00357.

Nalla, A.K., Casto, A.M., Huang, M.-L.W., Perchetti, G.A., Sampoleo, R., Shrestha, L., Wei, Y., Zhu, H., Jerome, K.R., Greninger, A.L., 2020. Comparative performance of SARS-CoV-2 detection assays using seven different primer-probe sets and one assay kit. J. Clin. Microbiol. 58 (6) https://doi.org/10.1128/JCM.00557-20.

O'Reilly, K.M., Allen, D.J., Fine, P., Asghar, H., 2020. The challenges of informative wastewater sampling for SARS-CoV-2 must be met: lessons from polio eradication. The Lancet Microbe 1 (5), e189–e190. https://doi.org/10.1016/S2666-5247(20)30100-2.

Österblad, M., Kirveskari, J., Hakanen, A.J., Tissari, P., Vaara, M., Jalava, J., 2012. Carbapenemase-producing Enterobacteriaceae in Finland: the first years (2008-11). J. Antimicrob. Chemother. 67 (12), 2860–2864. https://doi.org/10.1093/jac/dks299.

Pang, X., Gao, T., Ellehoj, E., Li, Q., Qiu, Y., Maal-Bared, R., Sikora, C., Tipples, G., Diggle, M., Hinshaw, D., Ashbolt, N.J., Talbot, J., Hrudey, S.E., Lee, B.E., 2022. Wastewater-based surveillance is an effective tool for trending COVID-19 prevalence in communities: A study of 10 major communities for 17 months in Alberta. ACS ES&T Water 2 (11), 2243–2254. https://doi.org/10.1021/acsestwater.2c00143.

Pärnänen, K., Narciso-da-Rocha, C., Kneis, D., Berendonk, T.U., Cacace, D., Do, T.T., Elpers, C., Fatta-Kassinos, D., Henriques, I., Jaeger, T., Karkman, A., Martinez, J.L., Michael, S.G., Michael-Kordatou, I., O'Sullivan, K., Rodriguez-Mozaz, S., Schwartz, T., Sheng, H., Sørum, H., Manaia, C.M., 2019. Antibiotic resistance in European wastewater treatment plants mirrors the pattern of clinical antibiotic resistance prevalence. Sci. Adv. 5 (3), eaau9124 https://doi.org/10.1126/sciadv.aau9124.

Pinholt, M., Bayliss, S.C., Gumpert, H., Worning, P., Jensen, V.V.S., Pedersen, M., Feil, E. J., Westh, H., 2019. WGS of 1058 Enterococcus faecium from Copenhagen, Denmark, reveals rapid clonal expansion of vancomycin-resistant clone ST80 combined with widespread dissemination of a vanA-containing plasmid and acquisition of a heterogeneous accessory genome. J. Antimicrob. Chemother. 74 (7), 1776–1785. https://doi.org/10.1093/jac/dkz118.

Pitkänen, T., 2023. Wastewater-based surveillance as pandemic preparedness tool (WastPan). Finnish Institute for Health and Welfare. https://thl.fi/en/web/thlfi-en/research-and-develop-ment/research-and-projects/wastewater-based-surveillance-as-pandemic-preparedness-tool-wastpan.

Pitkänen, T., Gunnar, T., 2022. THL is starting to analyse wastewater for coronavirus. Retrieved July 7, 2023, from. https://thl.fi/en/web/thlfi-en/-/thl-is-starting-to-analyse-wastewater-for-coronavirus.

Pitkänen, T., Ryu, H., Elk, M., Hokajärvi, A.M., Siponen, S., Vepsäläinen, A., Räsänen, P., Santo Domingo, J.W., 2013. Detection of fecal bacteria and source tracking identifiers in environmental waters using rRNA-based RT-qPCR and rDNA-based qPCR assays. Environ. Sci. Technol. 47 (23), 13611–13620. https://doi.org/10.1021/es403489b.

Pöyry, T., Stenvik, M., Hovi, T., 1988. Viruses in sewage waters during and after a poliomyelitis outbreak and subsequent nationwide oral poliovirus vaccination campaign in Finland. Appl. Environ. Microbiol. 54 (2), 371–374. https://doi.org/10.1128/aem.54.2, 1988.

Prevost, B., Lucas, F.S., Goncalves, A., Richard, F., Moulin, L., Wurtzer, S., 2015. Large scale survey of enteric viruses in river and waste water underlines the health status of the local population. Environ. Int. 79, 42–50. https://doi.org/10.1016/j.envint.2015.03.004.

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. Curr. Opin. Microbiol. 64, 91–99. https://doi.org/10.1016/j.mib.2021.09.013.

Santiso-Bellón, C., Randazzo, W., Pérez-Cataluña, A., Vila-Vicent, S., Gozalbo-Rovira, R., Muñoz, C., Buesa, J., Sanchez, G., Rodríguez Díaz, J., 2020. Epidemiological surveillance of norovirus and rotavirus in sewage (2016–2017) in Valencia (Spain). Microorganisms 8 (3), 458. https://doi.org/10.3390/microorganisms8030458.

Shrestha, S., Malla, B., Sangsanont, J., Sirikanchana, K., Ngo, H.T.T., Inson, J.G.M., Enriquez, M.L.D., Alam, Z.F., Setiyawan, A.S., Setiadi, T., Takeda, T., Kitajima, M., Haramoto, E., 2024a. Detection of enteroviruses related to hand foot and mouth disease in wastewater of Asian communities. Sci. Total Environ. 912, 169375 https://doi.org/10.1016/j.scitotenv.2023.169375.

Shrestha, S., Malla, B., Haramoto, E., 2024b. Estimation of norovirus infections in Japan: an application of wastewater-based epidemiology for enteric disease assessment. Sci. Total Environ. 912, 169334 https://doi.org/10.1016/j.scitotenv.2023.169334.

Southon, S.B., Beres, S.B., Kachroo, P., Saavedra, M.O., Erlendsdóttir, H., Haraldsson, G., Yerramilli, P., Pruitt, L., Zhu, L., Musser, J.M., Kristinsson, K.G., 2020. Population genomic molecular epidemiological study of macrolide-resistant streptococcus pyogenes in Iceland, 1995 to 2016: identification of a large clonal population with a pbp2x mutation conferring reduced in vitro β-lactam susceptibility. J. Clin. Microbiol. 58 (9) https://doi.org/10.1128/JCM.00638-20.

Statistics Finland, 2021. Causes of death in 2020. https://www.stat.fi/til/ksyyt/2020/ksyyt_2020_2021-12-10_en.pdf.
Thakali, O., Raya, S., Malla, B., Tandukar, S., Tiwari, A., Sherchan, S.P., Sherchand, J.B., Haramoto, E., 2022. Pilot study on wastewater surveillance of dengue virus RNA: lessons, challenges, and implications for future research. Environmental Challenges, 100614. https://doi.org/10.1016/j.envc.2022.100614.
THL, 2023. Infectious Disease Control and Vaccinations. https://thl.fi/en/about-us/organisation/departments-and-units/health-security/infectious-disease-control-and-vaccinations.
Tiwari, A., Hokajärvi, A.M., Santo Domingo, J.W., Kauppinen, A., Elk, M., Ryu, H., Jayaprakash, B., Pitkänen, T., Domingo, J.W.S., 2018. Categorical performance characteristics of method ISO 7899-2 and indicator value of intestinal enterococci for bathing water quality monitoring. J. Water Health 16 (5), 711–723. https://doi.org/10.2166/wh.2018.293.
Tiwari, A., Oliver, D.M., Bivins, A., Sherchan, S.P., Pitkänen, T., 2021. Bathing water quality monitoring practices in Europe and the United States. Int. J. Environ. Res. Public Health 18 (11), 5513. https://doi.org/10.3390/ijerph18115513.
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., 2022a. Monkeypox outbreak: wastewater and environmental surveillance perspective. Sci. Total Environ. 856 (Pt 2), 159166 https://doi.org/10.1016/j.scitotenv.2022.159166.
Tiwari, A., Lipponen, A., Hokajärvi, A.-M., Luomala, O., Sarekoski, A., Rytkönen, A., Österlund, P., Al-Hello, H., Juutinen, A., Miettinen, I.T., Savolainen-Kopra, C., Pitkänen, T., 2022b. Detection and quantification of SARS-CoV-2 RNA in wastewater influent in relation to reported COVID-19 incidence in Finland. Water Res. 215, 118220 https://doi.org/10.1016/J.WATRES.2022.118220.
Tiwari, A., Paakkanen, J., Österblad, M., Kirveskari, J., Hendriksen, R.S., Heikinheimo, A., 2022c. Wastewater surveillance detected Carbapenemase enzymes in clinically relevant gram-negative Bacteria in Helsinki, Finland; 2011–2012. Front. Microbiol. 13, 1–14. https://doi.org/10.3389/fmicb.2022.887888.
Tiwari, A., Kurittu, P., Al-Mustapha, A.I., Heljanko, V., Johansson, V., Thakali, O., Mishra, S.K., Lehto, K.-M., Lipponen, A., Oikarinen, S., Pitkänen, T., Heikinheimo, A., 2022d. Wastewater surveillance of antibiotic-resistant bacterial pathogens: A systematic review. Front. Microbiol. 13 https://doi.org/10.3389/fmicb.2022.977106.
Tiwari, A., Adhikari, S., Zhang, S., Solomon, T.B., Lipponen, A., Islam, M.A., Thakali, O., Sangkham, S., Shaheen, M.N.F., Jiang, G., Haramoto, E., Mazumder, P., Malla, B., Kumar, M., Pitkänen, T., Sherchan, S.P., 2023a. Tracing COVID-19 trails in wastewater: A systematic review of SARS-CoV-2 surveillance with viral variants. Water 15 (6), 1018. https://doi.org/10.3390/w15061018.
Tiwari, A., Paakkanen, J., Al-Mustapha, A.I., Kirveskari, J., Hendriksen, R.S., Heikinheimo, A., 2023b. Phenotypic and genotypic characteristics of beta-lactamase dominant with CARBA, AmpC, and ESBL-producing bacteria in municipal wastewater influent in Helsinki, Finland. Journal of Global Antimicrobial Resistance 33, 345–352. https://doi.org/10.1016/j.jgar.2023.04.015.
Tiwari, A., Krolicka, A., Tran, T.T., Räisänen, K., Ásmundsdóttir, Á.M., Wikmark, O.-G., Lood, R., Pitkänen, T., 2024. Antibiotic resistance monitoring in wastewater in the Nordic countries: A systematic review. Environ. Res. 246, 118052 https://doi.org/10.1016/j.envres.2023.118052.
Viikinmäki, 2021. Viikinmäki wastewater treatment plant. In: Helsinki Region Environmental Services Authority Helsinginseudun ympäristöpalvelut -kuntayhtymä. https://niini.fi/wp-content/uploads/2021/01/HSY0014_Viikinmaki_wastewater_treatment_plant.pdf.
Wettstone, E.G., Islam, M.O., Hughlett, L., Reagen, C., Shirin, T., Rahman, M., Hosan, K., Hoque, M.R., Brennhofer, S.A., Rogawski McQuade, E.T., Mira, Y., von Tobel, L., Haque, R., Taniuchi, M., Blake, I.M., 2023. Interactive SARS-CoV-2 dashboard for real-time geospatial visualisation of sewage and clinical surveillance data from Dhaka, Bangladesh: a tool for public health situational awareness. BMJ Glob. Health 8 (8), e012921. https://doi.org/10.1136/bmjgh-2023-012921.
WHO, 2015. Global Action Plan on Antimicrobial Resistance. World Health Organization, Geneva, Switzerland.
WHO, 2021. WHO integrated global surveillance on ESBL-producing E. coli using a “One Health” approach: implementation and opportunities.
Williams, B.A., Jones, C.H., Welch, V., True, J.M., 2023. Outlook of pandemic preparedness in a post-COVID-19 world. Npj Vaccines 8 (1), 178. https://doi.org/10.1038/s41541-023-00773-0.
Wu, Z., Che, Y., Dang, C., Zhang, M., Zhang, X., Sun, Y., Li, X., Zhang, T., Xia, Y., 2022. Nanopore-based long-read metagenomics uncover the resistome intrusion by antibiotic resistant bacteria from treated wastewater in receiving water body. Water Res. 226, 119282 https://doi.org/10.1016/j.watres.2022.119282.