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A review of wastewater-based epidemiology for antimicrobial resistance surveillance

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Abstract

Antimicrobial resistance (AMR) is recognized as one of the most serious threats to public health. Unparalleled population growth and accelerated rates of AMR emergence and dissemination have resulted in both novel resistance in pathogenic organisms and the re-appearance of infections that were formerly under control. Consequently, this has led to an increased quantity of infectious diseases. One of the main drivers of antimicrobial overuse is inappropriate prescribing in human and veterinary medicine. The ability to rapidly survey the spread of antimicrobial resistance within human populations is key for its prevention, intervention, and control. However, many constraints are present for current clinical surveillance systems and their capacity to determine AMR dynamics in the microbiome of healthy individuals as well as in clinical pathogens causing infections. Wastewater-based epidemiology (WBE) is an emergent technique that has the capacity to act as a supplementary measure for current infectious disease surveillance systems and as an early warning system for infectious disease outbreaks. The development of disease outbreaks to the community level can be monitored in real time through the analysis of population pooled wastewater. This review provides an introduction to using wastewater-based epidemiology to monitor AMR bacteria, as well as an overview of wastewater-based epidemiology and its components.

Keywords: Antimicrobial resistance, public health, wastewater, wastewater-based epidemiology

INTRODUCTION: ANTIMCROBIAL RESISTANCE

Antimicrobial resistance (AMR) is a global, overlooked pandemic[1]. From common infections such as urinary tract infections, sepsis, and sexually transmitted infections, high rates of AMR have been observed worldwide, indicating that the treatments we have are becoming ineffective[2-4]. AMR is a concern because once existing efficacious antimicrobials are exhausted, common infections and medical procedures may become lethal[2,5].

Epidemiological surveillance networks in Europe have documented that AMR bacteria have become much more prevalent during the past decade[6].

According to statistical models, there were a predicted 4.95 million deaths associated with bacterial AMR in 2019, including 1.27 million deaths directly caused by bacterial AMR[1]. The impact of AMR on death tolls, the economy, and the burden on healthcare systems will be catastrophic unless action is taken to mitigate this risk[7,8].

Antimicrobials can be defined as any drug or compound that exhibits antimicrobial activity - such that they impair the growth of microbial life forms (e.g., bacteria, viruses, fungi, protozoa, *etc.*)[4]. The discovery and use of antimicrobials have decreased the burden of infectious disease and allowed the innovation of complex medical procedures and surgeries in humans and animals.

AMR occurs when a microbe gains the ability to survive in the presence of an antimicrobial compound[4]. Evidence also shows that sub-lethal concentrations of antimicrobials can also favor resistant bacteria, which grow faster than susceptible bacteria at low antimicrobial concentrations[9]. AMR has occurred naturally over time in environmental bacteria exposed to antimicrobials produced by microorganisms, but human use and misuse of antimicrobials have accelerated AMR evolution. This review will focus on AMR bacteria.

Some bacteria are intrinsically resistant to specific antimicrobials, or resistance can be acquired through mutation of specific genes or through horizontal gene transfer (HGT) of mobile genes via transformation, transduction, or conjugation[10,11]. Molecular mechanisms of resistance to antimicrobials usually involve compound metabolism, target site alterations, or reduced cell membrane permeability/increased cell efflux[11,12]. There are many molecular mechanisms that use the aforementioned strategies to resist antimicrobial compounds and they are described in detail elsewhere[10-14]. Research in the discovery of novel resistance mechanisms is ongoing and is beneficial in directing research in the discovery of new antimicrobials[15].

Antimicrobial misuse and overuse in human and animal medicine and crop production are key drivers in the evolution of AMR[7,16,17]. AMR in humans is connected to AMR in animals and the environment because humans can be infected by pathogens found in their microbiomes[18] and resistance genes can also pass between microbe species via HGT[19]. AMR bacteria and genetic determinants are found in humans, food, animals, and the environment and can be transferred freely between these components. The following sections illustrate why AMR is a global concern with impacts on humans, animals, plants, and the environment.

The environment as a reservoir of antimicrobial resistance

Once antimicrobials are consumed by humans or animals, they are excreted into the environment either as parent molecules, metabolites, or a combination of both[20]. These chemicals often end up in wastewater treatment facilities and potentially contaminate groundwater, rivers, lakes, and agricultural land[17,21]. This is

of concern because once these chemicals are released into the environment, they have the potential to select for AMR[21-26]. People and animals may be exposed to microbial pathogens and AMR bacteria through recreational activities in contaminated water, drinking contaminated water, eating contaminated foods, or inhaling aerosols[17,21]. Importantly, AMR has been shown to evolve at sub-inhibitory concentration levels of antimicrobials[9,23,27,28]. For instance, it has been shown that environmentally relevant levels of heavy metals can select for antimicrobial resistance[29]. This is of particular concern because locations with even low concentrations of antimicrobial compounds could select for AMR in the environment[9].

The natural environment is a known reservoir of AMR E, which has been found in freshwater lake environments[30], on plastics[31], in sewers[32] and wastewater[33,34], in soil[35], and in groundwater[36]. Furthermore, animals themselves can act as reservoirs of resistance[19,37,38]. Areas where pharmaceutical[33,37,39], agricultural[40], municipal[41], and hospital waste[42] enter the environment and freshwater are of particular concern as they have increased AMR prevalence and provide routes for human exposure and transmission [Figure 1].

In brief, the acquisition of AMR bacteria in the environment is caused by three key mechanisms that may occur in combination[43]: (1) HGT of resistance genes between different bacterial species; (2) genetic mutation and recombination; and (3) selection pressure caused by antibiotics or other substances such as biocides or heavy metals, which may induce or accelerate the rates of (1) and (2)[44].

Many studies have made headway in determining the prevalence of AMR bacteria in the environment[34,43,45-49]. Most notably, a systematic review in 2015 found that AMR bacteria were detected in all (66/66) of the "contamination" sources (wastewater and manure) included in the review[21]. The review also included molecular evidence supporting AMR transmission from wastewater to the environment[21]. This paper illustrated that AMR bacteria are ubiquitous in the environment, and the authors emphasize that measuring the extent of AMR in the environment is important for the innovation of intervention strategies to limit the spread of AMR in the environment[21]. While ubiquitous, not all AMR is of equal concern, as different resistance genes confer resistance to different classes of antibiotics with differing clinical importance. In addition, some resistance genes, mobile genetic elements carrying multiple resistance genes, and AMR clinical pathogens have an "anthropogenic signature", meaning they have been selected for in humans or animal microbiomes, and therefore pose a more immediate transmission and infection risk (relative to resistance harbored by most environmental bacteria).

The long-term effects of the dissemination of antimicrobials in the environment are still unfolding, and the effect on the natural environment and the emergence of AMR in human and animal pathogens remains unclear. What is evident, however, is that the release of antimicrobials, AMR bacteria and the potential for subsequent evolution of AMR in various microbes can have serious consequences for both human and animal health.

Antimicrobial resistance surveillance

The first step in mitigating the problem of AMR is to examine its risk to human[50] and animal health and understand its drivers before creating public health policy to contain it. Comprehensive integrated AMR surveillance is needed to create evidence-based policy. The development of regional, national, and global collaborative surveillance networks is important in determining the risk AMR poses[51,52]. AMR surveillance can include recording antimicrobial prescribing in humans, infection rates in humans, antimicrobial use in agriculture, antimicrobial compound concentrations in the environment, and AMR microorganisms and/or gene concentrations in the environment, amongst others. Informed by these variables, integrated

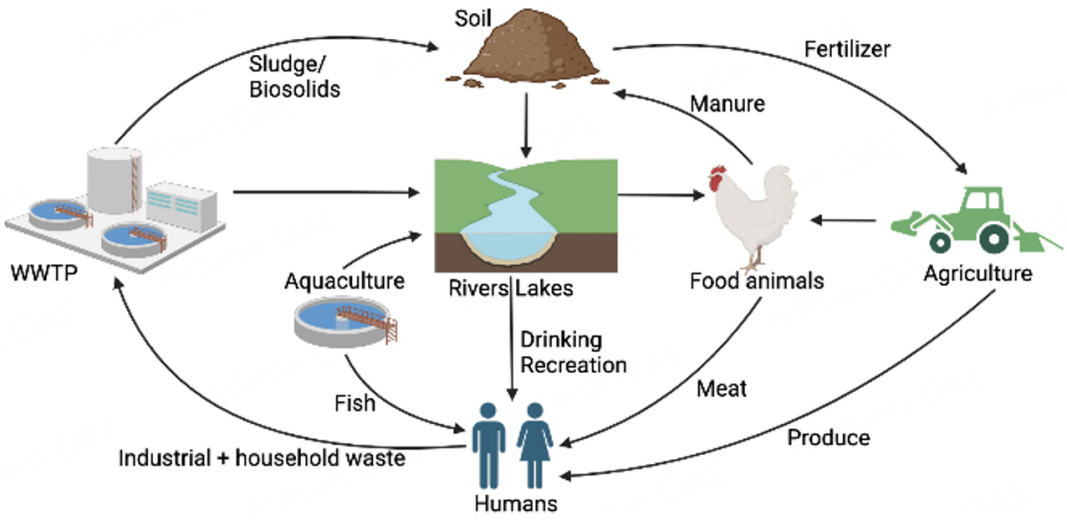

Figure 1. The environment as a reservoir for antimicrobial resistance. Created in BioRender.com[138].

surveillance programs can advise and inform policy on multiple drivers of AMR across the One Health continuum, including, but not limited to, antimicrobial use in medicine, livestock and crop production, infection control in hospitals, biosecurity on farms, and waste management.

Surveillance of antimicrobials and AMR in the environment is critical for public health authorities to evaluate the risk that AMR poses in different parts of the world, and to distinguish specific local drivers and risk factors. Policies can then be implemented, informed by evidence from surveillance and the latest research findings[51-56]. Theoretically, if certain resistance mechanisms (such as fluoroquinolone resistance) are high in a specific human population determined by wastewater surveillance, for example, public health authorities could warn against using the corresponding antimicrobial and suggest the use of an alternative[51]. Surveillance programs are important for evaluating risk and advising a course of action to mitigate further harm[4].

For example, in Australia, the Antimicrobial Use and Resistance in Australia (AURA) surveillance system retrieves and reports on data from hospitals, aged care facilities, and the community. AURA focuses on human health and uses data from five other national programs to create a report of patterns and trends of AMR across Australia[57]. The AURA program provides insight and suggestions for improving hospital care, aged care, and infection control. Notably, the sources AURA uses are mainly prescription data and not measurements of AMR in the environment or agriculture. Prescription data alone is not wholly reliable - as this does not mean the drugs are being taken or account for inappropriate disposal.

Another example of a surveillance system is the Global Antimicrobial Resistance and Use Surveillance System (GLASS)[58]. GLASS is a system put in place by the World Health Organization (WHO) and is the first international collaborative scheme to standardize AMR surveillance. GLASS implements a standard approach to the collection, analysis, interpretation, and sharing of data by countries and actively supports this by building and monitoring the status of existing and new surveillance systems. GLASS promotes a shift from traditional surveillance systems based solely on laboratory data to a system that encompasses epidemiological, clinical, and population-level data. GLASS has progressively incorporated data from surveillance of AMR in humans, such as the monitoring of resistance genes and the use of antimicrobial medicines, including AMR in the food chain and in the environment.

Comprehensive surveillance data is needed to inform evidence-based policy on AMR that can reduce the burden of AMR. Although many nations provide annual reports on prescription use and monitor resistant bacteria, national surveillance efforts are different across countries such that most incidence and prevalence data cannot be connected to epidemiological data[16,51,59]. Tacconelli and co-authors wrote a concise summary of the importance of surveillance systems across the world and emphasized the need to improve national AMR surveillance systems by including data from food, livestock, and the environment - in order to create a better narrative of the risk AMR imposes[51]. This integrated approach is called the One Health approach, and it has been promoted by organizations such as the WHO in their global action plan for combatting AMR[51,52,59-61].

An example of a surveillance system that has taken steps towards this One Health approach is the Canadian Integrated Program on Antimicrobial Resistance Surveillance (CIPARS). CIPARS is a national surveillance program which is maintained by the Public Health Agency of Canada's Centre for Food-borne, Environmental and Zoonotic Infectious Diseases and National Microbiology Laboratory in association with federal, provincial, and private industry partners. CIPARS collects and analyzes trends in antimicrobial use and AMR, in particular bacteria from humans, animals, and retail meat across Canada. The bacteria under scrutiny are known as enteric bacteria, and they can be passed between animals and humans. The program started by combining data on AMR from animal samples collected in abattoirs with data on AMR from sick animals and humans[59]. The CIPARS system has increased its level of complexity over time by adding collection points along the animal rearing system[49,59,62]. The program then added other types of data, such as AMR in farm samples and in retail meat samples, and data on the use of antimicrobials both in animal production and human health[59]. Another program that uses the One Health approach to surveillance is the National Antimicrobial Resistance Monitoring System for Enteric Bacteria (NARMS) implemented in the United States[63].

The Global Sewage Surveillance Project (GSSP) uses wastewater (sewage) samples from 102 countries to monitor the prevalence of infectious disease and AMR and has published data using samples from around the world[64]. Human disease surveillance is often impeded due to ethical problems with the sensitivity of data from clinical samples and healthy individuals. Wastewater has been suggested as an alternative for population-based surveillance and the anonymous nature of wastewater avoids many ethical concerns[65]. The rapid development in metagenome analyses offers the potential to rapidly detect emerging pathogens and related antimicrobial resistance genes. Since monitoring of pathogens and AMR in wastewater can provide timely information on pathogens of concern, the information can be used to assist policy managers with information on prevention strategies. This is one of the first coordinated global efforts to use wastewater in AMR surveillance, but others have also used wastewater to monitor AMR[34,46,48] on a smaller scale. Another notable program is the National Wastewater Surveillance System program in the United States, which was developed to track the presence of SARS-CoV-2 across the country[66] .

Using more types of data (such as information provided by wastewater analysis and data on antimicrobial usage and AMR in agriculture) in the surveillance of AMR will help create a more informed narrative on the prevalence and magnitude of AMR around the world. Using these data, policy makers can create evidence-based decisions on antimicrobial use and practice. Once work is done on standardizing data collection and reporting globally, data can be generated by an integrated One Health AMR surveillance system[5,51,52,54,59]. However, standardizing data collection is an ambitious goal for AMR surveillance because there are so many kinds of methods used for sample preparation and data collection.

WASTEWATER-BASED EPIDEMIOLOGY: A BRIEF INTRODUCTION

Wastewater-based epidemiology (WBE) is an approach that can be used to give comprehensive health information at a population or community level. WBE has become a growing field of scientific research, as wastewater contains the collective urine and faeces of whole communities and therefore contains a wealth of epidemiological information about chemical exposure, lifestyle, infectious disease, and wellbeing[55,67-69]. This approach can provide a ton of information on spatial and temporal consumption and serves as an intelligence tool for authorities[55,69]. This tool can be used to link exposure to environmental contaminants to health outcomes[69].

It is based on the concept that markers of chemicals and biologicals we consume or are exposed to (such as chemical compounds and biological microorganisms, defined as biomarkers) are excreted into wastewater either in their original or in a modified form (chemical metabolites)[68] [Figure 2]. In a recent review, Choi and colleagues described WBE as the normalization of analyte influent concentration to per capita mass loads using the daily flow and wastewater treatment plant (WWTP) population size[70]. WBE includes the extraction, detection, and analysis of chemical and/or biological markers in wastewater. This was first described by Daughton in 2001[71] and later tested by Zuccato[72]. Methods to improve WBE are continuously improving and evolving.

A huge number of global, long-term WBE monitoring initiatives have been created worldwide, such as in Europe[64,73], Australia[68,74-76], and the USA[77]. In the early days of WBE, research was focused on illicit drug consumption, including heroin, cocaine, and amphetamines[72,78,79], but has since diversified to include a large range of other factors such as alcohol[80-82], tobacco[76], SARS-CoV-2 (aetiological agent of COVID-19[83]), and psychoactive substances[84,85]. The success of WBE has been demonstrated globally and has encouraged discussion on the future use of the approach[55,69,86]. For example, a study in the Northern Territory of Australia by O'Brien *et al.* showed the use of WBE to assess the impact of policy-based interventions[87]. In this study, WBE was used to assess the successful outcome of setting a minimum unit price for alcohol to decrease alcohol consumption.

WBE is also used by surveillance systems previously mentioned to monitor AMR, such as the GSSP mentioned previously[88]. Monitoring the presence and prevalence of AMR bacteria and resistance genes in wastewater can provide information about the level of antimicrobial use and the emergence and spread of AMR within a local human population. By analyzing wastewater samples from different sources, such as hospitals, nursing homes, and communities, researchers can identify hotspots for AMR bacteria and genes. This information can then be used to inform public health strategies, including the development of targeted interventions to reduce antimicrobial use and prevent the spread of AMR infections. The role of WBE in policy making for the management of AMR is still emerging[89]. Surveillance systems such as NWSS will play a key role in preventing the spread of AMR by allowing the monitoring of trends and the identification of hotspots for resistance.

WBE can be used to monitor the use of antimicrobial agents in different populations and settings. For example, a study in South Africa found higher per capita antimicrobial usage in informal settlements than in sewerage connected communities[90]. Another study measured spatiotemporal trends in concentrations of antibiotics in Eastern China[91]. WBE can also monitor resistant pathogen distribution in the community. In one study, wastewater testing revealed geospatial-temporal trends of AMR pathogens in Australia[92]. WBE can be used to monitor the use of antimicrobials and AMR pathogens in different populations and settings.

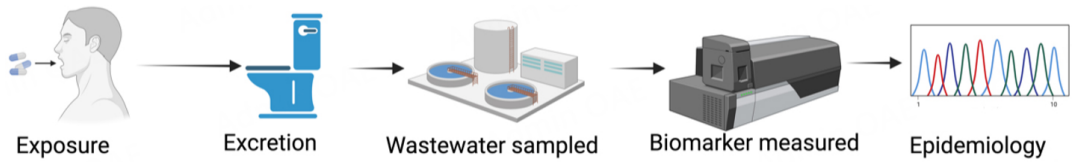

Figure 2. Wastewater-based epidemiology workflow. Created in BioRender.com[138].

Key considerations for WBE

Appropriate wastewater biomarkers

Biomarkers are compounds in wastewater that can be targets for WBE. Biomarkers can be sorted by their role as biomarkers of exposure, biomarkers of effect, biological biomarkers (e.g., metabolites, hormones), or by the disease they may suggest (e.g., cardiovascular biomarkers, obesity biomarkers)[69]. Examples of potential biomarkers include illicit drugs, alcohol, tobacco, UV filters, caffeine, pesticides, RNA, DNA, and antimicrobials. The choosing of a biomarker is a difficult task, as it needs to satisfy criteria as outlined in previous studies[69].

From a WBE perspective, a usable biomarker must be stable enough to allow for its measurement in WW - laboratory scale sewer reactor experiments can evaluate this[93]. Population biomarkers need to have low variance in the daily excretion. The selection of biomarkers can largely affect the results of the research and should be considered carefully.

A large number of resistance genes and antimicrobials can be potentially used as biomarkers, but not all are of equal importance[70]. Some guidelines for the selection of genes for wastewater-based monitoring were proposed by Lhat *et al.* and Berendonk *et al.*, who suggested the following: (i) clinically relevant genes posing a risk to human health; (ii) genes found in mobile elements, thus demonstrating potential for transfer; (iii) genes conferring resistance to high consumption antibiotics; (iv) genes conferring resistance to antibiotics that have been in use for a long time (e.g., tetracycline, sulfonamides); and (v) genes conferring resistance to newer, clinically relevant antibiotics such as the extended-spectrum beta-lactams[94,95]. Notably, sulfonamide and tetracycline resistance genes are among the most common resistance genes studied in wastewater, as both sulfonamide and tetracycline antimicrobials have been in use for a long time and cause resistance via multiple mechanisms[70].

Many new methods are currently being developed for the quantification of antimicrobials in wastewater, such as direct injection liquid chromatography-tandem mass spectrometry[96].

Wastewater sample collection

Wastewater samples are usually collected from municipal WWTPs as they serve communities located in specific sewerage catchment areas. Hence, wastewater from said community can be considered as its pooled excreta. Samples typically collected from WWTPs include wastewater influent and effluent as well as biosolids. Wastewater influent, which is collected at the inlet of the WWTP, can be analyzed to determine chemical or biological biomarkers that are excreted or discharged into the wastewater pipeline. This kind of sample can be used to determine community consumption of, or exposure to, a substance. Wastewater effluent samples, on the other hand, are samples that are collected at the exit of the WWTP after the wastewater has been treated. This type of sample is commonly used to estimate removal efficiencies during the treatment process and to monitor chemicals that are being discharged into the environment. Effluent can contain biological contaminants that can cause harm to humans and the environment. Therefore, it is important to analyze the risks associated with inadequate treatment and to understand the consequencesof

poor removal efficiencies.

In order to accurately determine the per capita amount of biomarkers from a sample, representative samples are collected over a specified time frame using autosamplers that collect high-frequency flow (preferred) or time-proportional wastewater influent samples[68,97]. Per capita daily consumption of a parent compound in each catchment is calculated using Equation (1)[72]

$$\text{daily chemical consumption}(i)\left(\frac{\text{mass/day}}{1000\text{ people}}\right) = (Ci * F * \frac{Ri}{Ei})/P \quad (1)$$

where Ci is the concentration of a given drug residue, i, measured in wastewater samples, F is the total wastewater volume during the sampling period, P is the number of people in the catchment, Ri is the ratio of molar mass of parent drug to its metabolite, and Ei is the average excretion rate of a drug residue, i[72].

Different methods can be employed to collect wastewater samples, including continuous and discrete sampling modes[97]. The most representative sampling mode is the continuous flow-proportional mode. In this mode, a side stream of wastewater enters that autosampler at a rate proportional to the flow rate of wastewater in the WWTP. Variants of this mode are described in detail in a review[97]. The least representative sampling technique is called the grab sample, as it only represents the amount of analyte present at a single time point[97]. An alternative sampling mode involves the use of passive samplers, which can absorb chemicals over a longer period, often days or weeks.

Once the wastewater is collected, a preservative such as hydrochloric acid may be added in order to minimize microbial degradation before samples are stored for chemical analyses - however, suitable preservation techniques can be biomarker-specific[70,98]. If the sample is being collected for genomic or culturing analysis, then it is mixed with 20% v/v of sterile glycerol and stored at -80 C in order to preserve the microbial community in the sample[46].

Wastewater analysis

Wastewater can be analyzed for AMR using several methods including analytical chemistry and molecular biology techniques[54]. When an appropriate chemical biomarker has been selected, samples can be analyzed to determine analyte concentrations. This often consists of a sample pre-treatment step, including filtration to remove solids in the sample matrix, as well as solid phase extraction (SPE) for clean-up and concentration of target analytes in a sample. Once the sample is "cleaned up", it can undergo chemical analysis, typically performed using quantitative analysis with liquid chromatography coupled to tandem mass spectrometry (LC-MS/MS).

If a sample is being analyzed using molecular microbiological methods, the sample will be filtered for solids and prepared via an appropriate DNA library preparation technique as described elsewhere[46]. These steps can vary depending on the specific technology being used (e.g., qPCR and/or metagenome sequencing) and the type of sample being analyzed, but some common steps include:

Sample collection and filtration: Depending on the source of the sample, it may need to be collected and filtered to remove any large solids or debris that could interfere with downstream processing.

DNA extraction: The DNA in the sample needs to be extracted and purified so that it can be used for downstream approaches, such as metagenome sequencing. Different extraction methods may be used

depending on the type of sample and the sequencing technology being used and are discussed in depth in other studies[99-101]. DNA quality and quantity is verified before progressing to:

Library preparation: The DNA needs to be fragmented and amplified using PCR to create a library of DNA fragments that can be sequenced. Different library preparation methods may be used depending on the sequencing technology being used.

Sequencing: The prepared library is then loaded onto the sequencing instrument, and the DNA fragments are sequenced using the appropriate sequencing chemistry.

After sequencing is complete, the resulting data is typically analyzed using specialized software and bioinformatics tools to identify and classify the different microbial species present in the sample, as well as their relative abundances, and functional capacity (such as relative abundance of *AMR* genes). Bioinformatics is needed for processing reads prior to assigning taxonomic rank in order to sort small species differences. This information can be used to gain insights into the microbial community structure and function, as well as potential links to human health or environmental processes.

Alternatively, samples may undergo qPCR to amplify specific gene targets of interest, or viable culturable bacteria in samples can be cultured[54].

Limitations and uncertainties of WBE

WBE has its own limitations that scientists and policy advisors need to be mindful of when describing data. Chemicals are not consistently stable under sewer conditions, as they may degrade or transform before entering the WWTP[102,103]. Therefore, sewer stability experiments may be required if data is not available in the literature. Another stability concern would be in-sample degradation, which can happen due to other compounds and microbes in the sample matrix. There are other concerns regarding the sampling mode (as explained above) and the flow measurement of the WWTP[68,104]. Furthermore, the matrix of wastewater can contain PCR inhibitors that can impair the accuracy of molecular-based methods of detecting *AMR* genes and microbes, particularly for qPCR[55]. In addition, population sizes in a catchment can normally only be estimated[105,106]. To apply Equation 1 (above) and determine the consumption of a compound, excretion data for the biomarker is needed. This information is not always readily available.

Even though WBE has its own set of limitations and uncertainties, technological advancements have improved the field. For example, one study advocates for biosensing techniques as a promising surveillance alternative. Another study showed improvement in qPCR methods for the detection of macrolide and tetracycline resistance[107]. Improvements in epidemiological modelling have also improved the field[108]. Although WBE has its own set of limitations and uncertainties, technological advancements in the field of biosensors[109], qPCR detection methods[107], and epidemiological modelling[108] have improved the field.

A benefit of WBE is that since WW samples are pooled samples from a community, the anonymity of the individual person is largely maintained. However, in some cases, it is necessary to manage the privacy of location data to prevent the stigmatization of certain groups of people. The ethical considerations of WBE for pharmaceuticals and drugs have been discussed elsewhere[65,110,111]. Generally, populations over > 10,000 are large enough to give anonymity and there is little risk in characterizing smaller societal groups[55].

Using metagenomic data comes with the risk that the individual person can be identified using archived wastewater samples due to the fact that metagenomic sequencing sequences all the DNA in the sample.

However, it is unlikely that the data would actually be traced back to the individual as researchers are focused on mapping human pathogens in wastewater[112,113]

Wastewater-based epidemiology and antimicrobial resistance

WBE poses a unique and innovative way of monitoring AMR in a community because it would allow for the simultaneous measurement of antimicrobial compound concentrations and AMR microbes in a community. Table 1 highlights key methodologies for detecting AMR and antimicrobials using WBE and references several studies that have detected antimicrobials and AMR organisms in wastewater in different capacities[46,48,64,67,114-116] . The table highlights the use of LC-MS, sequencing technologies, and culture-based methods for detecting AMR and antimicrobials and illustrates the pros and cons of each method. Combining the aforementioned detection methods with WBE methods[74] can also provide useful and unique information on socioeconomic determinants and temporal trends in the use of antimicrobials. Since the usage of some antimicrobials is seasonal, there are potentially compelling opportunities for trends to be established via wastewater analysis. Some studies have shown periodic patterns for several antibiotics, including clarithromycin, erythromycin, and ciprofloxacin, with more use observed in winter[117,118]. In addition, in locations where prescription information is not made available or antimicrobial medications can be bought over the counter easily, WBE can be a way of monitoring antimicrobial use in a community.

As mentioned before, WBE has been excellent at monitoring drug usage and has the potential to assist in the surveillance of AMR[46,51,55]. Using and developing a standardized methodology for characterizing AMR in wastewater will be important in quantifying AMR prevalence in communities. Recent literature on detecting antimicrobial residues in wastewater using analytical chemistry[119-121] is similar to how WBE has already been used to detect other drugs (as described in the previous section).

Another potential biological marker in wastewater for AMR is DNA fragments from bacteria. Most recently, wastewater has been used to monitor the prevalence and spread of COVID-19 (SARS-CoV-2 virus) in Australia[83] and around the world[122,123]. Government health authorities such as Queensland Health have been able to use this data to inform public health policy with respect to the pandemic[124]. Next-generation sequencing of wastewater samples can provide much data on the microbial communities in samples, including identification of the wide range of pathogens and resistance genes present. Analysis of the resistance genes present has been shown to provide key information on novel pathogens, as well as re-emerging infectious diseases and AMR[19,88,125]. Several studies have been published recently on the surveillance of AMR using WBE[46,64,126-128].

While standardization of protocols for sequencing remains a challenge, the improvements in technology combined with decreasing sequencing costs have the potential to improve both pathogen and resistance surveillance in wastewater.

WBE for AMR research: addressing gaps in the literature

Most surveillance systems for AMR focus on key pathogens and use passive laboratory reporting[46,51]. Integrating the power of WBE with the clinical and veterinary surveillance of AMR will help create a more informed understanding of the prevalence and diversity of AMR in microbial populations[5,19,51,55,129].

WBE could aid in providing this population-wide information on the prevalence of AMR in human populations. A large range of antimicrobial resistance genes (ARGs) have been studied and analyzed in wastewater, typically through qPCR[34,41,128,130-133]. Only a couple of studies have analyzed relationships between the levels of antimicrobials and the abundance of ARGs in wastewater. There have been some

Table 1. Methodology of using WBE to quantify AMR

Methodology	Description	Pros.	Cons.	Refs.
LC-MS	Liquid chromatography coupled with mass spectrometry (LC-MS) to elucidate antimicrobial compound concentrations in wastewater	By combining HPLC and MS, the strengths of both techniques can be used	Initial costs, requires skilled personnel to set up	[139]
Sequencing using Illumina	Next-generation sequencing of microbial genetic material in a wastewater sample using the Illumina platform	Technology used widely, lowest cost, wide range of instruments, lowest error rates	Long sequence runs, shortest read lengths, no real-time data access	[140,141]
Sequencing using Oxford Nanopore technology	Next-generation sequencing of microbial genetic material in a wastewater sample using the Oxford Nanopore platform	Fast sequencing, longest confirmed reads, small instrument footprint, lowest instrument and consumable cost, real-time data access	Highest error rate of all the platforms	[142]
Sequencing using Ion Torrent technology	Next-generation sequencing of microbial genetic material in a wastewater sample using the Ion Torrent platform	Fast run time, comparatively cheap, long reads possible	High error rate, lower overall data output	[143]
Sequencing using Pacific Biosciences technology	Next-generation sequencing of microbial genetic material in a wastewater sample using the Pacific Biosciences platform	Fast sequencing runs, long reads, real-time measurement of base incorporation	Largest instrument footprint, lower output per run, higher error rates	[144,145]
Metagenomic sequencing	Can be defined as the sequencing of all genomes in a sample	Gathers information on all genomes in a sample, discovery of novel organisms, no a priori data needed	DNA of environmental microorganisms cannot be extracted completely, the sequencing may miss low-abundance microorganisms, there is no "gold standard" for bioinformatic software	[146,147]
Sequencing using 16S region of microbial DNA	Sequencing of the microbial 16S rRNA region of genetic material using a sequencing technology	Targets and reads a region of the 16S rRNA gene which is found in all Bacteria and Archaea, relatively cheap, lots of computational pipelines available	Can only identify organisms that have a 16S rRNA gene, multicopy variation of the 16S rRNA gene, 16S rRNA gene variable regions cannot typically resolve species	[148,149]
Whole-genome sequencing	Sequencing of the genetic material of a single organism using sequencing technology. Can identify all the genes in a genome including ARGs, and contribute to high-resolution genome assembly and identification of bacterial species/strains	Provides a high-resolution, base-by-base view of the genome, lots of computational pipelines available	The processing involves a few extra steps compared to 16S rRNA sequencing, is more expensive, computationally intense	[46,150]
Measuring resistance genes using qPCR	Using quantitative polymerase chain reaction (qPCR) to quantify the quantity of *AMR* genes	Comparatively cheap (compared to most sequencing technologies), fast method of measuring resistance genes	Need prior sequence data of the specific target gene of interest, needs standard curve analysis, susceptible to impurities present in the sample	[151,152]
Using droplet digital PCR	Using droplet digital PCR (ddPCR) to quantify the quantity of *AMR* genes	Accurate absolute quantification of pathogens, less contamination, no need for standard curves, more resilient to inhibitory substances	Clinical application of ddPCR is still not popular, there are fewer references available	[144,153,154]
Culture-based methods	With or without antibiotic or selective media, allows for the identification of specific taxa	Relatively quick, cheap	Lacks resolution (number of taxa studied), ignores "unculturable" bacteria, low sensitivity (compared to molecular methods), works best for bacteria that replicate efficiently in rich media within 24 h, slow growing and viable but not culturable (VBNC) bacteria are not detected.	[54]

correlations observed between antimicrobials and respective resistance gene levels that have been antimicrobial dependent[48,133,134-136]. For example, Elder *et al.* observed positive correlations between fluoroquinolones and *qnr*S quantity between different locations ($r = 0.997$, $P < 0.004$)[137]. Further research is needed to elucidate the relationship (if any) between antimicrobial concentrations and AMR in wastewater. These data can be used to inform on the prevalence and risk of AMR in human populations, wastewater, and receiving environments.

CONCLUSIONS

WBE has emerged as a promising tool for the surveillance of AMR in human populations. WWTPs are a major point of entry for antimicrobial agents and resistant bacteria into the environment, making them an ideal site for monitoring AMR introduced to aquatic environments. This review discusses the potential of WBE for AMR surveillance, as well as the challenges and limitations associated with this approach. We highlight the importance of selecting appropriate sampling strategies and analytical methods to ensure the accuracy and reliability of the data.

One of the main advantages of WBE is that it provides a population-wide snapshot and trend analysis of AMR trends, as it captures wastewater from multiple sources, including households, hospitals, and industrial sites. This can help to identify hotspots of AMR and inform targeted interventions. WBE can help identify the changepoints in target concentrations after targeted interventions. This review also discusses the potential of WBE for monitoring the use of antimicrobial agents in different populations and settings. By analyzing the concentration and distribution of specific antimicrobials and their metabolites in wastewater, it is possible to estimate the consumption of these drugs in the population. We would like to highlight the importance of collecting and archiving representative samples now so that we can establish baseline data retrospectively, particularly as the costs of analyses are decreasing and the accuracy and scope of analyses are only improving.

However, there are also several challenges associated with WBE, such as variability in wastewater composition, dilution effects, and the presence of confounding factors such as environmental stressors, co-selective pressures, and developing models for fecal/urine shedding. In addition, the interpretation of WBE data requires a deep understanding of the local context and the factors that may influence AMR trends.

Overall, this review highlights the potential of WBE for AMR surveillance and calls for further research to optimize sampling and analytical methods, develop standardized protocols, and validate the data against clinical and environmental data.

DECLARATIONS

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Authors' contributions

Prepared the manuscript: Clarke LM
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Conflicts of interest

All authors declared that there are no conflicts of interest.

Ethical approval and consent to participate

Not applicable.

Consent for publication

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