Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

WASTEWATER MICROBIOLOGY AND PUBLIC HEALTH: PATHOGENS, ANTIMICROBIAL RESISTANCE, DETECTION, AND SUSTAINABLE TREATMENT STRATEGIES

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DOI: https://doi.org/10.5281/zenodo.23120712

Keywords
Wastewater microbiology, Public health, Antimicrobial resistance, Wastewater-based epidemiology, Sustainable wastewater treatment.

Article History
Received: 01 November 2025
Accepted: 17 December 2025
Published: 31 December 2025

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Abstract

Background: *Wastewater is an environment of complex microbes, including pathogenic microorganisms, antimicrobial-resistant bacteria, antibiotic-resistance genes, and other biological contaminants, with potential implications on human and environmental health.*

Objective: *This review aims to highlight the recent advancements in wastewater microbiology with emphasis on pathogenic microorganisms, antimicrobial resistance (AMR), wastewater microbial detection and surveillance methods, and sustainable wastewater treatment.*

Methods: *Recent peer-reviewed studies on bacterial, viral and protozoan pathogens associated with wastewater, AMR bacteria and resistance genes, wastewater-based epidemiology, molecular detection, metagenomics, and conventional and advanced treatment technologies were critically synthesized. Studies published between the years of 2022 and 2025 were given special attention.*

Results: *This study shows that wastewater contains many different types of microbial pathogens and AMR determinants from domestic, healthcare, agricultural and industrial sources. While culture-based methods are the gold standard for viable organism recovery and antimicrobial susceptibility testing, quantitative PCR, digital PCR, and metagenomic sequencing offer highly sensitive and comprehensive methods of detecting pathogens and resistance determinants. Wastewater based epidemiology has become an important population-level surveillance method. While conventional treatment can significantly decrease the amount of microbial contamination, it is essential to support the use of advanced or nature-based treatment technologies, as resistant organisms and ARGs can remain.*

Conclusion: *One Health approach of wastewater surveillance and multi-barrier treatment can enhance surveillance for infectious diseases, minimize environmental spread of AMR, and enhance public-health protection. Standardization of sampling, molecular techniques, treatment optimization and sustainable wastewater management should be considered in future research.*

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ISSN: (e) 3007-1607 (p) 3007-1593

INTRODUCTION

Wastewater is now seen as an important environmental source of microorganisms, antimicrobial resistance determinants, chemical contaminants, and biological information that signifies the health of the human population (Kumar and Pal, 2018). It receives a complex mixture of domestic sewage, hospital effluents, industrial effluents, agricultural effluents and other anthropogenic wastes giving rise to the highly dynamic microbial environment. Apart from the conventional FIOs, wastewater can also contain bacterial, viral and protozoan pathogens that can cause gastrointestinal, respiratory, urinary and other infections (Singh et al., 2024). This unchecked release of poorly managed wastewater in rivers, lakes, groundwater, agricultural fields, and coastal areas may thus provide routes for the spread of microorganisms and human exposure (Sassi et al., 2025). Thus, the treatment and control of wastewater are not just a challenge of environmental engineering but are also an important part of public-health protection and environmental microbiology (Kumar and Pal, 2018).

The microbiological composition of wastewater is especially significant since many pathogenic and opportunistic microorganisms present in wastewater can withstand wastewater collection and treatment systems, and may ultimately get into receiving environments (Owojori et al., 2024). A wide range of bacterial pathogens including Escherichia coli, Salmonella spp., Shigella spp., Campylobacter spp., Vibrio spp., Pseudomonas aeruginosa and pathogenic Enterococcus spp. is reported in wastewater systems (Zhang et al., 2025). In addition, the role of viral pathogens such as noroviruses, enteroviruses, hepatitis viruses, adenoviruses and other enteric viruses in relation to persistence and potential for transmission via contaminated water and environmental matrices (Lanrewaju et al., 2022) is important. The presence of protozoan pathogens like Giardia and Cryptosporidium, whose presence in the environment is associated with the resistant cysts, also complicates microbial risk assessment as the conditions under which these cysts are found are the ones that do not favour the survival of many bacterial pathogens (Sosah et al., 2025). In recent years, new metagenomic methods have also shown that wastewater is a source of much wider diversity, in terms of microorganisms, than can be identified by the monitoring of indicator organisms only.

Antimicrobial resistance (AMR) is one of the most worrying new public health issues related to wastewater microbiology. Healthcare facilities, domestic and industrial waste, pharmaceutical industries, animal husbandry and agricultural practices are all sources of antimicrobial resistant bacteria (AMRBs), antibiotic resistance genes (ARG) and antibiotic residues into wastewater (Fouz et al., 2020). These biological and chemical constituents are then introduced to and concentrated in wastewater treatment plants (WWTPs), where complex microbial communities can exist and resistant organisms and resistance determinants may survive (Ju et al., 2019). ARGs may reside within mobile genetic elements, like plasmids, transposons, and integrons, or in bacterial chromosomes and be horizontally transferred between microorganisms (Tokuda and Shintani, 2024). This environmental spread of resistance determinants is especially problematic because sewage can serve as a conduit for the transfer of resistance determinants from human, animal and environmental microbial communities. As a result, the awareness of wastewater as a part of the environmental AMR pathway has further accentuated the importance of integrated surveillance in the context of One Health (Patra and Dubey, 2025).

Volume 3, Issue 10, 2025

Frontier in Medical & Health Research

ISSN: (e) 3007-1607 (p) 3007-1593

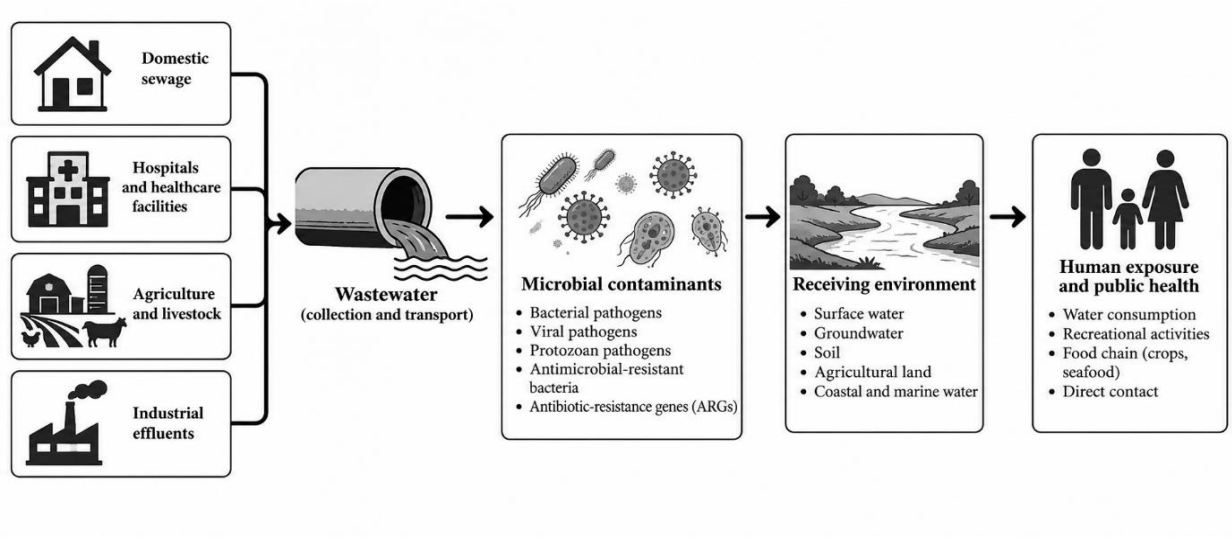

Figure 1. Major sources and pathways of microbial contamination in wastewater.

Conventional wastewater treatment systems offer significant protection against microbial contamination, but they have been developed primarily to remove organic matter, suspended solids, nutrients and conventional pollutants, and not specifically all pathogens or antimicrobial-resistance determinants. The microbial loads can be significantly reduced by primary sedimentation, secondary biological treatment, and tertiary processes; however, the efficiencies of these various treatments are dependent on the type of microorganism, the characteristics of the wastewater, treatment configuration, hydraulic conditions, temperature, and operating parameters. Furthermore, decreases in viable microorganisms do not equate to total elimination of extracellular DNA and ARGs. The use of the treatment may lead to the survival of resistant bacteria and the dissemination of resistance genes as a result of treatment and via treated effluent or associated sludge. Advanced treatment processes such as membrane bioreactors, ultraviolet/UV, ozonation, advanced oxidation processes and combined treatment processes have been gaining interest due to these limitations. Concurrently, microbial risk reduction systems are gaining more interest due to their lower energy consumption compared to other technologies, with nature-based technologies like constructed wetlands and biological filtration systems being investigated (Lou et al., 2021; Olds et al., 2018; Zhan et al., 2024).

The investigation of microorganisms associated to wastewater has undergone a revolution thanks to the creation of sensitive and rapid analytical technologies. Culture-based approach has its own merits as it is possible to obtain viable organisms and conduct phenotypic antimicrobial susceptibility testing, but it has its limitations, such as taking a long time to complete the process and not being able to culture many microorganisms in the environment in a routine laboratory setting (Morshed, 2024). The molecular approaches such as quantitative polymerase chain reaction (qPCR) and reverse-transcription qPCR enable sensitive detection and quantification of specific microbial pathogens and resistance genes (Liu et al., 2023). Absolute quantification and analytical sensitivity of selected targets can be further enhanced with digital PCR. In more recent times, whole genome sequencing and metagenomic techniques have helped to define the genes and genes for resistomes, virulence and microbial diversity without limiting analysis to a specific set of targets. The combination of these strategies offers a more holistic view of microbial risks and their dynamics in wastewater treatment facilities (Maestre-Carballa et al., 2024).

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Wastewater microbiology also has become significantly more relevant via wastewater-based epidemiology (WBE). Monitoring wastewater can offer population-level data on infectious agents that are circulating and mixing up biological material from many people in specific sewer catchments (Parkins et al., 2024). As of late, wastewater monitoring has been shown to be a useful tool for supplemental surveillance of SARS-CoV-2 transmission and novel variants during the COVID-19 pandemic (Organization, 2023). This is now the subject of study for other infectious diseases, antimicrobial resistance and other public health measures. Wastewater surveillance can provide an additional source of epidemiological evidence, as it can collect data from symptomatic, asymptomatic, and people not seeking health services. However, the interpretation of wastewater signals must consider the shedding pattern of pathogens, the properties of sewer systems, rainfall, population size, sampling protocols, analytical recovery, and the difference between nucleic-acid detection and the presence of infectious organisms (Parkins et al., 2024; Singh et al., 2024).

The growing convergence of environmental microbiology, molecular diagnostics, wastewater engineering, and public health highlights the need for an integrated approach to wastewater management (Renganathan et al., 2025). In addition to technological advances in treatment, however, there is a need for standardized surveillance, reliable detection methods, assessment of antimicrobial resistance, and interpretation of the population-level microbial signal to achieve effective microbial risk reduction (Tacconelli et al., 2018). Management of wastewater is particularly important in LMICs where microbial and AMR risk has been growing due to rapid population growth, weak sanitation systems, low treatment levels, water scarcity, and increased use of antimicrobials (Khalid et al., 2018). However, alongside the removal efficiency, treatment technologies must be assessed based on other factors including energy use, chemical requirements, simplicity of operation, cost, environmental impact, and local conditions (Azmi et al., 2025; Bhojwani et al., 2019; Zagklis and Bampos, 2022).

This review critically analyzes the wastewater microbiology and public health association, highlighting specific issues regarding pathogenic microorganisms, antimicrobial resistant bacteria and resistance genes, current detection and surveillance methods and options for sustainable wastewater treatment methods. The review also addresses the use of wastewater-based epidemiology, the shortcomings of existing monitoring strategies, obstacles in the wastewater treatment process, and innovative possibilities for incorporating molecular surveillance into multi-barrier treatment networks. This review seeks to create a synoptic view that integrates these components into the One Health approach to help understand how wastewater microbiology can play a role in prevention of infectious disease transmission, antimicrobial resistance, environmental health, and the creation of safer, more sustainable water-management practices.

2. Literature Review

In 2022, research significantly reinforced the significance of wastewater as an environmental reservoir of antimicrobial resistance (AMR) genes and bacteria. A systematic review by (Chau, 2022) suggests that wastewater-based surveillance could offer information on a community level that is complimentary to conventional clinical surveillance. The authors emphasized that the use of composite influent sampling and extended monitoring periods would enhance the correlation between wastewater and human AMR. Systematically reviewed wastewater surveillance of antibiotic resistant bacterial pathogens and highlighted the potential of wastewater surveillance in a One Health Approach in the same year, and also highlighted limitations in sensitivity, methodological heterogeneity and clinical interpretation (Tiwari et al., 2022). Other 2022 reviews found that ARGs like bla, sul, tet, and erm were commonly found in WWTPs and that traditional treatment was not always effective in removing ARGs. These results put wastewater under focus as a critical link between

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ISSN: (e) 3007-1607 (p) 3007-1593

environmental contamination, antimicrobial resistance, and public health.

In 2023, research shifted beyond identifying the presence of microorganisms in wastewater to wastewater as an active public-health surveillance tool. Wastewater as a source for information about pathogen spread, evolution, and outbreak risk; highlighting the ability to capture the population-level signal that may complement clinical surveillance (Barnes et al., 2023). A longitudinal study published in Water Research in China, which analyzed wastewater surveillance data alongside those of Salmonella and norovirus, as well as influenza A virus, during a large outbreak, showed the potential of wastewater data and predictive models to monitor multiple infectious diseases. Meanwhile, studies of AMR continued to show the importance of WWTPs for the persistence and dissemination of resistant bacteria and ARGs. The other reviews in this period covered advanced oxidation processes, membrane filtration, membrane bioreactors, and other advanced technologies for controlling AMR in water systems. Overall, 2023 studies illustrated how wastewater microbiology was evolving from the traditional monitoring of pollution to the integrated surveillance of pathogens, AMR monitoring, and treatment optimization (Levy et al., 2023).

Wastewater surveillance research grew significantly in 2024, both with regard to detection of pathogens and to antimicrobial-resistance monitoring. Singh et al. compiled a thorough review of the wastewater surveillance and its use for gastrointestinal and respiratory pathogens, including SARS-CoV-2 and compared culture based methods, PCR, digital PCR, sequencing and other molecular methods (Singh et al., 2024). The review highlighted that wastewater can collect signals from symptomatic, asymptomatic and presymptomatic persons, and is therefore important as an early warning system for the population. Wastewater was also identified as a significant pathway for pathogens, ARB, ARGs and antibiotics, with South Asia research indicating this is a problem in areas where untreated or inadequately treated wastewater persists. The authors noted that while conventional treatment can lower bacterial loads, it may be less effective at lowering ARGs, and indicated increased surveillance and policy action is required. In contrast, metagenomic sequences were able to yield a wider characterization of microbial communities and resistance determinants during the same time period than targeted assays could. In this context, the literature from 2024 reiterated the need for molecular surveillance, AMR monitoring, and advanced treatment within the framework of public health.

In the literature from 2025, wastewater surveillance was further integrated into a One Health approach, including for monitoring antimicrobial resistance. A global scoping and temporal review of AMR surveillance revealed that 136 (76.8%) of the 177 reports published between 2014 and October 2024 were published after 2019, reflecting the fast expansion of this research field (Popoola et al., 2025). The review also identified the use of quantitative PCR and sequencing-based methods for monitoring resistant bacteria and ARGs in wastewater is growing and that wastewater surveillance can be used to evaluate AMR within the entire sewer catchment and not just clinical samples. Notably, wastewater treatment plants were identified as sites where human, animal and environmental AMR pathways intersect, and the presence of resistant organisms and genes in receiving waters and agricultural systems was identified as a potential pathway for resistant organisms and genes to be released from the WWTP through treated effluent or sludge. The results highlighted the importance of using wastewater surveillance in conjunction with clinical and environmental data and not as a standalone approach. This time, therefore, there was a shift towards the wider surveillance networks, molecular typing and environmental AMR risk assessment.

Increased efforts have been focused on enhancing treatment barriers and managing the persistence of antimicrobial contaminants and resistance determinants in the wastewater in recent research conducted in 2025. In 2025, a critical review of hospital wastewater highlighted the presence of antibiotics, resistant bacteria and ARGs in hospital effluents and the significance of multi-

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ISSN: (e) 3007-1607 (p) 3007-1593

barrier treatment systems in minimizing environmental and public-health hazards (Sharma et al., 2025). A further review of antibiotics in wastewater by the systematic review methodology was conducted, and it concluded that conventional WWTPs can only remove antibiotic residues to varying extents and in an incomplete manner, and that advanced treatment and treatment using quaternary treatment processes can increase removal efficiency, but that oxidative treatment can lead to the formation of transformation products, which also need to be subjected to environmental assessment (Klimonda and Kowalska, 2025). Decentralized wastewater systems have also been identified as possible under-reported reservoirs for AMR/ARG dissemination in research on decentralized wastewater systems. The latest results highlight the need for more than just conventional biological treatment for future wastewater management and show that this will need molecular monitoring, source control, and risk-based assessment, among other technologies. Overall, the literature from 2025 is progressing towards integrated and sustainable treatment systems that can control pathogens, antibiotic residues, resistant organisms, and resistance genes (Okonkwo, 2023).

Table 1. Thematic analysis of recent literature on Wastewater Microbiology and Public Health

Year	Study / Authors	Major Theme	Methodology / Approach	Key Findings	Public-Health Relevance	Identified Gap
2022	Chau et al.	**AMR wastewater surveillance**	Systematic review of wastewater–human AMR studies	Wastewater surveillance showed potential for identifying associations between AMR in wastewater and human populations; composite influent sampling and longer surveillance periods were highlighted as important methodological features.	Provides population-level information that can complement clinical AMR surveillance.	Lack of standardized sampling, analytical protocols and reporting across studies (Chau et al., 2022).
2022	Tiwari et al.	**Antibiotic-resistant bacterial pathogens**	Systematic review of wastewater surveillance studies	The review identified substantial research interest in ARB/ARG surveillance and emphasized the potential of wastewater as a One Health	Supports environmental monitoring of resistant pathogens alongside clinical surveillance.	Clinical relevance, sensitivity and methodological uniformity remain insufficiently established (Tiwari et al., 2022).

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Year	Study / Authors	Major Theme	Methodology / Approach	Key Findings	Public-Health Relevance	Identified Gap
				surveillance matrix.		
2023	Levy et al.	**Wastewater-based epidemiology**	Review/perspective on wastewater surveillance	Wastewater was highlighted as a source of information about pathogen spread, evolution and outbreak risk, extending surveillance beyond conventional clinical testing.	Can provide population-level early-warning information and capture infections that may not be clinically diagnosed.	Interpretation is affected by shedding patterns, sewer dynamics and analytical limitations (Levy et al., 2023).
2023	Barnes et al.	**Multiple infectious pathogens**	Longitudinal wastewater sampling and molecular detection	Wastewater surveillance was increasingly applied to multiple pathogens rather than SARS-CoV-2 alone, demonstrating the potential for broader infectious-disease monitoring.	Supports integrated infectious-disease surveillance and outbreak preparedness.	Standardized multi-pathogen surveillance frameworks are still developing (Barnes et al., 2023)
2024	Singh et al.	**Pathogen detection and public health**	Narrative review of wastewater surveillance	Wastewater surveillance was identified as a useful population-level tool for monitoring gastrointestinal and respiratory pathogens, with PCR, digital PCR and sequencing among important detection approaches.	Provides an additional surveillance system for infectious diseases and early outbreak detection.	Sampling, concentration, detection sensitivity and interpretation require further standardization (Singh et al., 2024).
2024	Tokuda et al.	**Viral pathogens**	RT-qPCR, sequencing and	Recent research strengthened evidence that	Particularly useful where clinical testing	Detection of viral nucleic acid does not

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Year	Study / Authors	Major Theme	Methodology / Approach	Key Findings	Public-Health Relevance	Identified Gap
			wastewater-based epidemiology	wastewater can provide early signals of viral circulation and can complement clinical surveillance.	is incomplete or delayed.	necessarily demonstrate infectious virus (Tokuda and Shintani, 2024).
2024	Parkins et al.	**Microbial diversity and resistome**	Shotgun metagenomics / genomic analysis	Metagenomic approaches enabled simultaneous characterization of microbial communities, pathogens and resistance determinants beyond predefined PCR targets.	Provides broader assessment of environmental microbial hazards and AMR.	High cost, bioinformatics requirements and difficulties in interpreting viability and clinical significance. (Parkins et al., 2024)
2025	Popoola et al.	**AMR and One Health surveillance**	Global scoping and temporal review	**177 reports** were identified for wastewater AMR surveillance; **136 (76.8%) were published after 2019**, showing rapid growth of the field. qPCR and sequencing were prominent approaches.	Demonstrates the increasing role of wastewater in connecting human, animal and environmental AMR surveillance.	Research remains geographically uneven, with greater representation from high-income countries (Popoola et al., 2025).
2025	Rwnganathan et al.	**AMR mitigation and wastewater management**	Mixed-methods survey and interviews involving One Health stakeholders	Stakeholders identified wastewater monitoring deficits, infrastructure limitations and persistent AMR reservoirs; technological interventions were considered important but	Shows that AMR control requires coordination between healthcare, agriculture and environmental sectors.	Implementation barriers and differences between stakeholder priorities remain substantial (Renganathan et al., 2025).

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Year	Study / Authors	Major Theme	Methodology / Approach	Key Findings	Public-Health Relevance	Identified Gap
				influenced by cost, regulation and maintenance.		
2025	Sharma et al.	**AMR in vulnerable populations**	Scoping review; culture, AST, PCR and whole-genome sequencing	Enterobacterales, particularly *E. coli* and *Klebsiella* spp., were frequently detected, with substantial resistance reported to several antibiotic classes.	Wastewater may provide information about AMR circulation in institutional populations.	Limited sample sizes and relatively small number of eligible studies (Sharma et al., 2025).

The thematic analysis presented in Table 1 demonstrates the progressive expansion of wastewater research from conventional pathogen detection toward integrated public-health and One Health surveillance. Recent studies have increasingly focused on antimicrobial-resistant bacteria and ARGs, highlighting wastewater as an important environmental reservoir for resistance dissemination. Molecular techniques, particularly qPCR, digital PCR, and metagenomic sequencing, have improved the sensitivity and breadth of microbial detection. The literature also demonstrates the growing application of wastewater-based epidemiology for population-level monitoring of infectious diseases. However, substantial methodological differences in sampling, concentration, detection, and data interpretation remain. Overall, the evidence supports the integration of standardized wastewater surveillance with sustainable treatment strategies to strengthen public-health protection and environmental AMR control.

3. Discussion

The recent literature demonstrates that wastewater has evolved from being considered primarily a source of environmental pollution to an important interface between microbiology, antimicrobial resistance, environmental health, and public health. The findings from the studies summarized in Table 1 consistently show that untreated wastewater contains a variety of bacterial and viral pathogens, antimicrobial-resistant bacteria, ARGs sourced from domestic, healthcare, agricultural, and industrial sources. These micro-organisms increase the complexity of wastewater and make it a significant matrix to understand biological hazards circulation at the population level. This role has been further reinforced as wastewater-based epidemiology has been growing in use, providing community-level monitoring without a reliance on a full reliance on individual clinical testing. Nevertheless, wastewater signals are still dependent on wastewater flow, population-size, pathogen shedding properties, sampling frequency and analytical recovery.

One of the most significant issues raised by recent wastewater studies is the issue of antimicrobial resistance. ARGs and resistant bacteria can be introduced into wastewater from hospitals, residential communities, livestock operations, pharmaceutical production, and agricultural settings, and may survive through the wastewater treatment process (Olds et al., 2018). Mobile genetic elements like plasmids, integrons and transposons are important because they may allow for horizontal transfer of resistance determinants between bacterial populations. It has therefore become more common to include a One Health approach, in which measures for human, animal, and environmental AMR surveillance are not separate, but intertwined (Liu et al., 2023). However, the detection of an ARG does not

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ISSN: (e) 3007-1607 (p) 3007-1593

automatically mean the presence of a clinically resistant pathogen and requires molecular detection together with culture-based isolation, antimicrobial susceptibility testing and genomic characterisation (Owojori et al., 2024).



Figure 2. Integrated wastewater microbiology and public-health management framework.

A major advancement is the swift progress of molecular and genomic detection technologies. Although the conventional culture is useful for the recovery of viable microorganisms and for detection of phenotypic antimicrobial susceptibility, it can not successfully characterize the entire microbial community (Popoola et al., 2025). The detection of predefined pathogens and resistance genes is relatively fast and sensitive using qPCR and RT-qPCR, and absolute quantification of predefined pathogens is highly sensitive using digital PCR. Moreover, metagenomic sequencing allows for the comprehensive exploration of microbial diversity, pathogens, ARGs, virulence-associated genes, and potential genetic associations (Sharma et al., 2025). These methods are not directly comparable, however, and they have pros and cons. Detection of microbial nucleic acids alone is not sufficient to determine viability or infectivity, particularly when making public health risk assessments based on wastewater data. The literature also suggests wastewater treatment must be viewed as a multi-barrier system and not a single treatment process. While conventional biological treatment can significantly reduce microbial counts, ARGs and resistant bacteria can survive or act differently than conventional AR and may not be detected by conventional AR. The advanced processes such as membrane filtration, ultraviolet (UV) irradiation, ozonation, advanced oxidation and hybrid treatment systems may add further barriers to microbial hazards (Kumar and Pal, 2018). Meanwhile, sustainable solutions like constructed wetlands and other nature-based solutions could be especially applicable in decentralized and limited resource contexts. Therefore, future wastewater management should combine effective microbial treatment,

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ISSN: (e) 3007-1607 (p) 3007-1593

standardized molecular surveillance and AMR monitoring, with sustainable engineering principles. This integration may help improve early detection of an infectious threat, minimize environmental spread of AMR and aid to safer reuse of water and long-term public-health protection.

Conclusion

The microbiological properties of wastewater have gained significant importance in environmental health and public-health research as wastewater is a complex reservoir of pathogenic microorganisms, antimicrobial-resistant bacteria, antibiotic-resistance genes, and other biological contaminants. The studies reviewed in this paper show that untreated or poorly treated wastewater can spread microbial threats and antimicrobial resistance to the environment. The detection and characterization of pathogens and resistance determinants has been greatly improved with recent advances in quantitative PCR, digital PCR, whole genome sequencing and metagenomics. Wastewater-based epidemiology has also become a useful alternative population-level tool to support traditional clinical surveillance for infectious diseases, with its use continuing to grow concurrently.Concurrently, wastewater-based epidemiology has become a useful alternative population-based tool to support conventional clinical surveillance for infectious diseases. While there can be significant microbial reduction at conventional wastewater treatment plants, the resistance of these organisms and ARGs underscores the need for additional treatment barriers to remove resistant organisms and ARGs. The advanced technologies such as membrane filtration, ultraviolet treatment, ozonation, advanced oxidation and nature-based treatment offer promising solutions when properly conceived and tested. Wastewater management in the future should include microbial surveillance, monitoring of AMR, effective wastewater treatment, and sustainable wastewater engineering, as part of a One Health approach. To enhance comparability and reliability, it is important to have standardized sampling, analytical protocols, risk assessment, and long-term monitoring. These are all integrated approaches that can help ensure safer water reuse and minimize environmental spread of AMR and pathogens, and provide better protection of human and environmental health.

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