PROCEEDING

2nd Banten International Conference on Health Advancement & Research
binar@poltekkesbanten.ac.id
https://binar.poltekkesbanten.ac.id/
Vol. 1 No. 2 (2026)

LABORATORY SURVEILLANCE OF ANTIMICROBIAL RESISTANCE IN HOSPITAL WASTEWATER IN LOW- AND MIDDLE-INCOME COUNTRIES: A SYSTEMATIC REVIEW

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ABSTRACT

Antimicrobial resistance (AMR) poses a critical global health threat. Wastewater surveillance has recently emerged as a valuable public health tool for monitoring AMR in communities and healthcare settings. This narrative review comprehensively examines the role of hospital wastewater surveillance (HWWS) in monitoring antimicrobial resistance. Methods to detect resistant bacteria and antimicrobial resistance genes (ARGs) in wastewater systems, ranging from culture-based techniques to advanced molecular approaches, including polymerase chain reaction (PCR) and next-generation sequencing (NGS), are explored. The review synthesizes data on key antimicrobial resistance genes commonly detected in hospital effluents and explores how HWWS contributes to understanding the dynamics of resistance within healthcare settings. Furthermore, it identifies methodological challenges and inconsistencies in data reporting and outlines necessary standardization steps to enhance the effectiveness of HWWS programs. Opportunities for integrating HWWS with clinical and public health frameworks are presented, emphasizing the need for robust metadata and transparent reporting. This review provides a comprehensive approach to HWWS strategies, which could complement robust infection control and antibiotic stewardship interventions to combat AMR.

Keywords: Laboratory Surveillance; Resistance; Antimicrobial; Wastewater; Hospital

INTRODUCTION

Antimicrobial resistance (AMR) has emerged as one of the most pressing global public health challenges, with projections indicating that it will worsen significantly in the upcoming decades if urgent and targeted interventions are not implemented. In 2025, AMR caused an estimated 1.14 million deaths globally and was associated with approximately 4.71 million additional deaths. AMR transmission has been observed to occur through direct contact between hospital settings and patients. In particular, hospital wastewater (HWW) has been identified as a potential reservoir for pathogens that can lead to human infections acquired within the environment. Beyond human health, AMR threatens water safety, agriculture and ecology, making it a paradigm for a One Health issue (Chau, 2022).

The challenge of AMR has become more prominent during recent decades, also because of the slow rate of development of novel antimicrobials. Notably, most new agents represent modifications to existing drug classes rather than antimicrobial classes with new mechanisms of action. While AMR knows no borders and affects every country, low- and middle-income countries may bear the greatest burden, as they suffer from limited access to advanced-generation antimicrobials, weaker infection control infrastructure, and environmental mismanagement of pharmaceutical waste factors that illustrate the complexity of AMR (Hughes, 2026).

In 2025, Europe alone reported more than 800,000 infections caused by drug-resistant bacteria, resulting in more than 35,000 attributable deaths. The 2025 European Centre for Disease Prevention and Control (ECDC) annual report described persistently high resistance rates among clinically important bacterial pathogens: 14.3% of Escherichia coli (E. coli) isolates displayed resistance to third-generation cephalosporins, and 22% to fluoroquinolones. Among Klebsiella pneumoniae isolates, 10.9% exhibited carbapenem resistance. Additionally, for Pseudomonas aeruginosa (P. aeruginosa), 19.3% of isolates were resistant to piperacillin/tazobactam, while 15.2% of Staphylococcus aureus (S. aureus) isolates demonstrated methicillin resistance. The resistance rates in Acinetobacter spp. reached 36.3% for carbapenems across Europe. Regarding Greece, AMR rates remain significantly higher than the EU population-weighted average, meaning resistance to fluroquinolones in E.coli reaches 37.8%, P. aeruginosa resistance to piperacillin/tazobactam stands at 50.5%, methicillin-resistance S. aureus (MRSA) at 39%, and Acinetobacter spp. carbapenem resistance at 95.9%. This profound AMR burden in Greece underscores the urgency of developing innovative surveillance frameworks aiming to monitor emergence pathways and environmental dissemination routes (Lymperatou, 2025).

In this context, wastewater surveillance (WWS) has emerged as a promising complementary approach to tracking AMR. Although WWS gained significant prominence during the SARS-CoV-2 pandemic, it has been used for decades to track infectious diseases at the population level. In the context of AMR surveillance, this environment-based approach addresses many limitations of traditional event-based methods: it does not rely on cases of illness involving drug-resistant pathogens, does not require healthcare access, avoids privacy issues, and can reduce costs. The abundance of ARGs is believed to be an important indicator of AMR pollution in different environments, and the ARGs enriched in hospital wastewater (HWW) systems may reflect the AMR distribution in clinically related environments (Majumdar, 2026).

A specific and particularly relevant subset of WWS is hospital wastewater surveillance (HWWS), which focuses on the effluent generated within healthcare facilities. According to the World Health Organization (WHO), hospital wastewater refers to water discharged from all hospital-related activities, both medical and non-medical, including effluent from surgery rooms, diagnostic laboratories, patient wards, laundries, and kitchens. HWW may contain a mixture of pathogenic microorganisms, antibiotic residues, resistant bacteria strains, heavy metals, and chemical agents from disinfectants and pharmaceuticals. This complex composition makes HWW an important environmental reservoir for ARGs and a potential vector of AMR dissemination if inadequately treated prior to release into municipal systems (Mohammad, 2025).

Advances in detection technologies have led to great progress in the monitoring capacity of HWWS programs. Methods range from traditional culture-based techniques to advanced molecular tools such as polymerase chain reaction (PCR), next-generation sequencing (NGS), and microarray-based platforms. Each technique has its own advantages and disadvantages. For example, PCR offers rapid detection of known ARGs, and NGS provides a broad genomic preview, including novel resistance elements. The integration of those techniques into public health systems enables them to assess intervention efficacy and provide early warnings for potential outbreaks (Stange, 2025).

This narrative review aims to synthesize and discuss the existing literature on HWWS in the context of AMR, focusing on the most frequently identified ARGs and the methods to detect them, and explores the potential of HWWS to improve infection control and reduce the spread of AMR, highlighting current challenges and providing insights for further research.

MATERIALS AND METHODS

The research design used is a descriptive research method with a qualitative approach to literature study or literature review using the internet and manual search. Data was collected using databases and search engines Researchgate, Google Scholar and ScienceDirect. The search was carried out using the keywords "laboratory surveillance of antimicrobial resistance in hospital wastewater in low- and middle-income countries: a systematic review".

The inclusion criteria for this research are that the articles used as literature are research articles, both original articles and studies/reviews. Articles or literature discussing laboratory surveillance of antimicrobial resistance in hospital wastewater in low- and middle-income countries: a systematic review were published from 2022-2026. Researchers found articles that matched these keywords with details from Google Scholar (n = 1.400), Researchgate (n = 788), ScienceDirect (n = 212) and PubMed (n = 5) so that N = 2.405. The search results that were obtained were then checked for duplication using Mendeley and the same articles were found, so there were articles that were excluded or duplicated (n = 1.075). Researchers carried out screening based on the title (n = 810), then obtained abstracts (n = 105) then a complete copy was taken and assessed for suitability (n = 10) then screened based on inclusion and exclusion criteria on the entire text (full text) so that A total of (n = 8) were obtained which could be used in a systematic literature review. The results of article selection can be depicted in the Flow Diagram below.

RESULTS AND DISCUSSION

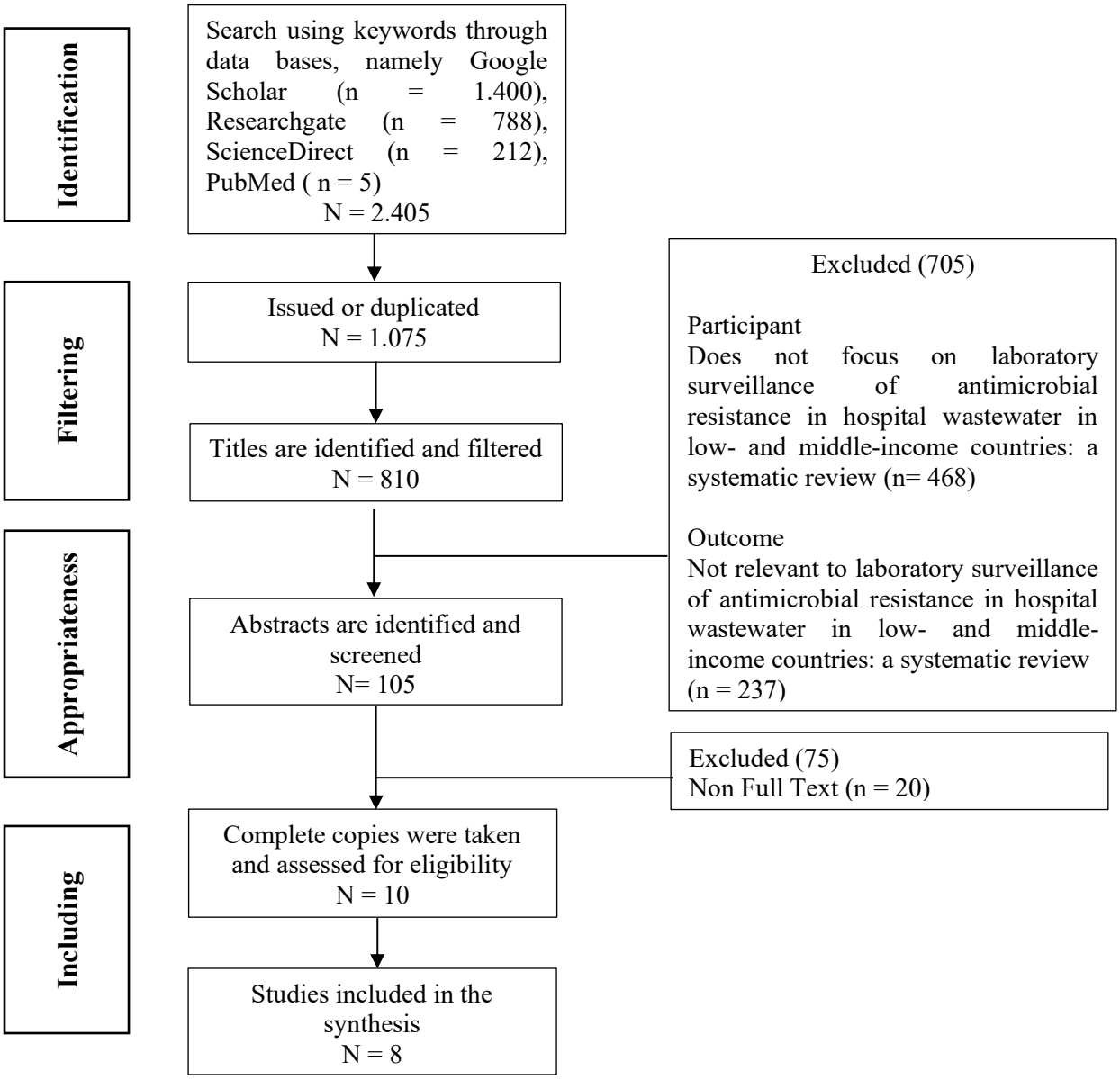


Figure 1. PRISMA Flow Chart

RESULTS

The study results showed that 8 articles met the criteria based on the systematic literature review topic. The results of study characteristics from 4 databases (Google Scholar, Researchgate, ScienceDirect and PubMed) are depicted in table 1 below:

Table 1. Summary of reference articles

No	Authors and Year	Research Title	Research Methods	Research Findings	Database
1.	Khushal Singh and Suparna Mitra (2026)	Operationalising Genomic Surveillance for Antimicrobial Resistance in Low- and Middle-Income Countries: A One Health Perspective from Bangladesh	Systematic Literature Review (SLR)	The AMR crisis is a ticking time bomb, especially for LMICs like Bangladesh, where antibiotic resistance rates are three to four times higher than in HICs. The complex interplay of human, animal, and environmental factors makes understanding and combating AMR exceptionally challenging. Traditional surveillance methods are insufficient to capture this complexity due to fragmented data and systemic weaknesses. Shotgun metagenomic sequencing is therefore not just a novel tool but an essential one for Bangladesh. It bypasses the need for culturing, providing a direct, unbiased snapshot of the entire "resistome" in any given environment, from a patient's gut to hospital wastewater or a fish farm. This is critical for a country where unregulated antibiotic use in agriculture and aquaculture, combined with inadequate sanitation, creates a vast and interconnected environmental reservoir of antibiotic resistance genes. Pioneering studies in Bangladesh have already demonstrated its power, directly linking the high abundance of ARGs in urban waters to human sewage, providing unequivocal evidence for targeted public health interventions like improving WASH infrastructure. By embracing shotgun sequencing, Bangladesh can reap substantial public health benefits. It can generate a comprehensive, high-resolution map of its national resistome, identifying hotspots and tracking the transmission of critical antibiotic resistance geneslike blaKPC in clinical isolates and mcr-1 in poultry across different sectors. This granular data can inform evidence-based policies, guide antimicrobial stewardship, and enable the early detection of emerging threats, such as the discovery of the mobile colistin resistance gene mcr-5.1 in hospital wastewater. As nation with a relatively homogenous community structure, findings from targeted sequencing studies can be more readily generalized across the country, making Bangladesh a powerful model for developing scalable AMR mitigation strategies that could benefit other LMICs. Despite its transformative potential, there is a significant lack of shotgun sequencing research in Bangladesh and across most LMICs, creating a critical blind spot in the global understanding of AMR. This gap is largely driven by prohibitive costs, inadequate laboratory infrastructure, and a severe shortage of trained bioinformaticians the same systemic challenges that fuel the AMR crisis itself. While data from HICs grows, the regions bearing the highest AMR burden	PubMed https://www.mdpi.com/2076-2607/14/3/527

No	Authors and Year	Research Title	Research Methods	Research Findings	Database
				remain the most under-studied. The AMR crisis is a global challenge that requires a global response. The evidence is clear: shotgun sequencing provides an unparalleled tool for understanding the complex drivers of antibiotic resistance in hotspots like Bangladesh. Investing in this technology and building local capacity is not merely a national priority for Bangladesh; it is a global public health imperative. By closing the research gap and empowering LMICs with these advanced surveillance tools, the global community can move from reactive crisis management to proactive, data-driven strategies to safeguard the efficacy of modern medicine for future generations.	
2.	Jiaan Carlo E. Santos, Daphne Janelyn L. Go, Robert D. Unciano, Paul K. Yu, Angelyn R. Lao, Ma. Luisa D. Enriquez, Llewelyn M. Espiritu, Anish M. S. Shrestha (2026)	Investigating the resistome, taxonomic composition, and mobilome of bacterial communities in hospital wastewaters of Metro Manila using a shotgun metagenomics approach	Literature Review	We profiled antibiotic resistance genes, bacterial communities, and mobile genetic elements in untreated hospital wastewater from three tertiary hospitals in Metro Manila using shotgun metagenomic sequencing. The resistome analysis revealed high abundances of genes known to confer resistance against sulfonamides (sul1, sul2), aminoglycosides (aadS), and macrolides/streptogramins (msrE, mphE). High-risk resistance genes were also detected, including those known to confer resistance to β-lactams (blaOXA, blaTEM, blaGES, blaNDM, blaKPC), colistins (mcr-5), and tetracyclines [tet(C), tet(A), tet(L), tet(M)]. Comparisons with hospital wastewater resistome profiles from regional neighbors and other lower-and-middle-income countries indicated broadly similar relative abundances of dominant resistance genes, with differences largely driven by low-abundance resistance genes. The bacterial community was dominated by the phylum Pseudomonadota, with high relative abundances of the genera Stenotrophomonas, Rhodococcus, and Pseudomonas, while ESKAPEE pathogens were detected at lower levels. A diverse array of mobile genetic elements many known to be associated with resistance to multiple drug classes and disinfectants was also observed. Overall, this study provides a valuable preliminary evidence base for future antimicrobial resistance and epidemiological surveillance efforts in the Philippines, particularly those employing wastewater-based approaches.	Researchgate https://journals.asm.org/doi/10.1128/spectrum.03963-25
3.	Arnab Majumdar, Dhritidipa Bagchi, Ioly Kotta-Loizou, Martin Buck (2026)	The One Health resistome: Integrating environmental, microbial, and human antimicrobial resistance surveillance and risk analysis in the digital age	Systematic Literature Review (SLR)	The One Health resistome framework has fundamentally transformed understanding of antimicrobial resistance from a purely clinical phenomenon to a complex ecological challenge spanning human, animal, and environmental domains. Digital technologies, whole-genome sequencing, metagenomics, machine learning, mobile diagnostics, and integrated surveillance platforms have revolutionised AMR detection, characterisation, and prediction capabilities, enabling unprecedented insights into	ScienceDirect https://www.sciencedirect.com/science/article/pii/S0304389426014093

No	Authors and Year	Research Title	Research Methods	Research Findings	Database
				resistance emergence, transmission pathways, and intervention opportunities. However, translating these technological advances into actionable global surveillance requires addressing persistent challenges: data heterogeneity and interoperability limitations constrain cross-sectoral integration; inadequate standardisation of environmental monitoring methods limits comparability; substantial resource gaps particularly affect low- and middle-income countries where AMR burden is highest; and temporal/spatial resolution constraints impede real time outbreak detection and source attribution. Emerging innovations, portable nanopore sequencing enabling field-based genomic surveillance, CRISPR-based point-of-care diagnostics providing hour scale resistance detection, artificial intelligence enhancing predictive analytics, and blockchain-federated learning addressing privacy preserving data sharing, offer pathways to overcome current limitations. Realising comprehensive One Health resistome surveillance demands coordinated international action: adopting universal data standards and interoperability frameworks; investing in open source, modular digital platforms with sustainable maintenance models implementing capacity-building programs training personnel in digital tool utilisation; establishing trustworthy governance structures ensuring equitable data access and benefit-sharing; and securing sustained political commitment to cross-sectoral coordination and funding. Only through such comprehensive, coordinated efforts can the global community transform AMR surveillance from fragmented retrospective reporting into integrated, predictive intelligence systems capable of preventing resistance amplification and safeguarding antimicrobial effectiveness for future generations.	
4.	Naomi Hughes, Sanchutha Sathiananthamoorthy, Chrysi Sergaki (2026)	Antimicrobial resistance surveillance through wastewater: Methodological considerations for metagenomic approaches and public health perspectives	Literature Review	In this Personal View, we aimed to advocate for community-level AMR surveillance using metagenomic technologies while highlighting areas that need to be addressed for producing standardised robust datasets. There remain issues with resource equity globally, for example, in low-income and middle-income countries, the proposed implementation of a metagenomic surveillance system will require high economic and personnel costs. However, failing to implement community-level AMR surveillance in equipped areas leaves us in an enhanced reactionary state to AMR, responding to threats after they emerge in clinical settings. Microbial evolution will continue to outpace the development of antimicrobials; however, real-time tracking of the spread and emergence of AMR in local and global populations can	Researchgate https://www.researchgate.net/publication/404916795_Antimicrobial_resistance_surveillance_through_wastewater_methodological_considerations_for_metagenomic_approache

No	Authors and Year	Research Title	Research Methods	Research Findings	Database
				shape stewardship and antimicrobial development. Sequencing approaches have undoubtedly shown immense potential for rapid and in-depth ARG detection and surveillance, but there are factors to consider when investigating resistance using the array of genomic tools available. These factors can influence the information and data obtained, in addition to their relevance and suitability to address research questions. A robust study design includes choosing the appropriate sample type and introducing measures to address specific biases associated with each stage of the study design. There is a need for standardisation of sequencing workflows and data analyses, in addition to implementation of quality control standards, which will facilitate the comparison and harmonisation of present and future datasets. This step alone can fortify the confidence in AMR data, provide better information on AMR patterns, and aid in the forecasting of potential outbreaks while also guiding experts on antimicrobial stewardship to curb and control the dire consequences of AMR in health. More specifically, the use of validated AMR physical reference reagents for sequencing methodologies will help to establish the ground truth about AMR and identify which sequencing and analytical approaches deviate from this truth. As far as sequencing technology and analysis have progressed, acknowledging the limitations in their ability to account for the complex AMR or tolerance mechanisms described is also important. To effectively and confidently track AMR through surveillance systems, it is imperative that collective work is carried out to improve the knowledge base in the AMR field while highlighting limitations when interpreting results. Perhaps, future work using advanced computational techniques in combination with phenotypic, genotypic, and other omic datasets could allow for explaining complex associations of and predict AMR from polymicrobial communities. Continuing efforts to further the knowledge of AMR mechanisms and their expression, in addition to characterising indirect, genomic measures of non-genotypic tolerance mechanisms, should be addressed by profiling real-time active gene expression of representative biological communities and moving beyond the characterisation of major pathogens to characterising less commonly reported keystone species and their effect on community-level AMR potential. Although NGS holds great promise for effective AMR surveillance, the limitations discussed in this Personal View ultimately impede the introduction of evidence-based public health measures. Governments interested in evidence-based health policy cannot create initiatives based on these beneficial technologies due to the variability in the datasets produced by differing methodologies. Even more, datasets cannot be shared across countries or even counties to better understand any epidemiological	s_and_public_health_perspectives

No	Authors and Year	Research Title	Research Methods	Research Findings	Database
				AMR data, as such data are often not comparable because of methodological variability. Clinicians who are ultimately responsible for patient lives will be unlikely to trust or base clinical decisions on NGS data as a result of its potential deviance from the ground truth. The scientific community needs to come to a consensus on how to use current metagenomic technologies and how to implement improvements and standardisation in the future, in addition to identifying away to include all stakeholders across industry, academia, and public health in these conversations. Overall, NGS has great potential, and the technology continues to outpace its applicability for AMR prediction in a standardised and reproducible way. As a field, the scientific community, needs to look to address the standardisation of metagenomic technologies for the production of reproducible and trustworthy datasets in a collaborative manner with a strategy to evolve our practices as our technology progresses.	
5.	Diamantina Lymperatou, Revekka Konstantopoulou, Michalis Mentsis, Natalia Atzemoglou, Christina Diamanti, Ioannis Tzourtzos, Katerina K. Naka, Michail Mitsis, Gartzonika Konstantina, Haralampos Milionis, Evangelia Ntzani and Eirini Christaki (2025)	Hospital Wastewater Surveillance and Antimicrobial Resistance: A Narrative Review	Narrative Review	Wastewater-based surveillance is now recognized as an important public health tool. Hospital wastewater-based surveillance has the potential to serve as a critical tool for understanding, tracking, and potentially mitigating the spread of AMR in highly endemic settings. By enabling comprehensive, real-time monitoring of ARGs and resistant bacteria from concentrated clinical sources, hospital wastewater surveillance provides a platform that closely reflects local prescribing practices and clinical AMR patterns. Unlike tradi-tional surveillance methods limited to symptomatic patients, this approach captures data on asymptomatic carriage, transmission dynamics, and emerging resistance mechanisms within institutions, complementing clinical datasets and reducing reporting bias. Evi-dence so far has demonstrated significant correlations between ARG profiles in hospital influent and regional clinical data, supporting the use of wastewater analysis for targeted interventions, outbreak detection, and evaluation of stewardship policies.	PubMed https://pubmed.ncbi.nlm.nih.gov/41471943/
6.	Mohammad Shehryaar Khan,	A Perspective on Wastewater and	Systematic Literature Review (SLR)	Geographical variations in infectious diseases create differences in public health priorities between high- and low-income countries. Low- and middle-income	Google Scholar

No	Authors and Year	Research Title	Research Methods	Research Findings	Database
	Christian Wurzbacher, Anna Uchaikina, Boris Pleshkov, Olga Mirshina and Jörg E. Drewes (2025)	Environmental Surveillance as a Public Health Tool for Low- and Middle-Income Countries		countries (LMICs) face resource constraints that limit adherence to international monitoring standards for wastewater-based epidemiology (WBE). The development of low-cost WBE programs, such as those to detect SARS-CoV-2, offers LMICs a promising tool for monitoring pathogens of local concern. In this work, we summarize important wastewater biomarkers for LMICs and their associated public health challenges, ranging from pathogens causing gastroenteritis to putative markers for plant diseases linked to food safety, as well as an- timicrobial resistance. We raise awareness of the great potential of WBE for LMICs and highlight the critical health markers, research needs, and strategies necessary to establish tailored wastewater surveillance programs.	https://www.mdpi.com/2076-2607/13/2/238
7.	Claudia Stange, Rogers Kalyetsi, Judith Owokuhaisa, Moses Ntaro, Arthur Leon, Paul R. Hunter, Andreas Tiehm, Edgar M. Mulogo (2025)	Monitoring of antimicrobial resistance in hospital, municipal, and treated wastewater in Mbarara, Uganda	Narrative Review	Monitoring the removal efficiency of AMR determinants by wastewater treatment can indicate whether the system is oper-ating optimally or whether steps should be taken to improve removal performance. This is particularly important because the release of AMR to the environment should be minimised to avoid adverse health effects to humans and animals that have contact with the waters into which the effluent is discharged. The present study has demonstrated the widespread distribution of ESBL and ARGs in the Mbarara population of Uganda. Our results suggest that AMR is much more common in Uganda, and probably much of Africa, than would be the case in higher income countries. Such high levels of resistance pose a significant threat to public health in Africa. Reducing the burden of AMR in Africa should be a top priority. As reported here, wastewater monitoring could be an important component of national strategic plans for AMR in African settings.	Researchgate https://www.sciencedirect.com/science/article/pii/S221371652500205X
8.	K.K. Chau , L. Barker, E.P. Budgell, K.D. Vihta, N. Sims, B. Kasprzyk-Hordern, E. Harriss, D.W. Crook, D.S. Read, A.S. Walker, N. Stoesser (2022)	Systematic review of wastewater surveillance of antimicrobial resistance in human populations	Systematic Review	In conclusion our review suggests that overall, wastewater-based surveillance has significant potential for monitoring population-level AMR, particularly for some species, despite high diversity in study design, methods and metadata. We found that no specific study feature or AMR detection method conferred a clear increase in the ability of a study to detect an association between wastewater and human AMR prevalence. However, based on limited available data, we would recommend that where feasible, genotypic AMR detection, composite sampling of influent with longitudinal timeframe >12 months, and contemporaneous sampling of wastewater and human compartments that are directly associated (i.e. the human population sampled contributes to the	Researchgate https://www.researchgate.net/publication/359197672_Systematic_review_of_wastewater_surveillance_of_antimicrobial_resistance_in_human_populations

No	Authors and Year	Research Title	Research Methods	Research Findings	Database
				wastewater sampled) are used to generate more robust data to better evaluate the strengths and limitations of this approach for surveillance purposes. Clear reporting of study methods and features are essential, and this will facilitate the iterative development of optimal practice guidelines for this emerging surveillance tool.	

DISCUSSION

Based on the results of the systematic review analysis, the data obtained are as follows:

1. Antimicrobial Resistance Genes That Are Identified in Hospital Wastewater

Key antimicrobial resistance genes detected in clinical isolates and hospital wastewater include those associated with extended-spectrum beta-lactamases (ESBLs) such as bla CTX-M, carbapenemases including bla_KPC, bla_NDM, and bla_OXA variants, and colistin resistance genes (mca-1 and variants), as well as vancomycin resistance genes such as vanA and vanB, reflecting the critical threat posed by multidrug-resistant pathogens. In a study that collected public metagenomic datasets of 71 hospital wastewater samples from 18 hospitals in 13 cities, the results revealed that the primary hosts of ARGs within HWS were found to be Escherichia coli and Klebsiella pneumoniae. In this review, we primarily examined both critical and common resistance traits detected in hospital isolates, such as those related to ESBL, carbapenemases, colistin resistance, and vancomycin resistance, and we also included those associated with tetracycline and sulfonamide resistance. Most common ARGs detected in HWW and their respective mechanisms of action are included in Table 2.

Table 2. The most common ARGs detected in hospital wastewater

Antibiotic Class	Key Resistance Genes	Mechanism
Carbapenems	*bla_KPC, bla_NDM, bla_VIM, bla_OXA-48, bla_IMP*	Carbapenemase production leading to β-lactam degradation
B-lactams	*bla_CTX-M, bla_SHV, bla_TEM, bla_OXA-ESBLs*	Hydrolyze third-generation cephalosporins
Colistin	*mcr-1 to mcr-10, pmrA, pmrB*	Modification of lipid A reducing colistin binding
Tetracyclines	*tetA, tetB, tetM, tetO, tetX, tetC, tetD, tetG*	Efflux pumps and ribosomal protection proteins
Sulfonamides	*sul1, sul2, sul3*	Altered dihydropteroate synthase
Vancomycin	*vanA, vanB, vanC, vanD, vanE, vanG, vanL, vanM, vanN*	Modified peptidoglycan precursors reducing binding

ARG = antimicrobial resistance genes; ESBL ss= extended-spectrum β-lactamase.

a. Carbapenem Resistance Related Genes

Carbepenem resistance in Enterobacterales is primarily driven by the production of carbapenemases. These enzymes are capable of hydrolyzing nearly all beta-lactam antibiotics, which greatly limits treatment options. Despite geographic variation, the most commonly encountered carbapenemases among the Enterobacterales are the Ambler class A serine carbapenemase, KPC, the class B metallo-β-lactamases (MBLs), including NDM, IMP, and VIM, and the class D OXA-48-like enzymes. The kpc gene, encoding the enzyme Klebsiella pneumoniae carbapenemase, is a widespread driver of carbapenem resistance among Enterobacteriaceae. The detection of kpc in hospital wastewater often indicates a high load of resistant bacteria, a situation frequently tied to the overuse and misuse of antibiotics, as well as suboptimal infection control implementation. Research shows that kpc genes are commonly found in hospital wastewater samples, underscoring the value of wastewater surveillance for tracing AMR patterns. The blaKpc is primarily associated with Klebsiella spp., Enterobacter spp., and Escherichia coli and has been found in other Enterobacteriaceae, Acinetobacter baumannii, Pseudomonas aeruginosa, as well as Aeromonadaceae. Quantitative real-time PCR (qPCR) and digital PCR (dPCR) analysis of hospital wastewater has also targeted several other carbapenemase genes like blaNDM, blaIMP, blaVIM, blaOXA-23-like, blaOXA-48-like, and blaOXA-58-like. The blaImp gene has been observed in Gram-negative bacteria and Enterococcaceae, while blaVIM-1 has been detected in Pseudomonas species and E. coli. The blaNDM-1 gene, which also confers broad betalactam resistance, has been identified in Enterobacteriaceae, Pseudomonas aeruginosa, and Acinetobacter baumannii. Notably, blaNDM-1 often coexists with the mcr-5.1 gene, which grants resistance to colistin, further complicating therapeutic choices (Stange, 2025).

b. 3rd Generation Cephalosporin Resistance Related Genes

ESBLs are enzymes that are responsible for the resistance of certain bacteria against a wide range of beta-lactam antibiotics, such as penicillins and cephalosporins. ESBL- producing bacteria, particularly Escherichia coli and Klebsiella pneumoniae, are frequently detected in hospital wastewater and are recognized as a major cause of healthcare-associated infections. The release of ESBL genes into wastewater can raise resistance levels within community settings, underscoring the importance of wastewater as an AMR hotspot. The notable presence of ESBL genes in hospital wastewater acts as an early warning sign for potential outbreaks, given that the resistant strains can easily propagate through environmental and water systems. Among the genes associated with extended-spectrum beta-lactamase resistance, several have been identified. The most common are bla_CTX-M, bla_SHV, bla_TEM and bla_OXAESBLs. The pooled prevalence of ESBL Enterobacteriaceae in wastewater was found to be 24.81% (95% CI, 19.28–30.77) in a metanalysis that included 57 observational studies, with blaCTX-M genes being the most prevalent (66.56%). The results of this metanalysis also showed that hospital wastewater may be a significant repository of ESBL genes. Furthermore, genes blaNdm-1 and blaCtx-m-15 have been linked to mobile genetic elements (MGEs), accelerating their horizontal transfer between environmental and clinical bacterial populations (Singh, 2026).

c. Colistin Resistance Related Genes

The identification of the mcr gene, conferring resistance to colistin, has generated significant concern in the medical field. Resistance to colistin is mediated primarily via the modification of the target site where colistin can make electrostatic interactions with Lipid A of lipopolysaccharides of the outer membrane. Colistin, often considered as the last line of defense against multidrug-resistant Gram-negative bacterial pathogens, is unfortunately losing its effectiveness due to the emergence of mcr genes, particularly mcr-1 and its variants, which have also been detected in hospital wastewater. The mcr-1 gene was described for the first time in China and subsequently various mcr-1 allelic variants and mcr-type genes were identified. Mcr-1 is the most prevalent with the mcr-1.1 allelic variant being the most distributed worldwide. E. coli is the main bacterial species carrying the mcr-1 gene, a phenomenon that can be attributed to the ubiquity of this species, the presence of mcr-1 in conjugative plasmids, and the extensive use of colistin in livestock. However, mcr genes have also been found in Klebsiella pneumoniae and other Enterobacteriaceae, Salmonella species, Aeromonadaceae, and Pseudomonas species. The ability of mcr genes to transfer between bacterial species aggravates the complexity of managing infections. In hospital wastewater, these genes are often associated with the selective pressure from antibiotic use, highlighting the critical need for strict monitoring and implementation of control practices. Of particular concern is the presence of mcr5.1 in wastewater, signaling the potential for multidrug-resistant and pan-drug-resistant (PDR) bacteria in hospitals, posing a severe risk to public health (Singh, 2026).

d. Vancomycin Resistance and Methicillin Resistance Related Genes

MRSA is the cause of both community-onset and hospital-associated infections and has been linked to outpatient visits, long-term care facilities, antibiotic exposure, chronic illness, intravenous drug use, and close contact with individuals carrying these risk factors. The primary determinant of methicillin resistance in S. aureus is the mecA gene, which encodes an altered penicillin-binding protein with a low affinity for β-lactam antibiotics (i.e., methicillin, oxacillin). MecA (and the newer variant mecC) is carried on a large mobile genetic element called SCCmec (Staphylococcal Cassette Chromosome mec), allowing horizontal transfer of methicillin resistance between staphylococcal species. In a study conducted in both Greece and Romania, the analysis of staphylococcal genes in MRSA strains within the Greek patients revealed the presence of three key genes: mecA, FemB, and SCCmec elements.The mecA gene, the hallmark determinant of MRSA, has been detected in both hospital effluents and municipal wastewater, demonstrating the release of resistant pathogens into the environment. Vancomycin resistance, primarily due to the van gene, presents significant challenges, especially in managing infections caused by Staphylococcus aureus. More than 100,000 deaths are caused by MRSA worldwide and at least 5400 deaths by VRE in the United States annually. The presence of VRE in hospital wastewater represents a serious public health threat, as these organisms can rapidly spread through environmental pathways. Research indicates that VRE can survive in water for extended periods, enabling its transmission to other pathogens and AMR spread. Vancomycin resistance genes, notably vanA, have been identified in Enterococcaceae, Staphylococcaceae, underscoring the need for environmental monitoring to track resistance and guide infection control practices (Santos, 2026).

e. Sulfonamide Resistance Genes

The extensive use of sulfonamides, which act by inhibiting bacterial folic acid synthesis, has driven selective pressure, leading to the spread of sulfonamide resistance genes, including sul1, sul2, and sul3, in healthcare environments. These genes, commonly detected in hospital wastewater, can enable isolates to bypass the drug's action, leading to bacterial survival despite sulfonamide exposure. The persistence of sul genes in hospital wastewater points to a substantial burden of sulfonamide resistance, frequently associated with high antibiotic usage in clinical environments (Santos, 2026).

f. Tetracycline Resistance Genes

Resistance to tetracyclines, mediated by genes such as tetA, tetB, and most commonly tetM, is also widely detected in hospital wastewater. Tetracyclines disrupt bacterial protein synthesis, yet the presence of tet genes in wastewater suggests selective pressure from the overuse of these antibiotics, raising concerns regarding their potential horizontal transfer to other clinically related pathogens (Santos, 2026).

2. Methods of ARGs Identification in Hospital Wastewater

Rapid detection of AMR traits is crucial to achieve guided treatment and limit the spread of resistant microorganisms. Various technical methods have been developed to identify resistance genes (ARGs), utilizing both culture-based and culture-free approaches, mainly molecular techniques and sequencing technologies (Chau, 2022).

a. Culture-Based Approaches

Culture-based techniques have been crucial in revealing AMR within hospital wastewater. These methods involve isolating and cultivating microorganisms from wastewater samples to isolate resistant strains and detect their resistance mechanisms. Standard isolation procedures include the use of selective media and enrichment cultures, with the last ones being used to enhance the population density of a particular group of microorganisms within the total microbial population of a sample. Minimum inhibitory concentration (MIC) defines in vitro levels of susceptibility of specific strains and helps determine microbial isolate susceptibility to drugs, offering essential information on AMR. Although accurate, these methods lack detection of non-culturable bacteria, which account for about 97% of the environmental microbiome. These techniques have been crucial in identifying pathogens like Klebsiella pneumoniae, Escherichia coli, and Enterococcus spp., which often have high levels of resistance. Despite their value, culture-based methods are narrowed by their limitations, such as the potential to overlook non-culturable or fastidious organisms. Additionally, the presence of resistant bacteria in complex microbial communities can make isolation more puzzling, and thus these methods are gradually replaced by molecular techniques (Chau, 2022).

b. Polymerase Chain Reaction (PCR)-Based Methods

To address the limitations of culture-based methods for detecting antimicrobial resistant pathogens and phenotypes, molecular techniques like PCR and microarrays have been developed to augment AMR detection in microbes. PCR is a technique that amplifies specific DNA sequences through repeated cycles of heating and cooling, leading to the creation of millions of copies of a targeted DNA fragment. Its advantages include speed, sensitivity, and specificity for detecting known mutations or pathogens, but it also has limitations as it only analyzes a small number of target sequences per reaction. Its simplicity and reliability make it popular in diagnostics and genetic testing. PCR remains the key method for detecting antimicrobial resistance genes (ARGs), as it obtains both high sensitivity and specificity. Real-time PCR is currently one of the most powerful methods for DNA amplification. Various PCR techniques, including conventional PCR, qPCR, and multiplex PCR, are used to isolate known ARGs in clinical samples. For instance, multiplex PCR enables simultaneous detection of multiple ARGs, which is particularly useful in multi-drug resistance cases. Additionally, qPCR can provide semi-quantitative data by evaluating bacterial load and gene expression levels, offering insights into gene responses after their exposure to antibiotics. For example, specific genes like blaCTX-M, blaKPC, qnrB, and mcr-1 can be easily detected with the use of qPCR. Although qPCR has many advantages by being a rapid, cost-effective, and sensitive method for detecting and quantifying ARGs, its application relies heavily on known target sequences that contain conserved primer sites. Reverse-transcription PCR (RT-PCR), which targets RNA instead of DNA, can also be utilized to examine gene expression. Moreover, droplet digital PCR (dd-PCR) enables precise absolute quantification and demonstrates strong resistance to inhibitors, making it a preferred alternative to address the limitations of

qPCR. While PCR is effective for detecting known ARGs, it has limitations when it comes to identifying novel resistance mechanisms. Last, although PCR-based methods are highly sensitive in detecting low-abundance genes, they are vulnerable to mutations in the primer target regions, which can lead to false-negative results (Chau, 2022).

c. Microarray Technology

Microarrays are essential tools for detecting multiple antimicrobial resistance genes (ARGs) in a single test. This technology quantifies and identifies AMR genes and their expression patterns by hybridizing probe DNA/RNA sequences to complementary target sequences on a microarray plate. It is particularly effective for the simultaneous multiplex detection of various ARGs, while its rapid performance and suitability for epidemiological or infection control studies that require the characterization of large sets of isolates makes it extremely useful. By analyzing the patterns of hybridization, microarrays can identify the existence of several resistance genes, especially those that are associated with β-lactamases, aminoglycosides, and tetracyclines. These systems are promising when it comes to genotyping, boasting high multiplexing capabilities that allow the detection of multiple β-lactamase genes within a single strain. Microarray systems like the Affymetrix GeneChip® and Check-Points® microarray are commonly employed for AMR surveillance in both environmental and clinical settings.

On the other hand, microarrays entail limitations that make them less than ideal for broad-scale wastewater surveillance. A primary challenge is their reliance on known genetic sequences for effective detection, meaning they can miss pathogens or ARGs that lack comprehensive genomic information. Additionally, wastewater samples contain various inhibitors and complex particulates, which can interfere with the accuracy of microarray detection. Sample processing and nucleic acid extraction steps can lead to data inconsistency, impacting the sensitivity and specificity needed for reliable results. These systems are highly efficient and capable of processing a large number of samples, but their main limitation is that, while they excel in throughput, they are not as effective in discovering previously unrecognized resistance genes (Majumdar, 2026).

d. Next-Generation Sequencing Techniques

NGS technologies, and particularly whole-genome sequencing (WGS), offer extensive insights into AMR by enabling the detection of both established and novel ARGs. NGS is a high-throughput technology that sequences millions to billions of DNA fragments simultaneously and can examine entire bacterial genomes, allowing for the identification of mutations linked to resistance mechanisms. It provides comprehensive genomic, transcriptomic, and epigenetic data, allowing for broad discovery and detailed analysis of complex genetic variations, unknown mutations, and large-scale genomic patterns. Another approach, targeted sequencing, focuses on specific genome regions likely to contain ARGs. Sequencing technologies such as Illumina® and Ion Torrent® allow for the rapid sequencing of pathogens directly from clinical samples, eliminating the need for culturing and thus accelerating diagnosis. Furthermore, Nanopore® sequencing (e.g., the MinION platform) provides real-time sequencing capabilities, with tailored workflows and improving accuracy. NGS is scalable, producing vast amounts of data for wholegenome, exome, or targeted segment sequencing, but generally takes longer and is more resource-intensive than PCR. In a study comparing high-throughput quantitative PCR (HT qPCR) and metagenomic sequencing for ARG screening in hospital wastewater, metagenomics offered a broad view of the resistome and was instrumental in evaluating ARG risk. Conversely, HT qPCR showed higher sensitivity in quantifying all targeted and clinically relevant ARGs. Currently, the use of NGS is primarily for microbial strain typing for epidemiological purposes; however, its potential to provide clinically valuable insights through microbial community analysis is increasingly recognized. The advantages and disadvantages of all the above techniques described are outlined in Table 3.

Table 3. Comparison between the techniques used to identify ARGs and monitor hospital wastewater surveillance.

Technique	Advantages	Disadvantages	Key Applications in Hospital Wastewater Surveillance
Culture-Based Approach	Reliable for identifying viable bacteria which have clinical significance (proof of existence)	Unable to detect non-culturable bacteria (only ~1–3% of environmental bacteria can be cultured)	Useful for detailed susceptibility testing and resistance pattern analysis
	Enables susceptibility testing to determine resistance patterns	Bacteria often die quickly when exposed to non-native environmental conditions	May miss non-culturable or difficult-to-culture organisms
qPCR (Quantitative PCR)	High sensitivity and specificity, detects hard-to-culture bacteria	Requires prior information on target sequence for assay design	Effective for rapid detection of target ARGs, even at low concentrations
	Can detect low-abundance ARGs, allowing early outbreak detection	Limited to preselected ARGs, unable to detect non-targeted or novel ARGs	Suitable for focused surveillance of known resistance genes in wastewater
Metagenomics	Detects a wide range of ARGs, including novel and divergent genes	More expensive per sample, especially if applied in small-scale applications	Comprehensive analysis for total ARG burden, capturing more gene diversity
	More cost-effective for large sample monitoring	May be less sensitive for low-abundance ARGs compared to targeted methods	Ideal for broader surveillance across multiple resistance genes and bacteria

Hospital drains and water interfaces are implicated in the nosocomial transmission of pathogens. The detection of antimicrobial resistance genes relies on a diverse array of technologies. Culture-based approaches are a reliable method for providing proof of viable bacteria of concern, but they have several limitations. PCR-based methods remain foundational for identifying known ARGs, while next-generation sequencing can provide more large-scale, in-depth genomic exploration. By integrating these methods into routine diagnostic workflows and environmental surveillance systems, it could facilitate a more comprehensive management of AMR globally (Majumdar,2026).

3. The Role of Hospital Wastewater Surveillance in Antimicrobial Resistance

Wastewater-based surveillance (WWS) has emerged as a transformative approach in public health epidemiology and can also be applied in monitoring AMR in healthcare settings. WWS captures resistome signals from the combined output of hospital activities and offers population-scale insights into AMR burden. Wastewater is believed to play a critical role in the dissemination of AMR due to the abundance of bacteria, including resistant ones, the potential for exchange of genetic elements between them, the dynamic confluence of water from different sources, and the AMR dynamics of communities, healthcare facilities, and industry. Therefore, WWS programs are increasingly recognized as significant tools in monitoring AMR, helping mitigate outbreaks, raising awareness, and providing valuable data for further research. These programs provide a population-level snapshot of antimicrobial use and resistance patterns, complementing traditional hospitaland clinic-based surveillance systems.

In healthcare settings, home to infections by resistant pathogens and high-level antimicrobial consumption, specific environmental conditions can support the persistence and spread of resistant bacterial strains (Figure 1). Several studies have highlighted the benefits of HWWS for AMR monitoring and control. HWWS can serve as an indicator of local AMR burden, reflecting the resistance profiles of hospital-acquired infections. In a study at Western General Hospital in Edinburgh, metagenomic analysis and multiple-site sampling were used to investigate the relationship between antimicrobial resistance gene prevalence and clinical activities, such as antimicrobial usage and hospitalization duration. Samples were collected over 24 h from various wastewater collection points within a tertiary hospital and compared with community sewage. Results indicated a higher abundance of ARGs in hospital wastewater compared to community influents. Although total antimicrobial usage did not show a direct correlation with elevated ARG levels, a modest

positive association was observed with certain ARG phenotypes. Furthermore, increased use of carbapenem and vancomycin was positively linked to the presence of ARGs associated with these specific antibiotics. Notably, Enterococcaceae and Staphylococcaceae isolates were the most prevalent. Overall, analyzing sewage samples offers a cost-effective method for monitoring antibiotic-resistant pathogens at a population level. A study conducted in Gothenburg, Sweden, revealed that E. coli resistance rates derived from hospital sewage and hospital patients strongly correlated, as did resistance rates in E. coli from municipal sewage and primary care urine samples. This approach could complement existing monitoring systems by addressing challenges related to limited sampling in clinical practice (Singh, 2026).

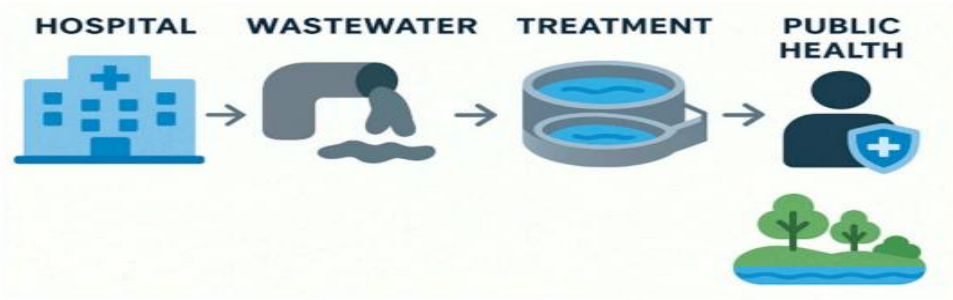


Figure 2. The spread of antimicrobial resistance genes from Hospital Wastewater

Moreover, wastewater surveillance can capture both pathogenic and non-pathogenic resistant bacteria, offering a comprehensive assessment of resistance gene reservoirs, including ARGs that may otherwise go undetected by following clinical surveillance alone. Hospital wastewater surveillance can also help monitor clinically significant pathogens and thus contribute to the early identification of outbreaks. The approach was successfully employed in large-scale wastewater-based surveillance during the COVID-19 pandemic, where it proved valuable in estimating infection prevalence and predicting outbreaks based on genetic material detected in wastewater. To address limitations impacting signal accuracy, such as wastewater composition variability and dilution from other effluents, near-source sampling (NSS) was employed. A study at the University of Arizona demonstrated that, when NSS was correlated with clinical samples, it provided a sensitive approach to identify acute cases within a defined population, such as hospital patients. This method could thus be valuable for monitoring shifts in disease dynamics and providing early warning of potential site-specific outbreaks. Similar approaches for AMR monitoring could enhance early warning capabilities for AMR in healthcare facilities, thereby improving public health responses to potential future outbreaks.

Despite its potential, HWWS faces limitations, including the lack of standardized guidelines and protocols for sample collection and analysis. Variations in sampling and analysis methods, timescales, and geographic locations can yield inconsistent results that are difficult to compare. Notably, when comparing antimicrobial resistance data from hospital wastewater treatment plants across different countries, several factors should be taken into consideration, like differences in environmental regulations, variable treatment technologies, and allocated financial resources that can have an impact on the efficiency of ARG removal and AMR containment. Moreover, HWWS alone cannot differentiate the exact sources of contamination within the healthcare facility. In addition, ARGs in wastewater could stem from non-human sources, such as veterinary settings or environmental reservoirs. Developing standardized protocols and harmonized procedures is therefore essential to enable comparable findings and to strengthen the role of WWS in managing the persistent threat of resistant pathogens (Singh, 2026).

4. Role of Hospital Wastewater Treatment Plants

Wastewater treatment plants (WWTPs) are designed to sequentially remove contaminants, including pathogenic microorganisms and organic matter, from wastewater before environmental discharge. Hospital WWT systems are constituted by several stages designed to minimize organic matter, microorganisms and chemical contaminants. Initial stages remove solids through screening and sedimentation, followed by biological treatment processes, such as biofilm reactors, that degrade organic pollutants via microbial metabolism. Finally, disinfection steps, commonly including chlorine, ultraviolet radiation, or advanced oxidation, are used for inactivation of the pathogenic organisms and reduction in microbial loads.

Despite being critical control points aimed at reducing bacterial load, WWTPs can also act as reservoirs and even amplifiers of antimicrobial resistance. Despite the above treatment steps, AMR can persist in treated effluents for many reasons. First, several resistant bacteria have the ability to adapt to treatment stressors, or they survive partial inactivation. Secondly, extracellular DNA, which contains ARGs may not be effectively removed, and therefore horizontal gene transfer to other bacteria in the environment can be facilitated. Furthermore, residual antimicrobials present in hospital wastewater exert selective pressure, resulting in the maintenance and spread of resistance determinants. Additionally, variation in treatment plant designs and operational parameters influences ARG removal efficiency. Conventional chlorination, one of the most common disinfection methods, is optimized to inactivate a broad spectrum of pathogens but does not specifically target or fully eradicate ARBs and extracellular ARGs. In certain contexts, chlorination may paradoxically facilitate persistence or even the enrichment of resistant bacteria and genes within treatment systems and downstream effluents. This can occur because chlorine disinfection is less effective against bacteria protected within biofilms or sludge aggregates and fails to degrade extracellular DNA harboring ARGs, thereby maintaining the environmental reservoir for horizontal gene transfer. Alternative or complementary treatments—such as ultraviolet disinfection, ozonation, membrane filtration, and advanced oxidation processes—offer varying degrees of ARG removal, yet their adoption depends on resource availability and regulatory frameworks, which differ globally.

Hence, hospital wastewater treatment plants (HWWTPs) have been identified as an emerging source of antimicrobial-resistant bacteria (ARB) that might cause horizontal gene transfer among microbial communities in water systems. Numerous studies have identified ARGs in various water sources originating from WWTPs. Indeed, a study in Mexico found carbapenemase-producing Klebsiella species surviving WWTP processes, highlighting the role these plants may inadvertently play in AMR dissemination. Meng Y. et al. describe in their article how they employed metagenomic sequencing of both influent and effluent hospital wastewater to assess bacterial composition and resistome. The results revealed that most ARGs decreased after treatment, but a subset persisted or increased in effluent. Another study, which was conducted in India, involved 24 hospital wastewater samples that were collected over a period of 6 months. In these samples, Gram-negative bacteria were cultured and identified, and antimicrobial resistance was tested, revealing high rates of carbapenem and cephalosporin resistance in the final treated effluent [65] Consequently, comprehensive monitoring of hospital WWTPs is essential to understand transmission dynamics of ARGs and to inform the design of enhanced treatment strategies. There is an urgent need for innovation towards the optimization of WWTP designs specifically targeting the effective mitigation of antimicrobial resistance risks associated with hospital wastewater discharges (Mohammad, 2025).

5. Future Perspectives

AMR remains a mounting global threat, necessitating coordinated, urgent action [66]. Wastewater has been shown to play an integral role in AMR development, as it serves as a substantial reservoir for ARBs and ARGs, with a significant correlation with human AMR prevalence, as shown in various studies, regardless of their heterogenous approaches. Prioritizing the development of standardized methodologies, alongside regulatory frameworks and interdisciplinary collaboration on wastewater AMR surveillance, will enable comparability, data sharing, and actionable reporting across regions. Parameters that should be included in these protocols are specifications regarding the time/location-matched sampling of wastewater and human populations, compound influent and effluent sampling, while sampling collection for >12months could provide data for longitudinal studies. Future research should focus on the validation and harmonization of sampling, data analysis, and reporting protocols; the integration of clinical, epidemiological, and environmental datasets; and the advancement of rapid, cost-effective surveillance platforms. The advancement of wastewater surveillance could support timely, evidence-based decision-making, enhance our ability to control AMR dissemination, enable the development of better treatment methods, and contribute to the preservation of antimicrobial efficacy for patients and communities worldwide (Lymperatou, 2025).

CONCLUSION

Wastewater-based surveillance is now recognized as an important public health tool. Hospital wastewater-based surveillance has the potential to serve as a critical tool for understanding, tracking, and potentially mitigating the spread of AMR in highly endemic settings. By enabling comprehensive, real-time monitoring of ARGs and resistant bacteria from concentrated clinical sources, hospital wastewater surveillance provides a platform that closely reflects local prescribing practices and clinical AMR patterns. Unlike traditional surveillance methods limited to symptomatic patients, this approach captures data on asymptomatic carriage,

transmission dynamics, and emerging resistance mechanisms within institutions, complementing clinical datasets and reducing reporting bias. Evidence so far has demonstrated significant correlations between ARG profiles in hospital influent and regional clinical data, supporting the use of wastewater analysis for targeted interventions, outbreak detection, and evaluation of stewardship policies.

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