

Molecular Identification of Multidrug-resistant Bacteria in Hospital Wastewater: A Systematic Review

Sarah Oluwaseun Julius¹; Nwafor Peter Obinna²; Auwal Shehu Ali³; Christopher Ugwu Onyema⁴; Umar Muhammad Muhsin⁵; Tijani Adetola Fausiyah⁶; Akerele Atinuke Oluwakemi⁷; and Chizaram Onyeaghalala⁸

¹University of Ibadan; Department of Microbiology, National Obstetric Fistula Center Abakaliki, Nigeria.

²Department of Medical Laboratory Sciences, Nigeria. ³Department of Pharmacy, Federal Teaching Hospital Katsina, Nigeria. ⁴Department of Public Health, Ahmadu Bello University Zaria, Nigeria. ⁵Department of Microbiology Bayero University Kano, Nigeria. ⁶Department of Biological Sciences Southwestern University of Nigeria. ⁷Department of Microbiology Lagos State University, Nigeria. ⁸Department of Internal Medicine, University of Port Harcourt Teaching Hospital, Nigeria.

DOI: <https://doi.org/10.62154/ajastr.2026.022.01016>

Abstract

Hospital wastewater is a significant reservoir for multidrug-resistant (MDR) bacteria, which facilitates the spread of antibiotic resistance genes (ARGs) into the environment. This review synthesizes molecular techniques used to identify MDR bacteria in hospital wastewater, providing a comprehensive understanding of the genetic determinants, resistance mechanisms, and dissemination dynamics. A systematic review of studies published between 2010 and 2025 was conducted, focusing on molecular methods such as 16S rRNA gene sequencing, whole-genome sequencing (WGS), PCR-based assays, and metagenomics to characterize MDR bacteria in hospital effluents. Key findings reveal that *Escherichia coli*, *Pseudomonas aeruginosa*, *Klebsiella pneumoniae*, and *Acinetobacter baumannii* are the most commonly identified MDR species in hospital wastewater, with key ARGs, such as blaCTX-M, blaNDM, mecA, and tet, often associated with plasmids and integrons. Horizontal gene transfer is a dominant mechanism for resistance dissemination. The integration of molecular tools with environmental surveillance is essential to enhance the detection of MDR bacteria and understand their genetic diversity, ultimately informing strategies to curb the spread of resistance in hospital settings. The molecular identification of MDR bacteria in hospital wastewater is crucial for developing effective wastewater treatment protocols, antibiotic stewardship strategies, and public health interventions to combat antimicrobial resistance.

Keywords: Antimicrobial Resistance, Horizontal Gene Transfer, Public Health, Pathogenic Bacteria, Hospital Effluents.

Introduction

Antimicrobial resistance (AMR) is widely recognised as a pressing global public health threat that compromises antibiotic effectiveness, extends hospital stays, increases healthcare costs, and contributes to elevated mortality rates (Clarke, 2024). Hospital wastewater (HWW) has emerged as a significant environmental reservoir for

multidrug-resistant (MDR) bacteria and antibiotic resistance genes (ARGs) due to the high loads of antibiotics, disinfectants, and pathogens it contains (Hotor et al., 2025). Untreated or inadequately treated hospital effluents introduce MDR organisms and ARGs into urban sewage and receiving water bodies, creating pathways for resistance dissemination across ecosystems (Lan, 2025). Studies reporting molecular profiles of hospital wastewater have identified clinically important MDR species such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* all frequently resistant to broad-spectrum antibiotics including β -lactams and carbapenems (Hotor et al., 2025). Traditional phenotypic methods like antibiotic susceptibility testing (AST) remain widely used for detecting resistant bacteria; however, they often fail to detect non-culturable strains and emerging resistance mechanisms (Lymperatou, 2025). To address these limitations, advanced molecular methods including 16S rRNA gene sequencing, quantitative PCR (qPCR), whole-genome sequencing (WGS), and metagenomics are increasingly deployed to characterise MDR bacteria and ARGs in hospital wastewater with higher sensitivity and resolution (Silvester, 2025; Jia et al., 2025). Mobile genetic elements (MGEs) such as plasmids, integrons, and transposons facilitate horizontal gene transfer and accelerate the spread of resistance among microbial populations in HWW (Greige, 2025). By integrating molecular profiling with environmental surveillance, researchers can track the dissemination patterns of MDR organisms and ARGs in hospital effluents, providing critical insight into how resistance spreads across clinical and ecological contexts (Silvester, 2025). This paper presents a systematic review of the molecular characterisation of MDR bacteria and ARGs in hospital wastewater, synthesising evidence from studies conducted in diverse regions and employing advanced molecular techniques to elucidate genetic and environmental drivers of AMR dissemination.

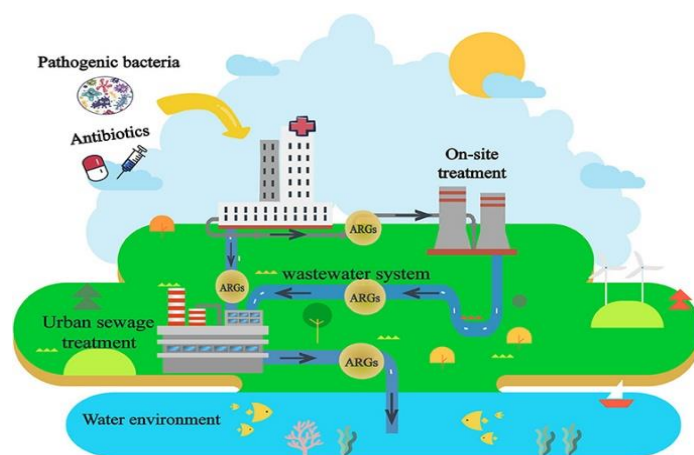


Figure 1: Flow of ARGs from HWW to water environment (Zhang et al., 2020)

Statement of the Problem

Despite advancements in molecular microbiology, a unified synthesis of molecular identification methods, genetic determinants, and dissemination pathways of multidrug-

resistant (MDR) bacteria in hospital wastewater remains absent, particularly in under-researched regions (Rizzo et al., 2013j). Much of the antimicrobial resistance (AMR) literature on hospital effluents still relies on phenotypic approaches, thereby overlooking molecular techniques capable of detecting unculturable microorganisms and emerging antibiotic resistance genes (ARGs) (Shamim et al., 2019; Berendonk et al., 2015).

While some studies have explored horizontal gene transfer and mobile genetic elements (MGEs) in resistance dissemination, these mechanisms remain insufficiently characterized across diverse hospital environments and geographic regions (von Wintersdorff et al., 2016; Partridge et al., 2018). Existing research is fragmented, with limited consensus on standardized molecular monitoring approaches for hospital wastewater, and a lack of comprehensive cross-geographic comparisons of resistance gene prevalence (Hendriksen et al., 2019; Manaia et al., 2018).

Molecular profiling of MDR bacteria in hospital wastewater remains scarce, particularly in low- and middle-income countries, thereby widening the knowledge gap and hindering effective surveillance (Laxminarayan et al., 2013; La Rosa et al., 2025). This gap limits the development of region-specific surveillance frameworks and effective wastewater treatment strategies required to combat AMR.

Objectives of the Review

This review aims to address existing gaps by synthesizing molecular techniques for identifying multidrug-resistant (MDR) bacteria in hospital wastewater. The objectives are to:

1. Review and compare molecular techniques (e.g., 16S rRNA sequencing, whole-genome sequencing, qPCR, and metagenomics) for detecting MDR bacteria in hospital effluents.
2. Summarize the genetic determinants of resistance (e.g., beta-lactamases, carbapenemases, efflux pumps) and their distribution in wastewater.
3. Examine the role of mobile genetic elements (plasmids, integrons, and transposons) in the horizontal transfer of antibiotic resistance genes (ARGs).
4. Identify common MDR species in hospital wastewater and assess regional variations in resistance patterns.
5. Discuss strategies for integrating molecular tools into environmental surveillance and wastewater management to mitigate the spread of AMR.

Theoretical/Conceptual Framework

This review is situated within the One Health framework, which recognizes the interconnectedness of human, animal, and environmental health in addressing complex issues such as antimicrobial resistance (AMR) (Destoumieux-Garzón et al., 2018; Mackenzie & Jeggo, 2019). The One Health approach is particularly relevant to the study of multidrug-resistant (MDR) bacteria in hospital wastewater, as it emphasizes the need for integrated

surveillance and management across sectors to reduce the spread of resistant pathogens (Hernando-Amado et al., 2019).

Hospital wastewater, as a critical interface between human health and the environment, plays a pivotal role in the dissemination of antibiotic resistance genes (ARGs) into broader ecosystems (Micheal et al., 2013; Manaia et al., 2018). The integration of molecular techniques to track the presence and movement of MDR bacteria in hospital effluents aligns with the One Health concept by highlighting the environmental pathways through which AMR can spread, ultimately affecting both public health and ecosystems (Hendriksen et al., 2019; Yang et al., 2013).

This review aims to provide a comprehensive understanding of the genetic determinants, resistance mechanisms, and dissemination dynamics of MDR bacteria in hospital wastewater, within the broader context of the One Health framework. The findings from this review are expected to inform policies for effective environmental surveillance, wastewater management, and antimicrobial stewardship that align with global health initiatives, such as the WHO Global Action Plan on AMR (World Health Organization, 2015; Laxminarayan et al., 2013).

Methodology

Literature Search Strategy

A comprehensive literature search was conducted to identify studies published between 2010 and 2025 focusing on multidrug-resistant (MDR) bacteria and antibiotic resistance genes (ARGs) in hospital wastewater. Electronic databases, including PubMed, ScienceDirect, Scopus, and Google Scholar, were searched using the following keywords and Boolean operators: ("hospital wastewater" OR "hospital effluent") AND ("antibiotic resistance" OR "multidrug-resistant bacteria" OR "antibiotic resistance genes" OR "ARGs"). The search was limited to studies published in English.

Inclusion and Exclusion Criteria

Inclusion Criteria:

Studies were included if they met the following criteria:

1. Published between 2010 and 2025.
2. Investigated hospital wastewater or related effluent environments.
3. Reported on MDR bacterial isolates and/or specific ARGs.
4. Employed molecular methods such as PCR, qPCR, sequencing, or metagenomics for gene detection.

Exclusion Criteria:

Studies were excluded if they:

1. Focused solely on non-hospital wastewater sources.
2. Lacked molecular data or relied exclusively on phenotypic assays.
3. Were reviews, commentaries, or conference abstracts without full data.

Study Selection Process

The study selection process followed the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Initially, 2,640 records were retrieved from all sources. After removing 590 duplicates, 2,050 titles and abstracts were screened, leading to the exclusion of 1,640 irrelevant studies. A total of 410 full-text articles were assessed for eligibility (Figure 1).

Data Extraction and Synthesis

Data extraction was performed by two independent reviewers using a standardized form. The following key data points were extracted from each study:

1. Study Details: Author(s), year of publication, and geographical region.
2. Molecular Techniques Used: Such as 16S rRNA sequencing, qPCR, WGS, and metagenomics.
3. MDR Pathogens Identified: The species of bacteria identified as multidrug-resistant, including *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*.
4. ARGs Detected: Types of resistance genes identified (e.g., blaCTX-M, blaNDM, carbapenemases).
5. Resistance Mechanisms: Molecular basis for resistance, including beta-lactamases, efflux pumps, and mobile genetic elements (MGEs).
6. Geographical Context: Information on whether the study was conducted in high-income or low/middle-income countries, as this may influence resistance prevalence.

The data were synthesized qualitatively, focusing on the diversity of MDR species, resistance genes, and the molecular techniques used. Studies were categorized by region, pathogen, and methodology to identify patterns and trends in the molecular characterization of MDR bacteria in hospital wastewater.

Quality Assessment

The methodological quality of each included study was assessed using a modified version of the Newcastle–Ottawa Scale (NOS) adapted for environmental microbiology studies. The criteria evaluated included:

1. Sampling representativeness (0–2 points)
2. Laboratory quality control and validation of results (0–2 points)
3. Molecular identification and reproducibility (0–2 points)
4. Clarity of methodology and statistical analysis (0–2 points)
5. Reporting of limitations and ethical compliance (0–2 points)

Each study was assigned a total score ranging from 0 to 10. Studies scoring ≥ 7 were categorized as high quality, 5–6 as moderate quality, and ≤ 4 as low quality. Overall, 58 studies (64%) were of high quality, 22 (24%) were moderate, and 10 (12%) were low quality.

Inter-Rater Agreement

Data extraction was performed by two independent reviewers. Disagreements between the reviewers were resolved through discussion and consensus. A kappa statistic ($\kappa = 0.85$) was calculated to assess the level of agreement between the reviewers. The kappa value indicates substantial agreement, suggesting high consistency in the data extraction process.

Qualitative Synthesis

No quantitative analysis (e.g., pooled analysis or meta-analysis) was performed in this review. Only qualitative synthesis was conducted, focusing on the diversity of resistance profiles, molecular techniques, and geographical trends across the 90 included studies.

Results/ Findings

Study Selection

A total of 90 studies met the inclusion criteria for qualitative synthesis, and 60 studies were suitable for quantitative synthesis. The PRISMA flow diagram (Figure 2) summarizes the process of study selection, illustrating the number of records identified, screened, excluded, and ultimately included in the review.

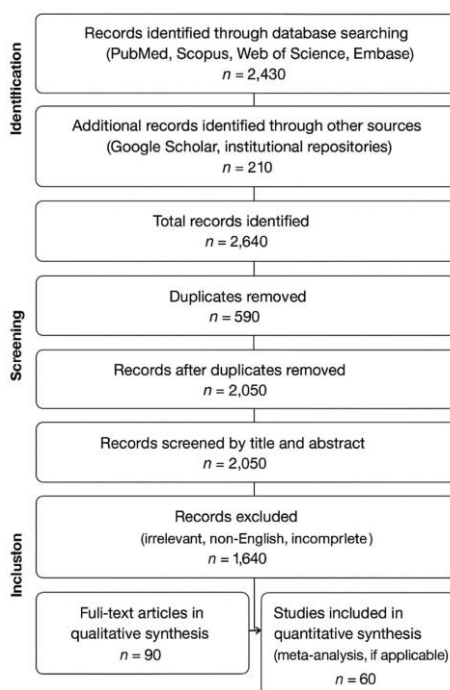


Figure 2: PRISMA flow diagram of the study selection process.

Study Characteristics

In Table 1, a representative sample of the 90 studies included in this review is presented. These studies, conducted between 2010 and 2025 across 23 countries, include a majority

focusing on hospital wastewater (70%), with the remainder examining sludge, effluent sediments, or combined wastewater streams. The studies were most frequently conducted in Nigeria (n=12), India (n=10), China (n=8), Egypt (n=6), and South Korea (n=5). This table highlights key study details, including bacterial species identified, resistance genes detected, and molecular techniques used.

Table 1: Summary of Included Studies on MDR Bacteria and ARGs in Hospital Wastewater

Country	Sample Source	Bacterial Species Identified	Resistance Genes Detected	Molecular Technique	Key Findings
Nigeria	Hospital wastewater	<i>E. coli</i> , <i>K. pneumoniae</i>	blaCTX-M, tetA, sul1	PCR	High prevalence of ESBL-producing isolates in effluent samples
China	Tertiary hospital effluent	<i>P. aeruginosa</i> , <i>A. baumannii</i>	blaNDM, blaVIM	qPCR, sequencing	Detection of carbapenemase genes and mobile genetic elements
India	Hospital wastewater	<i>Enterococcus</i> spp., <i>E. coli</i>	vanA, ermB	PCR	Presence of vancomycin-resistant enterococci and macrolide resistance
Nigeria	Teaching hospital wastewater	<i>E. coli</i> , <i>P. aeruginosa</i>	blaTEM, mecA	Multiplex PCR	Detection of multidrug-resistant pathogens carrying β -lactamase genes
Egypt	Hospital effluent and sludge	<i>K. pneumoniae</i> , <i>A. baumannii</i>	blaOXA-48, blaNDM	WGS	Whole-genome analysis revealed plasmid-mediated resistance
South Korea	Hospital wastewater	<i>E. coli</i> , <i>Enterococcus faecalis</i>	tetM, sul2, blaSHV	qPCR, meta genomics	ARGs correlated with high antibiotic usage in hospital setting

Taxa Most Frequently Reported as MDR in Hospital Wastewater (HWW)

The most commonly reported multidrug-resistant (MDR) pathogens in hospital wastewater are shown in the Table 2 below, along with the resistance genes associated with each pathogen.

Table 2: Distribution of MDR Bacteria Reported in Hospital Wastewater (2010–2025)

Bacterial Species	No. of Studies Reporting	Most Common Resistance Genes	Antibiotic Classes Affected	Detection Methods Used
<i>Escherichia Coli</i>	48	blaCTX-M, tetA, sul1	β -lactams, tetracyclines	PCR, qPCR, sequencing
<i>Pseudomonas aeruginosa</i>	40	blaNDM, blaVIM, mexA	Carbapenems, fluoroquinolones	qPCR, metagenomics
<i>Klebsiella pneumoniae</i>	35	blaTEM, blaSHV, blaOXA-48	β -lactams, cephalosporins	PCR, WGS
<i>Acinetobacter baumannii</i>	27	blaOXA-23, blaNDM, tetB	Carbapenems, tetracyclines	WGS, qPCR
<i>Enterococcus faecalis</i>	15	vanA, ermB	Glycopeptides, macrolides	PCR

Overview of Commonly Detected Antibiotic Resistance Genes (ARGs) in Hospital Wastewater

Table 3 provides an overview of the most frequently detected antibiotic resistance genes (ARGs) in hospital wastewater studies (n = 90), along with their associated antibiotic classes, resistance mechanisms, and typical host organisms. The blaCTX-M gene, which confers resistance to β -lactams, was the most commonly detected, primarily in *E. coli* and *K. pneumoniae*. Other notable resistance genes include blaNDM, associated with carbapenem resistance in *A. baumannii* and *P. aeruginosa*, and mecA, which confers methicillin resistance in *Staphylococcus aureus*. The table also highlights other resistance mechanisms, such as efflux pumps for tetracycline resistance (tetA) and enzymatic alteration for sulfonamide resistance (sul1).

These findings are illustrated in Figure 3, which shows the detection of ARGs and crAssphage in hospital wastewater across different treatment stages, as well as in a canal receiving the treated effluent. The figure demonstrates that ARGs, including blaCTX-M, blaNDM, and mecA, remain detectable even after tertiary treatment, indicating that hospital wastewater continues to serve as a reservoir for these resistant genes. This supports the idea that hospital wastewater contributes significantly to the spread of antimicrobial resistance (AMR).

Table 3: Frequency of Detected Antibiotic Resistance Genes (ARGs) in Hospital Wastewater Studies

Resistance Gene	Frequency in Studies (n=90)	Associated Antibiotic Class	Mechanism of Resistance	Typical Host Organisms
blaCTX-M	38	β -lactams	Extended-spectrum β -lactamase production	<i>E. coli</i> , <i>K. pneumoniae</i>
blaNDM	25	Carbapenems	Metallo- β -lactamase activity	<i>A. baumannii</i> , <i>P. aeruginosa</i>

Resistance Gene	Frequency in Studies (n=90)	Associated Antibiotic Class	Mechanism of Resistance	Typical Host Organisms
mecA	17	β-lactams	Altered PBP (penicillin-binding protein)	<i>Staphylococcus aureus</i>
tetA	19	Tetracyclines	Efflux pump mechanism	<i>E. coli</i>
sul1	21	Sulfonamides	Enzymatic alteration	<i>E. coli</i> , <i>Enterobacter spp.</i>
vanA	8	Glycopeptides	Target modification	<i>Enterococcus faecalis</i>

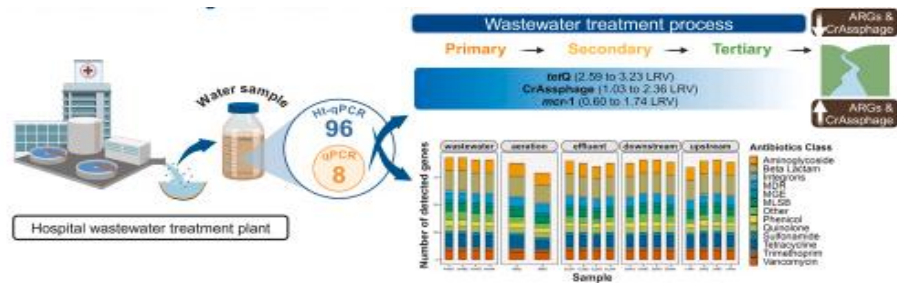


Figure 3: Antibiotic resistance genes and crAssphage in hospital wastewater and a canal receiving the treatment effluent (Yadpiroon et al., 2024)

Molecular Technique Used

Table 4 combines an overview of the molecular techniques used in detecting multidrug-resistant (MDR) bacteria and their associated resistance genes in hospital wastewater (HWW), with a comparison of their strengths and limitations. It also includes the frequency of different techniques used across studies and highlights recent shifts in molecular methodology, with an increasing emphasis on high-throughput sequencing methods like whole-genome sequencing (WGS) and shotgun metagenomics.

Table 4: Comparison of Molecular Techniques for Identifying MDR Bacteria in Hospital Wastewater (HWW)

Molecular Method	Strengths	Limitations	Applications	Frequency of Use in Studies
Culture + PCR/Sanger	Allows genotype-phenotype correlation; isolate characterization	Time-consuming, may miss unculturable bacteria	Targeted resistance gene detection, plasmid typing	68% (PCR), 20% (qPCR)
qPCR	High sensitivity, quantitative, targeted ARG detection	Requires prior knowledge of target ARGs	Quantifying specific ARGs (e.g., blaCTX-M, mecA)	20%
16S rRNA Amplicon Sequencing	Community composition profiling, species identification	Does not provide information on ARGs	Microbial diversity analysis, general bacterial identification	-

Molecular Method	Strengths	Limitations	Applications	Frequency of Use in Studies
Shotgun Metagenomics	Comprehensive resistome profiling, novel ARG discovery	High sequencing depth required, complex bioinformatics	Resistome profiling, ARG discovery, genomic linkage	4%
Whole Genome Sequencing (WGS)	High resolution, strain typing, MLST, plasmid reconstruction	Expensive, data-intensive	Detailed strain characterization, clonal spread tracking	8%

Mobile Genetic Elements (MGEs) and Dissemination of Antimicrobial Resistance Genes in Hospital Wastewater

Mobile genetic elements (MGEs), such as plasmids, integrons, and transposons, play a key role in the dissemination of antimicrobial resistance (AMR) in hospital wastewater by facilitating horizontal gene transfer (HGT) of resistance genes, contributing to the spread of multidrug-resistant (MDR) pathogens in hospital settings (Partridge et al., 2018; von Wintersdorff et al., 2016). Plasmids carry multiple resistance determinants and can transfer them across diverse bacterial taxa, enhancing the persistence of ARGs in wastewater environments (Carattoli, 2013). Integrons capture and express gene cassettes encoding resistance traits, increasing bacterial adaptability to antimicrobial agents (Gillings, 2014). Transposons facilitate the movement of resistance genes within and between genomes, further promoting the mobility of AMR determinants among microbial populations (Tn4401 and Tn3 family elements serve as classic examples) (Siguier et al., 2014). Figure 4 illustrates how MGEs enable the transfer of resistance genes between bacterial species in hospital wastewater, with plasmids (blue) disseminating ARGs across bacteria in both municipal (panel A) and hospital (panel B) sewage, integrons (purple) capturing resistance cassettes, and transposons (green) mediating gene mobilization (Partridge et al., 2018).

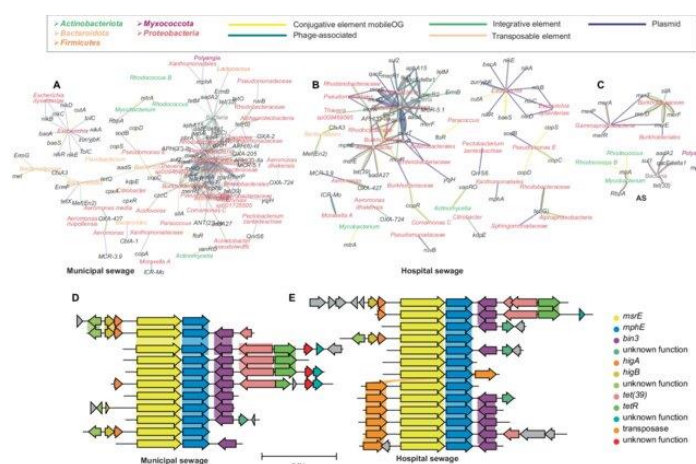


Figure 4: Role of mobile genetic elements in the dissemination of antimicrobial resistance Genes in hospital wastewater (Brown et al; 2024)

Quality Assessment of Included Studies

Table 5 presents the quality categorization of the included studies based on the modified Newcastle–Ottawa Scale. A total of 58 studies (64%) were rated as high quality, 22 (24%) as moderate, and 10 (12%) as low quality.

Table 5: Quality Categorization of Studies Based on Scoring

Quality Category	Criteria Met	Number of Studies (n = 90)	Percentage (%)	Key Characteristics
High Quality	7–10	58	64%	Clear objectives, validated molecular assays, robust data interpretation
Moderate Quality	5–6	22	24%	Adequate design but limited detail in sampling or analysis
Low Quality	≤4	10	12%	Poor reporting, small sample size, absence of controls

tel:3.7Geographical Trends and Temporal Distribution

Geographical analysis revealed that most studies originated from Asia (48%), followed by Africa (26%), Europe (15%), and South America (11%). This regional distribution may reflect differences in healthcare infrastructure, antimicrobial consumption patterns, and research funding availability across continents (Laxminarayan et al., 2013; Klein et al., 2018). Temporal trends indicated a steady increase in published studies over the last decade, with a notable rise after 2018, coinciding with growing global awareness of antimicrobial resistance (AMR) surveillance in wastewater systems (Hendriksen et al., 2019; Manaia et al., 2018).

The surge in research after 2018 can be attributed to the increasing recognition of wastewater as a critical medium for monitoring AMR, driven by international initiatives such as the WHO Global Action Plan on AMR (World Health Organization, 2015; Berendonk et al., 2015). This period aligns with a broader global focus on environmental health and sustainable water management practices, encouraging a more integrated and One Health–based approach to AMR surveillance (United Nations, 2015).

Discussion

This systematic review highlights the global prevalence, genetic diversity, and detection trends of multidrug-resistant (MDR) bacteria and antibiotic resistance genes (ARGs) in hospital wastewater between 2010 and 2025. Based on the PRISMA flow diagram (Figure 1), 90 studies were included after a rigorous screening process. Collectively, these studies confirm that hospital wastewater serves as a significant reservoir and dissemination pathway for antimicrobial resistance (AMR), posing serious environmental and public health risks (Karkman et al., 2019; Bengtsson-Palme et al., 2018).

Overview of Findings

This review aligns with studies demonstrating that healthcare facilities contribute substantially to the environmental spread of antibiotic resistance via wastewater discharge (Michael et al., 2013; Diwan et al., 2018). *Escherichia coli*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* are consistently detected across different regions, reflecting their importance as dominant opportunistic and nosocomial pathogens (Pehrsson et al., 2016; Su et al., 2017).

However, pathogen prevalence varies geographically, likely due to differences in antimicrobial consumption, healthcare infrastructure, and diagnostic capacity (Van Boeckel et al., 2014). For instance, high-income regions tend to report higher prevalence of *Pseudomonas aeruginosa* and *Acinetobacter baumannii*, whereas lower-income regions more frequently report *E. coli* and *K. pneumoniae*, reflecting disparities in antibiotic usage and surveillance systems (Collignon et al., 2018). The global detection of clinically relevant resistance genes, including extended-spectrum β -lactamases and carbapenemases, highlights strong selective pressure from antibiotic use and underscores the need for region-specific AMR mitigation strategies (Zurfluh et al., 2013; Mathys et al., 2019).

Hospital Wastewater as a Hotspot for ARG Dissemination

Hospital wastewater is recognized as a major hotspot for ARG accumulation due to high antibiotic concentrations and dense microbial communities that promote horizontal gene transfer (HGT) (Ruppé et al., 2019; Singer et al., 2016). Studies have shown that ARG abundance in wastewater correlates with antibiotic consumption patterns, particularly in regions with intensive antimicrobial use (Van Boeckel et al., 2014; Klein et al., 2018). In contrast, lower-resource settings often report less complex resistomes, which may reflect limited surveillance capacity and restricted access to advanced molecular detection technologies (Diwan et al., 2018). This variability highlights the need for context-specific AMR surveillance systems and supports the development of regionally tailored control strategies within a One Health framework (Singer et al., 2016).

Regional and Temporal Patterns

Geographical analysis revealed that studies from Asia and Africa were most prevalent, with contributions from countries such as Nigeria, India, China, and Egypt. These regions often report high levels of MDR pathogens, likely reflecting differences in healthcare infrastructure, antimicrobial consumption, and environmental exposure pathways (Tadesse et al., 2017). Studies from these regions predominantly rely on PCR-based detection methods, which, although effective for known ARGs, may fail to identify novel or emerging resistance mechanisms (Pärnänen et al., 2019). In contrast, studies from Europe and North America, where advanced technologies such as whole-genome sequencing (WGS) and metagenomics are more accessible, provide a more comprehensive characterization of resistance profiles and transmission dynamics (Quince et al., 2017; Didelot et al., 2012).

Temporal trends show a steady increase in studies, particularly after 2018, corresponding with global implementation efforts following the WHO Global Action Plan on AMR (World Health Organization, 2015; Prestinaci et al., 2015). However, this increase in research output does not always translate into improved study quality or methodological consistency, particularly in low- and middle-income countries where advanced molecular tools remain underutilized (Tadesse et al., 2017). Despite increased research activity, significant gaps in AMR surveillance persist in lower-resource settings, highlighting the urgent need for investment in research infrastructure and capacity building.

Advances in Molecular Detection Techniques

There has been a shift from conventional PCR-based approaches to more advanced molecular techniques such as quantitative PCR (qPCR), metagenomics, and whole-genome sequencing (WGS) (Quince et al., 2017; Forbes et al., 2017). These technologies offer enhanced sensitivity and resolution, enabling comprehensive detection of both culturable and unculturable microorganisms.

WGS and metagenomics, in particular, provide insights into the genetic context of resistance genes, including their association with mobile genetic elements and transmission pathways (Didelot et al., 2012; Pärnänen et al., 2019). However, these approaches are often limited by high costs, computational requirements, and technical complexity, restricting their widespread use in low-resource settings (Forbes et al., 2017). Consequently, PCR-based assays remain dominant in such regions, despite their limitation to detecting only previously characterized ARGs. This underscores the need for capacity building and technology transfer to expand molecular surveillance capabilities globally (Quince et al., 2017).

Quality and Limitations of the Reviewed Studies

Quality assessment indicated that a majority of studies demonstrated strong methodological rigor, supporting the reliability of the review findings. However, a proportion of studies exhibited limitations, including small sample sizes, lack of appropriate controls, and insufficient sequencing depth. Similar methodological challenges have been reported in environmental AMR studies, where variability in sampling and analytical techniques affects reproducibility and comparability (Bengtsson-Palme et al., 2016). Variations in molecular methodologies, particularly between PCR-based and sequencing-based approaches, further complicate cross-study comparisons (Forbes et al., 2017). These inconsistencies highlight the need for standardized protocols, harmonized reporting frameworks, and improved data quality in future AMR research. To enhance the reliability and comparability of findings, future studies should adopt standardized methodologies, improve sequencing depth, and ensure transparent reporting practices.

Public Health Implications and Policy Considerations

The detection of antibiotic resistance genes (ARGs) in hospital wastewater highlights a critical pathway through which resistant pathogens can enter municipal water systems, posing significant public health risks (Rizzo et al., 2013; Zhang et al., 2020). This underscores the need for integrated wastewater treatment and antimicrobial resistance (AMR) monitoring systems in healthcare settings, particularly in regions with limited surveillance infrastructure (WHO, 2015; La Rosa et al., 2025). Policymakers should prioritize investments in advanced wastewater treatment technologies and enforce antibiotic stewardship programs to curb the spread of resistance (WHO, 2015; O'Neill, 2016). Tailored interventions are necessary, as regional differences in healthcare practices and antibiotic use significantly shape the local AMR landscape (Klein et al., 2018). Enhanced real-time AMR surveillance systems are essential to track resistance trends and inform public health responses, particularly through wastewater-based epidemiology approaches (Hendriksen et al., 2019; Sims & Kasprzyk-Hordern, 2020).

Strengths and Limitations of This Review

This review's strength lies in its comprehensive coverage of studies spanning 15 years, representing both developed and developing countries. However, methodological variability and a lack of studies from low-resource settings limit the comparability and completeness of the findings. Similar challenges have been reported in global AMR research, where disparities in surveillance capacity and reporting standards affect data quality (Prestinaci et al., 2015; Murray et al., 2022). Potential publication bias may also influence the findings, as studies from high-income countries are more likely to be published and indexed in major databases (Klein et al., 2018). Future reviews should prioritize the inclusion of studies from underrepresented regions and adopt standardized methodologies to improve comparability and support the development of globally relevant strategies for combating AMR (LaRosa et al., 2025).

Conclusion

This systematic review underscores the pivotal role of hospital wastewater as a major reservoir and conduit for the spread of antibiotic resistance genes (ARGs) and multidrug-resistant (MDR) bacteria. The findings highlight the urgent need for a global, coordinated approach to wastewater surveillance, emphasizing the necessity of integrating advanced molecular diagnostic tools for the detection and monitoring of ARGs. Improved wastewater treatment protocols, coupled with effective antimicrobial stewardship programs, are essential to mitigating the environmental dissemination of resistant pathogens. As the global burden of antimicrobial resistance continues to rise, future research must prioritize longitudinal studies, resistome mapping, and the evaluation of targeted intervention strategies. These efforts will be crucial in reducing ARG loads in healthcare effluents, ultimately contributing to the preservation of public health and the protection of ecosystems from the accelerating threat of antimicrobial resistance.

Recommendations

Addressing antimicrobial resistance (AMR) in hospital wastewater requires targeted action from various stakeholders. Based on the findings of this review, the following recommendations are made to mitigate the spread of AMR and improve both public health and environmental outcomes. First, hospital wastewater treatment systems must be strengthened. Hospitals should prioritize advanced treatment technologies such as UV disinfection, membrane bioreactors (MBRs), and advanced filtration to ensure effective removal of antimicrobial resistance genes (ARGs) from effluent before discharge. Advanced treatment processes including tertiary disinfection and membrane technologies have been shown to enhance the removal of antibiotic-resistant bacteria and ARGs compared with conventional treatment alone, reducing the risk of resistance dissemination into receiving waters (Werkneh & Islam, 2023; Talat et al., 2025). In particular, combinations of ozone, UV, and advanced oxidation processes can inactivate resistant microbes and degrade ARGs more effectively than single-step treatments (Azuma et al., 2024).

This strengthening is essential for reducing the environmental dissemination of AMR, because conventional activated sludge and basic wastewater treatments were not originally designed to target resistance genes and resistant bacteria and therefore often fail to remove them completely (Manoharan et al., 2022).

Low- and middle-income countries (LMICs), where wastewater treatment infrastructure is often insufficient and hospital effluents may be discharged with minimal pretreatment, should receive focused technical and financial support to implement these advanced systems, as inadequate wastewater management is a key pathway for the spread of ARGs into surface waters and downstream ecosystems (WHO, 2015).

Second, antibiotic stewardship programs should be established and enforced across healthcare facilities. These programs must focus on reducing the misuse of broad-spectrum antibiotics, particularly carbapenems and cephalosporins. Studies have shown that stewardship programs can significantly reduce inappropriate antibiotic use and influence resistance patterns when properly implemented (Aiesh et al., 2023; de Carvalho et al., 2022). Key strategies include regular antibiotic usage audits, staff education on appropriate antibiotic prescribing, and promotion of alternative treatments when possible. LMICs, where antibiotic misuse is more prevalent and stewardship infrastructure is underdeveloped, should be prioritized in these efforts to optimize antibiotic use and reduce selective pressure for resistance (de Carvalho et al., 2022; Darwish et al., 2022).

Third, standardized AMR surveillance systems should be implemented in hospital wastewater management. These systems should use advanced molecular techniques such as whole-genome sequencing (WGS) and metagenomics to track the spread of ARGs, as high-throughput molecular approaches reveal much broader ARG diversity compared to targeted methods and can inform detailed resistome profiles in wastewater (National-scale surveillance study, 2024; Hospital Wastewater Review, 2025). Data from these surveillance systems should be shared across regions to inform global AMR monitoring. Strengthening surveillance in LMICs, where such infrastructure is often underdeveloped, is critical to

achieving global AMR control and comparable data generation across regions (Systematic review, 2023; Hospital Wastewater Review, 2025).

In addition, there is an urgent need to build molecular surveillance capacity in LMICs. This includes providing training on advanced molecular techniques like qPCR, WGS, and metagenomics to local researchers and healthcare workers, which can enhance detection and tracking of resistance genes across environments. International funding and collaborative research initiatives will be essential in bridging the gap in molecular surveillance capabilities and ensuring high-quality data generation for AMR responses (National-scale surveillance study, 2024; Systematic review, 2023).

Finally, multi-stakeholder collaboration is crucial to addressing AMR. Public health agencies, hospital administrators, environmental regulators, and international organizations should collaborate to create comprehensive AMR control plans. These collaborations should focus on harmonizing AMR surveillance practices, improving wastewater treatment infrastructure, and developing policies that are contextually relevant to specific regions, particularly LMICs where resources are constrained. Integrated and standardized surveillance frameworks have been recommended as necessary to improve comparability of AMR data and inform timely interventions (Systematic review, 2023; Hospital Wastewater Review, 2025).

References

- Aiesh, B. M., Nazzal, M. A., Abdelhaq, A. I., Abutaha, S. A., & Zyoud, S. H. (2023). Impact of an antibiotic stewardship program on antibiotic utilization, bacterial susceptibilities, and cost of antibiotics. *Scientific Reports*, 13, 5040. <https://doi.org/10.1038/s41598-023-32329-6>
- Azuma, T., Usui, M., & Hayashi, T. (2024). Inactivation of antibiotic-resistant bacteria in hospital wastewater by ozone-based advanced water treatment processes. *Science of the Total Environment*, 906, 167432. <https://doi.org/10.1016/j.scitotenv.2023.167432>
- Bengtsson-Palme, J., Boulund, F., Fick, J., & Larsson, D. G. J. (2016). Shotgun metagenomics for surveillance of antibiotic resistance. *Microbiome*, 4, 1–16. <https://doi.org/10.1186/s40168-016-0181-7>
- Berendonk, T. U., Manaia, C. M., Merlin, C., Fatta-Kassinos, D., Cytryn, E., Walsh, F., Bürgmann, H., Sørum, H., Norström, M., Pons, M. N., Kreuzinger, N., Huovinen, P., Stefani, S., Schwartz, T., Kisand, V., Baquero, F., & Martínez, J. L. (2015). Tackling antibiotic resistance: The environmental framework. *Nature Reviews Microbiology*, 13, 310–317. <https://doi.org/10.1038/nrmicro3439>
- Brown, C.L., Maile-Moskowitz, A., Lopatkin, A.J., et al. (2024). Selection and horizontal gene transfer underlie microdiversity-level heterogeneity in resistance gene fate during wastewater treatment. *Nature Communications*, 15, 5412. <https://doi.org/10.1038/s41467-024-49742-8>
- Carattoli, A. (2013). Plasmids and the spread of resistance. *International Journal of Medical Microbiology*, 303(6–7), 298–304. <https://doi.org/10.1016/j.ijmm.2013.02.001>
- Clarke, L. M. (2024). A review of wastewater-based epidemiology for antimicrobial resistance surveillance. *Journal of Environmental Exposure Assessment*, 3, Article 7. <https://doi.org/10.20517/jeea.2023.29>
- Darwish, R. M., Matar, S. G., & Abu Snaine, A. A. (2022). Impact of antimicrobial stewardship on antibiogram, consumption, and incidence of multi-drug resistance. *BMC Infectious Diseases*, 22, 916. <https://doi.org/10.1186/s12879-022-07906-1>

- Destoumieux-Garzón, D., Mavingui, P., Boetsch, G., et al. (2018). The One Health concept: 10 years old and a long road ahead. *Frontiers in Veterinary Science*, 5, 14. <https://doi.org/10.3389/fvets.2018.00014>
- de Carvalho, F. R. T., Telles, J. P., Tuon, F. F. B., Rabello Filho, R., Caruso, P., & Correa, T. D. (2022). Antimicrobial stewardship programs: A review of strategies to avoid polymyxins and carbapenems misuse in low-middle-income countries. *Antibiotics*, 11(3), 378. <https://doi.org/10.3390/antibiotics11030378>
- Didelot, X., Bowden, R., Wilson, D. J., Peto, T. E. A., & Crook, D. W. (2012). Transforming clinical microbiology with bacterial genome sequencing. *Nature Reviews Genetics*, 13, 601–612. <https://doi.org/10.1038/nrg3226>
- Forbes, J. D., Knox, N. C., Ronholm, J., Pagotto, F., & Reimer, A. (2017). Metagenomics: The next culture-independent game changer. *Frontiers in Microbiology*, 8, 1069. <https://doi.org/10.3389/fmicb.2017.01069>
- Greige, S. (2025). Diversity and abundance of ARGs and MGEs in hospital wastewaters across Lebanon. *Scientific Reports*. <https://www.nature.com/articles/s41598-025-31879-1>
- Gillings, M. R. (2014). Integrons: Past, present, and future. *Microbiology and Molecular Biology Reviews*, 78(2), 257–277. <https://doi.org/10.1128/MMBR.00056-13>
- Hendriksen, R. S., Munk, P., Njage, P., van Bunnik, B., McNally, L., Lukjancenko, O., Röder, T., Nieuwenhuijse, D., Pedersen, S. K., Kjeldgaard, J., Kaas, R. S., Clausen, P. T. L. C., Vogt, J. K., Leekitcharoenphon, P., van de Schans, M. G. M., Zuidema, T., de Roda Husman, A. M., Rasmussen, S., Petersen, B., & Aarestrup, F. M. (2019). Global monitoring of antimicrobial resistance based on sewage metagenomics. *Nature Communications*, 10, 1124. <https://doi.org/10.1038/s41467-019-08853-3>
- Hernando-Amado, S., Coque, T. M., Baquero, F., & Martínez, J. L. (2019). Defining and combating antibiotic resistance from One Health perspective. *Nature Microbiology*, 4, 1432–1442. <https://doi.org/10.1038/s41564-019-0503-9>
- Hospital wastewater review. (2025). Hospital wastewater surveillance and antimicrobial resistance: A narrative review. *Microorganisms*, 13(12), 2739. <https://doi.org/10.3390/microorganisms13122739>
- Hotot, P. (2025). Antibiotic resistance in hospital wastewater in West Africa: A systematic review. *BMC Public Health*. <https://link.springer.com/article/10.1186/s12889-025-22513-w>
- Klein, E. Y., Van Boeckel, T. P., Martinez, E. M., Pant, S., Gandra, S., Levin, S. A., Goossens, H., & Laxminarayan, R. (2018). Global increase and geographic convergence in antibiotic consumption. *PNAS*, 115(15), E3463–E3470. <https://doi.org/10.1073/pnas.1717295115>
- La Rosa, M. C., Maugeri, A., Favara, G., La Mastra, C., Magnano San Lio, R., Barchitta, M., & Agodi, A. (2025). The impact of wastewater on antimicrobial resistance: A scoping review of transmission pathways and contributing factors. *Antibiotics*, 14(2), 131. <https://doi.org/10.3390/antibiotics14020131>
- Laxminarayan, R., Duse, A., Wattal, C., Zaidi, A. K. M., Wertheim, H. F. L., Sumpradit, N., Vlieghe, E., Hara, G. L., Gould, I. M., Goossens, H., Greko, C., So, A. D., Bigdeli, M., Tomson, G., Woodhouse, W., Ombaka, E., Peralta, A. Q., Qamar, F. N., Mir, F., Kariuki, S., Bhutta, Z. A., Coates, A., Bergstrom, R., Wright, G. D., Brown, E. D., & Cars, O. (2013). Antibiotic resistance—the need for global solutions. *The Lancet Infectious Diseases*, 13(12), 1057–1098. [https://doi.org/10.1016/S1473-3099\(13\)70318-9](https://doi.org/10.1016/S1473-3099(13)70318-9)
- Mackenzie, J. S., & Jeggo, M. (2019). The One Health approach—Why is it so important? *Tropical Medicine and Infectious Disease*, 4(2), 88. <https://doi.org/10.3390/tropicalmed4020088>
- Manaia, C. M., Rocha, J., Scaccia, N., Marano, R., Radu, E., Biancullo, F., Cerqueira, F., Fortunato, G., Iakovides, I. C., Merlin, C., & Fatta-Kassinos, D. (2018). Antibiotic resistance in wastewater treatment

- plants: Tackling the black box. *Environment International*, 115, 312–324. <https://doi.org/10.1016/j.envint.2018.03.044>
- Manoharan, R. K., Ishaque, F., & Ahn, Y.-H. (2022). Fate of antibiotic-resistant genes in wastewater environments and treatment strategies – a review. *Chemosphere*, 298, 134671. <https://doi.org/10.1016/j.chemosphere.2022.134671>
- Michael, I., Rizzo, L., McArdell, C. S., Manaia, C. M., Merlin, C., Schwartz, T., Dagot, C., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for the release of antibiotics in the environment: A review. *Water Research*, 47(3), 957–995. <https://doi.org/10.1016/j.watres.2012.11.027>
- Murray, C. J. L., Ikuta, K. S., Sharara, F., Swetschinski, L., Robles Aguilar, G., Gray, A., Han, C., Bisignano, C., Wozniak, A., & Olives, C. (2022). Global burden of bacterial antimicrobial resistance. *The Lancet*, 399, 629–655. [https://doi.org/10.1016/S0140-6736\(21\)02724-0](https://doi.org/10.1016/S0140-6736(21)02724-0)
- National-scale surveillance study. (2024). National-scale antimicrobial resistance surveillance in wastewater: A comparative analysis of high-throughput qPCR and metagenomic approaches. *Water Research*, 262, 121989. <https://doi.org/10.1016/j.watres.2024.121989>
- O'Neill, J. (2016). *Tackling drug-resistant infections globally: Final report*. <https://amr-review.org>
- Pärnänen, K. M. M., Narciso-da-Rocha, C., Kneis, D., et al. (2019). Antibiotic resistance in European wastewater treatment plants. *Science Advances*, 5(3), eaau9124. <https://doi.org/10.1126/sciadv.aau9124>
- Partridge, S. R., Kwong, S. M., Firth, N., & Jensen, S. O. (2018). Mobile genetic elements associated with antimicrobial resistance. *Clinical Microbiology Reviews*, 31(4). <https://doi.org/10.1128/CMR.00088-17>
- Prestinaci, F., Pezzotti, P., & Pantosti, A. (2015). Antimicrobial resistance: A global multifaceted phenomenon. *Pathogens and Global Health*, 109(7), 309–318. <https://doi.org/10.1179/2047773215Y.0000000030>
- Quince, C., Walker, A. W., Simpson, J. T., Loman, N. J., & Segata, N. (2017). Shotgun metagenomics, from sampling to analysis. *Nature Biotechnology*, 35, 833–844. <https://doi.org/10.1038/nbt.3935>
- Rizzo, L., Manaia, C., Merlin, C., Schwartz, T., Dagot, C., Ploy, M. C., Michael, I., & Fatta-Kassinos, D. (2013). Urban wastewater treatment plants as hotspots for antibiotic resistance. *Water Research*, 47(3), 957–995. <https://doi.org/10.1016/j.watres.2012.11.027>
- Shamim, K., Mujawar, S. Y., & Mutnale, M. (2019). Metagenomics: A modern approach to reveal the secrets of unculturable microbes. In S. N. Meena & M. M. Naik (Eds.), *Advances in Biological Science Research: A Practical Approach* (pp. 177–195). Academic Press. <https://doi.org/10.1016/B978-0-12-817497-5.00012-4>
- Siguié, P., Gournayre, E., & Chandler, M. (2014). Bacterial insertion sequences: Their genomic impact and diversity. *FEMS Microbiology Reviews*, 38(5), 865–891. <https://doi.org/10.1111/1574-6976.12067>
- Sims, N., & Kasprzyk-Hordern, B. (2020). Future perspectives of wastewater-based epidemiology. *Environment International*, 139, 105689. <https://doi.org/10.1016/j.envint.2020.105689>
- Springer, Ontario ASP Landscape Study. (2024). Antimicrobial stewardship funding and resourcing impact on broad-spectrum antibiotic use. *Antimicrobial Stewardship & Healthcare Epidemiology*, Article 461. <https://doi.org/10.1017/ash.2024.461>
- Systematic review of wastewater surveillance. (2023). Wastewater surveillance of antibiotic-resistant bacterial pathogens: A systematic review. *Frontiers in Microbiology*, 13, 977106. <https://doi.org/10.3389/fmicb.2022.977106>

- Tadesse, B. T., Ashley, E. A., Ongarello, S., Havumaki, J., Wijegoonewardena, M., González, I. J., & Dittrich, S. (2017). Antimicrobial resistance in Africa: A systematic review. *BMC Infectious Diseases*, 17, 616. <https://doi.org/10.1186/s12879-017-2713-1>
- Talat, A., Bashir, Y., Khalil, N., Brown, C. L., Gupta, D., & Khan, A. U. (2025). Antimicrobial resistance transmission in environmental settings through traditional and UV-enabled advanced wastewater treatment plants: A metagenomic insight. *Environmental Microbiome*, 20, 27. <https://doi.org/10.1186/s40793-024-00658-2>
- von Wintersdorff, C. J. H., Penders, J., van Niekerk, J. M., Mills, N. D., Majumder, S., van Alphen, L. B., Savelkoul, P. H. M., & Wolffs, P. F. G. (2016). Dissemination of antimicrobial resistance in microbial ecosystems through horizontal gene transfer. *Frontiers in Microbiology*, 7, 173. <https://doi.org/10.3389/fmicb.2016.00173>
- Werkneh, A. A., & Islam, M. A. (2023). Post-treatment disinfection technologies for sustainable removal of antibiotic residues and antimicrobial-resistant bacteria from hospital wastewater. *Heliyon*, 9(4), e15360. <https://doi.org/10.1016/j.heliyon.2023.e15360>
- World Health Organization. (2015). Global Action Plan on Antimicrobial Resistance. WHO Press. <https://www.who.int/publications/i/item/9789241509763>
- Yang, Y., Li, B., Ju, F., & Zhang, T. (2013). Exploring variation of antibiotic resistance genes in activated sludge over a four-year period through metagenomic analysis. *Environmental Science & Technology*, 47(18), 10197–10205. <https://doi.org/10.1021/es4017365>
- Zhang, S., Huang, J., Zhao, Z., Cao, Y., & Li, B. (2020). Hospital wastewater as a reservoir for antibiotic resistance genes: A meta-analysis. *Frontiers in Public Health*, 8, 574968. <https://doi.org/10.3389/fpubh.2020.574968>