Pakistan Journal of Medical & Cardiological Review
https://pakjmcr.com/index.php/1/about
Online ISSN 3007-2387
Print ISSN 3007-2379

Genomic Surveillance and Epidemiological Modeling of Antimicrobial Resistance (AMR) in Urban Hospital Effluents

Muhammad Arshad Javed
Medical Laboratory Technologist, Department of Laboratory Medicine Purelab, Saqr Hospital United Arab Emirates Email: stap.aureus77@gmail.com
Muhammad Saeed
Medical Laboratory Technologist, Department of Laboratory Medicine Purelab, Fujairah Hospital United Arab Emirates
Email: saeed.06muhammad@gmail.com
Imran Zeb
Medical laboratory Technologist, Department of Laboratory Medicine Purelab, Fujairah Hospital United Arab Emirates Email: imranzeb1991@gmail.com
Dr. Afsheen Aqeel*
Department Of Microbiology, University Of Karachi Email: afsheenaqeel@gmail.com
Lawang Gurganari
Provisional Institute Of Teacher Education Email: Lawang.uob86@gmail.com
Sajida Parveen LMO
Shakh Zaid Hospital Quetta Email: Sajadamari@gmail.com

Author Details

Keywords: Antimicrobial Resistance, Hospital Wastewater, Genomic Surveillance, Whole-Genome Sequencing, Metagenomics, Epidemiological Modeling, Multi-Drug-Resistant Pathogens, Mobile Genetic Elements, One Health, Wastewater-Based Epidemiology, Carbapenem Resistance, Plasmid-Mediated Resistance

Received on 15 Mar 2026
Accepted on 10 Apr 2026
Published on 20 Apr 2026

Corresponding E-mail & Author*:

Dr. Afsheen Aqeel*
Department of microbiology, University of Karachi Email: afsheenaqeel@gmail.com

Abstract

Antimicrobial resistance (AMR) constitutes one of the most pressing global health crises, with hospital wastewater (HWW) serving as a critical environmental hotspot for the emergence, selection, and dissemination of multi-drug resistant (MDR) pathogens. This review synthesizes recent advances in genomic surveillance and epidemiological modeling of AMR in urban hospital effluents, focusing on high-income and low- and middle-income settings. Whole-genome sequencing (WGS) and metagenomic approaches reveal high abundances of clinically relevant resistance genes (blaNDM, blaOXA-48, mcr-1, qnrS) and mobile genetic elements (integrons, transposons, plasmids) in effluents, often mirroring patient case rates and mirroring hospital antibiotic usage patterns. Longitudinal sampling combined with phylogenetic analysis demonstrates clonal transmission of MDR Enterobacterales (e.g., carbapenem-resistant Klebsiella pneumoniae, ESBL-producing E. coli) from hospitals to downstream urban waterways and agricultural irrigation systems. Epidemiological models integrating compartmental SIR frameworks, agent-based simulations, and One Health network analysis quantify transmission risks, estimate secondary infection burdens, and evaluate intervention efficacy (advanced wastewater treatment, antibiotic stewardship). Key findings highlight the disproportionate contribution of

HWW to regional resistome expansion and underscore the urgent need for routine genomic surveillance, point-of-care diagnostics, and integrated wastewater-based epidemiology to inform national action plans and interrupt AMR dissemination cycles.

Introduction

The emergence and proliferation of antimicrobial resistance (AMR) have reached a critical threshold, positioning it as one of the most significant global health threats of the twenty-first century (Ferrara et al., 2024). Projections indicate that by 2050, AMR could result in approximately 10 million deaths annually, with a disproportionate burden falling on low- and middle-income countries (World Bank, 2017). In 2019 alone, bacterial AMR was associated with an estimated 4.95 million deaths, with 1.27 million deaths directly attributable to resistant pathogens (World Health Organization, 2025). Within this landscape, hospital wastewater (HWW) has been identified as a primary hotspot a complex environmental reservoir where high densities of antibiotic residues, patient-derived microbiota, and diverse genetic elements converge to facilitate the evolution and dissemination of multi-drug resistant (MDR) organisms (Hounmanou et al., 2025). Figure 1 illustrates the conceptual pathways through which antimicrobial resistance originating from hospital effluents disseminates into urban environmental systems.

Figure 1: Conceptual Pathway of Antimicrobial Resistance Dissemination from Hospital Effluents to the Urban Environment

The Ecological and Clinical Dimensions of Hospital Effluents

Hospital effluents are qualitatively and quantitatively distinct from municipal wastewater streams. While they typically represent less than 2% of the total volume of wastewater entering municipal treatment plants, their impact on the urban resistome is disproportionately high (Barchitta & Agodi, 2025). These effluents act as a concentrated "snapshot" of the clinical environment, reflecting the high-intensity use of antimicrobials and the high prevalence of nosocomial infections within the facility (Perry et al., 2021).

The physical and chemical composition of hospital wastewater creates a unique selective environment. It contains partially metabolized antibiotics, disinfectants, heavy metals, and other pharmaceuticals that exert continuous selective pressure on microbial communities (Zhang et al., 2020). This environment promotes the survival of resistant strains and facilitates horizontal gene transfer (HGT), allowing resistance determinants to move between pathogenic and commensal bacteria (Yan et al., 2020).

Characterization of High-Risk Pathogens and Resistance Profiles

The microbial profile of hospital effluents is dominated by clinically significant pathogens, particularly the "ESKAPE" group (*Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species). Surveillance data consistently shows that hospital wastewater contains significantly higher concentrations of these pathogens compared to urban influent (Rozman et al., 2020; Gowda & Aruna, 2025).

Table 1: Characterization of High-Risk Pathogens and Resistance Profiles in Hospital Wastewater

Pathogen Group	Resistance Mechanisms in HWW	Observed Prevalence/Abundance
ESBL Enterobacteriaceae	*blaCTX-M*, *blaSHV*, *blaTEM*	Up to 10^7 CFU/mL (Chagas et al., 2020)
CPE	*blaNDM*, *blaKPC*, *blaVIM*, *blaOXA-48*	Up to 10^5 CFU/mL (Chagas et al., 2020)
VRE	*vanA* gene cluster	87% prevalence in some HWW (Talebi et al., 2008)
MRSA	*mecA* gene	Significant presence in untreated HWW (Amin et al., 2025)
Colistin-resistant strains	*mcr-1* and chromosomal mutations	Emergent in pediatric hospital effluents (Lima study, 2024)

The presence of carbapenemase-producing organisms (CPOs) is particularly alarming, as carbapenems are often considered "last-resort" antibiotics. Research has identified high levels of OXA-48-type and VIM-positive isolates in hospital effluents, which can enter the municipal sewage system and eventually surface waters if not properly treated (Chelaru et al., 2024). Similarly, in resource-poor settings, untreated healthcare wastewater has been shown to contain NDM and OXA-48 producers, posing a direct threat to local communities (Gray, 2022).

Genomic Surveillance Methodologies: From Culture to Metagenomics

The transition from traditional microbiology to advanced genomic surveillance has revolutionized the ability to track AMR in hospital effluents. While culture-based methods remain essential for phenotypic characterization, they are limited by the "great plate count anomaly," failing to detect the approximately 97% of environmental bacteria that are non-culturable under standard laboratory conditions (Suleiman, 2025).

High-Throughput Quantitative PCR (HT-qPCR)

HT-qPCR has emerged as a robust tool for the rapid, sensitive quantification of a broad range of known antimicrobial resistance genes (ARGs). This technology allows for the simultaneous screening of hundreds of targets, providing absolute or relative abundance data that can be used to compare different wastewater sources (Knight et al., 2024).

A comparative study in Wales utilized HT-qPCR targeting 73 ARGs to monitor 47 wastewater treatment plants (WWTPs) and hospital discharge (Silvester et al., 2025). The results highlighted that HT-qPCR is superior for detecting low-abundance genes that are clinically significant, such as certain carbapenemases that might be missed by metagenomic sequencing at standard depths. However, its dependency on pre-designed primers means it cannot identify novel or divergent resistance genes (Zhao et al., 2026).

Shotgun Metagenomics and Metatranscriptomics

Shotgun metagenomics provides a non-targeted, comprehensive view of the entire microbial community (the microbiome) and its total collection of resistance genes (the resistome) (Shay et al., 2023). This approach is particularly valuable for identifying the genetic context of ARGs, such as whether they are located on chromosomes or mobile genetic elements (MGEs) like plasmids, integrons, and transposons (Manoharan et al., 2021).

Recent advances in "genome-resolved metagenomics" allow for the reconstruction of Metagenome-Assembled Genomes (MAGs) directly from wastewater samples. In Wales, researchers recovered nearly 4,000 MAGs from national wastewater surveillance, revealing that 13.6% of these genomes carried one or more ARGs (Silvester et al., 2025). This technique has successfully identified "microbial dark matter" previously uncharacterized bacterial hosts carrying high-concern genes like *blaNDM-5* (Hudson et al., 2018).

Metatranscriptomics adds another layer of resolution by sequencing the total RNA in a sample. This allows researchers to identify which ARGs are actively being expressed in the wastewater environment, providing insights into the functional resistance activity of the microbial community (Kumar & Yadav, 2024). For instance, metatranscriptomic analysis of hospital wastewater has revealed high abundances of transcripts for sulfonamide resistance (*sul1*, *sul2*) and clinically relevant beta-lactamases like *blaNDM-1* (Musundi et al., 2024).

Epidemiological Modeling of Resistance Dynamics

Modeling the transmission and persistence of AMR in urban hospital effluents requires the integration of diverse datasets, including clinical usage patterns, environmental factors, and bacterial population dynamics (Patra et al., 2025).

Mechanistic and Compartmental Models

Mechanistic models often employ compartmental frameworks (e.g., SIR models) to simulate the flow of resistance between different reservoirs. These models can incorporate "jumps" to account for the possibility of multiple infections from a single source or sudden changes in transmission dynamics (Jenner et al., 2020).

In hospital settings, mechanistic approaches can model the impact of clinical activity such as antimicrobial usage (AMU) and patient length of stay on the abundance of ARGs in wastewater (La Rosa et al., 2025). While some studies show that total AMU does not always correlate linearly with total ARG abundance, specific associations have been found. For example, increased use of carbapenems and vancomycin in hospital wards has been positively associated with higher counts of *bla* and *vanA* genes in the corresponding effluents (Alam et al., 2021).

Stochastic Modeling and Monte Carlo Simulations

Stochastic models are increasingly favored for studying wastewater dynamics because they account for the inherent randomness of bacterial shedding and sampling. These models are particularly useful for small populations, such as specific hospital wards, where deterministic assumptions of uniform mixing do not hold (Martin et al., 2023).

A key application of stochastic modeling in wastewater-based epidemiology (WBE) is the optimization of sampling protocols. By using Monte Carlo simulations, researchers can estimate the probability of detecting a pathogen given variation in flow rates, defecation distributions, and shedding intensities (Rallapalli et al., 2021).

Machine Learning and Computational Biosurveillance

The integration of artificial intelligence (AI) and machine learning (ML) into AMR surveillance represents a paradigm shift in how environmental and genomic data are utilized for risk assessment (Pennisi et al., 2025). As illustrated in Figure 6, genomic data derived from wastewater samples can be processed through computational models to forecast emerging resistance threats.

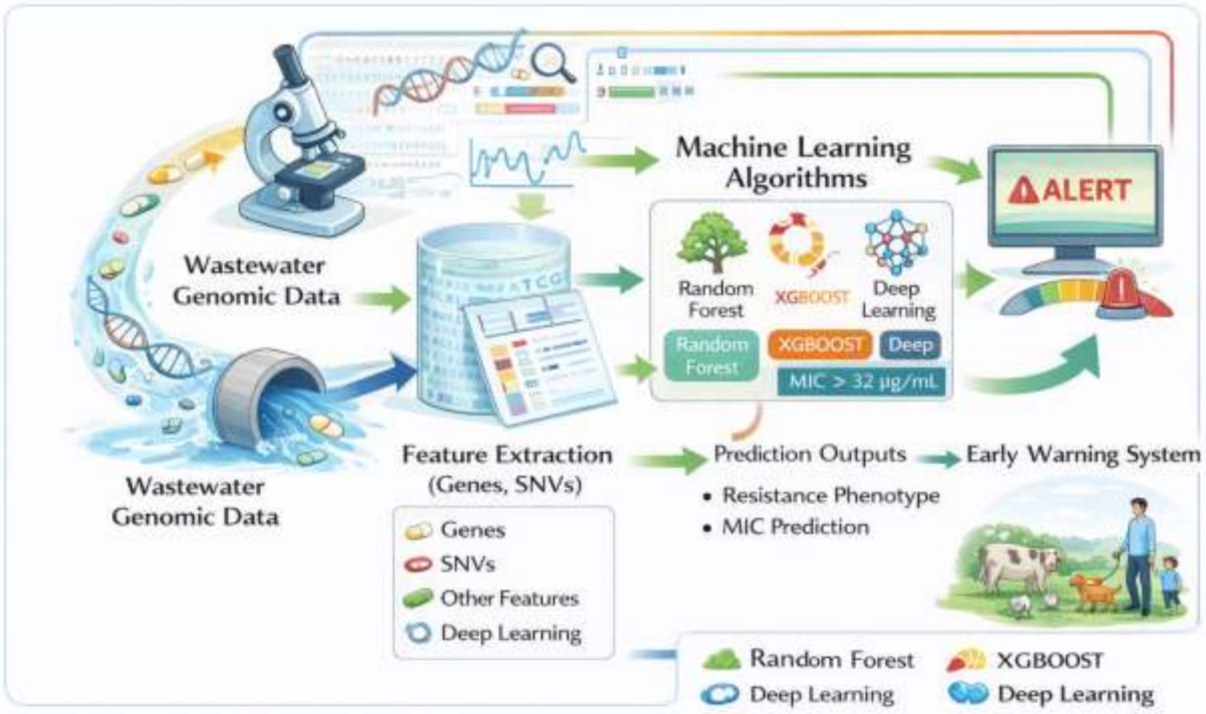

Figure 2: Machine Learning-Driven Biosurveillance Pipeline for Predicting Antimicrobial Resistance

Predictive Modeling for Resistance Phenotypes

Supervised machine learning algorithms (e.g., Random Forest, XGBoost) are increasingly used to predict antibiotic resistance phenotypes directly from genomic sequences. These models treat genes or single nucleotide variants (SNVs) as "features" to predict outcomes like the Minimum Inhibitory Concentration (MIC) of a pathogen (Kim et al., 2022). For instance, deep learning techniques have been applied to large datasets of *Mycobacterium tuberculosis* isolates to predict multi-drug resistance with high sensitivity (96.3%) (Liu et al., 2025).

In clinical settings, ML models trained on electronic medical records (EMRs) and previous susceptibility data have been implemented in ICUs to assist clinicians in choosing appropriate empiric therapies (Ferrari et al., 2024). Extending these models to wastewater data allows for population-level predictions of resistance trends, providing an early warning system for the emergence of new threats (Rojas et al., 2018).

The GRUMB Workflow and Simulation-Driven Surveillance

The Genome-Resolved Urban Microbiome Biosurveillance (GRUMB) workflow is a cutting-edge framework that integrates metagenomics with simulation-driven machine learning (Suleiman, 2025).

The GRUMB framework uses synthetic blending simulations to model "source-sink" dynamics, identifying how microbial communities change when hospital sewage (the donor) enters the municipal network (the sink) (Aminu et al., 2025). This allows for the identification of "tipping zones" thresholds of contamination at which the environmental identity of a site is compromised by clinical pathogens (Marwal et al., 2025).

Regional Case Studies and Environmental Impact

The dissemination of AMR from hospital effluents into the urban environment has been documented globally, revealing common patterns of spread and site-specific challenges (Nazir et al., 2025).

Regional Case Studies: Uruguay and Peru

In Montevideo, researchers combined metagenomic surveillance of urban beaches and sewage with an epidemiological investigation of a carbapenem-resistant outbreak in a major public hospital (Salazar et al., 2022). The study revealed that patient-derived bacteria and MDR clones were directly incorporated into the urban environment through the sewage system. This highlights the role of the "built environment" and inadequate infrastructure in facilitating the spread of nosocomial pathogens to public recreational areas (Martínez-Ruiz et al., 2026).

A genomic study in Lima compared *E. coli* and *Klebsiella* isolates from hospital wastewater and pediatric patients. The research found that MDR phenotypes were more prevalent among wastewater isolates (73.5%) than clinical isolates (56.8%), suggesting that hospital sewage serves as a reservoir for highly resistant strains (Perry et al., 2021).

Environmental Sentinels and Novel Genes

In Panama, surveillance of the Juan Díaz River showed that plasmid-associated resistance was more common in river isolates, while chromosomal integration was more frequent in wastewater (Hounmanou et al., 2025). In Spain, researchers used white storks as "environmental sentinels," discovering they carried the same high-risk ESBL and CP clones found in clinical settings (Yan et al., 2020).

Even in Norway a country with relatively low levels of clinical antibiotic use hospital effluents were identified as sources of significant genomic diversity. Researchers identified 1,130 unique ARGs, including 349 "novel" genes that were previously unknown (Alam et al., 2021). These genes were found to persist even after treatment at a municipal plant, demonstrating that hospitals can introduce new resistance mechanisms into the environment regardless of the local resistance burden (Victor et al., 2025).

Mitigation and Technological Interventions

Addressing the discharge of ARGs and ARB from hospital effluents requires a transition from conventional wastewater treatment to specialized technologies. Traditional municipal WWTPs are largely ineffective at eliminating the genetic burden of AMR (Barchitta & Agodi, 2025).

Table 2: Comparison of On-site Wastewater Treatment Technologies for AMR Removal

Technology	Mechanism of AMR Removal	Demonstrated Efficacy
Ultrafiltration (UF)	Physical size exclusion (20-25 nm pores)	Up to 6 log reduction of ARGs
Ozonation	DNA degradation through oxidation	High reduction of micro-pollutants
MBR	Biological and physical removal	Enhanced antibiotic residue removal

Ultrafiltration has been shown to be particularly effective, providing constant removal of pathogenic bacteria and ARGs regardless of their initial concentration. However, a significant drawback is the accumulation of resistant material in the back-flush retentate, which requires specialized handling to prevent secondary contamination (Chelaru et al., 2024).

Global Policy and Regulatory Frameworks

The recognition of hospital wastewater as a major conduit for AMR spread has prompted a shift in global policy toward a "One Health" approach (Al-Khalaifah et al., 2025).

WHO and Global Action Plans

The updated draft of the WHO Global Action Plan identifies environmental discharges from healthcare facilities as a primary driver of resistance development (WHO, 2025). The plan establishes core goals for 2030, including a 10% reduction in bacterial AMR-associated human deaths and the minimization of environmental pollution from AMR microbes and antimicrobial residues. It calls for the integration of environmental interventions into national AMR responses and investment in WASH infrastructure (Anderson et al., 2019).

EU Urban Wastewater Treatment Directive (2024)

Europe has taken a proactive stance with the 2024 update to the Urban Wastewater Treatment Directive (UWWTD), which mandates Member States to monitor AMR in urban wastewater (European Commission, 2024). This regulation requires reporting by 2030 and necessitates the development of standardized methodologies for sampling and analysis. Such mandates are expected to drive the adoption of advanced treatment for hospital effluents across the EU, setting a global precedent for environmental AMR regulation (European Environment Agency, 2025).

Conclusion

Hospital effluents represent a major but under-regulated conduit for the environmental dissemination of AMR, functioning as both a reservoir and amplifier of clinically critical resistance determinants that threaten community and ecosystem health. Genomic surveillance has matured into a powerful tool for real-time tracking of resistance emergence, clonal spread, and gene mobility, while epidemiological modeling provides quantitative foresight into transmission dynamics and the potential impact of control measures. The convergence of these approaches reveals that conventional wastewater treatment is insufficient against the selective pressure exerted by residual antibiotics and high pathogen loads in HWW. Achieving meaningful containment requires a multi-layered strategy: mandatory advanced treatment technologies (e.g., ozonation, membrane bioreactors), hospital-level antibiotic stewardship programs, routine WGS-based surveillance integrated into national AMR action plans, and international alignment on wastewater discharge standards. Without decisive implementation of these measures, the urban hospital–environment–community interface will continue to fuel the global AMR crisis, undermining the efficacy of last-resort antibiotics and jeopardizing progress toward the Sustainable Development Goals. Coordinated, evidence-driven action across clinical, environmental, and policy domains remains the most viable path to preserving antimicrobial effectiveness for future generations.

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