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Recent Advances in Hospital Wastewater Treatment Technologies for Controlling Antibiotic Resistance: A Systematic Review

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ABSTRACT

Introduction: Hospital wastewater is a key source of antibiotic-resistant bacteria and genes, driving the spread of environmental antimicrobial resistance (AMR). This review assessed the effectiveness of advanced treatment technologies in removing these resistance determinants from wastewater.

Materials and Methods: This study adhered to the PRISMA guidelines and the PECOS framework. A comprehensive systematic search of six major databases (PubMed, Scopus, Web of Science, Google Scholar, ProQuest, and ScienceDirect) was conducted between April and June 2025. Of the 412 records initially identified, 89 studies met the predefined inclusion criteria. Relevant data on treatment technologies, antibiotic-resistant bacteria (ARB), resistance gene (ARG), and geographical–economic contexts were extracted and synthesized qualitatively.

Results: Conventional treatment methods (e.g., activated sludge and chlorination) fail to fully remove ARB/ARGs and may even promote horizontal gene transfer via oxidative stress. In contrast, advanced technologies, such as MBR, advanced oxidation process (AOPs), and hybrid systems (MBR+ozone, MBR+GAC), achieve much higher removal efficiencies (>95%). Numerous critical ARGs (blaNDM, blaCTX-M, sul1, tetM, mcr-1, and vanA) have been detected in major pathogens (E. coli, P. aeruginosa, and Enterococcus spp.) in hospital wastewater worldwide. Research is largely focused on China, India, and Europe, while neglecting sludge and biofilms as important secondary reservoirs of ARGs, limiting accurate risk assessment.

Conclusion: Effective AMR control in hospital wastewater requires integrated treatment technologies, molecular monitoring, and a One Health approach. Smart, sustainable solutions are essential to reduce risks to public health and ecosystems.

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Introduction

Hospital wastewater provides a suitable environment for the proliferation of pathogenic bacteria, and its discharge into the environment can pose a significant threat to human health [1]. This problem is exacerbated by the lack of national regulations specifying limits for hospital wastewater discharge [2]. Wastewater from hospitals and other

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healthcare facilities contains a variety of microorganisms originating from patients, as well as antimicrobial agents used for the treatment of diseases [3, 4]. Currently, antibiotics are employed as potent drugs for common diseases, significantly contributing to the inhibition or suppression of bacterial growth. Nevertheless, the use and disposal of antibiotics in the environment have attracted considerable attention owing to the emergence of various antibiotic resistances. Approximately 30%–90% of antibiotics are not absorbed by the human body; instead, they are directly discharged into wastewater and subsequently accumulate in sewage treatment plants [5]. The consumption of antibiotics is rapidly increasing each year, and it is estimated that by 2030, antibiotic use will have risen by 200% [6]. Antibiotics are antimicrobial agents that can kill or inhibit the growth and proliferation of bacteria [7]. Antibiotics have revolutionized the field of medicine, and their increased use has exerted selective pressure on susceptible bacteria, favoring the survival of ARB and the proliferation of ARGs [8]. This situation creates a suitable environment for the interaction between bacteria and antibiotics, facilitating the spread of antibiotic resistance [9]. Numerous reports have confirmed that hospitals continue to release waste containing untreated or inadequately treated antibiotics into aquatic environments [10]. Without proper treatment, residual antibiotics can reach surface water, groundwater, sediments, and other compartments, affecting aquatic life and increasing risks to human health [11] .

The dynamic process of AMR evolution and emergence represents a growing concern for global public health. Research indicates that addressing the AMR problem requires a multifaceted approach, including an understanding of its evolution and dissemination in the environment [12]. Antibiotic resistance is an adaptive genetic trait exhibited or acquired by certain bacterial subpopulations, enabling them to survive and grow even when exposed to therapeutic doses of an antibiotic that would normally kill or inhibit them [13]. Water systems connect hospitals, communities, industries, and livestock and agricultural farms, through which waste generated in wastewater treatment plants (WWTPs) is collected. They are recognized as critical hotspots for the emergence of ARB, ARGs, and mobile genetic elements (MGEs), as the discharge of residual antibiotics into these treatment plants can enhance selective pressure [14, 15]. One of the sources of antibiotics in the environment is hospital wastewater. Multiple factors influence hospital wastewater generation, including water supply, bed availability, public services such as air conditioning, kitchens, and laundries, the types and numbers of units or departments, and management practices. All of these processes collectively affect the total volume of wastewater produced[16]. Hospitals are intensive consumers of antimicrobial agents and contribute significantly to the burden of AMR. Although antimicrobial use within hospitals can be monitored, its use in the broader community remains largely uncontrolled, posing challenges for tracking resistance trends [17]. Conventional wastewater treatment facilities are designed to remove contaminants such as total organic carbon and nutrients, including nitrate and phosphate. They are not specifically intended for the removal of micropollutants such as antibiotics and ARGs [18]. Consequently, significant amounts of antibiotics and ARGs are released into aquatic environments [19, 20]. Humans can acquire resistant bacteria through contaminated food and water, infected animals-via direct contact or consumption of meat or milk—contact with infected individuals, and the use of manure as fertilizer[21-25]. Even if antibiotic-resistant bacteria are damaged or eradicated during wastewater treatment, ARGs may still be discharged into the environment and transferred to other bacteria. Previous studies have indicated that ARGs remain abundant in wastewater treatment wetlands and municipal wastewater even after treatment [26]. Despite the growing body of literature on antimicrobial resistance in hospital wastewater, important gaps remain regarding the comparative evaluation of treatment technologies, their removal performance for ARB and ARGs, and their applicability across different geographical and operational settings.

This systematic review aimed to analyze and evaluate recent advancements in hospital

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wastewater treatment technologies, with a particular focus on their role in mitigating and controlling AMR. This study seeks to identify novel and effective solutions, highlighting the existing challenges and opportunities in this field, thereby contributing significantly to improving environmental health and reducing the threats posed by antibiotic resistance.

Materials and Methods

a) Study Design

This systematic review following the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines to ensure transparency, accuracy, and reproducibility. The specific study objectives included identifying, classifying, and evaluating the efficiency of treatment technologies (including physical, chemical, and biological) specifically designed for the removal or inactivation of antibiotic resistance determinants from hospital wastewater; analyzing the application of these technologies across different geographical and economic contexts; and identifying knowledge gaps and future research areas in this field. This systematic review included only original research articles, encompassing lab-scale, pilot-scale, and full-scale studies, while excluding review articles, case reports, books, and theoretical papers. Although a formal protocol was not registered in prospective databases such as PROSPERO, the review followed a pre-defined internal protocol to ensure methodological consistency.

b) Search Strategy

This study was performed in accordance with the PRISMA guidelines. A systematic literature search was conducted from April 9 to June 21, 2025, across six reputable scientific databases: PubMed, Scopus, Web of Science, Google Scholar, ProQuest, and Science Direct. All studies published between 2018 and 2025 were considered for inclusion. The keywords included ('Hospital wastewater' OR 'Hospital effluent' OR 'Healthcare wastewater' OR 'Clinical Wastewater') AND ('Treatment' OR 'Removal' OR 'Elimination' OR 'Degradation' OR 'Inactivation' OR 'Technology') AND ('Antibiotic Resistance' OR 'Antimicrobial Resistance' OR 'AMR' OR 'Antibiotic Resistance Bacteria' OR 'Antibiotic Resistance Genes' OR ARGs OR 'Mobile Genetic Elements'). The search was performed within the article titles, abstracts, and keywords. To ensure a comprehensive search, a combination of Medical Subject Headings (MeSH) and free-text keywords was used. The search strings were tailored to the specific requirements of each database. The core search components included:

- **Component 1 (Setting):** “Hospital wastewater”, “Hospital effluent”, “Healthcare wastewater”, “Clinical wastewater”.
- **Component 2 (Process):** “Treatment”, “Removal”, “Elimination”, “Degradation”, “Inactivation”, “Technology”.
- **Component 3 (Target):** “Antibiotic Resistance”, “Antimicrobial Resistance”, “AMR”, “Antibiotic Resistance Bacteria”, “Antibiotic Resistance Genes”, “ARGs”, “Mobile Genetic Elements”.

An example of the full search syntax used in PubMed is as follows

(“Hospital wastewater” [Mesh] OR “Hospital effluent” [tiab] OR “Healthcare wastewater” [tiab]) AND (“Waste Water Management” [Mesh] OR “Treatment” [tiab] OR “Removal” [tiab] OR “Technology” [tiab]) AND (“Antimicrobial Resistance” [Mesh] OR “Antibiotic Resistance Bacteria” [tiab] OR “Antibiotic Resistance Genes” [tiab]).

c) Eligibility Criteria

The inclusion and exclusion criteria for the studies in this systematic review were defined using the PECOS (Participants, Exposure, Comparison, Outcome, Study Design) framework. The inclusion criteria were as follows

- **Participants:** Hospital wastewater (or effluent from treatment plants receiving a significant share of hospital wastewater).
- **Exposure:** Application of a treatment technology or process (physical, chemical, biological, or combined) to remove or reduce antimicrobial resistance factors.

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- **Comparison:** Comparison of the treatment system performance (against influent quality, an alternative treatment technology, or a baseline scenario; however, this was not mandatory).
- **Outcome:** Quantitative measurement of efficiency focusing on indicators such as antibiotic-resistant bacteria (ARB), antibiotic genes (ARGs), and residual antibiotic concentrations.
- **Study Design:** Primary studies, including laboratory-, pilot-, and full-scale studies.

The **exclusion criteria** comprised:

- Studies that focus specifically on municipal wastewater (without considering the hospital share) or other wastewater types (e.g., industrial or agricultural).
- Studies that did not address the removal efficiency of ARB/ARGs or antibiotics.
- Articles for which full texts were unavailable.
- Articles not published in English were excluded.
- Review articles, books, case reports, theses, dissertations, and conference abstracts (to maintain data quality and consistency).
- Studies that only addressed conventional quality indicators (e.g., BOD and COD) and did not directly evaluate outcomes related to antibiotic resistance.

d) Study Selection Process

As illustrated in the PRISMA flow diagram (Figure 1), the initial search yielded 412 records. After removing duplicate entries (n = 250), 162 unique records were screened based on the title and abstract. Of these, 57 records were excluded because they did not meet the primary inclusion criteria. The remaining 105 full-text articles were independently assessed for eligibility, leading to the exclusion of 16 studies because they were irrelevant or duplicates. Finally, 89 studies were included in the qualitative synthesis

e) Data Extraction

Following the final study selection, data extraction was carried out systematically and compiled into a table with six columns. These columns included: "Key Points" (for recording the main findings and message of the article), "Bacteria" (including the pathogens and resistant bacteria under investigation), "Gene" (for listing the identified antibiotic resistance genes, such as various beta-lactamases and genes conferring resistance to tetracycline and sulfonamide), "Treatment Process" (including the evaluated technologies, such as membrane bioreactors, ozonation, chlorination, and constructed wetlands), "Country" (the location where the study was conducted), and "Reference." This process was implemented to ensure the integrity, accuracy, and comparability of the data extracted from the collection of studies.

f) Quality Assessment of Included Studies

Due to the methodological diversity of the included studies (from field monitoring to pilot-scale experiments and treatment trials), key quality assessment criteria included clarity of objectives and hypotheses, description of methodology (sampling methods, molecular analysis, and sequencing methods), presentation of results (clear and quantitative data reporting), and discussion of limitations and relevance of findings.

g) Data Synthesis and Analysis

Given the heterogeneous nature of the included studies concerning the types of treatment technologies, operational scales (lab-scale, pilot-scale, and full-scale), and reported efficiency indicators, conducting a quantitative meta-analysis was not feasible. Therefore, data were synthesized and analyzed descriptively and qualitatively within the framework of a systematic review. The main findings were grouped based on treatment technologies such as physical processes, advanced oxidation and disinfection processes, biological processes (e.g., bioreactors and attached growth systems), and hybrid systems. The removal efficiency, comparative analysis, potential for full-scale application for each technology category, and identification of knowledge gaps and promising areas for future studies were discussed and compared.

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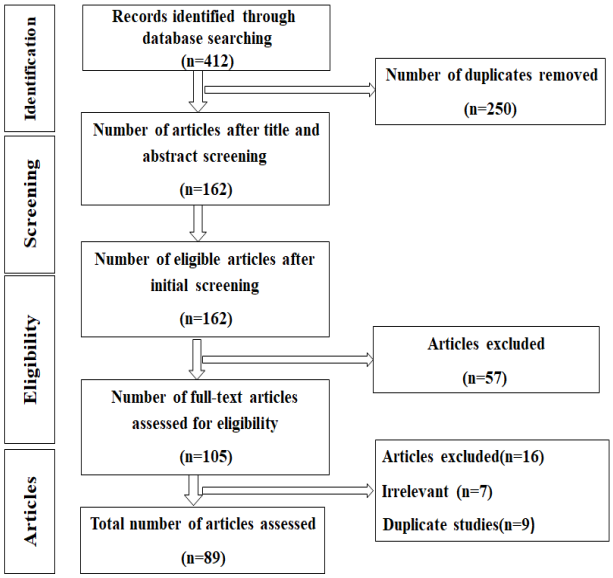

Figure 1: PRISMA flow diagram in this study.

Results

Figure 2 illustrates the temporal distribution of the studies reviewed from 2018 to 2025. The number of publications in the early years was relatively low and showed a marked increase starting in 2021, particularly in 2023 and 2024, which represent the periods of the highest research focus. This trend reflects the rapidly growing global attention to antibiotic resistance in hospital wastewater, highlighting the increasing emphasis on advanced treatment technologies and effective wastewater management to mitigate the dissemination of ARB and ARGs in recent years.

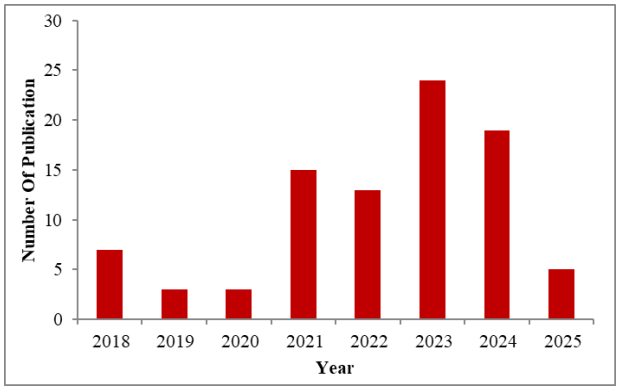

Figure 2: Number of studies reviewed on antibiotic resistance in hospital wastewater over time.

Table synthesizes key international studies on the removal of ARB and ARGs in hospital wastewater, underscoring the necessity of specialized and modern treatment technologies to mitigate environmental and public health risks. Evidence consistently shows that conventional methods, including chlorination and activated sludge, are inadequate for eliminating resistant agents and may even intensify the horizontal transfer of ARGs, as demonstrated in studies from Thailand and the United States (2,5,74,79). In contrast, advanced and integrated processes, such as MBRs, MBR-granular activated carbon (GAC) systems, nanofiltration with electrochemical oxidation, ozonation, ultraviolet irradiation, and innovative techniques such as electro-peroxone, photocatalysis, and microbial fuel cells, exhibit markedly higher removal efficiencies. Reports of complete elimination of indicator bacteria such as Escherichia coli and Shigella by MBRs in Uganda further reinforce the effectiveness of these technologies (1). alongside successful reductions in antibiotics such as azithromycin through combined treatment approaches.

A wide range of clinically relevant resistance genes, including blaNDM, blaCTX-M, mecA, sul1, tetM, mcr-1, and vanA, has been identified in gram-positive and gram-negative bacteria, such as E. coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterococcus spp., and Staphylococcus aureus. Their prevalence in hospital wastewater, particularly in low- and middle-income regions, highlights the ecological risks associated with uncontrolled dissemination. Accurate detection of ARGs through metagenomics, sequencing, and molecular analyses is essential for selecting effective treatment technologies and understanding persistence patterns, as some resistant bacteria and genes may survive or even proliferate during treatment. This integrated knowledge base is crucial for minimizing the environmental spread of antimicrobial resistance and for guiding the development of robust wastewater management strategy.

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Table1: Summary results of the reviewed studies.

Ref.	Country	Treatment Process	Gene	Bacterium	Key points
27	Uganda (Kampala City)	MBR, GAC, Solar-powered hybrid system	–	Escherichia coli (E. coli), Shigella	The MBR process completely removed *E. coli* and *Shigella*.
28	Thailand	Activated sludge with chlorination	–	Escherichia coli, Acinetobacter spp, Staphylococcus aureus (MRSA) Vancomycin-resistant Enterococcus (VRE)	Ineffective chlorination in eliminating bacteria and facilitating resistance gene transfer
29	Singapore	Combined Nanofiltration and Electrochemical Oxidation	–	–	Effective removal of Azithromycin with Nanofiltration and Electrochemical Oxidation
30	United States of America	Conventional Sewage Treatment Plant with Chlorine Final Disinfection	16S rRNA gene used for microbial source tracking (MST) sequencing.	Bacillus cereus, Bacillus pumilus, Chryseobacterium indologenes	Bacteria resistant to Ceftazidime and Meropenem are not eliminated by chlorination
31	Burkina Faso	Lack of investigation of a specific process	–	–	
32	Japan	Lab-scale CAS system	ARGs No proper names mentioned	–	The risk of persistent antibiotics and the need to identify ARGs
33	China	Pilot-scale SBR wastewater treatment system	Antibiotic resistance genes (ARGs) and metal resistance genes (MRGs) AdeF	Candidatus Competibacter	Changes in ARGs and MRGs levels depend on the type of treatment process
34	India	-	Beta-lactam, CAMP, and vancomycin resistance genes	Enterococcus, Pseudomonas, and Vibrio	Hazardous pathogens in hospital wastewater and their association with resistance genes such as ESBL and carbapenem
35	Saudi Arabia	MBR	bla_TEM, bla_SHV, bla_CTX-M, bla_OXA-48, bla_NDM-1	Clinical Gram-negative species	Presence of ESBL and carbapenem genes in isolates
36	Portugal	Biological treatment with UV, ozone, and sand filter	bla_VIM, bla_OXA-48, bla_KPC	Gram-negative species	Incomplete removal of genes from the environment
37	United States of America	Lack of investigation of a specific process	ARGs: blaZ (85%), mecA; MRGs: cadD, cadX	S. aureus, S. warneri, S. delphini	Co-occurrence and non-conjugative transfer of ARGs and MRGs

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
38	Poland	Biological-mechanical treatment with disinfection	VanA, vanB, vanC1, vanC2/C3	Enterococcus faecalis, Enterococcus faecium, Enterococcus hirae, Enterococcus durans, Enterococcus gallinarum, Enterococcus casseliflavus, Enterococcus avium	vanA and vanB, the main vancomycin resistance genes
39	India	Lack of investigation of a specific process	BlaTEM, blaSHV, blaCTX-M, mecA	Proteus vulgaris	Bacterial antibiotic degradation and gene transfer
4	China	Chlorine dioxide disinfection, 8 h HRT	blaTEM-1, blaNDM-1, sul1, tetM, and the horizontal gene transfer markers intI1 and 16S rRNA	-	The genes blaTEM-1, blaNDM-1, sul1, tetM, and intI1 are associated with resistance and gene transfer
40	Czech Republic	Lack of investigation of a specific process	Beta-lactamase encoding genes	Pseudomonas aeruginosa	P. aeruginosa with high antibiotic resistance and gene transfer
41	China	Lack of investigation of a specific process	NDM-5 antibiotic resistance gene	Enterococcus faecalis and other Gram-positive and Gram-negative bacteria across 12 different phyla	NDM-5, horizontal gene transfer, and high resistance prevalence in wastewater
42	South Korea	Activated sludge with supplements such as ozone, UV, and advanced filtration	Multidrug resistance genes, macrolide-lincosamide-streptogramin, beta-lactam, bacitracin; mobile genetic elements (plasmids, transposons, phages)	-	Risk of horizontal gene transfer in hospital wastewater
43	Poland	Continuous photocatalytic system	Sulfonamide resistance genes	-	Resistance gene increase and SMX removal via photocatalyst
44	India	Vermifiltration	blaCTX-M, mecA, mcr-1	-	Reduction of resistant bacteria
45	Nepal	Conventional biological treatment	sul1, tet(B), qnrS, blaCTX-M, blaNDM-1, intI1	Escherichia coli, Klebsiella pneumonia, Enterobacteriaceae	Key resistance and gene transfer genes: sul1, tet(B), qnrS, blaCTX-M, blaNDM-1, intI1
17	Romania	Lack of investigation of a specific process	-	E. coli	High antibiotic resistance in hospital wastewater
46	India	Lack of investigation of a specific process	blaNDM-1, blaCTX-M, blaTEM, mecA, tet(A), sul1, qnrS, vanA	E. coli, Klebsiella spp, Pseudomonas aeruginosa, Acinetobacter spp, Enterococcus spp, Staphylococcus aureus	Presence of genes associated with major antibiotic resistance groups
47	South Korea	BNR[1] and AD[2] processes	tetX, TEM, sul1, and the 16S rRNA gene as normalizers	Klebsiella, Enterococcus	Changes in tetX, TEM, and sul1 genes and the role of 16S rRNA in resistance

[1] Biological Nutrient Removal
[2] Anaerobic Digestion

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
					spread
48	China	Lack of investigation of a specific process	A wide range of ARGs	Pseudomonas and Enterobacteriaceae	ARG diversity and microbial contribution to resistance spread
49	China	Chlorination and advanced oxidation processes (UV/H_2O_2 and Fenton)	Mcr, tet(X)	Pseudomonas aeruginosa and Acinetobacter baumannii	Presence of resistance genes mcr and tet(X) and multidrug-resistant bacteria in hospital wastewater
50	China	MFCs[1]	tetA, tetC, tetG, tetM, tetW, sul1, sul2, qnrS, blaTEM, blaCTX-M, intI1	Pseudomonas, Bacillus, Acinetobacter, Enterobacter, Escherichia coli	Risk of resistance gene spread via fuel cells
51	China	Metagenomics with alternative disinfection: ozone, UV, or advanced filtration	blaCTX-M, blaNDM, mcr-1, tetM, sul1, vanA	Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii	Multiple resistance genes in hospital pathogens
52	China	E-peroxone method and SBR[2]	QnrA, qnrB, qnrS, qnrD, aac(6')-Ib-cr, qepA	Escherichia coli, Klebsiella pneumonia, Pseudomonas aeruginosa	Spread of quinolone resistance in hospital pathogens (Klebsiella and Pseudomonas)
53	China	Lack of investigation of a specific process	sul1, tetO, ermB, intI1, Tn916/1545	Trichococcus, Candidatus campbellbacteria	Role of Trichococcus in resistance spread and Candidatus campbellbacteria in resistance suppression
51	China	Electro-peroxone with SBR reactor	tet(X) and other multidrug resistance genes	Escherichia coli, Pseudomonas aeruginosa, Acinetobacter baumannii	Quinolone resistance in multidrug-resistant hospital bacteria (E. coli, Pseudomonas, Acinetobacter)
54	Iran	NLCs loaded with eugenol	-	Staphylococcus aureus (mcr, standard and wild), Enterococcus faecalis (standard and wild), Escherichia coli (wild), Pseudomonas aeruginosa (wild)	Reduction of hospital bacterial growth with NLC-eugenol
55	Canada	MBR and EO systems	-	-	Efficient hospital wastewater treatment
56	Vietnam	Sponge-MBR and ozonation	-	-	Antibiotic removal by Sponge-MBR and ozone; complete TET removal, SUL persistence

[1] Microbial Fuel Cells
[2] Sequencing Batch Reactor

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
57	India	SAFF[1] Reactor coupled with Tube Settler	-	-	Focus on removal of COD, BOD_5, nitrate, and phosphate
58	China (Ningbo city)	Aerobic + Sedimentation + Chlorination; Anaerobic + MBR + UV; Aerobic + Anaerobic + MBR + UV	-	-	Human enteric viruses: incomplete removal by chlorination, effective removal with MBR+UV
59	United States	Preliminary + Activated sludge + Secondary clarification + UV	Genes conferring resistance to ampicillin, ciprofloxacin, doxycycline, and sulfamethoxazole	-	Antibiotic reduction and detection of resistant bacteria
60	Germany and Denmark	Treatment with MBR, ozone, granular activated carbon filtration, and UV disinfection	-	--	Hormonal activity reduction with MBR, ozone, and GAC
61	Portugal	-	BlaTEM, blaSHV, blaCTX-M, blaCMY, mecA, vanA, mcr-1	-	Highest prevalence of blaTEM, lowest mecA and mcr-1 in hospital wastewater
62	Japan	Advanced ozonation methods (O_3, O_3/H_2O_2, O_3/UV, O_3/UV/H_2O_2)	Resistance genes to β-lactam, carbapenem, and tetracycline	E. coli, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus pneumoniae	Presence of critical hospital-resistant bacteria (CREC, CRPA, MRSA, PRSP)
63	Turkey	SCWO[2]	-	-	Over 90% removal of pharmaceutical pollutants using SCWO technology
64	India	chlorination	CTX-M, blaCTX-M-15, CTX-M Group 1, TEM, SHV, Class 1 integron, Dfr, Aad, plasmids, ICEs, transposons, IS, MITEs	Escherichia coli	Indicator E. coli with the most common ESBL gene (CTX-M) and resistance gene transfer via mobile elements

[1] Submerged Aerobic Fixed Film
[2] Supercritical Water Oxidation

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
65	Sweden	Ozonation	-	ESBL-producing Enterobacteriaceae	ESBL-producing Enterobacteriaceae in hospital wastewater; minimal population reduction after ozonation
66	Tanzania	Constructed Wetland	Sul1, Sul2, blaTEM, blaSHV, blaCTX-M, 16S rRNA	Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa	Prevalence of Sul and β-lactam resistance genes in hospital bacteria; highest in Klebsiella, lowest in E. coli
67	China	-	bacA, tetA, tetB, tetM, aph(3')-IIIa, aac(6')-Ib, ant(2'')-Ia, sul1, sul2	Arcobacter, Aeromonas, Enterococcus, Acinetobacter, Acidovorax	Prevalence of multiple resistance genes in hospital bacteria with key roles of Arcobacter, Aeromonas, and Acinetobacter
68	China	Direct chlorination and activated sludge	sul1, aadA, tet39, qacE1, bacA, lnuB, ermG, mefA, tetE, dfrb1, aph(3')-I, aadE	Bifidobacterium, Phocaeicola, Stenotrophomonaobacterium, Lactobacillus, Acinetobacters, Azoarcus, Enterobacter, Phascolarct	Resistance genes in hospital wastewater; ARGs in Bifidobacterium and Enterobacter, hazardous MDR Acinetobacter
69	China	-	sul1, aac(6′)-Ib′, AAC(6′)-30, acrA, acrD, acrF, cmlA5, floR, SHV-28, mdfA, mdtH, macA, macB, rosA, rosB, amA	Escherichia coli, Klebsiella pneumoniae, Pseudomonas aeruginosa, Enterobacter cloacae, Enterococcus faecium, Aeromonas caviae	
70	Qatar	-	blaVEB, blaKPC, blaGES, blaVIM-1, blaOXA-10, blaOXA-2, blaOXA-58, qnrB-1, qnrS, Aac(6')-Ib-cr, tetA, ermB, mefA, aadA1, blaSHV(238G240E), blaIMP-2, blaOXA-18	Escherichia coli, Morganella morganii, Salmonella enterica, Citrobacter freundii, Enterococcus faecalis, Enterococcus faecium, Clostridium perfringens, Clostridium difficile, Shigella dysenteriae, Streptococcus agalactiae	Widespread prevalence of β-lactamase genes and resistance to fluoroquinolones, aminoglycosides, tetracyclines, and macrolides in hospital bacteria
71	China	Constructed) (Wetlands	blaCTX-M, blaTEM, blaSHV, qnrS, sul1	Enterobacteriaceae, Escherichia coli, Coliforms	Effective removal of blaCTX-M and qnrS; sul1 remains with horizontal transfer risk; Enterobacteriaceae
72	Vietnam	-	blaCTX-M-1, blaTEM, blaSHV, mcr-1, mcr-2, mcr-3, mcr-4, mcr-5, mcr-6, mcr-7, mcr-8, mcr-9	Escherichia coli	Most common ESBL gene blaCTX-M-1 and key colistin resistance gene mcr-1
73	Algeria	-	OXA-23, VIM, cadA1, cadA2	Pseudomonas putida, Pseudomonas stutzeri, Pseudomonas fluorescens, Pseudomonas aeruginosa, Pseudomonas mendocina, Acinetobacter baumannii, Comamonas testosterone	Carbapenem- and cadmium-resistant NFGNB in hospital wastewater (Pseudomonas and Acinetobacter)

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
74	Egypt	Adsorption using nanoparticles: nZVI and CuONPs	aOXA-1, blaTEM, blaOXA-10, blaTEM-1, blaDHA-1, blaSHV-1, blaGES-1, qnrA, qnrS, qnrB-1, qnrB-4, qnrB-5, qepA	Acinetobacter baumannii, Helicobacter pylori, Escherichia coli, Pseudomonas aeruginosa, Clostridium beijerinckii, Shigella coli, Helicobacter cetorum, Lactobacillus gasseri, Bacillus cereus, Deinococcus radiodurans, Rhodobacter sphaeroides, Propionibacterium acnes, Bacteroides vulgatus	β-lactamase genes and quinolone resistance in hospital bacteria
75	Taiwan	Micron Bubble Ozone – OMB	tetA, blaTEM-1, sul1, mcr-1	-	Resistance genes in hospital wastewater with OMB; mcr-1 most persistent, sul1, blaTEM-1, and tetA reduced
76	United Kingdom	Tertiary UV disinfection	aminocoumarins, fluoroquinolones, glycopeptides, rifampicin, sulfonamides, MDR	Acinetobacter, Pseudomonas, Klebsiella spp, Escherichia coli, Enterococcus spp, Clostridium spp, Mycobacterium spp, Arcobacter spp, Paracoccus, Ottowia, Cloacibacterium, Actinobacteria	Persistence of certain antibiotics and ARGs; MDR associated with efflux pumps
77	France	-	tetW, tetQ, tetO, merA, blaTEM, bacA1, cblA	-	Tetracycline, β-lactam, bacitracin, and mercury resistance genes; abundance influenced by metals and surfactants
78	Thailand	Chlorination	blaNDM	Klebsiella pneumoniae, Escherichia coli, Enterobacter cloacae complex	blaNDM gene in K. pneumoniae, β-lactam resistance
79	Colombia	Sonochemical Process, Biological System	-	-	Effective removal of paracetamol and valsartan
80	Ethiopia	Sono-Photo-Fenton process (US/UV/Fe^{2+}/H_2O_2)	-	-	Combination of ultrasound, UV, and Fenton
81	Morocco	Electrocoagulation	–	Total coliforms, Escherichia coli, Enterococci, Clostridium	Reduction of indicator bacteria in hospital wastewater: Total coliforms 82%, E. coli 71.8%, Enterococci 80.8%, Clostridium 89.8%
82	Spain	Electrodisinfection, Photo-electrodisinfection	blaKPC, blaOXA-50, mecA	Klebsiella pneumoniae, Pseudomonas aeruginosa, Staphylococcus aureus	Klebsiella blaKPC low removal, Pseudomonas blaOXA-50 high removal, Staphylococcus mecA moderate removal
83	Egypt	$NiFe_2O_4$ nanocomposite	CYP450, 8-HDG, MDA, NO, TAC, ATP, Calcium, PC	–	Reduction of cellular and oxidative damage, improvement of antioxidant

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
		(AOPs)			capacity and metabolic function (CYP450, 8-HDG, MDA, NO, TAC, ATP, Calcium, PC)
84	United Kingdom	Immobilised Heterogeneous Photocatalysis	sul1 blaCTX-M qnrS ermB intI1	_	High resistance and variable abundance of genes; rapid transfer and high removal for some
85	Spain	Electrochemical ozonation using MIKROZON	aac(6′)-Ib, blaTEM, blaSHV, blaGPC	Klebsiella pneumoniae	Removal of resistance genes in hospital wastewater; aac(6′)-Ib highest, blaTEM and blaSHV moderate, blaKPC most persistent; Klebsiella reduced up to 6 log
86	China	Electro-peroxone	General reference to Args	E. coli	Removal of E. coli; indirect reduction of ARGs
87	Rwanda	Biochar adsorption	_	_	Focus on PPCPs; caffeine removal 65.5%; highest persistence of CBZ and DCF.
88	Netherlands	MBR + Ozonation + GAC[1] + UV	aph(III)a, blaKPC, blaSHV, blaOXA, mecA, ermB, ermF, qnrS, sul1, tetB, tetM, vanA, vanB, intI1	_	Resistance genes include those for carbapenems, cephalosporins, tetracyclines, sulfonamides, cotrimoxazole, macrolides, and integrative (intI1) genes.
89	Spain	AGS with SBR reactor	16SRNA, ITS, nosZ,AmoA	Hyphomicrobium,Dokdonella,Candidatus, Comamonadaceae,Acinetobacter, Accumulibacter, Diaphorobacter, Comamonas	Microbial community changes were analyzed using 16S rRNA (bacteria and archaea) and ITS (fungi).
90	Scotland	-	blaTEM, blaSHV, blaCTX-M, blaNDM, mecA, sul1, qnrS, intI1, ermB, tetM	Escherichia coli, Klebsiella pneumoniae, Acinetobacter baumannii, Enterococcus faecium/faecalis, Pseudomonas aeruginosa, Staphylococcus aureus	Focus on key resistance genes and their potential horizontal transfer
91	China	Anaerobic–aerobic treatment with sedimentation and chlorine disinfection.	blaVEB, blaNDM, blaOXA, blaTEM, blaCTX-M, tetA, tetB, tetM, ermB, ermF, aadA, aph(3’)-IIIa, sul1, sul2, qnrS, qnrB, vanA, vanB, cmlA, catB, mexF, acrB, and horizontal gene transfer markers, such as intI1 and IS613.	Bacteroides, Bacteroidetes, Firmicutes, Proteobacteria, Epsilonbacteraeota	Increased resistance and signs of horizontal gene transfer after treatment

[1] Granular Activated Carbon

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
92	China	Conventional activated sludge with chlorination and biocontact bio-contact oxidation.	blaNDM blaKPC blaCTX-M blaOXA blaTEM blaSHV mcr tet(X) tetA tetB tetM tetQ ermB ermF mefA aadA aph(3')-IIIa strA sul1 sul2 sul3 qnrS qnrB catA cmlA arr-3 vanA vanB mexF acrB fosA dfrA bacA	Klebsiella pneumoniae Escherichia coli Pseudomonas aeruginosa Acinetobacter baumannii Enterococcus faecium	Key genes with high persistence and horizontal transfer in effluents
93	Germany	Bio-contact oxidation, NaClO disinfection, Screening, Settling	blaKPC-2, blaOXA-48, blaOXA-232, blaVIM-1, blaNDM-5, blaGES-5, blaIMP-8,	Klebsiella pneumoniae, Escherichia coli, Enterococcus spp., Acinetobacter spp., Shigella spp., Stenotrophomonas spp., Wautersiella spp	Carbapenem genes: blaKPC-2 (highest resistance), blaOXA-48 (common in Germany), blaNDM-5, blaVIM-1, blaIMP-8; complete removal by conventional treatment is not possible.
94	Benin	Fixed bed adsorption with AC/$KMnO_4$ composite adsorbent	bla-CTX-M‹ bla-TEM, PVL, Trimethoprim/Sulfamethoxazole	Escherichia coli, Staphylococcus aureus, Salmonella typhi Vibrio cholerae O1, Pseudomonas aeruginosa, Enterococcus faecium	Key resistance genes: bla-CTX-M and bla-TEM (up to 5 log_{10} removal), PVL in S. aureus, and trimethoprim/sulfamethoxazole resistance in S. typhi (up to 3.82 log_{10} removal).
95	Thailand (Bangkok)	CAS and RBC with final chlorination.	–	–	Focus on antibiotics as indicators of selective pressure
96	India	ETP, CETP, ZLD, RO, MEE	–	–	Antibiotics as indicators of selective pressure for resistance emergence
97	China	CAS (A2/O), Ozonation (AOP)	Erm(35), Erm(B), Erm(F), Mph(A), tet(W/N/W), tetX, tetQ, tetO, tetM, GES-5, blaOXA, blaTEM, blaSHV, blaCTX-M, aadA, aph(3')-IIIa, strB, sul1, sul2, qnrS, qnrB, mef(A), lnu(A), catA, floR, kdpE, marA, acrA	Acinetobacter, Pseudomonas, Escherichia, Klebsiella, Enterobacter, Aeromonas, Bacillus, Staphylococcus, Streptococcus, Mycobacterium	Focus on antibiotic residues (ARs) in pharmaceutical wastewater.
98	Nigeria	Biological lagoon, waste stabilization pond and chlorination.	–	Fecal coliforms, total heterotrophic bacteria, Staphylococcus spp, Escherichia coli, other Enterobacteriaceae, Pseudomonas aeruginosa, Vibrio spp	High resistance and horizontal transfer in hospital wastewater
99	South Korea	Combined ultrasonication and terpinolene process	–	Enterobacter sp., Citrobacter freundii, Klebsiella pneumoniae	Presence of indicator bacteria with high resistance and persistence in hospital effluent

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
100	United States (Kokosing River, Ohio)	CAS[1] with chlorination	blaTEM, blaSHV, blaCTX-M, mecA, vanA, tetM, ermB, sul1	Escherichia coli, Klebsiella pneumoniae, Staphylococcus aureus, Enterococcus faecium, Pseudomonas aeruginosa	Focus on removing highly persistent hospital-associated MDR bacteria using combined processes
101	Benin, Burkina Faso, Finland	CAS, CW[2], chlorination, septic tank.	BlaGES, blaNDM, blaKPC, blaOXA-48, blaCTX-M, mcr-5, intI1, qacEΔ1, qnrVC	Acinetoacter, Pseudomonass aeruginosa, salmonella,Ecoli	Focus on key resistance genes against various antibiotics
102	Slovakia	CAS	-	Escherichia coli	Ampicillin (AMP): highest remaining resistance in the treatment plant effluent (58%). Cefotaxime (CTX): significant resistance in the effluent.
103	India	Treatment using MBBR and SBR with chlorination or UV disinfection.	tetA, tetC, tetG, ermB, ermF, qnrS, qnrD, sul1, sul2, blaTEM, blaSHV, blaOXA, aadA, aph(3')-IIIa, vanA, vanB, mefA, msrA, qacE, merA	Enterobacteriaceae, Pseudomonadaceae, Enterococcaceae, Moraxellaceae, Escherichia, Klebsiella, Pseudomonas, Acinetobacter, Enterococcus, Bacillus, Aeromonas, Bacteroides	The highest residual resistance was observed for AMP, and the lowest for TZP.
104	Scotland, United Kingdom	Lack of investigation of a specific process	bla_KPC, bla_NDM, bla_OXA, bla_TEM, bla_SHV, vanA, vanB, ermB, tetM	Enterococcus faecium (VRE), Klebsiella pneumonia, Escherichia coli	Highest resistance: blaTEM and ermB; highest removal: sul1 and tetA.
105	Japan	Ozone Treatment	blaCTX-M, blaKPC, blaNDM, blaVIM, blaOXA	Escherichia coli, Klebsiella spp, Raoultella ornithinolytica, Pseudomonas putida	Resistance to multiple antibiotics via efflux pumps and target modification
106	Brazil	Septic tank and aerobic filter	–	Enterobacteriaceae (e.g., E. coli), Non-Enterobacteriaceae (e.g., Pseudomonas), Streptococcaceae, Staphylococcaceae, Enterococcaceae	Highest resistance in Streptococcaceae and non-Enterobacteriaceae; highest prevalence in Enterobacteriaceae.
107	Brazil	MW/Fe^0 (microwave waves with zero-valent iron)	–	–	Incomplete antibiotic removal, spread of resistance genes
108	France	CAS	sul1, intI1, blaCTX-M, qnrS, tetM, ermB	Pseudomonas aeruginosa	Key genes: sul1 (persistent), intI1 (horizontal transfer), blaCTX-M, qnrS,

[1] Conventional Activated Sludge
[2] Constructed Wetlands

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Ref.	Country	Treatment Process	Gene	Bacterium	Key points
					tetM (incomplete removal), and ermB (partial removal).
109	Nigeria	Activated sludge/ Membrane Bioreactor+ Anaerobic DigestionTo	_	_	Focus on antibiotic concentrations as indicators of selective pressure:
110	France	CAS (aerobic, anoxic, and alternating anaerobic conditions).	_	_	Incomplete drug removal, persistence of resistance genes, and risk of environmental and health transfer.
111	Turkey	CAS + UV	ermB, ermC, ermF, qnrA, qnrB, qnrS, aac(6')-Ib-cr, sul1, sul2, dfr, tetA, tetM, tetO	_	erm, qnr, aac, sul, and dfr genes with incomplete removal and high risk; tet genes mostly removed
112	Iran	CAS	Viral genes analysis: ORF1ab, N gene	_	ORF1ab and N genes as stable SARS-CoV-2 environmental markers

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Discussion

A) Treatment technologies

The findings of this review show that hospital wastewater treatment technologies vary widely in terms of performance, cost, and efficiency in removing ARB and ARGs. Conventional approaches, such as CAS and chlorination, remain widely used but consistently demonstrate incomplete removal of resistant microorganisms and genes. Pathogenic bacteria such as E. coli and S. aureus can survive chlorination, and chlorine-induced oxidative stress facilitates HGT[28, 30]. Chlorination can also produce harmful DBPs[28]. These observations indicate that although conventional methods may serve as initial disinfection steps, they are insufficient on their own for effective control of antimicrobial resistance.

More advanced technologies, particularly MBR systems, have shown markedly superior results. Numerous studies have reported that MBRs at pilot or industrial scales remove indicator bacteria more effectively than conventional treatment[27]. Solar-powered hybrid MBR combined with GAC achieved complete removal of indicator bacteria in Uganda[27]. Coupling MBR with EO improves the removal of pharmaceutical compounds and reduces effluent toxicity[55]. AOPs, such as ozonation, EO, Fenton processes, and UV combined with H_2O_2, further enhance the removal of persistent contaminants. Ozone-based systems, including O_3, O_3 combined with H_2O_2, and O_3 combined with UV, effectively inactivate MDR bacteria[42, 62]. While electro-peroxone substantially decreases E. coli in hospital effluents[86].

Electro-oxidation (EO) and hybrid photoelectrochemical processes have emerged as highly effective advanced oxidation technologies for removing persistent antibiotics and refractory organic pollutants from hospital wastewater. These processes rely on the in situ generation of strong oxidizing species, particularly hydroxyl radicals (•OH), which can degrade pharmaceutical compounds resistant to conventional biological treatment [113].

Previous studies have demonstrated that photo-electro oxidation processes can achieve high amoxicillin removal efficiency accompanied by significant reductions in effluent toxicity, confirming the strong oxidative and mineralization capacity of EO-based systems. Operational parameters, including current density, reaction time, and electrolyte concentration, were found to significantly influence degradation kinetics and treatment performance[114].

Moreover, the modification of EO systems using activated carbon beds as porous electrodes has been shown to enhance mass transfer, increase reactive oxygen species (ROS) generation, and improve pollutant degradation efficiency and process stability. integration of adsorption with electrochemical oxidation significantly improved amoxicillin removal and reduced residual organic contamination[115]. Similarly, photoelectro-Fenton systems modified with porous cathode electrodes demonstrated enhanced degradation kinetics and toxicity reduction through intensified hydroxyl radical production and electrochemical reactions. These hybrid systems exhibited high capability for degrading resistant organic compounds that are difficult to eliminate by biological treatment alone[116].

Overall, EO-based and hybrid photoelectrochemical technologies represent promising and sustainable approaches for upgrading conventional hospital wastewater treatment systems, particularly when integrated with biological or membrane-based processes for enhanced removal of antibiotics and resistant contaminants. Nevertheless, some ARGs, including blaKPC, blaNDM, and mcr-1, display higher persistence than others[51, 62].

Integrated or hybrid systems achieve the highest overall removal efficiencies. Combinations such as MBR with ozonation or MBR with GAC yield over 95% reductions in pharmaceuticals and substantial decreases in ARGs[60, 88]. A sponge-MBR system combined with ozonation achieved complete removal of tetracyclines and major reductions in fluoroquinolones[56]. Only the MBR combined with a UV system achieved complete elimination of human enteric viruses [58].

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Complementary emerging methods, including NLC-eugenol, showmicrobial load reductions of 28%–40%[54]. MFCs reduce tet, sul, bla, and qnrS by more than 80%[50] and photocatalytic or nanoparticle-based systems highlight future directions [74, 83]. Overall, the literature indicates that conventional methods alone are inadequate; advanced and combined systems, especially MBR integrated with AOPs or GAC, consistently achieve the most effective reductions of ARB, ARGs, and pharmaceutical residues[27, 62, 88]. Research trends increasingly support multistage and hybrid configurations as sustainable strategies for limiting the spread of antibiotic resistance in hospital wastewater. However, despite these promising results, several limitations should be considered. Despite the high removal efficiencies reported for advanced treatment technologies, such as MBRs, advanced oxidation processes (AOPs), and their hybrid configurations, several practical limitations still restrict their full-scale application. These include high operational and maintenance costs, significant energy demand, membrane fouling, and the requirement for skilled operation. In addition, a considerable proportion of available evidence is derived from laboratory-scale or pilot-scale studies, which may not fully represent real hospital wastewater treatment conditions. Therefore, the scalability and long-term operational stability of these systems remain uncertain. Furthermore, variations in influent composition, antibiotic loads, and microbial communities can strongly influence treatment performance, making direct comparison across studies challenging. These limitations highlight the need for more full-scale investigations and standardized evaluation frameworks for assessing treatment efficiency against antibiotic-resistant bacteria (ARB) and ARGs.

B) Main Mechanisms Involved in Antibiotic and ARG Removal from Hospital Wastewater

Antibiotic removal from hospital wastewater occurs through biological, physicochemical, and oxidative mechanisms, depending on the antibiotic characteristics and the treatment technology applied. Conventional wastewater treatment processes alone are generally insufficient for the complete removal of antibiotics and ARGs, whereas advanced hybrid systems can significantly improve their removal efficiency [18].

Biodegradation is one of the main removal mechanisms in biological systems such as activated sludge, MBRs, and sponge reactors. Microorganisms degrade pharmaceutical compounds directly or through co-metabolism in these systems. The removal efficiency depends on the biodegradability of each compound, while some antibiotics remain resistant to biological degradation32 [32]. Longer sludge retention time (SRT), higher biomass concentration, and biofilm formation have been reported to improve the removal of antibiotics and ARGs18 [18, 56].

Adsorption and sorption significantly contribute to antibiotic removal, particularly for fluoroquinolones, which have a high affinity for sludge and adsorbent surfaces. Studies have shown that zero-valent iron and copper nanoparticles can effectively remove levofloxacin mainly through chemisorption mechanisms involving electrostatic interactions and surface complexation[74]. However, part of the removal observed in conventional systems results from the transfer of antibiotics into the sludge rather than complete degradation[18].

AOPs, including ozonation, electrochemical, electro-peroxone, and photocatalytic processes, remove antibiotics through the generation of ROS, such as hydroxyl radicals (•OH), which oxidize and mineralize organic pollutants [43, 55, 62, 85, 86]. Electro-oxidation and hybrid photoelectrochemical processes remove antibiotics mainly through in situ generation of ROS, particularly hydroxyl radicals, which enhance the oxidation and mineralization of refractory pharmaceutical compounds resistant to conventional biological treatment[1, 2, 4]. Modifications, such as porous activated carbon electrodes, can further enhance mass transfer and ROS generation, thereby improving antibiotic degradation efficiency and reducing residual toxicity[3].

These processes also contribute to the inactivation of antibiotic-resistant bacteria and

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pathogenic microorganisms62 [62, 85, 86]. In membrane systems, antibiotics are mainly removed through size exclusion, electrostatic repulsion, and adsorption onto membrane fouling layers. Because membranes mainly act as physical barriers, combining membrane technologies with biological and advanced oxidation processes provides higher removal efficiency55 [55, 56].

In addition to antibiotic removal, the elimination of ARGs is also essential. ARGs may be reduced through host bacteria removal, oxidation of extracellular DNA, and adsorption onto sludge or nanoparticles; however, their complete elimination remains more difficult than that of antibiotics themselves[18, 62, 74]. Overall, hybrid technologies integrating multiple removal mechanisms demonstrate the most effective performance in reducing pharmaceutical pollutants and limiting antimicrobial resistance dissemination.

C) Classification Based on ARGs

A systematic review shows that research on ARGs in hospital wastewater is globally distributed, with studies spanning Asia, Europe, North America, Latin America, Africa, and the Middle East. China, several European countries, and India represent major research hotspots, reflecting both the severity of AMR and strong scientific output. In Asia, most studies have centered on bla, sul, tet, and mcr, often evaluating advanced technologies such as MBR, nanofiltration, advanced oxidation, and microbial fuel cells under resource-limited conditions [27, 29, 46, 50]. European studies have targeted a broader gene spectrum, including blaTEM, mecA, and vanA, and have demonstrated the effectiveness of UV and ozone when combined with biological treatment[36, 38, 42, 61]. In North America, research has integrated biological systems with UV and applied advanced analytical techniques such as liquid chromatography, bioassays, and metagenomic assessments[30, 59].

In contrast, efforts in Latin America, Africa, and the Middle East remain limited but are steadily increasing. Studies from Uganda, Tanzania, Nigeria, Burkina Faso, Saudi Arabia, and Qatar primarily highlight infrastructural limitations and rely on simpler systems, such as bioreactors and combined processes, while still addressing ARG and ARB removal[27, 35, 66]. Emerging studies in Colombia have explored novel treatment strategies[79]. Overall, regional differences in bacterial profiles and ARG patterns reflect variations in antibiotic use, climate, microbial ecology, and health policy. Despite technological gaps, the expanding research output in developing regions indicates growing awareness and provides an opportunity to strengthen policy and surveillance frameworks. Collectively, global evidence underscores that effective control of ARGs requires adaptable, context-specific strategies supported by international collaboration in monitoring, knowledge exchange and treatment innovation.

D) Sampling Location

The reviewed studies reveal substantial variation in sampling design, with most research focused on measuring ARGs and ARBs in influent and final effluents, whereas solid fractions, such as primary sludge, activated sludge, and membrane biofilms, were rarely examined. This limited focus omits major reservoirs that govern the accumulation and transfer of ARGs, thereby constraining accurate assessment of AMR risks[68, 71, 74]. Several investigations sampled only influent and final treated water, including MBR or EO effluents, with no sludge or biofilm monitoring, which prevented full understanding of ARG fate despite demonstrating reductions in antibiotic concentrations[55-57]. Even laboratory studies using raw wastewater under controlled conditions, although useful for testing nanoparticle performance, offered limited real-world applicability[54].

In contrast, more comprehensive sampling designs that included intermediate treatment units as well as upstream and downstream river water enabled detailed tracking of microbial communities and ARG profiles across the treatment process and following discharge [59]. However, even in these cases, sludge fractions

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were not consistently sampled. Only one study incorporated seasonal sampling, which provided valuable temporal insights, even though its focus was on virus removal rather than ARG quantification [58]. Across many studies, activated sludge and biofilms were excluded despite their recognized role as reservoirs of ARGs [56, 57].

Overall, the findings indicate that relying solely on effluent monitoring underestimates the persistence and mobility of ARGs, as many ARGs accumulate in sludge and can facilitate continued resistance transfer32 [32, 47]. Studies that applied multi-compartment sampling, including sludge, clarified differences in ARG behavior during biological and chemical treatments and highlighted the potential for ARG retention in solid phases, particularly in systems such as MBR and advanced oxidation [27, 29, 45, 68]. Consequently, future research should incorporate multi-stage sampling from influent to effluent, including sludge and biofilms, together with temporal monitoring, to achieve more accurate evaluations of treatment technologies and better control of AMR dissemination [66, 68, 70, 72, 74, 76, 82].

E) Public Health Implications

The reviewed studies demonstrate a strong association between hospital wastewater treatment performance and public health risks. Incomplete removal of ARB and ARGs enables their release into aquatic environments, where humans may be exposed through contaminated water, food, or recreational contact. The persistence of mobile genetic elements, such as plasmids and integrons, amplifies this threat by enabling horizontal gene transfer and accelerating AMR dissemination [59]. Reports showing the survival of pathogens and viruses, even after chlorination, highlight the potential hazards of partially treated effluents [58]. Consequently, effective treatment is a critical barrier preventing the transfer of clinical resistance into community and environmental settings.

Several studies have emphasized that insufficient ARG removal significantly increases the likelihood of AMR spread in ecosystems and human populations[27, 28, 46]. Ineffective removal of genes such as blaCTX-M, sul1, vanA, and mcr-1 supports resistance transfer in aquatic and terrestrial habitats, including through the food chain[35, 45, 71]. Advanced systems, such as MBRs combined with AOPs, UV, ozone, or electrochemical methods, demonstrate higher removal efficiency and reduce the potential for ARG persistence and horizontal gene transfer[29, 50, 62]. Nevertheless, many ARGs continue to exist as free DNA or within mobile genetic elements, remaining detectable in WWTP effluents and entering receiving waters, where exposure pathways persist[74, 76, 82].

The continued presence of ARGs, including the sul1 and bla families, ESBL markers, and MDR determinants such as mcr, indicates that conventional treatment alone is insufficient[68, 71, 72]. In some cases, processes such as chlorination may alter gene concentrations, potentially increasing the risk [68]. Given the role of sludge and biofilms as ARG-rich reservoirs, proper management of solid fractions is essential for preventing secondary dissemination[76, 82]. Reducing ARG and ARB loads requires routine molecular monitoring through qPCR or metagenomics and the integration of advanced biological, oxidative, and filtration technologies within AMR control strategies[56, 64, 71]. Ultimately, safeguarding public health demands a coordinated One Health approach that integrates environmental surveillance, improved treatment and disinfection systems, safe sludge handling, and national monitoring policies to reduce environmental resistance loads and limit transmission to human populations[56, 64, 72].

F) Future Perspectives and Research Needs

The findings of this review indicate that, despite considerable progress in hospital wastewater treatment technologies, significant challenges remain regarding the effective removal of antibiotics, ARB, and ARGs. Given the limitations of conventional treatment systems, several strategies have been proposed to improve treatment performance. Advanced and hybrid technologies, including MBRs, sponge-MBR systems, and submerged fixed-film reactors, have demonstrated significantly higher removal efficiencies than

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conventional activated sludge systems because of enhanced biomass retention and longer sludge retention times[27, 55-57]. In addition, coupling biological systems with AOPs, such as ozonation, electro-peroxone, photoelectrodisinfection, and sono-photo-Fenton processes, can further enhance the degradation of pharmaceutical compounds and the inactivation of resistant microorganisms and ARGs through the generation of ROS[52, 62, 75, 80, 82].

Continuous molecular monitoring using metagenomics and qPCR should also be integrated into hospital wastewater surveillance programs to evaluate ARG persistence, transferability, and treatment efficiency[42, 51, 76]. As sludge and biofilms may act as secondary reservoirs for ARG accumulation and dissemination, appropriate sludge treatment and disposal strategies are essential[47, 76]. Furthermore, several studies have reported that chlorination alone is insufficient for the complete removal of resistant microorganisms and may even alter ARG abundance or promote the persistence of viable but non-culturable bacteria[30, 68, 71]. Future investigations should prioritize full-scale validation of advanced treatment technologies, standardized monitoring protocols, life-cycle and cost-effectiveness assessments, and the evaluation of long-term ARG fate in both liquid and solid treatment fractions. Addressing these knowledge gaps will facilitate the development of more sustainable and scalable approaches for mitigating antimicrobial resistance dissemination through hospital wastewater.

Conclusion

Studies have demonstrated that hospital wastewater is a major source of ARB and ARGs, posing significant threats to public health and the environment. The discharge of effluents containing residual antibiotics, pathogens, and mobile genetic elements exerts strong selective pressure on microorganisms, promoting resistance amplification, horizontal gene transfer, and the environmental dissemination of AMR. Conventional treatment methods, such as activated sludge and chlorination, only partially reduce the microbial load and are insufficient to fully remove ARGs and resistant bacteria and may even enhance resistance transfer via oxidative stress. Research indicates that advanced treatment technologies, including MBRs, AOPs, ozonation, electrochemical, and photocatalytic methods, and hybrid systems, demonstrate markedly superior performance in removing resistant contaminants and reducing antibiotic residues. Integrated approaches, particularly MBR combined with AOPs, ozonation, or GAC, achieve the highest removal efficiencies. Nevertheless, the persistence of stable genes, such as bla, mcr, and sul, underscores the necessity of multi-stage and combined treatment strategies.

Global studies reveal considerable variations in technology availability, target ARGs, and infrastructure levels, highlighting the importance of context-specific solutions and enforceable regional and national policies. Inadequate sampling designs in many studies-frequently excluding sludge, biofilms, and solid fractions-limit the accurate evaluation of treatment efficacy and increase the risk of secondary ARG dissemination. Multi-compartment monitoring, including the influent, intermediate units, effluent, sludge, biofilms, and receiving environments, is essential for precise assessment. From a public health perspective, incomplete removal of ARB and ARGs facilitates environmental transmission through water, soil, and the food chain. Even advanced systems cannot completely eliminate extracellular DNA, plasmids, and other mobile genetic elements, indicating that effective AMR control requires integrated approaches beyond wastewater treatment, including antibiotic stewardship and national resistance control strategies.

Ultimately, studies demonstrate that recent advances in hospital wastewater treatment offer valuable opportunities to mitigate environmental AMR loads. Effective control relies on the implementation of advanced technologies, comprehensive monitoring, coordinated policies, and proper waste management within an integrated, locally adapted framework. Transitioning toward smart, multi-stage, and

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hybrid systems represents the key pathway for effective antibiotic resistance control and protection of both human and environmental health.

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Conflict of Interest

The authors have no conflicts of interest to declare.

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Ethical Considerations

This study was conducted without the need for ethical approval.

Code of Ethics

This review article was conducted independently and was not registered as a university research project; however, all relevant ethical and scientific research principles were strictly followed.

Authors' Contributions

All authors contributed equally to the conception, design, writing, and revision of this manuscript.

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