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RESEARCH ARTICLE

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ANTIMICROBIAL RESISTANCE IN BACTERIA ISOLATED FROM HOSPITAL WASTEWATER

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ABSTRACT

Antimicrobial resistance (AMR) is a critical global public health problem, closely linked to the intensive use of antibiotics and the release of hospital effluents into the environment. To understand the role of hospital wastewater (HWW) in the dissemination of bacterial resistance to antimicrobials and its impact on public and animal health, a literature review was conducted using scientific databases. Studies show that HWW contains antibiotics at sublethal concentrations and a high load of multidrug-resistant pathogens, including Klebsiella pneumoniae, Acinetobacter baumannii, and Pseudomonas aeruginosa, which carry genes encoding carbapenemases or beta-lactamases. These pathogens persist after conventional purification treatments and are disseminated in the environment. It is concluded that AMR constitutes a critical threat to global public health that transcends the clinical setting and involves the environment and animal health. The evidence gathered indicates that HWW function as critical reservoirs and pathways for the dissemination of antimicrobial-resistant bacteria (ARBs) and antimicrobial resistance genes (AMRGs) into the environment. Therefore, a One Health approach is required to strengthen their treatment, environmental surveillance, and the rational use of antimicrobials.

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INTRODUCTION

Antimicrobial resistance (AMR) represents one of the most pressing global health challenges in the healthcare system. AMR has been reported as a critical global public health concern, with the potential to become the leading cause of death soon (Duran-Bedolla *et al*., 2024). The widespread use of antibiotics in clinical settings has led to the emergence and spread of resistant bacterial strains, posing serious threats to public health and healthcare systems (Endalamaw *et al*., 2024). In this regard, hospital wastewater has been reported as a critical, yet often overlooked, component in the spread of AMR; it serves as a major reservoir of antimicrobial-resistant bacteria and a conduit for the global spread of infections (Endalamaw *et al*., 2024). In this regard, the continuous discharge of antibiotics into the environment, often at sublethal concentrations within hospital effluent, has the potential to induce and sustain bacterial resistance antibiotics (Duran-Bedolla *et al*., 2024). This type of wastewater contains a cocktail of antibiotics, disinfectants, metabolized drugs, and antimicrobial-sensitive and antimicrobial-resistant bacteria from hospitalized patients (Lépesová *et al*., 2020). The main concern regarding the release of antimicrobials into the aquatic environment is associated with the development of antimicrobial resistance genes (AMRGs); in this context, the World Health Organization (WHO) has identified carbapenem-resistant Gram-negative bacteria and extended-spectrum beta-lactamase (ESBL) producers as a critical priority for study (Duran-Bedolla *et al*., 2024). Some pathogens, such as methicillin-resistant *Staphylococcus aureus*, carbapenem-resistant *Enterobacteriaceae*, and vancomycin-resistant *Enterococci*, can pose significant risks to human and animal health (Bakon *et al*., 2023). Antimicrobial resistance (AMR) is even more concerning due to the overuse of one antibiotic and its unsafe disposal, fostering the development of resistance to other classes of antibiotics (Manik *et al*., 2023). The aim of this review is to provide information on the role of hospital effluents in the spread of bacterial resistance, the reasons why they are considered favorable environments for this problem, and their impact on public and animal health.

Global importance of antimicrobial resistance: Antimicrobial resistance is a global public health problem related to the human and non-human use of antimicrobials. It is one of the issues addressed in the multisectoral approach known as One Health, a joint initiative of the WHO, the Food and Agriculture Organization of the United Nations, and the World Organisation for Animal Health (Wernli *et al*., 2017). According to the Pan American Health Organization (PAHO), hospital-acquired infections cause approximately 700,000 deaths annually. Without effective action, this figure is projected to reach 10 million deaths per year by 2050 (PAHO, 2021). Currently, the most serious life-threatening infections are caused by a group of antibiotic-resistant bacteria, which the Infectious Diseases Society of America has named the ESKAPE group. These bacteria cause severe infectious diseases, and their pathogenicity and antimicrobial resistance mechanisms are highly developed over time (Santajit and Idrawttana, 2016). The ESKAPE group of bacteria, which includes *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and *Enterobacter* species, is responsible for a significant proportion of difficult-to-treat infections, ranging from pneumonia and bloodstream infections to urinary tract and wound infections, particularly in immunocompromised patients or those undergoing invasive medical procedures (Akhter *et al*., 2024).

Hospital wastewater as a reservoir and dissemination route for antimicrobial-resistant bacteria: Hospital wastewater (HWW) contains various contaminants, including pharmaceutically active chemicals, microbiomes, antimicrobial-resistant bacteria, antimicrobial resistance genes, viruses, etc. (Sharma *et al*., 2025). However, the main chemicals present in hospital wastewater are pharmaceuticals, which are partially metabolized and excreted in urine (Zhang *et al*., 2020). Several groups of antibiotics, including macrolides such as azithromycin, clarithromycin, and erythromycin, as well as sulfonamides such as sulfamethoxazole, are frequently prescribed and persistent in the environment. These have been detected in hospital wastewater at concentrations of less than 1 µg/L, even after conventional treatment processes, due to their limited biodegradability and adsorption properties (Correia *et al*., 2015). Recent studies have reported concentrations of up to 300 ng/L for erythromycin, 245 ng/L for amoxicillin, and 500 ng/L for ciprofloxacin in treated effluents (PRAN-MA, 2023). Other researchers have observed a high frequency of antimicrobial-resistant bacteria (ARBs) and antimicrobial resistance genes (GRAMs), including genes encoding extended-spectrum beta-lactamases (ESBLs) and carbapenemases, which are clinically significant in hospital effluents (Cahill *et al*., 2019). Fatimazahra *et al*. (2023), in a study conducted on hospital wastewater (HWW), determined the concentration of 1.79 x 10^7 CFU/100 mL of coliforms, 7.15 x 10^5 CFU/100 mL of E. coli, 4.5 x 10^7 CFU/100 mL of fecal streptococci, 7.5 x 10^5 CFU/100 mL for Staphylococcus aureus, and 5.3 x 10^6 CFU/100 mL for *Pseudomonas aeruginosa*, highlighting the potential health risk associated with these effluents. For their part, Proia *et al*. (2018) and Azuma *et al*. (2019) observed the presence of antimicrobial-resistant bacteria (ARBs), as well as the presence of antimicrobial resistance genes (AMRGs), in hospital effluents, influents and effluents from wastewater treatment plants (WWTPs), and rivers receiving WWTP effluents. These findings suggest that the spread of ARBs and AMRGs is potentially being driven by the release of untreated or inadequately treated hospital effluents into the environment. In this regard, bacteria released by hospital effluents into WWTPs can exchange AMRGs with other strains through horizontal gene transfer (HGT), which involves plasmids, transposons, and integrons (Sharma *et al*., 2025). Typically, antibiotic-resistant bacteria replicate their AMRGs through vertical gene transfer (VGT), which allows for the maintenance of genetic stability within their population; However, genetic modification also occurs, creating diversity within populations (Del Casale *et al*., 2011; Paulson *et al*., 2016; Li *et al*., 2019). Furthermore, wild animals, interacting with habitats ranging from remote forests to urban and agricultural areas, are frequently exposed to sources of pollution such as industrial effluents, hospital waste, and veterinary products (Silva *et al*., 2025).

Main bacterial species isolated from hospital wastewater, antimicrobial resistance, and associated genes: Hospitals are critical reservoirs of antibiotic-resistant bacteria (ARBs), and their wastewater systems represent a significant pathway for the release of these microorganisms into the environment. Hocquet *et al*. (2016) demonstrated that total concentrations of *Escherichia col*i in hospital and community effluents are similar (≈10^5 to 10^6 CFU/mL), but the proportion of extended-spectrum β-lactamases (ESBLs-*E. coli*) is much higher in hospitals (3.8 to 39% vs. 0.1 to 19%). For *Pseudomonas aeruginosa*, concentrations were higher in hospital effluents (≈10^3 to 10^4 CFU/mL) than in urban effluents (≈10^2 CFU/mL), with a higher frequency of multidrug-resistant strains. For *Enterococcus* spp., although total concentrations were comparable (≈10^4 to 10^6 CFU/mL), the proportion of vancomycin-resistant *Enterococcus* spp. was higher in hospitals (up to 6.5%) than in the community (≈1% or less). Fekadu *et al*. (2015) more frequently identified, in hospital wastewater, strains of *Staphylococcus* spp., *Klebsiella* spp., *Escherichia coli*, *Bacillus* spp., *Proteus* spp., *Enterococcus* spp., *Salmonella* spp., *Shigella* spp., *Citrobacter* spp., as well as unidentified Gram-negative bacilli, demonstrating a high diversity of potentially pathogenic microorganisms present in this type of effluent. It was also reported that strains of *Staphylococcus* spp., *Escherichia coli*, *Salmonella* spp., and *Shigella* spp. showed susceptibility to antibiotics such as penicillin, ampicillin, tetracycline, vancomycin, gentamicin, ciprofloxacin, erythromycin, doxycycline, and ceftriaxone. Similar results were reported by Tellez-Carrasquilla *et al*. (2023), who studied 40 Gram-negative bacilli obtained from a hospital effluent and observed that 80% were *Enterobacteriaceae* and 20% were non-Enterobacterial bacilli.

The genera identified were *Citrobacter freundii* (30%), *Klebsiella pneumoniae* (25%), *Escherichia coli* (17.5%), *Aeromonas hydrophila/caviae* (10%), *Vibrio cholerae* (5%), and, in smaller proportions, *Acinetobacter baumannii complex*, *Enterobacter cloacae complex*, *Kluyvera intermedia*, *Raoultella planticola*, and *Pseudomonas aeruginosa* (2.5% each). These same authors observed high percentages of resistance to most of the antibiotics evaluated, with particularly high resistance to carbapenems and third and fourth generation cephalosporins. Endalamaw *et al*. (2024) identified colonies of *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Escherichia coli*, *Citrobacter* spp, *Acinetobacter* spp, and *Enterobacter* spp in samples of these effluents. It was observed that 100% of the Klebsiella pneumoniae isolates were resistant to ceftazidime, 52.4% of the E. coli isolates were resistant to both ceftazidime and tetracycline, and 64% of the S. aureus isolates were resistant to erythromycin. Garza-González *et al*. (2019) identified a high percentage of carbapenem-resistant Gram-negative bacteria from samples of various hospital effluents; more than 50% of the isolates were *Acinetobacter baumannii*, 40% were *Pseudomonas aeruginosa*, and 12% each were *Klebsiella* spp. and *Enterobacter cloacae*. High levels of multidrug resistance (MDR) were observed in *A. baumannii* (53%) and *Klebsiella pneumoniae* (22%). Furthermore, in the Gram-positive bacteria group, 21% of the *Staphylococcus aureus* isolates were methicillin-resistant and 21% of the enterococci were vancomycin-resistant. Based on the results analyzed previously, it can be said that the reported isolates can be classified as multidrug-resistant, with *Staphylococcus* spp, *E. coli*, and *K. pneumoniae* being the microorganisms most frequently reported in healthcare settings. This indicates that hospitals contribute disproportionately to the burden of resistant bacteria in the urban healthcare system. Kehl *et al*. (2022), from hospital wastewater samples, obtained isolates of *Escherichia coli*, *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Pseudomonas aeruginosa* producing the carbapenemases *blaOXA-48, blaVIM, blaNDM, and blaKPC.* The most frequently identified colonies were *Klebsiella pneumoniae*, which harbored blaNDM genes, often in combination with blaOXA-48 or genes encoding OXA-48-like enzymes. Similarly, Cahill *et al*. (2019) isolated *Enterobacter cloacae complex*, *Klebsiella pneumoniae*, *Citrobacter freundii*, *Klebsiella*

Table 1. Antimicrobial-resistant bacterial genera and species observed in hospital wastewater.

Genera and species of bacteria	Antimicrobial resistance	Antimicrobial resistance genes	References
Escherichia coli	Beta-lactams Carbapenems Aminoglycosides Tetracyclines Sulfonamides	*blaOXA-48, blaVIM, blaNDM, blaKPC, blaTEM, blaCTX-M blaSHV*	Hoquet *et al*. (2016), Fedaku *et al*. (2015), Tellez-Carrasquilla *et al.* (2023), Endalamaw *et al*. (2024), Garza-González *et al*. (2019), Kehl *et al*. (2022), Cahill *et al*. (2019)
Staphylococcus aureus	Beta-lactams Macrolides Aminoglycosides Tetracyclines	*blaNDM, blaOXA-48, blaVIM MecA, blaZ*	Fedaku *et al*. (2015), Garza-González *et al*. (2019), Endalamaw *et al*. (2024)
Klebsiella pneumoniae	Beta-lactams Carbapenems Polymyxins	*blaNDM-1, blaOXA-10, blaOXA-48, blaSHV-11, blaTEM-1B blaCTX-M-15, tetA, blaKPC, blaVIM, blaIMP*	Tellez-Carrasquilla *et al*. (2023), Endalamaw *et al*. (2024), Cahill *et al*. (2019), Garza-González *et al*. (2019), Kehl *et al*. (2022)
Acinetobacter baumannii	Beta-lactams Carbapenems Phenicols	*blaKPC, blaVIM*	Garza-González *et al*. (2019), Endalamaw *et al*. (2024), Tellez-Carrasquilla *et al*. (2023)
Pseudomonas aeruginosa	Beta-lactams Carbapenems Quinolones Aminoglycosides Sulfonamides Fosfomycin Phenicols	*blaVIM, blaGES-1, blaGES-5 blaOXA-10, blaCARB-2, blaKPC, blaVIM*	Hoquet *et al*. (2016), Garza-González *et al*. (2019), Kehl *et al*. (2022), Tellez-Carrasquilla *et al*. (2023), Endalamaw *et al*. (2024)
Enterobacter	Beta-lactams Carbapenems Phenicols Tetracyclines	*blaVIM, blaOXA-48, blaNDM blaGIM, blaTEM, blaCTX-M, blaSHV, blaKPC, blaIMP*	Tellez-Carrasquilla *et al*. (2023), Endalamaw *et al*. (2024), Garza-González *et al*. (2019), Cahill *et al*. (2019), Kehl *et al*. (2022)

oxytoca, and *Escherichia coli* from hospital wastewater, carrying the carbapenemases OXA-48 (the most frequent), IMP, KPC, VIM, NDM, and combinations of IMP and OXA-48. Susceptibility testing showed a high prevalence of resistance to β-lactams (including third and fourth generation cephalosporins and carbapenems), aminoglycosides, and fluoroquinolones, demonstrating the widespread circulation of antimicrobial resistance genes in hospital wastewater systems. Table 1 summarizes the results of studies on antimicrobial-resistant bacterial genera and species observed in hospital wastewater. Taken together, these studies confirm that hospital wastewater acts as a critical point of dissemination and genetic exchange, representing a direct threat to the effectiveness of conventional antibiotic treatments.

CONCLUSIONS

Antimicrobial resistance has become a critical threat to global public health, transcending the clinical setting and impacting the environment and animal health. Hospital wastewater serves as a critical reservoir and pathway for the dissemination of antimicrobial-resistant bacteria and antimicrobial resistance genes into the environment. The discovery of genes encoding carbapenemases, such as *blaOXA*-48, *blaNDM*, *blaVIM*, and *blaKPC*, in multiple bacterial strains belonging to the ESKAPE group, along with the high prevalence of multidrug-resistant strains, underscores the epidemiological complexity of these effluents. In this context, strengthening hospital wastewater treatment strategies is essential.

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