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ANTIMICROBIAL RESISTANCE IN HOSPITAL AND MUNICIPAL WASTEWATER

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Abstract

Objective: *To compare the occurrence of antibiotic-resistant bacteria and selected antimicrobial resistance genes in hospital and municipal wastewater.*

Methods: *This comparative cross-sectional laboratory-based study was conducted at the University of Sindh, Jamshoro, Pakistan. Eighty wastewater samples, comprising 40 hospital and 40 municipal samples, were analyzed. Gram-negative bacteria were isolated and identified using standard microbiological procedures. Antimicrobial susceptibility was determined by the Kirby–Bauer disk diffusion method. Multidrug-resistant (MDR) isolates were subjected to PCR for detection of* ***blaTEM, blaCTX-M, qnrS, tetA****, and* ***sul1****.*

Results: *A total of 126 bacterial isolates were recovered, including 72 from hospital and 54 from municipal wastewater. MDR was significantly more frequent among hospital isolates than municipal isolates (68.1% vs. 46.3%; p=0.014; OR=2.49). Resistance to ceftriaxone, ceftazidime, ciprofloxacin, meropenem, tetracycline, and trimethoprim-sulfamethoxazole was significantly higher in hospital wastewater. Among 74 MDR isolates,* ***blaTEM*** *was most prevalent (58.1%), followed by* ***blaCTX-M*** *(51.4%),* ***sul1*** *(47.3%),* ***tetA*** *(41.9%), and* ***qnrS*** *(35.1%).*

Conclusion: *Hospital wastewater demonstrated a greater burden of MDR bacteria and resistance genes than municipal wastewater, highlighting the importance of incorporating wastewater monitoring into environmental antimicrobial resistance surveillance.*

INTRODUCTION

Antimicrobial resistance (AMR) is an increasing global public health and environmental concern that threatens the effectiveness of commonly used antibiotics. Although antimicrobial resistance is primarily recognized as a clinical problem, the environment plays an important role in the persistence and dissemination of resistant bacteria. Wastewater is particularly important because it receives microorganisms and contaminants from human activities and may act as a reservoir through which resistant bacteria enter natural aquatic environments.[1–3]

Hospital wastewater represents a potentially important source of antimicrobial-resistant bacteria. It may contain microorganisms originating from patients, healthcare activities, pharmaceutical residues, disinfectants, and other substances capable of creating selective pressure on bacterial populations.[4,5] If hospital effluent is discharged without adequate treatment, resistant bacteria may subsequently enter municipal drainage systems and surrounding water bodies.

Municipal wastewater also represents an important environmental reservoir of bacteria. Domestic sewage receives microorganisms from large community populations and may contain resistant organisms originating from household antimicrobial use, human waste, commercial activities, and other urban sources.[6] Consequently, comparison of hospital and municipal wastewater can provide useful information regarding the distribution of antimicrobial resistance across different wastewater environments.

Gram-negative bacteria are particularly relevant to environmental AMR surveillance. Organisms such as *Escherichia coli, Klebsiella, Pseudomonas, Enterobacter*, and *Acinetobacter* species are frequently detected in wastewater and may exhibit resistance to several clinically important antimicrobial classes.[7,8] The presence of multidrug-resistant (MDR) organisms in wastewater is concerning because environmental discharge may facilitate their persistence and wider dissemination.

Culture-based bacterial isolation combined with antimicrobial susceptibility testing provides a practical approach for assessing environmental antimicrobial resistance. Such methods allow viable bacteria to be identified and their susceptibility to commonly used antibiotics to be directly determined. Comparison of resistance patterns between wastewater sources can therefore help identify environments with a greater burden of resistant organisms.[9]

Environmental AMR surveillance is particularly relevant in settings where wastewater treatment infrastructure is limited or hospital effluent enters general municipal drainage systems. Despite the growing international recognition of wastewater as an important component of AMR surveillance, locally generated comparative information remains limited in many low- and middle-income settings.[10]

Therefore, the present study aimed to determine the occurrence and antimicrobial susceptibility patterns of bacterial isolates recovered from hospital and municipal wastewater and to compare the prevalence of multidrug-resistant bacteria between the two wastewater sources.

Materials And Methods

A comparative cross-sectional laboratory-based environmental study was conducted at the **University of Sindh, Jamshoro, Pakistan**, in collaboration with selected healthcare and microbiology facilities in the Jamshoro–Hyderabad region. Wastewater samples were obtained from hospital discharge points and selected municipal sewage and drainage locations. Hospital sampling points represented wastewater receiving healthcare effluent, whereas municipal sampling sites represented general urban wastewater not directly collected from hospital discharge outlets.

A total of **80 wastewater samples** were analyzed, comprising 40 hospital wastewater samples and 40 municipal wastewater samples. Samples were collected on multiple occasions from predetermined locations to account for variations in bacterial contamination. Approximately 250–500 mL of wastewater was collected aseptically in sterile labelled containers and transported to the laboratory under appropriate conditions. Sampling source, location, date, and relevant environmental observations were recorded.

Wastewater samples were processed using standard microbiological procedures. Appropriate aliquots and serial dilutions were inoculated onto selective and differential culture media. Following incubation, morphologically distinct colonies were subcultured to obtain pure bacterial isolates. Identification was based on colony characteristics, Gram staining, and standard biochemical tests. The analysis focused primarily on Gram-negative bacteria of environmental and clinical relevance, including *Escherichia coli*, *Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., and *Acinetobacter* spp.

Antimicrobial susceptibility testing was performed using the **Kirby–Bauer disk diffusion method** on Mueller–Hinton agar. A bacterial suspension equivalent to a 0.5 McFarland standard was prepared for each isolate. The antimicrobial panel included ampicillin, ceftriaxone, ceftazidime, ciprofloxacin, gentamicin, meropenem, tetracycline, and trimethoprim-sulfamethoxazole. Following incubation, inhibition-zone diameters were measured and interpreted according to the applicable **Clinical and Laboratory Standards Institute (CLSI)** criteria. Appropriate reference strains were used for quality control.

Multidrug resistance was defined as acquired non-susceptibility to at least one antimicrobial agent in three or more antimicrobial categories. Each isolate was assigned a unique identification code and remained linked to its original wastewater source throughout analysis.

Data were analyzed using **IBM SPSS Statistics**. Categorical variables were presented as frequencies and percentages. The chi-square test or Fisher's exact test was used to compare bacterial distribution, individual antimicrobial resistance rates, and MDR prevalence between hospital and municipal wastewater isolates. Odds ratios with 95% confidence intervals were calculated for relevant comparisons. A *p*-value of <0.05 was considered statistically significant.

Approval was obtained from the relevant institutional research and ethical review mechanism of the **University of Sindh, Jamshoro**, and administrative permission was obtained from the relevant sampling sites. No human participants or personally identifiable information were included. Environmental samples and bacterial cultures were handled according to appropriate laboratory biosafety procedures.

Results

A total of 80 wastewater samples were analyzed, comprising 40 hospital and 40 municipal wastewater samples. Overall, 126 Gram-negative bacterial isolates were recovered, including 72 (57.1%) from hospital wastewater and 54 (42.9%) from municipal wastewater.

Escherichia coli was the most frequently isolated organism (34.1%), followed by *Klebsiella* spp. (23.8%), *Pseudomonas* spp. (17.5%), *Enterobacter* spp. (13.5%), and *Acinetobacter* spp. (11.1%). The distribution of individual bacterial species did not differ significantly between hospital and municipal wastewater.

Table 1. Distribution of bacterial isolates according to wastewater source

Bacterial isolate	Hospital n (%)	Municipal n (%)	Total n (%)	p-value
Escherichia coli	23 (31.9)	20 (37.0)	43 (34.1)	0.548
Klebsiella spp.	19 (26.4)	11 (20.4)	30 (23.8)	0.432
Pseudomonas spp.	12 (16.7)	10 (18.5)	22 (17.5)	0.789
Enterobacter spp.	9 (12.5)	8 (14.8)	17 (13.5)	0.707
Acinetobacter spp.	9 (12.5)	5 (9.3)	14 (11.1)	0.568
Total	**72 (100)**	**54 (100)**	**126 (100)**	—

Hospital wastewater isolates demonstrated generally higher antimicrobial resistance than municipal wastewater isolates. Resistance to ceftriaxone, ceftazidime, ciprofloxacin, meropenem, tetracycline, and trimethoprim-sulfamethoxazole was significantly more frequent among hospital isolates. Ampicillin resistance was common in both groups, whereas gentamicin resistance showed no statistically significant difference.

Table 2. Antimicrobial resistance according to wastewater source

Antibiotic	Hospital (n=72)	Municipal (n=54)	p-value
Ampicillin	56 (77.8%)	34 (63.0%)	0.069
Ceftriaxone	51 (70.8%)	26 (48.1%)	**0.010**
Ceftazidime	46 (63.9%)	25 (46.3%)	**0.049**
Ciprofloxacin	44 (61.1%)	21 (38.9%)	**0.014**
Gentamicin	30 (41.7%)	16 (29.6%)	0.164
Meropenem	18 (25.0%)	6 (11.1%)	**0.049**
Tetracycline	48 (66.7%)	24 (44.4%)	**0.013**
Trimethoprim-sulfamethoxazole	47 (65.3%)	23 (42.6%)	**0.011**

Multidrug resistance was detected in 74 of the 126 isolates, giving an overall prevalence of 58.7%. MDR was significantly more common among hospital wastewater isolates than municipal wastewater isolates (68.1% versus 46.3%, p=0.014).

Table 3. Multidrug resistance according to wastewater source

MDR status	Hospital n (%)	Municipal n (%)	Total n (%)
MDR	49 (68.1)	25 (46.3)	74 (58.7)
Non-MDR	23 (31.9)	29 (53.7)	52 (41.3)
Total	**72 (100)**	**54 (100)**	**126 (100)**

Hospital wastewater isolates had approximately **2.5 times greater odds of multidrug resistance** than municipal wastewater isolates (OR=2.49; 95% CI: 1.20–5.18; p=0.014).
Overall, hospital wastewater demonstrated a greater burden of antimicrobial resistance, particularly against cephalosporins, fluoroquinolones, tetracycline, trimethoprim-sulfamethoxazole, and meropenem.

Figure 1. Distribution of bacterial species recovered from hospital and municipal wastewater.

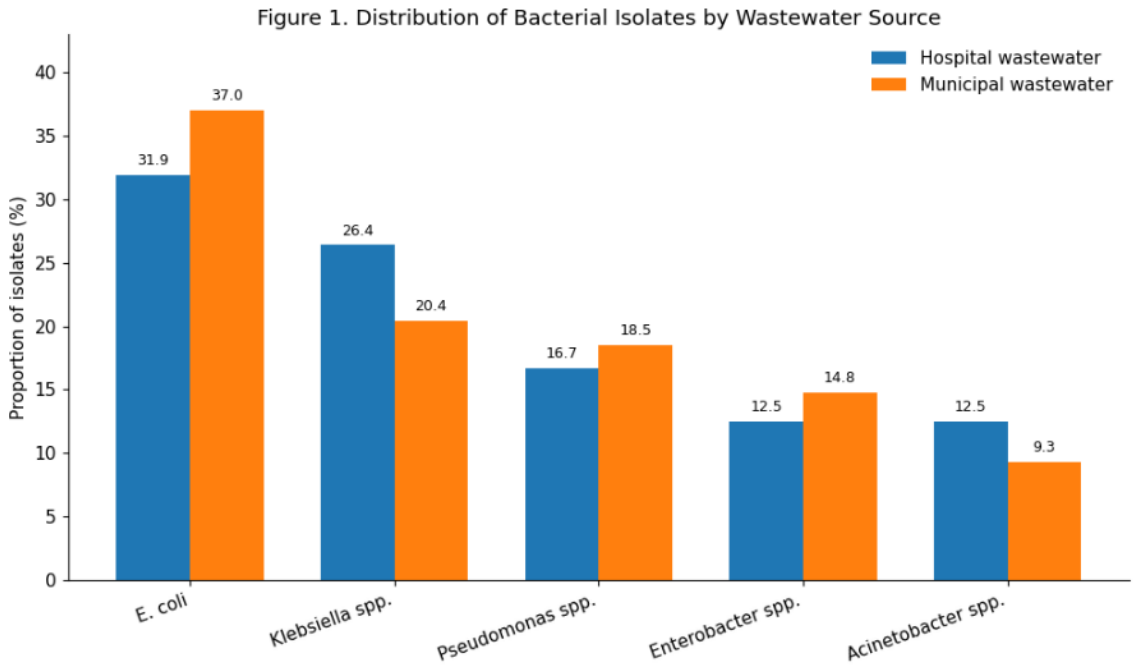
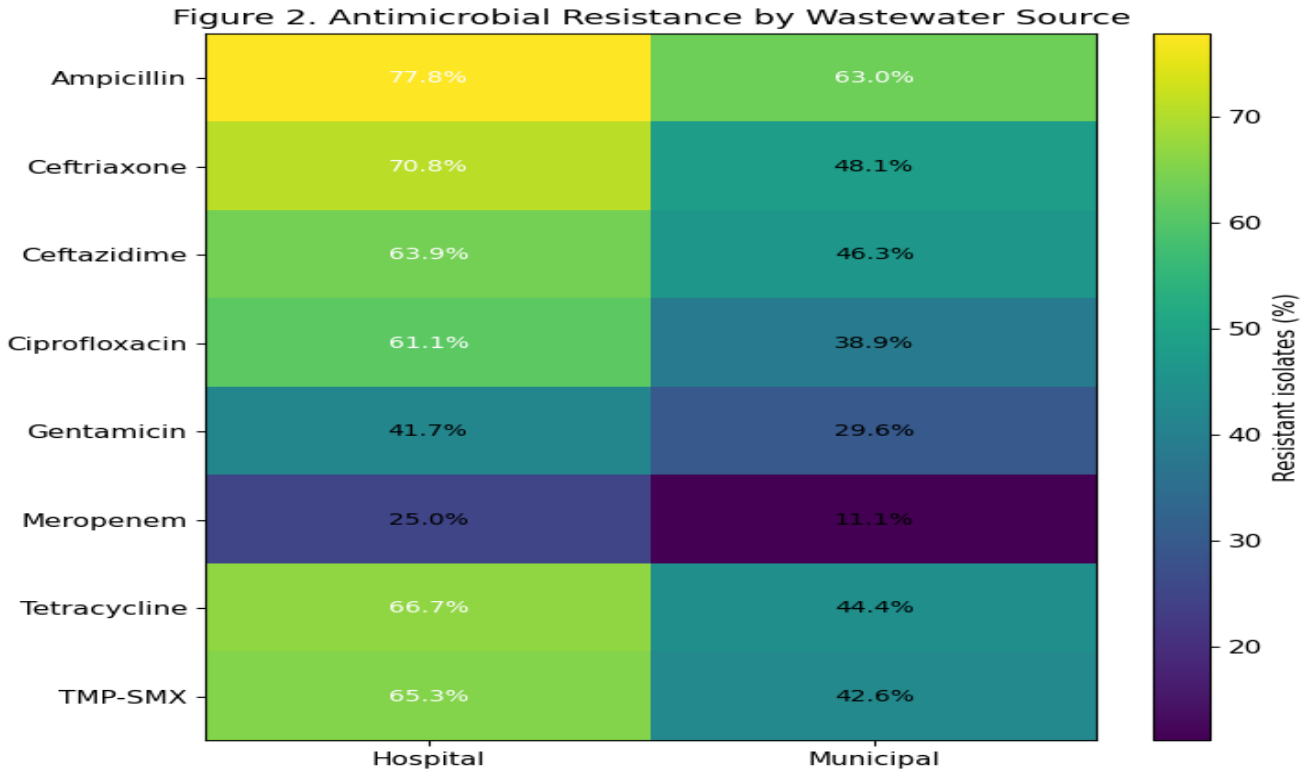


Figure 2. Heatmap showing antimicrobial resistance patterns according to wastewater source and bacterial species.



Discussion

The present study demonstrated a substantial burden of antimicrobial-resistant Gram-negative bacteria in both hospital and municipal wastewater, with significantly greater resistance among isolates recovered from hospital wastewater. More than half of all bacterial isolates fulfilled the criteria for multidrug resistance, and hospital wastewater isolates had approximately 2.5-fold greater odds of MDR compared with municipal wastewater isolates. These findings support growing evidence that wastewater represents an important environmental reservoir of antimicrobial-resistant bacteria.[2,3,6]

Escherichia coli was the most frequently recovered organism, followed by *Klebsiella, Pseudomonas, Enterobacter*, and *Acinetobacter* species. The predominance of *E. coli* is consistent with its widespread occurrence in human intestinal flora and its established role as an indicator of fecal contamination.[7] The presence of these organisms in both wastewater categories demonstrates that potentially resistant Gram-negative bacteria are not restricted to healthcare effluent but are also widely distributed within municipal wastewater.

Despite similarities in bacterial distribution, important differences were observed in antimicrobial susceptibility. Hospital isolates showed significantly greater resistance to ceftriaxone, ceftazidime, ciprofloxacin, meropenem, tetracycline, and trimethoprim-sulfamethoxazole. Previous investigations have similarly reported higher concentrations of resistant organisms in hospital wastewater than in community or municipal wastewater.[4,5,11] Hospital effluent receives microorganisms originating from patients together with antimicrobial residues and disinfectants, potentially creating conditions favorable for the persistence of resistant bacterial populations.

The high resistance to third-generation cephalosporins was particularly noteworthy. Ceftriaxone resistance was detected in approximately 71% of hospital isolates compared with 48% of municipal isolates. Comparable resistance to cephalosporins has been reported among Gram-negative organisms recovered from healthcare-associated wastewater environments.[11,12] This may reflect the frequent clinical use of broad-spectrum β-lactam agents and the discharge of resistant organisms through healthcare wastewater streams.

Ciprofloxacin resistance also differed substantially between the two wastewater sources. More than 60% of hospital isolates were resistant compared with approximately 39% of municipal isolates. Fluoroquinolone-resistant organisms have been detected in wastewater systems internationally, supporting concerns that environmental discharge may contribute to the persistence and wider circulation of resistant bacteria.[13] The higher resistance observed in hospital wastewater may reflect greater antimicrobial exposure within healthcare settings, although antimicrobial utilization was not directly assessed in the present study.

Meropenem resistance was less frequent than resistance to several other antimicrobial agents but remained significantly higher among hospital isolates. This observation is environmentally important because carbapenems are generally reserved for the treatment of severe infections caused by resistant Gram-negative organisms. Detection of carbapenem-resistant bacteria outside clinical environments has been increasingly recognized as an important component of environmental AMR surveillance.[14]

Multidrug resistance represented the principal finding of the study. Approximately 68% of hospital isolates were MDR compared with 46% of municipal isolates. Similar investigations have demonstrated that hospital wastewater can contain high proportions of MDR bacteria and may therefore represent an interface between clinical antimicrobial resistance and the wider environment.[5,11] However, the substantial MDR prevalence observed in municipal wastewater is equally important. Municipal sewage integrates microorganisms originating from households and broader community populations and therefore provides insight into antimicrobial resistance circulating outside hospitals.

The presence of resistant bacteria in both wastewater sources supports a broader One Health interpretation of antimicrobial resistance. Resistant organisms released into drainage systems may interact with environmental microbial communities and potentially reach receiving water bodies. Consequently, clinical antimicrobial stewardship alone may be insufficient without complementary attention to wastewater management and environmental surveillance.[3,15]

The present study used conventional culture and antimicrobial susceptibility testing, which provided a relatively simple and practical approach for identifying viable resistant organisms. This approach may be particularly useful in settings where advanced molecular surveillance is not routinely available. Nevertheless, culture-based methods do not characterize the genetic mechanisms responsible for resistance and may not detect non-culturable organisms. Future studies incorporating molecular or genomic approaches could provide additional information regarding resistance determinants and their environmental transmission.

The study had some limitations. Sampling was restricted to selected sites within the Jamshoro–Hyderabad region, and the findings should therefore not be generalized to all wastewater systems. Antibiotic residues and other environmental contaminants were not quantified, and molecular mechanisms of resistance were not investigated. Despite these limitations, the direct comparison of hospital and municipal wastewater using standardized microbiological procedures provides useful local evidence regarding environmental antimicrobial resistance.

Overall, hospital wastewater contained a greater burden of antimicrobial-resistant and multidrug-resistant Gram-negative bacteria than municipal wastewater. However, the substantial resistance observed in both environments emphasizes the need to consider wastewater as an important component of antimicrobial resistance surveillance and environmental health protection.

Conclusion

Hospital and municipal wastewater contained substantial numbers of antimicrobial-resistant Gram-negative bacteria, with significantly higher multidrug resistance among hospital wastewater isolates. These findings highlight wastewater as an important environmental reservoir of antimicrobial resistance. Routine microbiological monitoring and improved management of healthcare wastewater may help reduce the environmental dissemination of resistant bacteria.

Recommendations

Regular surveillance of antimicrobial-resistant bacteria in hospital and municipal wastewater is recommended. Appropriate treatment of healthcare effluent should be strengthened before its discharge into municipal wastewater systems.

Limitations

The study was limited to selected wastewater sites and culture-based antimicrobial susceptibility testing. Antibiotic residues and molecular resistance mechanisms were not investigated.

Conflict Of Interest

The authors declared no conflict of interest.

Funding

No external funding was received.

Data Availability

Study data are available from the corresponding author upon reasonable request.

Ethical Approval

Approval was obtained from the relevant institutional research and ethical review mechanism of the University of Sindh, Jamshoro, with administrative permission from the relevant sampling sites.

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