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Research Article

ISOLATION AND IDENTIFICATION OF ESKAPEE PATHOGENS IN HOSPITAL WASTEWATER OF KATHMANDU VALLEY

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ABSTRACT

The ESKAPEE pathogens—comprising *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, *Enterobacter* species, and *Escherichia coli*—represent a critical group of antibiotic-resistant bacteria frequently detected in hospital and municipal wastewater systems. This study evaluated the physicochemical parameters, isolation profiles and antibiotic resistance patterns of ESKAPEE pathogens in hospital wastewater (HWW) within the Kathmandu Valley, Nepal. A cross-sectional study was conducted between March 2024 and May 2024, during which nine HWW samples were collected via the grab sampling method from seven major hospitals. Bacterial isolation and identification were performed through cultural and biochemical analysis, followed by antibiotic susceptibility testing (AST) using the Kirby–Bauer disk diffusion method. The dissolved oxygen (DO) concentration varied widely among the samples, ranging from 0.813 mg/l to 3.6585 mg/ml, which was below the WHO recommended standard. The pH values also exhibited considerable variation among the samples, with 4.4 and 9.5 representing the lowest and highest values, respectively. Out of 19 bacterial isolates, the most prevalent pathogens were *Escherichia coli* (32%), *Klebsiella pneumoniae* (26%), and *Enterococcus faecium* (21%). Linezolid and gentamicin demonstrated the highest efficacy against Gram-positive isolates, whereas amikacin and chloramphenicol were most effective against Gram-negative bacteria. Conversely, ampicillin exhibited the highest overall resistance rate followed by oxacillin and co-trimoxazole. Notably, 42% of the isolates were classified as multidrug-resistant (MDR). These findings underscore the critical need for continuous surveillance and enhanced wastewater management strategies to mitigate the environmental dissemination of MDR pathogens and safeguard public health in the Kathmandu Valley.

Keywords: Antibiotic susceptibility, ESKAPEE pathogens, Hospital wastewater, Multidrug resistance

INTRODUCTION

Hospital wastewater (HWW) is the water used for a wide range of activities in hospitals, including those related to patient care, medical procedures, cleaning and other activities that occur in hospitals. There are various types of contaminants in the hospital wastewater including formaldehyde, glutaraldehyde, radioactive waste, soap, disinfectants, pathogens, dyes, blood and urine (Khan et al., 2020). Medical waste (solid and liquid) is a serious global challenge, and is an environmental as well as a human health concern (Ahmed et al., 2014). Hospital-generated waste serves as a reservoir of numerous pathogenic bacteria, viruses and fungi, each contributing to potential public health hazards and environmental contamination. HWW containing high concentration of antibiotics and other drugs provides an ideal environment for the development and dissemination of antibiotic-resistant bacteria, making it a major source of environmental contamination (Odoyo et al., 2023).

The acronym "ESKAPE" refers to a group of six rapidly emerging and critical priority antibiotic-resistant pathogens; *Enterococcus faecium*, *Staphylococcus aureus*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, *Pseudomonas aeruginosa* and *Enterobacter* spp (Ballante et al., 2023). In certain contexts, *Escherichia coli* is additionally included, giving rise to the expanded term ESKAPEE (Mahmood et al., 2016; Santajit and Indrawattana, 2016). These organisms are of critical concern due to their remarkable ability to evade the effects of multiple classes of antimicrobial agents, primarily through the acquisition and dissemination of multidrug resistance (MDR) mechanisms (Santajit and Indrawattana, 2016). ESKAPEE pathogens are widely distributed in wastewater, aquatic environments, soil and crops, where they can survive for extended periods. Their capacity to disseminate antibiotic resistance genes and their extensive resistance to multiple antibiotics pose serious concerns for public health and environmental safety (Ngoi et al., 2021; Pandey et al., 2021; Mustafa et al., 2022). ESKAPEE bacteria have different levels of resistance mechanisms; enzymes that inactivate certain antibiotics, change the binding sites for antibiotics, high efflux activity and form biofilm (Santajit & Indrawattana, 2016). The transfer of pathogens from HWW has been documented in several studies as a potential cause of hospital-acquired infections (HAI) Pandey et al., 2021; Ruekit et al., 2022; (Momenah et al., 2023). The presence of ESKAPEE pathogens in HWW poses an immediate public health threat to those directly exposed, including healthcare workers, patients, visitors and also to the general public. In highly populated settings such as Kathmandu, the risk is amplified as contaminated water supplies and food products may facilitate broader community transmission including MDR (Mustafa et al., 2022).

The physiochemical and microbiological properties of HWW can vary and fluctuate due to location and other factors depending on hospital practices and treatment processes, complicating effective management. In Kathmandu, the HWW has a very high load of pathogenic microorganisms, which pose a serious threat of spreading diseases if not properly treated (Pandey et al., 2021). Pathogen (bacteria, viruses and fungi) can be resistant to the conventional treatment process, which indicates the need for more effective disinfection measures for better health protection. Despite increasing evidence of this dissemination, wastewater treatment systems in developing regions are often inadequate, allowing resistant pathogens to persist and spread into natural water bodies. Additionally, there is limited data on the prevalence and antibiotic susceptibility patterns of ESKAPEE pathogens. This lack of localized evidence hinders the development of effective control and treatment strategies. Therefore, there is a critical need to investigate the occurrence and resistance profiles of these pathogens in HWW to better understand their role in environmental contamination and public health risk. The insights gained from this study will pave the way for future research on pathogens management and antimicrobial resistance in HWW and also highlight areas where additional studies are needed, contributing to the broader scientific understanding of wastewater-related health risks.

MATERIALS AND METHODS

Study design

A laboratory based cross-sectional analysis was conducted between March 2024 and May 2024 at Nepalese Farming Institute, Old-Baneshwor and Tri-Chandra Multiple Campus, Ghantaghar, Kathmandu. Sample collection was limited by the study duration, resulting in a total of nine wastewater samples. Seven different hospitals located across Kathmandu Valley were selected for collection of wastewater samples.

Method of sample collection

Samples were collected using the Grab sampling method. For laboratory analysis, sterile 100 mL bottles were filled while sterile 250 mL BOD bottles were used for physicochemical analysis. pH and temperature were measured in situ at the time of sample collection. After collection, the bottles were properly labeled and transported to the laboratory in an ice box within 2–3 hours while maintaining cold chain. If immediate processing was not possible, the samples were stored in a refrigerator until analysis.

Analysis of physicochemical parameters

Physicochemical parameters such as temperature, pH and dissolved oxygen (DO) were analyzed following the Standard Methods for the Examination of Water and Wastewater as recommended by the American Public Health Association (APHA, 2017).

Temperature was measured using a mercury thermometer by immersing the bulb directly into the water sample and recording the reading. The pH of the samples was determined using a pH meter. Dissolved oxygen (DO) was analyzed using the standard Winkler's iodometric method.

Analysis of microbial parameters

Enumeration of bacteria load

Serial dilution of the hospital wastewater (HWW) samples was performed up to 10^{-3} to reduce the microbial load and facilitate the isolation of discrete colonies. The spread plate technique was employed for the enumeration of bacterial populations. Selective and differential media were employed for the isolation and enumeration of target bacterial groups, including Bile Esculin Azide (BEA) agar for *E. faecium*, Mannitol Salt Agar (MSA) for *S. aureus*, MacConkey Agar (MA) for *K. pneumoniae*, Cetrimide Agar (CA) for *P. aeruginosa*, and Eosin Methylene Blue (EMB) agar for *E. coli* (Ben-David

& Davidson, 2014; O'Toole, 2016; Gutierrez et al., 2018).

Identification of ESKAPEE

Agar plates were examined to determine total bacterial counts and assess colony morphology. Isolates were further subjected to Gram staining, motility assessment, and catalase and oxidase tests for preliminary identification, enabling differentiation among members of the *Enterobacteriaceae*, *P. aeruginosa* and *A. baumannii*. Gram-negative isolates were subsequently characterized using a series of standard biochemical assays. *S. aureus* was identified based on selective growth on MSA followed by coagulase and DNase tests. The methylene blue milk test was employed to confirm the presence of *E. faecium*.

Antibiotic susceptibility testing

The modified Kirby–Bauer disc diffusion method on Mueller–Hinton Agar (MHA), as recommended by the Clinical and Laboratory Standards Institute (CLSI), was employed for antimicrobial susceptibility testing (AST) of both Gram-positive and Gram-negative bacterial isolates (CLSI 2020). A standardized inoculum for each isolate was prepared using the 0.5 McFarland turbidity standard. The inoculum was then uniformly inoculated onto MHA plates using sterile swabs to obtain a confluent lawn culture. After allowing the surface to dry, antibiotic discs representing different classes were aseptically placed on the agar surface, followed by incubation at 37°C for 24 hours. The diameters of the zones of inhibition (ZOI) around each antibiotic disc were subsequently measured and recorded. The antibiotics tested against Gram-negative isolates were ampicillin (10µg), ceftazidime (30µg), gentamicin (10µg), ciprofloxacin (5µg), cotrimoxazole (25µg), cefotaxime (30µg), piperacillin/tazobactam (100/10µg), amikacin (30µg), tetracycline (30µg), nalidixic acid (30 µg), chloramphenicol (30µg), imipenem (10µg) while those tested against Gram-positive isolates were cotrimoxazole (25µg), penicillin-G (10µg), clindamycin (2µg), tetracycline (30µg), erythromycin (15µg), ciprofloxacin (5µg), gentamicin (10µg), oxacillin (1µg), cefoxitin (30µg) and linezolid (30µg).

MDR isolates were defined as those resistant to one antibiotic from two or more antibiotic classes.

Screening for methicillin resistance was performed by cefoxitin disc diffusion method and interpreted according to CLSI (2020). Isolates with diameter of ZOI ≥ 22mm were identified as Methicillin sensitive *S. aureus* (MSSA) and isolates with ZOI ≤ 21mm identified as Methicillin resistant *S. aureus* (MRSA).

Detection of Extended-Spectrum β-Lactamase (ESBL) producers

Cefotaxime (30 µg) and Ceftazidime (30 µg) discs were used for the initial screening of ESBL production among Gram-negative isolates of ESKAPEE bacteria. Isolates were considered potential ESBL producers when the ZOI was ≤22 mm for Ceftazidime and ≤27 mm for Cefotaxime. Isolates suspected of ESBL production during screening were further confirmed using the combined disc method. In this procedure, a standardized lawn culture of each isolate was prepared on MHA and discs of Cefotaxime and Ceftazidime, both alone and in combination with clavulanic acid (10 µg), were applied. An increase of ≥5 mm in the ZOI for either antibiotic in combination with clavulanic acid compared to the antibiotic alone was interpreted as confirmatory evidence of ESBL production, in accordance with CLSI guidelines (CLSI, 2020).

Data analysis

Microsoft Excel was used for data entry and statistical analysis. The findings were summarized and presented using appropriate statistical tables and graphs.

RESULTS AND DISCUSSION

The temperature of hospital wastewater ranged from 19°C to 24°C (Table 1). Similar variations have been reported in studies from Peru and other regions (Custodio et al., 2022; Hossain et al., 2023). The observed differences in wastewater temperature are likely influenced by gAnswer eographical location, climatic conditions, seasonal variation and wastewater management practices. The pH values ranged from 4.4 to 9.5 (Table 1). While most samples fell within the acceptable range recommended by national and international standards, one sample was highly acidic and another was slightly alkaline. But study by Hossain et al (2023) reported pH range of 5.39-8.35 in HWW of Dhaka city, Bangladesh. Variations in pH may be attributed to differences in hospital activities, chemical discharges, cleaning agents, pharmaceutical residues, and wastewater treatment practices.

Dissolved oxygen concentrations ranged from 0.813 to 3.659 mg/L, indicating poor oxygenation of the wastewater (Table 1). Although some values were slightly higher than those reported in previous studies (Adanlokonon et al., 2018; Al-Khafaji & Al-Rekabi, 2021), all samples exhibited low DO concentrations, suggesting a high organic load and microbial activity. Such conditions may impair natural self-purification processes and reduce the efficiency of aerobic wastewater treatment systems.

Table 1. Physicochemical characteristics of hospital wastewater samples collected from Kathmandu Valley hospitals

S.N.	Sample code	Temperature	pH	DO (mg/l)
1.	S1	21°C	7.4	2.0325
2.	S2	19°C	4.4	3.6585
3.	S3	23°C	7.3	2.439
4.	S4	22°C	7.7	1.626
5.	S5	19°C	9.5	3.2520
6.	S6	20°C	7.5	0.813
7.	S7	24°C	6.8	0.813
8.	S8	23°C	5.4	2.0325
9.	S9	22°C	8.2	1.2195

The highest total bacterial counts were observed on EMB agar in samples S7 (2.52×10^6) and S8 (1.16×10^6), while BEA agar showed a maximum count of 2.06×10^6 in sample S8. No bacterial growth was detected in any of the culture media used for samples S1 and S9 (Table 2). The absence of detectable growth on the selective media may indicate effective wastewater treatment; however, it could also result from low bacterial concentrations, sampling variability or limitations of culture-based detection methods.

Table 2. Total bacterial counts (CFU/mL) on selective culture media in hospital wastewater samples from Kathmandu Valley

Sample code	BEA	MSA	MA	CA	EMB
S1	No growth	No growth	No growth	No growth	No growth
S2	3.04×10^5	9×10^2	3.7×10^4	1.8×10^3	1.07×10^4
S3	3.4×10^5	No growth	2.9×10^5	2.04×10^4	3.2×10^5
S4	1.9×10^5	No growth	1.9×10^5	No growth	3.2×10^5
S5	3.48×10^4	2.2×10^3	4.3×10^5	3×10^2	2.4×10^5
S6	No growth	6.4×10^3	4.1×10^5	1.9×10^5	1.0×10^5
S7	3.2×10^5	1.6×10^4	2.88×10^4	No growth	2.52×10^6
S8	2.03×10^6	4.3×10^3	3.7×10^5	1.1×10^5	1.16×10^6
S9	No growth	No growth	No growth	No growth	No growth

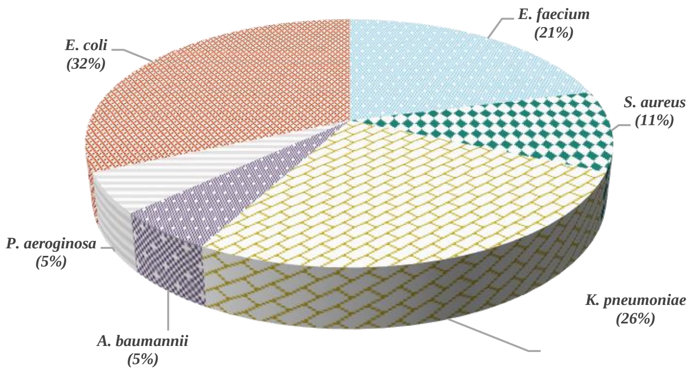

Figure 1. Distribution of ESKAPEE pathogens isolated from hospital wastewater samples collected from Kathmandu Valley hospitals

A total of 19 ESKAPEE bacterial isolates were recovered from the processed HWW samples. A substantial proportion of the bacteria isolated from HWW samples were Gram-negative organisms, accounting for 68% (n = 13/19), while the remaining 32% (n = 6/19) were Gram-positive bacteria. Among the identified pathogens, *E. coli* was the most frequently isolated species, representing 32% (n = 6/19), followed by *K. pneumoniae* at 26% (n = 5/19) and *E. faecium* at 21% (n = 4/19). Additionally, *S. aureus* was detected in 11% (n = 2/19) of isolates, whereas both *A. baumannii* and *P. aeruginosa* were each confirmed at 5% (n = 1/19). No *Enterobacter* spp were recovered from the samples (Fig. 1).

E. coli has consistently been reported as the most prevalent pathogen in various environmental and clinical studies. For instance, a European surveillance report (Zarb et al., 2012) indicated that *E. coli* accounted for approximately 70% of invasive Gram-negative bacterial isolates, with a notable prevalence in community-acquired infections, particularly urinary tract infections (UTIs). Similarly, a study investigating wastewater from clinical and non-clinical sources identified 43 ESKAPEE isolates among 92 selected bacterial isolates, with *E. coli* and *P. aeruginosa* predominating in hospital effluent, while *K. pneumoniae* was mainly associated with pharmaceutical wastewater. Gram-positive cocci, including *S. aureus* and *Enterococcus* spp, were predominantly recovered from HWW, highlighting their strong association with clinical environments (Mustafa et al., 2022). In addition, a study conducted in poultry slaughterhouse wastewater reported ESKAPEE pathogens in 87.5% of samples, with *E. coli* as the most prevalent organism, followed by *A. baumannii, S. aureus* and *K. pneumoniae* (Marutescu et al., 2023).

Overall, the comparative analysis indicates that the present findings are consistent with previously reported trends on the distribution of ESKAPEE pathogens in wastewater environments. The dominance of *E. coli* underscores its persistence and epidemiological significance in both community and healthcare-associated settings, while *K. pneumoniae* and *E. faecium* further contribute to the observed burden of clinically important resistant pathogens.

Table 3 Antibiotic Susceptibility pattern of *E. faecium* and *S. aureus*

Antibiotics	**Class**	***E. faecium* (n=4)**		***S. aureus* (n=2)**	
		Resistant	**Sensitive**	**Resistant**	**Sensitive**
Penicillin	Penicillin	0	4 (100%)	2 (100%)	0
Tetracycline	Tetracyclines	2 (50%)	2 (50%)	0	2 (100%)
Cotrimoxazole	Sulfonamides	4 (100%)	0	1 (50%)	1 (50%)
Erythromycin	Macrolides	4 (100%)	0	1 (50%)	1 (50%)
Ciprofloxacin	Fluoroquinolones	3 (75%)	1 (25%)	0	2 (100%)
Oxacillin	Penicillin	4 (100%)	0	1 (50%)	1 (50%)
Linezolid	Oxazolidines	0	4 (100%)	0	2 (100%)
Clindamycin	Lacosamide	4 (100%)	0	0	2 (100%)
Gentamicin	Aminoglycosides	1 (25%)	3 (75%)	0	2 (100%)
Cefoxitin	Cephalosporins	4 (100%)	0	0	2 (100%)

All four *E. faecium* isolates demonstrated 100% susceptibility to penicillin and linezolid, whereas complete resistance (100%) was observed against oxacillin, erythromycin, clindamycin and cefoxitin (Table 3). Similar resistivity pattern was reported by other studies (Sattari-Maraji et al., 2019). The universal susceptibility to linezolid agrees with previous reports, indicating that linezolid remains an effective therapeutic option against *E. faecium* infections (Horner et al., 2021).

Both *S. aureus* isolates exhibited complete susceptibility to ciprofloxacin, gentamicin, clindamycin, tetracycline, cefoxitin, and linezolid. Resistance was observed only against oxacillin, with one isolate (50%) classified as resistant (Table 3). These findings indicate that most tested antibiotics remain effective against *S. aureus* isolates recovered from HWW. Similar to our finding, Yakut et al., (2024) reported that *S. aureus* isolates were fully susceptible to linezolid and vancomycin in the wastewater samples. Another study conducted on clinical isolates; *S. aureus* showed a susceptibility rate of 78.1% to ciprofloxacin (Derakhshan et al., 2021). While this is lower than the reported 100% susceptibility in the wastewater samples, it still reflects a significant level of effectiveness for these antibiotics against *S. aureus* in certain contexts, indicating that susceptibility can vary based on source of the isolates.

Table 4 Antibiotic Susceptibility pattern of *K. pneumoniae* and *E. coli*

Antibiotics	**Class**	***K. pneumoniae* (n=5)**		***E. coli* (n=6)**	
		Resistant	**Sensitive**	**Resistant**	**Sensitive**
Cotrimoxazole	Sulfonamides	0	5(100%)	0	6(100%)
Ciprofloxacin	Fluoroquinolones	1(20%)	4(80%)	2(33.33%)	4 (66.67%)
Cefotaxime	Cephalosporin	3 (60%)	2(40%)	2(33.33%)	4 (66.67%)
Ceftazidime	Cephalosporin	3 (60%)	2(40%)	3(50%)	3(50%)
Piperacillin-Tazobactam	Beta-Lactamase	2 (40%)	3(60%)	1(16.67%)	5(83.3%)
Imipenem	Carbapenems	1(20%)	4(80%)	4 (66.7%)	2 (33.33%)
Amikacin	Aminoglycosides	0	5(100%)	0	6(100%)
Ampicillin	Penicillin	5(100%)	0	5(83.3%)	1 (16.66%)
Gentamicin	Aminoglycosides	0	5(100%)	0	6(100%)
Tetracycline	Tetracycline	0	5(100%)	2(33.33%)	4 (66.67%)
Nalidixic acid	Quinolones	2(40%)	3(60%)	5(83.3%)	1 (16.67%)
Chloramphenicol	Phenicol	0	5(100%)	0	6(100%)

All *K. pneumoniae* isolates were susceptible to cotrimoxazole, amikacin, gentamicin, tetracycline, and chloramphenicol. In contrast, complete resistance to ampicillin was observed among all isolates (Table 4). These findings suggest that aminoglycosides and selected non-β-lactam antibiotics remain effective treatment options against *K. pneumoniae* isolates recovered from hospital wastewater. Research has consistently shown that *K. pneumoniae* isolates often display elevated resistance rates to ampicillin. For instance, a study conducted on *K. pneumoniae* from HWW in Egypt revealed that the presence of different beta lactamases genes in all isolates resulted in resistance to ampicillin (Abdelwahab et al., 2021). A study investigating carbapenem-resistant *K. pneumoniae* isolated from hospital wastewater in Romania reported that 91.4% of the isolates harbored the *bla*$_{\text{KPC}}$ gene, a major determinant of resistance to carbapenems and several other β-lactam antibiotics (Popa et al., 2021). This finding suggests that β-lactam resistance is a common characteristic among *K. pneumoniae* isolates recovered from healthcare-associated environments. Furthermore, the detection of carbapenemase-producing *K. pneumoniae* in HWW highlights the potential role of healthcare effluents as reservoirs and dissemination pathways for clinically significant antimicrobial resistance. The presence of these highly resistant pathogens in wastewater is of particular concern because infections caused by carbapenem-resistant strains are associated with limited treatment options and poor clinical outcomes. Therefore, effective wastewater treatment and continuous surveillance are crucial to reduce the environmental release and spread of carbapenem-resistant *K. pneumoniae* and other antimicrobial-resistant bacteria.

The *E. coli* isolates exhibited complete susceptibility to cotrimoxazole, amikacin, gentamicin and chloramphenicol. The highest resistance was observed against ampicillin and nalidixic acid (83.3% each) followed by imipenem (66.7%) (Table 4). Similar resistivity has been observed among *E. coli* isolated from HWW in various studies (Paulshus et al., 2019; Daoud et al, 2020). These findings indicate the presence of clinically relevant antimicrobial resistance among environmental *E. coli* isolates and emphasize the importance of continuous surveillance. The elevated resistance to ampicillin may reflect both the accumulation of antibiotic residues and the enrichment of resistant bacterial populations within hospital wastewater.

Although six isolates were initially suspected to be ESBL producers based on preliminary screening, none were confirmed by the phenotypic confirmatory test. Therefore, no ESBL-producing *E. coli* or *K. pneumoniae* isolates were detected in the present study. In contrast to the findings of the present study, a higher prevalence of ESBL-producing isolates has been reported in HWW from different countries (Korzeniewska et al., 2013; Paulshus et al., 2019; Gaspar et al., 2021). The observed variation may be attributed to differences in antibiotic prescribing practices, infection control measures, wastewater management systems and the local epidemiology of antimicrobial resistance. Furthermore, differences in study design, sampling strategies, bacterial species investigated, sample size, and laboratory methods used for ESBL detection may have contributed to the variation in prevalence rates.

Of the 19 isolates recovered, 8 (42.1%) were identified as MDR, whereas 11 (57.9%) were non-MDR isolates. A study conducted in Nepal reported an alarmingly high prevalence of MDR pathogens, with rates reaching up to 91.3% in some cases, particularly in developing regions where antibiotic misuse is rampant (Shilpakar et al., 2021). Another study found that 42.5% of isolates from patients with HAIs were MDR, closely aligning with this findings (Wang et al., 2019). This study's rate suggests a stabilization compared to the highest reported figures but still reflects a significant public health challenge. A study comparing HWW to community wastewater found lower resistance rates in community settings, indicating that hospital environments are hotspots for MDR bacteria. For instance, community wastewater showed lower isolation rates of MDR *E. coli* and *K. pneumoniae* compared to those found in HWW (Gaşpar et al., 2021).

The highest MDR isolates were *E. faecium* (37.5%) followed by *E. coli* (25%) and the rest *K. pneumoniae*, *A baumannii*, *P. aeruginosa* were 12.5% each (Fig. 2). In a study in Ghana, 55.4% of bacteria isolated from HWW were MDR with the predominant ones being *E. coli* (30.6%), *K. pneumoniae* (11.2%) (Addae-Nuku et al., 2022). Seguni et al., (2023) reported that out of 517 isolates, a significant portion (186 *E. coli* and 101 *K. pneumoniae*) were found to be MDR, highlighting a much higher prevalence compared to this result. However, it is still significant and indicates a need for ongoing surveillance and effective management strategies to reduce the prevalence of MDR bacteria in HWW.

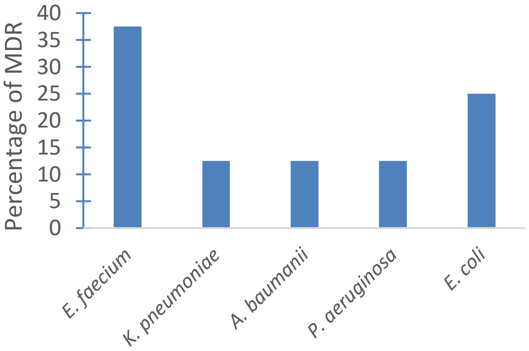

Figure 2. Distribution of multidrug-resistant ESKAPEE isolates recovered from hospital wastewater samples

CONCLUSION

Hospital wastewater in Kathmandu Valley was found to harbor multiple ESKAPEE pathogens with a high prevalence of multidrug resistance, underscoring a serious public health concern. The detection of MDR organisms such as *K. pneumoniae*, *A. baumannii*, *P. aeruginosa*, *E.s faecium*, *E. coli*, highlights the role of hospital effluents as reservoirs of resistant bacteria. The high resistance rates indicate reduced effectiveness of commonly used antibiotics against these pathogens. Substandard physicochemical parameters, including pH and dissolved oxygen levels, further indicate inadequate wastewater treatment. Overall, these findings stress the urgent need for improved HWW treatment, strict infection control practices and rational antibiotic use to prevent the environmental dissemination of antimicrobial resistance and protect public health.

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AUTHORS CONTRIBUTION

Conceptualization: GS, SM; Methodology: GS, BK. JNA, SM; Validation: SP, SM; Investigation: GS, BK; Data analysis: SP, SM; Writing-original draft: GS, BK, JNA; Writing-review & editing: SM; Supervision: SM

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CONFLICTS OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this article.

ETHICAL STATEMENT

This is an original work and has not been previously published or submitted for publication elsewhere. Ethical statement was not required for this research work as the sample used was hospital waste water.

DATA AVAILABILITY STATEMENT

The data that supports the findings of this study are available from the corresponding author, upon reasonable request.

SUPPLEMENTARY INFORMATION

None

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