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Original Article

Antimicrobial Resistance Pattern of Bacterial Isolates from ICU Patients in a Tertiary Care Hospital

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ABSTRACT

Objective: To determine the frequency and antimicrobial resistance (AMR) pattern of bacterial isolates among intensive care unit (ICU) patients in a tertiary care hospital.

Methodology: This cross-sectional descriptive study was carried out in the Microbiology Laboratory of Provincial Headquarter Hospital, Gilgit, from October 2025 to March 2026 after ethical approval. After taking informed consent, 150 culture specimens received from ICU patients were tested by non-probability consecutive sampling. Each specimen was inoculated onto the appropriate culture media, then incubated and carefully identified based on colony morphology, Gram stain, and biochemical tests. The antimicrobial sensitivity testing (AST) was performed by the Kirby-Bauer disc diffusion method. The antibiotic zone diameters were reported following the Clinical and Laboratory Standards Institute (CLSI) guidelines 2025. The statistical analysis was done by the Statistical Package for the Social Sciences (SPSS) version 27.

Results: The most common pathogen isolated was *Staphylococcus* (31.6%), succeeded by *Escherichia coli* (17.3%), *Klebsiella pneumoniae* (15.3%), *Pseudomonas aeruginosa* (13.3%), *Acinetobacter baumannii* (10.2%), *Enterococcus species* (9.2%), and *Proteus* species (3.1%). *Staphylococcus aureus*, Coagulase-negative *Staphylococcus* species (CoNS) and *Enterococcus* species were sensitive to vancomycin and linezolid. *Staphylococcus aureus* and CoNS showed less resistance to gentamicin (35.7% versus 23.5%) and doxycycline (28.6% and 17.6%). Carbapenems showed 100% susceptibility in all Gram-negative isolates. Piperacillin-tazobactam, amikacin and nitrofurantoin showed better sensitivities as compared to other antibiotics. *Escherichia coli, Klebsiella pneumoniae, Proteus* and *Pseudomonas* showed 29.4%, 33.3%, 0% & 38.5% resistance to piperacillin-tazobactam and 17.6%, 20%, 33.3% & 46.1% to amikacin. Resistance to nitrofurantoin was 27.3% for *E. coli*, 0% for *Klebsiella pneumoniae*, and *Proteus* species. *Acinetobacter baumannii* exhibited higher antibiotic resistance.

Conclusion: Gram-negative bacteria constituted 59.2%, whereas Gram-positive bacteria made up 40.8% of the isolates. Around 71.4% isolates of *Staphylococcus aureus* were methicillin-resistant and 70.6% *Escherichia coli* & 73.3% *Klebsiella pneumoniae* were extended-spectrum beta-lactamase (ESBL) producers. A higher antimicrobial resistance was seen in the isolates.

Keywords: *Drug resistance. Bacterial infections. Anti-bacterial agents. Intensive care units.*

INTRODUCTION

Intensive care units (ICUs) have a central role in managing critically ill patients. However, these units also present a major challenge for infection control because patients are often immunocompromised, require prolonged hospital stays and extensive antibiotic therapy.[1,2] Environmental surfaces and fomites in ICUs can act as important reservoirs for infectious microorganisms. These fomites include medical equipment, bed rails, doorknobs, countertops, ventilators, catheters, and other frequently touched surfaces.[3] Patients in ICU often require invasive devices which makes them more vulnerable to infections. In addition, multidrug-resistant (MDR) organisms may arise and spread as a result of regular usage of antibiotics in these patients.[1] Healthcare-associated infections (HAIs) remain a significant public health issue in ICUs across the world. These infections not only increase morbidity and mortality but also prolong hospital stays and add substantial financial burden to health care systems.[3] The World Health Organization estimates 42.7 million cases of HAIs each year, emphasizing the significant global impact of these infections. In the United States, the estimated annual cost associated with HAIs ranges from $96 to $147 billion, whereas in Africa, the annual economic burden is estimated at nearly $13 billion. Studies have consistently shown that patients admitted to ICUs are at a much higher risk of developing HAIs compared with patients in general hospital wards.[4] Patients admitted to ICUs are particularly vulnerable to infections caused by *Staphylococcus aureus*, *Enterococcus* species, *Escherichia coli (E. coli)*, *Klebsiella pneumoniae*, *Acinetobacter baumannii*, and *Pseudomonas aeruginosa*. These organisms are a growing concern worldwide due to their resistance to multiple antibiotics and their association with high morbidity and mortality rates.[5] Detecting infections early in ICU patients can be difficult because common clinical signs such as fever and tachycardia are often nonspecific in critically ill

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individuals. For this reason, laboratory tests such as complete blood count (CBC), C-reactive protein (CRP), and microbiological cultures are essential for accurate diagnosis, appropriate antimicrobial therapy, and infection prevention measures.[6] Effective cleaning, disinfection, and adherence to infection control guidelines are therefore essential to reduce contamination and prevent healthcare-associated infections.[7] The purpose of this study was to ascertain the frequency and AMR pattern of bacterial isolates among ICU patients in a tertiary care facility. Such efforts can help lower the burden of HAIs, decrease the emergence of MDR organisms, and improve prognosis in ICU patients.

METHODOLOGY

This cross-sectional descriptive study was carried out in the Microbiology Laboratory of Provincial Headquarter Hospital, Gilgit, from October 2025 to March 2026 after ethical approval (Letter No. 1103/PHQ/2025, 03-10-2025). The sample size of 143 (rounded off to 150) was calculated by using 95% confidence interval, 6% margin of error, and 84% assumed prevalence of multidrug-resistant pathogens from the ICU.[8]

After taking written informed consent from patients or their attendants, 150 culture specimens received from ICU patients, irrespective of age or gender, were tested using a non-probability consecutive sampling. The clinical samples received included blood, urine, sputum, pus, and catheter tips. Samples with improper labeling, or evidence of contamination or repeat samples from the same patient were excluded. Each specimen was inoculated onto the appropriate culture media under aseptic conditions, then incubated and carefully identified by an experienced microbiologist based on colony morphology, Gram stain, and biochemical tests. Among biochemical tests, catalase, coagulase and bile esculin hydrolysis tests were done for Gram-positive cocci. For Gram-negative bacteria, citrate utilization, triple sugar iron (TSI), urease, motility, indole, and oxidase tests were used. The antimicrobial sensitivity testing (AST) was performed by the Kirby-Bauer disc diffusion method. The antibiotic zone diameters were reported as sensitive, intermediate, and resistant following the Clinical and Laboratory Standards Institute (CLSI) guidelines 2025.[9] All the required information was recorded on a predesigned proforma.

The classes of antibiotics that were applied for Gram-positive bacteria were penicillin (P), ampicillin (AMP), cefoxitin (FOX), ciprofloxacin (CIP), levofloxacin (LEV), gentamicin (CN), trimethoprim-sulfamethoxazole (SXT), erythromycin (E), clindamycin (DA), doxycycline (DOX), vancomycin (VA), and linezolid (LZD). Cefoxitin was used to report methicillin sensitivity in *Staphylococcus*.[9]

The antibiotic panel used for Gram-negative bacteria included amoxicillin-clavulanic acid (AMC), cefotaxime (CTX), ceftazidime (CAZ), ceftriaxone (CRO), gentamicin (CN), amikacin (AK), ciprofloxacin (CIP), trimethoprim-sulfamethoxazole (SXT), imipenem (IPM), meropenem (MEM) and piperacillin-tazobactam (TZP). Only urinary isolates were subjected to nitrofurantoin susceptibility testing, in line with CLSI recommendations. The double-disc synergy test (DDST) was used to identify extended-spectrum beta-lactamase (ESBL). Amoxicillin-clavulanic acid was placed in the center with cefotaxime and ceftazidime discs placed 20 mm (center-to-center) from AMC. Increased zone diameters of CTX and CAZ caused by synergy of AMC disc was considered as ESBL production. Antibiotics not recommended for a particular organism according to the CLSI guidelines were excluded from that organism's testing panel.[9]

STATISTICAL ANALYSIS

The statistical analysis was done by the Statistical Package for the Social Sciences (SPSS) version 27. Categorical variables such as the most common isolates, types of specimens, predominant bacteria isolated from various specimens, and their antibiotic resistance pattern were presented as frequency and percentage. The Chi-square test was done to evaluate the relation between specimen type and organisms isolated, with a p-value <0.05 showing statistical significance.

RESULTS

Out of 150 clinical specimens, 98(65.3%) showed bacterial growth. Gram-negative bacteria constituted 58(59.2%) isolates, whereas Gram-positive bacteria accounted for 40(40.8%) isolates. The most common pathogen isolated was *Staphylococcus* species (31.6%), succeeded by *Escherichia coli* (17.3%), *Klebsiella pneumoniae* (15.3%), *Pseudomonas aeruginosa* (13.3%), *Acinetobacter baumannii* (10.2%), *Enterococcus* species (9.2%), and *Proteus* species (3.1%) (Table 1).

The majority of the clinical specimens were blood samples (35.7%), followed by sputum (28.6%), urine (24.5%), pus (6.1%), and catheter tips (5.1%). A significant relation existed between clinical

specimens and isolated organisms (p <0.0001), indicating that the distribution of bacterial isolates differed significantly across various specimens. The distribution of bacterial isolates according to specimen type is shown in Figure 1.

Among 14(14.3%) isolates of *Staphylococcus aureus*, 10(71.4%) were methicillin-resistant *Staphylococcus aureus* (MRSA), while 4(28.6%) were methicillin-sensitive (MSSA). Among 17(17.3%) CoNS, 6(35.3%) were methicillin-resistant, and 11(64.7%) were methicillin-sensitive. Eight (88.9%) isolates of *Enterococcus* species were resistant to penicillin and ampicillin. All isolates of *Staphylococcus aureus*, CoNS, and *Enterococcus* species were sensitive to vancomycin and linezolid. High resistance rates to other antibiotics were observed among Gram-positive bacteria (Table 2).

Among Gram-negative bacteria, 12(70.6%) isolates of *E. coli* and 11(73.3%) isolates of *Klebsiella pneumoniae* were ESBL producers, whereas none of the *Proteus* species produced ESBL. Carbapenems demonstrated 100% susceptibility against all Gram-negative isolates. Piperacillin-tazobactam, amikacin, and nitrofurantoin exhibited comparatively better antimicrobial activity than other antibiotics tested. Among Gram-negative bacteria, *Acinetobacter baumannii* demonstrated the highest level of antimicrobial resistance (Table 3).

Table 1: Distribution of Gram-Positive and Gram-Negative Bacterial Isolates among ICU Patients

Gram Stain Classification	Bacterial Isolates	Frequency (Percentage)
Gram-Positive Bacteria	**CoNS**	17(17.3%)
	Staphylococcus aureus	14(14.3%)
	***Enterococcus* species**	9(9.2%)
	Total	40(40.8%)
Gram-Negative Bacteria	***E. coli***	17(17.3%)
	Klebsiella pneumoniae	15(15.3%)
	Pseudomonas aeruginosa	13(13.3%)
	Acinetobacter baumannii	10(10.2%)
	***Proteus* species**	3(3.1%)
	Total	58(59.2%)

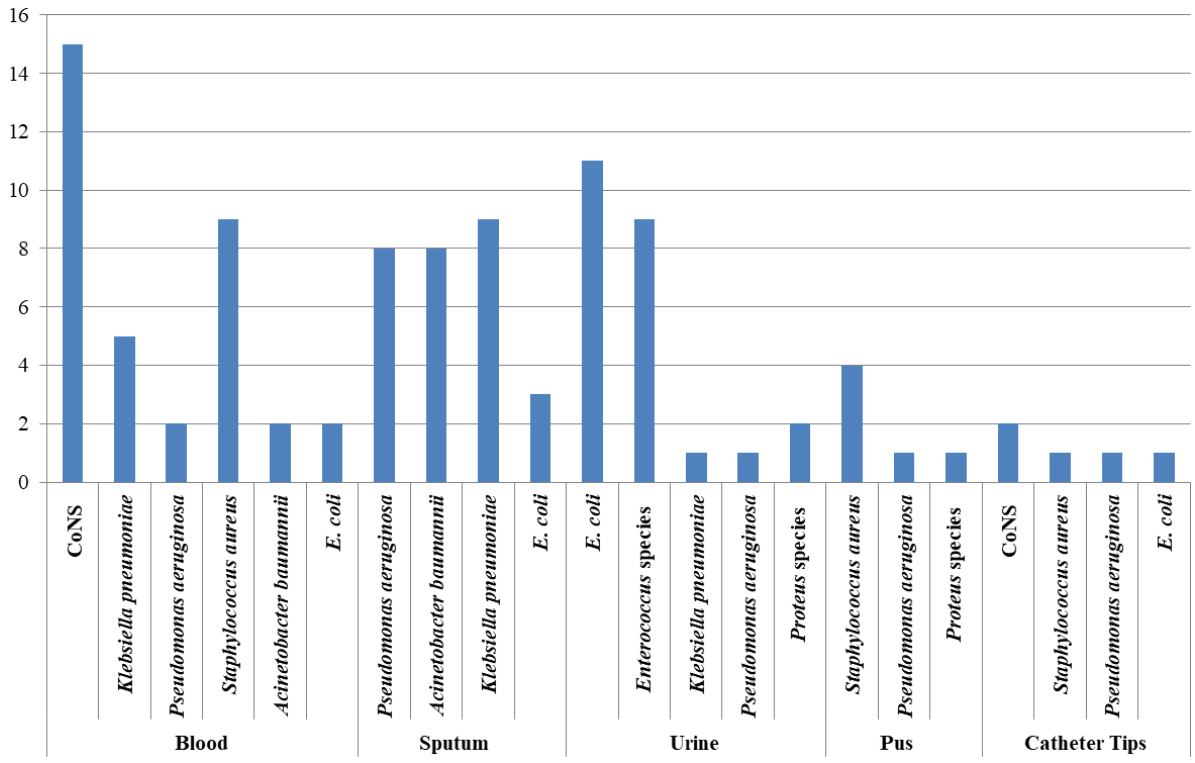

Figure 1: Bar Chart showing the Distribution of Bacterial Isolates from Different Clinical Specimens

Table 2: Antimicrobial Resistance Pattern in Gram-Positive Bacteria

Gram-Positive Bacteria	VA	LZD	CIP	LEV	CN	DOX	SXT	E	DA	F**
Staphylococcus aureus **(n=14)**	0(0%)	0(0%)	10(71.4%)	8(57.1%)	5(35.7%)	4(28.6%)	8(57.1%)	13(92.9%)	10(71.4%)	---
CoNS (n=17)	0(0%)	0(0%)	10(58.8%)	9(52.9%)	4(23.5%)	3(17.6%)	8(47.1%)	14(82.3%)	7(41.2%)	---
Enterococcus **species (n=9)**	0(0%)	0(0%)	6(66.7%)	6(66.7%)	NA*	NA*	NA*	NA*	NA*	2/9(22.2%)

**NA means Antibiotics not recommended for those organisms*
***Only for urinary isolates*

Table 3: Antimicrobial Resistance Pattern in Gram-Negative Bacteria

Gram-Negative Bacteria	AMC	CAZ	CTX	CRO	SXT	CIP	CN	AK	IPM	MEM	TZP	F**
E. coli **(n=17)**	12(70.6%)	12(70.6%)	12(70.6%)	12(70.6%)	14(82.4%)	11(64.7%)	5(29.4%)	3(17.6%)	0(0%)	0(0%)	5(29.4%)	3/11(27.3%)
Klebsiella pneumoniae **(n=15)**	11(73.3%)	11(73.3%)	11(73.3%)	11(73.3%)	13(86.7%)	10(66.7%)	4(26.7%)	3(20%)	0(0%)	0(0%)	5(33.3%)	0/1(0%)
Proteus species **(n=3)**	2(66.7%)	1(33.3%)	1(33.3%)	1(33.3%)	1(33.3%)	2(66.7%)	1(33.3%)	1(33.3%)	0(0%)	0(0%)	0(0%)	0/2(0%)
Pseudomonas aeruginosa **(n=13)**	NA*	5(38.5%)	NA*	NA*	NA*	11(84.6%)	8(61.5%)	6(46.1%)	0(0%)	0(0%)	5(38.5%)	NA*
Acinetobacter baumannii **(n=10)**	NA*	8(80%)	NA*	NA*	NA*	10(100%)	8(80%)	7(70%)	0(0%)	0(0%)	6(60%)	NA*

**NA means Antibiotics not recommended for those organisms*
***Only for urinary isolates*

DISCUSSION

The spread of multidrug resistant pathogens in an ICU setting is a serious concern for all tertiary care hospitals globally.[10] Continuous ICU surveillance is essential for monitoring healthcare-associated infections and antimicrobial resistance patterns, thereby supporting infection prevention and antimicrobial stewardship strategies.[11] In our study, the majority of the clinical specimens were blood (35.7%) followed by sputum (28.6%), urine (24.5%), pus (6.1%) and catheter tips (5.1%). Similarly, in another study, most of the positive cultures were from blood (56%), 27% from respiratory samples and 17% from urine.[12] Altaf et al. from Karachi reported positive cultures from blood (30%), urine (26%), respiratory samples (34%), pus, catheter tips and body fluids (10%).[13] In contrast, the most common specimen was urine (48.7%) followed by sputum (19.7%), blood (17.1%), catheters (9.1%) and endotracheal tube (5.3) in a study conducted in 2025.[14] Kumar et al. revealed that cultures were positive in 65.4% of the respiratory samples, 22.2% of the blood, and 12.35% of the urine samples.[15] Saleem et al. reported that the frequency of respiratory samples was highest (43%) followed by urine (24%), blood (15%), and pus (8.6%) among positive ICU samples.[16]

Our results showed that Gram-negative bacteria constituted 59.2%, whereas Gram-positive bacteria made up 40.8% of the isolates. The most common pathogens isolated were *Staphylococcus* (31.6%), *E. coli* (17.3%), *Klebsiella pneumoniae* (15.3%), *Pseudomonas aeruginosa* (13.3%), *Acinetobacter baumannii* (10.2%), *Enterococcus* species (9.2%), and *Proteus* species (3.1%). Chakraborty et al. also reported a predominance of Gram-negative bacteria (66%) as compared to Gram-positive bacteria (28%).[12] Another study revealed the highest prevalence of *E. coli* (39.5%), followed by *Klebsiella pneumoniae* (30.3%), *Enterococcus faecalis* (6.6%), *Acinetobacter baumannii* (6.6%), *Pseudomonas aeruginosa* (3.9%), MSSA (3.9%), *Proteus* (2.6%), and *Enterobacter* (1.3%).[14] A study reported *Acinetobacter baumannii* as the most prevalent pathogen from ICUs (36.6%). The other pathogens were *Klebsiella pneumoniae* (15.5%), *Pseudomonas aeruginosa* (11.5%), and *E. coli* (11.8%).[17] In another study, *Acinetobacter* spp. (22%) was most prevalent, followed by isolation of *E. coli* (14%), *P. aeruginosa* (10%), *S. aureus* (10%), and *Enterococcus spp.* (8%).[13] *Klebsiella* (32.69%) was most common in a study conducted in Kurnool, India, followed by *Acinetobacter* (26.4%), *Pseudomonas aeruginosa* (19.2%), *E. coli* (9.13%), *Staphylococcus aureus* (6.73%), *Enterobacter* species (3.84%), and *Citrobacter* species (1.92%).[18] According to our study, CoNS (42.9%) and *Staphylococcus* (25.7%) were the most common pathogens from blood culture, *Klebsiella*

pneumoniae (32.1%), *Pseudomonas aeruginosa* (28.6%) and *Acinetobacter baumannii* (28.6%) from sputum, *E. coli* (45.8%) and *Enterococcus* (37.5%) from urine, *Staphylococcus aureus* (66.6%) from pus, and CoNS (40%) from catheter tips. Just like our study, CoNS was most frequent in blood samples. The organisms isolated from respiratory samples were *Pseudomonas*, *Klebsiella, E. coli*, *Acinetobacter*, and CoNS. Pus samples grew *Staphylococcus aureus*, *Pseudomonas*, *Klebsiella and E. coli.*[19] In another study, the most common pathogens from respiratory specimens were *Pseudomonas aeruginosa* (27%) and *Acinetobacter baumannii* (22%). *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* (22% each) were frequent in blood, and *Enterococcus* was most common in urine specimens. [15] In our study, 71.4% isolates of *Staphylococcus aureus* were MRSA. Another study revealed that 57.1% of ICU pathogens were MRSA isolates.[18] All strains of *Staphylococcus aureus* were MRSA according to Chakraborty et al.[12] *Staphylococcus aureus* showed 57.1% resistance to trimethoprim-sulfamethoxazole, 35.7% aminoglycoside resistance, and 71.4% ciprofloxacin resistance in our study. None of them were resistant to vancomycin and linezolid. Another study revealed 55% resistance to co-trimoxazole, 30% to aminoglycosides, and 65% to fluoroquinolones. All of them were sensitive to vancomycin and linezolid. In our study, *Enterococcus* showed 88.8% ampicillin resistance and 66.7% ciprofloxacin resistance. All the strains were sensitive to vancomycin and linezolid. Around 96% of the *Enterococcus* species were ampicillin-resistant, 98% were resistant to fluoroquinolones and 33% to vancomycin in another study.[12] Around 70.6% of *E. coli* and 73.3% of *Klebsiella pneumoniae* were ESBL producers in our study. Similarly, 72.4% of *E. coli* and *Klebsiella pneumoniae* were ESBL producers in a study.[20] In another study, 56% of the *Klebsiella pneumoniae* were ESBL producing.[16] In our study, 70.6% of *E. coli* and 73.3% of *Klebsiella pneumoniae* were resistant to beta-lactams and third-generation cephalosporins, 82.4% and 86.7% to trimethoprim-sulfamethoxazole, 64.7% and 66.7% to ciprofloxacin and 17.6% & 20% to amikacin, respectively. *Proteus* showed 66.7% resistance to beta-lactams & ciprofloxacin and 33.3% to third-generation cephalosporins, amikacin & trimethoprim-sulfamethoxazole. Around 38.5% of *Pseudomonas* were resistant to ceftazidime, 84.6% to ciprofloxacin, and 46.1% to amikacin. All isolates of *Acinetobacter baumannii* were resistant to ciprofloxacin, 80% to ceftazidime, and 70% to amikacin. A much higher antibiotic resistance was seen in other studies. Another alarming concern in these studies was carbapenem resistance in all the isolates, whereas in our study, carbapenem resistance was not seen. According to Chakraborty et al, *Escherichia coli* showed 95% resistance to cephalosporins, beta-lactams & fluoroquinolones, 85% to co-trimoxazole, and 75% to carbapenems & aminoglycosides. Ninety four percent of *Klebsiella pneumoniae* were resistant to beta-lactams, 96% to third-generation cephalosporins, 92% to fluoroquinolones (92%), 88% to carbapenems, 83% to co-trimoxazole, and 36% to aminoglycosides. *Acinetobacter* was 100% resistant to second-generation cephalosporins, 97% to third-generation cephalosporins, 91% to beta-lactams, 97% to fluoroquinolones, 89% to carbapenems, and 66% to aminoglycosides. *Pseudomonas* spp. was 100% resistant to third-generation cephalosporins. 89% to carbapenems, 74% to beta-lactams & fluoroquinolones (74%), and 63% to aminoglycosides. *Proteus* species showed only cephalosporin resistance (100%).[12] In another study, 90% of *Klebsiella pneumoniae* were resistant to beta-lactams, 70-80% to third-generation cephalosporins, 89.7% to ciprofloxacin, and 85.7% to amikacin and 56.7% to imipenem. Sixty percent of *E. coli* were resistant to beta-lactams & cephalosporins, 90% to ciprofloxacin, 10% to amikacin, and 20% to imipenem. *Acinetobacter* showed 100% resistance to ciprofloxacin, 97% resistance to ceftazidime, 85.7% to amikacin, and 88.6% to imipenem. *Pseudomonas* was 80% resistant to ceftazidime, 64% to ciprofloxacin, 28% to amikacin, and 44% to imipenem.[16] In our study, carbapenem resistance was not detected in any Gram-negative bacteria. In contrast, studies have reported 15% and 38% carbapenem resistance from Karachi, Pakistan.[21,22]

CONCLUSION

Gram-negative bacteria constituted 59.2%, whereas Gram-positive bacteria made up 40.8% of the isolates. Around 71.4% isolates of *Staphylococcus aureus* were methicillin-resistant and 70.6% *E. coli* & 73.3% *Klebsiella pneumoniae* were ESBL producers. A higher antimicrobial resistance was seen in the isolates. These findings are a serious concern in clinical practice because they limit the options available for effective treatment and can lead to treatment failure and longer hospital stays.

LIMITATIONS & RECOMMENDATIONS

The single-centered study and limited sample size may affect the generalizability of the results. Multicenter studies with a larger sample size should be conducted for broader and more representative results. Only bacterial pathogens were included, while fungal pathogens were not studied.

Higher AMR among bacterial isolates highlights the importance of regular microbiological surveillance, and adherence to infection prevention practices in intensive care settings. It also underscores the necessity for comprehensive antimicrobial stewardship initiatives to ensure judicious use of antimicrobials, help prevent the spread of resistant bacteria, and improve outcomes for critically ill patients.

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Authors' Contributions:

Authors approved the draft and are accountable in ensuring that questions related to accuracy or integrity of the work are duly investigated and resolved.

A.A: Conceptualization, data collection, manuscript drafting.
S.N: Laboratory work, data interpretation, manuscript writing.
A.H.A: Statistical analysis, critical revision.
U.A: Literature review, data management.
H.A: Quality control, data acquisition.
N.R: Supervision, manuscript review and final approval.

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