

MONITORING ANTIMICROBIAL RESISTANCE AMONG INTENSIVE CARE UNIT GRAM-NEGATIVE ISOLATES: INSIGHTS FROM A RETROSPECTIVE STUDY

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ABSTRACT

Objectives: To determine the distribution and resistance profiles of gram-negative bacteria (GNB) in intensive care units (ICUs) at Tertiary care center Adesh Medical College and Hospital, Shahabad, Kurukshetra, Haryana.

Methods: A record-based retrospective study was conducted from January 2023 to December 2024. In total, 540 clinical specimens and isolates, 58 (9.7%) Gram-positive cocci, and 302/540 (55.9%) from the general ICU, neonatal ICU, respiratory ICU, neurology ICU, and coronary care unit were analyzed for pathogens and their antibiotic susceptibility patterns.

Results: Among GNB isolates, 302/540 (55.9%) were isolated in the year 2023, and 238 (44.1%) were isolated in the year 2024. Predominantly isolated from the medicine ICU, with 109 isolates in 2023 and 87 in 2024, followed by the neuro ICU. The predominant clinical sample from which GNB were isolated was a respiratory sample for both years, with 132/302 (43.7%) for 2023 and 131/238 (55%) for 2024. *Klebsiella* spp. were the most frequently isolated organism, accounting for 47.7% in 2023 and 36.6% in 2024, with 231 isolates (42.8%) across both years. *Acinetobacter* spp. showed a marked increase, rising from 13.9% in 2023 to 26.9% in 2024. *Klebsiella* displayed a high resistance pattern to cephalosporin and a high rising trend of carbapenems from 19.4% in 2023 to 71.2% in 2024, followed by *Acinetobacter* spp., *Escherichia coli*, and *Pseudomonas* spp. multidrug-resistant (MDR) bacteria decreased sharply from 36.42% in 2023 to 11.76% in 2024, with concomitant increase of extensively drug-resistant (XDR) bacteria from 16.89% in 2023 to 55.4 % in 2024.

Conclusion: In this study, GNB caused the majority of infections in patients admitted to the ICU, with *Klebsiella pneumoniae* being the predominant pathogen. A shift of resistance among isolates from MDR to XDR, pointing toward more severe and difficult-to-treat infections in ICU settings

Keywords: Gram-negative organisms, Intensive care units, Multidrug-resistant, Extensively drug-resistant, Pan-drug-resistant.

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INTRODUCTION

Patients admitted to intensive care units (ICUs) often suffer from critical and life-threatening conditions that require invasive interventions and prolonged hospital stays. These factors significantly increase the risk of colonization by pathogenic organisms and the development of multidrug-resistant (MDR) infections, largely due to healthcare-associated infections (HAIs).

Antibiotic resistance has been termed "The Silent Tsunami of Modern Medicine," reflecting its growing threat to global health. Annually, bacterial infections are responsible for approximately 700,000 deaths worldwide. Between 2016 and 2023, there has been a 10.6% rise in global antibiotic consumption. Factors such as longer life expectancy, increased disease burden, improved access to health care, and environmental influences are fueling the spread of antimicrobial resistance (AMR) [1].

In India, ICU-related nosocomial infections have been reported at rates ranging from 11% to 60% in various studies. Common (HAIs) include device-associated infections such as ventilator-associated pneumonia, central line-associated bloodstream infections, and catheter-associated urinary tract infections, along with surgical site infections. The transmission of resistant pathogens between patients is a key contributor to the spread of these infections. Notably, the incidence of ICU-acquired infections in developing nations is 2–3 times higher than that observed in high-income countries. ICU patients are at a higher risk of HAIs because they are more exposed to invasive procedures, immunocompromised, and often require prolonged and intensive care [2].

The prevalence of infections due to gram-negative bacteria (GNB), such as extended-spectrum beta-lactamases (ESBLs) and metallo-beta-lactamase (MBL) producing *Escherichia coli* and *Klebsiella pneumoniae*, and drug-resistant Gram-positive organisms is high in ICUs [3].

MDR bacteria pose a major threat to existing therapeutic strategies, particularly in critical care settings. These organisms exhibit resistance to multiple antibiotic classes, significantly reducing the options for effective treatment. Infections caused by MDR pathogens are associated with higher morbidity, mortality, prolonged hospital stays, and increased health-care costs. The emergence of extensively drug-resistant (XDR) and pan-drug-resistant (PDR) strains further complicates management, as few or no effective antibiotics remain. This crisis of growing resistance undermines the success of modern medical procedures such as organ transplantation, chemotherapy, and major surgeries, which depend heavily on reliable antimicrobial therapy.

The purpose of the present study was to identify the prevalence of GNB isolated from the clinical samples of patients admitted to various ICUs and to understand their trends of AMR patterns at a tertiary care hospital in Haryana, India.

METHODS

Study

This was a retrospective cross-sectional study conducted at Adesh Medical College and Hospital, Village Mohri (Shahabad), District Kurukshetra, Haryana. Data on clinical samples of ICU patients showing bacterial growth on culture and sensitivity testing were retrieved from

the medical records after due approvals, for the period from January 2023 to December 2024.

Inclusion

The data of all the clinical samples received from ICU patients in which growth of GNB was reported were included in this study.

Exclusion criteria

All clinical samples of ICU patients in which no gram-negative pathogen was reported were excluded from the study. Duplicate isolates and follow-up cultures were also excluded from the study.

Methods and/or techniques used for the study

Data from the patients admitted to ICUs with bacterial growth from clinical samples were further analyzed for the antimicrobial susceptibility profiles.

Antimicrobial testing

The microbiology laboratory of this tertiary care center carried out bacterial identification using standard protocol and performed antibiotic susceptibility testing (AST) using standard drug susceptibility methods such as the disk diffusion method and broth microdilution method (for colistin), as per Clinical and Laboratory Standards Institute guidelines [4].

The antibiotic panel for *Enterobacterales* included cefuroxime, ceftazidime, aztreonam, amoxicillin-clavulanate, piperacillin-tazobactam, meropenem, imipenem, amikacin, gentamicin, ciprofloxacin, fosfomycin (for urine isolates only), nitrofurantoin (for urine isolates only), and colistin.

The antibiotic panel for *Acinetobacter* included ceftazidime, cefepime, aztreonam, ampicillin-sulbactam, piperacillin-tazobactam, meropenem, imipenem, amikacin, gentamicin, ciprofloxacin, co-trimoxazole, and colistin.

The antibiotic panel for *Pseudomonas* spp. and other non-fermenters included cefepime, ceftazidime, aztreonam, piperacillin-tazobactam, meropenem, imipenem, amikacin, gentamicin, ciprofloxacin, and colistin.

AMR pattern

The antibiotic resistance pattern of GNB was assessed based on the following definitions: Bacteria that were resistant to at least three classes of drugs were labeled as MDR. Bacterial isolates resistant to almost all the antibiotics, leaving susceptibility to one or two classes of antibiotics, were labeled as XDR, whereas pathogens that were resistant to all the classes of antibiotics were labeled as PDR [5].

Statistical analysis

The data were entered into Microsoft Excel and analyzed using Statistical Package for the Social Sciences version 27.

Ethical approval

The study was reviewed and approved by the Institutional ethics committee, vide letter no. AMCH/IEC-BHR/2025/02/05, dated 28-02-2025.

RESULTS

A total of 598 bacterial isolates were obtained from the clinical specimens of patients admitted to various ICUs from January 2023 to December 2024. Out of 598 bacterial isolates, 540 (90.30%) were GNB and 58 (9.7%) were Gram-positive cocci (GPC).

Among GNB isolates, 302/540 (55.9%) were isolated in the year 2023, and 238 (44.1%) were isolated in the year 2024.

The majority of GNB pathogens were isolated from the medicine ICU, with 109 isolates in 2023 and 87 in 2024, followed by the neuro ICU (Fig. 1).

As shown in Table 1, the predominant clinical sample from which GNB were isolated was a respiratory sample for both years, with 132/302 (43.7%) for 2023 and 131/238 (55%) for the year 2024.

Table 2 shows the year-wise distribution of GNB isolated from ICU patients during 2023 and 2024, with a total of 540 isolates collected over the 2 years (302 in 2023 and 238 in 2024). *Klebsiella* spp. were the most frequently isolated organism, accounting for 47.7% in 2023 and 36.6% in 2024, with 231 isolates (42.8%) across both years. *Acinetobacter* spp. showed a marked increase, rising from 13.9% in 2023 to 26.9% in 2024, indicating a growing prevalence and accounting for 16.3% of the total of 88 isolates. *E. coli* decreased slightly, from 18.5% in 2023 to 17.3% in 2024, making up 18% of the total 97 isolates. A similar trend was observed for *Pseudomonas* spp., with 15.2% resistance in 2023 and 14.3% in 2024, totalling 14.8% overall in 80 isolates. *Proteus* spp. had a modest presence, slightly declining from 3.7% to 2.5%, with 17 total isolates. *Serratia* spp. had 2 isolates (0.7%) in 2023 and 1 (0.4%) in 2024, making up a total of three isolates. *Enterobacter* spp. was rare, with one isolate in 2023 and two in 2024, making up 0.5% of total isolates. Only two *Citrobacter* spp. were isolated in 2024, and only one isolate of *Stenotrophomonas maltophilia* was isolated in 2024.

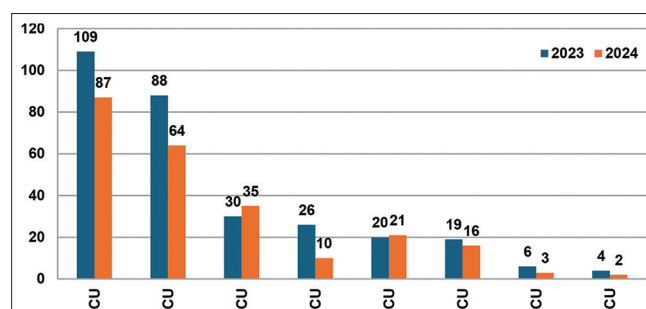


Fig. 1: Year-wise distribution of Gram-negative bacterial isolates from various intensive care units

Table 1: Year-wise distribution of various clinical samples showing growth of GNB isolates

Sample	2023 (%)	2024 (%)
Respiratory samples (BAL, sputum, and ET secretions)	132 (43.7)	131 (55)
Urine	62 (20.5)	38 (16)
Blood	53 (17.5)	31 (13)
Pus	26 (8.6)	24 (10.1)
Ascitic fluid	9 (3)	4 (1.7)
Wound swab	9 (2)	5 (2.1)
CSF	7 (2.3)	2 (0.8)
Tissue	4 (1.4)	3 (1.3)
Total	302 (100)	238 (100)

GNB: Gram-negative bacteria, CSF: Cerebrospinal fluid, BAL: Bronchoalveolar lavage, ET: Endotracheal

Table 2: Year-wise distribution of GNB isolated from ICU patients

Bacterial isolates	2023	2024	Total
<i>Klebsiella</i> spp.	144 (47.7)	87 (36.6)	231 (42.8)
<i>Acinetobacter</i> spp.	42 (13.9)	64 (26.9)	106 (19.6)
<i>Escherichia coli</i>	56 (18.5)	41 (17.3)	97 (18)
<i>Pseudomonas</i> spp.	46 (15.2)	34 (14.3)	80 (14.8)
<i>Proteus</i> spp.	11 (3.7)	6 (2.5)	17 (3.2)
<i>Serratia</i> spp.	2 (0.7)	1 (0.4)	3 (0.5)
<i>Citrobacter</i> spp.	-	2 (0.8)	2 (0.4)
<i>Stenotrophomonas maltophilia</i>	-	1 (0.4)	1 (0.2)
<i>Enterobacter</i> spp.	1 (0.3)	2 (0.8)	3 (0.5)
Total	302 (100)	238 (100)	540 (100)

ICU: Intensive care units, GNB: Gram-negative bacteria

Table 3 shows the antibiotic resistance of the four gram-negative pathogens isolated. For *Klebsiella* spp., high resistance was observed across most antibiotics in both years. Maximum resistance was shown toward cephalosporins, with 95% in both 2023 and 2024 for cefuroxime and 95% in 2023 and 93% in 2024 for ceftazidime. Although there was a slight variation in the percentage of resistance for almost all the antibiotics over the study period but for carbapenems (imipenem and meropenem), there was a drastic increase in the resistance from 19.4% in 2023 to 71.2% in 2024. Only one isolate showed resistance to colistin in each of 2023 and 2024.

For *E. coli*, moderate to high resistance to many drugs was observed, but maximum resistance was observed for cefuroxime with 89.3% in 2023 and 95.1% in 2024. *E. coli* also showed a drastic increase in resistance from 7.1% in 2023 to 34.1% in 2024 for carbapenems (imipenem and meropenem).

Acinetobacter spp. showed very high resistance across nearly all antibiotics. Maximum resistance was reported for ceftazidime and aztreonam (95.2% in 2023 and 98.4% in 2024 for both antibiotics), followed by cefepime (95.2% in 2023 and 96.8% in 2024). *Acinetobacter* spp. also showed a drastic increase in the resistance for carbapenems (47.6% in 2023–96.8% in 2024), piperacillin-tazobactam (54.8% in 2023–84.3% in 2024), and ampicillin-sulbactam (48% in 2023–70.3% in 2024). Only one isolate showed resistance to colistin in each of 2023 and 2024.

Pseudomonas spp. showed variable resistance across drugs. However, maximum resistance was observed for ceftazidime (58.7% in 2023

and 67.6% in 2024), followed by cefepime (58.7% in 2023 and 70.5% in 2024). Drastic variation was observed for piperacillin-tazobactam (13% in 2023 and 41.1% in 2024) and carbapenems (21.7% in 2023 and 55.8% in 2024).

The resistance pattern data for the years 2023 and 2024 show a significant shift in the types of AMR among bacterial isolates. MDR bacteria decreased sharply from 36.42% in 2023 to 11.76% in 2024, with a concomitant increase of XDR bacteria from 16.89% in 2023 to 55.46% in 2024. PDR bacteria showed a slight increase from 0.66% to 0.84% from 2023 to 2024 (Fig. 2).

In our study, it was observed that 49.3% of *Klebsiella* spp. were MDR in 2023, and this percentage decreased to 11.4% in 2024. *E. coli* had an MDR prevalence of 30.3% in 2023 and 29.2% in 2024, whereas *Acinetobacter* spp. had 35.7% in 2023, but it decreased to 6.2% in 2024. For *Pseudomonas* spp., the prevalence of MDR isolates was 10.8% in 2023 and 2.9% in 2024. *Proteus* spp. had an 18.1% prevalence of MDR in 2023, whereas it was 16.6% in 2024 (Table 4).

The XDR GNB prevalence rates increased over the 2-year study period. The XDR prevalence of *Klebsiella* spp. increased from 13.2% to 66.6%, *E. coli* from 7.1% to 24.4%, *Acinetobacter* spp. from 13.2% to 75%, whereas *Pseudomonas* spp. from 15.2% to 44.1%. *Proteus* spp. had the least variation in percentage of XDR, with 18.1% in 2023 and 16.6% in 2024 (Table 4).

Pan-drug resistance was shown by *Klebsiella* spp. and *Acinetobacter* spp. The percentage of PDR in *Klebsiella* spp. was 0.6% in 2023 and 1.1% in 2024, and for *Acinetobacter* spp. was 2.3% in 2023 and 1.5% in 2024 (Table 4).

DISCUSSION

Infections in the ICU are a major concern in hospital setups, but are unavoidable due to intrinsic factors associated with admitted patients, increased admission rates, use of invasive medical devices and procedures, which then act as reservoirs for pathogenic isolates. Other factors, such as increased age, associated morbidity, and prolonged admission, also play a crucial role in the pathogenesis, transmission, and mortality of patients. In the present study, it was observed that GNB (90.3%) were the predominant pathogens isolated from the clinical samples of the ICU, whereas GPC were much less (9.7%). A similar observation was seen in the study by Chidambaram *et al.*, which reported 89.36% GNB isolates from ICU patients [6].

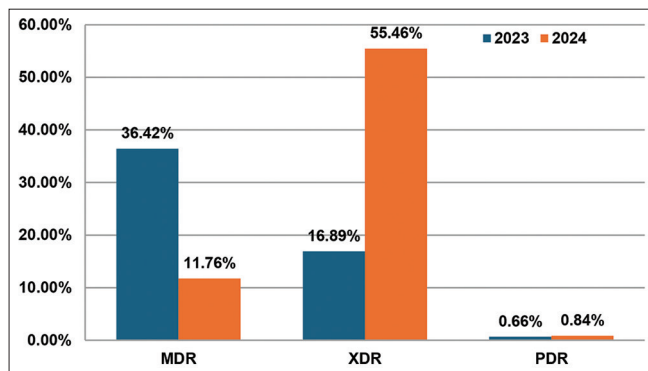


Fig. 2: Resistance pattern of Gram-negative bacterial isolates. MDR: Multidrug resistance, XDR: Extensive drug resistance, PDR: Pan-drug resistance

Table 3: Antibiotic resistance in predominant Gram-negative bacterial isolates

Antibiotics	<i>Klebsiella</i> spp.		<i>Escherichia coli</i>		<i>Acinetobacter</i> spp.		<i>Pseudomonas</i> spp.	
	R (%) 2023 n=144	R (%) 2024 n=87	R (%) 2023 n=56	R (%) 2024 n=41	R (%) 2023 n=42	R (%) 2024 n=64	R (%) 2023 n=46	R (%) 2024 n=34
Cefuroxime	137/144 (95.1)	83/87 (95.4)	50/56 (89.3)	39/41 (95.1)	-	-	-	-
Ceftazidime	137/144 (95.1)	81/87 (93.1)	35/56 (62.5)	38/41 (92.6)	40/42 (95.2)	63/64 (98.4)	27/46 (58.7)	23/34 (67.6)
Cefepime	-	-	-	-	40/42 (95.2)	62/64 (96.8)	27/46 (58.7)	24/34 (70.5)
Aztreonam	126/144 (87.5)	75/87 (86.2)	47/56 (83.9)	32/41 (78)	40/42 (95.2)	63/64 (98.4)	25/46 (54.3)	19/34 (55.8)
Ampicillin-clavulanate	135/144 (93.7)	62/87 (71.2)	47/56 (83.9)	30/41 (73.1)	-	-	-	-
Piperacillin-tazobactam	72/144 (50)	61/87 (70.1)	16/56 (28.6)	20/41 (48.7)	23/42 (54.8)	54/64 (84.3)	6/46 (13)	14/34 (41.1)
Ampicillin-sulbactam	-	-	-	-	20/42 (48)	45/64 (70.3)	-	-
Meropenem	28/144 (19.4)	62/87 (71.2)	4/56 (7.1)	14/41 (34.1)	20/42 (47.6)	62/64 (96.8)	10/46 (21.7)	19/34 (55.8)
Imipenem	28/144 (19.4)	62/87 (71.2)	4/56 (7.1)	14/41 (34.1)	20/42 (47.6)	62/64 (96.8)	10/46 (21.7)	19/34 (55.8)
Amikacin	87/144 (60.4)	61/87 (70.1)	23/56 (41)	17/41 (41.5)	32/42 (76.2)	57/64 (89)	18/46 (39.1)	20/34 (58.8)
Gentamicin	90/144 (62.5)	61/87 (70.1)	24/56 (42.8)	18/41 (43.9)	32/42 (76.2)	58/64 (90.6)	19/46 (41.3)	20/34 (58.8)
Ciprofloxacin	127/144 (88.2)	76/87 (87.3)	44/56 (78.5)	40/41 (97.5)	41/42 (97.6)	62/64 (96.8)	25/46 (54.3)	22/34 (64.7)
Fosfomycin (U)	21/24 (87.5)	10/12 (83.3)	30/36 (83.3)	18/20 (90)	-	-	-	-
Co-trimoxazole	-	-	-	-	42/42 (100)	56/64 (87.5)	-	-
Nitrofurantoin (U)	22/24 (91.6)	11/12 (91.6)	30/36 (83.3)	19/20 (95)	-	-	-	-
Colistin*	1/144 (0.6)	1/87 (1.1)	0 (0)	0 (0)	1/42 (2.3)	1/64 (1.5)	0 (0)	0 (0)

*For colistin, a minimum inhibitory concentration ≥ 4 μ g/mL was taken as a resistant isolate

Table 4: Prevalence of MDR, XDR, and PDR among gram-negative isolates

Name of isolate	2023			2024		
	MDR Number (%)	XDR Number (%)	PDR Number (%)	MDR Number (%)	XDR Number (%)	PDR Number (%)
<i>Klebsiella</i> spp. 2023 (n=144) 2024 (n=87)	71/144 (49.3)	19/144 (13.2)	1/144 (0.6)	10/87 (11.4)	58/87 (66.6)	1/87 (1.1)
<i>Escherichia coli</i> 2023 (n=56) 2024 (n=41)	17/56 (30.3)	4/56 (7.1)	0 (0)	12/41 (29.2)	10/41 (24.4)	0 (0)
<i>Acinetobacter</i> spp. 2023 (n=42) 2024 (n=64)	15/42 (35.7)	19/42 (13.2)	1/42 (2.3)	4/64 (6.2)	48/64 (75)	1/64 (1.5)
<i>Pseudomonas</i> spp. 2023 (n=46) 2024 (n=34)	5/46 (10.8)	7/46 (15.2)	0 (0)	1/34 (2.9)	15/34 (44.1)	0 (0)
<i>Proteus</i> spp. 2023 (n=11) 2024 (n=6)	2/11 (18.1)	2/11 (18.1)	0 (0)	1/6 (16.6)	1/6 (16.6)	0 (0)

In the present study, the maximum number of gram-negative pathogens was isolated from respiratory specimens, 43.7% in 2023 and 55% in 2024. A similar observation was reported by Sader *et al.*, where 61.4% of pathogens were isolated from respiratory specimens [7]. However, in another study conducted by Pattnaik *et al.*, urine was the most common source of pathogens (42.43%), followed by respiratory specimens (25.65%) and blood specimens (10.53%) [8].

In the present study, among the GNB isolates, the predominant pathogen isolated was *Klebsiella* spp. (47.7% in 2023 and 36.6% in 2024) followed by *Acinetobacter* spp. (13.9% in 2023–26.9% in 2024), *E. coli* (18.5% in 2023–17.3% in 2024), *Pseudomonas* spp. (15.2% in 2023–14.3% in 2024). These findings are consistent with those reported by Chidambaram *et al.*, where *Klebsiella* spp. accounted for (47.87%), followed by *E. coli* (10.64%), *Pseudomonas* spp. (11.70%), and *Acinetobacter* spp. (11.17%) [6]. However, a study conducted in Iran by Sader *et al.* found *E. coli* as the most prevalent isolate (18.8%), followed by *Klebsiella* spp. (14.4%), *Proteus* spp. (3.2%), and *Acinetobacter* spp. (2.9%) [7].

The variation in prevalence rates observed across studies may be attributed to differences in geographical settings, hospital environments, antimicrobial stewardship practices, and patient demographics. The rising trend of *Acinetobacter* spp. in our setting, from 13.9% in 2023 to 26.9% in 2024, is particularly concerning due to their high potential for multidrug resistance and association with adverse clinical outcomes.

This study also evaluated the trends in antibiotic resistance among GNB over a 2-year timeframe. The antibiotic resistance profiles of the predominant bacteria, *Klebsiella* spp. exhibited consistently high levels of resistance to several antibiotic classes. The highest resistance was observed against cephalosporins (cefuroxime and ceftazidime), with resistance rates of over 95% in both 2023 and 2024. A concerning trend was noted in the resistance to carbapenems (imipenem and meropenem), which showed a drastic increase from 19.4% in 2023 to 71.2% in 2024, suggesting the possible emergence of carbapenemase-producing strains. Colistin resistance remained rare, with only one isolate each year exhibiting resistance. These findings are consistent with a study by Chidambaram *et al.*, where 79.26% of *Klebsiella* isolates were resistant to third-generation cephalosporins, 34.44% to quinolones, and 48.34% to aminoglycosides. Another Indian study by Moolchandani *et al.* reported a high degree of resistance of *Klebsiella* spp. to cephalosporins (67.6–89.9%) and quinolones (74.1–90.1%), and aminoglycosides (48.2–76.2%) [9].

In the present study, *E. coli* exhibited high resistance to cefuroxime (89.3% in 2023 and 95.1% in 2024) and ciprofloxacin (78.5% in 2023, increasing to 97.5% in 2024). Furthermore, in this study, resistance to carbapenems rose notably from 7.1% in 2023 to 34.1% in 2024, raising concerns regarding limited therapeutic options. Similar findings were observed in a study by Singh *et al.*, which reported 44% *E. coli* isolates being resistant to third-generation cephalosporins and 83% to ciprofloxacin [10].

Acinetobacter spp. demonstrated an alarmingly high resistance profile across all major classes, especially to cephalosporins, aztreonam, and fluoroquinolones, with resistance exceeding 95% in both years. The resistance to piperacillin–tazobactam also increased significantly from 54.8% in 2023 to 84.3% in 2024. Resistance to co-trimoxazole remained high (100% in 2023 and 87.5% in 2024), whereas colistin

resistance was detected in a single isolate each year. In our study, carbapenem resistance was very high, with a drastic rise from 47.6% in 2023 to 96.8% in 2024. This indicates the potential dominance of MDR or XDR *Acinetobacter* spp. in the ICU environment.

Aboshakwa *et al.* and Huber *et al.* studied the resistance pattern of *Acinetobacter* spp. in ICU settings. Both studies reported similar high-level resistance among major classes of drugs [11,12]. Another study by Reddy *et al.* showed that 88.7% of *Acinetobacter baumannii* isolates were resistant to aminoglycosides, whereas 80.9% were resistant to either β -lactams or β -lactam/ β -lactamase inhibitor combination, 77.3% were cephalosporin resistant, 75.3% were carbapenem resistant, and 0.5% were colistin resistant [13].

In the present study, *Pseudomonas* spp. exhibited comparatively lower resistance rates than *Acinetobacter* spp. but still posed a challenge. Resistance to ceftazidime (58.7% in 2023 and 67.6% in 2024) and cefepime (58.7% in 2023 and 70.5% in 2024) increased over 2 years, and carbapenem resistance rose from 21.7% in 2023 to 55.8% in 2024, indicating an upward trend. Resistance to other antibiotics amikacin (39.1% in 2023 and 58.8% in 2024), gentamicin (41.3% in 2023 and 58.8% in 2024), and fluoroquinolones (ciprofloxacin 54.3% in 2023 and 64.7% in 2024) also showed an increasing trend, although still lower compared to *Acinetobacter* spp. which showed comparatively higher resistance to amikacin (76.2% in 2023 and 89% in 2024), gentamicin (76.2% in 2023 and 90.6% in 2024) and fluoroquinolones (ciprofloxacin 97.6% in 2023 and 96.8% in 2024). Resistance to retrospective ICU study by Dash *et al.* found a significant upward trend in resistance among *Pseudomonas* spp. with meropenem resistance rising from 51.4% to 68.8%, ceftazidime from 48.6% to 60.4%, amikacin from 45.7% to 56.3%, ciprofloxacin from 57.1% to 66.7%, piperacillin–tazobactam from 62.9% to 70.8% [14]. These trends closely mirror our findings with cephalosporins and carbapenems showing increased resistance, alongside aminoglycosides and fluoroquinolones.

In our study, the organism-wise distribution of MDR, XDR, and PDR gram-negative isolates showed notable year-wise variations. Among the MDR isolates in 2023 and 2024, *Klebsiella* spp. exhibited a marked decline from 49.3% to 11.4%. A similar trend was observed in *Acinetobacter* spp. (35.7% in 2023–6.2% in 2024) and *Pseudomonas* spp. (10.8–2.9%). *E. coli* showed a relatively stable MDR pattern (30.3% in 2023 and 29.2% in 2024), whereas *Proteus* spp. showed a marginal decline (18.1–16.6%). However, this decrease is likely at the cost of an increase in the XDR pattern of the various isolates. *Klebsiella* spp. rose significantly from 13.2% in 2023 to 66.6% in 2024. Similarly, XDR *Acinetobacter* spp. increased from 13.2% to 75%, and *Pseudomonas* spp. from 15.2% to 44.1%. XDR *E. coli* also showed an increase from 7.1% to 29.2%, whereas *Proteus* spp. maintained the same frequency (18.1% in 2023 and 16.6% in 2024).

It was observed that the PDR isolates showed a slight increase. *Klebsiella* spp. showed a minimal rise from 0.6% in 2023 to 1.1% in 2024. *Acinetobacter* spp. had a slight decline in PDR isolates from 2.3% to 1.5% over the same period. No PDR strains were noted for *E. coli*, *Pseudomonas* spp., or *Proteus* spp. in either year.

When compared to other studies, our findings demonstrate differing patterns. Pattnaik *et al.* reported much higher MDR rates: 71.63% in

Acinetobacter spp., 71% in *Klebsiella* spp., and 70.04% in *E. coli* [8]. Their reported XDR rates were also higher in *Klebsiella* spp. (57.65%) and *Acinetobacter* spp. (50.35%). PDR rates in their study were notably higher, with 2.84% in *Acinetobacter* spp., 1.71% in *Pseudomonas* spp., 7.4% in *Proteus* spp., and 0.4% in *Klebsiella* spp. [8].

Basak *et al.* reported overall MDR prevalence at 33.5%, XDR at 12.1%, and no PDR strains, indicating a relatively moderate resistance profile [15]. Oliveira *et al.* observed MDR rates of 36%, XDR at 8.1%, and PDR at 0.9%, which aligns partially with our 2023 data but contrasts with the spike in XDR in 2024 [16]. Similarly, Adrizain *et al.* noted MDR at 28.7% and XDR at 4.7% in the pediatric ICU [17].

Our findings reflect an evolving resistance pattern, with a concerning increase in XDR isolates, particularly among *Klebsiella* spp. and *Acinetobacter* spp. in 2024. This shift underscores the urgent need for robust antimicrobial stewardship programs (AMS), continuous surveillance, and targeted infection control interventions to curb the rising threat of XDR gram-negative pathogens.

Limitations

This study has several important limitations that should be acknowledged. First, it was a retrospective observational study, relying solely on existing laboratory records, which inherently limits the availability of comprehensive clinical information such as patient comorbidities, duration of ICU stay, severity of illness, and prior antibiotic exposure. These factors could have provided a deeper understanding of the risk factors associated with AMR.

Second, the study did not assess the clinical outcomes of patients infected with MDR, XDR, or PDR organisms. This omission restricts the ability to correlate AMR patterns with morbidity, mortality, and length of hospital stay.

The treatment regimens administered to the patients were also not analyzed. Evaluating the appropriateness and effectiveness of empirical versus culture-directed therapy could have offered insights into the impact of AMR on therapeutic outcomes.

Furthermore, the study did not perform molecular or genetic characterisation of the resistant isolates. Identifying specific resistance genes or mechanisms (e.g., ESBL, MBL, and carbapenemases) would have provided a more precise understanding of resistance trends and could guide targeted infection control strategies.

Finally, the study employed a limited AST panel focusing on commonly used antimicrobials, which may have missed emerging resistance to less frequently prescribed agents.

CONCLUSION

In this study, GNB caused the majority of infections in patients admitted to the ICU, with *K. pneumoniae* being the predominant pathogen during the study period. The trend showed an increase in resistance toward the majority of classes of antibiotics throughout the study period, with a shift of resistance among isolates from MDR to XDR, pointing toward more severe and difficult-to-treat infections in ICU settings. Infection control practices and implementing AMS are the two keys to controlling the spread of resistant bacteria, especially in ICUs. Thus, early and accurate microbiological diagnosis, culture-guided therapy, and periodic resistance pattern, along with robust infection control practices, are recommended.

AUTHOR'S CONTRIBUTIONS

All authors equally contributed to this article.

CONFLICTS OF INTEREST

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