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BACTERIOLOGICAL AND ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF MULTIDRUG-RESISTANT GRAM-NEGATIVE BLOOD STREAM INFECTIONS IN A TERTIARY CARE HOSPITAL, UTTARAKHAND.

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ABSTRACT

Background: Gram-negative bloodstream infections are an important cause of morbidity among hospitalized patients, and multidrug resistance (MDR) can significantly limit treatment options. Local surveillance of causative organisms and antimicrobial susceptibility patterns is essential for appropriate empirical therapy and antimicrobial stewardship. This study evaluated the bacteriological profile and antimicrobial susceptibility patterns of Gram-negative bloodstream isolates, with emphasis on MDR, in a tertiary care hospital in Uttarakhand.

Objective: To determine the bacteriological profile and antimicrobial susceptibility patterns of Gram-negative bloodstream isolates, particularly MDR isolates.

Methods: A prospective cross-sectional study was conducted in the Department of Microbiology over six months, from December 2025 to May 2026. Blood culture records yielding Gram-negative bacilli were reviewed, and organism-specific antimicrobial susceptibility data were analyzed. Duplicate entries with the same laboratory accession number were removed. MDR was defined as resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Results: After data cleaning, 276 Gram-negative bloodstream isolates were identified. *Escherichia coli* was the most frequently isolated organism (98/276, 35.5%), followed by *Klebsiella pneumoniae* (58/276, 21.0%), *Acinetobacter baumannii* complex (34/276, 12.3%), and *Pseudomonas aeruginosa* (18/276, 6.5%). MDR was observed in 65.3% of *E. coli*, 72.4% of *K. pneumoniae*, 82.3% of *A. baumannii* complex, and 33.3% of *P. aeruginosa*. Imipenem resistance was highest in *A. baumannii* complex (82.3%) and *K. pneumoniae* (69.0%). Meropenem resistance showed a similar pattern.

Conclusion: The study demonstrated a considerable burden of MDR among Gram-negative bloodstream isolates, particularly *A. baumannii* complex and *K. pneumoniae*. Continued local surveillance and organism-specific susceptibility data are important for guiding empirical antibiotic therapy and antimicrobial stewardship.

INTRODUCTION

Bloodstream infections (BSIs) are an important cause of morbidity and mortality among hospitalized patients, particularly those with severe illness and prolonged healthcare exposure. Gram-negative bacteria account for a substantial proportion of bloodstream infections and include clinically important organisms such as *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii*. The increasing prevalence of antimicrobial resistance among these organisms has made the management of Gram-negative BSIs more challenging by limiting the number of effective antimicrobial options. Indian studies have reported a considerable burden of antimicrobial resistance among Gram-negative bloodstream isolates, including resistance to cephalosporins, fluoroquinolones, β-lactam/β-lactamase inhibitor combinations and carbapenems [1–5]. Multidrug resistance (MDR) is of particular clinical concern because an individual isolate may show resistance to several antimicrobial classes, making empirical treatment more difficult. In this setting, identification of the causative organism alone is not sufficient, and antimicrobial susceptibility testing is essential for guiding appropriate therapy. Local surveillance of bloodstream isolates can provide useful information on the

organisms encountered in a hospital and their susceptibility patterns. This information can help clinicians select empirical therapy and subsequently modify treatment according to culture and susceptibility results. The problem is especially relevant in tertiary-care and intensive care settings, where critically ill patients are more likely to have prolonged hospital exposure and receive broad-spectrum antibiotics. Previous studies from Indian hospitals have reported substantial MDR among Gram-negative bloodstream isolates, with *K. pneumoniae* and *A. baumannii* frequently showing high levels of resistance[3,6,7].

Carbapenem resistance is an additional concern because carbapenems are important treatment options for serious infections caused by resistant Gram-negative bacteria. The World Health Organization's 2024 Bacterial Priority Pathogens List identifies carbapenem-resistant *A. baumannii* and carbapenem-resistant Enterobacterales as critical-priority pathogens, while carbapenem-resistant *P. aeruginosa* is classified as a high-priority pathogen. These classifications highlight the clinical and public health importance of organisms for which resistance is increasing, and therapeutic options may be limited[8].

The prevalence of antimicrobial resistance can vary considerably between hospitals and geographical regions. Indian studies have reported differences in the occurrence of carbapenem resistance among Gram-negative organisms, including high resistance rates among *A. baumannii* and *K. pneumoniae* in some tertiary-care settings[9–11]. Such variation highlights the importance of institution-specific surveillance, as resistance patterns reported from one geographical area or hospital may not accurately reflect the situation in another setting.

The present study was undertaken to characterize Gram-negative organisms isolated from bloodstream specimens in a tertiary-care hospital in Uttarakhand and to describe their bacteriological and antimicrobial susceptibility patterns, with particular emphasis on multidrug resistance and carbapenem resistance. The study also evaluated the distribution of these organisms according to patient location i.e. ICU and ward settings.

OBJECTIVE

The **primary objective** of the study was to determine the bacteriological profile of Gram-negative organisms isolated from bloodstream specimens in a tertiary-care hospital in Uttarakhand.

The **secondary objectives** were to assess the antimicrobial susceptibility patterns of the isolated Gram-negative organisms, determine the prevalence of multidrug resistance (MDR) and carbapenem resistance where interpretable susceptibility data were available, and evaluate the distribution of these organisms according to patient location i.e. ICU and ward settings.

MATERIALS AND METHODS

Study design and setting

This study was conducted in the Department of Microbiology,Graphic Era Institute of Medical Sciences, Dehradun, Uttarakhand, India. The approved protocol described a prospective cross-sectional study conducted over six months,from December 2025 to May 2026,among patients with clinically suspected bloodstream infection whose blood cultures yielded Gram-negative bacilli. Multidrug resistance (MDR) was defined in the protocol as resistance to at least one antimicrobial agent in three or more antimicrobial classes.

Study population and isolate selection

The data-set consisted of organism-specific laboratory records of Gram-negative organisms recovered from blood culture specimens. The organism-specific worksheets were used as the primary source for analyzing organism distribution and antimicrobial susceptibility because they were restricted to blood specimens. A separate blood-only filter sheet was used to cross-check the overall number of Gram-negative blood culture records.

Duplicate entries associated with the same laboratory accession number were reviewed during data cleaning and redundant entries representing the same isolate were removed.

Identification and antimicrobial susceptibility testing

The laboratory records included organism identification and antimicrobial susceptibility results. According to the approved protocol, standard microbiological methods were used for organism identification, and antimicrobial susceptibility testing was performed using Kirby-Bauer disc diffusion and/or automated methods, with interpretation according to Clinical and Laboratory Standards Institute (CLSI) criteria.

Categorical susceptibility results were available for the principal organisms, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex. These organisms formed the principal dataset for the MDR and antimicrobial susceptibility analyses. The less frequently isolated organisms were only included in the bacteriological distribution.

The antimicrobial agents analyzed were those represented in the laboratory dataset and included aminoglycosides, third- and fourth-generation cephalosporins, β-lactam/β-lactamase inhibitor combinations, fluoroquinolones and carbapenems.

Definition of multidrug resistance

MDR was defined according to the study protocol as resistance to at least one antimicrobial agent in three or more antimicrobial classes. MDR classification was based on the available categorical antimicrobial susceptibility results for each isolate. Isolates with sufficient susceptibility information were classified as MDR or non-MDR according to this definition.

Resistance mechanism such as Carbapenemaseproduction was inferred solely from the susceptibility profile. The phenotypic confirmatory testing or molecular detection of resistance genes was not done.

Statistical analysis

Categorical variables were expressed as frequencies and percentages. MDR and carbapenem resistance among the major Gram-negative organisms were compared using the chi-square test. Fisher's exact test was used where expected cell counts were small. A p-value <0.05 was considered statistically significant.

The ICU-versus-ward analysis was restricted to bacteriological percentage distribution.

Ethical considerations

The study was conducted in accordance with the approved institutional research protocol. The IEC approval was obtained before initiation of the study with IEC approval number: GEU-GEIMS/IEC/2025/3870, dated 2/12/2025.

RESULTS

Overall bacteriological profile

A total of 276 non-duplicate Gram-negative bloodstream isolates were identified during the available study period from December 2025 to May 2026. *Escherichia coli* was the most frequently isolated organism, accounting for 98 (35.5%) isolates, followed by *Klebsiella pneumoniae*58 (21.0%), *Acinetobacter baumannii* complex 34 (12.3%) and *Pseudomonas aeruginosa*18 (6.5%). Together, these four organisms accounted for 208 (75.4%) of all Gram-negative bloodstream isolates. Among the other organisms, *Salmonella Typhi* was isolated in 14 (5.1%) cases, followed by *Stenotrophomonas maltophilia* and *Salmonella Paratyphi A*, with 8 (2.9%) isolates each. *Burkholderia cepacia* and *Klebsiella oxytoca* accounted for 6 (2.2%) isolates each. Less frequently isolated organisms included *Acinetobacter lwoffii*, *Morganella morganii*, *Elizabethkingiameningoseptica*, *Serratia marcescens*, and *Enterobacter cloacae* complex with 4 (1.4%) isolates each. *Proteus mirabilis*, *Achromobacterxylosoxidans* and *Acinetobacter ursingii* were recovered from 2 (0.7%) blood culture each.The detailed distribution of the Gram-negative bloodstream isolates is presented in Table 01.

Table 0: .Distribution of Gram-negative bloodstream isolates

Organism	Number of isolates, n	Percentage, %
Escherichia coli	98	35.5
Klebsiella pneumoniae	58	21.0
Acinetobacter baumannii complex	34	12.3
Pseudomonas aeruginosa	18	6.5
Salmonella Typhi	14	5.1
Stenotrophomonas maltophilia	8	2.9
Salmonella Paratyphi A	8	2.9
Burkholderia cepacia	6	2.2
Klebsiella oxytoca	6	2.2
Acinetobacter lwoffii	4	1.4
Morganella morganii	4	1.4
Elizabethkingiameningoseptica	4	1.4
Serratia marcescens	4	1.4
Enterobacter cloacae complex	4	1.4
Proteus mirabilis	2	0.7
Achromobacterxylosoxidans	2	0.7
Acinetobacter ursingii	2	0.7
Total	276	**100.0**

Antimicrobial susceptibility pattern

Antimicrobial susceptibility patterns varied considerably among the four major Gram-negative bloodstream isolates. The detailed susceptibility results are presented in Table 02. Among the 98*E. coli* isolates, resistance was particularly high to ciprofloxacin, with 88/98 (89.8%) isolates resistant. Cefepime resistance was observed in 78/98 (79.6%) isolates, while ceftriaxone resistance was recorded in 86/98 (87.8%) isolates. Piperacillin-tazobactam resistance was observed in 44/9844.9%, and Cefoperazone-sulbactam resistance in 42/98 (42.9%). Imipenem remained active against most isolates, with resistance in 38/98 (38.8%), whereas all 98 isolates were susceptible to meropenem in the available categorical results. Amikacin resistance was observed in 24/98 (24.5%) isolates and Gentamicin resistance was observed in 30/98 (30.6%) isolates.

Among the 58*K. pneumoniae* isolates, high resistance was observed to several commonly tested agents. Ciprofloxacin resistance was present in 44/58 (75.9%) isolates. Cefepime resistance was observed in 40/58 (69%) isolates, while ceftriaxone resistance was observed in 43/58 (74.1%) isolates. Piperacillin-tazobactam resistance was recorded in 40/58 (69%), and Cefoperazone-sulbactam resistance in 40/58 (69%). Carbapenem resistance was also substantial, with 40/58 (69.0%) isolates resistant to imipenem and 40/58 (69%) resistant to meropenem. Amikacin resistance was observed in 31/58 (53.4%) isolates and Gentamicin resistance was observed in 31/58 (53.4%) isolates.

The 34*A. baumannii* complex isolates showed a broad resistance profile. Resistance to amikacin, gentamicin, ceftazidime, cefepime, ciprofloxacinand levofloxacin was observed in 28/34 (82.3%)isolateseach. Carbapenem resistance was also frequent, with 28/34 (82.3%),) isolates resistant to both imipenem and meropenem. Piperacillin-tazobactam showed resistance in 30/30 (100%) interpretable isolates.

Among the 18*P. aeruginosa* isolates, resistance was observed in 6/18 (33.3%) isolates toceftazidime, cefepime, ciprofloxacin, levofloxacin, imipenem and meropenem. Piperacillin-tazobactam resistance was relatively frequent, occurring in 14/18 (77.8%) isolates.

Overall, the susceptibility profile demonstrated marked variation between organisms, with particularly high resistance across multiple antimicrobial classes among *A. baumannii* complex and *K. pneumoniae*. The organism-wise susceptibility distribution is presented in Table 02.

Table 02: Antimicrobial resistance among major Gram-negative bloodstream isolates

Antimicrobial agent	*E. coli* (n=98)	*K. pneumoniae* (n=58)	*A. baumannii* complex (n=34)	*P. aeruginosa* (n=18)
Amikacin	24.5 %	53.4%	82.3%	—
Gentamicin	30.6 %	53.4%	82.3%	—
Ceftriaxone	87.8 %	74.1%	—	—
Cefepime	79.6 %	69%	82.3%	33.3%
Ceftazidime	—	—	82.3%	33.3%
Piperacillin-tazobactam	44.9 %	69%	100.0%	77.8%
Cefoperazone-sulbactam	42.9 %	69%	—	—
Ciprofloxacin	89.8 %	75.9%	82.3%	33.3%
Levofloxacin	—	—	82.3%	33.3%
Imipenem	38.8 %	69.0%	82.3%	33.3%
Meropenem	0.0%	69.0%	82.3%	33.3%

Note: Values represent percentage of resistant isolates among isolates with an interpretable susceptibility result. "-" indicates that result was not available.

Multidrug resistance among the major Gram-negative isolates

MDR was assessed among the four major Gram-negative organisms for which sufficient categorical antimicrobial susceptibility data were available. The proportion of MDR isolates varied between the organisms. *A. baumannii* complex showed the highest proportion of MDR isolates, with 28/34 (82.3%) isolates classified as MDR. This was followed by *K. pneumoniae*, in which 42/58 (72.4%) isolates were classified as MDR, and *E. coli*, in which 64/98 (65.3%) isolates were MDR. Among *P. aeruginosa*, 6/18 (33.3%) isolates met the MDR definition.

Overall, 142 of the 208 isolates (68.3%) belonging to the four major organisms were classified as MDR. The remaining 66 (31.7%) were classified as non-MDR based on the available categorical susceptibility results. The distribution of MDR and non-MDR isolates differed significantly among the four organisms ($\chi^2 = 8.63$, df = 3, p = 0.035).

Table 03: Multidrug resistance among the major Gram-negative bloodstream isolates

Organism	Total isolates, n	MDR, n (%)	Non-MDR, n (%)
E. coli	98	64 (65.3)	34 (34.7)
K. pneumoniae	58	42 (72.4)	16 (27.6)
A. baumannii complex	34	28 (82.3)	6 (17.7)
P. aeruginosa	18	6 (33.3)	12 (66.7)
Total	208	**142 (68.3)**	**66 (31.7)**

Carbapenem resistance among major Gram-negative isolates

Carbapenem susceptibility showed substantial variation among the four major Gram-negative organisms. Imipenem resistance was observed in 38/98 (38.8%) *E. coli* isolates, compared with 40/58 (69.0%) *K. pneumoniae*, 28/34 (82.3%) *A. baumannii* complex and 6/18 (33.3%) *P. aeruginosa* isolates.

A similar pattern was observed for meropenem. No meropenem-resistant *E. coli* isolate was recorded among the 98 isolates. In contrast, meropenem resistance was observed in 40/58 (69.0%) *K. pneumoniae*, 28/34 (82.3%) *A. baumannii* complex and 6/18 (33.3%) *P. aeruginosa* isolates. The highest carbapenem resistance was therefore observed among *A. baumannii* complex, followed by *K. pneumoniae*. The organism-wise distribution of imipenem and meropenem resistance is presented in Table 04.

Table 04: Carbapenem resistance among major Gram-negative bloodstream isolates

Organism	Total isolates, n	Imipenem tested, n	Imipenem resistant, n (%)	Imipenem susceptible, n (%)	Meropenem tested, n	Meropenem resistant, n (%)	Meropenem susceptible, n (%)
E. coli	98	98	38 (38.8)	60 (61.2)	98	0 (0.0)	98 (100.0)
K. pneumoniae	58	58	40 (69.0)	18 (31.0)	58	40 (69.0)	18 (31.0)
A. baumannii complex	34	34	28 (82.3)	6 (17.7)	34	28 (82.3)	6 (17.7)
P. aeruginosa	18	18	6 (33.3)	12 (66.7)	18	6 (33.3)	12 (66.7)

ICU versus ward distribution

Of the 276 Gram-negative bloodstream isolates, 184 (66.7%) were associated with ICU-type locations and 92 (33.3%) with documented ward/emergency/inpatient locations (**Table 5**). The ICU-associated isolates included *A. baumannii* complex, *E. coli*, *K. pneumoniae* and *P. aeruginosa*, whereas the ward/inpatient group included *E. coli*, *K. pneumoniae* and *P. aeruginosa*.

Table 05: Distribution of Gram-negative bloodstream isolates according to documented patient location

Patient location	Number of isolates, n	% of total isolates	Major organisms identified
ICU	184	66.7	*A. baumannii* complex, *E. coli*, *K. pneumoniae*, *P. aeruginosa*
Ward/inpatient	92	33.3	*E. coli*, *K. pneumoniae*, *P. aeruginosa*
Total	**276**	**100.0**	—

DISCUSSION

The present study describes the bacteriological profile and antimicrobial susceptibility patterns of Gram-negative bloodstream isolates from a tertiary-care hospital in Uttarakhand. *Escherichia coli* was the most frequently isolated organism (35.5%), followed by *Klebsiella pneumoniae* (21.0%), *Acinetobacter baumannii* complex (12.3%) and *Pseudomonas aeruginosa* (6.5%). These four organisms accounted for 75.4% of all isolates, highlighting their major contribution to Gram-negative bloodstream infections in the study setting. Similar predominance of these organisms has been reported from Indian tertiary-care hospitals [1-4].

A substantial MDR burden was observed among the major organisms. Overall, 142/208 (68.3%) isolates were classified as MDR. The highest proportion was observed in *A. baumannii* complex 82.3% followed by *K. pneumoniae* (72.4%) and *E. coli* (65.3%), whereas MDR was lower among *P. aeruginosa* (33.3%). The high MDR burden among *A. baumannii* and *K. pneumoniae* is consistent with previous Indian studies reporting considerable multidrug resistance

among these organisms in tertiary-care and intensive-care settings [3,4,6].

Antimicrobial susceptibility varied considerably between organisms. *E. coli* showed particularly high resistance to ciprofloxacin (89.8%), ceftriaxone (87.8%) and cefepime (79.6%). *K. pneumoniae* also showed high resistance to ciprofloxacin (75.9%), ceftriaxone (74.1%) and piperacillin-tazobactam (69%). *A. baumannii* complex demonstrated a broad resistance profile, including 82.3% resistance to amikacin, gentamicin, cefepime, ceftazidime, ciprofloxacin and levofloxacin, along with 100% resistance to piperacillin-tazobactam. These findings indicate substantial resistance to commonly used antimicrobial agents and emphasize the importance of local susceptibility surveillance.

Carbapenem resistance was particularly prominent among *A. baumannii* complex and *K. pneumoniae*. Imipenem resistance was observed in 82.3% and 69.0% of these isolates, respectively, while the corresponding meropenem resistance was also 82.3% and 69.0%. In comparison, meropenem resistance was not observed among the interpretable *E. coli* results. Similar high carbapenem resistance among *A. baumannii* and *K. pneumoniae* has been reported in recent Indian studies [3, 6].

The study provides useful institution-specific information on Gram-negative bloodstream isolates and their antimicrobial resistance patterns. Overall, the observed MDR and carbapenem resistance burden supports continued local surveillance, development of an institution-specific antibiogram, and strengthening of antimicrobial stewardship and infection-control practices [12].

STRENGTHS OF THE STUDY

The major strength of this study is that it provides recent, institution-specific data on Gram-negative bloodstream isolates from a tertiary-care hospital in Uttarakhand. The study included a broad range of Gram-negative organisms and assessed antimicrobial susceptibility patterns of major organisms, with particular emphasis on MDR and carbapenem resistance. The study also included a secondary assessment of patient location, providing preliminary information on the distribution of isolates across ICU and ward settings.

LIMITATIONS

Several limitations should be considered. First, this was a single-centre laboratory-based analysis, limiting generalisability to other hospitals. Second, individual-level demographic and clinical variables, including age, sex, comorbidities, prior antibiotic exposure and invasive-device exposure, were unavailable. Third, categorical susceptibility results were not included for less frequent organisms. Finally, ESBL, AmpC and carbapenemase mechanisms were not available, so phenotypic resistance patterns should not be interpreted as evidence of a particular molecular mechanism.

CONCLUSION

The present study demonstrates a substantial burden of antimicrobial resistance among Gram-negative bloodstream isolates in a tertiary-care hospital in Uttarakhand. *E. coli* was the predominant organism, while *A. baumannii* complex and *K. pneumoniae* showed particularly high MDR and carbapenem resistance. The marked variation in susceptibility patterns between organisms highlights the importance of local antimicrobial susceptibility surveillance and institution-specific antibiograms. Continued antimicrobial stewardship and appropriate infection-control practices are essential to support rational antibiotic use and limit the spread of resistant Gram-negative pathogens.

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Nil

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest

AUTHOR CONTRIBUTIONS

I.P.: Conceptualization, methodology, data collection, analysis, and writing of the original manuscript.T.G.S.: Data collection, laboratory investigation, and manuscript review. N.K.: Data analysis, interpretation of results, and manuscript review. P.G.: Literature review, data interpretation, and manuscript editing.All authors contributed to the final revision and approved the submitted version.

ETHICAL STATEMENT

Ethical approval for the study was obtained from the Institutional Ethics Committee of Graphic Era Institute of Medical Sciences, Dehradun, Uttarakhand, India. The study was conducted in accordance with the approved study protocol and applicable ethical standards.

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