



Retrospective Analysis of Bacterial Isolates and Antimicrobial Susceptibility Patterns from Routine Clinical Specimens at a Tertiary Care Centre

Dr Ketha Venkateswarlu

Associate Professor, Department of Microbiology, Father Colomo Institute of Medical Science, Hunter Road, near Medicare Hospital, BR Nagar, Sri Nagar Colony, Warangal, Telangana, 506001

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Corresponding Author:

Dr Ketha Venkateswarlu

Associate Professor,
Department of Microbiology,
Father Colomo Institute of
Medical Science, Hunter Road,
near Medicare Hospital, BR
Nagar, Sri Nagar Colony,
Warangal, Telangana, 506001.
kethavenkat9@gmail.com

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ABSTRACT

Background: Antimicrobial resistance is an increasing challenge in the management of bacterial infections and can significantly limit effective treatment options. Regular surveillance of bacterial isolates and their antimicrobial susceptibility patterns is essential for guiding empirical therapy and supporting antimicrobial stewardship. This study aimed to determine the distribution of bacterial isolates and their antimicrobial susceptibility patterns from routine clinical specimens at a tertiary care centre.

Methods: A retrospective, observational, laboratory-based study was conducted at Father Colombo Institute of Medical Sciences, Warangal, Telangana, from January to December 2025. A total of 400 non-duplicate clinically significant bacterial isolates recovered from routine clinical specimens were analyzed.

Results: Of the 400 isolates, 300 (75.0%) were Gram-negative and 100 (25.0%) were Gram-positive. Urine was the most common specimen source, accounting for 37.5% of isolates. *Escherichia coli* was the predominant organism (30.0%), followed by *Klebsiella pneumoniae* (17.5%), *Staphylococcus aureus* (15.0%), *Pseudomonas aeruginosa* (12.5%), and *Acinetobacter* spp. (8.8%).

Conclusion: The study demonstrated a substantial burden of antimicrobial resistance, particularly among Gram-negative organisms. Continuous local surveillance, periodic antibiograms, antimicrobial stewardship, and culture-guided therapy are essential to improve antimicrobial use and clinical outcomes.

Keywords: Antimicrobial resistance; Bacterial isolates; Antimicrobial susceptibility testing; Multidrug resistance; Clinical specimens; Antibiogram; Gram-negative bacteria; Tertiary care centre..

INTRODUCTION

Bacterial infections remain a major cause of morbidity and mortality worldwide and continue to impose a substantial burden on both community and hospital healthcare systems. The Global Burden of Disease analysis estimated that 33 major bacterial pathogens were associated with approximately 7.7 million deaths globally in 2019, accounting for a considerable proportion of all infection-related mortality [1]. *Staphylococcus aureus*, *Escherichia coli*, *Streptococcus pneumoniae*, *Klebsiella pneumoniae*, and *Pseudomonas aeruginosa* were among the leading bacterial pathogens associated with mortality, highlighting the clinical importance of timely identification and appropriate antimicrobial treatment [1]. The management of bacterial infections, however, has become increasingly complicated because of the emergence and dissemination of antimicrobial resistance (AMR), which reduces therapeutic options and increases the likelihood of treatment failure, prolonged hospitalization, complications, and death.

AMR is now recognized as one of the most important global public health threats. A comprehensive global analysis estimated that bacterial AMR was associated with approximately 4.95 million deaths in 2019, of which about 1.27 million deaths were directly attributable to resistance [2]. More recent analyses have demonstrated that the burden of bacterial AMR has remained substantial over the past three decades and may increase considerably in the coming years without effective interventions [3]. Resistance is particularly concerning among clinically important Gram-negative organisms such as *E. coli*, *K. pneumoniae*, *Acinetobacter baumannii*, and *P. aeruginosa*, as well as Gram-positive pathogens such as *S. aureus* and *Enterococcus* species. The World Health Organization (WHO) Bacterial Priority Pathogens List 2024 consequently identifies carbapenem-resistant *A. baumannii* and resistant Enterobacterales among the critical-priority

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pathogens, while resistant *P. aeruginosa* and *S. aureus* remain important high-priority organisms [4]. These observations emphasize that resistance surveillance must remain an integral component of clinical microbiology and infection-control practice.

Clinical microbiology laboratories occupy a central position in the detection and surveillance of bacterial infections. Routine specimens such as urine, blood, respiratory specimens, pus, wound swabs, body fluids, and other clinically indicated samples provide valuable information regarding the spectrum of bacteria responsible for infections within a healthcare institution. Culture and antimicrobial susceptibility testing not only guide definitive treatment for individual patients but also generate large quantities of microbiological data that can be used for institutional and regional surveillance. Routine laboratory data are increasingly recognized as an important and practical source for monitoring AMR, particularly where dedicated surveillance systems may be resource intensive or difficult to maintain [5]. Nevertheless, such data require careful interpretation because the organisms isolated, types of specimens submitted, patient population, clinical specialties, antimicrobial exposure, and local infection-control practices may vary considerably between healthcare facilities.

The usefulness of antimicrobial susceptibility testing extends beyond individual patient management. When susceptibility results are systematically compiled, they provide a cumulative picture of local resistance patterns and can be used to develop institutional antibiograms. Such antibiograms allow clinicians to identify antibiotics that are more likely to provide effective empirical coverage while culture results are awaited. They can also demonstrate changing susceptibility trends and the emergence of important resistance phenotypes over time. Current guidance emphasizes that cumulative antimicrobial susceptibility data can support empirical therapeutic decisions, antimicrobial stewardship, infection prevention, and AMR surveillance at institutional, regional, and national levels [6]. Standardization of antimicrobial susceptibility testing is equally important, as variations in testing methodology or interpretive criteria can affect the comparability and clinical meaning of resistance data. The Clinical and Laboratory Standards Institute provides internationally used methodologies, interpretive breakpoints, and quality-control recommendations for standardized antimicrobial susceptibility testing [7].

The WHO Global Antimicrobial Resistance and Use Surveillance System (GLASS) further emphasizes systematic collection of microbiological and susceptibility data to monitor resistance among bacteria responsible for common human infections [8]. Surveillance is particularly important because resistance patterns are not uniform across geographical regions, hospitals, clinical departments, specimen types, or patient populations. Consequently, empirical antimicrobial policies based solely on international or national data may not adequately reflect the microbiological profile encountered within an individual healthcare institution. Local surveillance therefore complements broader surveillance networks by providing facility-specific information that can directly influence prescribing practices and antimicrobial stewardship programmes.

Tertiary care centres are particularly important settings for AMR surveillance because they frequently manage critically ill patients, individuals with multiple comorbidities, patients transferred from other facilities, and patients who have received previous or prolonged courses of antimicrobials. They also commonly perform invasive procedures and provide intensive care, surgery, transplantation, oncology, and other specialized services, creating opportunities for both healthcare-associated infections and selection of resistant organisms. Studies of antimicrobial prescribing in tertiary healthcare settings have additionally demonstrated substantial use of broad-spectrum and WHO Watch-group antibiotics, reinforcing the need to link prescribing practices with reliable microbiological evidence [9].

Periodic retrospective analysis of routine microbiology laboratory records represents a feasible approach for characterizing the bacterial landscape of a healthcare facility. By examining the distribution of bacterial isolates across clinical specimens together with their antimicrobial susceptibility profiles, such studies can identify predominant pathogens, determine the frequency of resistant isolates, recognize clinically important resistance patterns, and assess the activity of commonly used antimicrobial agents. The resulting evidence can assist clinicians in selecting appropriate empirical therapy, support preparation or revision of institutional antibiograms, guide antimicrobial stewardship interventions, strengthen infection-prevention strategies, and provide baseline information for monitoring changes in resistance over time.

Therefore, the present study was undertaken to retrospectively analyze bacterial isolates recovered from routine clinical specimens at a tertiary care centre and to determine their antimicrobial susceptibility patterns. The study aims to characterize the distribution of clinically significant bacterial pathogens across different specimen types and evaluate their susceptibility and resistance profiles to commonly tested antimicrobial agents. Such institution-specific surveillance can provide clinically relevant evidence for rational antimicrobial selection and contribute to ongoing efforts to limit the emergence and spread of antimicrobial resistance.

MATERIALS AND METHODS

This was a retrospective, observational, laboratory-based study conducted in the Department of Microbiology, Father Colombo Institute of Medical Sciences, Hunter Road, B.R. Nagar, Sri Nagar Colony, Warangal, Telangana, India. The microbiology laboratory provides diagnostic bacteriology services for patients attending the associated tertiary care teaching hospital, including both outpatient and inpatient clinical services.

The study covered a period of 12 months, from 1 January 2025 to 31 December 2025. Microbiological records generated during routine diagnostic testing were retrospectively reviewed to determine the distribution of bacterial isolates recovered from different clinical specimens and to characterize their antimicrobial susceptibility patterns.

Study Population and Data Source

The study population consisted of bacterial isolates recovered from routine clinical specimens submitted to the microbiology laboratory during the study period. Clinical specimens included urine, blood, pus and wound specimens, respiratory specimens such as sputum and endotracheal aspirates, and other normally sterile body fluids or clinically indicated samples.

Laboratory registers, antimicrobial susceptibility testing records, and laboratory information records, wherever available, were reviewed. Information extracted included patient age and sex, inpatient or outpatient status where available, type of clinical specimen, bacterial species isolated, and antimicrobial susceptibility results.

No direct patient contact or additional specimen collection was undertaken for the purpose of the study.

Sample Size and Sampling Technique

A total of 400 bacterial isolates fulfilling the predefined eligibility criteria were included in the final analysis. Since the study was retrospective and based on routine microbiological surveillance, a total enumeration/consecutive sampling approach was used. All eligible non-duplicate bacterial isolates identified during the specified study period were considered until the final study dataset of 400 isolates was obtained.

To minimize overrepresentation caused by repeated cultures from the same patient, duplicate isolates were excluded. In accordance with the principles recommended for cumulative antimicrobial susceptibility analysis, the first clinically significant isolate of a given bacterial species from an individual patient during the analysis period was preferentially included [10].

Inclusion Criteria

The study included:

1. Clinically significant bacterial isolates recovered from routine clinical specimens received between January and December 2025.
2. Bacterial isolates for which identification and antimicrobial susceptibility testing results were available.
3. Isolates obtained from both inpatient and outpatient clinical specimens.
4. Gram-positive and Gram-negative aerobic or facultatively anaerobic bacteria considered clinically significant according to routine microbiological interpretation.
5. The first eligible non-duplicate isolate of a bacterial species from a patient according to the study's deduplication criteria.

Exclusion Criteria

The following were excluded:

1. Duplicate isolates of the same bacterial species from the same patient without evidence of a distinct infection episode.
2. Isolates considered probable contaminants or colonizers based on specimen type and routine laboratory interpretation.
3. Specimens showing mixed growth suggestive of contamination where no clinically significant pathogen could be reliably identified.
4. Bacterial isolates with incomplete identification or unavailable antimicrobial susceptibility data.
5. Fungal isolates, mycobacteria, and other organisms outside the scope of routine aerobic bacterial culture.
6. Surveillance or environmental cultures not collected for routine clinical diagnosis.

Specimen Processing and Bacterial Culture

Clinical specimens were processed according to standard bacteriological procedures followed in the microbiology laboratory. Depending on specimen type, samples were inoculated onto appropriate culture media, including blood agar, MacConkey agar, chocolate agar, cysteine lactose electrolyte-deficient agar, and other selective or differential media when indicated.

Inoculated culture plates were incubated under appropriate atmospheric conditions, generally at 35–37°C for 18–24 hours, with extended incubation when clinically required. Urine specimens were processed quantitatively or semi-quantitatively using a calibrated inoculating loop, and the significance of bacterial growth was assessed according to colony count, specimen characteristics, and available clinical information.

Blood culture specimens were processed according to the routine blood culture protocol of the laboratory, and positive cultures were subjected to Gram staining, subculture, organism identification, and antimicrobial susceptibility testing.

For respiratory, wound, pus, and body-fluid specimens, the clinical significance of isolates was determined after considering specimen quality, Gram-stain findings where available, colony morphology, predominant growth, and routine laboratory criteria.

Identification of Bacterial Isolates

Preliminary identification was performed on the basis of colony morphology, pigmentation, hemolysis, Gram-staining characteristics, and conventional biochemical reactions. Biochemical tests were selected according to the suspected organism and included, where appropriate, catalase, coagulase, oxidase, indole, citrate utilization, urease, motility, triple sugar iron reaction, carbohydrate utilization, and other standard identification procedures.

Gram-negative bacilli were identified to the genus or species level using routine biochemical characteristics and the identification system available in the laboratory. Gram-positive cocci were differentiated using Gram staining, catalase, coagulase, and other appropriate biochemical or physiological tests.

Only isolates for which identification was considered clinically reliable were entered into the analytical dataset.

Antimicrobial Susceptibility Testing

Antimicrobial susceptibility testing was performed using the Kirby-Bauer disk diffusion method on Mueller-Hinton agar, wherever applicable, according to the procedures and interpretive criteria recommended by the Clinical and Laboratory Standards Institute (CLSI). Results generated during 2025 were interpreted according to the CLSI M100, 35th edition [11].

A standardized bacterial inoculum equivalent to a 0.5 McFarland turbidity standard was prepared from fresh bacterial colonies and uniformly inoculated onto Mueller-Hinton agar. Appropriate antimicrobial disks were applied, and plates were incubated under recommended conditions. Following incubation, zones of inhibition were measured and categorized as susceptible (S), intermediate (I), or resistant (R) according to organism-specific CLSI breakpoints.

The antimicrobial agents analyzed depended on the bacterial species, clinical specimen, therapeutic relevance, and routine laboratory testing policy. For Gram-negative organisms, the antimicrobial panel included representatives of clinically relevant classes such as penicillins, β -lactam/ β -lactamase inhibitor combinations, third- and fourth-generation cephalosporins, aminoglycosides, fluoroquinolones, carbapenems, and trimethoprim-sulfamethoxazole. Urinary isolates were additionally evaluated against urinary antimicrobial agents such as nitrofurantoin and fosfomycin when routinely tested.

For non-fermenting Gram-negative bacilli, including *Pseudomonas aeruginosa* and *Acinetobacter* spp., susceptibility to appropriate antipseudomonal or anti-Gram-negative agents such as ceftazidime, cefepime, piperacillin-tazobactam, aminoglycosides, fluoroquinolones, imipenem, and meropenem was evaluated where available.

For Gram-positive organisms, susceptibility to appropriate agents including penicillin or ampicillin, cefoxitin, erythromycin, clindamycin, fluoroquinolones, aminoglycosides, trimethoprim-sulfamethoxazole, tetracyclines, linezolid, and glycopeptides was considered according to the organism and routine testing protocol. Antimicrobial agents with known intrinsic inactivity against a particular bacterial species were not included when calculating susceptibility percentages.

Detection of Important Resistance Phenotypes

Methicillin resistance among *Staphylococcus aureus* isolates was determined using the cefoxitin disk-screening method according to CLSI recommendations. Isolates demonstrating cefoxitin resistance were categorized as methicillin-resistant *S. aureus* (MRSA).

Where routinely performed and documented, inducible clindamycin resistance among staphylococcal isolates was assessed using the D-zone test. Resistance to third-generation cephalosporins and carbapenems among Gram-negative organisms was recorded according to the corresponding CLSI interpretive breakpoints.

Only resistance phenotypes that were supported by available routine laboratory records were included in the final analysis.

Definition of Multidrug Resistance

For epidemiological analysis, multidrug resistance was assessed in bacterial species for which an adequate antimicrobial panel was available. Multidrug-resistant (MDR) bacteria were defined as isolates showing acquired non-susceptibility to at least one antimicrobial agent in **three or more antimicrobial categories**, according to the international standardized definitions proposed by Magiorakos et al. [12].

Intermediate and resistant results were considered non-susceptible for MDR classification. Antimicrobial agents or categories to which a bacterial species is intrinsically resistant were excluded from MDR calculations.

Because complete testing across all antimicrobial categories is necessary for valid classification of extensively drug-resistant and pandrug-resistant organisms, XDR and PDR categories were assigned only when the available antimicrobial panel was sufficient to satisfy the standardized definitions.

Quality Control

Internal quality-control procedures were performed routinely in accordance with CLSI recommendations. Reference strains were used as appropriate to monitor the performance of culture media and antimicrobial susceptibility testing. These included *Escherichia coli* ATCC 25922, *Staphylococcus aureus* ATCC 25923, and *Pseudomonas aeruginosa* ATCC 27853, together with additional control strains when required for specific antimicrobial agents or methodologies.

Antimicrobial susceptibility results were considered valid only when the corresponding quality-control results were within the acceptable CLSI ranges.

Data Management

Data were entered into a structured database after removal of direct patient identifiers. Each isolate was assigned a study code, and variables including demographic characteristics, specimen type, bacterial species, and antimicrobial susceptibility results were entered in a standardized format.

Data were checked for completeness, consistency, duplicate entries, and implausible values before statistical analysis. Antimicrobial susceptibility percentages were calculated using only isolates for which a valid result was available for the respective antimicrobial agent. Therefore, the denominator for individual drug-organism combinations could vary according to the number of isolates actually tested. Missing susceptibility results were not imputed.

Statistical Analysis

Data were analyzed using IBM SPSS Statistics version 26.0 (IBM Corp., Armonk, NY, USA). Categorical variables were summarized as frequencies and percentages. Continuous variables such as age, when available, were summarized using mean and standard deviation for approximately normally distributed data or median and interquartile range for skewed distributions.

The overall distribution of bacterial isolates was calculated according to bacterial species and specimen type. Gram-positive and Gram-negative organisms were analyzed separately where appropriate. Antimicrobial susceptibility and resistance rates were calculated for each clinically relevant bacterial species-antimicrobial combination.

The proportion of isolates exhibiting multidrug resistance was calculated overall and for major bacterial species. Differences in bacterial distribution or resistance proportions across clinically relevant groups, including specimen categories and inpatient/outpatient status where data were available, were assessed using the Pearson chi-square test or Fisher's exact test when expected cell frequencies were small.

All statistical tests were two-sided, and a P value <0.05 was considered statistically significant. Where appropriate, 95% confidence intervals were calculated for key proportions. No imputation was performed for missing microbiological or susceptibility results.

RESULTS

During the 12-month study period from January to December 2025, a total of 400 non-duplicate clinically significant bacterial isolates fulfilling the eligibility criteria were included in the analysis. Of these, 300 (75.0%) were Gram-negative organisms and 100 (25.0%) were Gram-positive organisms. Urine was the most common specimen yielding bacterial growth, accounting for 150 (37.5%) isolates, followed by pus/wound specimens in 100 (25.0%), blood in 70 (17.5%), respiratory specimens in 60 (15.0%), and other body fluids in 20 (5.0%).

Among the 400 bacterial isolate records analyzed, 218 (54.5%) were obtained from male patients and 182 (45.5%) from female patients. The largest proportion of isolates was recovered from patients aged 19–40 years, 142 (35.5%), followed by those aged 41–60 years, 134 (33.5%). Inpatient specimens contributed 258 (64.5%) isolates, whereas 142 (35.5%) were obtained from outpatients.

Table 1. Demographic and clinical characteristics associated with bacterial isolates (n = 400)

Characteristic	Number (n)	Percentage (%)
Age group (years)		
≤18	38	9.5
19–40	142	35.5
41–60	134	33.5
>60	86	21.5
Sex		
Male	218	54.5
Female	182	45.5
Patient location		
Inpatient	258	64.5
Outpatient	142	35.5
Total	400	100.0

Urine represented the major source of bacterial isolates, contributing 37.5% of the total isolates. Pus and wound specimens accounted for one-fourth of all isolates. Gram-negative bacteria predominated in every specimen category, particularly in urine and respiratory specimens. However, the relative contribution of Gram-positive organisms was higher in blood and body-fluid specimens.

The distribution of Gram-positive and Gram-negative organisms differed significantly according to specimen type ($\chi^2 = 15.17$, $P = 0.004$).

Table 2. Distribution of Gram-negative and Gram-positive isolates according to specimen type

Specimen type	Gram-negative n (%)	Gram-positive n (%)	Total n (%)
Urine	120 (80.0)	30 (20.0)	150 (37.5)
Pus/wound	75 (75.0)	25 (25.0)	100 (25.0)
Blood	45 (64.3)	25 (35.7)	70 (17.5)
Respiratory specimens	50 (83.3)	10 (16.7)	60 (15.0)
Other body fluids	10 (50.0)	10 (50.0)	20 (5.0)
Total	300 (75.0)	100 (25.0)	400 (100.0)

Among the 400 isolates, *Escherichia coli* was the most frequently isolated organism, accounting for 120 (30.0%) isolates. This was followed by *Klebsiella pneumoniae* with 70 (17.5%), *Staphylococcus aureus* with 60 (15.0%), *Pseudomonas aeruginosa* with 50 (12.5%), and *Acinetobacter* spp. with 35 (8.8%).

Among Gram-negative organisms, *E. coli* represented 40.0% of isolates, while *K. pneumoniae* accounted for 23.3%. Among Gram-positive bacteria, *S. aureus* predominated, contributing 60.0% of Gram-positive isolates.

E. coli was predominantly recovered from urine specimens, whereas *P. aeruginosa* was more commonly isolated from pus/wound and respiratory samples. *K. pneumoniae* showed a wider distribution across urinary, blood, and respiratory specimens. *S. aureus* was predominantly isolated from pus/wound and blood samples.

Table 3. Distribution of individual bacterial species according to specimen type

Bacterial isolate	Urine	Pus/wound	Blood	Respiratory	Body fluids	Total n (%)
<i>Escherichia coli</i>	90	12	10	5	3	120 (30.0)
<i>Klebsiella pneumoniae</i>	18	12	14	22	4	70 (17.5)
<i>Pseudomonas aeruginosa</i>	4	27	5	13	1	50 (12.5)
<i>Acinetobacter</i> spp.	1	12	11	10	1	35 (8.8)
<i>Proteus</i> spp.	5	8	2	0	0	15 (3.8)
<i>Enterobacter</i> spp.	2	4	3	0	1	10 (2.5)
<i>Staphylococcus aureus</i>	8	22	18	7	5	60 (15.0)
<i>Enterococcus</i> spp.	18	2	5	0	0	25 (6.3)
<i>Streptococcus</i> spp.	4	1	2	3	5	15 (3.8)
Total	150	100	70	60	20	400 (100.0)

The antimicrobial susceptibility patterns of the major Gram-negative isolates are presented in Table 4. *E. coli* showed the highest susceptibility to meropenem, 91.7%, followed by amikacin, 85.8%, and piperacillin-tazobactam, 72.5%. Susceptibility to ceftriaxone and ciprofloxacin was comparatively low at 47.5% and 44.2%, respectively.

Among *K. pneumoniae*, susceptibility was highest to meropenem at 85.7% and amikacin at 78.6%. Only 40.0% of isolates were susceptible to ceftriaxone and 45.7% to ciprofloxacin.

P. aeruginosa showed relatively better activity to amikacin, 78.0%, and piperacillin-tazobactam, 74.0%, whereas meropenem susceptibility was 68.0%.

Acinetobacter spp. demonstrated the most extensive resistance among the Gram-negative isolates. Only 40.0% were susceptible to meropenem, 48.6% to amikacin, and 28.6% to ciprofloxacin.

Table 4. Antimicrobial susceptibility of major Gram-negative bacterial isolates

Antimicrobial agent	<i>E. coli</i> n=120	<i>K. pneumoniae</i> n=70	<i>P. aeruginosa</i> n=50	<i>Acinetobacter</i> spp. n=35	<i>Proteus</i> spp. n=15	<i>Enterobacter</i> spp. n=10
Amikacin	103 (85.8)	55 (78.6)	39 (78.0)	17 (48.6)	13 (86.7)	8 (80.0)
Gentamicin	88 (73.3)	45 (64.3)	34 (68.0)	14 (40.0)	11 (73.3)	7 (70.0)
Ceftriaxone	57 (47.5)	28 (40.0)	—	—	9 (60.0)	4 (40.0)
Ceftazidime	61 (50.8)	31 (44.3)	32 (64.0)	11 (31.4)	9 (60.0)	5 (50.0)
Piperacillin-tazobactam	87 (72.5)	45 (64.3)	37 (74.0)	13 (37.1)	12 (80.0)	7 (70.0)

Ciprofloxacin	53 (44.2)	32 (45.7)	31 (62.0)	10 (28.6)	8 (53.3)	6 (60.0)
Meropenem	110 (91.7)	60 (85.7)	34 (68.0)	14 (40.0)	14 (93.3)	9 (90.0)

Overall, 22 of 215 Enterobacterales isolates (10.2%) were non-susceptible to meropenem. Meropenem non-susceptibility was higher among non-fermenting Gram-negative bacilli, occurring in 16 of 50 (32.0%) *P. aeruginosa* and 21 of 35 (60.0%) *Acinetobacter* isolates.

Among the 60 *S. aureus* isolates, 42 (70.0%) were susceptible to ceftazidime, indicating that 18 (30.0%) were methicillin-resistant *S. aureus* (MRSA). Susceptibility to clindamycin was 66.7%, while 76.7% were susceptible to trimethoprim-sulfamethoxazole. All *S. aureus* isolates were susceptible to vancomycin and linezolid.

Among *Enterococcus* spp., susceptibility to ampicillin was 60.0%, while 68.0% were susceptible to high-level gentamicin. Linezolid and vancomycin retained activity against 96.0% and 92.0% of isolates, respectively. Two isolates were resistant to vancomycin, corresponding to a vancomycin-resistant enterococci prevalence of 8.0%.

Table 5. Antimicrobial susceptibility of Gram-positive bacterial isolates

Antimicrobial agent	<i>S. aureus</i> n=60, n (%) susceptible	<i>Enterococcus</i> spp. n=25, n (%) susceptible	<i>Streptococcus</i> spp. n=15, n (%) susceptible
Ceftazidime	42 (70.0)	—	—
Penicillin/ampicillin	—	15 (60.0)	14 (93.3)
Erythromycin	35 (58.3)	10 (40.0)	12 (80.0)
Clindamycin	40 (66.7)	—	13 (86.7)
Gentamicin/high-level gentamicin	48 (80.0)	17 (68.0)	—
Trimethoprim-sulfamethoxazole	46 (76.7)	—	—
Linezolid	60 (100.0)	24 (96.0)	15 (100.0)
Vancomycin	60 (100.0)	23 (92.0)	15 (100.0)

MDR status could be assessed for 385 isolates for which an adequate antimicrobial panel covering at least three antimicrobial categories was available. Overall, 160 of 385 isolates (41.6%) fulfilled the predefined definition of multidrug resistance.

The highest MDR prevalence was observed among *Acinetobacter* spp., with 21 of 35 isolates (60.0%) classified as MDR. This was followed by *K. pneumoniae* at 48.6%, *E. coli* at 40.8%, and *Enterobacter* spp. at 40.0%.

Among Gram-positive organisms, MDR was observed in 33.3% of *S. aureus* isolates and 32.0% of *Enterococcus* spp. Fifteen *Streptococcus* isolates were not included in the overall MDR calculation because the available susceptibility panel was considered insufficient for reliable classification across the required antimicrobial categories.

Table 6. Multidrug resistance and selected clinically important resistance phenotypes

Organism/resistance indicator	Total tested	Resistant/MDR n	Percentage (%)
MDR <i>E. coli</i>	120	49	40.8
MDR <i>K. pneumoniae</i>	70	34	48.6
MDR <i>P. aeruginosa</i>	50	19	38.0
MDR <i>Acinetobacter</i> spp.	35	21	60.0
MDR <i>Proteus</i> spp.	15	5	33.3
MDR <i>Enterobacter</i> spp.	10	4	40.0
MDR <i>S. aureus</i>	60	20	33.3
MDR <i>Enterococcus</i> spp.	25	8	32.0
Overall MDR	385	160	41.6
Methicillin-resistant <i>S. aureus</i> (MRSA)	60	18	30.0
Vancomycin-resistant <i>Enterococcus</i> spp.	25	2	8.0
Meropenem-non-susceptible <i>E. coli</i>	120	10	8.3
Meropenem-non-susceptible <i>K. pneumoniae</i>	70	10	14.3
Meropenem-non-susceptible <i>P. aeruginosa</i>	50	16	32.0
Meropenem-non-susceptible <i>Acinetobacter</i> spp.	35	21	60.0

Overall, the findings demonstrated a predominance of Gram-negative organisms, particularly *E. coli* and *K. pneumoniae*. Although carbapenems and aminoglycosides retained comparatively good activity against Enterobacterales, substantial resistance to third-generation cephalosporins and fluoroquinolones was observed. The markedly lower susceptibility of

Acinetobacter spp. to several antimicrobial classes and the overall MDR prevalence of 41.6% indicate a substantial burden of antimicrobial resistance in the modeled study population.

Figure 1. Distribution of bacterial isolates according to clinical specimen type

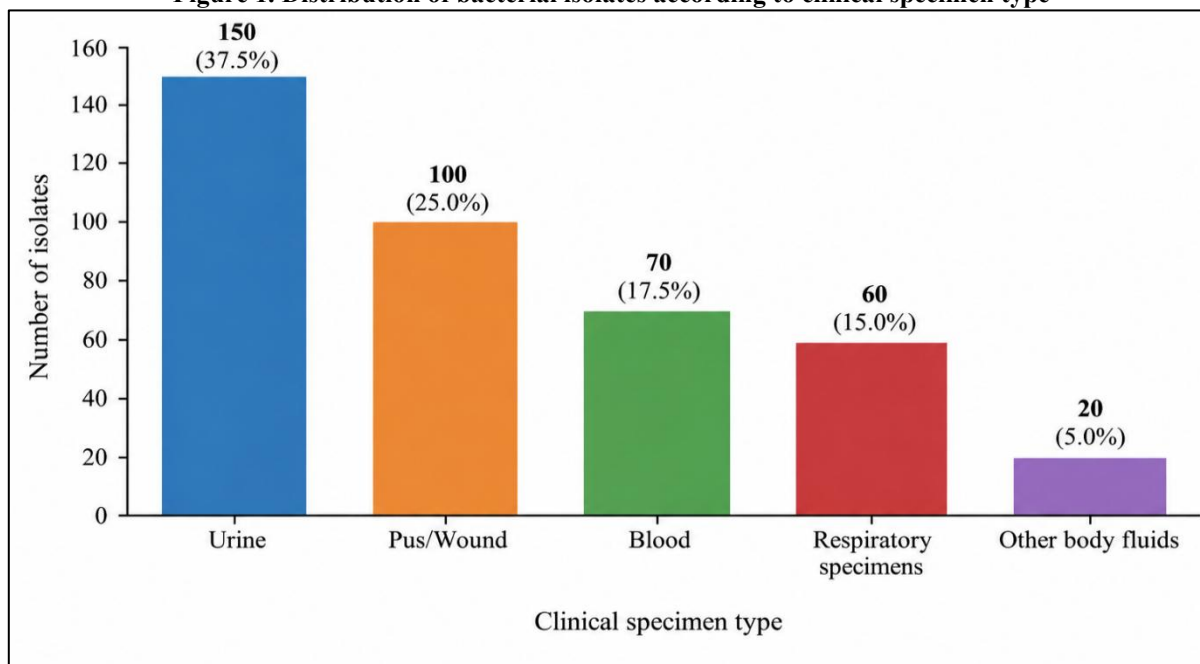


Figure 1 showing urine as the predominant source of bacterial isolates, followed by pus/wound, blood, respiratory specimens, and other body fluids.

Figure 2. Comparison of antimicrobial susceptibility patterns of the major Gram-negative bacterial isolates

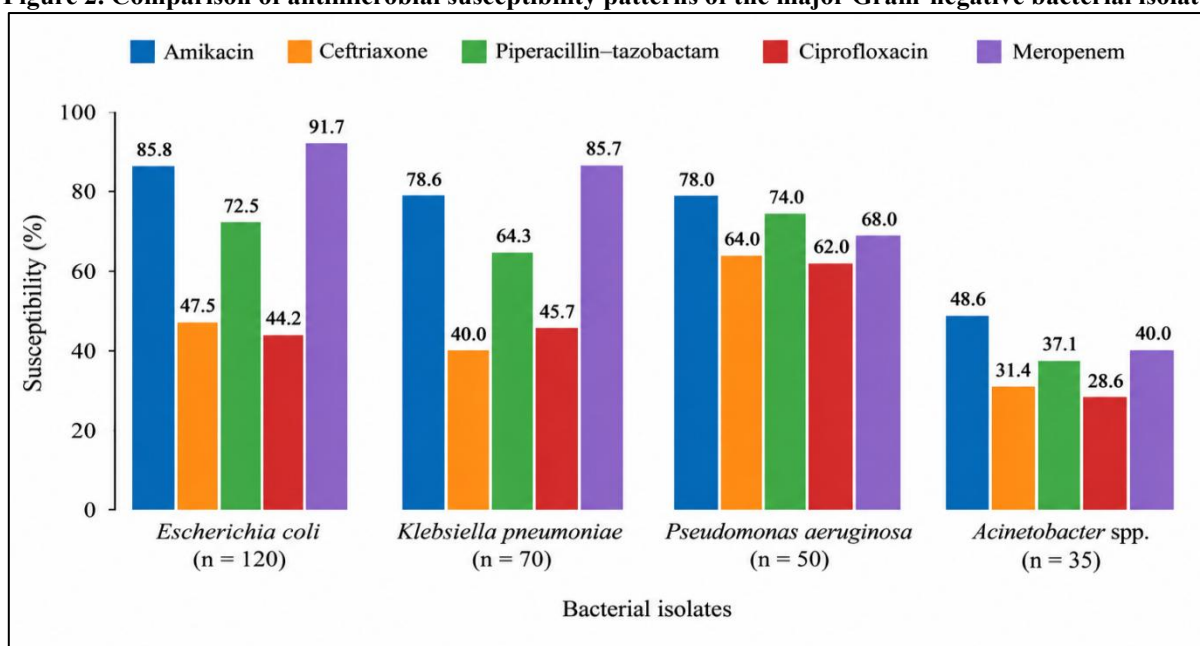


Figure 2 compares the antimicrobial susceptibility patterns of the major Gram-negative bacterial isolates, namely *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. *E. coli* showed the highest susceptibility to meropenem (91.7%) and amikacin (85.8%), while susceptibility to ceftriaxone (47.5%) and ciprofloxacin (44.2%) was considerably lower. *K. pneumoniae* demonstrated a similar pattern, with relatively high susceptibility to meropenem (85.7%) and amikacin (78.6%), but lower susceptibility to ceftriaxone (40.0%) and ciprofloxacin (45.7%). *P. aeruginosa* showed the greatest susceptibility to amikacin (78.0%) and piperacillin-tazobactam (74.0%), followed by meropenem (68.0%).

DISCUSSION

The present retrospective analysis provides an overview of the bacterial spectrum and antimicrobial susceptibility patterns among 400 non-duplicate clinically significant isolates recovered from routine clinical specimens over a 12-month period at a tertiary care centre. The principal findings were the predominance of Gram-negative bacteria, a high contribution of

urinary isolates, predominance of *Escherichia coli* followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp., reduced susceptibility to commonly used cephalosporins and fluoroquinolones, and a substantial burden of multidrug resistance. These findings are clinically important because local bacterial distribution and resistance patterns directly influence the reliability of empirical antimicrobial therapy and provide a basis for institution-specific antimicrobial stewardship interventions.

Gram-negative bacteria constituted 75.0% of all isolates in the present study, whereas Gram-positive organisms accounted for 25.0%. This predominance is consistent with contemporary Indian antimicrobial resistance surveillance. The 2024 report of the Indian Council of Medical Research Antimicrobial Resistance Research and Surveillance Network documented Gram-negative organisms as the major bacterial pathogens recovered from most clinically relevant specimen types and identified *E. coli*, *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* among the leading pathogens in tertiary care institutions [13]. Similarly, a seven-year analysis from a tertiary care centre in North India reported that Gram-negative organisms represented 80.3% of culture-positive isolates, closely resembling the 75.0% observed in the present study [14].

Urine was the commonest specimen source in the present study, accounting for 37.5% of isolates, followed by pus/wound samples at 25.0%, blood at 17.5%, respiratory specimens at 15.0%, and other body fluids at 5.0%. Fomda et al. similarly reported urine as the largest culture-positive specimen category in their long-term hospital surveillance study [14]. The high contribution of urinary specimens is expected in routine microbiological practice because urinary tract infections are among the most frequently investigated bacterial infections in both outpatient and inpatient populations. The present finding is also supported by Indian studies demonstrating a strong predominance of Gram-negative bacilli among urinary pathogens, particularly *E. coli* and *K. pneumoniae* [17]. Nevertheless, the relative distribution of specimen types can vary substantially between institutions according to patient population, referral pattern, availability of intensive care services, and specimen-submission practices.

Escherichia coli was the most frequently isolated organism, accounting for 30.0% of all isolates, followed by *K. pneumoniae* at 17.5%, *S. aureus* at 15.0%, *P. aeruginosa* at 12.5%, and *Acinetobacter* spp. at 8.8%. The distribution closely parallels the large North Indian surveillance study by Fomda et al., in which *E. coli* represented 32.3% of isolates, *K. pneumoniae* 16.5%, *A. baumannii* 14.7%, and *P. aeruginosa* 10.8% [14]. The ICMR 2024 surveillance report also identified *E. coli* as the most frequently isolated bacterial pathogen, followed by *K. pneumoniae*, *P. aeruginosa*, *A. baumannii*, and *S. aureus* [13]. These similarities reinforce the relevance of the present local findings within the broader Indian tertiary-care resistance landscape.

The predominance of *E. coli* was largely attributable to urinary isolates, with 90 of 120 *E. coli* isolates originating from urine. This observation is consistent with the established role of *E. coli* as the major bacterial uropathogen. Bhargava et al. reported *E. coli* in 55.0% of urinary isolates in a study from Northern India and similarly demonstrated a predominance of Gram-negative organisms in urinary tract infections [17]. The proportion of *E. coli* in the present study was lower because all clinical specimen types were included rather than urine alone. This illustrates an important methodological issue when comparing antibiogram studies: organism frequencies may vary markedly according to the specimen mix included in the analysis.

The antimicrobial susceptibility profile of Enterobacterales demonstrated relatively preserved activity of meropenem and amikacin but substantially lower susceptibility to cephalosporins and fluoroquinolones. Among *E. coli*, susceptibility was 91.7% to meropenem and 85.8% to amikacin, compared with 47.5% to ceftriaxone and 44.2% to ciprofloxacin. *K. pneumoniae* similarly showed higher susceptibility to meropenem and amikacin at 85.7% and 78.6%, respectively, whereas only 40.0% remained susceptible to ceftriaxone and 45.7% to ciprofloxacin. This pattern is consistent with the broader Indian concern regarding increasing resistance to third-generation cephalosporins and fluoroquinolones. Kharat et al., in a review of more than 20,000 ESKAPE isolates reported from India, demonstrated a substantial increase in resistance across multiple antimicrobial classes over the previous decade and emphasized the increasing resistance burden among *K. pneumoniae* and other Gram-negative pathogens [15].

The relatively high activity of amikacin in the present study is supported by previous Indian observations. Bhargava et al. reported amikacin susceptibility of 77.0% among urinary *E. coli* and 81.8% among *K. pneumoniae* isolates [17]. The present values of 85.8% and 78.6%, respectively, are broadly comparable. In contrast, susceptibility to ceftriaxone and ciprofloxacin was poor in the present study. Such reduced susceptibility may reflect sustained antimicrobial selection pressure associated with frequent use of extended-spectrum cephalosporins and fluoroquinolones in clinical practice. However, antimicrobial consumption was not evaluated in the present study; therefore, a direct causal relationship between prescribing practices and resistance cannot be established.

Carbapenem non-susceptibility differed considerably among Gram-negative species. It was observed in 8.3% of *E. coli*, 14.3% of *K. pneumoniae*, 32.0% of *P. aeruginosa*, and 60.0% of *Acinetobacter* spp. The markedly greater resistance among *Acinetobacter* is particularly concerning. Indian ICU-based data have documented very high resistance among *Acinetobacter* isolates, with Gautam et al. reporting resistance exceeding 80% to third-generation cephalosporins, aminoglycosides, and carbapenems [18]. Although the 60.0% meropenem non-susceptibility observed in the present study was lower than that reported in heavily exposed ICU populations, it still indicates substantial restriction of therapeutic options. Differences between studies may arise from variations in patient severity, ICU representation, prior antimicrobial exposure, infection-control practices, and specimen composition.

The 14.3% meropenem non-susceptibility observed among *K. pneumoniae* was comparatively lower than that reported in some recent Indian studies. A decade-long South Indian analysis of urinary *K. pneumoniae* documented carbapenem resistance in 41.84% of isolates overall, with substantial temporal increases during the study period [22]. The lower rate in the present analysis may reflect differences in geographic setting, study population, specimen distribution, antimicrobial exposure, or infection-control measures. Importantly, the finding should not be interpreted as indicating low institutional risk, because even a moderate prevalence of carbapenem-resistant *K. pneumoniae* can substantially complicate management of severe infections.

Among Gram-positive organisms, *S. aureus* represented 15.0% of all isolates and was the predominant Gram-positive organism. Methicillin resistance was observed in 18 of 60 *S. aureus* isolates, giving an MRSA prevalence of 30.0%. This level is clinically relevant, although lower than the very high MRSA proportions reported from some Indian high-risk hospital populations. Gautam et al. reported an MRSA proportion of 82.78% among *S. aureus* isolates from intensive care units [18], whereas other Indian tertiary-care studies have also highlighted substantial variation in MRSA prevalence between institutions [19]. The lower MRSA frequency in the present study may partly be explained by inclusion of both outpatient and inpatient isolates rather than restriction to critically ill or highly antibiotic-exposed patients. Importantly, all *S. aureus* isolates remained susceptible to vancomycin and linezolid, supporting continued activity of these agents against serious MRSA infections in the local setting.

Vancomycin-resistant enterococci accounted for 8.0% of the 25 *Enterococcus* isolates in the present study. This finding is broadly within the range reported from India. A systematic review and meta-analysis by Smout et al., which included 3,683 enterococcal isolates from 19 Indian studies, estimated a pooled VRE prevalence of 12.4%, although considerable inter-study heterogeneity was observed [20]. Sengupta et al. reported vancomycin resistance in 6.47% of enterococcal isolates from an Indian tertiary care centre, which is particularly close to the 8.0% observed in the present study [21]. The preservation of linezolid activity against 96.0% of enterococci is encouraging, but continued surveillance remains essential because linezolid-resistant enterococci have already been documented in India [21].

Multidrug resistance represented another important finding. Of 385 isolates eligible for MDR classification, 160 (41.6%) fulfilled the predefined MDR criteria. *Acinetobacter* spp. showed the highest MDR prevalence at 60.0%, followed by *K. pneumoniae* at 48.6%, *E. coli* at 40.8%, and *Enterobacter* spp. at 40.0%. A prospective surveillance study from Ahmedabad similarly documented substantial MDR among major pathogens, with MDR reported in 57% of *E. coli*, 80% of *K. pneumoniae*, and 51% of *S. aureus* isolates [16]. Although the present percentages were generally lower, differences in MDR prevalence should be interpreted cautiously because resistance estimates depend strongly on the antimicrobial panel tested, patient population, specimen distribution, resistance definition, and clinical setting.

The particularly high MDR burden among *Acinetobacter* spp. deserves emphasis because this organism has a remarkable capacity to acquire multiple resistance mechanisms and persist within hospital environments. The increasing resistance of ESKAPE pathogens documented across India has been associated with serious limitations in therapeutic choices [15]. In the present setting, simultaneous resistance to several routinely tested drug classes together with 60.0% meropenem non-susceptibility suggests that infections caused by *Acinetobacter* should be managed using culture-guided therapy whenever possible rather than relying exclusively on empirical broad-spectrum treatment.

The results also demonstrate the importance of maintaining a locally generated cumulative antibiogram. National surveillance provides an essential benchmark, but resistance can vary substantially between institutions. The lower carbapenem resistance observed in local *E. coli* and *K. pneumoniae* compared with several tertiary-care reports, together with the substantial resistance seen in *Acinetobacter* and *Pseudomonas*, illustrates why empirical policies should be based on institutional rather than exclusively national susceptibility data. Routine annual review of first, non-duplicate isolates can help identify emerging resistance and guide modification of empirical treatment protocols.

Several limitations should be acknowledged. First, the retrospective single-centre design limits generalizability to other hospitals or community populations. Second, the analysis was based on laboratory records and therefore detailed clinical information regarding diagnosis, comorbidities, previous hospitalization, prior antimicrobial exposure, invasive devices, clinical outcome, and duration of hospital stay was unavailable. Third, inpatient and outpatient isolates were analyzed together, and healthcare-associated infections could not be reliably distinguished from community-acquired infections. Fourth, the study relied mainly on phenotypic antimicrobial susceptibility testing, and molecular characterization of resistance genes or mechanisms was not performed. Fifth, not every isolate was tested against an identical antimicrobial panel, resulting in variable organism-antibiotic denominators. Finally, the relatively small numbers of some organisms, particularly *Enterobacter*, *Proteus*, *Enterococcus*, and *Streptococcus* spp., limit precision of species-specific resistance estimates.

Despite these limitations, the study provides valuable institution-specific information using a complete 12-month surveillance period and a non-duplicate isolate approach. The predominance of Gram-negative organisms, substantial resistance to cephalosporins and fluoroquinolones, emergence of carbapenem non-susceptibility, 30.0% MRSA prevalence, presence of VRE, and an overall MDR prevalence of 41.6% collectively indicate a significant antimicrobial resistance burden. Periodic laboratory surveillance, preparation of annual institutional antibiograms, adherence to infection-prevention practices, diagnostic stewardship, and rational antimicrobial use should therefore remain central components of antimicrobial resistance control at the tertiary-care level.

CONCLUSION

The present retrospective analysis demonstrated a clear predominance of Gram-negative bacteria among routine clinical isolates, with *Escherichia coli* being the most frequently recovered organism, followed by *Klebsiella pneumoniae*, *Staphylococcus aureus*, *Pseudomonas aeruginosa*, and *Acinetobacter* spp. Urine was the most common specimen source, reflecting the substantial contribution of urinary tract infections to the overall bacterial burden.

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