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Research Article

Antimicrobial Susceptibility Profile of Gram-Positive Cocci and Gram-Negative Bacilli Isolated at a Tertiary Care Teaching Hospital: A Retrospective Observational Study

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Abstract

Background: Antimicrobial resistance (AMR) complicates the treatment of bacterial infections. Local surveillance of antimicrobial susceptibility is needed to track resistance trends and guide empirical therapy.
Aims: To describe the distribution of Gram-positive cocci (GPC) and Gram-negative bacilli (GNB) isolated from clinical specimens and their antimicrobial susceptibility patterns at Khaja Banda Nawaz Teaching and General Hospital (KBNTGH), Kalaburagi.
Materials and Methods: This retrospective observational study reviewed culture and susceptibility records from January 2025 to June 2026. A total of 504 isolates (100 GPC and 404 GNB) were included. Identification used conventional phenotypic methods, and susceptibility was tested by the Kirby–Bauer disc diffusion method and interpreted with CLSI 2025 criteria. Data were analysed descriptively.
Results: Urine was the commonest specimen type (34.3%). GNB accounted for 80.2% of isolates, and *Pseudomonas* spp. (24.8%), *Klebsiella* spp. (24.0%), *Escherichia coli* (22.8%) and *Acinetobacter* spp. (21.8%) predominated. Of 100 *Staphylococcus aureus* isolates, 62% were methicillin-resistant (MRSA). Among GPC, resistance was highest to penicillin (93%). Among GNB, resistance was highest to ampicillin (98.8%) and amoxicillin–clavulanate (91.6%), whereas piperacillin–tazobactam (83.3% susceptible), cefoperazone–sulbactam (71.8%) and meropenem (71.3%) showed the highest susceptibility. Vancomycin results are not reported, because disc diffusion is not a CLSI-recommended method for vancomycin testing of *S. aureus*.
Conclusion: Substantial resistance to commonly used antimicrobials was observed among these clinical isolates. Periodic antibiogram surveillance, rational prescribing and antimicrobial stewardship are needed to guide empirical therapy. As a single-centre retrospective study with pooled Gram-negative data, these findings require confirmation, including species-specific analysis and MIC-based testing of vancomycin.

Keywords: Antimicrobial resistance; Antibiogram; Disc diffusion; Gram-negative bacilli; Methicillin-resistant *Staphylococcus aureus*.

Introduction

Antimicrobial resistance (AMR) is a major global threat to the prevention and treatment of bacterial infection [1]. Excessive and inappropriate antimicrobial use drives the selection and spread of resistant organisms, contributing to treatment failure, higher mortality and a growing healthcare burden [1]. Several resistant pathogens, including methicillin-resistant *Staphylococcus aureus* (MRSA) and resistant Gram-negative bacilli, have therefore been prioritised for research and surveillance [2].

Surveillance is central to the response to AMR. The Global Antimicrobial Resistance and Use Surveillance System (GLASS) offers a framework for collecting and interpreting resistance data to inform public health action [3]. At the hospital level, cumulative antibiograms summarise susceptibility results and support empirical prescribing and antimicrobial stewardship [4].

Tertiary care hospitals treat patients with diverse infections and frequently encounter organisms resistant to several antimicrobial classes [5]. *S. aureus* and Gram-negative bacilli such as *Pseudomonas*, *Klebsiella*, *Escherichia coli* and *Acinetobacter* species are therefore central to routine microbiological surveillance. Reliable results depend on standardised susceptibility testing, performed and interpreted according to Clinical and Laboratory Standards Institute (CLSI) criteria [6,7]. MRSA remains an important pathogen in both healthcare and community settings, and invasive MRSA disease carries substantial morbidity [8,9]. Cefoxitin disc testing serves as a surrogate marker for methicillin resistance in *S. aureus* when it is performed and interpreted according to CLSI standards [6]. Because resistance patterns vary between healthcare settings, locally generated data are needed to guide empirical therapy and public health action [3]. This study therefore describes the distribution of GPC and GNB isolated from clinical specimens and their antimicrobial susceptibility patterns at KBNTGH, Kalaburagi. The specific objectives were to describe the distribution of isolates by specimen type and organism, to determine the proportions of MRSA and methicillin-susceptible *S. aureus* (MSSA), and to identify agents with comparatively higher or lower resistance.

Materials and Methods

Study design and place of study. This was a retrospective, observational, record-based study conducted at the Central Laboratory, Department of Microbiology, Khaja Banda Nawaz Teaching and General Hospital, Kalaburagi, Karnataka, India, a tertiary care teaching hospital. Culture and antimicrobial susceptibility records were reviewed.

Duration. Records of isolates processed from January 2025 to June 2026 (18 months) were analysed.

Study population and sample size. Bacterial isolates recovered from clinical specimens during the study period, with complete identification and susceptibility records, were eligible. All 504 eligible isolates were included, comprising 100 GPC and 404 GNB. No a priori sample size calculation was performed, as all eligible records from the study period were included.

Specimens and bacterial identification. Clinical specimens included urine, blood, pus, sputum, swabs, stool, and pleural or other body fluids. Specimens were processed using routine microbiological procedures. Gram staining and conventional phenotypic identification methods were used as appropriate.

Antimicrobial susceptibility testing. Susceptibility testing was performed by the Kirby–Bauer disc diffusion method [10] according to CLSI performance standards [6,7]. Results were categorised as susceptible, intermediate or resistant using the CLSI 2025 criteria adopted in the laboratory. Methicillin resistance in *S. aureus* was determined using the cefoxitin disc. Vancomycin susceptibility of *S. aureus* was also obtained by disc diffusion. Because CLSI does not recommend disc diffusion for this purpose, these vancomycin results are excluded from the reported findings. The number of isolates tested varied by antibiotic, and percentages were calculated on the number tested for each agent.

Data analysis. Data were compiled in a spreadsheet and summarised descriptively, with frequencies and percentages used to describe the distribution of isolates and susceptibility results. No comparative statistical tests were performed.

Results

All findings are descriptive. No comparative hypothesis tests were performed, and no *p* values are reported.

Distribution of clinical specimens. Of the 504 isolates, urine yielded the largest number (173; 34.3%), followed by blood (134; 26.6%), pus (99; 19.6%) and sputum (50; 9.9%) (Table 1). Together, urine and blood accounted for 60.9% of isolates.

Table 1. Distribution of bacterial isolates by clinical specimen type (n = 504)

Specimen type	No. of isolates	Percentage (%)
Urine	173	34.3
Blood	134	26.6
Pus	99	19.6
Sputum	50	9.9
Swab	37	7.3
Stool	8	1.6
Pleural fluid / other	3	0.6
Total	**504**	**100.0**

The table gives the number and share of isolates from each specimen type. Urine and blood specimens predominated, so the susceptibility

findings mainly reflect urinary and bloodstream isolates.

Distribution of organisms. GNB accounted for 404 isolates (80.2%) and GPC for 100 (19.8%). Among GNB, *Pseudomonas* spp. (24.8%), *Klebsiella* spp. (24.0%), *E. coli* (22.8%) and *Acinetobacter* spp. (21.8%) together accounted for 93.3% of GNB isolates (Table 2). All 100 GPC isolates were *S. aureus*: 62 (62%) were MRSA and 38 (38%) were MSSA.

Table 2. Distribution of Gram-negative bacilli and *Staphylococcus aureus* isolates (n = 504)

Group	Organism / phenotype	No.	% of group
GNB	Pseudomonas spp.	100	24.8
GNB	Klebsiella spp.	97	24.0
GNB	Escherichia coli	92	22.8
GNB	Acinetobacter spp.	88	21.8
GNB	Proteus spp.	18	4.5
GNB	Enterobacter spp.	5	1.2
GNB	Citrobacter spp.	2	0.5
GNB	Shigella spp.	2	0.5
GNB	**Total GNB**	**404**	**100.0**
GPC	MRSA (S. aureus)	62	62.0
GPC	MSSA (S. aureus)	38	38.0
GPC	**Total GPC**	**100**	**100.0**

GNB percentages use 404 GNB isolates as the denominator, and MRSA/MSSA percentages use the 100 *S. aureus* isolates. The four leading genera account for most GNB isolates, while MRSA makes up the majority of *S. aureus* isolates.

Antimicrobial susceptibility of Gram-positive cocci. Among the 100 *S. aureus* isolates, resistance was highest to penicillin (93%), followed by erythromycin (64%), cefoxitin (62%), ciprofloxacin (60%) and clindamycin (60%) (Table 3; Figure 1). Levofloxacin (91% susceptible) and cefoperazone–sulbactam (88% susceptible) showed the highest susceptibility among the agents reported. The cefoxitin resistance rate of 62% equals the MRSA proportion in Table 2. This is expected, because methicillin resistance was defined by the cefoxitin disc, and the concordance supports the internal consistency of the data.

Table 3. Antimicrobial susceptibility pattern of *Staphylococcus aureus* isolates (n = 100), ordered by resistance rate

Antibiotic	Sensitive (S) %	Intermediate (I) %	Resistant (R) %
Penicillin (P)	2	5	93
Erythromycin (E)	28	8	64
Cefoxitin (CX)	38	0	62
Ciprofloxacin (CIP)	33	7	60
Clindamycin (CD)	35	5	60
Azithromycin (AZM)	56	9	35
Amoxiclav (AMC)	61	5	34
Cotrimoxazole (COT)	68	6	26
Gentamicin (GEN)	62	18	20
Amikacin (AK)	72	12	16
Cefoperazone–sulbactam (CFS)	88	0	12
Levofloxacin (LE)	91	2	7

Note: Vancomycin was tested by disc diffusion and is omitted from this table. CLSI does not recommend disc diffusion for vancomycin testing of *S. aureus*, so these results are not reported as valid findings. MIC-based testing is required before vancomycin activity can be described.

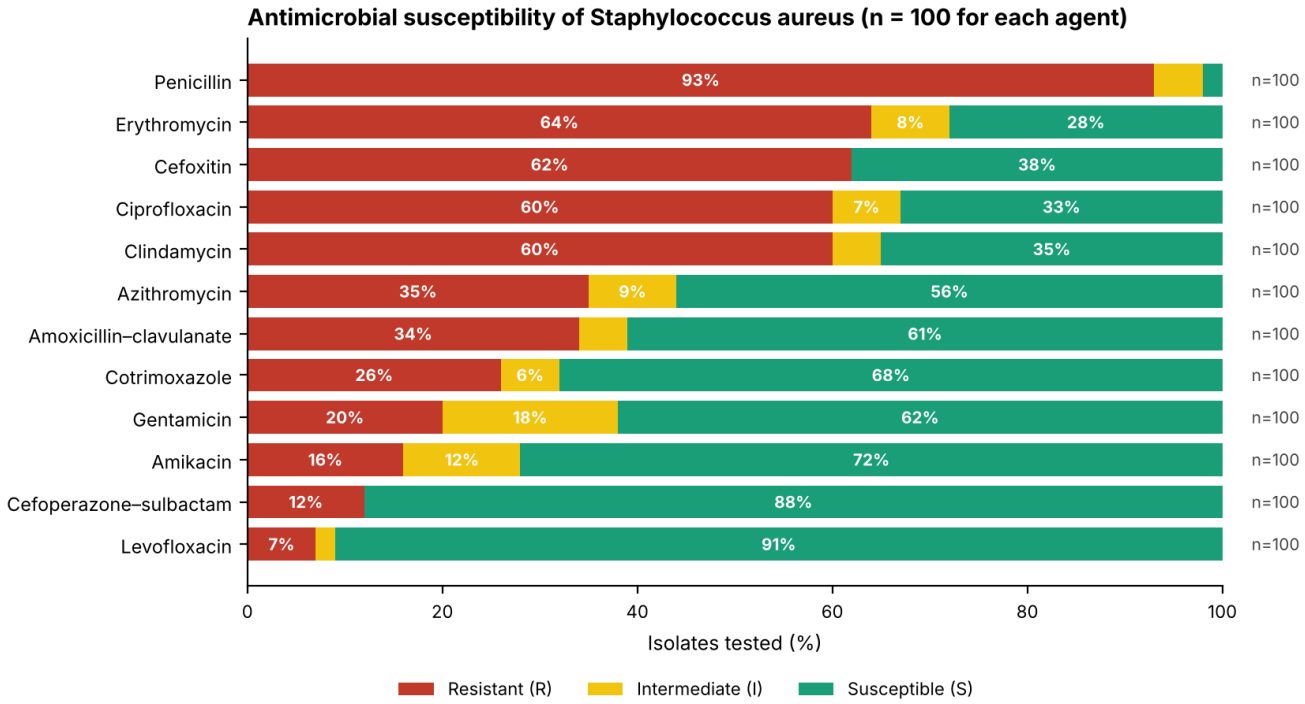


Figure 1. Antimicrobial susceptibility of 100 *S. aureus* isolates to 12 antibiotics. Bars show the percentage of isolates classed as resistant (red), intermediate (yellow) and susceptible (green); n = 100 for every agent. Vancomycin is excluded (see Table 3 note). Antibiotics are sorted by resistance rate.

Interpretation of Table 3 and Figure 1: the two present the same data. Resistance is concentrated in penicillin, erythromycin, cefoxitin, ciprofloxacin and clindamycin, whereas levofloxacin and cefoperazone–sulbactam show the highest susceptibility. Near-universal penicillin resistance is expected in *S. aureus*.

Antimicrobial susceptibility of Gram-negative bacilli. Among the 404 GNB isolates, resistance was highest to ampicillin (98.8%) and amoxicillin–clavulanate (91.6%), followed by ceftriaxone (85.1%), nitrofurantoin (72.0%) and cotrimoxazole (63.8%). Piperacillin–tazobactam (83.3% susceptible), cefoperazone–sulbactam (71.8%), meropenem (71.3%) and imipenem (59.5%) showed the highest susceptibility. Because each agent was tested on a different number of isolates, complete results for each agent, with the number tested and the percentages susceptible (S), intermediate (I) and resistant (R), are given in Table 4. Figure 2 presents the same distributions graphically.

Table 4. Antimicrobial susceptibility of Gram-negative bacilli by agent. Percentages are calculated on the number of isolates tested with each agent (total GNB isolates, n = 404).

Antimicrobial agent	No. tested (n)	Susceptible, n (%)	Intermediate, n (%)	Resistant, n (%)
Ampicillin (AMP)	404	1 (0.2)	4 (1.0)	399 (98.8)
Amoxicillin–clavulanate (AMC)	403	14 (3.5)	20 (5.0)	369 (91.6)
Ceftriaxone (CTR)	268	18 (6.7)	22 (8.2)	228 (85.1)
Nitrofurantoin (NIT)	132	35 (26.5)	2 (1.5)	95 (72.0)
Cotrimoxazole (COT)	290	91 (31.4)	14 (4.8)	185 (63.8)
Cefotaxime (CTX)	114	20 (17.5)	22 (19.3)	72 (63.2)
Cefepime (CPM)	354	78 (22.0)	58 (16.4)	218 (61.6)
Ceftazidime (CAZ)	344	112 (32.6)	31 (9.0)	201 (58.4)
Fosfomycin (FOS)	66	36 (54.5)	2 (3.0)	28 (42.4)
Ciprofloxacin (CIP)	257	122 (47.5)	31 (12.1)	104 (40.5)
Gentamicin (GEN)	391	220 (56.3)	46 (11.8)	125 (32.0)
Amikacin (AK)	396	191 (48.2)	89 (22.5)	116 (29.3)
Imipenem (IMP)	398	237 (59.5)	78 (19.6)	83 (20.9)
Cefoperazone–sulbactam (CFS)	280	201 (71.8)	33 (11.8)	46 (16.4)
Tobramycin (TOB)	258	127 (49.2)	103 (39.9)	28 (10.9)
Meropenem (MRP)	394	281 (71.3)	78 (19.8)	35 (8.9)
Piperacillin–tazobactam (PIT)	396	330 (83.3)	35 (8.8)	31 (7.8)

Note: Species-specific susceptibility data are not presented in this pooled analysis (see Discussion).

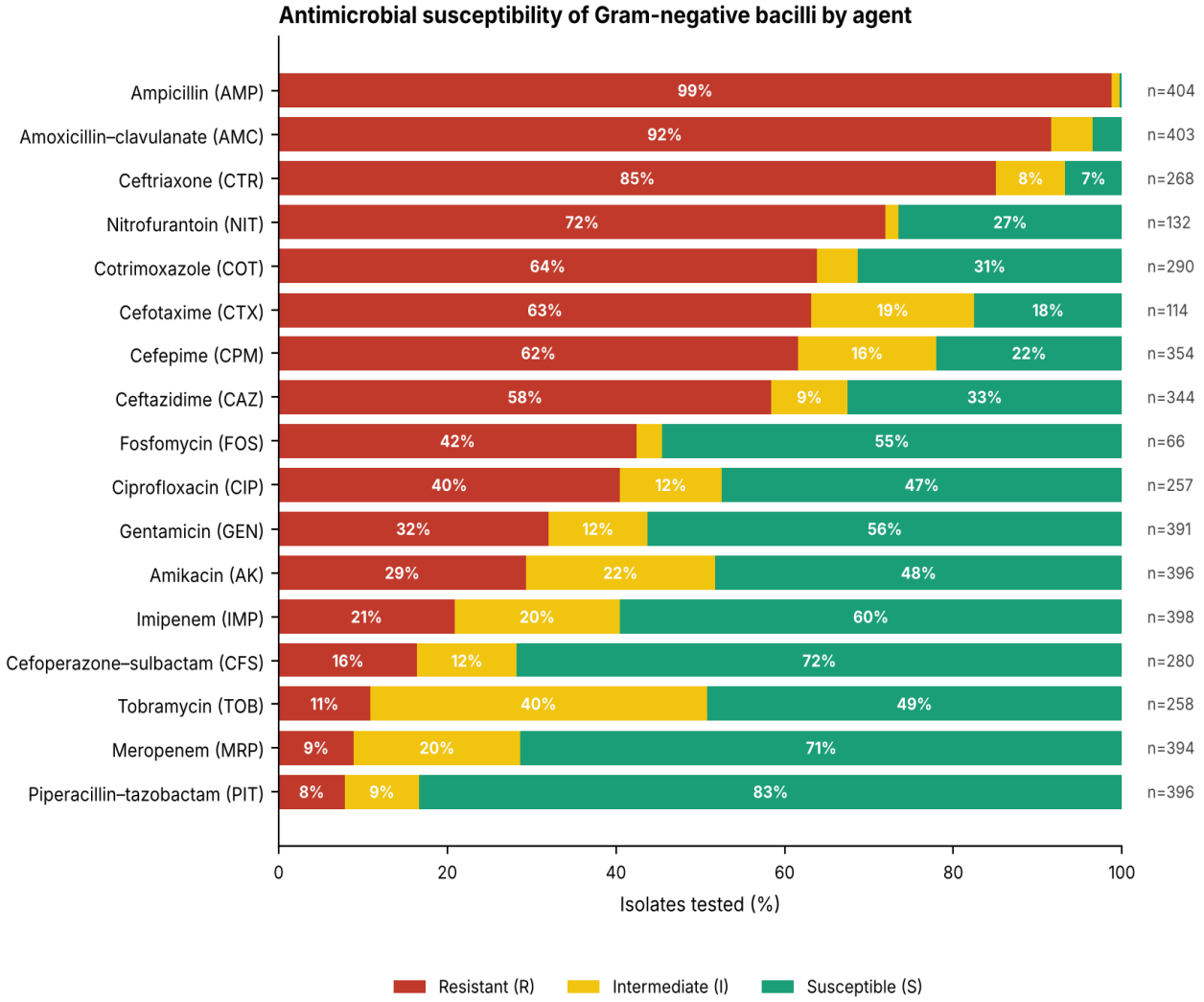


Figure 2. Antimicrobial susceptibility of 404 Gram-negative bacilli by agent. Bars show the percentage of isolates resistant (red), intermediate (yellow) and susceptible (green). The number to the right of each bar gives the number of isolates tested with that agent.

Interpretation of Figure 2: aminopenicillins were largely ineffective, and third-generation and related cephalosporins (ceftriaxone, cefotaxime, cefepime and ceftazidime) showed resistance of 58–85%. Piperacillin–tazobactam, meropenem, tobramycin, cefoperazone–sulbactam and imipenem had the lowest resistance rates (8–21%). Tobramycin, however, had a large intermediate fraction (39.9%), which limits its interpretive value. These pooled proportions describe the isolate population as a whole and cannot be used to select an agent for a specific organism.

Discussion

This retrospective study of 504 clinical isolates from a tertiary care teaching hospital found that GNB made up 80.2% of isolates and that resistance to several commonly used antimicrobials was substantial. Because resistance patterns differ between institutions, these local findings provide a baseline for antibiogram review and stewardship [3].

Pseudomonas, *Klebsiella*, *E. coli* and *Acinetobacter* species together accounted for 93.3% of GNB isolates. These organisms are leading causes of healthcare-associated infection and are recognised for resistance to multiple drug classes [11]. The high resistance to ampicillin (98.8%) and amoxicillin–clavulanate (91.6%), together with resistance to ceftriaxone (85.1%), indicates that these agents are unlikely to be reliable empirical choices for the pooled isolate population. This statement describes the population as a whole and should not be read as guidance for a particular infection. Standardised reporting is needed to

compare these patterns meaningfully with other institutions [12].

Piperacillin–tazobactam (83.3%), cefoperazone–sulbactam (71.8%) and meropenem (71.3%) showed the highest susceptibility among the GNB agents tested. These findings support susceptibility-guided therapy rather than the assumption that any single agent will remain effective across organisms. Carbapenem use should nevertheless be restricted through stewardship programmes to limit selection pressure [13].

The pooled GNB analysis may conceal species-specific differences, and this is the most important limitation of the present report. Carbapenem and beta-lactamase inhibitor combination results can differ substantially between *Pseudomonas*, *Acinetobacter*, *Klebsiella* and *E. coli*. The pooled percentages in Table 4 should therefore not be used to choose therapy for a specific organism. Species-level analysis would identify the organisms contributing most to resistance and would improve the usefulness of the institutional antibiogram [4], and it is the priority for the next phase of this work.

MRSA accounted for 62% of *S. aureus* isolates, a high proportion that underlines the importance of cefoxitin screening, infection prevention and stewardship [8,9]. Because methicillin resistance was defined by the cefoxitin disc, the agreement between cefoxitin resistance (62%) and the MRSA proportion reflects the CLSI-based classification used in this study [6] rather than independent confirmation. Penicillin resistance of 93% is consistent with the widespread penicillinase production in *S. aureus*. The observed percentages describe the isolates in this study and should not automatically be generalised to all patients at the institution.

Vancomycin was tested by disc diffusion. CLSI does not recommend disc diffusion for vancomycin testing of *S. aureus*, and MIC-based methods are the accepted reference standard [6]. For this reason, vancomycin results are not reported as findings in this study, and they should not be used to infer vancomycin activity at this institution. Vancomycin susceptibility of *S. aureus* should be determined by MIC or broth microdilution in future work.

Repeat isolates from the same patient may also distort susceptibility summaries, and a defined first-isolate protocol should be applied in future analyses [4].

Antimicrobial stewardship promotes appropriate selection, dosing, duration and review of therapy. Combined with infection prevention and periodic surveillance, these measures help limit the spread of resistance [3,13]. Multidrug-resistance categories based on standard definitions [5] were not assessed in this study and represent an important extension of this work.

Limitations of the study. The study was conducted at a single institution, which limits generalisability. Its retrospective design depended on existing laboratory records, and clinical data such as age, sex, ward, inpatient or outpatient status and outcome were not available, so susceptibility results could not be linked to patient characteristics. No formal deduplication protocol was applied, so repeat isolates may have contributed to the reported distribution. Denominators differed between antibiotics, and pooled GNB results do not provide species-specific resistance patterns. Vancomycin susceptibility was determined by disc diffusion, a method not recommended for *S. aureus*, and these results were therefore excluded. These limitations should be considered when interpreting the findings.

Conclusion

This retrospective analysis of 504 clinical isolates from a tertiary care teaching hospital showed a predominance of Gram-negative bacilli, led by *Pseudomonas*, *Klebsiella*, *E. coli* and *Acinetobacter* species, and a 62% MRSA proportion among *S. aureus* isolates. Resistance to ampicillin, amoxicillin–clavulanate, ceftriaxone and penicillin was high, whereas piperacillin–tazobactam, cefoperazone–sulbactam and meropenem showed the highest susceptibility among the Gram-negative agents tested. These findings reinforce the value of local antibiogram surveillance, rational antimicrobial prescribing and institutional stewardship [4,13]. Species-specific analysis, MIC-based testing of vancomycin for *S. aureus*, and multicentre prospective studies are needed before these findings guide treatment policy.

Declarations

Conflict of interest: The authors declare that they have no conflict of interest.

Source of funding: None.

Ethical clearance: Was obtained

Informed consent: Not required for this retrospective analysis of existing laboratory records.

Data availability: The dataset analysed in this study is available from the corresponding author on reasonable request.

Use of Artificial Intelligence: Artificial intelligence-based assistance was used solely for grammatical correction and language improvement. No AI tools were used for data collection, statistical analysis, interpretation of results, or generation of research findings. The authors reviewed and approved the final manuscript and take full responsibility for its content.

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