

Original Article

Etiological profile and antimicrobial susceptibility pattern of blood culture isolates for bloodstream infection

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ABSTRACT

Objectives: The objective of the study is to identify and study the antimicrobial susceptibility pattern of various causative agents of bloodstream infection (BSI) in a tertiary care hospital.

Materials and Methods: This was a retrospective observational study conducted from October 2022 to September 2023. Paired blood culture samples were collected aseptically and incubated in BD BACTEC FX-40, a fully automated blood culture system, for 5 days. When the bottle was “flagged positive,” gram staining was done, followed by subculture onto Blood agar and MacConkey agar. Species identification and antibiotic susceptibility testing were performed using the VITEK 2 Compact system and interpreted according to Clinical Laboratory Standard Institute guidelines.

Statistical analysis: The data were analyzed using the Statistical Package for the Social Sciences version 20.0.

Results: 10,449 blood cultures were received during the 1-year study period, out of which 13.2% (1378 samples) showed growth of pathogenic organisms. Gram-negative bacilli (810/1,378, 58.8%) were the most common etiological agents, and *Klebsiella pneumoniae* was the most predominant pathogen recovered. *Candida* spp. were recovered in (150/1378, 10.9%) isolates. A high rate of antimicrobial resistance (AMR) was noted, including (26/46, 56.5%) methicillin-resistant *Staphylococcus aureus*, (9/125, 7.2%) vancomycin-resistant enterococci, and (269/536, 50.2%) carbapenem-resistant enterobacterales. The current study also highlights high resistance among non-fermenting Gram-negative bacteria.

Conclusions: Gram-negative bacilli are the most common cause of BSIs in our setup. A high level of AMR is observed among the pathogens. The formulation of an effective antimicrobial stewardship program and its strict compliance is the need of the hour.

Keywords: Antimicrobial susceptibility, Antimicrobial stewardship, Bloodstream infections, *Klebsiella pneumoniae*, Non-fermenting Gram-negative bacteria

INTRODUCTION

Bloodstream infections (BSIs) represent a significant global burden, contributing substantially to both morbidity and mortality. They are identified by the presence of a positive blood culture in a patient exhibiting systemic signs of infection and originate either primarily or result from an established infection at another body site.^[1] In critically ill patients, each hour of delay between symptom onset and the initiation of antibiotic therapy raises the mortality rate by 9%. Therefore, prompt identification of the causative agents responsible for these infections, along with their

antimicrobial susceptibility profiles, is crucial for effective patient management.

Patients admitted in critical care units are particularly vulnerable to colonization by highly resistant pathogens due to factors such as overuse of broad-spectrum antibiotics, compromised immune system, presence of indwelling devices, and need for invasive procedures. An effective Antimicrobial Stewardship Program (AMSP) ensuring appropriate use of antibiotics is vital for effectively managing these infections. Baseline data about the microorganisms responsible for such infections and their antimicrobial susceptibility profile protect against the irrational use of antibiotics and aid in the prevention of antimicrobial resistance (AMR). Therefore, this study was conducted to identify the organisms responsible for BSIs in our setup and to assess the antimicrobial susceptibility patterns of the isolated strains.

MATERIALS AND METHODS

Study design

This retrospective observational study was carried out in the Department of Microbiology at Mahatma Gandhi Medical College and Hospital, Jaipur, Rajasthan, a superspecialty tertiary care teaching hospital. This center provides specialized services and caters to a wide geographical region in Rajasthan and nearby states as well as serving as an emerging center for medical tourism.

Duration

The study was conducted for 1 year, from October 2022 to September 2023.

Sample collection and processing

Paired blood culture samples were collected aseptically and incubated in the BD BACTEC FX-40, a fully automated blood culture system, for 5 days. During the incubation period, when the bottle “flagged positive,” gram staining was performed, and a critical alert was made to the clinician. Species identification and antibiotic susceptibility testing were performed using the VITEK 2 Compact system. Results were interpreted according to Clinical Laboratory Standard Institute guidelines.

Microbes presumed to be introduced during specimen collection or processing, not associated with causing illness in the patient, such as diphtheroids, *Bacillus* spp., *Propionibacterium* spp., and *Micrococcus* spp., were defined as contaminants. Coagulase-negative staphylococci (CoNS) were considered pathogenic only when isolated from paired blood cultures.

Statistical analysis

Descriptive statistics for categorical variables were presented as frequencies and percentages, while continuous variables were expressed as means and standard deviations. The data analysis was conducted using the Statistical Package for the Social Sciences version 20.0.

RESULTS

Ten thousand four hundred forty-nine blood samples were received in the microbiology laboratory during the study period. Among these, 1378 samples (13.2%) showed growth of pathogenic organisms, comprising 1,228 bacterial isolates (89.1%) and 150 *Candida* isolates (10.9%). A male-to-female ratio of 2.27:1 was observed, and the highest positivity rate was found in the 46–60 age group. The mean age of patients with positive blood cultures was 51 years, ranging from 18 to 91 years. The majority of samples were received from intensive care unit (ICU) patients (99.4%).

Figure 1 summarizes the results of the study, showing that Gram-negative bacilli were the most common isolates (810/1,378, 58.8%). The most common organism recovered was *Klebsiella pneumoniae* (309/1,378, 22.4%), followed by CoNS (240/1,378, 17.4%). *Candida tropicalis* was the most common fungal isolate (43/150, 28.7%).

Gram-positive cocci showed the highest susceptibility to linezolid, vancomycin, and daptomycin [Table 1].

Tables 2 and 3 present the antimicrobial and antifungal susceptibility data of the most common Gram-negative and fungal isolates, respectively.

A high rate of AMR was noted, including (26/46, 56.5%) methicillin-resistant *Staphylococcus aureus* (MRSA), (9/125, 7.2%) vancomycin-resistant enterococci (VRE), and (269/536, 50.2%) carbapenem-resistant enterobacterales (CRE).

DISCUSSION

The current study aimed to identify the spectrum of microorganisms responsible for BSIs and to analyze their antimicrobial susceptibility profiles. In the present study, blood culture positivity was detected in 13.2% of the samples, with the majority of culture-positive cases (99.4%) found among in-patients. This rate is consistent with findings from several studies conducted in India^[2,3] and abroad.^[4,5] Higher culture positivity was observed by Pal and Sujatha^[6] and Vasudeva *et al.*^[7] Differences in blood culture results can be linked to factors such as blood specimen volume, timing of collection, culture techniques, and variations in the epidemiology of causative agents. This study reveals a higher prevalence of BSI in males, with a male-to-female ratio of 2.27:1 and the highest positivity rate observed in the

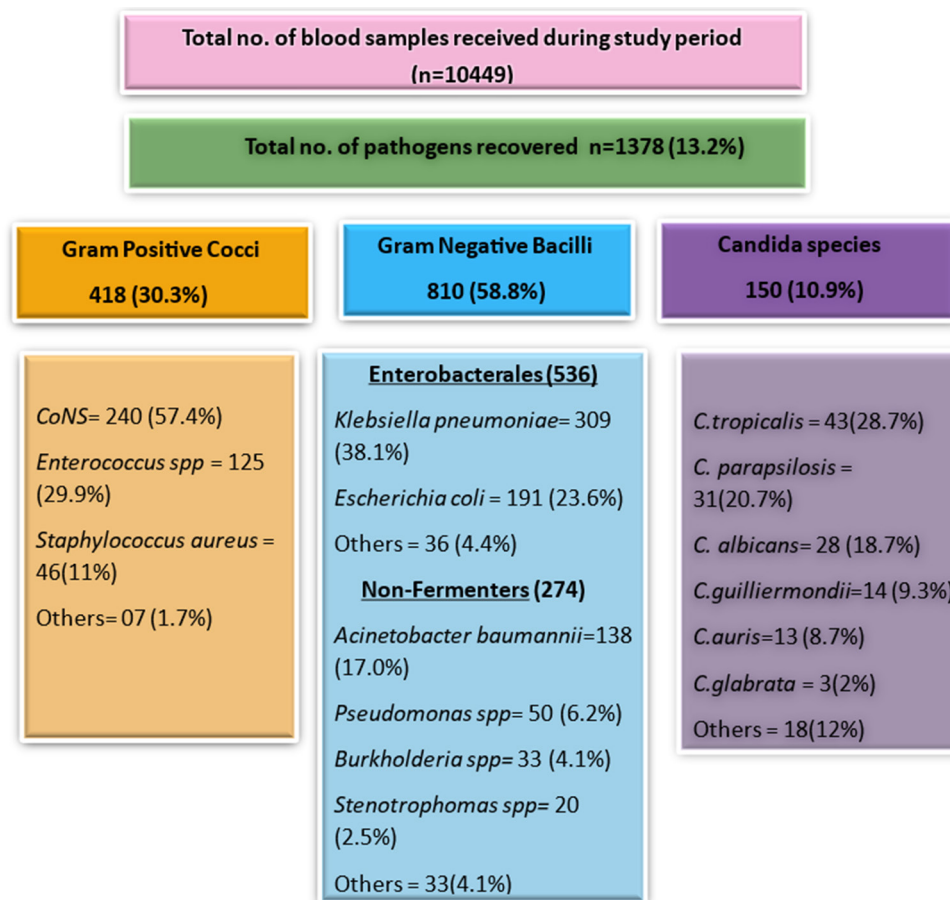


Figure 1: Spectrum of most common microorganisms isolated from blood culture during the study period.

Table 1: Antimicrobial susceptibility pattern of common Gram-positive isolates (%).

Organism	P	OX	GEN	HLG	CIP	LE	TE	COT	E	CD	VAN	TEI	LZ	DAP
CoNS (n=240)	0.83	4.16	49.16	NT	21.25	22.5	76.66	51.25	19.16	32.91	100	91.66	100	100
<i>Staphylococcus aureus</i> (n=46)	15.21	43.47	82.60	NT	8.69	15.21	89.13	80.43	34.78	67.39	100	95.65	100	100
<i>Enterococcus spp.</i> (n=125)	12	NT	IR	18.4	4.8	6.4	11.2	IR	2.4	IR	92.8	93.6	96.8	100

P: Penicillin, OX: Oxacillin, GEN: Gentamicin, HLG: High-level gentamicin, CIP: Ciprofloxacin, LE: Levofloxacin, TE: Tetracycline, COT: Cotrimoxazole, E: Erythromycin, CD: Clindamycin, VAN: Vancomycin, TEI: Teicoplanin, LZ: Linezolid, DAP: Daptomycin, NT: Not tested, IR: Intrinsically resistant, CoNS: Coagulase-negative staphylococci

46–60 age group. Several other studies have also documented similar results.^[8]

Gram-negative BSIs were more common, a trend consistent with findings from other studies. Among Gram-negative pathogens, enterobacterales accounted for the maximum cases (66.2%) with a predominance of *Klebsiella spp.* (38.1%), followed by *Escherichia coli* (23.6%), [Figure 1] which corroborates with a study done by Banik et al.^[1]

The antibiotic susceptibility pattern of common Gram-negative pathogens is summarized in Table 2. They exhibited low

susceptibility to fluoroquinolones, aminoglycosides, and third-generation cephalosporins, except for *E. coli*, which showed moderate susceptibility to fluoroquinolones. The CRE prevalence in our study is 50.2%, which is higher than the prevalence reported by Modi et al. (29.07%).^[9] Similarly, a high prevalence was observed in the study by Bajaj et al.^[10] with 67.3%. These results highlight a significant rise in carbapenem resistance, likely driven by the overuse and misuse of carbapenems.

Non-fermenting Gram-negative bacteria (NFGNB) accounted for 33.8% of the isolates in our study, among which

Table 2: Antimicrobial susceptibility pattern of common Gram-negative isolates (%).

Organism	CAZ	CPM	ATR	MRP	IPM	P/T	CFS	GEN	AK	CIP	LE	MIN	CL (I)	COT	CXM	CTR	ETP	AMC
<i>Klebsiella</i> spp. (n=309)	NT	10.35	NT	12.29	25.24	20.06	21.03	27.50	28.15	14.23	NT	NT	100	23.30	23.94	34.62	28.47	26.86
<i>Escherichia coli</i> (n=191)	NT	37.69	NT	57.59	59.68	39.79	52.87	62.82	78.53	3.66	NT	NT	100	38.21	13.61	29.84	55.49	24.6
<i>Acinetobacter</i> spp. (n=138)	6.52	5.07	5.07	7.97	5.07	7.24	15.21	7.97	7.24	6.52	6.52	42.02	100	26.08	IR	IR	IR	IR
<i>Pseudomonas</i> spp. (n=50)	44	42	0	36	40	28	40	NT	NT	32	28	IR	100	IR	IR	IR	IR	IR
<i>Burkholderia cepacia</i> (n=33)	100	IR	IR	69.69	IR	IR	18.18	IR	IR	24.24	21.21	69.69	IR	100	NT	IR	IR	IR
<i>Stenotrophomonas maltophilia</i> (n=20)	NT	NT	IR	IR	IR	IR	NT	IR	IR	NT	70	85	IR	75	IR	IR	IR	IR

CAZ: Cefazidime, CPM: Cefepime, ATR: Aztreonam, MRP: Meropenem, IMP: Imipenem, PT: Piperacillin-tazobactam, CFS: Cefoperazone sulbactam, GEN: Gentamicin, AK: Amikacin, CIP: Ciprofloxacin, LEV: Levofloxacin, MIN: Minocycline, CL: Collistin, COT: Cotrimoxazole, CXM: Cotrimoxazole, CTR: Ceftriaxone, ETP: Ertapenem, AMC: Amoxicillin clavulanate, NT: Not tested, IR: Intrinsically resistant, I: Intermediate

Acinetobacter spp. was the most common (50%), followed by *Pseudomonas* spp. (18.1%), *Burkholderia cepacia* (12%) and *Stenotrophomonas maltophilia* (7.2%) [Figure 1]. These findings are consistent with those of Manjunath *et al.*^[11] who reported a similar isolation rate of 28.3% of NFGNB. Our findings indicated varying drug susceptibilities for *B. cepacia*, with trimethoprim-sulfamethoxazole and ceftazidime being 100% effective options. Similar resistance patterns were seen in studies done by Kady *et al.*^[12] and Omar *et al.*^[13] Drugs of choice for *S. maltophilia* include trimethoprim-sulfamethoxazole, fluoroquinolones, and minocycline. Our findings showed 70–100% susceptibility to these drugs, consistent with the study done by Bacchani *et al.*^[14] [Table 2]

Among 418 Gram-positive cocci, CoNS predominated (57.4%), followed by *Enterococcus* spp. (29.9%) and *S. aureus* (11%) [Figure 1]. *Staphylococcus hemolyticus* was the most common CoNS (47.9%) isolated, which is concordant with studies done by Gopalakrishnan and Sureshkumar^[15] and Edwards *et al.*^[16] Isolation from paired blood culture and correlation with clinical signs and biomarkers were carefully ascertained before reporting CoNS as a true pathogen. The high prevalence of CoNS in our study can be linked to our position as a tertiary care hospital that caters to a substantial population of immunocompromised patients and those with comorbidities.

MRSA prevalence in our BSI isolates is 56.5%. They are known to vary from 25% in the western part of the country to 50% in the southern regions.^[17] Chaturvedi *et al.*^[18] reported similar findings, highlighting the high prevalence rates of MRSA. In contrast, research conducted by Banik *et al.*^[11] and Vasudeva *et al.*^[7] indicated lower rates of MRSA. Nonetheless, MRSA remains a global threat to public health. Vancomycin is the primary treatment choice for MRSA infections. However, the emergence of vancomycin-resistant *S. aureus* also poses a growing concern. In the present study, *S. aureus* showed 100% susceptibility to vancomycin, daptomycin, and linezolid, consistent with other contemporary studies^[1,3,7,18] [Table 1].

Among 224 erythromycin-resistant *Staphylococcal* isolates, 66% exhibited inducible clindamycin resistance (ICR), significantly higher than our previous study by Shrigaur *et al.* (30.6%).^[19] *Staphylococcus* spp. also exhibited moderate susceptibility to gentamicin (82.6%), cotrimoxazole (80.4%), and tetracycline (89.1%), while showing high resistance to ciprofloxacin (91.3%), consistent with findings by Gade and Qazi^[20] [Table 1].

The prevalence of *Enterococcus* spp. among Gram-positive bacteria was 29.9%, with *Enterococcus faecium* and *Enterococcus faecalis* comprising 85% (107/125) and 12.8% (16/125), respectively. High-level gentamycin resistance was observed in 81.6% of isolates. Susceptibility rates observed for linezolid, vancomycin, and teicoplanin were 96.8%,

Table 3: Antifungal susceptibility pattern of most common *Candida* spp. (%).

Organism	Fluconazole	Voriconazole	Caspofungin	Micafungin	Amphotericin B	5-Flucytosine
<i>Candida tropicalis</i> (n=43)	97.67	93.02	100	100	100	90.69
<i>Candida parapsilosis</i> (n=31)	35.48	90.32	93.54	93.54	93.54	93.54
<i>Candida albicans</i> (n=28)	75	85.71	92.85	92.85	78.57	78.57

92.8%, and 93.6%, respectively [Table 1]. VRE prevalence in the current study was found to be 7.2%. Over the past decade, the prevalence of VRE in India has risen, with reported rates ranging from 1% to 8.7%.^[21]

Candida spp. accounted for 10.9% of BSIs in our setup, with *C. tropicalis* being the most common (28.7%) [Figure 1]. Globally, non-albicans *Candida* represents 60% of candidemia cases^[22,23] and our study similarly reported 58%. Known risk factors for candidemia include prolonged health care and antibiotic exposure, ICU admission, and immunocompromised states.^[24] Our isolates showed high susceptibility to echinocandins, the preferred treatment for candidemia [Table 3], consistent with findings in our previous studies.^[25]

This study has a few limitations inherent to its retrospective design, with poor control over confounding factors. Furthermore, this is a single-center study, therefore data may not be generalizable. Yet, it provides vital insight into the current epidemiology of BSIs and their antimicrobial susceptibility profile. This data shall help in developing empirical therapy guidelines and providing a baseline for timely and effective management of such infections, thereby reinforcing the principles of antimicrobial stewardship.

CONCLUSIONS

BSIs are an important cause of morbidity and mortality among hospitalized patients. Gram-negative organisms have been found to be the most common cause, with *K. pneumoniae* being the most common isolate. A high level of AMR is observed among the pathogens, which necessitates the formulation and strict compliance of effective AMSP.

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Author's contributions

Purohit S: Conceptualization, Data curation, Formal analysis, Investigation, Methodology, Resources, Writing the original draft; Kaur P: Data acquisition, Writing the original draft, Review, Revising the manuscript; Lamba M: Data

analysis, Editing, Revising the manuscript; Jangid M: Data acquisition; Sharma C: Data acquisition; Rajni E: Design, Conceptualization, Data analysis and interpretation, Drafting Editing, Gave the final approval for publishing, overall supervision.

Ethical approval

The research/study was approved by the Institutional Review Board at Mahatma Gandhi Medical College and Hospital, number MGMC&H/IEC/JPR/2023/1730, dated September 30th, 2023.

Declaration of patient consent

Patient's consent is not required as there are no patients in this study.

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Nil.

Conflicts of interest

There are no conflicts of interest

Use of artificial intelligence (AI) assisted technology for manuscript preparation

The authors confirm that there was no use of artificial intelligence (AI)-assisted technology for writing and editing the manuscript and no images were manipulated using AI.

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