Original Article

Bacteriological profile and antibiotic sensitivity pattern of bloodstream infections from a teaching institution in Eastern Uttar Pradesh, North India

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ABSTRACT

Introduction: Bloodstream infections (BSIs), encompassing both self-limiting bacteremia and potentially fatal septicaemia, make up the majority of healthcare-associated ailments worldwide. The organisms encountered are mostly multidrug-resistant (MDROs), leading to increased hospital stays. Our study aims to collect data about blood culture isolates from a medical college in eastern Uttar Pradesh, India. **Materials and Methods:** A retrospective analysis of blood culture isolates obtained at our laboratory for ten months from patients with clinical suspicion of sepsis or infection with the possibility of haematogenous spread was done. We only considered consecutive and patient-specific, non-duplicate isolates. Blood samples were initially incubated in BacT/ALERT® and then manually processed once they flagged positive. **Results:** A total of 1,033 blood samples were received, of which 217 (21%) showed the growth of a pathogenic organism. The positivity rate varied significantly across different age groups, locations, and departments (P value < 0.001). It was higher among in-patients, those with central venous access, and patients with diabetes mellitus (DM). *Staphylococcus aureus* [n = 105, 48.38%] was isolated most commonly, with a high prevalence of methicillin resistance (83%). *Enterococcus* demonstrated a high degree of resistance. MDROs accounted for 68% of the detected Gram-negatives. **Discussion:** This study comprehensively analyses blood culture results from a diverse group of patients and emphasizes the association between risk factors and positive blood cultures. Gram-positive and Gram-negative isolates demonstrated low sensitivity to common antibiotics, urging vigilant monitoring and specific therapy. **Conclusion:** Our study reveals important insights guiding clinical practices, antimicrobial stewardship, and infection control strategies.

Keywords: Bacteremia, Gram-positive bacteria, sepsis

Introduction

Bloodstream infections (BSIs) make up the majority of healthcare-associated ailments worldwide.[1] Around 11.3 million sepsis cases and 2.9 million fatalities occurred in India in 2017.[2] Additionally, 7.3% of Asians had community-onset BSIs (range, 2.0 to 48.4%), according to a recent meta-analysis.[3] The Surviving Sepsis Campaign recommends collecting blood cultures within the first hour of suspected sepsis. Knowing the bacterial sensitivity patterns is crucial for initiating empirical therapy.[4,5]

Distribution and sensitivity trends in bacteria fluctuate across geographical locations and various medical facilities.[6] Our

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study aims to analyse data about the prevalence and antibiotic susceptibility patterns of blood culture isolates.

Materials and Methods

Study area and period: This research aims to retrospectively analyse the blood culture isolates obtained at the Department of Microbiology, All India Institute of Medical Sciences (AIIMS), located in Raebareli, Uttar Pradesh. Our medical college is a teaching institution with a 600-bed capacity. It caters to the district's and surrounding areas' medical requirements, ensuring top-notch healthcare services. The study covers ten months, starting from May 2022 and ending in February 2023, and was approved by the institutional ethics committee. This study comprised blood culture specimens obtained from inpatients and outpatients (paediatric and adult) for bacteriological inquiry as a standard part of their patient care. A comprehensive inquiry into the records of all relevant cases was carried out using the laboratory register and the medical records department at the hospital.

Inclusion and exclusion criteria: The attending clinician requested blood cultures based on clinical symptoms that were deemed indicative of sepsis or infection with the possibility of haematogenous spread. The diagnosis of BSI required the presence of positive blood cultures in conjunction with systemic indications of infection.[7] To identify sepsis in newborns, medical professionals searched for distinct clinical manifestations such as fever (≥38.0°C), respiratory distress, convulsions, hypothermia (≤36.5°C), lethargy, poor feeding, vomiting, jaundice, purulent infections in the umbilical region.[8] Patients who were excluded from the analysis had autoimmune/chronic diseases, including tuberculosis and sarcoidosis, weakened immune systems, steroid use, heat stroke, or suspected viral and parasitic infections. We only considered consecutive and patient-specific non-duplicate isolates.

Specimen collection and processing: By the hospital's established protocol for sample collection, the blood samples were obtained after adhering to aseptic precautions. Notably, before the blood collection, the skin was thoroughly cleansed with a 2% chlorhexidine solution, as stipulated by the protocol. This practice aims to maintain the sample's integrity and minimize the risk of contamination. Optimal sites for blood sampling included the antecubital and median cubital fossae. If the patient had central venous access, blood was collected from one of the lumens after thoroughly cleaning the port hub with 2% chlorhexidine. For each sample, 8-10 ml and 2–4 ml of blood were obtained from adult and paediatric patients, respectively. The blood samples were promptly added to BacT/ALERT® aerobic blood culture bottles, adult (BacT/ALERT® FA plus and BacT/ALERT® FN plus) or paediatric (BacT/ALERT® PF plus), as required, made by BioMérieux, France followed by immediate transport to the Microbiology laboratory wherein these were incubated in a BacT/ALERT® 3D Microbial Detection System

an automated blood culture analyser (BioMérieux, France). The samples were processed in compliance with the manufacturer's recommended standard criteria.

The BacT/ALERT-positive broths were immediately inoculated onto blood agar and MacConkey agar (Hi-Media, Mumbai). These inoculated agar plates were incubated at 37°C for 18 to 24 hours. The culture was deemed contaminated when diphtheroids, Bacillus spp., *Micrococcus* spp., and viridans streptococci were detected. The bacterial growth on the agar plates in the case of pathogens was determined and characterized based on the colony morphology, Gram staining, and traditional biochemical testing procedures, all conducted using established laboratory techniques.[9,10] Antibiotic susceptibility testing was carried out using the Kirby-Bauer disk diffusion method, employing antibiotic discs (Hi-Media) and interpreting the results based on the guidelines provided by the Clinical and Laboratory Standards Institute (CLSI).[11] In this study, multidrug resistance (MDR) is defined as resistance to three or more classes of tested antibiotics.[12] The Staphylococcal isolates underwent additional testing for methicillin resistance as per CLSI.[11]

Quality Control and Data Analysis: The strains, namely *Escherichia coli* ATCC 25922, *Enterococcus faecalis* ATCC 29212, *Pseudomonas aeruginosa* ATCC 27853, and *Staphylococcus aureus* ATCC 25923, were employed to ensure quality control in biochemical assays and assessment of antibiotic sensitivity. The percentage of susceptible isolates for each antibiotic was calculated by dividing the number of susceptible isolates by the total number of isolates. The data was compiled into a single chart using Microsoft Excel and analysed.

Results

During the study period, a total of 1,033 blood samples were collected. Among the patients, 54.70% were male, and about one-third belonged to the paediatric age group. Out of all the blood samples, 78.61% were obtained from patients admitted in the in-patient department (IPD) and 21.39% from patients of the out-patient department (OPD). The General Medicine department contributed to over one-third of the overall samples. Departments like Dermatology, Urology, Obstetrics and Gynaecology, and Ophthalmology had sent less than ten samples in the 10-month study period and were clubbed together in the analysis. About 15.39% of the patients had central venous access, and a quarter of them had a history of diabetes mellitus (DM). Patients exhibited a diverse array of signs and symptoms, so those involving the same system were combined in the data presentation. The most common presentation of the patients was fever (46%) followed by respiratory signs and symptoms (12.58%). Table 1 shows the demographic distribution of the patients.

Of the 1,033 samples, 217 (21%) showed growth of a pathogenic organism, while 26 (2.5%) showed growth of contaminants. The remaining 790 samples (76.5%) did not show any growth, as per Figure 1. The 26 samples which showed contamination were excluded from further analysis.

Table 1: Distribution of the patients on the basis of demographic and clinical profile (*n* = 1033)		
Number of patients	Parameter	Percentage (%)
Gender		
Male	565	54.70%
Female	468	45.30%
Age groups (years)		
0-10	186	18.01%
11-20	161	15.59%
21-30	140	13.55%
31-40	95	9.20%
41-50	105	10.16%
51-60	128	12.39%
61-70	143	13.84%
>70	75	7.26%
Location		
OPD	221	21.39%
IPD	812	78.61%
Departments		
Gen Medicine	386	37.37%
Trauma & Emer.	263	25.46%
Paediatrics	240	23.23%
Paediatric Surgery	46	4.45%
Neurosurgery	38	3.68%
Gen Surgery	22	2.13%
Others	38	3.68%
Central venous access		
Present	159	15.39%
Absent	874	84.61%
History of Diabetes Mellitus		
Present	259	25.07%
Absent	774	74.93%
Clinical Presentation		
Fever	476	46.08%
Respiratory symptoms (shortness of breath, tachypnoea, cough)	130	12.58%
Meningeal signs (headache, vomiting)	118	11.42%
Urinary symptoms (Burning micturition, frequency)	88	8.52%
Gastro-intestinal symptoms (pain in abdomen, diarrhoea)	87	8.42%
Cellulitis	49	4.74%
Sepsis	48	4.65%
Others	37	3.58%

Figure 1: Growth in blood culture

Staphylococcus (CoNS) [n = 55, 25.34%], accounting for almost three-quarters of all isolates. The most commonly isolated Gram-negative organisms were *Escherichia coli* and *Acinetobacter baumannii* complex, each with 15 (6.91%) isolates. Figure 2 illustrates the distribution of different organisms isolated from blood cultures.

Our research found a high prevalence of methicillin resistance among *Staphylococcus aureus* (83%) and *Coagulase-Negative Staphylococci* (81%). The sensitivity of all *Staphylococcus* isolates to Penicillin, Ampicillin-Sulbactam, Quinolones, Clindamycin, and Erythromycin was less than 50%; however, it was 100% for Vancomycin and Teicoplanin, and only one *Staphylococcus aureus* isolate was resistant to Linezolid. *Enterococcus* demonstrated a higher degree of resistance to Vancomycin and Teicoplanin (33%), and Linezolid (50%). Its sensitivity to all the other antibiotics tested was less than 50%, except for Doxycycline (50%). [Figure 3]

Due to the limited number of Gram-negative isolates, their sensitivity pattern was calculated as a group. Some drugs were not tested against all isolates because they are not recommended for those organisms. Netilmicin and Aztreonam were only tested against *Pseudomonas aeruginosa* (n = 4). Similarly, Cefotaxime, Ceftriaxone, Ertapenem, and Amoxicillin-clavulanic acid were not tested against *Pseudomonas aeruginosa* and *Acinetobacter baumannii* complex (n = 31). Please refer to Figure 4 for further details.

The high prevalence of multidrug-resistant organisms (MDROs) among Gram-negative bacteria found in blood isolates has reached an alarming rate of up to 68%. This poses a severe threat to public health as the situation is further complicated because all *Klebsiella pneumoniae*, *Klebsiella oxytoca*, and *Citrobacter freundii* isolates are MDROs. Moreover, over half of the *Escherichia coli* and *Acinetobacter baumannii* complex isolates are MDROs, as shown in Figure 5.

Discussion

Monitoring the blood-borne organisms in a specific area is essential to identify infectious pathogens and understand their sensitivity

Table 2 presents the distribution of positive blood cultures based on demographic parameters. The positivity rate was similar in both genders but varied significantly across different age groups, locations, and departments (*P* value < 0.001). It was notably higher among IPD, patients with central venous access, and those with a history of DM (Odds ratio >1).

The results of the blood cultures taken from patients revealed that the frequency of Gram-positive bacteria (76.9%) isolated was over three times that of Gram-negative bacteria (23.1%). *Staphylococcus aureus* [n = 105, 48.38%] was the most commonly isolated organism, followed by *Coagulase-Negative*
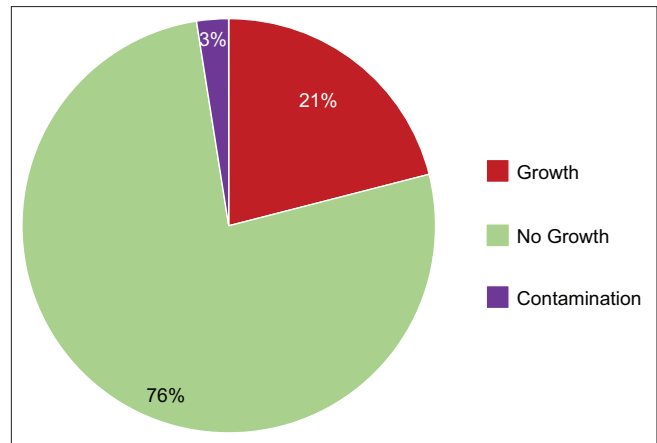
patterns. By doing so, we can appropriately use antibiotics and curtail the spread of antibiotic resistance.[13,14] The current study

comprehensively analyses blood culture results from a diverse group of patients. It reveals the prevalence of BSIs, the distribution of causative organisms, and antibiotic susceptibility patterns.
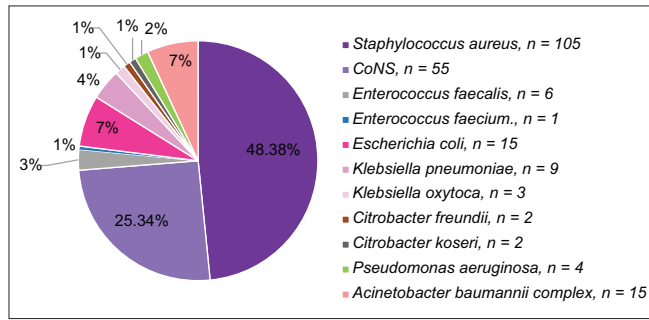
In our study, out of a total of 1033 blood culture samples, 217 (21%) came positive, similar to a study in Nepal by Pokhrel *et al.*[15] (20.5%). However, other studies had slightly higher (Ejaz *et al.*:28.26%) or lower (Kajumbula *et al.*: 14%).[16,17] The American Society of Microbiology (ASM) suggests an adequate positive blood culture rate of 6–12%.[18] A study in five Belgian tertiary care hospitals showed a positive blood culture rate of 9.8–12.9%.[19]

The observed variability in positive blood culture results can be attributed to several factors, including the quantity or volume
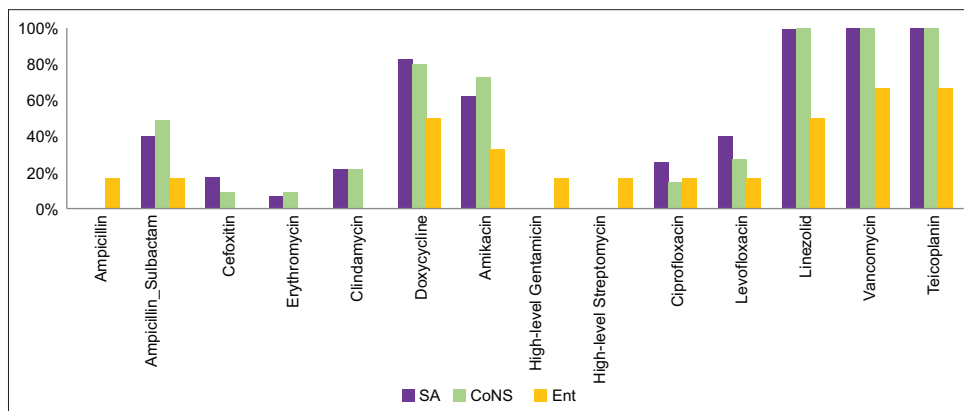

Figure 2: Distribution of various organisms isolated from blood cultures

Figure 3: Sensitivity pattern of Gram-positive bacteria

	Penicillin	Ampicillin	Cefoxitin	Erythromycin	Clindamycin	Doxycycline	Amikacin
SA	12%	NA	17.14%	6.66%	21.90%	82.86%	61.90%
CoNS	5.45%	NA	9.09%	9.09%	21.81%	80%	72.72%
Ent	16.67%	16.67%	NA	NA	NA	50%	33%

	High-level Gentamicin	High-level Streptomycin	Ciprofloxacin	Levofloxacin	Vancomycin	Teicoplanin	Linezolid
SA	NA	NA	25.71%	40%	100%	100%	99%
CoNS	NA	NA	14.54%	27.27%	100%	100%	100%
Ent	16.67%	16.67%	16.67%	16.67%	66.67%	66.67%	50%

SA: *Staphylococcus aureus*; CoNS: Coagulase-Negative Staphylococcus; Ent: *Enterococcus faecalis*. NA: Not Applicable
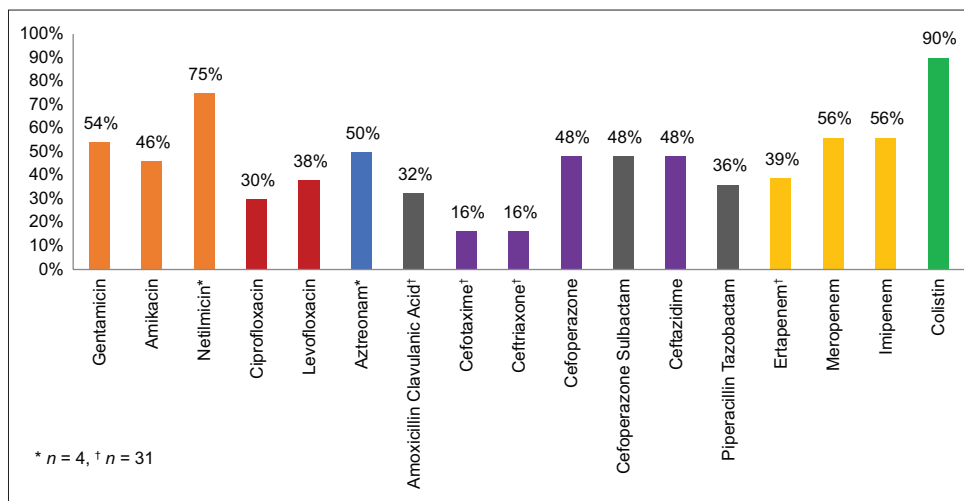

Figure 4: Sensitivity pattern of Gram-negative bacteria

Table 2: Distribution of positive blood culture across various demographic parameters	Growth	No Growth	Total
Gender			
Male	119 (21.6%)	432 (78.4%)	551 (100%)
Female	98 (21.5%)	358 (78.5%)	456 (100%)
P=0.97			
Age groups (years)			
0-10	28 (15.38%)	154 (84.62%)	182 (100%)
11-20	18 (11.32%)	141 (88.68%)	159 (100%)
21-30	25 (18.52%)	110 (81.48%)	135 (100%)
31-40	26 (27.96%)	67 (72.04%)	93 (100%)
41-50	33 (31.73%)	71 (68.27%)	104 (100%)
51-60	35 (28.23%)	89 (71.77%)	124 (100%)
61-70	30 (21.28%)	111 (78.72%)	141 (100%)
>70	22 (31.88%)	47 (68.12%)	69 (100%)
P≤0.001			
Location			
OPD	23 (10.5%)	196 (89.5%)	219 (100%)
IPD	194 (24.62%)	594 (75.38%)	788 (100%)
P≤0.001; Odds ratio: 2.78			
Departments			
Gen Medicine	57 (15.2%)	318 (84.8%)	375 (100%)
Trauma & Emer.	105 (41.5%)	148 (58.5%)	253 (100%)
Paediatrics	29 (12.29%)	207 (87.71%)	236 (100%)
Paediatric Surgery	9 (20%)	36 (80%)	45 (100%)
Neurosurgery	6 (15.79%)	32 (84.21%)	38 (100%)
Gen Surgery	4 (18.18%)	18 (81.82%)	22 (100%)
Others	7 (18.42%)	31 (81.58%)	38 (100%)
P≤0.001			
Central venous access			
Present	39 (25.66%)	113 (74.34%)	152 (100%)
Absent	178 (20.82%)	677 (79.18%)	855 (100%)
P=0.18; Odds ratio: 1.31			
History of Diabetes Mellitus			
Present	71 (28.17%)	181 (71.83%)	252 (100%)
Absent	146 (19.34%)	609 (80.66%)	755 (100%)
P=0.003; Odds ratio: 1.63			
Clinical Presentation			
Fever	360 (77.25%)	106 (22.75%)	466 (100%)
Respiratory symptoms (shortness of breath, tachypnoea, cough)	99 (77.34%)	29 (22.65%)	128 (100%)
Meningeal signs (headache, vomiting)	89 (79.46%)	23 (20.54%)	112 (100%)
Urinary symptoms (Burning micturition, frequency)	68 (79.07%)	18 (20.93%)	86 (100%)
Gastro-intestinal symptoms (pain in abdomen, diarrhoea)	70 (82.35%)	15 (17.65%)	85 (100%)
Cellulitis	38 (79.17%)	10 (20.83%)	48 (100%)
Sepsis	38 (79.17%)	10 (20.83%)	48 (100%)
Others	28 (82.35%)	6 (17.65%)	34 (100%)
P=0.97			

of blood culture samples collected for analysis.[20] Only cultures showing monomicrobial growth were included in our analysis.

The demographic distribution of the study population reflects a balanced representation of both genders, with a slight male predominance. The preponderance of paediatric patients in the study, constituting about one-third of the total, was a unique observation, allowing us to analyse the patterns of BSIs in this group. It is essential to highlight that negative blood cultures do not necessarily mean no infection is present. Physicians sending one set of blood cultures, the presence of anaerobes, fungi, or viruses could account for the negative cultures in sepsis patients.[21] The male-to-female ratio in our study was 1.2:1, but blood culture positivity rates were similar in both genders, around 20%. Similar observations have been documented by Ombelet *et al*. and Banik *et al*.[22,23]

Approximately, 80% of our samples were from patients admitted to the IPD. The positivity rate among these samples was more than twice as high as those received from the OPD, and this
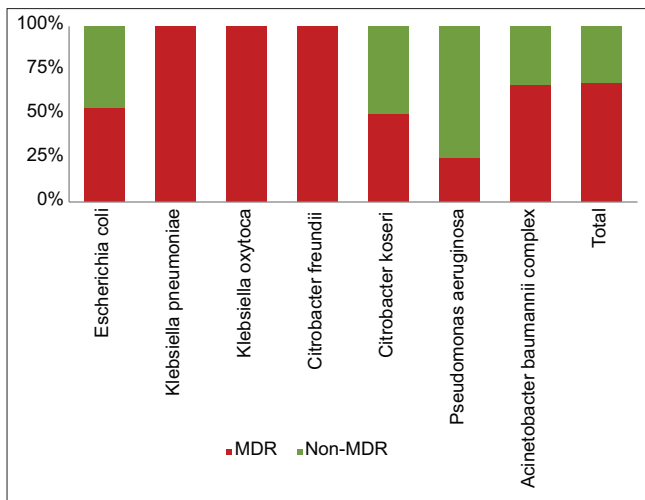
Figure 5: Percentage of MDROs among various Gram-negative isolates

difference was statistically significant. It is worth noting that our findings contradict those of Tarai *et al.*[24] This increased rate could be explained by the vulnerable inpatients contracting hospital-acquired sepsis, which needs to be explored in further studies. Although the General Medicine department had the highest number of patients in the sample pool (n = 386, 37.37%), the Trauma and Emergency department had the highest percentage of positive cases (48.8%) among all the departments. This was because, in our centre, patients with septicaemia are initially admitted to the Trauma and Emergency department, where they undergo initial investigations, are stabilized, and then are transferred to other departments, based on their age and underlying conditions, like General Medicine (positivity rate 26.1%), and Paediatrics (13.7%).

The presence of central venous access and a history of DM significantly increase the risk of positive blood culture (Odds ratio >1), highlighting their association with BSIs. In our study, 25.66% of patients with central venous access (n = 152) developed positive blood culture, which was similar to the findings of Kaur *et al.* (21.73%)[25] but relatively high compared to the findings of Lona-Reyes *et al.*[26] (7.35%, 15/204). The relation of bacteremia with DM is well studied. Though a study by Stoeckle *et al.* reported only 25.8 episodes of bacteremia/1000 admissions among diabetic patients, their relative frequency of BSI was much higher than non-diabetic patients (Odds ratio = 4.4).[27] Ghonim *et al.*[28] reported a high incidence of bacteremia among diabetic patients (66.67%), but their sample size was small (n = 60). Our study observed a blood culture positivity rate of 28.17% among diabetic patients with an Odds ratio of 1.63.

The majority of the patients presented with only fever (46%), followed by respiratory symptoms like cough and shortness of breath (12.58%); however, positivity rate of blood culture did not differ significantly among the varied clinical presentations of patients, suggesting any infection can spread to blood, and there is no fixed clinical presentation for BSIs. Primary physicians should

also be sceptical about patients presenting with trivial symptoms like fever or cough as they may progress to BSIs.

The contamination rate observed (2.5%) in our study is below the desired level proposed by Hall. The contaminants primarily consist of isolates from the genera *Bacillus* spp., *Corynebacterium* spp., and *Micrococcus* spp., as well as polymicrobial growth.[29] The overall positivity rate of blood cultures was 21%, with Gram-positive bacteria being the most common (76.9%). This discovery aligns with a comparable investigation in a tertiary hospital in Ghana, where Gram-positive bacteria outnumbered the Gram-negative. *Staphylococcus aureus* was the most frequently isolated organism (n = 105). The prevalence of Gram-positive cocci has increased as the leading cause of BSIs since the early 1980s with the advent of modern medical treatment.[21] Coagulase-negative Staphylococci (*S. epidermidis* and *S. haemolyticus*) were the second most common Gram-positive cocci to be isolated. It is expected to find coagulase-negative staphylococci in blood cultures, which can be due to the normal bacterial flora present on the skin.[1] This typically occurs when the skin is not adequately disinfected. Our research discovered an increased isolation of coagulase-negative staphylococci in blood cultures, likely caused by the same contamination.

It is important to note that *Staphylococcus* isolates had low sensitivity to commonly prescribed antibiotics such as Penicillin, Ampicillin-Sulbactam, Quinolones, Clindamycin, and Erythromycin, heightening the need to tailor antibiotic therapy based on local resistance patterns. These bacteria exhibited good sensitivity to Doxycycline (>80%). Among the *Staphylococcus aureus* isolates, 83.65% were MRSA, a cause for great concern. This percentage is higher than documented in other studies.[1,30] This observation suggests that infection control practices need to be more stringent and robust in our institute. Herein, we want to highlight the importance of following proper contact precautions when handling IPD patients, especially in critical areas more prone to hospital-acquired infections.

Currently, Vancomycin and Teicoplanin are viable treatment options for these Gram-positive infections, but it is essential to follow proper guidelines regarding antibiotic usage to limit the spread of resistance. Only 50% of the Enterococci isolates exhibited susceptibility to doxycycline and Linezolid. This finding aligns with a series of investigations undertaken in Ethiopia and India.[31,32] Four of the Enterococcus species isolates were resistant to critical antibiotics such as Vancomycin and Teicoplanin, thus posing a challenge as effective antibiotics against Enterococci are limited, highlighting the need for continuous surveillance and developing novel therapeutic strategies.

Only 23.1% of isolates were Gram-negative bacteria, with *Escherichia coli* and *Acinetobacter baumannii* complex being the most prevalent (n = 15 each), closely followed by *Klebsiella spp.* (n = 12). This observation is in line with other studies.[1,5]

Upon analysing cumulative GNB data, the most significant susceptibility is to Colistin (90%), followed by

Carbapenems (56%) and Aminoglycosides (50%) in general. Cephalosporins proved effective only against certain bacterial strains (16%). The cephalosporin resistance pattern of the Gram-negative isolates was similar to that reported by Agnihotri Bhat *et al.*[33] Pseudomonas species exhibited high sensitivity to Netilmicin (75%) and Aztreonam (50%), in addition to Colistin (90%). Such a high resistance rate to Carbapenems could be attributed to the indiscrete use of this antibiotic class in our region. The increasing prevalence of MDR in Gram-negative bacterial blood isolates is a severe issue, with the current rate at 68%. This is in agreement with other studies.[1,31,34] *Klebsiella* sp., *Escherichia coli*, and *Acinetobacter baumannii* complex isolates are particularly MDROs, highlighting the need for formulation of antibiotic policy and strict implementation of antibiotic stewardship practices to prevent the proliferation of resistant strains further.

Due to the paucity of isolates, individual analysis of isolates according to wards and Intensive Care Units (ICUs) was not done. The percentage of MDR organisms was slightly higher in the ICUs, but it was not statistically significant. This highlights the importance of implementing infection control measures and antibiotic policies at all levels and not just for critically ill or vulnerable patients of the ICUs.

Limitations

While the study provides valuable observations, certain limitations should be acknowledged.
1. As the study is retrospective, causation or temporality cannot be established. Moreover, the results of this single-centre study cannot be generalized to the whole Indian population. Multicentric studies with a larger sample size would increase the robustness of the findings.
2. This study's antibiotic sensitivity testing method was restricted to disc diffusion sensitivity testing for all antibiotics except Vancomycin and Colistin.
3. The number of individual Gram-negative organisms was too low to be analyzed separately.

Conclusion

In conclusion, this study contributes valuable data on the epidemiology of BSIs and antibiotic resistance patterns among bacterial isolates. The findings underscore the complexity of BSIs and the importance of tailoring clinical practices based on local epidemiological data. The identification of isolates obtained from patients can aid primary healthcare physicians in the selection of appropriate antibiotics for early empirical introduction, thus preventing patients from progressing to sepsis and other complications. Further multicentric trials are necessary to expand our knowledge and database. These trials will enhance our understanding of the antibiotics required to combat infections caused by these isolates, in addition to providing a more comprehensive database to support clinical decision-making. The study also highlights the need for a multifaceted approach to infection control, which includes strict adherence to aseptic practices during invasive procedures, judicious use of indwelling devices, and effective management of underlying medical conditions like DM. The demographic diversity of our study population strengthens the generalizability of the study's findings to a broader patient population.

Conflicts of interest

There are no conflicts of interest.

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