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IP International Journal of Medical Microbiology and Tropical Diseases

Journal homepage: https://www.ijmmtd.org/

Original Research Article

Bacterial isolates & their resistance patterns from blood cultures, KIMS

N Padmaja[1,*], Srilalitha .V[1], K.Ramya Sree[1]

[1] *Dept. of Microbiology, Konaseema Inst. of Medical Sciences Research Foundation, Amalapuram, Andhra Pradesh, India*

ARTICLE INFO

Article history:
Received 20-01-2022
Accepted 24-03-2022
Available online 07-06-2022

Keywords:
Bloodstream infections
Bacteriological profile
Resistance patterns
Kirby-Bauer disk diffusion
antibiotic therapy

ABSTRACT

Introduction: Bloodstream infections (BSI) remain one of the important causes of morbidity and mortality. Microorganisms present in circulating blood result in serious sequelae like shock, DIC, and death. Blood culture is a vital tool for their detection. Drug sensitivity patterns help in rationalizing the treatment. The present study was undertaken to determine the bacterial profile & resistance patterns in our hospital.
Aim: To study the bacterial isolates and their resistance patterns from blood cultures.
Materials and Methods: A retrospective study was conducted from August 2019 to January 2020 in the Dept. of Microbiology, KIMS, Amalapuram. Blood samples were collected from clinically suspected patients with aseptic techniques, processed, isolated and identified according to standard microbiological techniques. Antibiotic sensitivity testing was done according to Kirby-Bauer disk diffusion method.
Results: A total of 57 pathogens were isolated from 380 suspected patients of BSI. Gram-negative bacilli (52.7%) (GNB) were predominant organisms isolated followed by Gram-positive cocci (47.3%) (GPC).*Staphylococcus aureus* (20) and *Klebsiella* pneumoniae (19) were the predominant pathogens isolated. Among GPC, *S.aureus* was highly resistant to Penicillin (85%), Ampicillin (85%), Amoxyclav (75%), and Ciprofloxacin (50%). All Staphylococcal species were sensitive to Methicillin, Vancomycin, Linezolid. Among GNB, *Klebsiella* exhibited high resistance to Cefixime (95%), Amoxyclav (84%), Ceftazidime (79%). Among 30 GNB, 12(40%) were ESBL producers. Most of the GNB were sensitive to Imipenem.
Conclusion: Appropriate treatment of BSI should be based on the current knowledge of local bacterial resistance patterns of the hospital. Hence this kind of study will help in formulating management guidelines and antibiotic policy.

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1. Introduction

Bloodstream infections (BSI) remain one of the important causes of morbidity and mortality worldwide.[1] Bacteremia refers to the presence of bacteria in the blood and Septicemia is a condition where bacteria circulate, multiply, form toxic products and cause high fever.[2] Several mechanisms play a role in the removal of microorganisms from the bloodstream. Patients who are debilitated, immunocompromised/ immunodeficient are at increased risk because circulating bacteria may not be cleared from blood due to impairment in defense mechanisms.[3] A variety of bacteria have been recovered from the bloodstream, both Gram-positive and Gram-negative. The most common ones are members of Enterobacteriaceae, *Staphylococcus aureus*, Streptococcus pneumoniae, Enterococci, *Pseudomonas aeruginosa*.[3]

Microorganisms present in the circulating blood - whether continuously, intermittently, or transiently are a threat to every organ in the body.[2] The major categories of BSI are intravascular i.e, they originate within CVS.

* *Corresponding author*.
E-mail address: masalapadmaja@gmail.com (N. Padmaja).

https://doi.org/10.18231/j.ijmmtd.2022.032
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eg: infective endocarditis, catheter-associated bacteremia, and extravascular where the organisms multiply at the primary site and are drained by lymphatics reaching the bloodstream, eg: genitourinary tract (25%), respiratory tract (20%), abscess (10%), surgical site infections (5%), biliary tract (5%).[4] The usual presentation of septicemia is fever, chills, malaise, tachycardia, hyperventilation, prostration, and hypertension.[5]

Major complications are the development of shock, disseminated intravascular coagulation, and acute renal failure.[5] Early diagnosis plays a crucial role in managing BSI and hence, prompt detection of such infections is a critical function of the microbiology laboratory.[6] Blood culture is a vital tool for the detection of BSI and remains the gold standard for bacteremia detection.[6] Prompt initiation of appropriate antimicrobial therapy is demonstrably important for preventing morbidity and mortality.[3] Empiric antimicrobial therapy is based on knowledge of the microbial profile and their antimicrobial sensitivity patterns, clinical and epidemiological data.[6] Irrational use of drugs has led to an increase of MDR bugs and thus worsened the condition.[7] Hence, the present study was undertaken to describe the bacteriological profile and drug resistance pattern from blood culture specimen to guide clinicians to initiate empiric antibiotic therapy.

2. Materials and Methods

The present study is a retrospective analysis of blood culture isolates received in the Department of Microbiology, KIMS & RF, Amalapuram, Andhra Pradesh from August 2019 to January 2020.

2.1. Inclusion criteria

Patients of all age groups with fever due to infective causes were included in the study.

2.2. Exclusion criteria

Patients with a history of chronic diseases, patients on immunosuppressive therapy, immunodeficient patients were excluded from the study.

2.3. Sample collection

Blood samples were collected according to the sample collection protocol of the hospital. First, the skin was disinfected with 2% chlorhexidine, and then blood samples were collected from antecubital or median cubital vein before the start of antimicrobial therapy.

5ml of blood from adults and 2 ml from paediatric cases were collected and inoculated into adult and pediatric blood culture bottles containing Brain Heart Infusion broth (1 in 10 dilutions) and they were incubated at 37^0C in the incubator for 24 hours. After overnight incubation, periodic subcultures were done on Macconkey Agar, Blood agar, and Nutrient agar on Day 1,2, and finally on Day 7.

Any growth obtained was processed and identified by Gram staining, colony morphology, and standard biochemical tests.[8] All negative bottles were incubated for 7 days and subculture was done at the end of 7th day before reporting them as Negative.[9]

2.4. Antibiotic susceptibility testing

AST was done on Mueller Hinton Agar according to Kirby-Bauer's Disc Diffusion method and interpreted according to CLSI Guidelines using Hi-Media antibiotic discs.[9]

3. Results

A total of 380 blood culture samples were processed. Out of 380 samples processed, 57 samples (15%) showed bacterial growth. Rest 323 (83%) samples showed no growth.

Table 1:

Total number of cases	Culture positive	Culture negative
380	57(15%)	323(85%)

Out of 57 culture-positive blood samples, isolates of Gram-positive cocci were 27 (47.3%) and those of Gram-negative Bacteria were 30 (52.7%).

Table 2: Distribution of organisms isolated from blood culture

Organism	Number	Percentage
Gram-positive	27	47.3%
Gram-negative	30	52.7%

S.aureus is predominant organism, isolated in 20 samples (35.08%) followed by *Klebsiella* in 19 samples (33.3%), Pseudomonas in 7 cases (12.28%), CONS in 7 cases (12.28%), *E.coli* in 3 cases (5.26%) and *Acinetobacter* in 1 case (1.75%).

Table 3: Percentage of isolates from blood culture

S.No	Name of the organism	Number (n)	Percentage (%)
1	*Staphylococcus aureus*	20	35.08
2	*Coagulase-negative Staphylococcus*	7	12.28
3	*Pseudomonas aeruginosa*	7	12.28
4	*Klebsiella sps*	19	33.3
5	*Escherichia coli*	3	5.26
6	*Acinetobacter*	1	1.75
7	Total	57	

Antibiotic susceptibility patterns for Gram-positive cocci and Gram-negative bacilli were interpreted according

to CLSI Guidelines. Both *S.aureus* and CONS were highly resistant to Penicillin, Ampicillin, Amoxyclav, and Ciprofloxacin. All *S.aureus* isolates were highly sensitive to Methicillin, Vancomycin, Teicoplanin, Linezolid, Clindamycin, and Cefoperazone-sulbactam.

Among Gram negative bacteria, *Klebsiella* and *E.coli* showed high resistance to Cefixime (95% &67%) amoxycillin/clavulanic acid (84% & 100%) and Ceftazidime (79% & 67%). *Klebsiella* isolates also exhibited high resistance to third generation cephalosporins (74%) and low resistance to piperacillin tazobactum (31%) and cefoperazone sulbactum (26%). Pseudomonas isolates showed high resistance to cefixime (100%), cefoperazone (86%) and amoxyclav (86%). *Acinetobacter* showed resistance to ciprofloxacin and cefixime.

3.1. ESBL producers

Out of 30 Gram-Negative isolates, 12 were ESBL producers (40%). Out of 12 ESBL producers, 11 were *Klebsiella* isolates and 1 was *E.coli*.

4. Discussion

The present study was conducted to demonstrate the distribution of microbial isolates causing bloodstream infections and their drug resistance patterns to the commonly used oral and parenteral antimicrobial agents.

The susceptibility to antimicrobials and the bacteriological profile is a constantly evolving feature. This necessitates the need for effective management of bloodstream infections.

In our study, the rate of positive blood culture was found to be 15%. This finding is comparable to studies conducted by Qureshi et al,[10] Mehta MP et al,[11] and Vijaya Devi et al[4] who reported a culture-positive rate of 16.6%, 16.4%, and 16.8% respectively. The low rate of isolation could be due to prior antibiotic therapy before reaching the tertiary center and the other reason could be self-medication and availability of antibiotics without any prescripition. However slight higher rate (27.9%) of positive blood culture was reported by Latif et al[12] Such differences in the prevalence of bloodstream infections could be due to blood culture systems. geographical location and difference in infection control practices.

In the present analysis, Gram-negative bacteremia accounted for 52.7% while 47.3% isolates were Gram-Positive bacteria. It was by the study conducted by A.Vijaya Devi et al[4] and Fatima Fasih et al[5] in which the Gram-negative isolates were higher as compared to Gram-Positive isolates. Kalantar et al[13] documented an increased number of Gram-positive isolates (65%) compared to Gram-negative (42%). The differences in the pattern of bacterial isolates could be differences in the study plan, variation in etiological agents, and seasonal variations.

Staphylococcus aureus was the predominant Gram-positive isolate (47.3%) and *Klebsiella species* was the predominant Gram-negative isolate (33.3%) in our study. Several studies like Laxmikant et al[14] revealed that *S.aureus* is the common cause of bacteremia and septicemia particularly in Post-op Surgical Site Infections, CVP lines, etc.

In our study, *Coagulase Negative Staphylococcus* was isolated from 7 cases (12.28%). CONS, the usual skin commensals, are being increasingly considered as bloodstream pathogens over the past two decades.[5] Presence of long-standing intravascular catheters, increasing use of medical devices like prosthetic heart valves, vascular grafts could be the mode of spread of bloodstream infections by CONS. Two studies, Valencia et al[15] and Wattal et al[16] have reported CONS as the most common isolate causing Bloodstream infections in ICU patients.

The most common Gram-negative organism isolated in the present study is the *Klebsiella species*. Similar findings were observed by Fatima Fasih et al (2019) and Tariq et al in 2014.[5,17] However, *E coli* followed by *Klebsiella sps* was found to be the most common among Gram-negative bacteria in a study by Fayyaz et al and N Vasu deva et al.[7,18]

Few studies like Amit Banik et al (2018)[6] and Laxmikant et al (2020)[14] have documented *Acinetobacter* baumanii as the predominant Gram-negative isolate. In contrast, *Acinetobacter sps* (1.75%) was isolated from only one case in our study.

Antibiotic susceptibility testing in the present study showed high resistance towards Penicillin (85%), Ampicillin (85%) in *S.aureus* isolates. CONS isolates were highly resistant to ciprofloxacin (71.4%) and ampicillin (71.4%). All Gram-positive cocci isolates were susceptible to Methicillin, Vancomycin, Teicoplanin, Linezolid, and Cefaperazone-sulbactam. Out of 20 Staphylococcus species, none were Methicillin-resistant which is comparable to the study done by Sweta et al[20] in 2016. However, the incidence of MRSA was between 29-57% in other studies Sheshta et al 2014,[1] Banik et al (2018).[6]

Among the Gram-negative isolates, all *Klebsiella* isolates exhibited high resistance to Cefixime (95%), Ceftazidime (79%), and Amoxycillin/Clavulanic Acid (84%), lowest resistance against Cefoperazone -Sulbactam (26%), Piperacillin-Tazobactum (32%), and no resistance to Imipenem. Out of 30 Gram-Negative isolates,[12] were ESBL producers (40%). Out of 19 *Klebsiella* pneumoniae isolates, 9 were ESBL producers. The prevalence of ESBL producer GNB was 32% in a study done by Prabhu et al (2010).[19]

The isolation of Non-Fermenters like *Pseudomonas aeruginosa* (12.28%) and *Acinetobacter* (1.75%) is of concern in the present study. These bacteria are associated with a high degree of resistance to antibiotics. In the current scenario, constant antibiotic surveillance and cautious use of antibiotics are the need of the hour.

Table 4: Percentage of antimicrobial resistance patterns of gram-positive cocci.

Antibiotics	*S.aureus*		CONS	
	Number (n)	Percentage (%)	Number (n)	Percentage (%)
Penicillin	17	85.0	5	71.4
Ampicillin	17	85.0	5	71.4
Amoxycillin/clavulanic acid	15	75.0	3	42.8
Methicillin/Oxacillin	-	-	-	-
Ceftriaxone	4	20.0	1	14.2
Cefotaxime	4	20.0	1	14.2
Cefuroxime	6	30.0	2	28.5
Cefoperazone sulbactam	-	-	-	-
Teicoplanin	-	-	-	-
Vancomycin	-	-	-	-
Linezolid	-	-	-	-
Ciprofloxacin	10	50.0	5	71.4
Clindamycin	-	-	-	-

Table 5: Percentage of antimicrobial resistance patterns of gram-negative bacilli

Antibiotics	*Klebsiella* n=19	Pseudomonas n=7	*Escherichia coli* n=3	*Acinetobacter* n=1
Amoxycillin/clavulanic acid	16(84.2%)	6(85.71%)	3(100%)	-
Ciprofloxacin	10(52.6%)	3(42.8%)	2(66.6%)	1(100%)
Amikacin	12(63.2%)	4(57.1%)	-	-
Gentamycin	10(52.6%)	3(42.8%)	-	-
Ceftriaxone	14(73.7%)	4(57.1%)	1(33.3%)	-
Cefotaxime	14(73.7%)	4(57.1%)	1(33.3%)	-
Cefixime	18(94.7%)	7(100%)	2(66.6%)	1(100%)
Cefoperazone	15(78.9%)	6(85.71%)	1(33.3%)	-
Ceftazidime	15(78.9%)	5(71.4%)	2(66.6%)	-
Piperacillin tazobactum	6(31.5%)	3(42.8%)	-	-
Imipenem	-	-	-	-
Cefoperazone sulbactam	5(26.3%)	3(42.8%)	-	-

5. Conclusion

Early commencement of antimicrobial therapy can play a vital role in reducing mortality and morbidity in bloodstream infections. Appropriate empirical treatment is based on continuous knowledge of causative agents and their resistance patterns. Our study has provided information about likely pathogens in bloodstream infections and their resistance patterns in the rural setup. This can help in formulating Antibiotic policy and can avoid indiscriminate antibiotic use.

6. Acknowledgments

Technical and material resource support was provided by the Dept of Microbiology, KIMS&RF.

7. Conflict of Interest

The authors declare no relevant conflicts of interest.

8. Source of Funding

None.

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Author biography

N Padmaja, Professor & HOD

Srilalitha .V, Assistant Professor

K.Ramya Sree, Senior Resident

Cite this article: Padmaja N, Srilalitha .V, Sree KR. Bacterial isolates & their resistance patterns from blood cultures, KIMS. *IP Int J Med Microbiol Trop Dis* 2022;8(2):154-158.