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IP International Journal of Medical Microbiology and Tropical Diseases

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

Original Research Article

Study of prevalence and antimicrobial susceptibility pattern of blood culture isolates from a tertiary care hospital in South India

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ARTICLE INFO

Article history:
Received 03-06-2023
Accepted 17-05-2023
Available online 23-09-2023

Keywords:
Bloodstream infections
bacteremia
Extended Spectrum ßLactamases
MetalloßLactamase

ABSTRACT

Background: Bloodstream infections progress rapidly to life threatening septicemia and sepsis and hence appropriate rapid management is crucial.
Materials and Methods: A prospective study was carried out in the department of microbiology where patients with clinical suspension of bacteremia were enrolled and blood culture was performed. The isolates were identified by phenotypic characters and antimicrobial susceptibility performed.
Results: A total of 1200 blood samples were collected out of which 279 were positive for aerobic bacterial growth. *Staphylococcus aureus* was the most commonly isolated organism followed by *Klebsiella, Escherichia coli* and *Pseudomonas aeruginosa*. Gentamicin (81.6%), Levofloxacin (75%), Meropenem (88.5%) and *Piperacillin-tazobactam* (93.8%) showed higher sensitivities when compared to Cephalosporins (55%-68%) and penicillin.
Conclusion: Gram-negative bacteria have high resistance rates and discordant antibiotic treatment can lead to multi-organ involvement. Proper timely institution of empirical treatment and adherence to antimicrobial stewardship guidelines coupled with aggressive management is crucial in saving lives.

1. Introduction

Blood Stream Infections (BSI) are defined as presence of bacteria in the blood; which can range from inapparent bacteraemia to fulminant septic shock with high mortality.

The invasion of microorganisms in the bloodstream-intermittently or continuously pose a major threat to every organ in the body can lead to serious consequences like shock, multi-organ failure, Disseminated Intravascular Coagulation and death.

Sepsis is defined as the presence of viable multiplying bacteria in the bloodstream and production of toxins. Patients with bloodstream infections manifest clinically with systemic signs of infection such as fever, leukocytosis and raised inflammatory markers. BSI could be primary due to spread from infective endocarditis, Urinary Tract Infection (UTI), or Community acquired pneumonia, or secondary resulting from device associated infections or surgical interventions.

Blood culture is the yard-stick for identification of these pathogens. However empirical antimicrobial therapy is crucial in preventing unchecked progression to sepsis.[1]

The choice of Automated Manual Transmission (AMT) depends on the likely source of infection and the causative organism. Empirical therapy can be narrowed down to a single antibiotic agent once the causing agent is isolated and the antimicrobial susceptibility testing is performed.[2]

It is important to consider the resistance pattern of each species for better treatment of infections caused by resistant microorganisms.

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https://doi.org/10.18231/j.ijmmtd.2023.029

2. Materials and Methods

2.1. Study duration and sample

A prospective study was carried out between January 2022 to February 2023 in a Tertiary care Hospital in Hyderabad, India. A total of 1200 patients with clinical suspicion of bacteraemia were enrolled after obtaining the consent.

2.2. Inclusion and exclusion criteria

Patients aged≥ 2 years were included while neonates and those with history of Antibiotic intake <72 hours earlier were excluded from the study. Patients suspected to have viral or parasitic infections, tuberculosis and those with autoimmune disease and on steroids were excluded.

2.3. Specimen collection and processing

Blood was collected via venipuncture from a peripheral vein (preferably Antecubital vein) under strict aseptic conditions and inoculated appropriately into labelled adult and paediatric blood culture bottles, respectively. These were loaded into BD BACTEC-9050 Blood Monitoring System and incubated at 37°C. Positively flagged Blood Cultures were further processed via direct Gram's stain and inoculation onto solid media.

A subculture was performed onto Nutrient, MacConkey and 5% Sheep Blood Agar plates and incubated overnight at 37°C. The isolate was identified based on colony morphology, Grams staining and biochemical properties as per standard protocols.[3] Antimicrobial susceptibility testing of the isolated bacteria was performed using Kirby-Bauer disk diffusion method and the results interpreted as per the CLSI 2022 guidelines.[4] Quality control was carried out by testing each new batch of media and antibiotics using standard strain. Cefozolin was used as a surrogate marker for testing cefpodoxime, cefuroxime and cefdinir.[4]

The Gram-Positive cocci, *Staphylococcus* species were tested for methicillin resistance using 30μg Cefoxitin disc with served as a surrogate marker for Methicillin resistance. A zone size of ≤21 was considered as *MecA* positive, MRSA.[4] Quality control was performed using control MRSA strain ATCC 43300.

Gram Negative bacilli obtained were subjected to detection of ESBL production using Cefotaxime 30μg and Cefotaxime-clavulanate 30/10μg disc.[4] Quality control was performed using *Klebsiella pneumoniae* ATCC 700603.

Gram Negative isolates were screened for imipenem resistance. Imipenem resistant isolates were further tested for Metallo-ß-Lactamase production using Imp-EDTA combined disc as described by Yong et. al.[5]

The data and the results were obtained in the Microsoft Excel and statistically analysed. Multi-drug resistant bacteria were identified and percentages of MDR were calculated.

3. Results

279 (23.25%) aerobic bacterial isolates were obtained from paediatric and adult patients with clinical suspicion of bacteraemia. The spectrum of isolates obtained and their frequency are depicted in Table 1 and diagrammatic presentation in Figure 1. 108 (38.71%) isolates were Gram positive cocci while 171(61.29%) were Gram negative bacilli. 5 fungal isolates were also obtained during the study.

Table 1: Frequency of bacterial isolates.

Organism	**Frequency (n)**	
Gram positive bacteria	**n=108**	**Percentage**
Staphylococcus aureus	63	58.3%
Coagulase Negative Staphylococci	14	12.9%
Enterococcus species	26	24%
Streptococcus pyogenes	2	1.8%
Streptococcus viridans	3	2.7%
Gram negative bacteria	**n=171**	**Percentage**
Escherichia coli	38	22.22%
Klebsiella species	42	24.56%
Enterobacter	14	8.18%
Pseudomonas aeruginosa	38	22.22%
Acinetobacter species	34	19.88%
Salmonella species	4	2.3%
Citrobacter freundii	1	0.5%
Fungi	**n=5**	
Candida species	5	

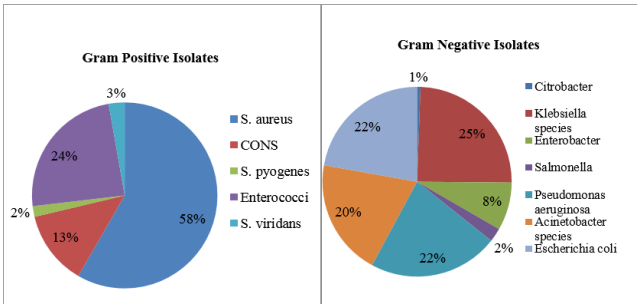

Fig. 1: Frequency of bacterial isolates.

Staphylococcusaureus was the most common isolate followed by *Klebsiella pneumoniae*,*Escherichia coli*, *Pseudomonas aeruginosa*, *Acinetobacter* and *Enterococcus* species. Antimicrobial susceptibility pattern for Gram positive and Gram-negative isolates was interpreted and is displayed in Tables 2 and 3. 24.37% of all isolates were MDR. A bacterial isolate is said to be multi-drug resistant when it tests resistant to at least one (1) antimicrobial agent from three separate antimicrobial classes.[6] *Klebsiella spp.* and *Enterobacter spp.* showed 28.56% multi-drug resistant strains each.

Table 2: Drug sensitivity profile of Gram-negative isolates. (%)

	***Escherichia coli* (n=38)**	***Klebsiella spp.* (n=42)**	***Enterobacter Cloaca* (n=14)**	***Acinetobacter* spp. (n=34)**	***Pseudomonas aeruginosa* (n=38)**
Ampicillin	26.67	-NT-	-NT-	-NT-	–
Ampicillin-sulbactum	35.29	61	-NT-	56.25	–
Cefoxitin	29.41	44.44	–	–	–
Cefozolin	45.45	41.67	-NT-	–	–
Co-trimoxazole	58.82	46.15	66.67	83	–
Aztreonam	52.94	64.29	66.67	-NT-	58.33
Amikacin	50	70.00	75	88.89	81.81
Gentamicin	90	75.00	–	80.00	–
Levofloxacin	60	81.25	–	77.78	81.25
Cefotaxime	47.06	53.33	66.67	80	–
Cefatazidime	43.75	61.54	66.67	58.33	61.11
Cefepime	55	62.50	57.14	81.25	84.61
Meropenem	78.26	77.78	100	93.33	93.33
Piperacillin-tazobactam	93.75	83.33	100	100	92.30
MDR%	21	28.5	28.5	17.6	10.5

Table 3: Drug-sensitivityprofile of Gram-positive isolates (%)

	***Staphylococcus* spp.**			
	Coagulase positive		Coagulase negative (n=16)	***Enterococcus* (n=26)**
	Methicillin sensitive(n=38)	Methicillin Resistant (n=25)		
Penicillin	25	25	19.14	50
Azithromycin	57.71	50	21.15	–
Clindamycin	58.33	75	55.76	–
Erythromycin	16.67	12.5	31.57	–
Doxycycline	100	90	92.59	–
Ciprofloxacin	91.66	75	76	81.81
Rifampicin	100	100	84	–
Cotrimoxazole	90	85	66.67	–
Vancomycin	100	100	95	100
Linezolid	100	100	100	100
High-level gentamicin	–	–	–	80
MDR %	14.2	25.4	–	–

4. Discussion

Blood stream infections are a life-threatening condition and timely diagnosis and rapid appropriate institution of antimicrobials is crucial in saving lives. Antibiotic resistance rates keep constantly increasing and has become a major public health threat; responsible for 1.27 million deaths worldwide.[7] 20% of the cases of BSI have been reported to receive discordant empirical antimicrobial therapy; i.e. administration of antibiotics to the patient which later tested resistant on performing antimicrobial susceptibility testing.[8] Infection with an antibiotic resistant pathogens (e.g. *Pseudomonas aeruginosa*) is more associated with discordant therapy with increased mortality rates; even in patients without sepsis.[8]

Hence adherence to antibiogram is necessary to as local bacterial communities and their antibiotic susceptibility patterns differ widely and appropriate empirical treatment is crucial.

In our study Gram negative bacteria predominated as the causative agents of BSI; accounting for 61% of the bacterial isolates and 39% Gram Positive cocci. Banik et al[9] reported 57% Gram negative isolates while Khurana et. al had GNB amounting to 77% in 2013; 85% in 2014 and 81% in 2015 & 2016.[10]

Staphylococcus aureus was the single most common isolate amounting to 58.3% of all Gram-positive isolates. 39.68% of *S. aureus* were Methicillin Resistant *Staphylococcus aureus* (MRSA). Similarly, 42% *S. aureus* 59% methicillin resistance was noted by Banik. et. al.[9]

Vancomycin, linezolid, doxycycline and rifampicin showed good action for both MSSA and MRSA; however, it has been recommended that Rifampicin should not be used as single drug therapy for treatment.[4]

Doxycycline is has 90% oral bio-availability as it is highly lipophilic and can easily cross various biological membranes to reach target site in both Gram positive and negative bacteria.[11] Doxycycline should be preferred to Linezolid as although both have high oral bioavailability but Doxycycline is more cost-effective. Vancomycin is available as I.V. preparations and as contrary to previous recommendations,[11] recent studies highlight the advantage of oral preparations to treat bloodstream infections in patients with uncomplicated bacteraemia.[12] And hence doxycycline can be used as alternative to Vancomycin and linezolid in immunocompetent patients.

Coagulase Negative *Staphylococci* are usual skin commensals but are being increasingly recognised as pathogenic bacteria in certain settings especially in the presence of any risk factor; namely presence of central venous catheter, immunocompromised status, and metabolic disorders. 12.9% of the Gram-positive isolates in our study comprised CONS while other studies by Vasudeva et. al.[13] reported 30% of GPC isolates to be CONS and Banik et. al.[9] reported 23.31% CONS.

We considered CONS as the pathogenic organism based on clinical presentation, procalcitoninlevels and wherever possible reisolating the same strain on subsequent blood culture. Improper technique of blood collection; coupled with existence of long-standing intravascular devices are recognised as possible routes of spread of CONS into the bloodstream. CONS were highly susceptible to doxycycline, Ciprofloxacin, Linezolid and Vancomycin while penicillin, azithromycin, erythromycin, and clindamycin showed poor response. All Gram-positive isolates were susceptible to Linezolid.

While the incidence of Enterococcal Bloodstream infections varies considerably[12,13]ranging from 5% as observed by Banik et al.[9] to 8% in Meshram et al[14] in we had a higher incidence of Enterococcal Blood stream infections; contributing to 24% of GPCs and 9.3% of total bacterial isolates with high susceptibility towards linezolid, vancomycin, ciprofloxacin and high-level gentamicin; however considerable resistance towards penicillin was demonstrated by Enterococcal isolates.

61% of the total isolates were Gram-negative bacteria. The Gram-negative isolates obtained showed high resistance towards penicillin and ampicillin-sulbactam indicating reduced efficacy of these drugs.

Klebsiella was the most common GNB isolated. Vasudeva et al[13] had *E. coli* as the commonest GNB while Banik et al[9] has *Acinetobacter* followed by *Klebsiella spp*.

Klebsiella pneumoniae and *Escherichia coli* showed decreased susceptibility to cephalosporins as well as cephamycins. *Piperacillin-Tazobactam* and Gentamicin had good sensitivities against GNB. 39% of the isolates showed production of Extended spectrum ß-Lactamases, with 52% of the *Escherichia coli* being ESBL producers. Significant number of isolates were positive for Metallo-ß-Lactamaseproduction with 21.7% of *E. coli* and 22.22% of K. Pneumoniae being positive for MBL production.

Previous studies highlight high frequency of prevalence of MDR *Klebsiella pneumoniae* with 30-day mortality rate as high as 45% in patients with K. pneumonia BSI.[15,16] 28% of *Klebsiella* and 21% *E. coli* were detected to be multi drug resistant strains. *Klebsiella pneumoniae* is notorious for its ability to accumulate and transfer resistance determinants, and it has been well-recognized as a leading causative agent of hospital-based infections over the previous decades.

Non-Enterobacteriaceae Gram Negative bacilli have been implicated to cause 43-58% of BSI.[10] In our study these amounted to 43.8% of the total isolates with high incidence of *Pseudomonas aeruginosa*, i.e., 50.6% of the non-Fermenting bacteria (n=38) and *Acinetobacter* spps. Being 45.3%; (n=34) P. aeruginosa is a major cause of Hospital-associated infections; in our study the isolates were moderately resistant to Aztreonam and ceftazidime and showing good susceptibility (>80%) to Aminoglycosides; fluoroquinolones (i.e. levofloxacin), carbapenems and *Piperacillin-tazobactam*. Micek et al.[17] in their study entitled "*Pseudomonas aeruginosa* bloodstream infection: importance of appropriate initial antimicrobial treatment" state that mortality in patients with P. aeruginosa infections is statistically higher in patients receiving discordant empirical therapy and is reduced in patients who receive combination therapy than monotherapy.[17]

Acinetobacter species in our study were sensitive to Meropenem (93%), *piperacillin-tazobactam* (100%) and 4^{th} generation cephalosporins (84%). One isolate of Carbapenem-Ampicillin Sulbactam Resistant (CASR) *Acinetobacter baumannii* was isolated. Infection with CASR *A. baumannii* was observed to be associated with increased severity of infection and high mortality.[18,19]

5. Conclusion

Bloodstream infections are a medical emergency and antibiotics are central and pivotal in saving lives. Judicial usage of antibiotics while preventing antibiotic misuse is a challenge especially in severely ill patients. Adherence to Hospital antibiogram and antimicrobial stewardship policy can help tackle this situation.

6. Conflict of Interest

The authors declare that there is no conflict of interest.

7. Source of Funding

None.

Acknowledgements

None.

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Cite this article: Anjum I, Mustafa M. Study of prevalence and antimicrobial susceptibility pattern of blood culture isolates from a tertiary care hospital in South India. *IP Int J Med Microbiol Trop Dis* 2023;9(3):145-149.