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Original Research Article

A study of bacteriological profile and antimicrobial susceptibility pattern of bacteria isolated from blood stream infections in a tertiary care hospital

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ABSTRACT

Background: Blood cultures are procedures that aid in evaluation of microorganisms present in the blood and guide the treatment of various pathogens leading to blood stream infections and the most dreaded complication sepsis
Aim: To study the distribution of bacterial flora and antimicrobial susceptibility in blood stream infections (BSI) in adults and pediatric patients including neonates.
Materials and Methods: This retrospective study was conducted in the department of Microbiology, ESIC Medical College and hospital, Hyderabad over a period of 1 year, from January 2020 to December 2020. During this period, 1681 samples received from various in patient department were processed according to standard laboratory guidelines and findings were observed.
Statistical analysis: WHO net software was used to analyze the data.
Results: On analysis of all the samples, 208(12.4%) samples were culture positive. Among them Gram positive bacteria were 55.2 % and Gram negative were 44.8%. Coagulase negative Staphylococcuswas the predominant isolate among Gram positiveGram-positive,resistance to35%34.3xtensivelyXDR)Maximumresistanceprescription and usage.
Conclusion: Early diagnosis and appropriate treatment of bacterial infections can make difference between life and death. It would reduce mortality from septicemia and improve patient management.

1. Introduction

Bloodstream infections (BSI) are infectious diseases defined by the presence of viable bacterial or fungal microorganisms in the bloodstream that elicit or have elicited an inflammatory response characterized by the alteration of clinical, laboratory and hemodynamic parameters.[1]

Bloodstream infection is a major cause of morbidity and mortality despite the availability of broad spectrum and effective antimicrobials and major advances in supportive care. Bacterial endocarditis accounts for approximately 3–8% of cases of bloodstream infections.[2]There is a risk for BSI patients to develop sepsis, caused by a dysregulated host immune response.[3]They are responsible for prolonged hospital stays, high healthcare costs, and significant mortality.[4]

Early detection of pathogens and determination of their susceptibility are essential for the optimization of treatment.[5]Blood culture has been long recognized as a gold standard for definitive diagnosis of bacterial and fungal infections worldwide.[6]

The distribution of microorganism and the susceptibility pattern to antibiotics even within the same hospital seem to vary with time. Therefore, continuous surveillance of blood stream infection etiology is of paramount importance to help in regularly updating the antibiogram and as a

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guide to clinicians for starting a presumptive prophylaxis and empirical treatment so as to decrease morbidity and mortality. Additionally, since the antibiotic pipeline being practically dry, routine surveillance keeps a check on development of multi-drug resistant bugs in the hospital environment in the era of rampantly increasing multi drug resistance globally. The present study was undertaken to understand the bacteriological flora in cases of BSIs and the antibiotic susceptibility pattern of the isolated strains in a tertiary care hospital.

2. Aims and Objectives

1. To determine the distribution of bacteriological isolates causing blood stream infections in suspected cases of bacteremia and septicemia
2. To determine the antibiotic susceptibility pattern of bacterial isolates and to aid in formulating an empirical therapy accordingly.

3. Materials and Methods

This was a retrospective Laboratory record-based study which was conducted at the Department of Microbiology ESIC Medical College and Hospital. The data of blood samples received from inpatients between January to December 2020 was collected. The ethical clearance was sought for analysis of all clinical samplesantibiogram prior to initiating this study.

3.1. Inclusion criteria

1. All Blood samples from in patients received at the study site for culture and sensitivity.
2. Pediatric and adult age groups were included.

3.2. Exclusion criteria

1. Duplicate samples sent additionally yielding the same organism were excluded.
2. Blood cultures yielding mixed flora and contaminants were excluded.

Blood samples were collected according to standard operational procedures.[7,8]2 sets of adults and pediatric BACTEC bottles were collected per patient and processed by automated BACTEC system as per manufacturer's protocols. Under strict aseptic conditions, 1ml of venous blood was inoculated in 10 ml of sterile BACTEC bottle supplied by the manufacturers. BACTEC system would undergo daily temperature maintenance checks and annual maintenance as per schedule. Any alert beep sounded by the equipment was noted and samples flagged would be sub cultured periodically. Before inoculating the blood sample onto the plate, gram stain was done and presumptive organism was telephonically communicated to the treating doctor in charge for empirical therapy. Subcultures were done at timely intervals. The clinical data of the patient was recorded in laboratory records. Periodic subcultures were done on sheep blood agar and Mac Conkey agar at 24hrs, 48hrs, 72hrs, 5^{th} day and 7^{th} day as per standard protocol. The growth obtained was identified by colony morphology, gram stain of the isolated colonies and standard biochemical identification tests.[9] Antimicrobial susceptibility testing was performed by Kirby–Bauer disk diffusion method and interpreted using clinical laboratory standard institute (CLSI) guidelines 2020.[4] A provisional report was given at 48hrs followed by a final report at

3.3. Antibiotic susceptibility testing

The antibiotics tested for Gram-positive bacteria from blood isolates were as follows: For Staphylococcus aureus -penicillin (10U), ampicillin (10μg), cefoxitin (30μg), high level gentamicin (HLG) (120μg), levofloxacin trimethoprim/sulfamethoxazole (1.25/23.75μg) clindamycin (2μg), erythromycin (15μg), linezolid (30μg), vancomycin (30μg discs), teicoplanin (30μg), tetracycline (30μg). Vancomycin E strip was used for Staphylococci isolates.

3.4. For streptococcus and enterococcus species

Penicillin (10U), cefoxitin (30μg), trimethopim-sulfamethoxazole, gentamicin (10μg and 120μg), ciprofloxacin (5μg), linezolid (30μg), teicoplanin (30μg), chloramphenicol (30μg). Vancomycin (30μg discs) was used for Enterococcus and Streptococci

3.5. For Gram-negative bacteria following drugs were tested

Ampicillin (10μg), piperacillin(100μg), amoxicillin/clavulanic acid (20/10μg), ceftazidime/avibactam (E-strip used), ticarcillin/clavulanic acid(75/10μg), piperacillin/tazobactam (100/10μg), cefuroxime (30μg), ceftazidime (30μg), ceftriaxone (30μg), cefotaxime (30μg), cefepime (30μg), cefoxitin (30μg), cefixime (5μg), aztreonam (30μg), imipenem (10μg), meropenem (10μg), amikacin (30μg), gentamicin-High (10μg), netilmicin (30μg), ciprofloxacin (5μg), levofloxacin (5μg), trimethoprim/sulfamethoxazole (1.25/23.75μg), erythromycin (15μg), tetracycline (30μg).

Quality control strains were used for culture and susceptibility testing at weekly intervals. The reference strains used for Antibiotic susceptibility testing were Escherichia coli (ATCC 25922), Staphylococcus aureus (ATCC 25923), Pseudomonas aeruginosa (ATCC 27853), and Enterococcus faecalis (ATCC 29212).

Detection of Methicillin Resistant Staphylococcus aureus (MRSA) was done by cefoxitin disk diffusion method by placing 30μg cefoxitin disks on the bacterial lawn culture of S.aureus. After overnight incubation, the

zone of inhibition was measured. An inhibition zone of diameter less than or equal to 21 mm indicates MRSA. S.aureus ATCC 25923 was used as quality control strain.[8]

Extended spectrum beta lactamase (ESBL) producers were detected by combination disk method using cefotaxime (30μg) and cefotaxime/clavulanate (30/10μg) (Himedia- Mumbai, India) and ceftazidime /clavulanate (30/10μg). An increase of 5 mm in the zone of inhibition in a disk containing clavulanate compared to the drug alone was considered as positive for ESBL producers.

As per standardized international terminology created by European Centre for Disease Control (ECDC) and Centre for Disease Control and Prevention (CDC), Atlanta, the multidrug-resistant (MDR), extensively drug-resistant (XDR) bacteria have been defined.[10]

The rare strains of non-fermenting gram negative bacilli were identified and susceptibility was carried out by VITEK 2 automated bacterial identification system.same.WHONET isCategorical variables have been mentioned in numericals and percentages.

4. Results

A total of 1681 blood samples were received from various in patient locations in the hospital. 208 samples were shown to flag positive and culture yielded a pathogenic organism, followed by the antibiotic susceptibility testing which was carried out. For formulation of the Antibiogram only the 1^{st} isolate from the patient was considered. Culture positivity was seen in 12.4% of the samples received. Forty two isolates (1.4%) were contaminants recovered during the process of culture of samples. Out of 208 culture positive samples, 108 (52%) were male and 100 (48%) were female. Gender-wise ratio of 1.08:1 was observed and skewed in favor of male. Mean age of distribution was 38 +/- 24 (Range 0 - 85yrs). The predominant age group affected between 45-54 years (19.2%) About 13 (6 %) samples showed polymicrobial growth while 195 (94%) were monomicrobial. Total number of pathogenic isolates were 221.

Majority of the blood culture positive samples were received from the ICU's being 105 (50.5%), followed from wards 103 (49.5%). Amongst the ICU's majority of the samples were sent from the MICU 51 (24.5%) followed by 15 (7.2%) from NICU. Among the wards majority of the samples received were from Medical ward 38 (18.2%) followed by Oncology Wards 17 (8.17%).

Among the culture positives gram positive organisms were 55.2 % (n= 221) and gram negative organisms 44.7% (n=221). Among the Gram-positive isolates, the predominant isolate was Coagulase negative Staphylococcus species (CONS) (N=67) followed by Staphylococcus aureus (N=38). (Table 1) 30% of CONS were Methicillin resistant (MRCONS) and 82% of the Staphylococcus aureus strains were found to be MRSA. The susceptibility pattern of Staphylococcus aureus is depicted in Table 2. which showed least resistance to vancomycin, teicoplanin, tetracycline, linezolid and clindamycin. None of the Staphylococcus aureus isolates showed vancomycin and teicoplanin resistance. Coagulase negative staphylococcal strains (CONS) showed least resistance to vancomycin, tigecycline, linezolid, quinolones and tetracycline.

Among the Enterococcus isolates least resistance was demonstrated to linezolid, teicoplanin, vancomycin, and high level gentamicin.

In this study the gram negative bacteria isolated showed high susceptibility to piperacillin tazobactam (83%), netilimycin (76%), tetracycline (75%), meropenem (71%) and cotrimoxazole (71%). Moderate susceptibility was seen to imipenem (64%), ticarcillin clavulanate (63%). Distribution of susceptibility pattern is shown below in Table 3. Maximum resistance was seen to beta lactam antibiotics. 35% of the strains were ESBL producers.

All the Salmonella isolates were resistant to quinolones (100 %) while 96% of them were susceptible to chloramphenicol.

Amongst the Non- fermenting gram negative bacilli high susceptibility was seen to minocycline and colistin (100%) in Acinetobacter spp. Pseudomonas species was highly susceptible to Anti Pseudomonal cephalosporins (86%). Strains of Elizabethkingea meningoseptica, Brevundimonas diminuta, and Chrysobacterium indologens were isolated, which were multidrug resistant.

5. Discussion

Bloodstream infection (BSI) is potentially life-threatening condition with a case fatality rate of 30-40%. In view of suspected BSI empirical therapy must be started by the clinician without any delay based on the type of infection, underlying disease, patient age, infecting pathogen, and site of acquisition of infection.[11]

This study aims at determining the bacterial profile and assess their antimicrobial trends to formulate an antibiogram that would aid in effective treatment of BSIs.

In this study the culture positivity was seen in 208 (12.4%) samples. This finding is seen to be consistent with many other Indian studies and International studies.[12–15] On the contrary, higher culture positivity has been reported by some authors.[16,17] Variation in culture positivity rates could be due to difference in geographical location, nature of population, epidemiological difference of the etiological agents, also factors such as volume or number of blood culture samples.[9] The low rate of isolation in this study could be due to patients taking over counter medications, incomplete treatment without follows up as Peripheral health care center (PHC's) before getting admitted.

The contamination rate in this study was 1.4%. The rate of contamination observed is below the target level

Table 1: Distributions of bacterial isolates from positive Blood cultures

Organism	Number of isolates (n=221)	Percentage
Acinetobacter baumannii	28	12.6
Burkholderia cepacia	1	0.45
Citrobacter freundii	1	0.45
Enterococcus sp.	19	8.59
Escherichia coli	25	11.3
Klebsiella aerogenes	1	0.45
Klebsiella pneumoniae ss. pneumoniae	19	8.59
Moraxella (Branh.) catarrhalis	1	0.45
Proteus mirabilis	1	0.45
Pseudomonas aeruginosa	7	3.16
Salmonella Typhi	12	5.42
Brevundimonas diminuta	1	0.45
Elizabethkingia meningoseptica	1	0.45
Serratia marcescens	1	0.45
Staphylococcus aureus ss. aureus	32	14.47
Staphylococcus epidermidis	1	0.45
Staphylococcus, coagulase negative (CONS)	67	30.3
Streptococcus viridans alpha-haemolyticus	2	0.9
Streptococcus beta-haemolyticu s Group A	1	0.45

Table 2: Susceptibility pattern of the Gram positive organisms isolated from positive blood culture

Organism	Number of isolates	AMP %S	FOX %S	CIP %S	CLI %S	ERY %S	NAL %S	TEC %S	TCY %S	SXT %S	VAN %S	GEH %S	LVX %S	PEN %S	LNZ %S
Streptococcus, beta-haem. Group A	1	100			100	100					100				
Enterococcus sp.	19	18.2		18.2		33.3		86.7	42.9		84.2	75.0	28.6	22.2	100
Staphylococcus aureus ss. aureus	32	11.1	18.8		62.5	39.3	50	100	66.7	27.8	100	0.0	38.9	8.3	93.3
Staphylococcus, coagulase negative	68	34.8	30.3		48.9	43.1	75	97.6	87.2	40.6	100	62.5	52.9	34.1	93.5
Streptococcus viridans, alpha-hem.	2	50	0.0		0.0	50		100	100	100	100				

PEN- Penicillin, AMP-Ampicillin, FOX- Cefoxitin, GEH- High level gentamycin, NAL – Nalidixic acid, LVX- Levofloxacin, SXT- Cotrimoxazole- CLI-Clindamycin, ERY – Erythromycin, LNZ- Linezolid, VAN- Vancomycin, TEC- Teicoplanin, TCY- Tetracyclin

Table 3: Antibiotic susceptibility pattern of Gram negative Bacteria

Susceptibility pattern	AMP	AMC	CXM	CAZ	CZA	CRO	FEP	CIP	ERY	IPM	MEM	SXT	NET	TZP	TCY	TCC
Sensitivity - S%	33	62	28	54	75	50	57	55	58	64	71	71	76	83	75	63
Resistance -R%	67	38	72	46	25	50	43	45	42	26	29	29	24	16	25	37

AMP-Amplicillin, AMC- Amoxicillin – clavulinate, CXM – Cefuroxime, CAZ- Ceftazidime, CZA- Ceftazidime- Avibactam, CRO – Ceftriaxone, FEP-Cefipime, CIP-Ciprofloxacin, ERY-Erythromycin, IPM-Imipenem, MEM- Meropenam, SXT-Cotrimoxazole, NET- Netilimycin, TZP- Piperacillin tazobactam, TCY- Tetracycline, TCC- Ticarcillin clavulinate

suggested by Hall et al.[18] This correlate well with other studies by Palewar et al.[9,19]

Gender-wise ratio of 1.08:1 was observed skewed in favor of males which was in accordance with studies done by Palewar et al and Baniker et al.[9,12] The recent review of data in the National Hospital Discharge Survey (U.S) which states incidence of sepsis, severe sepsis, and septic shock is higher in men than in women.[9] In the current study the highest blood culture positivity the mean age of distribution was found to be 38+/-24 which in accordance with a study conducted in Iran.[20]

Most common isolate among the gram-positive bacteria (GPC) was Coagulase negative Staphylococcus (CoNS) followed StaphylococcusaureusIndian.[21–24] The higher isolates of CONS were isolated from neonatal units and Oncology Unit.few clinical departments were recognized as possible modes of spread of BSI by CoNS.[9]

Within Staphylococcus spp., MRSA was most susceptible to the action of vancomycin, and teicoplanin followed by linezolid. These findings are similar to various other studies.[9,12,24,25] The MSSA isolates were highly susceptible to tetracyclines, clindamycin, and quinolones. Among the infections caused by CoNS higher susceptibility was seen for quinolones, tetracycline, teicoplanin and vancomycin. Similar findings were reported by Banik et al and Ashok et al.[12,16] Enterococcus spp. were highly susceptible to Linezolid, Teicoplanin, vancomycin, and high-level gentamycin. These findings are similar to a study done by Palewar et al except for high level gentamycin where higher resistance (44-60%) was seen.[9]

All Gram-negative bacteria showed low sensitivity to beta lactam drugs. 34.3% of isolates were Multi drug Resistant (MDR) and 9% strains were XDR. Among the MDR strains majority were Escherichia coli(n=13) followed by Klebsiella spp (n=11). Beta-lactam drugs are rapidly becoming ineffective for treating BSIs due to indiscriminate and non-judicious usage.[12] These medications have been used rampantly over the counter by self-medication and improper dosage schedule leading to increased resistance that has been reported by other studies.[12,17]

All Gram-negative bacteria showed good susceptibility to piperacillin-tazobactam, meropenem, tetracycline and netilmicin. These findings match with other Indian studies.[9,12,16] In this study ceftazidime – avibactam showed good susceptibility.

Among the Non – fermenting gram-negative bacteria the predominant isolates were Acinetobacter baumannii followed by Pseudomonas aeroginosa. One strain each of Elizabethkingia meningoseptica (NICU), Brevundimonas diminuta (Oncology ward) was isolated and two strains of Chrysobacterium indolgens from NICU were isolated. The strains of Elizabethkingia meningoseptica were susceptible to cotrimoxazole and vancomycin. The data on antibiotic susceptibility of E. meningosepticum is limited because it is rarely isolated from clinical specimen and there are no standard guidelines on antibiotic susceptibility testing and reporting and interpretation of the susceptibility data.[26]The case of Brevundimonas diminuta did well on a combination of ceftazidime and tobramycin. The treatment of Brevundimonas spp. infections is frequently difficult, as these bacteria can be resistant to many different antibiotics including β-lactams and fluoroquinolones. There have been no controlled trials of antimicrobial therapy for Brevundimonas spp. infections in humans therefore therapy should be informed by the results of in vitro susceptibility testing on isolates.[27]The Strains of non-fermenting gram-negative bacteria were Multi-drug resistant strains, which showed favorable treatment when attempted with Minocycline & Colistin. Pseudomonas spp. isolates were sensitive toward ceftazidime and cefoperazone. One case of Burkholderia cepacia was encountered from PICU (Pediatric ICU). This case did well on cotrimoxazole treatment. Most of the non-fermenting gram-negative bacilli isolated showed higher resistance to carbapenems, Beta lactam + beta lactam inhibitor combination and aminoglycoside, which was contrary to study done by Katyal et al.[24]

Effective treatment of bloodstream infections should be based on early diagnosis and appropriate and targeted antimicrobial therapy.

The antibiogram must be updated locally based on the hospital flora and optimal utilization policies and guidelines must be framed to the limit the further development of pan drug resistance.

6. Conclusion

This study gives an insight into the prevalence of various isolates from a tertiary care center in South India. Repeated revisions of organism isolated followed by their Antibiogram is imperative in the ever-growing era of drug resistance and keeping in view the static pipe line of antimicrobials. It is crucial to monitor the epidemiology of Blood stream infections in order to improve the antibiotic utilization policies like antibiotic restriction, combination therapy, antibiotic usage according to the standard antimicrobial susceptibility testing and antibiotic recycling may aid to reduce incidence of blood stream infections and also to prevent the emergence of resistance. A strong antibiotic stewardship program and stringent infection control policies are vital in the epoch of escalating antibiotic resistance.

7. Conflict of Interest

None.

8. Source of Funding

None.

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