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Study of Microbiological Profile and Antibiotic Susceptibility of Blood Stream Infections in Tertiary Care Hospital

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A B S T R A C T

Blood stream infections (BSIs) can lead to life-threatening sepsis and are globally associated with high morbidity and mortality. Early diagnosis plays a crucial role in managing BSI. Objective is to identify the pathogens causing blood stream infections and to know their antibiotic sensitivity pattern. This was a retrospective study of 1 year duration. A total of 1332 blood samples from clinically diagnosed cases of blood stream infections received in the microbiology laboratory were included in the study. Blood samples were processed and isolates were identified by standard biochemical tests and antibiotic susceptibility testing was done by Kirby Bauer disc diffusion methods as per CLSI guidelines. Out of 1332 blood samples received, 204 (15.3%) samples showed growth and 1128 (84.68%) samples showed no growth, with total percentage of culture positivity being 15.3%. Among 204 positive cultures, 202 (99%) showed bacterial growth and 2 (0.98%) were Candida spp. Bacteremia due to Gram-positive pathogens was more common compared to Gram-negative pathogens. The present study provides information about pathogens responsible for blood stream infections and their antibiotic susceptibility. Antibiotic susceptibility pattern of isolates provides useful guidelines to clinicians in initiating empiric therapy and help in management of blood stream infections.

Introduction

Blood stream infections (BSIs) can lead to life-threatening sepsis and are globally associated with high morbidity and mortality.[1] Blood stream infections (BSI) are defined by the presence and active multiplication of microorganisms in the blood stream.[2] BSI by the place of acquisition is categorized either community associated or hospital associated. Blood stream infections occur when bacteria enter the blood stream from either a primary focus of infection in an organ (UTI, Pneumonia, meningitis...), a wound or via an indwelling or implanted device.[3] Health care associated (HCA) BSIs can occur as complications following medical and surgical procedures or the insertion of an intravascular or indwelling device.[4] Blood stream infections (BSIs) have serious

consequences such as shock, disseminated intravascular coagulation, multiple organ failure, and even death. Early diagnosis plays a crucial role in managing BSI, and hence, prompt detection of such infections is a critical function of clinical microbiology laboratories. [5]

BSI can be caused by both Gram-positive and Gram-negative microorganisms as well as fungi. Common Gram negative bacteria are *Escherichia coli, Klebsiella spp, Enterobacter spp, Proteus spp, Salmonella typhi, Pseudomonas aeruginosa, Acinetobacter spp, Haemophilus influenza, Brucella spp* and *Neisseria meningitidis*. Common Gram positive bacteria are *Staphylococcus aureus*, Coagulase negative *Staphylococci* (CONS), *Enterococci* and alpha haemolytic (viridans) streptococci. [6]

Blood culture is the gold standard for the detection of blood stream infection.[7] One of the main complication in the treatment of BSI is the increasing resistance of bacteria to antibiotics.

Emerging drug resistance among blood stream pathogens limit therapeutic options and complicate patient's management. [8] Today the only way to reduce mortality due to blood stream infection is early diagnosis and appropriate antimicrobial therapy at the earliest.

The aim of the present study to identify the pathogens causing blood stream infection and to know their antibiotic sensitivity pattern, thus providing useful guidance to clinicians to antibiotic therapy.

Materials and Methods

This was a retrospective study conducted for a period of one year in a tertiary care hospital. A total of 1332 blood samples from clinically diagnosed cases of blood stream infections received in the microbiology laboratory were included in the study.

Blood samples were collected from clinically suspected bacteremia cases before the administration of antibiotics under aseptic precautions and inoculated into brain heart infusion broth. A volume of 5–10 ml from adults and 2–3 ml from pediatric patients were obtained for culture. The culture bottles were incubated at 37°C aerobically for 18-24 hours and periodic subcultures were done onto MacConkey agar and blood agar on day 2, day 4 and finally on day 7 and samples were reported as no growth after 7 days of aerobic incubation. Bacterial growth on the subcultures was identified by colony morphology, Gram staining, and standard biochemical tests. [6] Antibiotic susceptibility testing was done by Kirby Bauer disc diffusion methods as per CLSI guidelines**.** Cefoxitin disc diffusion method used to identify Methicillin resistant *Staphylococcus aureus* (MRSA) and Methicillin resistant Coagulase negative Staphylococci (MRCONS) among *Staphylococcus aureus* and Coagulase negative Staphylococci respectively.[9] Extended spectrum beta-lactamases (ESBL) in Gram-negative bacilli were studied by phenotypic method with ceftazidime (30µg) and ceftazidime + clavulanic acid (30µg+10µg) as per CLSI guidelines.[9] MDR (Multi drug resistant) was defined as non-susceptibility to at least one agent in three or more antimicrobial categories. [9]

Results and Discussion

In the present study, 1332 blood samples were received and processed for aerobic culture. Out of 1332 blood samples, 204 (15.3%) samples showed growth, 1128(84.68%) samples showed no growth and with total percentage of culture positivity being 15.3%.

Out of 1332 blood samples received, 793 (59.53%) were from male and 539(40.46%) were female, maximum from the age group of <1year (37.31%), followed by 1-10 year (19.29%), 21-30years (7.73%) and 31-40 years (7.28%) respectively (Figure 1).

The ward wise distribution of samples includes 1254(94.19%) received from IPD, 72(5.40%) from ICU and 6(0.45%) from OPD.

Among 204 positive cultures, 202(99%) showed bacterial growth and 2(0.98%) were Candida spp. Out of 202 (99%) bacterial growth, Gram positive organisms were 128(62.74%) and Gram negative organisms were 74(36.27%) respectively (Table 1).

Among Gram positive isolates (128), the most predominant isolate was Coagulase negative Staphylococci (CONS) 114(89%) followed by *Staphylococcus aureus* 8(6.25%) and *Enterococcus* faecalis 6(4.68%) (Table 2).

Methicillin resistance Coagulase negative Staphylococci (MRCONS) was found in 82.45% of total CONS isolates and Methicillin resistance *Staphylococcus aureus* (MRSA) in 50% of total *Staphylococcus aureus* isolates.

Among Gram negative isolates (74), the predominant isolate was *Acinetobacter* spp 25(33.78%) followed by *Klebsiella* spp 22(29.72%), *Citrobacter freundii* 9 (12.16%), *Escherichia coli* 5 (6.75%), *Pseudomonas aeruginosa* 5(6.25%), NFGNB 4 (5.4%), *Enterobacter aerogenes* 3(4%) and *Salmonella typhi* 1 (1.35%) (Table 3).

Antibiotic sensitivity pattern of Gram positive and Gram negative organisms was studied. CONS was 100% sensitive to Vancomycin and Linezolid, followed by Clindamycin (61.12%), Tetracycline (56.14%), Amikacin and Gentamicin (47.76% each). *Staphylococcus aureus* was 100% sensitive to Vancomycin and Linezolid, followed by Clindamycin (62.5%) and Ciprofloxacin, Oflaxacin, Levofloxacin, Amikacin, Gentamicin and Tetracycline (50% each) respectively. *Enterococcus* spp showed 100% sensitive to Vancomycin and Linezolid, followed by 50% Amoxy-Clav and Penicillin (Table 4).

Out of total 114 CONS isolates, 94 were MRCONS and among 8 *Staphylococcus aureus* isolates, 4 were MRSA and the incidence of MRCONS and MRSA being 82.45% and 50% respectively.

The most effective antibiotic against Gram positive organisms were Vancomycin and Linezolid (100% each) followed by Clindamycin (60.12%) and Tetracycline (56.14%).

The most effective antibiotic against *Acinetobacter* spp was Colistin (100%), followed by Imipenem and Meropenem (68.96% each), Oflaxacin and Levofloxacin (65.50% each), Ciprofloxacin (58.62%), Amikacin and Pipercillin-Tazobactam (51.72% each). *Klebsiella* spp showed 100% sensitive to Colistin, followed by 81.8% to Imipenem and Meropenem. Citrobacter spp was 100% sensitive to Colistin, followed by 88.88% to Imipenem and Meropenem, 77.77% to Amikacin and Oflaxacin, 55.55% to Pipercillin-Tazobactam, Ciprofloxacin and Levofloxacin. *Escherichia coli* showed 100% sensitive to Colistin, followed by Imipenem and Meropenem (80% each), Amikacin (60%) (Table 5).

MDR was found in 60.80% of Gram negative isolates and ESBL were 20.27%. MDR was found high among Enterobacteriaceae. Carbapenem resistance was seen more among Nonfermenters (31%) as compared to Enterobacteriaceae (20.2%)

The most effective antibiotic against Gram negative organisms were Colistin followed by Imipenem, Meropenem, Amikacin and Oflaxacin.

In the present study, majority of the isolates showed high resistance to commonly used antibiotics belongs to Penicillins (Ampicillin &Amoxy-Clav), Cephalosporins (Cefotaxime, Ceftriaxone and Ceftazidime), Fluoroquinolones (Ciprofloxacin,Ofloxacin and Levofloxacin) and Aminoglycosides (Gentamicin and Amikacin).

Blood stream infections constitute one of the most serious conditions and associated with high morbidity and mortality as a result, timely detection, identification and antimicrobial susceptibility testing of blood stream pathogens are important.

The gold standard for diagnosis of BSIs is blood culture. [7] The present study gives information about pathogens causing blood stream infections. It also provides information about antibiotic sensitivity pattern that plays an important role in management of septicaemia cases.

In this study, total 1332 blood samples were received, out of which 204 (15.3%) samples showed growth and 1128(84.68%) showed no growth, with culture positivity being 15.3%.

Fig.1 Age distribution of patients

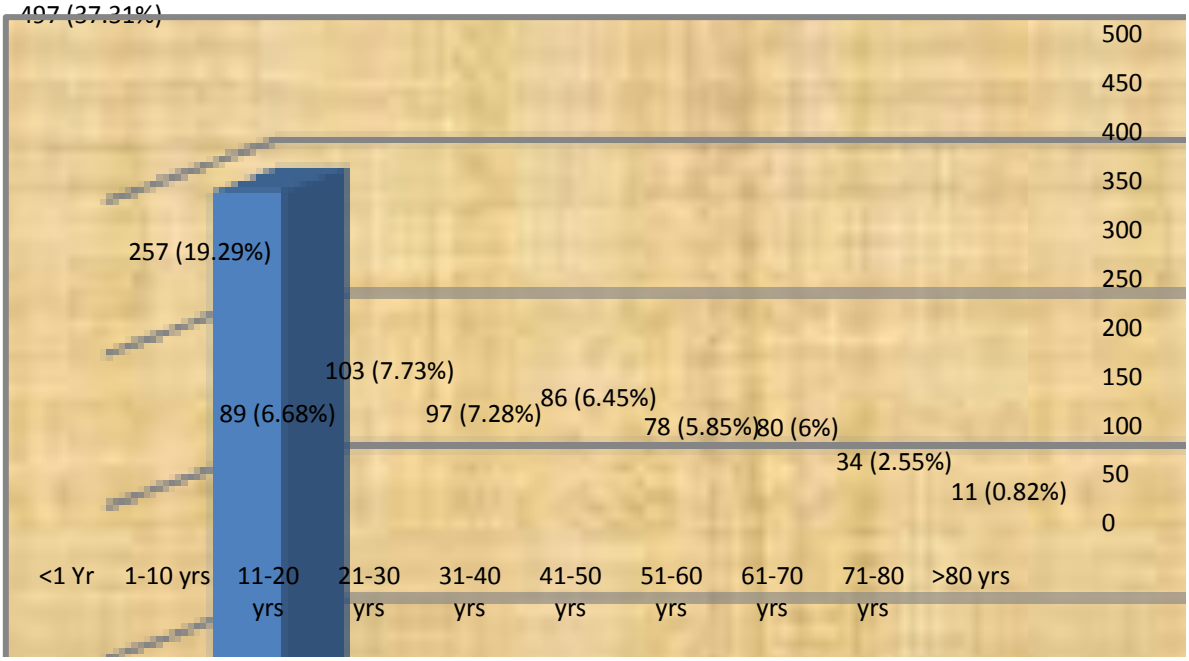

Table.1 Pathogens isolated from samples

Isolates	No. of isolates	Percentage
Gram positive bacteria	128	62.74%
Gram negative bacteria	74	36.27%
Candida spp	2	0.98%
Total	**204**	**100%**

Table.2 Gram positive bacteria isolated from the samples

Isolates	No. of isolates	Percentage
Coagulase negative Staphylococci (CONS)	114	89%
Staphylococcus aureus	8	6.25%
***Enterococcus* faecalis**	6	4.68%
Total	**128**	**100%**

Table.3 Gram negative bacteria isolated from the samples

Isolates	No. of isolates	Percentage
***Acinetobacter* spp**	25	33.78%
***Klebsiella* spp**	22	29.72%
Citrobacter freundii	9	12.16%
Escherichia coli	5	6.75%
Pseudomonas aeruginosa	5	6.75%
NFGNB	4	5.41%
Enterobacter aerogenes	3	4%
Salmonella typhi	1	1.35%
Total	**74**	**100%**

Table.4 Antibiotic sensitivity pattern of Gram positive isolates

	CONS n=114(%)	Staph.aureus n=8(%)	Enterococci spp n=6(%)
Vancomycin	100%	100%	100%
Linezolid	100%	100%	100%
Clindamycin	61.12%	62.50%	-
Tetracyclin	56.14%	50%	-
Gentamicin	47.36%	50%	-
Amikacin	47.36%	50%	-
Ciprofloxacin	43%	50%	16.66%
Oflaxacin	43%	50%	16.66%
Levofloxacin	43%	50%	16.66%
Erythromycin	21%	37.50%	-
Cotrimoxazole	14%	12.50%	-
Chloramphenicol	13.15%	25%	-
Cefotaxime	7.90%	37.50%	-
Amoxy-Clav	5.26%	12.50%	50%
Penicillin	0%	0%	50%

Table.5 Antibiotic sensitivity pattern of Gram negative isolates

	Acinetobacter spp n=25(%)	*Klebsiella* spp n=22(%)	*Citrobacter* spp n=9(%)	*E. coli* n=5(%)	*Pseudomonas aeruginosa* n=5(%)	NFGNB n=4(%)	*Enterobacter aerogenes* n=3(%)
Colistin	100%	100%	100%	100%	100%	100%	100%
Imipenem	68.96%	81.80%	88.68%	80%	80%	75%	66.66%
Meropenem	68.96%	81.80%	88.68%	80%	80%	75%	66.66%
Ofloxacin	65.50%	27.27%	77.77%	0%	40%	50%	0%
Levofloxacin	65.50%	27.27%	55.55%	20%	60%	50%	0%
Ciprofloxacin	58.62%	22.72%	55.55%	0%	60%	50%	0%
Amikacin	51.72%	22.72%	77.77%	60%	80%	75%	0%
Pipercillin-Tazobactam	51.72%	22.72%	55.55%	40%	60%	75%	66.66%
Gentamicin	44.82%	22.72%	44.44%	40%	80%	75%	0%
Tobramycin	31%	13.63%	22.22%	0%	40%	50%	0%
Amoxy-Clav	24.13%	20%	22.22%	0%	20%	25%	0%
Cefipime	24.13%	9%	33.33%	0%	40%	50%	0%
Cefotaxime	17.24%	4.54%	22.22%	0%	0%	25%	0%
Ceftriaxone	20.68%	4.54%	11.11%	0%	0%	25%	0%
Ceftazidime	10.34%	4.54%	22.22%	0%	50%	25%	0%
Ampicillin	6.89%	0	5%	0%	0%	0%	0%

Blood culture positivity in our study is in comparison with the studies conducted by Pragnya Paramita Jena *et al.*,[10] (16.2%), Roy *et al.*,[11] (16.4%), Shilpi Gupta *et al.*,[12] (16.5%), Banik A *et al.*,[13] (14.24%) and China D *et al.*,[14] (13.9%). In the study of Sultana *et al.*, [15] and Sharma *et al.*,[16] culture positivity rate was 49.28% and 33.9% respectively, which is higher to our study. In contrast low blood culture positivity of 9.9% by Mehta *et al.*,[17], 7.9 % by Anbumanni *et al.*,[18], 5.17% by Barati *et al.*,[19] and 5.6% by Mehdinejad *et al.*,[20] were reported in similar other studies. The variation in the positivity rate among studies may be due to most of the patient are given antibiotics before they come to the hospital or may be due to self-medication which is more common. [21] Difference in positivity rate from place to place is also due to different blood culture systems used in laboratories, amount and number of blood culture taken, the study design, and difference in the infection control policies between countries.[21]

In the present study, among 204 positive cultures, 202(99%) showed bacterial growth and 2(0.98%) were Candida spp. This is in comparison with studies conducted by Pragnya Paramita Jena *et al.*,[10] (96.85% & 3.14%), Banik *et al.*,[13] (96.66% and 3.33%), Qazi *et al.*,[22] (98.4% and 1.5%) and Shilpi Gupta *et al.*,[12] (96.68% and 3.31%).

In the present study, bacteremia due to Gram-positive pathogens was more common compared to Gram-negative pathogens. Out of 202 (99%) bacterial growth, Gram positive organisms were 128(62.74%) and Gram negative organisms were 74(36.27%). The predominance of Gram positive bacteria in BSIs was also reported by Banik *et al.*,[13] (GPB 60.37% and GNB 36.29%), Pavani *et al.*,[23](GPB 61.7% and GNB 38.3%), Khaleel

et al.,[24] (GPB59.85% and GNB40.15 %), Gohel *et al.,*[25] (GPB 58.3% and GNB 40.2%), Bhavna Bhadauria *et al.,*[26] (GPB 57.28% and GNB 42.74%), Ashima Katayi *et al.,*[27] (GPB 57.14% and GNB 42.85%), Dagnew *et al.,*[28] (GPB69% and GNB31%) and Wasihun *et al.,*[29] (GPB 72.2% and GNB 27.8%) respectively.

In the present study, among Gram positive isolates (128), the most predominant isolate was CONS 114(89%) followed by *Staphylococcus aureus* 8(6.25%) and *Enterococcus* spp 6(4.68%). This finding is in accordance with studies conducted by Karlowsky *et al.,*[30] (CONS 42%, *Staph. aureus* 16.2% and *Enterococcus* spp 8.3%), Ashima Katayi *et al.,*[27] (CONS 55.5%, Staph.aureus 34% and *Enterococcus* spp 10.4%), Alam *et al.,*[31] (CONS 63.5%, *Staph. aureus* 23.1% and *Enterococcus* spp 5.8%), Nazir *et al.,*[32] (CONS 67.9%, *Staph. aureus* 24.5%& *Enterococcus* spp 7.5%), Pragnya Paramita Jena *et al.,*[10] (CONS 40.5%, *Staph.aureus* 7.87% and *Enterococcus* spp 3.1%) where CONS reported as the most common isolate causing BSIs.

CONS were mainly recognized as mere contaminants till 1970's; however, several studies have now reported an increasing incidence of infection by this group of bacteria. [32] Over the past two decades, CONS, the usual skin commensals are increasingly being considered blood stream pathogens in select settings. Coagulase negative *Staphylococcus* is the third most common cause of BSI and the most common cause of nosocomial BSI. [33] Incidence of nosocomial bacteremia due to CONS is increasing due to frequent use of vascular access devices. Improper methods of blood collection and the presence of long standing intravascular catheters are recognized as possible modes of spread of BSI by CONS. [13] Some authors have demonstrated that coagulase-negative *Staphylococcus* adheres to the catheter surface, and produces slime, which are risk factors for BSI. [34] According to Souvenir *et al.,* clinical significance of CONS was defined as at least two blood cultures positive for CONS within 5 days or one positive blood culture plus clinical evidence of infection, which includes abnormal leukocyte count and temperature or blood pressure. [35]

In this study, among Gram negative isolates (74), *Acinetobacter* spp was the most predominant organism isolated (39.18%), followed by *Klebsiella* spp (29.72%), *Citrobacter* spp (12.16%), *E.coli* (6.75%), *Pseudomonas aeruginosa* (6.25%), Enterobacter spp (4%) and S.typhi (1.35%). These findings are consistent with other studies conducted by Pragnya Paramita Jena *et al.,*[10], Banik *et al.,*[13], Ashima Katayi *et al.,*[27]and Nazir *et al.,*[32] where *Acinetobacter* spp and *Klebsiella* spp have been found to be predominant isolates among Gram negative organisms.

The reason for high rate of isolation of *Acinetobacter* spp among Gram-negative bacteria may be because of acquisition of infection during hospital stay, as it is one of the commonest pathogen seen in nosocomial infections. Also, their ubiquitous nature in the hospital environment and inadequate infection control practice has continuously raised the incidence of *Acinetobacter* infections over the past two decades. [36]

Apart from Gram positive and Gram negative organisms, *Candida albicans* were isolated in two positive blood cultures (0.98%). Similar observation was made by Qazi *et al.,*[22].

The results of antibiotic sensitivity of Gram-positive bacteria showed CONS, *Staphylococcus aureus* and *Enterococcus* spp were 100% sensitive to Vancomycin and

Linezolid followed by Clindamycin and Tetracycline and were least sensitive to Penicillins, Cephalosporins and Fluoroquinolones and this finding similar to other studies. The incidence of MRCONS was 82.45% and MRSA was 50%. Methicillin resistance rate was higher in CONS as compared with *Staphylococcus aureus*, which is similar to study by Mathur *et al.*,[37] and Mir *et al.*,[38].

These organisms are notorious since they do not respond to the broad class of beta lactam antibiotics and acquire resistance to newer antibiotics quite rapidly. This effectively complicates the management of such BSIs. [10] In the present, the most effective antibiotic against Gram positive organisms were Vancomycin and Linezolid followed by Clindamycin and Tetracycline.

Among Gram negative bacteria, *Acinetobacter* spp, *Klebsiella* spp, *Citrobacter* spp, *E. coli* and NFGNB showed 100% sensitive to Colistin followed by Imipenem and Meropenem (80% each), Oflaxacin and Levofloxacin (60% each) and Amikacin (55%) and least sensitive to Ampicillin, Amoxicillin+clavulanic acid combination and Cephalosporins and this is similar to other studies.

In our study MDR was found in 60.80% of Gram negative isolates and is in comparison with study conducted by Shilpi Gupta *et al.*,[12] and Nazir *et al.*,[32] and ESBL were 20.27% which is similar to study conducted by Anathan *et al.*,[39] (25.4%). MDR was found high among Enterobacteriaceae. Carbapenem resistance was seen more among Nonfermenters (31%) as compared to Enterobacteriaceae (20.2%) and this may be due to inappropriate empirical use of Carbapenem as the first line treatment.

The greatest threat with MDR and Carbapenem resistant Gram negative bacteria is that the infections are usually untreatable due to the limited options of the antibiotics available, resulting into increased mortality. Worldwide, their incidence is rising with variations due to regional and geographical differences as stated by Jadhav *et al.*,[40]. With the shortage of newer drugs availability and increasing resistance, use of limited option drugs such as colistin by clinicians could soon lead to the condition of so called pan drug resistance.[12] In the present study, the most effective antibiotic against Gram negative organisms were Colistin followed by Imipenem, Meropenem, Amikacin and Oflaxacin.

The information of predominant organisms and their sensitivity among sepsis patients is essential for making the right choice of antibiotics in the management of sepsis. Hence, blood cultures must be obtained from all suspected cases of bacteraemia or sepsis before prescribing antibiotics. The main factors causing the increase in antimicrobial resistant bacteria are poor infection control practices and inappropriate use of antibiotics. Strict infection control measures along with antibiotic policy for judicious antibiotic therapy should be implemented in the hospitals as control measures against blood stream infections and to check the emergence of resistance. [41]

Blood stream infections are an important nosocomial infection responsible for morbidity and mortality in the patients. The present study provides information on the spectrum of pathogens causing blood stream infections and their antimicrobial susceptibility profile, helping the clinicians in early diagnosis and guiding in the management of blood stream infections. The study identified both Gram-positive and Gram-negative bacteria to be responsible for blood stream infections and most of them were found to be MDR. Inappropriate antibiotic use and poor infection control

practices contributes to the emergence of antimicrobial resistance in bacteria. The key to control of antibiotic resistant pathogens is to strictly adhere to infection control practices and mandates antibiotic policy for rational use of antibiotics. Also, Routine surveillance of antimicrobial resistance in frequently encountered bacterial pathogens will be useful for deciding on empirical treatment strategies and also devising an effective antimicrobial stewardship program in hospitals.

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