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Research Article

PREVALENCE, CO-MORBIDITY FACTORS, CHARACTERIZATION AND ANTIMICROBIAL RESISTANCE PATTERN OF ISOLATES ASSOCIATED WITH BLOOD STREAM INFECTION

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Abstract- Background: Blood stream infection (BSI) remains one of the important causes of morbidity and mortality throughout the world. Aim: To study the prevalence, co-morbidity factors, characterization and antimicrobial resistance pattern of isolates associated with blood stream infection. Materials and Methods: A retrospective study carried out on blood culture samples and catheter tip cultures in a tertiary care teaching hospital from January 2016 to December 2016 according to standard protocol. Data Analysis was done by using *WHO NET ANTIBIOTIC RESISTANCE SURVEILLANCE SOFTWARE.* Result: A total of 3269 blood samples were processed, out of which 327 showed growths of organisms. The most vulnerable age group was above 70 years where the incidence was 22.1% and the lowest incidence was observed among age group 1 to 10 years (3.4%). Respiratory infection was the predominant co-morbidity factor. Among the isolates, Gram positive bacteria accounted for 54. 9 % with *Staphylococcus aureus* topping the list (39.1%) and 43.3 % of were MRSA; all the strains showed 0% resistance to vancomycin and linezolid. *E. coli* was the predominant Gram negative bacteria (13.3%) followed by *Acinetobacter* (8.2%). Conclusion: Blood stream infection (BSI) is one of the most important causes of morbidity and mortality. *S. aureus* and *E. coli* are the most common organisms isolated in this study. Increasing rates of antimicrobial resistance pose a great problem in treating these infections leading to high morbidity and mortality.

Keywords- Blood stream infection, Co-morbidity factors, Characterization, Antibiotic resistance, MRSA.

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Introduction

Blood stream infection (BSI) remains an important cause of morbidity and mortality throughout the world [1]. BSI occurs when bacteria enter the bloodstream through a wound, infection, surgical procedure or intravascular catheters [2]. Immunosuppression, prolonged illness, drainage of an abscess, and prolonged IV needle placement increase the chances of BSI [3]. Organisms likely to cause bacteremia include *Staphylococci, Streptococci, Pseudomonas, Esherichia coli and* non-*albicans Candida* [4]. BSI by *Staphylococcus aureus, Candida albicans*, MRSA and Vancomycin –resistant Enterococci are associated with significant mortality [5, 6]. Increasing rates of antimicrobial resistance and changing patterns of antimicrobial usage change outcome of BSI. [7] This study was undertaken to analyze the incidence, co-morbidity factors, bacteriological profile and antimicrobial resistance pattern of the isolates in BSI.

Materials and Methods:

After getting approval from Institutional ethics committee a retrospective cross sectional study was carried out, based on review of records of 3296 patients from whom blood culture samples and catheter tip cultures were collected and processed in diagnostic section of Department of Microbiology, from January 2016 to December 2016, meeting inclusion and exclusion criteria. The basic socio-demographic information and data regarding age, gender, microbial isolates from blood culture and catheter tip samples and their antibiotic resistance pattern were collected using predesigned Performa, according to standard protocol.

Patients with fever (>38°C); chills or rigors, with isolation of one or more recognised bacterial or fungal pathogens from one or more blood cultures and isolation of potential contaminants from two or more blood cultures drawn on separate occasions within a 48 hour period were included in the study.[8] Isolation of potential contaminants not associated with signs and symptoms were excluded from the study.

The blood samples were collected aseptically. The skin over the venipuncture site was cleaned with soap & water followed by disinfection with 70 % ethanol and povidone/iodine or 2% tincture of iodine. Using disposable syringes 5 ml of blood from adults or 1-3 ml from children was drawn and introduced into the BACTEC blood culture bottle immediately and the bottles shaken well. The samples were immediately placed in BACTEC automated blood culture system.All BACTEC positive samples were subjected to Gramstain and inoculated onto Blood and McConkey agar plates and incubated at 37°C for 48 hours. The bacterial isolates were identified based on the study on colony morphology, gram stain and biochemical reactions. For Gram positive organisms biochemical tests like catalase and coagulase as well as novobiocin/ optochin discs and for Gram negative fermenters indole, methyl red, voges-prausker, citrate, urease, TSI agar, oxidase, mannitol motility, nitrate reduction test and esculin hydrolysis were used; additional tests such as fermentation of 10% lactose, decarboxylation of arginine, ornithine and lysine, oxidative fermentation of (OF) of Hugh-Leifson- glucose, lactose, sucrose, mannitol, maltose and xylose and arabinose fermentation tests were done for non-fermenters.

The fungal isolates were identified based on cultural characteristics on SDA and Chrome agar, Gram stain and biochemical reactions for sugar assimilation and fermentation and test for germ tube formation.

Antibiotic susceptibility testing for the bacterial isolates was performed on Muller Hinton agar using agar disc diffusion method. The antimicrobials for Gram positive organisms were ciprofloxacin (5 µg), erythromycin (15 µg), gentamycin (10µg), penicillin (10 IU), oxacillin (1µg), clindamycin (2µg), vancomycin (30µg), linezolid (30µg) and tetracycline (30 µg). Against the Gram negative isolates ampicillin (10µg), gentamycin (10µg), amikacin (30µg), cefotaxime(30µg), ceftazidime (30µg), ceftazidime+ clavulanic acid (30µg+10µg), cefoperazone + sulbactum (75µg+10µg), cefipime (30µg), ciprofloxacin (5 µg), piperacillin (100µg), piperacillin+ tazobactam (100µg+10µg), imipenem (10 µg) , meropenem (10 µg), colistin (10µg) polymixinB (300 units), co-trimoxazole (25µg) and chloramphenicol (30µg) were used. The antimicrobials used against the *Candida* isolates were nystatin (100 units), Amphotericin B (20µg), Fluconazole (25µg), Voriconazole (1µg), Itraconazole (10µg) and Ketoconazole (10µg).

The resistance and susceptibility were interpreted according to CLSI guidelines. *Escherichia coli* (ATCC 25922), *Staphylococcus aureus* (ATCC 25923) and *Pseudomonas aeroginosa* (ATCC 27853) were used as reference strains. All the BACTEC negative samples that did not show growth after seven days of incubation were reported as negative. *Data Analysis was done by using WHO NET ANTIBIOTIC RESISTANCE SURVEILLANCE SOFTWARE*

Result

Out of the total of 3269 blood samples taken up for the study 327(10.0%) were positive for growth of organisms. Of these, the incidence was 55% and 45% in male and female patients respectively [Fig-1]. In relation to age factor, the most vulnerable age group was above 70 years where the incidence was 22.1% and those above the age of 50 constituted 58.2 % [Fig-2].

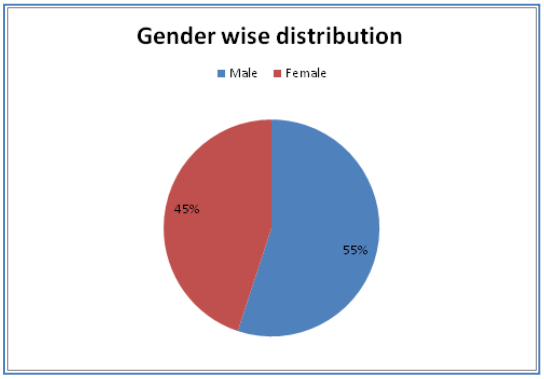

Fig-1 Gender wise distribution

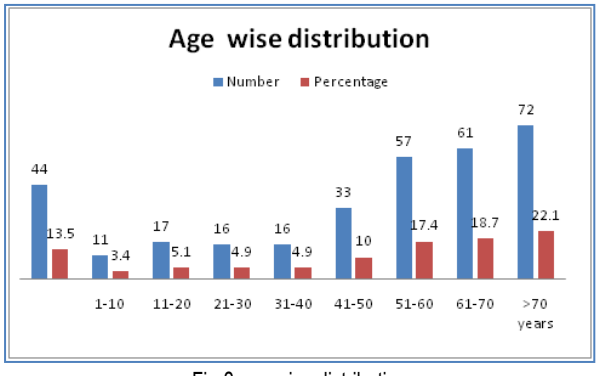

Fig-2 age wise distribution

Among the underlying co-morbidity factors, COPD, bronchial asthma and other chronic lung diseases constituted the major contributing factor in 45 patients (13.9%) followed by renal diseases including patients on dialysis in 33 cases (10.1%), bacterial infections including abscess anywhere in the body in 15 (4.6%), diabetes mellitus in 14 (4.3%), cardiovascular conditions like cardiac failure in 13 (4.0 %), liver disease in 12 (3.7%), malignancy in 10 (3.1%), CNS disorders in 9 (2.6%), hypertension, concomitant viral diseases, and catheter in situ in 8 (2.4%) each and hematological disorders in 3 (0.9%) patients. [Table-1]

Table-1 *Co-morbidity Factors Associated with BSI*

Respiratory diseases	45 (13.9)
Renal diseases	33 (10.1)
Bacterial infections	15 (4.6)
Diabetes mellitus	14 (4.3)
CVS conditions	13 (4.0)
Liver diseases	12 (3.7)
Malignancy	10 (3.1)
CNS disorders	9 (2.6)
Hypertension	8 (2.4)
Viral infections	8 (2.4)
Catheter in situ	8 (2.4)
Hematological disorders	3 (0.9)
GIT diseases	3 (0.9)
Autoimmune diseases	2 (0.6)
Organophosphorus poisoning	2 (0.6)
No apparent co-morbidity factors	142 (43.5)

Figures in parenthesis indicates percentage

Out of these 327 culture positive samples, 314 and 9 showed single bacterial and fungal growths respectively; 4 samples showed polymicrobial growth. Among the bacterial isolates, Gram positive cocci and Gram negative bacilli were recovered from 181(55.4%) and 139 (42.5%) samples respectively. *Staphylococcus aureus* was the predominant organism having isolated from 129 (39.0%) cases out of which 55 isolates turned out to be MRSA. Among the Gram negative isolates *E. coli* was the predominant organism having recovered from 43 cases (13.0%). The other bacterial isolates were *Klebsiella pneumoniae* in 27 (8.2%), *Acinetobacter* in 26(7.9%), *Enterococci* in 23 (7.0%), *CONS* in 22 (6.6%), *Pseudomonas aeruginosa* in 19 (5.7%), *Salmonella typhi* in 14 (4.2%), *Streptococcus pneumoniae* in 7 (2.1%), *Candida parapsilosis* in 5(1.5%), *Citrobacter* species in 4(1.2%), *Salmonella paratyphi* in 3 (0.9%), *Candida albicans* in 3(0.9%), *Candida glabrata* in 2(0.6%) samples each. *Klebsiellaoxytoca, Enterobacter* and *Serratia marcescens* and *Candidatropicalis* were isolated from one sample each (0.3%). [Fig-3] Antimicrobial resistance pattern of both Gram positive and Gram Negative bacteria determined based on CLSI guidelines is shown in [Table-2&3].

Table-2 *Antibacterial resistance pattern of Gram positive cocci in BSI*

Organism Antibiotics	*Staphylococcus aureus(129)*	*CONS (22)*	*Enterococcus (23)*	*Strepto-pneumonia(07)*
Pencillin G	88.4	88.2	52.4	28.6
Gentamycin	13.2	11.8	23.8	0
Ciprofloxacin	41.1	17.6	38.1	0
Clindamycin	24	11.8	-	-
Erythromycin	48.1	17.6	-	14.3
Vancomycin	0	0	0	0
Linezolid	0	0	0	0
Tetracycline	5.4	0	23.8	0
Oxacillin	43.3	54.5	-	-

Total number of isolates given in parenthesis

Discussion

In a study conducted by Dagnew et al the overall prevalence of bacteria isolated from blood culture of bacteremia suspected patients was 18.2% whereas in our study it was 10.0%. [1]

The co-morbidity factors reported by various authors include hypertension, diabetes mellitus, malignancy, COPD, renal failure, bronchial asthma, immune suppression, antibiotic therapy and prolonged illness and central catheter was an important risk factor. [4, 9, 10]

In the study of Parameswaran R. *et al*. 64% of the pathogens causing CRBSI was Gram-positive and 36% was Gram-negative. According to various studies the commonest pathogens responsible for BSI were *S. aureus, Enterococci, Pseudomonas aeruginosa, co-agulase negative staphylococci*, *E. coli*, *Klebsiella*

pneumoniae, *Acinetobacter baumanii* and *yeast* [2, 3, 11]

Bloodstream infection and invasive candidiasis are substantially more common than realized and probably result from multiple factors, including unrestrained antibiotic drug use, indwelling devices and increasing populations of immunocompromised patients. Multiple studies have shown the incidence of bloodstream infections with *Candida* spp. to be 1.2–26 cases/100,000 population. Lack of routine diagnostic testing for fungal diseases exacerbates the problem of antimicrobial drug empiricism, both antibiotic and antifungal. [12].

Table-3 *Antibacterial resistance pattern of Gram Negative bacilli in BSI*

Organism Antibiotics	*E.coli* (43)	*Klebsiella* (27)	*Salmonella* (17)	*Citrobacter* (4)	*Enterobacter* (1)	*Serratia* (1)	*Psedomonas* (19)	*Acinetobacter* (26)
Ampicillin	93.9	100	33.3	100	100	100	-	-
Ceftazidime	59.2	85.2	-	100	100	0	11.1	100
Cefotaxime	79.6	70.4	16.7	100	100	0	-	-
Cefipime	79.6	81.5	-	75	100	0	11.1	100
Amikacin	14.3	29.6	-	25	50	0	5.6	44.4
Gentamicin	16.3	29.6	-	25	50	0	5.6	29.6
Cefta+ Clav	59.2	64		75	75	0	0	0
Ciplox	40.8	51.9	38.5	25	100	0	0	85.2
Piperacillin Tazobactum	32.7	33.3	-	75	50	0	0	0
CefoperazoneSulbactum	40.0	45.2	-	75	50	0	5.6	85
Imipenem	0	11.1	-	0	50	0	0	11.0
Meropenem	2	3.7	-	0	0	0	0	11.0
Polymixin B	-	-	-	0	0	0	0	0
Colistin	-	-	-	0	0	0	0	0
Cotrimoxazole	-	-	0	-	-	-	-	-
Chloramphenicol	-	-	9.1	-	-	-	-	-
Piperacillin	-	-	-	-	-	-	0	0

In our study the rate of isolation of *Candida* species was 3.3%. In a study by TAN BH et al, out of 1910 blood isolates evaluated, among the *Candida* species, *Candida albicans* was most frequently isolated (41.3%), followed by *Candida tropicalis* (25.4%), *Candida glabrata* (13.9%) and *Candida parapsilosis* (12.1%). [13] Blood stream infection with *Candida albicans*, MRSA and Vancomycin –resistant *Enterococcus faecium* has been associated with significant attributable mortality [6].

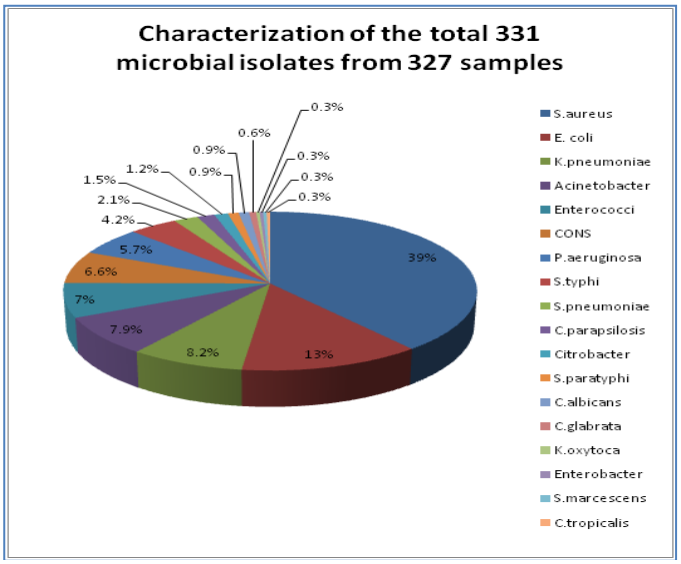

Fig-3 Characterization of the isolates

A study by Fowler et al showed incidence of complicated SAB in 43% of 724 adult hospitalized patients and the strongest predictor of complicated SAB was a positive follow-up blood culture result at 48 to 96 hours[14]. Therefore it is important to do repeat blood culture 48-96 hours later to assess the status of BSI in patients with *S. aureus* bacteremia.

No strains of Gram positive cocci exhibited resistance to vancomycin and linezolid. However, 43.3 % of *S. aureus* strains turned out to be MRSA. In a study by M. Kaur et al, 87.5 % of *S. aureus* strains isolated from CVC-BSI were MRSA. [4] *E. coli* showed 93.9 % resistance against ampicillin whereas *Klebsiella, Citrobacter* and *Enterobacter* were 100% resistant.

E. coli showed least resistance against amikacin (14.3%), Gentamycin (16.3%) and piperacillin-tazobactum (32.7%). However, an important organism causing BSI, *Salmonella typhi* showed only 9.1% resistance to chloramphenicol. Members of Enterobacteriaceae group were 42.5% and 25.5% Amp C and ESBL producers. In our study all strains of Candida species isolated from BSI showed 0% resistance to the antifungal agents used for susceptibility tests. Blood stream infection with *Candida albicans*, MRSA and Vancomycin –resistant *Enterococcus faecium* has been associated with significant attributable mortality [6].

Conclusion

Blood stream infection (BSI) remains one of the most important causes of morbidity and mortality throughout the world. Among the underlying co-morbidity factors, COPD, bronchial asthma and other chronic lung diseases constituted the major contributing factors in 45 patients (13.9%) followed by renal diseases including patients on dialysis in 33 cases (10.1%).

The most vulnerable age group was above 70 years where the incidence was 22.1% and those who are above the age of 50 constituted 58.2 %. *Staphylococcus aureus* was the predominant organism having isolated from 129 (39.0%) cases out of which 55 isolates turned out to be MRSA followed by *E. coli*. Increasing rates of antimicrobial resistance pose a great problem in treating these infections leading to high morbidity and mortality. Therefore early diagnosis and initiation of appropriate antimicrobial therapy including antifungal, is of utmost importance to improve patient outcome.

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Author Contributions: All authors equally contributed

Abbreviations:
BSI: Blood stream infection

Conflict of Interest: None Declared

Ethical approval: This article does not contain any studies with human participants or animals performed by any of the authors.

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