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Microbiology

KEY WORDS: Blood stream infections; Bacterial profile; Antibiogram

STUDY OF MICROBIAL PROFILE AND ANTIBIOGRAM OF BLOOD STREAM INFECTIONS (BSIS) IN ADULTS WITH SEPTICEMIA

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ABSTRACT

Blood stream infections are the most important cause of health care associated infections. The infections caused by multidrug resistant organisms are more likely to prolong hospital stay, increase risk of death and requires treatment with more expensive antibiotics. Present study was intended to determine pattern of etiological agent responsible for BSI's in tertiary care hospital, to find out the primary source of BSIs and to get an updated knowledge about their antibiotic sensitivity pattern.

Material and methods – Blood culture samples of total 1017 patients with clinical diagnosis of septicemia were processed. Identification of the isolates and antibiotic sensitivity testing was done according to standard bacteriological technique.

Result - Out of 1017 samples processed, 192(18.87%) showed growth of microbes. Among them predominant organisms were gram negative bacilli in 135 (70 %) samples, gram positive cocci were isolated in 57 (29.68 %) samples. High degree of resistance was seen in both gram negative and gram positive isolates. 36.1% of GNR showed ESBL production while 31 % isolates of *Staphylococcus aureus* were MRSA.

Introduction -

Invasion of the blood stream by microorganisms constitute one of the most serious situation in infectious diseases.[1] It is one of the most common health care associated infections[2].Infections of the genito-urinary tract, respiratory tract, and gastrointestinal tract often result in BSIs[3]. Risk factors contributing to these infections are many but leading causes are intravascular catheters (IVCs), debilitating conditions of the patients due to underlying disease, infection and invasive, diagnostic or therapeutic procedures. Wide range of organisms, both gram positive and negative are associated with the blood stream infection[4]. Sensitive bacterial strains are now being replaced by multidrug resistant strains of Salmonella, Klebsiella, Pseudomonas, Acinetobacter and Citrobacter spp[5].Increase in incidence also seen among gram positive isolates such as methicilline resistance among *Staphylococcus aureus* and vancomycin resistance in *Enterococci.* The infections caused by multidrug resistant organisms are more likely to prolong hospital stay, increase risk of death and requires treatment with more expensive antibiotics. Surveillance is important in monitoring the spectrum of microbes that cause BSIs.[6] The present study was aimed to determine the bacterial agents associated with BSI, to find the primary source and their antimicrobial resistance.

MATERIAL AND METHODS –

Adults (>18years) admitted with a clinical diagnosis of septicemia were included in this study. The study period was from January 2013 to December 2016. History of patients had been taken regarding age, sex, symptoms, underlying disease and invasive procedures. Processing of blood samples was done by standard microbiological techniques by Conventional Blood culture methods standard microbiological techniques.[7] Identification of the isolates was done according to colony characterization, morphology and biochemical tests.[7]Antibiotic sensitivity testing was performed by modified Kirby-Bauer disc diffusion technique[8] as per CLSI guidelines. Commercially available antibiotics disks (Himedia, India) with proper diameter and potency were used. All the isolates were tested for their sensitivity to microbial agents using recommended CLSI formulary practices for the purpose of reporting to the clinician. The reference strains used as control were 1) *Escherichia coli* (ATCC 25922) 2) *Escherichia coli* (ATCC 35218) for -lactam/lactamases inhibitor combinations 3) *Pseudomonas aeruginosa* (ATCC 27853) and *Staphylococcus aureus* (ATCC 25923). In this study MRSA detection was done by cefoxitin disk diffusion test. Testing for Extended Spectrum -lactamases (ESBLs) was done by phenotypic method with ceftazidime (30µg) and ceftazidime + clavulanic acid (30µg+10µg).

RESULTS

Table 1 Age & Sex: Distribution of blood culture received

Sr .No	Age Group	Male	Female	Total
1	16-30	108	82	190 (18.68%)
2	31-45	134	104	238 (23.4%)
3	46-60	160	112	272 (26.74%)
4	61-75	110	84	194 (19 %)
5	>76	71	52	123 (12%)
	Total	583 (57.3%)	434 (42.7%)	1017(100%)

Table 2 : Total blood culture positivity in adults with septicemia

BLOOD CULTURE RESULTS	NUMBER OF CASES (%)
POSITIVE	192 (18.87%)
NEGATIVE	825 (81.12%)
TOTAL	1017 (100%)

Table 3: Microbial isolates from blood culture by conventional method (n=192)

Organism	**Total**	**Percentage**
Gram positive cocci		
Staphylococcus aureus	29	16.7
Staphylococcus epidermidis(CoNS)	20	10.9
Enterococcus fecalis	05	2.6
Streptococcus pneumoniae	03	1
Total	**57**	**29.68**
Gram negative bacilli		
Klebsiella pneumoniae	43	22.3
Kiebsiella aerogenes	05	2.6
Escherichia coli	37	19.3
Salmonella enterica serovar Typhi	05	2.6
Citrobacter frundii	05	2.6
Enterobacter aerogenes	05	2.6
Acinetobacter baumannii	07	3.6
Pseudomonas aeruginosa	25	13
Total	**132**	**68.75**
Candida albicans	03	1.56

Table 4 : Distribution of cases based on clinical diagnosis

Diagnosis	Cases	Percentage
Respiratory Infections	172	16.9
Genitourinary Infections	208	20.4
Surgical site Infections	56	5.5
Gastrointestinal Infections	40	3.9
Skin & Soft tissue infections	120	11.7
Central Nervous System Infections	42	4.1
Undetermined source including primary bacteremia	379	37.2
Total	1017	100

Table 5: Antimicrobial sensitivity of gram positive organisms

Organisms	Antibiotics													
	P	CX	E	G	AK	TB	VA	LZ	C	T	OF	CO	CL	LE
Staphylococcus aureus (n=29)	0	9	20	10	26	24	29	29	20	8	14	19		
Staphylococcus epidermidis (n=20)	7	15	11	9	15	18	20	20	18	3	9	9		
Enterococcus faecalis (n=05)	3	-	5	-	-	-	5	5	3	-	-	-		
S.pneumoniae (n=03)		3	3			3							3	3

Table 6: Antimicrobial sensitivity of Enterobacteriaceae isolates (n= 100)

Organisms	Antibiotics															
	A	AC	CZ	CS	CPM	CFZ	CX	CE	CF	PC	PIT	IPM	G	TB	AK	AZ
K. pneumoniae n = 43	10	11	0	0	11	32	12	20	20	21	39	43	22	35	20	10
K. aerogenes n = 05	0	03	0	0	03	0	0	0	03	03	05	04	03	05	05	0
E. coli n= 37	06	09	06	06	10	09	12	11	24	06	25	37	20	28	30	12
Citrobacter freundii n = 05	0	05	0	0	05	05	0	05	05	05	05	05	05	05	05	0
Enterobacter aerogenes n = 05	0	03	0	0	0	0	0	0	03	03	03	05	0	05	05	0
Salmonella enterica serovar Typhi n = 05	04	-	-	4	-	-	05	04	05	-	-	-	-	-	-	05

Table 12: ESBL production among Enterobacteriace isolates (n= 100)

Method 0f detection of ESBL	***K.pneumoniae***	***K. aerogenes***	***E.coli***	***Citrobacter spp***	***Enterobacter spp***	Total
Phenotypic confirmatory disk diffusion method	23 (53.48 %)	0	12(32.43%)	0	0	35(35%)

Table 13: Antibiotic sensitivity of *Pseudomonas aeruginosa and Acinetobacter*

Organisms	Antibiotics									
	CFZ	CE	CS	CPM	PC	G	PIT	IP	AK	CF
Pseudomonas aeruginosa n=25	06	14	07	07	07	14	20	25	22	20
Acinetobacter baumannii n=07	0	05	05	05	05	05	07	07	07	05

Discussion

Bloodstream infection is a challenging problem. Wide application of new medical technologies like rampant usage of indwelling devices, may change the epidemiology and outcome of BSIs. Therefore, timely detection, identification, and antimicrobial susceptibility testing of blood-borne pathogens are one of the most important functions of diagnostic microbiology laboratory. The results of the study showed the microbial profile of the blood stream infections as well as the resistance pattern of the isolates. Out of 1017 blood samples received 57.3% were from males and 42.7%were from females, maximum from the age group of 46-60years (26.7%), followed by age group of 31-45(23.4%).Among culture positive samples, male contributes to 51% (98/192) of the cases and female for 48.9(94/192).Our study is comparable with the observations made by Uslan DZ et al[12] and McDonald JR et al[13].

Out of 1017 clinically suspected adult septicemia cases, blood cultures were positive in 192 (18.87%) cases whereas in 825 (81.1 %) were negative. Blood culture positivity in our study was similar to the finding of the study conducted by Arora and Devi et al[8] who reported 20.02% culture positivity. Similar findings 19.3% by Ayobola et al[14] & 20.5% by Garg et al[15] further supports our findings. In contrast low blood culture positivity of 13.9% by China D et al[16], 9.9% by Mehta et al[17] ,7.9 % by Anbumanni et al[18] &5.17% by Barati M et al[19]and 5.6% by Mehdinejad M et al[20] were reported in similar other studies. Factors which explain variation in blood culture positivity antibiotic intake, number of blood cultures taken etc.

In present study gram negative bacilli were found to be the more common cause of adult septicemia 132 (68.7%), gram positive cocci were found in 57 (29.7%) cases, while Candida albicans was isolated in 1.5% cases. Predominance of gram negative bacteria was reported as 80.96% by Mehta et al[17], 86.5% by Mehdinejad M et al[20], 91.8% Barati M et al[19], 77.1% by Ayobola et al[14] and 67.5% by Garg et al[15]. In other studies of BSIs, Pavani G et al[21] reported only 38.3% gram negative isolates. Khaleel M et al[11]reported 40.15 % gram negative isolates.

Among gram negative isolates, *Klebsiella pneumonia* 22.3% was the commonest followed by *E.coli* 19.3%. which is the similar to the study by Mehdinejad M et al[20],Latif et al[21], and Mehta et al[17], Arora et al[8] and Barati et al[19]. *Salmonella enterica serovar typhi* was isolated in 2.6% of cases which is similar to the observations of Anbumani et al[18] 2.5%. Chinna D et al[16]& Mehta et al[17] has reported *Salmonella enterica serovar typhi* in 14.6% and 13.8% respectively.

In our study *Pseudomonas aeruginosa* was isolated in 13% of positive blood culture samples. Similar observations were made by Qureshi M et al[23], Arora et al[8]and Asghar A.H.et al[24]. *Acinetobacter baumanni* were isolated in 3.6% of samples which is similar to the study of Pavani G et al[21] and Latif et al[22]

Among gram positive isolates *Staphylococcus aureus* 29 (16.7%) was the commonest followed by *Staphylococcus epidermidis* 20(10.9%), *Enterococcus faecalis* (2.6%) and *Streptococcus pneumonia* (1.5%).This is in accordance to studies of Ayobola et

al[14]and Mehta et a[8]l(2005) who reported *S.aureus* 14.6%and 13.86% of total blood isolates respectively. A high iolation rate of *S.aureus* was reported by Chinna D et al[16] 37.2% and Anbumanni et al[18]36.4%.

Apart from gram positive and gram negative organisms,*Candida albicans* were isolated in three positive blood cultures. Similar observation was made by Latif et al[22].

In present study all isolates of *S.aureus* were resistant to penicillin. Methicilline resistance was seen in 31.03% isolates of *S. aureus* which is similar to findings of Anbumanni et al[18] 30% and Latif et al[22] 31.5%. All gram positive isolates were sensitive to vancomycin and linezolid. Similar sensitivity pattern to vancomycin and linezolid was reported by Mehta et al[17] ,Anbumanni et al[18] ,36.4% by Mehdinejad M. et al[20] and Chinna D et al[16].

In present study maximum isolates of enterobacteriaecae were sensitive to imipinam followed by amikacin, tobramycin and piperacilline-tazobactam. High resistance showed to ampicilline , amoxclav,1[st]and 2[nd] generation cephalosporins. Arora et al[8] (2007) Barati M et al[19] (2009) and Kumar S.et al[27] (2004) also reported same antibiotic resistance pattern in gram negative isolates from BSIs.

In present study 35% (35/100) of gram negative isolates were ESBL producer, among these ESBL production was highest among *K.pneumoniae* 53.48% followed by *E coli* 32.43% which is similar to the ESBL production reported by Arora et al[8] 34.35% and Anathan et al[28] 25.4%. All isolates of Pseudomonas and Acinetobacter spp were sensitive to imipenam. Next effective antibiotics against them were amikacin, piperacilline-tazobactam which is similar to the finding of Chinna Det al[16]and Anubanni N et al[17].

In present study genitourinary infections (20.4%) followed by respiratory infections (17%), skin and soft tissue infections were the common clinically suspected primary source of infections. Undetermined primary bacteremia accounts for 37.2%of the clinically suspected BSIs. Siegman-igra Y et al[25], McDonald JR et al[13]and Son JS et al[26] also found that urinary tract infections followed by pneumonia are the main source of BSIs.

Conclusion

The present study showed prevalence of multi-drug resistant isolates in BSIs and this limits the therapeutic options. It implies that blood cultures must always be done in all cases of suspected bacteremia and septicemia and once the sensitivity pattern of the isolate is known de-escalation of the high-end antimicrobials should be considered to reduce the antimicrobial pressure. Moreover stringent hospital infection control measures and a good antibiotic policy for the hospital is the need of the hour.

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