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Original Article

Microbiology Section

Evaluation of Bacteriological Profile and their Antimicrobial Susceptibility Pattern in Blood Stream Infections in a Multispeciality Hospital

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ABSTRACT

Introduction: Bloodstream Infections (BSIs) are one of the major cause of morbidity and mortality worldwide. To decrease the mortality from septicaemia early diagnosis and appropriate treatment of BSIs is most important. Early diagnosis of a BSI will markedly improve patient management.

Aim: To identify various pathogenic organisms causing BSIs and determine their susceptibility to various antibiotics.

Materials and Methods: A total of 1367 blood samples were received in the bacteriology laboratory for culture out of which 274 samples showed culture positivity. Among the positive cases 32 cases of Multidrug Resistance (MDR) were found. MDR cases show resistance to ≥3 classes of antibiotics. Enterobacteriaceae family showed highest MDR cases. Blood cultures were repeated for confirmation of results. Simple descriptive analysis of data was done and results presented in frequencies and percentages.

Results: Out of 274 positive samples obtained, Coagulase Negative Staphylococci (CoNS) constituted maximum proportion of isolates (66%) followed by *Pseudomonas* species (12%), *Escherachia coli* (6.2%), *Klebsiella* species (3.2%), *Citrobacter* spp. (2.9%), *Staphylococcus aureus* (2.9%) and *Enterococcus* (2.9%).

Conclusion: The present study highlighted the bacteriological aetiology of BSIs along with their antibiogram that may provide necessary information for the formulation of antibiotic policy in effective management of such cases.

Keywords: Bacteremia, Blood culture, Coagulase negative *staphylococcus*, Multidrug resistance, Septicaemia

INTRODUCTION

Approximately 200,000 cases of bacteraemia and fungemia occur annually with mortality rates ranging from 20-50% [1]. Blood cultures in which contamination has been effectively ruled out and viable bacteria are observed in it then bacteremia is considered [2,3]. The most common bacteria that cause bacteremia include members of *Staphylococcus* spp., *Streptococcus* spp., *Enterococcus* spp., *Escherichia coli, Klebsiella* spp., *Pseudomonas* spp., *Enterobacter* spp., *Haemophilus* spp., and *Neisseria* genera [4-6]. Septicaemia can lead to serious complications such as shock, disseminated intravascular coagulation, multiple organ failure, etc. Thus, BSIs are one of most serious pathologies, so early detection and identification of blood stream pathogen is important [7]. Provisional diagnosis of septicaemia can be carried out by clinical assessment using a combination of symptoms and signs. But identifying the causative pathogen by bacteriologic culture is necessary for definitive diagnosis of septicaemia [8]. Early diagnosis of bacteremia can be obtained by blood culture and antimicrobial susceptibility test which helps in identifying the most appropriate effective antibiotic which can be a choice of drug to be administered and thus helps in early recovery and reducing mortality due to septicaemia. This can reduce turnaround time and improve patient management.

In a particular area knowing the epidemiology and antibiotic susceptibility pattern of a various pathogenic organisms will help to determine the antibiotic of choice and thus assist in proper management. This study will identify the most common pathogens causing BSIs and will provide their antimicrobial susceptibility pattern.

MATERIALS AND METHODS

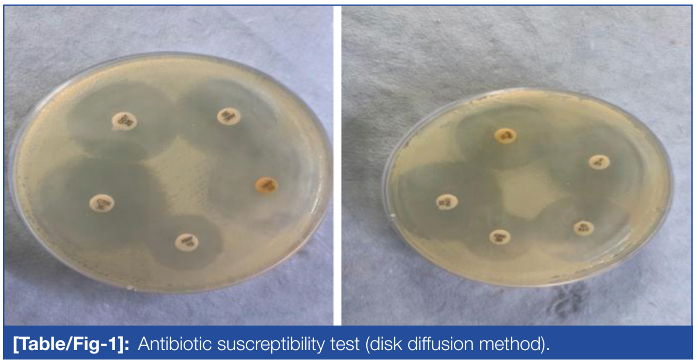
This retrospective study was conducted in the Department of Microbiology, Central Laboratory, School of Medical Sciences and Research, Sharda Hospital, Greater Noida, Uttar Pradesh, India. This study was conducted for a period of six months from May 2019 to October 2019. Institutional Ethical Committee provided approval for this study (Ref no. - SU/SMS&R/76-A/2019/35). All the isolates from blood culture positive samples collected over period of six months were included in the study. All the blood samples received in bacteriological laboratory for culture were used for study. Automated method i.e., (BACT-ALERT system) was used to culture the bottles received in the bacteriology laboratory. The bottles were put inside the automated machine, when there was a signal the bottles were removed and the blood from the positive blood culture bottles was subjected to subculture on 5% sheep blood agar and MacConkey agar. The growth on culture plates was identified on the basis of colony morphology, Gram stain, and various biochemical tests. Blood cultures were repeated for confirmation of results. Antimicrobial susceptibility testing was performed for all blood cultures isolated on Muller Hinton agar by Kirby-Bauer disc diffusion method as recommended in the CLSI (Clinical and Laboratory Standards Institute guidelines) 2019. Commercially, available antibiotics disks (Himedia) were used for antimicrobial susceptibility testing [Table/Fig-1]. The antibiotics used for Gram positive bacteria were levofloxacin (5 ug), clindamycin (2 ug), ciprofloxacin (5 ug), linezolid (30 ug), penicillin (10 units), erythromycin (15 ug), gentamicin (10 ug), vancomycin, cefotaxime (30 ug) and the antibiotics used for gram negative bacteria were cefotaxime (30 ug), cefepime (30 ug), cefuroxime (30 ug), levofloxacin (5 ug), ampicillin (10 ug), gentamicin (10 ug), imipenem (10 ug), meropenem (10 ug), amoxiclav (30 ug), ceftriaxone (30 ug).

STATISTICAL ANALYSIS

Simple descriptive analysis of the distribution of sample, age, gender, and antimicrobial susceptibility data were done, and the results were obtained and presented as frequencies and percentages.

RESULTS

A total of 1367 blood samples were received in the bacteriology lab for culture out of which 274 samples showed culture positivity. Out of 274 positive samples obtained, 156 were male patients and



[Table/Fig-1]: Antibiotic suscreptibility test (disk diffusion method).

118 were females. The maximum number of samples was obtained from patients between age group 0-10 years followed by the age group 40-50 and 60-70 years [Table/Fig-2].

Age group (Years)	Males	Females	Total
0-10	79	59	138
10-20	09	10	19
20-30	14	05	19
30-40	08	07	15
40-50	16	08	24
50-60	07	15	22
60-70	17	07	24
70-80	06	06	12
80-90	0	01	01
Total	156	118	274

[Table/Fig-2]: Age and sex wise distribution of samples.

CoNS constituted maximum proportion of isolates followed by *Pseudomonas* species and *E. coli*. [Table/Fig-3]. Most of the cases were positive for coagulase negative organisms and amongst all the antibiotics, vancomycin (100%) emerged as most sensitive drug against CoNS followed by linezolid (90%), and clindamycin (55%). Strains of CoNS showed high resistance for erythromycin (76.2%) followed by penicillin (65.7%) and ciprofloxacin (59.6%) [Table/Fig-4].

Organisms	Number	%
Coagulase negative *staphylococcus*	181	66.0
Staphylococcus aureus	08	2.9
Enterococcus	08	2.9
Escherichia coli	17	6.2
Klebsiella spp.	09	3.2
Citrobacter spp.	08	2.9
Pseudomonas spp.	33	12.0
Acinetobacter spp.	07	2.5
Sphingomonas spp.	02	0.72
Burkholderia cepacia	01	0.36

[Table/Fig-3]: Distribution of organisms in positive samples.

Amongst all the antibiotics, linezolid and vancomycin emerged as most sensitive drug against *Staphylococcus* spp. Strains of *Staphylococcus* spp. showed high resistance for Penicillin. Linezolid was found most sensitive drug against *Enterococcus* spp. Strains *Enterococcus* spp. showed high resistance for ampicillin and penicillin. *E.coli* was found most susceptible to gentamicin, meropenem, imipenem and cotrimoxazole. Strains of *Escherichia coli* showed high resistance for cefuroxime and ceftriaxone [Table/Fig-5].

Imipenem and meropenem emerged as most sensitive drug against *Klebsiella* spp. Strains of *Klebsiella* spp. showed high resistance for cefepime and ceftrioxone. Ampicillin and gentamicin were found most effective against *Citrobacter* spp. Strains of *Citrobacter* spp. showed high resistance for cefepime. Piperacillin+Tazobactum were most efficient against *Pseudomonas* spp.

Antibiotics	(Number of sensitive samples)	Sensitive %	Number of resistant samples	Resistant %
Vancomycin	181	100%	0	0
Linezolid	164	90%	17	9.3%
Gentamicin	139	77%	42	23%
Ciprofloxacin	73	40%	108	59%
Levofloxacin	99	54%	82	45%
Erythromycin	43	23%	138	76%
Penicillin	62	34%	119	65%
Clindamycin	101	55%	80	44%
Cefotaxime	85	46%	96	53%

[Table/Fig-4]: Antibiotic susceptibility pattern of coagulase negative *Staphylococcus* (CoNS) (n=181).

Antibiotics	Sensitive N%	Resistant N%
Ampicillin	08 (47%)	09 (53%)
Amoxyclav	09 (53%)	08 (47%)
Cefepime	13 (76%)	04 (24%)
Cefotaxime	08 (47%)	09 (53%)
Ceftriaxone	06 (35%)	11 (65%)
Cefuroxime	06 (35%)	11 (65%)
Gentamicin	16 (94%)	01 (6%)
Imipenem	14 (82%)	03 (18%)
Levofloxacin	11 (65%)	06 (35%)
Meropenem	13 (76%)	04 (08%)
Cotrimoxazole	14 (82%)	03 (18%)

[Table/Fig-5]: Antibiotic susceptibility pattern of *Escherichia coli* species.

Strains of *Pseudomonas* spp. showed high resistance for Aztreonam [Table/Fig-6]. Gentamicin and Levofloxacin were observed most sensitive drug against *Acinetobacter* spp. Strains of *Acinetobacter* spp. showed high resistance for tetracycline and ceftriaxone.

Antibiotics	Sensitive N%	Resistant N%
Aztreonam	12 (37%)	21 (63%)
Ceftazidime	16 (48%)	17 (51%)
Ciprofloxacin	25 (75%)	08 (24%)
Levofloxacin	29 (87%)	04 (12%)
Meropenem	25 (75%)	08 (25%)
Piperacillin	25 (75%)	08 (24%)
Piperacillin-Tazobactum	29 (87%)	04 (12%)
Tobramycin	22 (66%)	11 (33%)
Ticarcillin	21 (63%)	12 (37%)

[Table/Fig-6]: Antibiotics suscetivility pattern in *Pseudomonas* spp.

MDR was observed in approximately 32 positive culture isolates which constituted 11.6% of total positive cultures. Gram negative bacilli constituted higher percentage (27, 10%) than gram positive organism (5, 1.8%) among MDR isolates. Among gram negative organisms *Pseudomonas* spp. were most common isolates to demonstrate MDR [Table/Fig-7].

DISCUSSION

In this study, gram positive bacteria were more common isolates than gram negative organism. Gram positive bacteria accounted for 71% of the isolates. This was also observed by Prabhu K et al., who found 64% of isolates were gram positive organisms [9]. Among gram positive bacteria CONS was isolated in most samples (92%) than *Staphylococcus aureus* (4%). Jamal WY et al., found that most common isolate in BSI was CoNS (46%) [10]. In a similar study done by Valles J et al., CoNS accounted for 49.8% of the isolates [11]. Wattal C et al., reported CoNS as the most common isolate causing BSIs in ICU patients [12]. It has

Organisms	Number	Percentage
CoNS	0	100
Citrobacter spp.	4	12.5
Klebsiella spp.	4	12.5
Pseudomonas spp.	9	28
E.coli	6	18
Citrobacter spp.	2	6.2
Acinetobacter spp.	2	6.2
Staphylococcus spp.	3	9
Enterococcus spp.	2	6.2

[Table/Fig-7]: Distribution of multi drug resistant organism.

been demonstrated by some studies upto 85% of CoNS represent contamination rather than true bacteremia [13]. In order to avoid this repeat blood cultures were done for confirmation. In the present study, gram negative bacilli accounted for 28.1 of BSIs, similar observations were done by Prabhu K et al., who observed 35% of the culture isolates were gram negative bacilli [9]. *Pseudomonas* spp. are most common and accounted for 42.6% among gram negative organisms.

In this study, males were more commonly involved accounting for 56.9% of total number of positive cases and females account for approximately 43% of total number of positive cases. The result was consistent with the study done by Kaur A and Singh V who reported high culture positivity in men 65.2% [14]. Hussein A et al., reported 66.6% positivity in men and 33.3% in women [15]. Zenebe T et al., found that women (59.8%) were effected more than men (40.2%) [16].

From amongst the bacteria isolated in present study, CoNS showed 100% sensitivity to vancomycin and 90% sensitivity to linezolid. Vancomycin and Linezolid were sensitive to all species of CoNS in the study by Asangi SY et al., and Singh L et al., [17,18]. The organisms were found highly resistant to erythromycin (76%) followed by penicillin (65%). The second common isolate among gram positive bacteria was *Staphylococcus* spp. which showed 100% sensitivity to linezolid and vancomycin, followed by clindamycin (75%). The organisms were found highly resistant to penicillin (87%) followed by erythromycin (75%) and cefoxitin (62%). The antimicrobial susceptibility testing revealed that resistance to penicillin was frequent in *Staphylococcus* spp. (87%) and CONS (65%) this was consistent with the study of Roy I et al., who observed 89% of *Staphylococcus* isolated were resistant to pencillin and none of isolates were resistant to vancomycin [19]. Among gram negative organisms *Pseudomonas* spp. was the most common isolate in which Piperacillin+Tazobactum (87%) emerged as most sensitive drug followed by piperacillin (75%), imipenem, meropenem (75%) and ciprofloxacin (75%). Patel PH et al., observed 100% susceptibility to imipenem of *Pseudomonas* spp. [20]. However, Pourakbari B et al., had reported resistance to most of antibiotics by *Pseudomonas* spp. Imipenem (92%) emerged as most sensitive drug against *Klebsiella* spp. followed by cotrimoxazole (67. Strains of *Klebsiella* showed high resistance for ceftriaxone (78%), amoxyclav (55%) followed by Ampicillin (33%). *Citrobacter* spp. was highly sensitive to imipenem (75%) and appear resistant to ampicillin (62%) [21]. *Sphingomonas paucimobilis* was detected in 2 samples. It was highly sensitive to imipenem (100%) which was consistant with study of Bayram N et al., [22]. Carbapenems were the most effective therapy. *Burkholderia cepacia* was found in single culture constituting a mere 0.36% of total number of isolates Gautam V et al., isolated 39 isolates of *Burkholderia cepacia* from various specimens which accounted for 0.35% of total number of isolates [23]. It showed sensitivity to co-trimoxazole, imipenem, levofloxacin and appeared resistant to cephalosporins, ampicillin and gentamicin. In this study, 32 isolates showed MDR which constitute 11.6% of total number of positive cultures with gram negative organisms most commonly involved. Among gram negative organisms *Pseudomonas* spp. mainly show MDR. Tam VH et al., observed that in *P. aeruginosa* bloodstream isolates approximately 10-17% showed MDR [24]. The number of isolates obtained in this study has been compared with other previous studies [Table/Fig-8] [25-30].

Various studies/Year	CoNS	*Staphylococcus* spp.	*E. coli*	*Klebsiella* spp.	*Acinetobacter* spp	*Pseudomonas* spp.	*Enterococcus*	*Burkholderia* spp.
Present study	66%	2.9%	6.2%	3.2%	2.5%	12%	2.9%	0.3%
Mathur P et al., 2014 [25]	4%	14.5%	4%	18%	21.5%	8%	1.5%	2%
Gohel K et al., 2014 [26]	4.5%	38.6%	15.2%	9.8%	1.5%1	5.3%	3.8%	4.5%
Singhal T et al., 2016 [27]	8%	8%	26%	22%	10%	8%	8%	1.2%
Prabash K et al., 2010 [28]	10.5%	12.6%	10.9%	4.5%	11.5%	30.3%	4.1%	--
Garg A et al., 2007 [29]	20.7%	8,3%	11%	7.3%	12.6%	16%	3.7%	--
Khurana S et al., 2017 [30]	6.8%	7.1%	5.1%	15.2%	24.1%	9.6%	1.4%	10.6%

[Table/Fig-8]: Comparison of present study isolates with other Indian studies [25-30].

Thus, the present study clearly indicates that most common isolates from BSIs were CoNS which appeared highly sensitive to vancomycin and linezolid whereas, among gram negative organisms *Escherichia coli* were commonly isolated. MDR isolates also constituted a significant number and were commonly found among gram negative organisms.

Limitation(s)

This study was conducted for shorter period of time that is six months and thus chances for identifying risk factors for mortality were reduced. Another limitation of this study was its retrospective analysis which may be prone to selection bias which was omitted by selecting all samples in present study.

CONCLUSION(S)

This study formed a useful reference for clinical microbiologists, physicians and others attempting to monitor the prevalence of BSIs and for the treatment of patients with such infections. Utilising various methods for reduction of development of antibiotic resistance is utmost requirement which can be established by adopting specific antibiotic utilisation strategies e.g., reducing antibiotic usage, developing combination therapy, using antibiotics only after standard antimicrobial susceptibility testing and recycling of antibiotics. Using methods for proper infection control and proper channelization of antibiotic programs are most important and are of prime requirement.

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