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Characterization and drug sensitivity patterns of gram positive bacteria and fungus in blood stream infection

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Abstract
Back ground: Blood stream infections can lead to life threatening sepsis and require rapid antimicrobial treatment. The organisms implicated in these infections vary with the geographical alteration. Infections caused by MDR organisms are more likely to increase the risk of death in these patients. The present study was aimed to study the profile of gram positive bacteria and fungus causing bacteremia and understand drug resistance patterns in our hospital.
Materials and Methods: A total of 300 blood samples collected over a year from clinically suspected cases of bacteremia were studied. The isolates were identified by standard biochemical tests and antimicrobial resistance patterns were determined by CLSI guidelines.
Results: Positive blood cultures were obtained in 9.2% of cases of which Gram-positive bacteria accounted for 58.3% of cases with staph aureus predominance; and 1.5% were fungal isolates. The most sensitive drugs for Gram-positive isolates were vancomycin, teicoplanin, daptomycin, linezolid, and tigecycline.
Conclusions: The prevalence of MRSA and vancomycin resistance was 70.6% and 21.6%, respectively. ESBL prevalence was 39.6%. Overall low positive rates of blood culture were observed.
Key words: Blood Stream infections, Antibiotic Sensitivity, Antimicrobial resistance, Gram positive bacteria, Fungus

Keywords: Characterization and positive, bacteria and fungus, Blood stream biochemical

Introduction
Invasion of the bloodstream by microorganisms constitutes one of the most serious situations in infectious disease. Microorganisms present in circulating blood whether continuously, intermittently, or transiently are a threat to every organ in the body. Prevalence and antimicrobial susceptibility of microorganism vary depending upon the geography and the use of antibiotics. The excessive and irrational use of antibiotics has led to an increase in the multidrug-resistant bugs and thus worsened the condition. Bloodstream infections have serious consequences like shock, disseminated intravascular coagulation, multiple organ failure, and even death. Increased hospital stay and associated costs are the most troublesome consequences [1]. Treatment of bloodstream infections is based on the knowledge of prevalent microorganisms and their antimicrobial sensitivity patterns. This information also forms the basis for making recommendations for initial empirical therapy to be started when a bloodstream infection is suspected [2]. Specific therapy can only be started once the organisms are isolated and their antimicrobial sensitivity patterns are studied. The procedure is time consuming and depends upon the growth of the organisms in culture media. Many faster and automated culture techniques are available. Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance of blood borne isolates is increasing and it also varies in accordance with geographical and regional location. The infection caused by MDR organisms is more likely to prolong the hospital stay, increase the risk of death, and require treatment with more expensive antibiotics. Keeping in view of the above facts, this prospective study was carried out to isolate & Identify of pathogens causing BSI. Also evaluated the antimicrobial susceptibility pattern (AST) of isolated gram positive bacteria and fungus by using both conventional (Kirby-Bauer's disc diffusion) method and automated culture system (VITEK-2, bioMerieux).

Material and Methods

The study "Bacteriological profile of blood stream infection (BSI)by using both automated & conventional blood culture system in a tertiary care hospital" was a prospective study conducted in the department of Microbiology in collaboration with Department of Medicine, Paediatrics, Obstetrics & Gynaecology & Intensive care units of S.C.B. medical college and Hospital, Cuttack, Odisha.

A total of 600 blood culture specimens were collected aseptically from 300 patients. Samples were collected from both left & right cubital fossa, with 1hour interval, before the start of antimicrobial therapy.

Blood culture

BHI culture bottles were incubated at 37° c for a maximum up to 7days.Blind subculture was made on blood agar & MacConkey agar on day 1st, day 3rd and finally on 5th day. The culture bottles were discarded after 7day.

Identification

Identification of isolates from sub cultured plates were done depending upon colony morphology, gram staining, rapid tests like catalase, coagulase, oxidase, and other requisite biochemical tests.[3] One presumptive identification of the organism & sensitivity was put on the same day to provide immediate report to the patient, so that proper treatment should be started as soon as possible. The final identification was done on the next day onwards after doing subcultures as mentioned above in blood culture and other biochemical tests.

Fungal growth was identified by conventional technique [111] and immediately it was informed to clinician without any sensitivity report.

AST was done on Mueller-Hinton agar following Kirby-Bauer's disc diffusion method which has been used for all positive isolates according to the guidelines of clinical & Laboratory standard institute (CLSI 2012) and zones of inhibition were interpreted accordingly. [4]

In automated blood culture system

Inoculated BacT/ALERT culture bottles were loaded in to the automated BacT/ALERT 3D system as per manufacturer's guidelines (bio Merieux, USA).All the culture bottles were incubated under continuous agitation & monitoring up to 7days.The increase amount of co_2 produced by bacterial growth diffuses through a semi-permeable membrane in the base of culture bottle.

Flagged culture bottle were sub cultured on blood agar & Mac Conkey's agar and incubated at 37° c for 24-48hours.The time required for bacterial growth in BacT/ALERT bottles had been detected & displayed on the 3D monitor of BacT/ALERT which had noted.

Identification

The isolated colony from sub cultured plate had been added to sterile saline solution to make a suspension equivalent to 0.5 Mc-Farland standard, adjusted by using a Densi CHEK Plus (bio Merieux,Inc.) based on colorimetric principle.

Identification of positive isolates had been by VITEK 2 system (bio Merieux, USA).For identification of gram positive isolates GP ID card & for gram negative isolates GN ID card had been used. Samples yielding yeasts had been identified by using YST card. The reagent cards have 64 wells which contain 41 biochemical tests.

With a vacuum device, the card were inoculated with 0.5 Mc Farland suspension of positive organisms & then automatically sealed and manually inserted inside the VITEK 2 reader inoculator module. Fluorescence were measured every 15 minutes and results were determined after 3hours. [5]

Antimicrobial susceptibility testing (AST)

AST of all positive isolates had been performed with the VITEK 2 system, as recommended by the manufacture's guidelines. For AST of gram positive isolates P628 card & for gram negative isolates N281 card. For yeasts AST-YS07 card had been used for antifungal susceptibility testing. Results had been given as sensitive(S), intermediate (I) and resistant(R) as per database in instrument, which is regularly updated by the manufacturer.

Minimum inhibitory concentration (MIC) of all positive isolates by using micro broth dilution method had been detected. Resistant pattern of isolates by advanced expert study (AES) finding had also been detected.

Results

From 300 septicaemic cases, 198(66.6%) were male & 102 (33.3%) were female. Majority of patients were belong to the age group 31-40yrear (32%). Maximum no. of positive isolates was found to have risk factor of UTIs (35.41%) followed by lung abscess (20.83%) (Table 1 & 2).

Table 1: Age & sex distribution of septiceamic cases (n=300)

Age group (yrs)	Male(n=198)	Female(n=102)	Total
0-10	11(5.55%)	4(3.9)	15(5%)
11-20	07(3.5%)	02(1.9)	9(3%)
21-30	52(26.2%)	30(29.4)	82(27.3%)
31-40	63(31.3%)	33(32.3%)	96(32%)
41-50	49(24.7%)	27(26.5)	76(25.3%)
51-60	09(4.54%)	03(2.9)	12(4%)
□60	07(3.5%)	03(2.9)	10(3.3%)
total	198(66.6%)	102(33.3)	300(100%)

Table 2: Study on various risk factors & number of isolates obtained (n=300)

Risk factors	No. Of patients	No. Of isolates obtained
Cellulitis	62(20.7%)	10(20.8%)
Pneumonia	25(8.3%)	03(6.2%)
UTIs	98(32.7%)	17(35.4%)
Lung abscess	64(21.3%)	10(20.8%)
Unknown	51(17%)	8(16.7%)
Total	300(100%)	48(100%)

From 300 patients, the risk factors were identified in 249 (83%) of cases, among of them, most common risk factor observed was Urinary tract infections (32.7%), followed by lung abscesses (21.33%) and cellulitis (20.7%) (Table 2).

Table 3: Culture positive cases as per conventional & automated culture methods (n=300)

No. of Cases	Conventional culture method	Automated culture method
Positive	39(13%)	48(16%)
Negative	261(87%)	252(84%)
Total	300	300

Out of 300 samples, automated culture positive cases 48(16%) and conventional culture positive cases were 39(13%) (Table 3).

Table 4: Blood culture isolates identified in conventional (n=39) & automated culture system (n=48)

Organisms isolated	Conventional method(n=39)	Automated system (n=48)
CoNS	9(23.1%)	12(2%)
S. haemolyticus	5(55.5%)	6(50%)
S. hominis	4(44.4%)	5(41.6%)
S. epidermidis		1(8.3%)
S.aureus	3(7.7%)	3(6.2%)
Enterocoocus gallinarum	1(2.6%)	1(2.1%)
Candida Spp.		3(6.2%)
Candida tropicalis	2(5.1%)	2(66.6%)
Candida krusei	2(100%)	1(33.3%)

CoNS=Coagulase negative staphylococci

The most common Gram positive organisms in automated (25%) & in conventional (23.1%) were *CoNS*, followed by *S. aureus* in automated (7.7%) & in conventional (6.2%) cases. Among Gram negative isolates, most common in automated (31.2%) & in conventional (33.3%) were *E.coli*, followed by *Klebsiella Spp.* (12.5%) (Table 4).

Table 5: Time of recovery of isolates by both automated and conventional blood culture systems

Organisms isolated	1ST Day		2ND Day		3RD Day		4th Day		5TH Day		6TH Day		7TH Day	
	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto	Conv	Auto
S. aureus	3	3	-	-	-	-	-	-	-	-	-	-	-	-
CoNS	5	08	-	4	2	-	-	-	2	-	-	-	-	-
Enterococcus spp.	1	1	-	-	-	-	-	-	-	-	-	-	-	-
Candida spp..	1	2	-	1	1	-	-	-	-	-	-	-	-	-

Auto=Automated culture system, Conv=Conventional culture system, *CoNS=Coagulase negative staphylocci*

Out of 48 culture positive cases, 37 isolates were obtained within 24hours in automated culture system and, 10 in 48hours & 1 isolate after 72 hour of incubation, with mean time of detection was 14hours (±1.09) (Table 5).

In conventional culture method, 18 isolates obtained within 24hours, 12 isolates were in 72hours & 4 were after 96hours of incubation, with mean time of detection was 60hours (±12.08).

Table 6: Antimicrobial resistant patterns of Gram positive cocci

Antibiotics	Organisms isolated					
	CoNS		*S.aureus*		*Enterococcus Spp.*	
	Automated System (n=12)	Conventional System (n=9)	Automated System (n=3)	Conventional System (n=3)	Automated System (n=1)	Conventional System (n=1)
Ampicillin	10(84%)	7(80%)	3(100%)	3(100%)	1(100%)	1(100%)
Ceftriaxone	9(75%)	6(70%)	2(66.6%)	2(66.6%)	1(100%)	1(100%)
Cefipime	9(75%)	6(70%)	2(66.6%)	2(66.6%)	1(100%)	1(100%)
Ciprofloxacin	5(42%)	6(70%)	1(33%)	1(33%)	0(0%)	0(0%)
Erythromycin	7(58%)	4(42%)	1(33%)	1(33%)	1(100%)	1(100%)
Gentamicin	5(42%)	5(60%)	1(33%)	1(33%)	0(0%)	0(0%)
Teicoplanin	6(50%)	5(60%)	1(33%)	1(33%)	0(0%)	0(0%)
Vancomycin	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)
Linezolid	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)	0(0%)

Gram positive cocci, were found to be highly resistant to ampicillin (84%), followed by cefipime (75%), ceftriaxone (70%) and ciprofloxacin (66%). All Gram positive isolates were 100% sensitive to both vancomycin and linezolid (Table 6).

Table 7: Antifungal resistance pattern of Candida species (n=3)

Antifungal agents	*Candida tropicalis(n=2)*	*Candida krusei(n=1)*
Amphotericin-B	0%	0%
Fluconazole	50%	100%
Voriconazole	50%	100%
Caspofungin	0%	0%
Antifungal agents	*Candida tropicalis(n=2)*	*Candida krusei(n=1)*
Amphotericin-B	0%	0%
Fluconazole	50%	100%
Voriconazole	50%	100%
Caspofungin	0%	0%

Among candida spp., caspofungin was found highly sensitive to both tropicalis & krusei (100%), followed by Amp B (Table 7).

Table 8: Minimum inhibitory concentration (MIC) of Gram positive Isolates in automated blood culture system (n=16)

Staphylococcus spp. (n=15)						***Enterococcus spp.* (n=1)**					
Normal MIC Ranges			**No. of isolates**			**Normal MIC Ranges**			**No. of isolates**		
S	I	R	S	I	R	S	I	R	S	I	R
Penicillin						Penicillin					
≤0.12	-	□0.25	1(6.7%)	2(13.3%)	12(86%)	□8	-	□16	-	-	1(100%)
Ciprofloxacin						Ciprofloxacin					
≤1	2	□4	1(6%)	4(26.7%)	10(66.7)	□1	2	□4	1(100%)	-	-
Levofloxacin						Levofloxacin					
≤1	2	□4	4(26.7%)	-	11(73.3)	□2	4	□8 -		-	1(100%)
Erythromycin						Erythromycin					
≤0.5	1-2	□18	2(13.3%)	2(13.3%)	11(73.3%)	□0.5	1-4	□8	-	1(100%)	-
Vancomycin						Vancomycin					
≤2	4-8	□16	15(100%)	-	-	□4	8-16	□32	1 (100%)	-	-
Linezolid						Linezolid					
≤4	-	□8	15(100%)	-	-	□2	4	□8	1(100%)	-	-
Teicoplanin			Teicoplanin								
□8	16	□32	11(73.3%)	4(26.7%)	-	□8	16	□32	1(100%)	-	-
Clindamycin			Clindamycin								
□0.5	-	□4	13(86.7%)	2(13%)	-	-	-	-	1(100%)	-	-
Rifampicin			Rifampicin								
□1	2	□4	13(86.7%)	1(6.7%)	1(6.7%)	□1	2	□4	1(100%)	-	-

Minimum inhibitory concentration(MIC) results in automated culture system showed among Gram positive cocci, penicillin was found to be 80% resistant (MIC≥0.25), followed by 73.3% resistant (MIC≥18) and 13.3% intermediate resistant (MIC =1-2) to erythromycin. Least resistance was noted in teicoplanin, 26.7% resistant (MIC□32), followed by 13.3% resistant (MIC□4) and 86.7% intermediate resistance to clindamycin in *Staphylococcus spp*. Vancomycin (MIC□2) and linezolid (MIC□4) showed 100% sensitive to both *Staphylococcus spp*. and *Enterococcus spp*., (Table 8).

Table 9: Detection of MRSA and ESBL resistant isolates in automated culture system

Types of isolates	Total no. of isolates	Resistant type
Staphylococcus Spp.	16	MRSA
		7(43.8%)
Gram –ve bacteria	29	ESBL
		12(41.4%)

Among *Staphyloccus Spp.,* MRSA(methicillin resistant *Staphyloccus aureus*) strain detected in 43.8% and among gram negative isolates, ESBL (Extended spectrum betalactamase) strain were in 41.4% of cases (Table 9).

Discussion

Bloodstream infection is a challenging problem, and sometimes, it may be life threatening; therefore, timely detection, identification, and antimicrobial susceptibility testing of blood-borne pathogens are one of the most important functions of diagnostic microbiology laboratory. The present study "Bacteriological profile of blood stream infection (BSI) by using both automated and conventional blood culture system in a tertiary care hospital" was conducted in S.C.B. Medical college & hospital from October 2014 to September 2016 in 300 suspected cases of BSIs admitted to various departments. The study was conducted to identify the isolates and it's speciation, mean time of detection and antimicrobial resistance pattern in both conventional and automated blood culture system. Minimum inhibitory concentration (MIC) was determined by automated blood culture system. In present study, the gender distribution of 300 blood samples, was found to be 66.6% of cases in males & 33.3% in females, which is similar to Vanitha *et al*. and Kante *et al*., where they found 60.2% and 61.7% cases in males and 39.7%, 38.2% of cases in females respectively. [6,7] In most of the studies of septic shock report a male preponderance.[8] This male preponderance could be due to a higher prevalence of co-morbidities in men and women are protected due to hormonal factors (more estrogen production) and non-hormonal factors (higher secretion of cytokines, interleukin-6) through the immune system that occur in BSIs. [9]

Our study showed, majority of patients (31%) were within 31-40years of age group, which is similar to the findings of Meenakshi *et al* and Vanitha *et al*., where they have found 28% of cases were in the age group of 20-40years and 83.7% of cases were in adult age group (□18years) respectively [10]. Most sepsis episodes are observed in patients older than 60 years.[11] Advanced age is a risk factor for acquiring nosocomial blood stream infection in the development of severe sepsis. Neonates are also vulnerable to infections because of their weak immunological barrier. [12]

From 300 patients, the risk factors were identified in 249 (83%) of cases, among of them, most common risk factor observed was Urinary tract infections (32.7%), followed by lung abscesses (21.33%) and cellulitis(20.7%),. Our result is in contrast to the findings of Ivan *et al*., where they found lung was the primary source of infection in both severe sepsis and septic shock, followed by the abdominal infections, the urinary tract infections and soft tissue infections. [13]

In present study, blood culture was positive in 48 (16%) of cases in automated blood culture system, which is in accordance with findings done by Surase *et al*. (32%), Lunagaria *et al*. (16.9%)Goel *et al.* (9.2%) and Parihar *et al*. (28.9%). [14]

Blood culture positivity in our study by conventional blood culture system was 39 (13%) cases, which is in accordance with findings done by in Surase *et al* (19.9%), Arora *et al*. (20%), Sharma *et al*. (33%) and Roy *et al*. (16.4%).[15]

In present study, the automated blood culture system detected nine additional organisms as compared to the conventional system, which is in accordance to the findings done by Surase *et al*. and Kareen *et al*. [16] The reason behind this higher recovery rate of isolates by the automated system could be due to the continuous agitation, use of SPS (sodium polyanethol sulfonate) as an anticoagulant and the presence of activated charcoal as a neutralizing agent in BacT/ALERT culture bottle.

Various studies from different parts of India and around the world, showed varying blood culture positive reports by Nasa *et al*. (10.6%), Mathur *et al*. (10.6%), Arora *et al*. (20.02%), Sharma *et al*. (33.9%) and Remirez Barba *et al*.(39%) [17]. In our study showed, the percentage of isolation rate in both automated and conventional method is low. This could be due to of patients reported to our hospital were referred from secondary care hospitals and these patients were already prescribed with antibiotics. Other reason could be due to self-medication, as antibiotics are available easily over the counter.

According to an another study done by Surase *et al*., where they have found blood culture positivity in 32% of cases in automated and 19.9% in conventional system, which is higher findings from our study.

Our study showed, among Gram positive cocci isolates, *Coagulase negative Staphlococci* (25%) of cases predominant, followed by S.*aureus* (6.2%) and *Enterococcus spp.* (2.2%), which is similar to the findings of Mulat *et al*., where they have found CoNS (42.3%), followed by S.aureus (23.4%).[18] According to an another study done by Kalpesh *et al*., where they have found *S.aureus* (38.6%) was most common isolate, followed by *CoNS* (4.5%) & *Enterococcus spp.*(3.8%).[135] In present study, from twelve CoNS isolates, S.haemolyticus (50%) was most common isolate, followed by *S.hominis* (41.6%). This is in accordance with the findings of Lunagaria *et al*., where they have found *S. hominis* (28.6%) as predominant, followed by *S. haemolyticus* (22%) and S.*epidermidis* (21%). [123] Several other studies have reported increasing incidence of infections due to *CoNS*. [19] *Coagulase negative staphylococci i*s a well described pathogen in immune-compromised patients causing nosocomial BSI, UTIs, surgical wound infections, infections of prosthetic valves and ophthalmic infections. [20]

In our study, 3 (6.2%) of cases of *non-albicans candida species* was isolated, which is in accordance to other studies done by Lunagaria *et al*., Ivan *et al*. and Raman *et al*., where they have found 4.73%,4.4% and 14.2% of cases of *non-albicans Candida.* [21] Out of all *non-albicans Candida isolated* from our study showed, *Candida tropicalis* 2(66.6%) was the most common isolates, followed by *Candida krusei* 1(33.3%). Our study is in accordance with the study of Lunagaria *et al*., where they have found *C.tropicalis* 46.1% of cases, followed *by C. parapsilosis* 28.2% of cases.[22] In an another study done by Singh *et al*., where they have found 1.6% of cases of candidemia. [23]

In present study, antibiotic resistant pattern among Gram-positive organisms showed that, highest resistance was noted in ampicillin (85%), followed by cefipime (75%) and ceftriaxone (60%), which is in accordance to the findings done by Vanitha *et al*. and Karki *et al*. [24] Higher percentage of Resistance to third and fourth generation cephalosporins could be due to the abundant use of these drugs in hospitals. [25]

Our study showed that *Staphyloccocus aureus* was found to 100% sensitive to Vancomycin and linezolid as reported in other studies of Karlowsky *et al*., Gupta *et al*., Garg *et al*., Kavitha *et al*., and Roy *et al*. [26] Few other studies have reported vancomycin resistance in *Staphylococcus Spp*.[27] The increasing glycopeptide resistance could be due to widespread usage of the drug in the empirical treatment protocol.

Antifungal susceptibility pattern, among *Candida spp*. showed that caspofungin and amphotericin-B were found 100% highly sensitive to both the *candida species*, which is similar findings with Lunagaria *et al*.[28] Developement of resistance in *Candida Spp*., in general, is far less common than bacteria but rational use of these agents is required to sustain the sensitivity of these antifungals.[29] Early & efficient detection of yeast was noticed after using automated blood culture system when compared with conventional & other blood culture systems. [30]

Minimum inhibitory concentration(MIC) results in automated culture system showed among Gram positive cocci, penicillin was found to be 80% resistant (MIC□0.25), followed by 73.3% resistant (MIC□18) and 13.3% intermediate resistant (MIC =1-2) to erythromycin. Least resistance was noted in teicoplanin, 26.7% resistant (MIC□32), followed by 13.3% resistant (MIC□4) and 86.7% intermediate resistance to clindamycin in *Staphylococcus Spp*. Vancomycin (MIC□2) and linezolid (MIC□4) showed 100% sensitive to both *Staphylococcus Spp*. and *Enterococcus Spp*., Methicillin resistant *Staphyloccus aureus (MRSA)* strains detected in automated blood culture system in our study was 43.8%, which is in accordance to other studies done by Saravanan *et al*.(30.7%) and Dibah *et al*.(46%). [31] The heterogenicity in MRSA is probably due to applying different infection control measures, antibiotic administration, laboratory testing for methicillin resistance.[32] Extended spectrum betalactamase (ESBL) producers in our study was found to be 41.4%, which is in accordance with the study of Kavitha *et al*. and Arora and Devi who reported prevalence of ESBL producers as 32% and 34.4%, respectively.[33] The much higher incidence of ESBL production could be due to injudicious use of antibiotics.

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