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Isolates and their Antibiogram from Blood Stream Infection in a Tertiary Care Hospital, Uttarakannada, India

V.N. Venkatesh* and Swapna Kotian

Department of Microbiology Karwar Institute of Medical Sciences,
Karwar M. G road Karwar -581301, India
**Corresponding author:*

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A B S T R A C T

The aim of the work was to study the blood culture isolates and their antibiogram. A total of 1070 blood samples were collected from suspected cases of blood stream infection from August 2015 to July 2016, they were processed aerobically and Antibiogram pattern was studied. Cultures were positive in 277 (25.89%) cases. All the positive cultures were obtained after 48 hours of incubation. Gram negative bacteria were 114 (41.16%) of the total isolates with *Pseudomonas aeruginosa* 28 (10.11%) and Gram positive were 118 (42.60%) amongst which coagulase negative *Staphylococcu*s species 48 (17.33%) being the commonest isolate, *Candida* species isolated in 46(16.61%) of cases. In Gram negative isolate highest resistance was seen for cefazolin 63(86.30%) followed by aztreonam 60 (82.19%), amoxicillin + clavulanic acid 57(78.08%) and cefaperazone sulbactum 53 (72.60%) in contrast *S. aureus* strains isolated showed highest resistance to cotrimoxazole 40 (93.02%) followed by penicillin 29(67.44%) and erythromycin 27(62.79%). Blood cultures provide a valuable guide in identifying etiological agent and selecting appropriate antibiotic. Thus helps to achieve a high level antibiotic activity against the off ending bacterial organism.

Introduction

Blood stream infection is the most common health care associated infection and an important cause of mortality and morbidity around the globe (Diekma *et al*., 2003). The illness associated with the infection may range from mild self limiting to life threatening sepsis requiring rapid and aggressive antimicrobial treatment (Young *et al*., 1995). Blood stream infection may be transient bacteraemia which is an indication of true systemic infection or contamination from skin (Ladhani *et al*., 2004; Ayoola *et al*., 2002). Microbiological culture of blood remains gold standard for the diagnosis of bacterial agents and antibiotic susceptibility providing essential information for the evaluation of broad range of diseases like endocarditis, pneumonia, pyrexia of unknown origin and helpful particularly in patients with suspected sepsis allowing for successful recovery of bacteria in 99% patients with bacteraemia (Yagupsky *et al*., 1990). Antimicrobial resistance is growing threat worldwide in health care setting and possesses a major risk for human health. Resistance to antibiotics limits the success in therapy and prevention of disease (Dagnachew *et al*., 2014; Singh *et al*., 2013;

Opintan *et al*., 2015). Among the resistance shown Extended Spectrum Beta lactamase (ESBL) producing enterobacteriacea pose a major threat among drug resistant bacteria (Paterson *et al*., 2005). Keeping all these facts in view the present study was carried out with aim to determine the microbial profile of blood stream infection and their Antibiogram to different antibiotics, which would enable determination of empiric antimicrobial strategies guiding in infection control and rational use of antibiotics in this region.

Materials and Methods

The present study was carried out from August 2015 to July 2016 in the Department of Microbiology Karwar Institute of Medical Sciences Hospital, Karwar. After the approval from Institutional Ethical Committee and obtaining a written informed consent from the patients, a total of 1070 samples were collected from suspected cases of blood stream infection from patients belonging to all the age groups with detailed history. Antibiotic usage empirically before or after admission was noted. Blood samples from culture were collected following aseptic precautions. The venous site was cleaned with 70% alcohol and with allowing it to dry for 1-2 minutes, asset of two samples were collected giving a hour interval from different anatomical sites. The collected blood was inoculated into blood culture bottles containing Brain Heart Infusion broth (BHI) with 0.025%of Sodium polyanethol sulphonate as anticoagulant (Himedia, a commercial firm).

The blood culture bottles were then incubated 37^0C aerobically. After overnight incubation, the bottles were observed for turbidity and broths were subcultured with aseptic precautions onto blood agar, Mac Conkey agar and Chocolate agar. The plates were incubated at 37^0C overnight. If there was no growth observed on the plates the next day, the samples were further incubated and subsequently subcultured till seventh day. If growth was observed it was identified based on gram staining, colony characteristics and standard bio chemical tests (Elmer *et al*., 2006). Antibiotic susceptibility test were performed against locally available antibiotics by using disk diffusion methods in accordance with Clinical and Laboratory Standards Institute (CLSI) criteria (Clinical and Laboratory Standards Institute, 2007).

Drug resistant strains belonging to gram negative bacilli (GNB) oxidase negative were studied for Extended Spectrum Beta-Lactamases (ESBL) by double disk diffusion test that is by enhancement of the inhibitory zone between clavulanate impregnated disk Augmentin (Amoxicillin 20– Clavulanic 10µg) and disk impregnated with Cefotaxime (30µg) placed 20 mm apart (centre to centre). To check for *Methicillin resistant S. aureus* (MRSA), Cefoxitin (30µg) disk diffusion method was used. *Escherichia coli* ATCC 25922, *Klebsiella oxytoca* ATCC 700324, *Pseudomonas aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *Enterococcus fecalis* ATCC 29212 were included as control strains.

Statistical analysis was done manually. Qualitative variables were expressed as percentages and the culture reports were issued.

Results and Discussion

During the study period a total of 1070 blood culture samples were collected among which 672 (62.80%) were males and 408 (38.13%) were females as seen in table 1. Of these 1070 samples a positivity of 159 (57.40%) was seen in males and 118 (42.60%) was seen in females as depicted in table 2. Out of the 277 positive samples 114 (41.16%) were GNB

which include *E. coli* 25 (9.03%), *K. pneumoniae* 24(8.66%), *Salmonella species* 13 (4.69%), *Acinetobacter species* 12 (1.44%), *K. oxytoca* 8 (2.89%), *Enterobacter species* 4(1.44%) and *P. aeruginosa* 28 (10.11%). Among Gram Positive Cocci (GPC) isolated CoNS 48 (17.33%), *S. aureus* 43 (15.52%), *Enterococcus* species 19 (6.86%) and *Micrococcus* 8 (2.89%). In addition 46 *Candida* species (16.61%) were isolated as seen in table 2.

Highest blood culture positivity was seen in 0-10 years age group cases with 234 (84.48%) positivity out of total 277 cases. Among the GNB *Pseudomonas aeruginosa* 28 (11.97%) followed by *Klebsiella pneumoniae* 20 (8.55%) was the commonest isolate and in GPC CoNS 44(18.80%) followed by MSSA 43 (18.38%) were the commonest isolates. 13 (4.70%) isolate were from 11-20 years age group with Salmonella species 5(38.46%) being the commonest isolate followed by *E. coli* 4 (30.77%) and *Acinetobacter* species 4 (30.77%). 10 cases (3.61%) were from 41-50 years age group with *E. coli* 6 (60%) being the commonest isolate. 21-30 years had 8 (2.89%) positive case with Salmonella species 8 (100%) being isolated. 7 cases (2.35%) belonged to 31-40 years with *E. coli* 7(100%) being isolated as seen in table 3.

S. aureus strains isolated showed highest resistance to Cotrimoxazole 40 (93.02%) followed by Penicillin 29(67.44%) and Erythromycin 27(62.79%). All the strains of *S. aureus* isolated were sensitive to Cefoxitin as seen in table 4. Of the 19 strains of *Enterococcus* highest resistance was observed for Cotrimoxazole 13(68.42%) followed by Erythromycin 12(63.16%) and for Penicillin 11 (57.89%) and Ticarcillin Clavulanic acid 11 (5.89%).

Gram negative bacilli oxidase negative showed 100% sensitivity to Polymyxin B 300 and Colistin. Highest resistance was seen for Cefazolin 63 (86.30%) followed by Aztreonam 60 (82.19%), Amoxicillin – Clavulanic acid 57 (78.08%), Cefaperazone sulbactum 53 (72.60%). The most sensitive antibiotic was Meropenem 4 (5.48%) as observed in table 6. 19 (26.03%) of the total GNB isolates were found to be Extended Spectrum Beta Lactamase producer. Among 28 *Pseudomonas aeruginosa* isolated 100% sensitivity was seen for Polymyxin B and Colistin. Highest Resistance was seen for Cefaperasone – Sulbactum 20(71.43%), Ceftazidime 13(46.43%) and Cefepime 13(46.43%). Least resistance was observed for Amikacin 1(3.57%) and Meropenem 1(3.57%) table 7. Of the 13 Salmonella species isolated highest resistance was observed for Ciprofloxacin 6 (42.86%) and Ceftazidime 5(38.46%) as seen in table 8.

Changing trend in microbiology, epidemiology of the infecting agent and the clinical and prognostic significance of bacteraemia has been observed over the last 20 years. The timely detection of bacteraemia can have a profound influence on the clinical outcome (Aranson *et al*., 1987). The study demonstrates the microbial isolate distribution causing bacteraemia and their susceptibility pattern to the most commonly used oral and parentral antimicrobial agents. In our study, a total of 277 (25.89%) isolates were identified which is in accordance with few previously conducted studies by Qureshi *et al.*, (2011), Ravi *et al.*, (2012) and Jambo *et al.*, (2010). This is quite low compared to studies conducted by Jain *et al.*, where a positivity of 52. 63% was noted (Roy *et al*., 2002). The reason for low rate in our study could be due to prior empirical antibiotic treatment before the collection of sample for the culture resulting in negative cultures. The other reason for low rate could be due to infection by anaerobic organisms which cannot be detected by routine aerobic culture.

The most common age group showing positive culture was 0- 10 years group with 234 (84.48%) positivity among which 213 (91.03%) were neonates as this group is most vulnerable for infection due to their developmental status and physical examination findings are less reliable in neonates (Elbashier *et al*., 1998; Berkley *et al*., 2005; Berger *et al*., 1998). They are vulnerable to infection because of their weak immunological barriers. Lack of infection control procedures, inadequate sterilization of multiuse instruments, understaffing and crowded nurseries in developing country provide means for transmission of neonatal infections (Stapleton *et al*., 2015). Higher incidence in children were also quoted by other studies (Murty *et al*., 2007).

Predominance of gram positive organism was seen in comparison to gram negative bacilli. Recent reports too have shown that gram positive organisms particularly the cocci are assuming greater significance in causing bacteraemia concomitant with increasing incidence of nosocomial blood stream infection. Such change happened parallel to the evolution of medical care, more so with increasing number of critically ill and immune compromised individuals who require aggressive medical support and indwelling devices. A total of 118(42.60%) belonged to the Gram Positive Cocci group with the highest isolation of MSCoNS 48(17.33%), CoNS previously considered as a contaminant is being recognised increasingly as a cause of bacteraemia. The ascendance of this group of *Staphylococci* has created increased interpretative difficulties for the clinicians since great majority of CoNS isolates continues to represent contamination rather than true bacteraemia as it is a common skin habitant and may indeed contaminate poorly collected blood cultures leading to difficulty in determining bacteraemia from contamination (Robert *et al*., 1991; Weinstein *et al*., 1997; Behrman *et al*., 2004; Naas *et al*., 2016). In our study 48(17.33%) was isolated which is similar to the rate of isolation in studies conducted by Tariq Mahamud (2014) showing 26.34% and by Murthy *et al.,* 25% but is low when compared to studies conducted by T Naas *et al.,* (30) showing a positivity of 54.76% and Hanan *et al.,* (2005) having 55.4% rate of isolation. Haini Chen *et al.,* have suggested qualitative culture to aid interpretation and determine vascular relation (2002).

S. aureus was isolated in 43 (15.52%) while in some of the other studies by Bernadette *et al.,* 5.7%, Atul garg *et al.,* 8.3% (2007), Hanan *et al.,* 9.5% (2005) and Tariq Mahmud 26.34% (2014) showed a varying rate of isolation. Friedland *et al.,* reported in 36% of children having Staphylococcal septicemia had silent endocarditis and some cases of 'no focus' could be related to cardiac lesions and according to one study it was seen that 57% of cases where *S. aureus* was repeatedly isolated will have a cardiac pathology and all such patients with *S. aureus* bacteraemia should be thoroughly evaluated for the presence of any cardiac pathology as the cardiac vegetation serves as important source of persistent *S. aureus* bacteraemia. *Enterococcus* species was isolated in 19 (6.86%) cases and it was reported earlier that *Enterococcal* BSI is more common in older age group with instrumentation and prior to or with antimicrobial therapy (Madani *et al*., 1999).

E. coli is the most common enterobacteriacea causing gram negative bacteraemia as see in other studies but our study revealed *Klebsiella* species as the predominant etiological agent. *Klebsiella* species has been isolated as the main etiological agent in many other studies conducted by Tariq Mahmud 16.10% (2014), Nass *et al.,* 27.5% (2016), Alaah Al *et al.,* 46.8% (2005) and DS Murthy 35% (2007).

The resistance of *Klebsiella* species to antimicrobials may be an essential factor in their higher emergence in nosocomial infection (Alaa *et al*., 2005).

In the recent years increased incidence of systemic fu gal infection especially by *Candida* species has been noted in hospitalised intensive care unit patients. With an increased use of broad spectrum antimicrobials, endotracheal tubing, invasive lines in these patients it is known to be easy for *Candida* to bypass the natural barriers of infection and contribute to deep seated infection. Maternal factors also contribute to septicaemias. In the present study *Candida* species was isolated in 46(16.61%). Antifungal susceptibility testing was not done due to lack of facilities. *S. aureus* as found to be resistant to many antibiotics with highest resistance to Cotrimoxazole 93.02% followed by Penicillin 67.44%, Erythromycin 62.79%, Amoxicillin Clavulanic acid 55.81% and Linezolid 19 (44.19%). Similar resistance pattern have also been seen in studies conducted previously by Bibek Bhatt *et al.*, (2015), Atul Garg *et al.*, (2007) and Roy *et al.*, (2002). *Enterococcus* species was highly resistant to Cotrimoxazole in 13 (68.42%), Erythromycin 12(63.16%), Penicillin 11(57.87%), Ticarcillin Clavulanic acid 11(57.89%) and Amikacin 8(42.11%), Clindamycin 8(42.11%), Teicoplanin 8 (42.11%).

Table.1 Gender distribution

	Positive (n=277)	Negative	Total (n=1070)
Male	159(57.40%)	520	672(62.80%)
Female	118(42.60%)	296	408(38.13%)
Total	277	816	1070

Table.2 Spectrum of isolates

Isolates	**Number (%) (n=277)**
Gram Negative Bacilli	
1. *Pseudomonas aeruginosa*	28(10.11%)
2. *Escherichia coli*	25(9.03%)
3. *Klebsiella pneumoniae*	24(8.66%)
4. *Salmonella species*	13(4.69%)
5. *Acinetobacter species*	12(4.33%)
6. *Klebsiella oxytoca*	8(2.89%)
7. *Enterobacter species*	4(1.44%)
Gram Positive Bacilli	
1. *Staphylococcus aureus*	43(15.52%)
2. *Coagulase Negative Staphylococcus species*	48(17.33%)
3. *Enterococcus species*	19(6.86%)
4. *Micrococcus species*	8(2.89%)
Fungal isolate	
1. Candida species	46(16.61%)
Total	277

Table.3 Nutritive value of ripe mango per 100g Age wise distribution

Age in years	1	2	3	4	5	6	7	8	9	10	11	12	Total
0-10	8	20	12	4	0	8	28	43	44	8	19	40	234
11-20	4	0	0	0	5	4	0	0	0	0	0	0	13
21-30	0	0	0	0	8	0	0	0	0	0	0	0	8
31-40	7	0	0	0	0	0	0	0	0	0	0	0	7
41-50	6	0	0	0	0	0	0	0	4	0	0	0	10
51-60	0	0	0	0	0	0	0	0	0	0	0	5	5
>60	0	0	0	0	0	0	0	0	0	0	0	0	0
Total	25	20	12	4	13	12	28	43	48	8	19	45	277

1- *Escherichia coli*
2 – *Klebsiella pneumoniae*
3 – *Klebsiella oxytoca*
4- *Enterobacter species*
5- *Salmonella species*
6- *Acinetobacter species*
7- *Pseudomonas species*
8- MSSA
9-MSCoNS
10-*Micrococcus species*
11-*Enterococcus species*
12-*Candida species*

Table.4 Resistance pattern of *Staphylococcus aureus*

Sl. No	**Antibiotic**	**Number (%)(n=43)**
1.	Cotrimoxazole	40(93.02%)
2.	Penicillin	29(67.44%)
3.	Erythromycin	27(62.79%)
4.	Amoxicillin – Clavulanic	24(55.81%)
5.	Linezolid	19(44.19%)
6.	Ciprofloxacin	16 (37.21%)
7.	Teicoplanin	11(25.58%)
8.	Vancomycin	8(18.60%)
9.	Amikacin	8(18.60%)
10.	Chloramphenicol	8(18.60%)
11.	Clindamycin	8(18.60%)
12.	Gentamicin	5(11.63%)
13.	Rifampacin	4(9.30%)

Table.5 Resistance pattern of *Enterococcus* species

Sl.No	**Antibiotics**	**Number (%)(n=19)**
1.	Cotrimoxazole	13(68.42%)
2.	Erythromycin	12(63.16%)
3.	Ticarcillin – Clavulanic	11(57.89%)
4.	Penicillin	11(57.89%)
5.	Amikacin	8(42.11%)
6.	Clindamycin	8(42.11%)
7.	Teicoplanin	8(42.11%)
8.	Rifampacin	5(62.32%)
9.	High level Gentamicin	0
10.	Vancomycin	0

Table.6 Resistance pattern of Enterobacteriacea (Oxidase Negative)

Sl.No	Antibiotic	Number (%)(N=73)
1.	Cefazolin	63(86.30%)
2.	Aztreonam	60(82.19%)
3.	Cefuroxime	59(80.82%)
4.	Amoxicillin –Clavulanic acid	57(78.08%)
5.	Cefotaxime	56(76.71%)
6.	Cefaperazone – sulbactum	53(72.60%)
7.	Ticarcillin Clavulanic acid	53(72.60%)
8.	Cefepime	52(71.23%)
9.	Gentamicin	47(64.38%)
10.	Piperacillin Tazobactum	40(54.79)
11.	Cotrimoxazole	36(49.32%)
12.	Ciprofloxacin	17(23.29%)
13.	Amikacin	13(17.81%)
14.	Meropenem	4(5.48%)
15.	Polymyxin B	Nil
16.	Colistin	Nil

Table.7 Resistance pattern of *Pseudomonas aeruginosa*

Sl.No	Antibiotic	Number (%)(n=28)
1.	Cefaperazone sulbactum	20(71.43%)
2.	Ceftazidime	13(46.43%)
3.	Cefepime	13(46.43%)
4.	Piperacillin Tazobactum	8(28.57%)
5.	Ticarcillin – Clavulanic acid	7(25%)
6.	Aztreonam	6(21.43%)
7.	Gentamicin	5(17.86%)
8.	Cotrimoxazole	3(10.71%)
9.	Ciprofloxacin	2(7.14%)
10.	Amikacin	1(3.57%)
11.	Meropenem	1(3.57%)
12.	Polymyxin B	Nil
13.	Colistin	Nil

Table.8 Resistance pattern of *Salmonella* species

Sl.No	Antibiotic	Number (%)(n=13)
1.	Ciprofloxacin	6(42.86%)
2.	Ceftazidime	5(38.46%)
3.	Cefazolin	4(30.77%)
4.	Cefotaxime	4(30.77%)
5.	Clindamycin	4(30.77%)
6.	Aztreonam	3(23.08%)
7.	Chloramphenicol	Nil
8.	Cotrimoxazole	Nil
9.	Meropenem	Nil
10.	Azithromycin	Nil

A combination of third generation Cephalosporin with Aminoglycosides have been usually considered from Gram negative bacteraemia but in the recent days it was seen that at least 60-70%of the gram negative organisms are resistant to most of these antibiotics (Mehta *et al*., 2005). In the present study among the various antibiotics used for the susceptibility testing for gram Negative oxidase negative organisms resistance was seen for many antibiotics in varying percentage with least resistance to Meropenem 4 (5.48%) and Ciprofloxacin (23.29%) and Cotrimoxazole 36 (49.32%). For oxidase positive bacilli least resistance was observed for Amikacin 1 (3.57%), Meropenem 1 (3.57%), Ciprofloxacin 2 (7.14%) and Cotrimoxazole 3 (10.71%).

ESBL producing enterobacteriacea have become well recognised in many hospitals worldwide. The extended spectrum beta lactamase enzyme showing plasmid mediated resistance as a consequence of point mutation in the TEM or SHV gene represents a widening threat to the utility of the antimicrobials (Canton *et al*., 2008; Shukla *et al*., 2004; Paterson *et al*., 2005; Nwadioha *et al*., 2010). These ESBL producing GNB's were also known to be multidrug resistant and show high resistance to commonly used anti microbials like Ampicillin, Gentamicin, third generation Cephalosporin and Fluroquionolones. Previous studies have suggested that in patients with serious infection due to Ceftazidime resistance third generation cephalosporin's could not be used. The lack of data in patients undergoing therapy with combination of beta lactam plus a beta lactamase inhibitor limits their usage and concomitant resistance to ciprofloxacin restricts empiric use of these agents in circumstances when an extended spectrum beta lactamase producing organism is suspected (Schiappa *et al*., 1996). For antimicrobials like Penicillin, Cephalosporin's, aminoglycosides in enterobacteriacea and *Pseudomonas aeruginosa* resistance was seen based on decreased entry of drugs (Bhatta *et al*., 2015).

In conclusion, blood stream infection is an important cause of morbidity and mortality in our patients. *Klebsiella species, E. coli, P. aeruginosa* and *Staphylococcus species* remain the principal bacteria responsible for infection. Blood cultures provide a valuable guide to the clinician in identifying etiological agent and selecting appropriate antibiotic. There should be an effective and rational use of antibiotic especially in tender age group in order to achieve a high level antibiotic activity against the offending bacterial organism.

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