

Bacteriological Profile and Antimicrobial Resistance Pattern of Bloodstream Infections in A Tertiary Care Hospital Set Up

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Abstract: Bloodstream infections (BSIs) are one of the common causes of infective morbidity and mortality. Early detection of causative pathogens and their antimicrobial resistance helps to start the appropriate antimicrobial therapy thereby reducing the number of complications. The present study was conducted to determine the bacteriological profile of bloodstream infections and their antibiotic resistance pattern in our tertiary care setup. Blood samples were received in the department of Microbiology, Hind institute of medical sciences, Barabanki. The sample processing and identification was done as per the standard protocol. Antibiotic sensitivity testing was done using Kirby Bauer disk diffusion method as per Clinical Laboratory Standard Institute (CLSI) guidelines. A total of 521 samples were processed, out of which 174(33.4%) isolates were positive for bacterial growth. From 174 bacterial culture positive isolates, 62% gram positive and 38% gram negative bacteria were isolated respectively. The most common bacteria isolated was Coagulase negative *Staphylococcus* spp (CoNS) (33%) followed by *Staphylococcus aureus*(25%), *Escherichia coli*(10%) and *Acinetobacter* spp. (10%). All gram positive cocci were completely sensitive to linezolid and teichoplanin. The present study observed high prevalence of antimicrobial resistance both in gram positive and gram negative bacteria. Hence, it is important to know the current trend of antimicrobials and formulate the antibiotic policy in the hospital so that the clinicians can start the proper empirical treatment and prevent the injudicious use of antibiotics.

Keywords: bloodstream infections, antibiotic resistance, Coagulase negative *Staphylococcus*.

INTRODUCTION

Blood stream infections (BSIs) are the most common cause of infective morbidity and mortality ranging from 15% to 50%.[1-3]There are several factors responsible for BSIs including hospital acquired infections due to indwelling catheters and other intravascular devices[4-6].

Bacteremia is defined as cultivable bacteria in bloodstream. Various micro-organisms have been isolated from the BSI like *Escherichia coli*, *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, *Acinetobacter* spp, Coagulase negative *staphylococcus* spp(CoNS), *Staphylococcus aureus*, *Enterococcus faecalis* and *Candida* spp[7]. It is important to diagnose and treat BSI as they can lead to sepsis and septic shock [8]. Early detection of pathogenic bacteria by blood culture detection methods can help prevent these complications and reduces the hospital stay. It also helps clinicians to determine the appropriate empirical

treatment before the blood culture result is available [9,10]. Increasing prevalence and antimicrobial resistance among the isolated micro-organisms from blood cultures is the main concern among the clinicians in the current scenario [11,12].

The present study has been conducted to know the bacteriological profile of bloodstream infections and their antimicrobial resistant pattern in our tertiary care setup.

MATERIALS AND METHODS

A total of 521 samples were received from various inpatient departments of our hospital. Out of the total samples collected, 415 were collected as paired blood culture from two different sites and 106 were collected from single site. The samples were processed in Bacteriology laboratory, Department of Microbiology, Hind Institute of Medical Sciences, Barabanki during the period from July 2016 to June

2017. It was a prospective cross-sectional study conducted after Ethical committee approval.

Sample Processing

Blood samples were collected in brain heart infusion broth in a ratio of 1:5 to 1:10 (blood to broth) before the start of antimicrobial therapy. Inoculated blood culture bottles were incubated overnight at 37°C and sub-cultured on sheep Blood agar and Mac Conkey agar at 2nd day and 7th day. If no growth was present on media then declared negative after 7days of incubation. Samples with growth on sheep Blood agar and MacConkey were processed and identification was done as per standard protocol [13]. Antibiotic sensitivity test was performed using Kirby Bauer method and organism was reported as susceptible or resistant to the antibiotics as per CLSI guidelines 2016 [14]. All the samples with single growth and turbid BHI broth with both culture and broth showing same micro-organism on gram staining were included in the study.

The antibiotics used (potency in µg/disc; Himedia) for gram negative bacteria were ampicillin (10), piperacillin (100), gentamicin (10), amikacin (30), tobramycin (10), ceftazidime (30), ceftriaxone

(30), cefepime (30), aztreonam(30), amoxicillin-clavulanate (10/10), piperacillin-tazobactam (100/10), ticarcillin-clavulanate (75/10), ciprofloxacin (5), levofloxacin (5), imipenem (10), meropenem (10), ertapenem (10) and doripenem (10).

For gram positive bacteria, penicillin(10Units), cefoxitin(30), teichoplanin(30), erythromycin(15), clindamycin(2), vancomycin(30), linezolid(30) and high level gentamicin(120) were added.

RESULTS

A total of 521 blood samples were collected, out of which 329 were sterile (without growth) whereas 174 samples have isolated micro-organism with single growth. Other than sterile and bacterial isolates, 8 samples were contamination (more than one micro-organism grown) and in 10 samples *Candida* was isolated. Out of the total samples collected, most of the samples were from paediatric age group accounting for 414 samples from which 146 have isolated the bacteria. Sample distribution among the different age group i.e., age ≤18 years, age between 19-50 years and age > 50 years is depicted in Table-1.

Table-1: Distribution of samples among different age groups

Age group	Age ≤ 18 yrs	Age between 19 to 50yrs	>50yrs	Total
Samples without growth (sterile)	259(49.7%)	49(9.4%)	21(4%)	329(63.1%)
Samples with bacterial growth	146(28%)	21(4%)	7(1.3%)	174(33.4%)
Contamination	4(0.7%)	2(0.4%)	2(0.4%)	8(1.5%)
<i>Candida</i> spp	5(0.9%)	3(0.6%)	2(0.4%)	10(1.9%)
Total samples	414(79.5%)	75(14.4%)	32(6.1%)	521(100%)

The percentage positivity of bacterial growth was 33.4% (174/521) with maximum number of isolates from age ≤ 18 years accounting for 84% (146/174) of the positivity whereas 28%(146/521) among overall samples. *Candida* accounts for 1.9 % (10/521) positivity.

Out of 174 bacterial culture positive isolates, gram positive bacteria were mostly isolated accounting

for 62% (108/174) and gram negative bacteria 38%(66/174) respectively. Among all bacteria isolated, the most common bacteria was Coagulase negative *Staphylococcus* spp(CoNS) which accounts for 32.8%(57/174) of the total positive samples. The percentage distribution of other bacteria in blood culture positive samples is depicted in Figure-1.

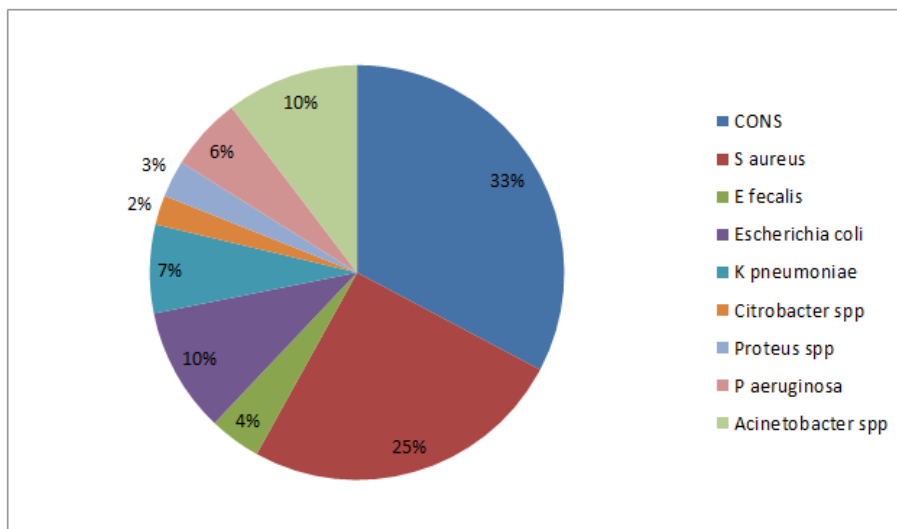


Fig-1: Percentage distribution of micro-organism in blood culture

Among the gram positive bacteria, CoNS was the most common bacteria isolated (52.8%,57) followed by *Staphylococcus aureus*(40.7%,44) and *Enterococcus* spp(6.5%;7).The most common bacteria isolated among gram negatives was *Acinetobacter* sp(27%,18) and *Escherichia coli*(25.7%,17) followed by *Klebsiella pneumonia*(18%,12), *Pseudomonas aeruginosa*(15%,10) and *Proteus* spp(7.6%,5) and *Citrobacter* spp(6%,4).

Among the gram negative bacteria, *Enterobacteriaceae* showed resistance in decreasing order with aztreonam(89.5%), ampicillin, ceftriaxone and ceftazidime(81.6%), amoxicillin/ clavulanate(65.8%), ticarcillin/ clavulanat(68.4%), piperacillin/ tazobactam (52.6%), gentamicin(52.6%), ciprofloxacin(44.7%), levofloxacin(36.8%), cefepime and amikacin(31.6%), meropenem and ertapenem (5.3%) and imipenem (2.6%).[Figure-2]

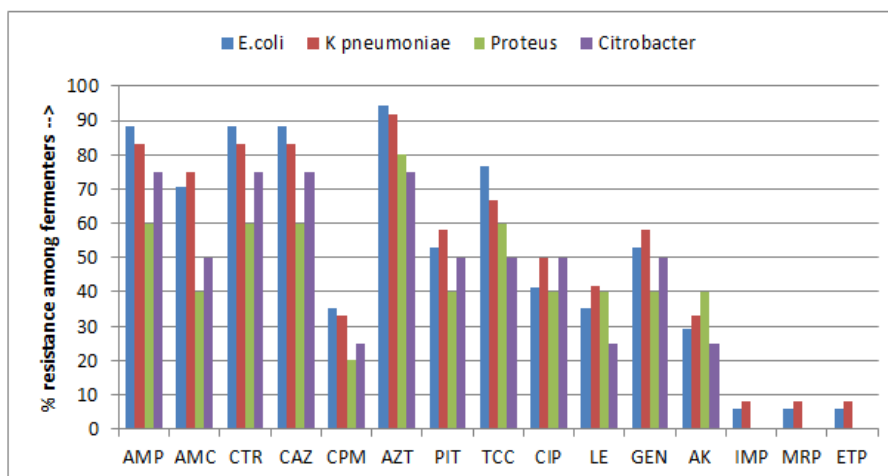


Fig-2: Antibiotic resistance pattern of fermenters among gram negative bacteria in bloodstream infections

Among non-fermenters, resistance pattern in decreasing order was as follows: piperacillin, aztreonam (90%), ceftazidime (82.1%), ticarcillin/ clavulanate (64.3%), piperacillin /tazobactam (57.1%),

ciprofloxacin (46.4%), gentamicin and tobramycin (46.4%), cefepime(42.8%), levofloxacin (35.7%), amikacin(28.6%), meropenem and doripenem(14.3%) and imipenem(10.3%).[Figure-3]

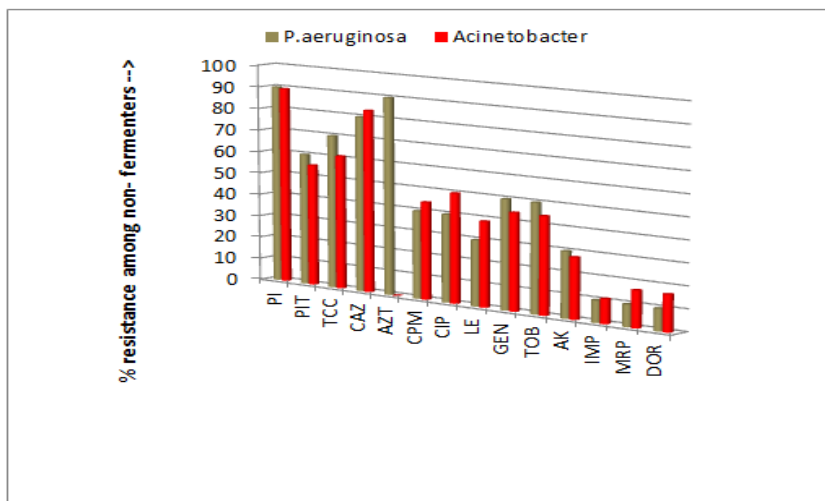


Fig-3: Antibiotic resistance pattern of non-fermenters among gram negative bacteria in bloodstream infections

Among the gram positive bacteria, resistance pattern was as follows: penicillin (82.4%) erythromycin (45.4%), ciprofloxacin (25%) and tetracycline (2.8%). Oxacillin resistance was tested by using cefoxitin (30µg) disc which gives 31.8% resistance in *S. aureus* and 35.1% in CoNS. Clindamycin resistance was 50% in *S. aureus* and CoNS. Vancomycin was 2.3% resistant in

S. aureus, 1.7% in CoNS and 28.6% in *Enterococcus*. All gram positive were sensitive to teichoplanin and linezolid. Ampicillin (57.1%) and levofloxacin (28.6%) resistant in *Enterococcus*. Gentamicin resistance was nearly 28% in *S. aureus* and CoNS whereas *Enterococcus* showed 42.8% resistance in high-level gentamicin [Figure-4].

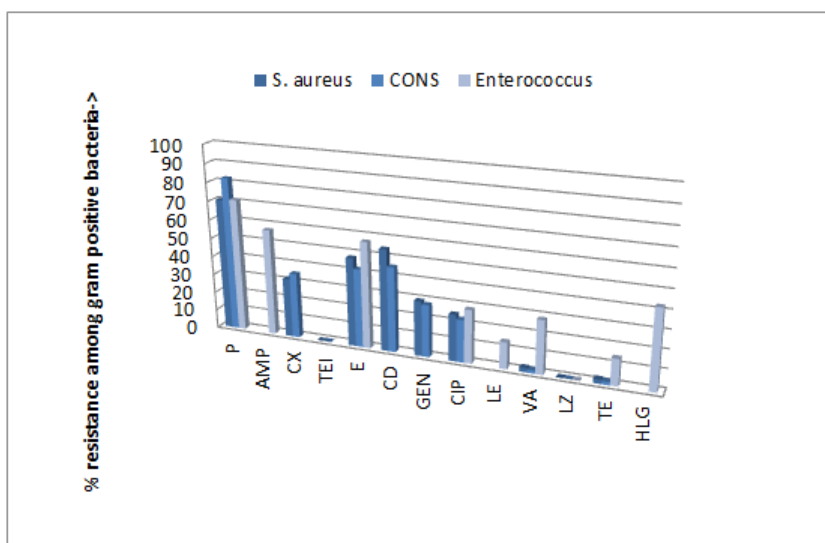


Fig-4: Antibiotic resistance pattern among gram positive bacteria in bloodstream infections

DISCUSSION

Due to irrational use of antibiotics, antimicrobial resistance to broad spectrum antibiotics is increasing which has become a great challenge for the clinicians to treat blood stream infections. The present study tried to depict the status of prevalent micro-organisms and their antibiotic resistance profile which can help to start the appropriate empirical treatment.

Out of 521 blood samples, we have seen 174 bacterial culture positives which accounts for 33.4% positivity rate, nearly corroborating with the study conducted by Parihar *et al.*, (29%) from Rajasthan [15], Ramana *et al.*, (42%) from South India [16], Mittal *et al.*, (54.6%) from Udaipur [17]. Other studies like Pandey *et al.*, showed 12% positivity from Nepal [18], Dash *et al.*, reported 17.2% from East India [7] and Fayyaz *et al.* documented 16% from Pakistan [19].

The present study showed high prevalence of gram positive bacteria (62%,108/174) which were more prevalent compared to gram negative bacteria (38%,66/174).*Candida* was 1.9%(10/521) positive out of the total blood samples. Mittal *et al.* reported 60.6% gram positive bacteria, 28.2% gram negative bacteria and 11.1% *Candida* respectively from the total culture positive samples.[17] Similarly Fayyaz *et al.* [19]. Parihar *et al.*, [15] and Jain *et al.*, [20] reported high prevalence of gram positive bacteria compared to gram negative bacteria, but studies like Gupta *et al.*, and others have determined that gram negative bacteria are slightly more prevalent than gram positive bacteria [10,21,22].

CoNS was the most common bacteria isolated overall (33%) which was similar to the study conducted by Singh *et al.* (25.9%) and Garg *et al.*, (20.7%) [10,23]. Other bacteria isolated in the present study were *S. aureus*, *Acinetobacter* and *E.coli*.

Among the gram positive bacteria also, CoNS was the most common bacteria isolated (52.8%) followed by *S.aureus* (40.7%) and *Enterococci*(6.5%) [7,19,22,24,25]. Some studies, have reported *S. aureus* to be the most common gram positive bacteria isolated [15,20,21].

Increased number of CoNS may be due to increased intravascular devices which is thought to be the entry route of bloodstream infection [7,20]. It could be stated that we have more number of paediatric culture positive patients and studies have reported that CoNS is an important cause of blood stream infection among the newborns due to premature birth [10]. CoNS is also a common blood contaminant so clinicians are advised to be careful while collecting blood culture. Practice of collecting paired blood culture sample is useful to rule out skin contamination if the same micro-organism is isolated from both sites simultaneously. Though some studies report even the single blood culture positive for CoNS to be significant [26].

Among the gram negative bacteria, *Acinetobacter* was the most common organism (27%) followed by *Escherichia coli* (25.7%) similar to Alam *et al.* which reports 31% *Acinetobacter* and 23.3% *E. coli* [22]. This shows that both fermenters and non-fermenters are equally important for causing bloodstream infection. There are also studies reporting fermenters like *Escherichia coli* (38.5%) and *Klebsiella* (33.8%) as the most common gram negative bacteria [21].

Apart from the causative pathogen, another important aim of this study was to determine the antibiotic resistant pattern of the causative pathogens in our hospital. The degree of resistance to antimicrobials

varies from one micro-organism to another and also differs from one region to another.

The present study showed that gram positive bacteria have high resistance to penicillin and oxacillin. They were also resistant to macrolide, fluoroquinolones and gentamicin. This increased resistance was also observed in the earlier studies conducted [21, 25, 27]. 1.7% of vancomycin resistance was reported in CoNS in the present study whereas Ramana *et al.*, reported 20% vancomycin resistance in CoNS [16]. The present study revealed that gram positive cocci were 100% sensitive to linezolid and teichoplanin which corroborates with the findings of other studies in relation to linezolid but teichoplanin resistance was reported by Dash *et al.* [7,19].

In the present study, 28.6% vancomycin resistance was determined in *Enterococci* whereas Gupta *et al.* reported that all *Enterococci* were sensitive to vancomycin [21]. This study has reported 42.8% resistance to high level gentamicin whereas Devi *et al.*, reported no case resistant to high level gentamicin [25].

Methicillin resistant staphylococci (MRS) are increasing nowadays. The present study reported 31.8% Methicillin Resistant *Staphylococcus aureus* (MRSA) infection which was similar to the findings with other studies like Gupta *et al.* [21] with 26.5% MRSA and Ahmedey *et al.* [28] with 35% MRSA. MRCoNS documented in the present study were 35.1% which was similar to Singh *et al.* study reporting 31% MRCoNS, however they documented 57.1% MRSA [10]. Devi *et al.* reported 61% MRCoNS and 33.3% MRSA respectively [25].

In the present study, gram negative bacteria were highly resistant to β -lactams, β -lactams- β -lactamase inhibitors combination, monobactams and third generation cephalosporins. These isolates were also resistant to aminoglycosides and fluoroquinolones reporting them as multidrug resistant isolates [21, 27, 29, 30]. Though amikacin, cefepime, imipenem and other carbapenems were relatively sensitive [7, 19].

CONCLUSION

Early detection of bloodstream infections helps prevent the complications like septicemia and septic shock thereby reducing the mortality rate. The study of prevalence of micro-organisms causing blood stream infection and their antimicrobial resistance pattern can help the clinicians to choose the appropriate antibiotic for empirical therapy. An effort should be taken to promote the antibiotic policy in every hospital to know the preliminary status of antibiotic resistance in their local population. This practice is a good step towards the control of drug resistance occurring due to injudicious antibiotic use.

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