

A Study on the Bacterial Profile of Bloodstream Infections in Rims Hospital

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Abstract:

Introduction: Bacteremia refers to the presence of viable bacteria in blood as evidenced by a blood culture positivity. The rapid and reliable detection of bacteremia by culturing blood is one of the most important functions of a clinical microbiology laboratory. The present study has been taken up to have a better understanding of bacteria causing blood stream infections and their antibiotic sensitivity pattern so as to be a useful guide for clinicians instituting empirical therapy.

Methods: The study was carried out in the Department of Microbiology, RIMS, Imphal over a period of two years from November 2010 to October 2012. During the study period, 280 patients samples from both the inpatients as well as outpatients attending various departments were processed and bacterial pathogens were identified by conventional methods. Subsequently, antimicrobial testing was carried out by Kirby Bauer disc diffusion method as per CLSI recommendations.

Results: Out of the 280 clinically diagnosed cases of bacteremia, 47 (16.78%) were culture positive, all monomicrobial (100%). Gram negative bacteria constituting 23/47 (51.06%) while gram positive isolates were 23/47 (48.93%). Out of the forty seven isolates. *Salmonella Typhi* (29.78%) was the most common organism, followed by CoNS (27.65%), *Staphylococcus aureus* (19.14%), *Escherichia coli* (10.63%) and *Acinetobacter spp* (6.38%). This study showed that vancomycin, imipenem, gentamicin and ceftriaxone are the most effective antibiotics against most of the bacterial pathogens isolated in the study.

Key words: Bloodstream, Bacteremia, RIMS

I. Introduction

Bacteremia refers to the presence of viable bacteria in blood as evidenced by a blood culture positivity.[1] Brill reported the first case of bacteremia (due to *Bacillus pyocyaneus*, now *Pseudomonas aeruginosa*) in 1899. Ten years later, fewer than 40 cases had been reported worldwide, with less than 30 added cases in the 15 years following that. [2]

The major categories of bloodstream infections are intravascular i.e., those that originate within the cardiovascular system eg. infective endocarditis, mycotic aneurysm, catheter associated bacteremia and extravascular i.e., those that result from bacteria entering the blood circulation through the lymphatic system from another site of infection. Other organisms such as fungi may also cause intravascular and extravascular infections. However, bacteria account for the majority of significant vascular infections.[3] Over the past 20-30 years, there have been significant changes in the causation and epidemiology of blood stream infections. While *Staphylococcus aureus* and *E.coli* continue to be the most common causative agents, there have been substantial increase in blood stream infection due to coagulase negative staphylococcus (CoNS).[4] *S.epidermidis* was the most common blood stream infection isolate in studies from Western countries among pediatrics population followed by *S.aureus*.[5]

The rapid and reliable detection of bacteremia by culturing blood is one of the most important functions of a clinical microbiology laboratory. It essentially comprises aseptic collection of blood from patient, most preferably before antibiotic administration, culture of this in a liquid medium, a means of detecting the presence of bacteria growing in the medium, a final phase of subculture on a solid media for identification and sensitivity testing.[6]

The isolation of a bacterium from the blood of a patient is valuable firstly in indicating the urgent need for antibacterial therapy, secondly in revealing the species of bacterial agent against which therapy should be directed and finally in providing a culture for the performance of in vitro drug sensitivity tests. [7]

One of the alarming recent trends in infectious disease has been the increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community acquired infections. Numerous classes of antimicrobial agents have become less effective as a result of emergence of antimicrobial resistance, often as a result of the emergence of the selective pressure of antimicrobial usages. [8]

Among the most important emerging resistance problems are methicillin resistance in staphylococcus (MRSA), penicillin resistance in streptococcus, vancomycin resistance in enterococci (VRE and eventually staphylococci), resistance to extended spectrum cephalosporins (ESBL) and fluoroquinolones among enteriobacteriaceae, and carbapenem resistance in *P.aeruginosa*. [9] The emergence of MDR salmonella strains with resistance to fluoroquinolones and third-generation cephalosporins is a serious development, which results in severe limitation of the possibilities for effective treatment of human infections. [10]

The changing spectrum of microbial pathogens together with antimicrobial resistance trends and clinical significance necessitate continuous monitoring of antimicrobial resistance. Surveillance of antimicrobial resistance is critical in guiding physicians towards appropriate choice of antimicrobial agents to treat these blood stream infections.

Keeping this in mind, the present study has been taken up to have a better understanding of bacteria causing blood stream infections and their antibiotic sensitivity pattern so as to be a useful guide for clinicians instituting empirical therapy.

II. Aims And Objects

1. To study the bacterial profile of bloodstream infections in patients attending RIMS hospital.
2. To study the drug sensitivity pattern of the isolated bacteria to the commonly used antibiotics.

III. Materials And Methods

Study Design: Cross sectional study

Setting: Department of Microbiology, RIMS, Imphal.

Duration Of Study: 2 years (November 2010 to October 2012).

Study Population: Clinically diagnosed cases of bacteremia attending RIMS hospital.

Sample Size: A total of 280 samples were collected for the study. Blood samples were collected from patients, most preferably before antibiotic administration. The details of the patients were recorded.

Method Of Collection Of Blood: [3]

In most cases, blood samples were collected before antimicrobial treatments have been started. In patients receiving antibiotics, the timing of collection of blood samples was just before the next dose of the antibiotic. Using a sterile needle and syringe by aseptic precaution about 5ml of blood was drawn and in case of children, only 2.5ml of blood was collected.

Processing Of Specimens [11,12,13]

The blood was injected into each of the blood culture bottles containing Bile broth and Brain Heart Infusion broth with utmost precaution and aseptic procedure and were incubated at 35-37°C and subcultures were made daily on blood agar and MacConkey agar medium till a significant growth were isolated for a period of seven days.

The colonies were then subjected to a series of tests for identification of the isolates based on the gram stain, motility test and various biochemical tests.

Antibiotic susceptibility testing [14,15]

Antibiotic susceptibility was carried out by Kirby Bauer disc diffusion method as per CLSI recommendations in Mueller Hilton Agar (MHA). ATCC strains available in Microbiology Department, RIMS were put to use for quality control purpose. Antibiotic panels proposed to be used were: gentamicin (10µg), cotrimoxazole (1.25/23.75 µg), ciprofloxacin (5 µg), imipenem (10 µg), cefixime (5 µg), ceftriaxone (30 µg), ceftazidime+clavulanic acid, erythromycin (15 µg), cefotaxime (5 µg), vancomycin (30 µg), ceftazidime (30 µg), linezolid (30 µg), clindamycin (2 µg), nalidixic acid (30 µg), azithromycin (30 µg), chloramphenicol (30 µg).

Detection of Methicillin resistance in Staphylococcus

Staphylococcus species were further screened for methicillin resistance by using 30 µg ceftazidime disc. If the zone diameter ≤ 22 mm, then it was considered Methicillin resistant *S.aureus* (MRSA) and Methicillin resistant CoNS (MR-CoNS) if ≤ 24 mm.

Detection of Extended Spectrum Beta-Lactamase production (ESBL) in Gram negative bacilli¹

All gram-negative bacteria found resistant to 3rd generation cephalosporins, ceftazidime and ceftriaxone were further tested for ESBL production. Confirmatory test was done by potentiated disc diffusin method using ceftazidime alone on one end and ceftazidime with clavulanic acid kept 20 mm apart. An increase in the zone of inhibition of ≥ 5mm around the ceftazidime-clavulanic acid combination was taken as positive.

Statistical Analysis: Data so collected was analyzed by using Software Statistical Package for Social Sciences (SPSS) 16. The statistical testing was carried out by employing Chi square test.

Ethical Issue: Ethical approval was obtained from Institutional Ethical Committee, RIMS, Imphal before the beginning of the study.

IV. Results And Observation

A total of 280 samples of blood collected during a period of two years from clinically diagnosed cases of bacteremia were included in the study. The data collected were then analyzed.

The age of the study subjects ranged from 2 years to 80 years with mean age of 29.25. The female patients (51.07%) outnumbered the male patients (48.92%).

Out of 280 patients, 47 (16.78%) were culture positive, Gram negative bacteria constituted 23/47 (51.06%) while gram positive isolates were 23/47 (48.93%)

Salmonella Typhi (29.78%) was the most common organism, followed by CoNS (27.65%), Staphylococcus aureus (19.14%) , Escherichia coli (10.63%) and Acinetobacter spp (6.38%). There was one isolate each of Klebsiella spp, Enterococcus spp. and Proteus mirabilis. CoNS was the most common isolate among the gram positive cocci (figure 1)

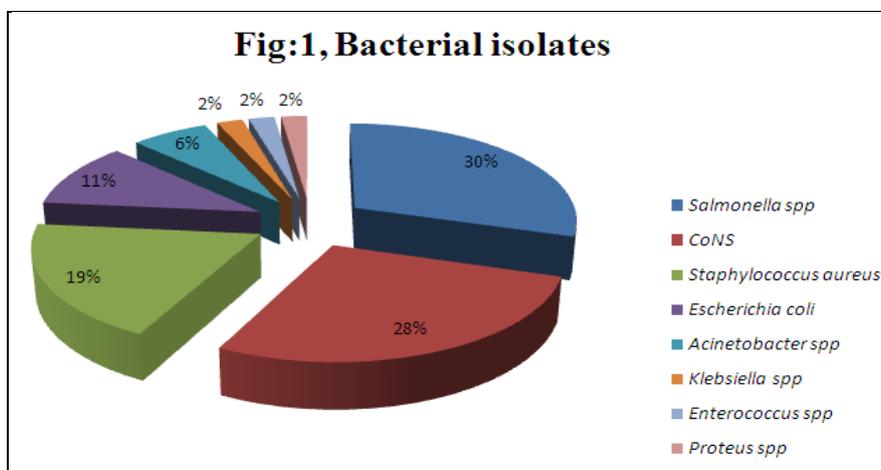


Table 1 shows that all gram positive bacteria were 100% sensitive to vancomycin and linezolid. CoNS showed 70% sensitivity to clindamycin and 75% to gentamicin, while sensitivity was much lower in case of erythromycin (46%), ciprofloxacin (30%), co-trimoxazole (20%) and amoxicillin+clavulanic acid (15%) (figure 10). S.aureus showed high degree of sensitivity to gentamicin (90%) and clindamycin (78%) and reduced sensitivity to cefoxitin and erythromycin (66% each). A much higher degree of resistance was observed against amoxicillin+clavulanic acid (90%) and ciprofloxacin (78%) . Methicillin resistance was observed in 61% of CoNS and 33.3% of S.aureus . Only one species of Enterococcus was isolated and it was resistant to cefoxitin, erythromycin, clindamycin, amoxicillin+clavulanic acid, ciprofloxacin and gentamicin (high level gentamicin resistance).

Table-1

Drug	CoNS, n=13	S.aureus, n=9	Enterococcus spp, n=1
Amoxicillin + clavulanic acid	2 (15.40%)	1 (11.10%)	0 (00%)
Ciprofloxacin	4 (30.80%)	2 (22.20%)	0 (00%)
Co-trimoxazole	3 (20%)	3 (37.50%)	Not done
Cefoxitin	5 (38.50%)	6 (66.70%)	0 (00%)
Gentamicin	9 (75%)	8 (88.90%)	0 (00%)
Erythromycin	6 (46.20%)	6 (66.70%)	0 (00%)
Vancomycin	13 (100%)	9 (100%)	1(100%)
Clindamycin	9 (69.20%)	7 (77.80%)	0 (00%)
Linezolid	13 (100%)	9 (100%)	1 (100%)

All the gram negative isolates were 100% sensitive to imipenem and gentamicin, except for *Proteus* spp. Also, a high degree of sensitivity was observed with ceftriaxone. *E.coli* showed only 20% to 40% sensitivity to amoxicillin+clavulanic acid, ciprofloxacin and ceftazidime. However, sensitivity was increased upto 60% when combined with a beta-lactamase inhibitor (ceftazidime+clavulanic acid) as shown in table 2.

Table-2

Drug	<i>E.coli</i> (n=5)	<i>Acinetobacter</i> spp.(n=3)	<i>Proteus</i> spp (n=1)	<i>Klebsiella</i> spp (n=1)
Amoxicillin+ Clavulanic acid	1 (20%)	1 (33%)	0 (00%)	1 (100%)
Ciprofloxacin	1 (20%)	1 (33%)	0 (00%)	1 (100%)
Gentamicin	5 (100%)	3 (100%)	0 (00%)	1 (100%)
Ceftriaxone	3 (60%)	1 (33%)	1 (100%)	1 (100%)
Ceftazidime	2 (40%)	0 (00%)	0 (00%)	0 (00%)
Ceftazidime + Clavulanic acid	3 (60%)	0 (00%)	1 (100%)	0 (00%)
Imipenem	5 (100%)	3 (100%)	1 (100%)	1 (100%)
Co-trimoxazole	2 (40%)	0 (00%)	0 (00%)	0 (00%)

All the *Salmonella* isolates showed 100% sensitivity to ceftriaxone, chloramphenicol, and cefotaxime. They also exhibit a high degree of sensitivity to azithromycin (93%), co-trimoxazole (93%) and ampicillin (71.4%). High degree of resistance was observed against the commonly used drugs like ciprofloxacin, nalidixic acid (93% each) and cefixime (71.4%) (table 3).

Table-3

ANTIBIOTICS	Sensitive (%)	Resistant (%)
Ciprofloxacin	1 (7.1%)	13 (92.9%)
Ceftriaxone	14 (100%)	0 (00%)
Chloramphenicol	14 (100%)	0 (00%)
Ampicillin	10 (71.4%)	4 (28.6%)
Azithromycin	13 (92.9%)	1 (7.1%)
Co-trimoxazole	13 (92.3%)	1 (7.1%)
Cefotaxime	14 (100%)	0 (00%)
Cefixime	4 (28.6%)	10 (71.4%)
Nalidixic acid	1 (7.1%)	13 (92.9%)

V. Discussion

The present study demonstrated the distribution of microbial isolates causing bloodstream infections and their antibiotic susceptibility pattern to the commonly used oral and parenteral antimicrobial agents.

This study revealed a blood culture positivity rate of 16.8% which was comparable to those conducted by Mehta MP et al [16] and Qureshi M et al [17] who reported a culture positive rate of 16.4% and 16.6% respectively. The low rate of isolation may be explained by the fact that many of the patients probably received antibiotic therapy before they come to the tertiary care hospital and the other reason is that in most of the cases self medication is very common as the medicines are available over the counter. The present study showed a predominance of gram negative bacteremia 24(51%) over gram positive bacteremia 23 (49%). Similar findings were also observed by Qureshi M et al [17] and Decousser JW et al [18].

The most predominant gram negative bacteria isolated in this study was *Salmonella* spp. Phetsouvanh R [19] et al also had reported *Salmonella* enteric serovar Typhi as the most common organism isolated but they observed a higher prevalence rate (50.90%).

The most common gram positive bacterium isolated in this study was CoNS. This finding is consistent with other surveillance studies. CoNS are one of the most common cause of nosocomial bloodstream infections and also the most common blood contaminant.[6] Because only one blood culture was obtained from each of our study patients, it was not possible to determine whether the patients who had CoNS isolated had a true bacteremia or the finding was due to skin contamination. It is also possible that recovery of CoNS could have resulted from other factors such as prolonged use of invasive intravascular devices, prolonged hospital stay, or other underlying co-morbidities. Studies conducted by Warren D, et al [20] have identified CoNS (37%) as leading pathogens in bloodstream infections. The increased isolation of CoNS in the blood culture may also reflect a change from regarding these organisms as normal skin.

Due to increased and indiscriminate use of antibiotics, there has been emergence of antibiotic resistance among microorganisms. This also holds equally good for bloodstream pathogens. This phenomenon of drug resistance varies from microorganism to microorganism and from one place to another. Therefore, the second important purpose of this study was to assess the degree of antimicrobial susceptibility pattern among the key pathogens causing bloodstream infections to the commonly used antibiotics in the hospital. The present

study observed that vancomycin and linezolid appeared to maintain activity against gram positive isolates in 100% of the cases. A high sensitivity to vancomycin (100%) was observed by Qureshi M et al [17], Pfaller MA et al [8] and Atul G et al [21]

VI. Conclusion

On analysis of the results and observation on the study, the following conclusions can be draw

The findings in this study revealed that out of the 280 clinically diagnosed cases of bacteremia, growth was obtained in 47 (16.78%) of the cases, all monomicrobial. The proportion of positive bacterial isolates had no statistical significance to gender and different age groups. However, a statistical significant higher culture positivity rate was observed in those samples collected before antibiotic administration ($p=0.018$).

In this study, all the gram positive bacteria were highly sensitive to vancomycin, linezolid, gentamicin and clindamycin. High degree of resistance were observed to amoxicillin+clavulanic acid, ciprofloxacin and co-trimoxazole. Thirty three percent of the S.aureus isolates were MRSA.

Majority of the gram negative isolates were sensitive to imipenem, gentamicin and ceftriaxone. However, high degree of resistance was noted against amoxicillin+clavulanic acid, ceftazidime and co-trimoxazole. Two-fifth of the E.coli isolates were ESBL producers.

Salmonella spp showed high activity against chloramphenicol, ceftriaxone, cefotaxime, co-trimoxazole, azithromycin and ampicillin but were resistant to ciprofloxacin and cefixime.

Overall antibiotic susceptibility pattern of the isolates clearly suggest existence of drug resistant pathogens of bloodstream infections in our set up. Indiscriminate use of antibiotics in our set up could be one of the principle reasons for this scenario. Rational and proper use of antibiotics would definitely contribute in controlling infections.

Given these findings and the fact that the study on bloodstream infections in our set up is limited, much more in this area needs to be carried out. This will go a long way in understanding proper nature of bloodstream infections as well as its causative agents. This type of study will also help in formulating management guidelines and antibiotic policy for effective management and proper antibiotic therapy in our patients with bacteremia.

ESBL production in our study was comparable (34%) to those observed by Usha A et al [21]. In our study, all i.e 3 (100%) Acinetobacter isolates showed sensitivity to imipenem and gentamicin. Thirty three percent of these isolates were sensitive to amoxicillin+clavulanic acid, ciprofloxacin and ceftriaxone. All the isolates were resistant to ceftazidime, ceftazidime+clavulanic acid, and co-trimoxazole.

Rahbar M et al [22] observed that Acinetobacter spp showed maximum resistance to amoxicillin (89.70%), ampicillin (82%) and cefuroxime (72.70%). Vanitha RN et al [10] found 100% sensitivity to imipenem. However, Alonso R et al [23] reported that Acinetobacter spp were resistant to carbapenems.

Only one species of Proteus vulgaris was isolated in the present study. It was sensitive to ceftriaxone, ceftazidime+clavulanic acid and imipenem and resistant to amoxicillin+clavulanic acid, ciprofloxacin, gentamicin, ceftazidime and co-trimoxazole.

Also, only one Klebsiella spp was isolated which was relatively more sensitive to the antibiotics tested, but was found resistant to ceftazidime, ceftazidime+clavulanic acid and co-trimoxazole.

Alonsa R et al [23] found that 93% of the Klebsiella spp were resistant to 3rd generation cephalosporins. Al-Charrakh AH et al [24] also reported that about 59% to 83% of the Klebsiella spp were resistant to 3rd generation cephalosporins, gentamicin, chloramphenicol and co-trimoxazole, although all the isolates were highly sensitive to rifampicin.

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