Etiology of blood culture from septicemia cases and their antibiotic susceptibility pattern at a tertiary care hospital

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Abstract

Background: Septicemia contributes significantly to morbidity and mortality especially in hospitalized patients. The situation further deteriorates with increasing rate of multidrug resistance. Therefore, the early and accurate diagnosis and start of empirical treatment is a success key to cure such infection. However, for successful treatment, geographical epidemiological etiology is must. Blood culture is the gold standard for the diagnosis of septicemia.

Methods: In the present study, etiological and antimicrobial susceptibility profile of blood culture collected from in-patients, out-patients, and ICU patients in three months at a tertiary care hospital was done. Blood culture positive isolates were identified using standard microbiological, biochemical tests. The antimicrobial susceptibility was determined by Kirby-Bauer disc diffusion method. Oxacillin disc diffusion test was used to identify MRSA.

Results: A total of 217 blood culture were positive out of which 110 (50.6%) were from pediatric group. Gram positive cocci (60.8%) outnumbered Gram negative bacilli (39.2%). Among Gram positive, *S. aureus* (51.5%) was the most common isolate followed by CoNS (39%). Among Gram negative, *Klebsiella spp.* (32%) was most common followed by *Acinetobacter spp.* (21%). Among isolates, 35% were MRSA and 30% were ESBL producer.

Conclusion: The results indicate emergence of high rate of antimicrobial resistance among septicemia patients. The results warrant continuous monitoring of etiology and antimicrobial pattern so as to build geographical epidemiological data. This may help clinicians to select appropriate empirical treatment to control such infections especially in hospitalized patients.

Keywords: Blood culture, Bacteria, Antimicrobial susceptibility, Routine monitoring

Introduction

Blood infections such as septicemia and bactermia leads to life threatening health care associated infections. These infections causes significant morbidity and mortality, worldwide.[1-2] The burden of these infections is as high as 20–50% especially in children's in developing countries.[3] Serious injuries, chronic antibacterial therapy, malnourishment, chronic medical problems, and immuno-suppressants are most predisposing factors, which makes children's more susceptible to blood infections.[4] The onset of blood infections needs immediate attention with rapid and accurate antimicrobial treatment. This needs the correct and earliest identification of etiological agents. Bacteria belonging to groups of *Staphylococci, Streptococci, Enterococci* or *Enterobacteriaceae* are the most common pathogens associated with blood infections.[5] Further, it has been observed that morbidity and mortality of blood infections is higher in case of Gram negative bacilli as compared to Gram positive species.[6] Situation get further complicated with greater septic shock and mortality rate in case of high grade bacteremia or poly-microbial infections.[7]

Emergence of antimicrobial resistance has further added to increase in morbidity and mortality due to blood infections. Development of multidrug resistance is a major concern in blood infections. The prevalence of antimicrobial resistance in septicemia is increasing day by day.[8-9] However, it has been observed that the pattern of antimicrobial resistance among septicemia pathogens varies with geographical and regional location. Since antimicrobial therapy is initiated empirically before the results of blood culture are available, therefore, identification of blood infections pathogens and their antimicrobial susceptibility is of utmost importance for the selection of empirical therapy.[10-11] Further, it is also important epidemiologically to collect data on etiology and antimicrobial surveillance as same changes geographically. Such epidemiological data help clinicians to select right antibiotics according to common pathogen and their antibiogram prevailing in given area.[12-13] In the present study, profile of bacterial pathogens of septicemia patients attending Lady Hardinge Medical College, New Delhi was assessed. The antibiotic susceptibility profile of blood culture isolates was also done.

Material and Methods

Sample collection and culture: The present study was carried out between over a period of three months in the Department of Microbiology, Lady Hardinge Medical College, New Delhi. Blood was collected from clinically diagnosed septicemia cases following strict aseptic precautions. Blood was inoculated aseptically into brain heart infusion broth (1:10 dilution). The culture bottles were incubated at 37°C aerobically and periodic subcultures were done onto MacConkey's agar,

blood agar and chocolate agar after overnight incubation on day 1, day 3, day 5 and on day 7 for isolation. The growth obtained was identified by conventional biochemical test.[14]

Antibiotic Susceptibility: Antibiotic susceptibility testing of isolated microorganisms was done by routinely used antibiotic susceptibility method i.e. Kirby disc diffusion method. Briefly, Mueller Hinton agar plate was divided in two parts. On one part standard strain was plated while on other half, test strain was plated. Antimicrobial discs of different antibiotics were placed on both sides. Methicillin resistance in *Staphylococcus aureus* was detected by using Mueller Hinton agar with 30µg cefoxitin and 1µg oxacillin disk using CLSI guidelines. *S. aureus* ATCC 25923 was used as standard reference for oxacillin susceptibility.[15]

Results

In the present study, a total of 217 blood samples showed growth of various microorganisms (Table 1). The majority of bacteria were isolated from blood culture of hospitalized (55.7%) followed by ICU (29.4%) and 14.7% from outpatients. *S. aureus* (31.3%) was the most common pathogen isolated from blood culture in all three sections. CoNS (23.96%) were second most common pathogen associated with septicemia cases among Gram positive cocci. Among Gram negative bacilli, *Klebsiella spp.* (12.9%) was the most prevalent organism followed by *Acinetobacter spp.* (8.2%), *E. coli* (5%). *Pseudomonas aeruginosa*, a serious opportunistic pathogen was also isolated in 4.6% cases. 3 *Salmonella typhi* were also isolated.

Table 1: Bacteriological profile of microorganisms isolated and identified from blood culture of septicemia cases in different units

Bacterial isolates	Total number	ICU	Inpatient	Outpatient
S. aureus	68 (31.3%)	16	43	9
CoNS	52 (23.96%)	19	25	8
Enterococcus spp.	9 (4.1%)	4	3	2
Streptococci spp.	3 (1.3%)	1	2	-
Klebsiella spp.	28 (12.9%)	8	15	5
E. coli	11 (5%)	4	6	1
Enterobacter spp.	8 (3.6%)	4	4	-
Salmonella spp.	3 (1.3%)	-	2	1
Citrobacter spp.	1(0.4%)	-	1	-
Acinetobacter spp.	18 (8.2%)	4	9	5
Pseudomonas spp.	10 (4.6%)	3	6	1
Nil fermenter	6 (2.7%)	1	5	-
Total	217	64	121	32

The antibiotic susceptibility profile of blood culture Gram positive cocci and Gram negative bacilli isolates was done. The results showed emergence of high rate of antibiotic resistance in both Gram positive and Gram negative bacteria. Out of total 68 *S. aureus* significantly higher rate (35%) of methicillin resistant *S. aureus* (MRSA) was observed. Among Gram positive cocci, majority of isolates showed susceptibility towards gentamicin, amikacin, tetracycline, and vancomycin. A total of 30% isolates were also found to be extended spectrum β lactamase (ESBL) producer (Table 2).

Table 2: Antibiotic resistance profile of Gram positive cocci isolated from blood culture of septicemia cases

Bacterial isolates	Antibiotics used								
	P	OX	G	V	CIP	AK	CTX	COT	T
S. aureus (n=68)	45	24	24	0	33	20	33	40	16
CoNS (n=52)	33	38	17	0	28	25	38	33	20
Enterococci (n=9)	6	NT	6	0	6	6	NT	3	0
Streptococci (n=3)	0	NT	NT	NT	NT	NT	NT	NT	NT

P- penicillin, OX- oxacillin, G- gentamicin, V-vancomycin, CIP- ciprofloxacin, AK- amikacin, CTX- cefotaxime, COT- cotrimoxazole, T- tetracycline, NT- not tested.

Among Gram negative bacteria, *Enterobacteriacae* group (except *S. typhi*) showed high rate of resistance towards ampicillin, amoxycillin, ceftriaxome, ceftazidime, ciprofloxacin, and cefipime. All 3 *S. typhii* showed resistance to ciprofloxacin whereas one each showed resistance towards ceftroaxome, ceftazidime, gentamycin and

cotrimoxazole, respectively. Out of total 34 non fermenters, significantly higher rate of resistance was observed as majority of isolates were resistant towards all the antibiotics tested (Table 3).

Table 3: Antibiotic resistance profile of Gram negative bacilli isolated from blood culture of septicemia cases

Antibiotics	*Enterobacteriacae* except *S. typhi* (n=51)	*S. typhi* (n=3)	Non fermenters (n=34)
Ampicillin	40	0	27
Amoxycillin Clavulanic Acid	35	0	27
Ceftriaxome	33	1	24
Ceftazidime	34	1	28
Gentamicin	19	1	12
Amikacin	20	0	23
Ciprofloxacin	33	3	23
Chloramphenicol	20	0	14
Cotrimoxazole	25	1	20
Pieracillin Tazobactam	Not tested	Not tested	20
Imipenam	15	Not tested	18
Cefipime	35	1	30

Discussion

Blood infections or septicemia remains one of the most important causes of morbidity and mortality especially in developing countries. Symptoms based diagnosis has limited specificity in identifying exact causes. Blood culture is gold standard to identify organisms and antibiotic susceptibility since early and appropriate antimicrobial treatment result in decreased mortality and morbidity among blood infection patients. However, to initiate such therapy, knowledge about pathogen and their antimicrobial susceptibility profile is required.[10-12] Thus, in the era of antimicrobial resistance, defining the pathogen distribution and drugs resistance provides the basis for empirical therapy. Therefore, in the present study, the etiological and antimicrobial profile of blood culture isolates was ascertained in a health care hospital.

In the present study, 217 blood samples showed bacterial growth on culture over a period of three months. 55.7% inpatients showed positive blood culture while 29.4% ICU patients were positive for blood culture. Only 14.7% outpatients were positive for blood culture. Among Gram positive cocci, *S. aureus* was most common isolates among all 3 patients followed by CoNS. On the other hand, *Klebsiella spp.* was the most prevalent organism followed by *Acinetobacter spp.*, and *E. coli*. Three *Salmonella typhi* were also isolated from 2 inpatients and 1 outpatient sample. The results of the present study corroborated with the previous findings where high rate of *S. aureus*, CoNS, *Klebsiella spp.* and *Acinetobacter spp*. was observed on blood culture.[16-19] *S. aureus* and *Klebsiella* as major isolates in blood culture has also been demonstrated.[20] However, slight differences in prevalence of blood infection isolates across geographical areas could be due to number of factors such as blood culture systems, geographical presence, diverse patient's, different etiological agents, season, and clinical practices.[11,21-22]

The antibiotic susceptibility of all the isolates was also determined with standard procedure. The susceptibility results showed high rate of resistance among Gram positive and Gram negative isolates. However, high rate of MRSA (35%) isolates and ESBL (30%) observed in present study is a matter of concern. High rate of vancomycin resistance and ESBL producers among blood culture isolates has also been reported earlier and was stressed upon selection of antibiotics for treatment.[20] Another study reported a resistance rate as high as 59% and suggested that multi drug resistance could be due to uncontrolled use of antibiotics. Moreover, poor clinical practices add up to problem.[23] To reduce the same, specific strategies with antibiotics restriction, combination, and/or cycling may be adopted.

In the present study, 110 (50.6%) isolates were from pediatric wards like NICU, PICU, pediatric surgery, and pediatric nursery ward's. The high rate of blood culture among pediatric patients associated with high rate of antibiotic resistance indicates that serious attention needs to be paid to treat septicemia. In a another study, high rate of *S. aureus* and *E. coli* was observed.[24] The study suggested for the need of epidemiological data on etiological agents and their antibiotic susceptibilities. Further, in a study carried out on 82569 blood samples, it was concluded that ongoing antibiotic susceptibility surveillance is essential to identify the resistance and susceptibility profiles.[25] Similar results have also been reported by number of other studies. It has been concluded that the empirical treatment should be based on prevalence of bacterial isolates and their antibiotic profiles.[26]

Emergence of multi drug resistance in close relation to septicemia alerts for the need for continuous screening for antibiotics susceptibility. Various studies have highlighted the high rate of morbidity and mortality associated with multi drug resistance in septicemia cases.[8,10-11] It has been observed that drug resistance is emerging at alarming level among blood infection isolates and hence the epidemiological data collected will proved to be a useful guide for physicians to select and start correct empirical therapy.[9,27]

The successful treatment of sepsis cases generally relies on early diagnosis and appropriate antimicrobial therapy.[28-29] However, the use of antibiotic should be based upon local epidemiology since it can result in reduced morbidity and mortality. Routinely, the antibiotics combinations are selected for empiric treatment so as to cover the wide range of pathogens. It may vary from aminoglycoside paired with a broad-spectrum β-lactam for nosocomial infections. Ceftriaxone and cefotaxime has been shown effective against *E. coli* and *Klebsiella*.[29] Ceftriaxone has also been proved effective in Gram negative infections especially in nosocomial ones.[30] It was concluded that few classes of antibiotics showed reduced susceptibilities to *Enterobacteriaceae* and *P. aeruginosa*. While older antibiotics such as ceftriaxone still retain high susceptibility against many pathogens.[25] Although selective resistance pressure due to uncontrolled antibiotics use is important, but the clinical practices are also important in spread of resistant pathogens. However, the severe nature of sepsis downgrades the importance of epidemiological data for designing the national antimicrobial strategies. The results of present study indicate that in direction to decrease antibiotic resistance among bacteria, stringent actions need to be taken.

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