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Bacteriological Profile and Antimicrobial Resistance of Blood Culture Isolates from a University Hospital

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Abstract

Context: Blood stream infections are an important cause of mortality and morbidity and are among the most common health-care associated infections. Illness associated with blood stream infection ranges from self-limiting infections to life-threatening sepsis that require rapid and aggressive antimicrobial treatment.

Aims: The objective of the study was to describe the pattern of bacterial isolates from the blood cultures in a university hospital and determine their antibiotic resistance, so that the study can provide guidelines for choosing an effective antibiotic therapy in cases of septicaemia.

Settings and design: This is a retrospective study of 2,400 blood samples collected from clinically suspected cases of bacteraemia reviewed over a period of 2 years.

Methods and material: The isolates were identified by standard biochemical tests and antimicrobial susceptibility testing determined by National Committee for Clinical Laboratory Standards (NCCLS) guidelines.

Results: Positive cultures were obtained in 493 (20.5%) cases. Among culture positive isolates, Gram-negative bacteria accounted for 67.5% cases; most common being Pseudomonas spp. (16%) followed by Salmonella typhi and S. paratyphi A (14.2%). Of the pathogenic Gram-positive isolates, Staphylococcus aureus (8.3%) was the predominant isolate followed by Enterococcus faecalis (3.7%). Maximum Gram-negative isolates were sensitive to cefoperazone-sulbactam combination (81%). Vancomycin sensitivity was reported in 100% Staph. aureus and 83.3% Enterococcus faecalis.

Conclusions: This study provides information on antibiotic resistance of blood isolates. It may be a useful guide for physicians initiating empiric therapy and will help in formulation of antibiotic therapy strategy in this part of the country.

Key words: Septicaemia, Bacteraemia, Antibiotic resistance, Blood culture.

Introduction

Blood stream infections are an important cause of mortality and morbidity and are among the most common health-care associated infections[1]. Illness associated with blood stream infection ranges from self-limiting infections to life-threatening sepsis that require rapid and aggressive antimicrobial treatment[2]. A wide spectrum of organisms have been described and this spectrum is subject to geographical alteration. Patients who are granulocytopenic or inappropriately treated may have a mortality rate that approaches 100%. Moreover, fatalities among patients infected with Gram-negative bacilli are higher than those among patients who have Gram-positive cocci as causative agents of their bacteraemia[3-6]. Increasing antimicrobial resistance is a worldwide concern. The prevalence of resistance in both out-patients and hospitalised patients with septicaemia is increasing, and it varies in accordance with geographical and regional location. In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available. Keeping in mind the high mortality and morbidity associated with septicaemia, a right choice of empiric therapy is of utmost importance. Therefore, the present study was undertaken to describe the antibiotic resistance of blood culture isolates as it may be a useful guide for clinicians initiating the empiric antibiotic therapy.

Material and methods

In this retrospective study, a total of 2,400 blood samples from the clinically suspected cases of bacteraemia were reviewed for a period of two years from October 2002 to September 2004. All the samples were collected at Sir Sunderlal Hospital – a 926 bedded, tertiary care, teaching hospital providing a full range of medical, surgical and super-speciality facilities. Processing of samples was done at the department of Microbiology, Institute of Medical Sciences,

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5 ml of blood was collected from each adult patient by nursing personnel, male orderlies, or physicians, using strict aseptic precautions, and inoculated immediately into 50 ml of 'Brain Heart Infusion' (BHI) broth with 0.025% of sodium polyanethol sulphonate as anticoagulant (HI media, a commercial firm). In paediatric cases 1 - 2 ml of blood was inoculated in 5 - 10 ml of BHI broth. The broths were subcultured on 5% sheep blood agar and MacConkey agar after overnight incubation. A negative result was followed-up by examining the broth daily and doing a final subculture at the end of seventh day. Positive growth was identified by Gram staining, colony characteristics, and standard biochemical tests[7]. Antimicrobial susceptibility testing was performed by Kirby-Bauer disk diffusion method as per NCCLS guidelines[8]. The antibiotic discs used were Ampicillin (10 mg), amoxycillin/clavulanic acid (20/10 mg), Penicillin (10 units), Vancomycin (30 mg), Erythromycin (5 mg), cephalexin (30 mg), ceftazidime (30 mg), ceftriaxone (30 mg), gentamicin (10 mg), tobramycin (10 mg), amikacin (30 mg), netilmicin (30 mg), ciprofloxacin (5 mg), chloramphenicol (30 mg), tetracycline (30 mg), trimethoprim/sulfomethoxazole (1.25/23.75 mg) and cefoperazone/sulbactam (75/30 mg). These were procured from Hi-media, Mumbai; the reference strains used as control for disc diffusion testing were *E. coli* ATCC 25922, *P. aeruginosa* ATCC 27853, *S. aureus* ATCC 25923 and *E. faecalis* ATCC 29212.

All collected data was later on statistically analysed and presented.

Results

During the two-year study period 2,400 blood cultures were analysed. 493 microorganisms were isolated from 466 patients. Of all the isolates, 74.8% were isolated from hospitalised patients while the remaining 25.2% were from those who attended out-patients departments. This corresponds to a rate of 5.8 cases/1,000 hospital admissions. Most infections were due to a single organism, while 22 (4.5%) were of polymicrobial aetiology. In seventeen of these episodes, two different microbes were detected while in five patients three microbes were present. All the polymicrobial infections were from hospitalised patients.

Gram-negative bacteria were encountered more often – 332 (67.5%) – than Gram-positive organisms. The common Gram-negative organisms were *Pseudomonas spp.* (16%) followed by *Salmonella typhi and S. paratyphi A* (14.2%), *Acinetobacter spp.* (12.6%), *Escherichia coli* (11%), *Klebsiella pneumoniae* (7.3%), and *Citrobacter spp* (5%). Among the Gram-positive bacteria, coagulase-negative *Staphylococcus* was the predominant isolate (20.7%) followed by *Staphylococcus aureus* (8.3%) and *Enterococcus faecalis* (3.7%). The distribution of bacterial species of the 493 isolates collected are reported in Table I.

The *S. aureus* strains showed no resistance to vancomycin and resistance to amikacin was also relatively uncommon (19.5%). In *Enterococcus faecalis*, resistance to vancomycin by disc diffusion was seen in 16.6% isolates (Table II).

The most common bacterial isolate from OPD was *Salmonella spp.* Ceftriaxone and ciprofloxacin were very effective with low resistance of 8% and 30.3% respectively, among other enterobacteriaceae members high resistance was noted with tetracycline (89.5%) and ceftriaxone (65.2%). Among aminoglycosides least resistance was noted with amikacin (29.5%). Overall, the most sensitive drug was cefoperazone-sulbactum combination with a low resistance of 19% (Table III).

Discussion

The results of our retrospective study demonstrate the distribution of microbial isolates causing septicaemia and their susceptibility pattern to most commonly used oral and parenteral antimicrobial agents. The incidence of septicaemia in Europe and USA has varied from 3.4 - 28/1,000 hospital admissions[9-11]. A report from Kuwait indicated an incidence of septicaemia to be 10.9/1,000 hospital admissions[12]. An incidence of 5.8/1,000 hospital admissions in our study is comparable with those reported elsewhere.

In most cases of septicaemia, a single microorganism was isolated from blood, while in 4.5% of cases two or more microorganisms were isolated. Septicaemia of polymicrobial aetiology was found only in hospitalised patients. The polymicrobial blood stream infections have been reported by various workers with an incidence ranging from 4.7 - 18.7%, most of which were hospital acquired[5,12].

Table I: Incidence and distribution of microorganisms isolated from blood cultures.

	OPD	Med	Paeds	ICU	Surg	Gynae	Total (n = 493)
CONS	20	32	34	5	6	5	102 (20.7%)
Pseudomonas spp	23	16	32	3	3	2	79 (16%)
Salmonella typhi and S. paratyphi A	32	8	25	3	1	1	70 (14.2%)
Acinetobacter spp.	13	16	21	7	3	2	62 (12.6%)
E.coli	12	9	18	9	3	3	54 (11%)
S. aureus	5	12	13	1	9	1	41 (8.3%)
Klebsiella pneumoniae	6	16	8	6	0	0	36 (7.3%)
Citrobacter spp.	7	4	11	2	0	1	25 (5%)
Enterococcus faecalis	6	5	5	1	0	1	18 (3.7%)
Others	1	1	2	1	1	0	6 (1.2%)

OPD: Out patient department, Med: Medicine ward, Paeds: Paediatric ward, ICU: Intensive care unit, Surg: Surgery ward, Gynae: Obstetrics and gynaecology ward, CONS: Coagulase-negative Staphylococcus, Others: include Proteus spp and Enterobacter spp.

Table II: Resistance pattern of Gram-positive isolates.

	Pn	Ox	Ap	Gm	Hgm	Am	Nt	Cf	Em	Vm	Mx
S. aureus	33 80.5%	31 75.6%	NT	23 56%	NT	8 19.5%	11 26.5%	20 48.8%	21 51.2%	0	16 39%
Enterococcus faecalis	NT	NT	84 4.5%	3 NT	16.7%	NT	NT	3 NT	NT	16.6%	NT

Pn = Penicillin, Ox = Oxacillin, Ap = ampicillin, Gm = gentamicin, Hgm = High-strength gentamicin, Am = Amikacin, Nt = Netilmicin, Cf = ciprofloxacin, Em = Erythromycin, Vm = Vancomycin, Mx = Cefoperazone-sulbactam combination, NT = Not Tested.

Table III: Resistance pattern of Gram-negative isolates.

	Enterobacteriaceae except *S. typhi* (n = 125)	*Salmonella typhi* (n = 66)	Non fermenters (n = 141)
Ampicillin	98 (78.4%)	36 (54.5%)	NT
Amoxycillin-clavulanic acid	93 (74.4%)	30 (45.5%)	NT
Cephalexin	91 (72.8%)	30(45.5%)	NT
Ceftriaxone	38 (30.4%)	5 (7.6%)	112 (79.4%)
Ceftazidime	NT	NT	64 (45.4%)
Gentamicin	56 (44.8%)	22 (33.4%)	56 (39.7%)
Tobramycin	NT	NT	68 (48.2%)
Amikacin	37 (29.6%)	NT	45 (31.9%)
Netilmicin	60 (48.0%)	NT	62 (43.9%)
Ciprofloxacin	53 (42.5%)	20 (30.3%)	63 (44.7%)
Chloramphenicol	NT	46 (69.7%)	NT
Cotrimoxazole	NT	48 (72.7%)	NT
Tetracycline	103 (82.4%)	NT	NT
Cefoperazone-sulbactam	23 (18.4%)	NT	28 (19.8%)

NT = Not tested.

Early clinical suspicion, rigorous diagnostic measures, aggressive initiation of appropriate antimicrobial therapy, comprehensive support care and measures aimed at reversing predisposing causes (e.g., amelioration of an underlying disease, removal of foreign bodies, drainage of abscess) are the cornerstones of successful management of patients with sepsis syndrome[13]. Early initiation of appropriate antimicrobial treatment is critical in decreasing mortality and morbidity among patients with blood stream infections due to Gram-negative organisms[14]. The initiation of such therapy is almost always empirical requiring knowledge of likely pathogen and their usual antimicrobial susceptibility patterns[15,16].

The results of our study demonstrate that blood culture positivity rate in clinically suspected septicaemia cases was 20.5%. Overall, 67.5% of septicaemia was caused by Gram-negative bacilli and remaining 32.5% by Gram-positive bacteria; this was in accordance with other studies[5,17,18].

Like many other studies[19,20] Coagulase-negative Staphylococcus were the most common blood culture isolates; however, given that CONS isolated from blood are often contaminants (>85% are clinically insignificant)[13], their antibiotic susceptibility was not determined. The most frequent pathogenic microorganisms included *Pseudomonas spp.* (16%) followed by *Salmonella spp.* (14.2%), which is similar to another study from north India[18].

S. aureus was frequently found to be penicillin resistant (80.5%). Antimicrobial resistance to erythromycin, gentamicin, ciprofloxacin were above 45%, but none of the strains showed resistance to vancomycin and it could be used in multidrug resistant strains. Similar results have been reported by other workers[17,18].

In the current study, among the antibiotics used for susceptibility testing for Gram-negative isolates, ceftriaxone was very effective against *Enterobacteriaceae*, whereas for non-fermenters like *Pseudomonas spp.* and *Acinetobacter spp.* amikacin was more active. However, the combination of cefoperazone-sulbactam put up for all Gram-negative isolates showed the highest activity among all antibiotics used for these isolates.

The present observation that ceftriaxone was most effective *in vitro* against *Enterobacteriaceae* family has been well documented by other authors as well[20-23]. Aminoglycosides, such as amikacin used singly has also exhibited increased susceptibility pattern[16,24].

None of the antibiotics used singly showed high susceptibility to all the Gram-negative bacilli, so a combination of two or more drugs is recommended to cover the broad range of possible pathogens which may be difficult to distinguish clinically. This may prevent the emergence of resistance as they may have additive or synergistic antimicrobial activity[19].

In conclusion, these data provided much needed information on the prevalence of antimicrobial resistance amongst pathogens causing blood stream infections. The rise in antibiotic resistance in blood isolates emphasises the importance of sound hospital infection control, rational prescribing policies, and the need for new antimicrobial drugs and vaccines. Our results seem helpful in providing useful guidelines for choosing an effective antibiotic in cases of septicaemia and for choosing salvage therapy against hospital resistant strains.

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