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# Prevalence of gram negative organisms isolated from blood culture and their antimicrobial susceptibility pattern : A five-year retrospective study from a tertiary referral hospital

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## Summary

Early diagnosis and proper management of septicaemia can bring down the mortality and morbidity substantially. Current study was aimed at the bacteriological profile of septicaemia cases and their antibiotic susceptibility pattern with special reference to gram negative isolates for planning strategy and management of these cases. The aim of the study was to determine the prevalence of gram negative blood culture isolates and their susceptibility pattern to the commonly used antibiotics. From January 1998 to December 2002, total 4968 cases of clinically suspected samples for bacteremia were processed and susceptibility to commonly treated antibiotics were analyzed according to National Committee for Clinical Laboratory Standard (NCCLS) criteria. *Pseudomonas* spp. were found to be most prevalent (27%) followed by *Salmonella* Typhi (23%), E.coli (14%), *Citrobacter* spp. (12%) *Acinetobacter* spp. (11%) *Klebsiella* spp. (7%), *Proteus* spp. (3.5%) , *Enterobacter* spp. (2.5%) and *Edwrdsiella* spp. (0.1%).

A combination of cefoperazone/sulbactam was found to be the most potent antimicrobial agent. Other antibiotics like ceftriaxone, amikacin, gentamicin and ciprofloxacin were also effective compared to the other drugs tested invitro. For the effective management of bacteremia cases, study of the bacteriological profile with their antibiogram plays a significant role. If facilitates the proper treatment and prevents the possible spread of multi drug resistant bacteria.

**Key-words** : Gram negative bacteria, bacteremia, septicemia, drug resistance.

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## INTRODUCTION

Micro organisms present in the circulating blood, whether continuously or intermittently, are a threat to every organ in the body. Microbial invasion of the blood stream can have serious implications, including shock, organ failure, disseminated intravasular coagulation and death (1). In spite of great advances in antimicrobialn therapy, life support measures and early detection of risk factors, septicaemia continues to be a major cause of mortality and morbidity among people world wide. Blood stream infections are know to be the most common infections in all age groups. A very wide spectrum of organisms has been described for the cases of septicaemia and this spectrumis subject to geographical alterations. More over the organisms isolated are ofter resistant to multiple antibiotics which makes the treatment more difficult and complicated.

For the last five years gram negative bacteria were the most common blood stream pathogens. The current incidence of gram negative bacteremia has been estimated between 70,000 to 3,30,000 cases per yers, with most estimates over 200,000(2). This represents approximately 1% to 3% of all hospitalised patients. Mortality rates among patients who are appropriately treated range from 10% to 38%. Patients who are granulocytopenic or inappropriately treated my have mortality rate that approaches 100%. More over fatalites among patients infected with gram negative bacilli are higher than those among patients who have gram positive cocci as causative agents of their bacteremia (3-6).

Early studies were made in this regard inIndia, where there was an overall predominance of gram negative organisms from blood culture (7-9). An emergence of methicillin resistant *staphylococcus aureus* and vancomycin resistant *Enterococci* in blood culture was also reported (10). Thus it is te urgent need of the hour to know te antimicrobial susceoptibility pattern of the blood isolates.

Early diagnosis and proper management of septicaemia can bring down the mortality and morbidity substantially. So our study was aimed at the bacteriological profile of the septicaemia cases and their antimicrobial sensitivity pattern with special reference to gram negative isolates for planning strategy and management of these cases.

## MATERIALS AND METHODS :

In our study, from January 1998 to December 2002, specimens were taken to estimate the prevalence of the blood culture isolates with special reference to gram negative organisms and to determine the antibiogram of the isolated organisms.

The study includes 4968 cases of clinically suspected bacteremia. Blood samples (5 ml) were collected from each patient using proper aseptic precautions and inoculated immediately in to 50 ml of Brain Heart Infusion both (Hi media laboratories, Mumbai) with 0.025% sodium polyanethol sulfonate as anti coagulant. After overnight incubation at 37°C, subculture was made on to MacConkey agar and Blood agar. The subculture was repeated on 7th day if first subculture was negative. The isolate obtained was further processed as per standard procedure to identify the pathogen (11,12). Anti microbial susceptibility testing was performed by Kirby-Bauer disc diffusion method as per the NCCLS recommendations (13). The antibiotics used were

ampicillin (10μg), amoxycillin/clavulanic acid 20/10 μg), cefalexin (30μg), cefuroxime (30μg), ceftazidime (30μg), ceftriaxone (30μg), gentamicin (10μg), tobramycin (10μg), amikacin (30μg), netilmicin (30μg), ciprofloxacin (5μg), chloramphenicol (30μg), tetracycline (30μg), trimethoprim/sulfomethoxazole (1.25/23.75μg), piperacillin (100μg), carbenicillin (100μg) [Hi media] and cefoperazone/sulbactam (75/30μg), [Pfizer].

The drug used for the member of *Enterobacteriaceae* other than *Salmonella* Typhi were ampicillin, amoxycillin/clavulanic acid, cefalexin, cefuroxime, gentamicin, ciprofloxacin, tetracycline, ceftriaxone, tobramycin, amikacin, netilmicin and cefoperazone/sulbactam. For *Salmonella* Typhi, tobramycin, amikacin, netilmicin and cefoperazone/sulbactam were replaced by chloramphenicol and trimethoprim/sulfomethoxazole. For *Pseudomonas* spp. and *Acinetobacter* spp. ceftriaxone, ceftazidime, gentamicin, tobramycin, amikacin, netilmicin, ciprofloxacin, cefoperazone/sulbactam, carbenicillin and piperacillin were used. The diameter of the zone of inhibition for each antibiotic was measured and interpreted as resistant, intermediate and susceptible according to NCCLS criteria (13).

## RESULTS :

A total of 4968 samples were studied from January 1998 to December 2002 and a total of 1050 (21%) were pathogenic isolates. Out of which 487 (46%) were found to be gram positive orgtanisms which included *Staphylococcus aureus*, coagulase negative *Staphylococcus*, *Enterococcus faecalis* and *Streptococcus* spp. From 530 (50.5%) samples gram negative organisms and from 33 (3%) samples fungi (*Candida* spp.) were isolated. Gram negative organisms included mainly members of *Enterobacteriaceae* family and non fermenting gram negative bacilli.

*Pseudomonas* spp. was found to be most prevalent (27%) followed by *Salmonella* Typhi (23%), *E.coli* (14%), *Citrobacter* spp. (12%), *Acinetobacter* spp. (11%) *Klebsiella* spp. (7%), *Proteus* spp. (3.5%), *Enterobacter* spp. (2.5%) and *Edwardsiella* spp. (0.1%).

For *Pseudomonas* spp. combination of cefoperazone/sulbactam proved to be the most effective (82%) followed by amikacin (64%) while for *Acinetobacter* spp. ceftazidime and amikacin showed 58% sensitivity.

For *Salmonella* Typhi ceftriaxone (92%), ciprofloxacin (72%) and gentamicin (66%) were the most effective, whereas amoxycillin/clavulanic acid and cefuroxime also showed moderate activity. In other members of the *Enterobacteriaceae*, the combination of cefoperaxone/sulbactam (72%) was the most potent drug, followed by ceftriaxone (68%), amikacin (62%) and netilmicin (52%).

A total of 35 isolates [*Pseudomonas* spp. (15 nos.), *Citrobacter* spp. (1 no.)] were resistant of all the antibiotics tested invitro.

## DISCUSSION :

For the effective management of bacteremia cases, study of the bacteriological profile with their antibiotic sensitivity pattern plays a significant role. In the current study a low blood culture isolation rate (21%) might be due to several reasons, e.g. administration of antibiotics before blood collection or the possibility of infection with anaerobes, which can not be ruled out and being a tertiary referral hospital, partially treated patients were usually admitted. The

rate of isolation could be improved if blood is collected after withdrawing all antibiotics for 72 hours. Similar rate of isolation have also been seen in previous studies (8).

In previous studies, gram negative bacilli constituted the majority (80%) of the total isolates and *Pseudomonas* spp. and *Klebsiella* spp. were more dominate among all followed by *E.coli* and negative bacilli (8,9).

In our study, gram negative organism formed 50.5% of the total pathogenic isolates, among them *Pseudomonas* spp. and *Salmonella* Typhi were more prevalent. Prevalence of *Salmonella* Typhi is quite unlike the previous studies conducted in this country where the prevalence has been found to be ranging from 9% to 12% (9). Frequency of the third major pathogen *E.coli* (14%) remains similar to that of the earlier studies (7,8,14). Our study shows that *Pseudomonas* spp. and *Salmonella* Typhi were dominant throughout the study period, though there was an increase of *Citrobacter* spp. and *E.coli* infection in the year 2000. High frequency of *Pseudomonas* spp. indicates nosocomial blood stream infection possibly due to interventions like prolonged vascular catheterization. Similarly, high prevalence of *Salmonella* Typhi throughout the study period indicates its endemicity. The alarming rate of prevalence is a cause of serious concern and needs public awareness regarding hygiene and sanitation.

In the current study, among the antibiotics used singly for susceptibility testing for gram negative isolates, ceftriaxone was the most effective against *Enterobacteriaceae*, whereas for nonfermenters like *Pseudomonas* spp. and *Acinetobacter* spp. amikacin was more active. However the combination of cefoperaxone/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 invitro against *Enterobacteriaceae* family has been well documented by other authors as well (8, 15-18). A similar susceptibility pattern for *Salmonella* Typhi was observed in the previous studies with high activity of ceftriaxone (88%) follwed by ciprofloxacin (79%) (19). For *Pseudomonas* spp. and *Acinetobacter* spp. higher efficacy of amikacin was evidenced by other too (20, 21).

In our study the gram negative isolates did not show high susceptibility to any single antibiotic tested in vitro. This may be due to indiscriminate use of the drugs, genetic background of the isolates and due to some environmental factors which lead to the occurrence of the resistant organism in this region. 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 (22).

*Brill* reported the first case of bacteremia in 1899 (1) where *Pseudomonas aeruginosa* was the causative organism, hundred years later, the *Pseudomonas* spp. continues to be the extremely important causes of blood stream infection in this region. It is highly alarming that there is an increase in resistance of the blood isolates against commonly tested antibiotic and none of the single antibiotic could prove to be effective

if used empirically. The high frequency of drug resistance can be avoided by using drug to which most organism are susceptible. In our study cefoperazone/sulbactam, ceftriaxone gentamicin, ciprofloxacin showed to be more effective, so the combined therapy of these drugs will be good alternative to treat blood stream infections.

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**Table 1. Invitro antimicrobial susceptibility of Pseudomonas spp. and Acinetobacter spp.**

| Antibiotic | Organisms | | | |
|---|---|---|---|---|
| | *Pseudomonas spp.* | | *Acinetobacter spp.* | |
| | N* | %† | N | % |
| Cetftriaxone | 17 | 12 | 11 | 19 |
| Ceftazidime | 77 | 54 | 34 | 58 |
| Gentamicin | 74 | 52 | 18 | 31 |
| Tobramvcin | 80 | 56 | 32 | 55 |
| Amikacin | 91 | 64 | 34 | 58 |
| Netilmicin | 78 | 55 | 28 | 48 |
| Ciprofloxacin | 70 | 49 | 26 | 45 |
| Cefoperazone/sulbactam | 116 | 82 | 30 | 52 |
| Carbenicillin | 69 | 48 | 23 | 39 |
| Piperacillin | 71 | 50 | 18 | 31 |

* No. of susceptible organism.
† Approximate percentage of susceptible organism.

**Table 2. Invitro antimicrobial susceptibility of Enterobacteriaceae family.**

| Antibiotic | Organisms | | | | | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| | E. coli | | Klebsiella Spp. | | Proteus spp. | | Enterobacter spp. | | Citrobacter spp. | | Edwardsiella spp. | | Salmonella Typhi | |
| | N | % | N | % | N | % | N* | %† | N | % | N | % | N | % |
| Amplicillin | 20 | 27 | 5 | 14 | 5 | 28 | 2 | 15 | 14 | 21 | 0 | – | 51 | 47 |
| Amoxycillin/clavulanic acid | 21 | 28 | 5 | 14 | 6 | 33 | 0 | – | 15 | 23 | 0 | – | 70 | 57 |
| Cefalexin | 22 | 29 | 5 | 14 | 5 | 28 | 3 | 23 | 8 | 28 | 1 | 100 | 54 | 44 |
| Cefuroxime | 25 | 33 | 1 | 3 | 5 | 28 | 3 | 23 | 10 | 15 | 1 | 100 | 68 | 56 |
| Ceftriaxone | 52 | 69 | 22 | 61 | 14 | 78 | 10 | 77 | 41 | 64 | 1 | 100 | 114 | 92 |
| Gentamicin | 37 | 49 | 14 | 38 | 10 | 55 | 6 | 96 | 24 | 37 | 1 | 100 | 81 | 66 |
| Tobramycin | 32 | 43 | 21 | 58 | 12 | 67 | 10 | 77 | 27 | 42 | 1 | 100 | – | – |
| Amikacin | 40 | 53 | 27 | 75 | 14 | 78 | 9 | 69 | 37 | 58 | 1 | 100 | – | – |
| Netilmicin | 37 | 49 | 21 | 58 | 12 | 67 | 8 | 61 | 30 | 47 | 1 | 100 | – | – |
| Ciprofloxacin | 27 | 36 | 18 | 50 | 9 | 50 | 9 | 70 | 24 | 37 | 0 | – | 88 | 72 |
| Tetracycline | 8 | 10 | 3 | 8 | 0 | – | 0 | – | 5 | 8 | 0 | – | – | – |
| Cefoperazone/sulbactam | – | – | 32 | 89 | 16 | 89 | 11 | 87 | 29 | 45 | 1 | 100 | – | – |
| Chloramphenicol | – | – | – | – | – | – | – | – | – | – | – | – | 36 | 30 |
| Trimethoprim/Sulfomethoxazole | – | – | – | – | – | – | – | – | – | – | – | – | 50 | 41 |

*No. of susceptible organism.
=Approximate percentage of susceptible organism.