Bacteriological Profile and Antimicrobial Susceptibility of Blood Culture Isolates from Tertiary Care Hospital, Navi Mumbai

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Abstract

Introduction: Blood stream infections (BSI) constitute a significant public health problem with increasing morbidity and mortality in hospitalized patients. Illnesses associated with blood-stream infections range from self-limiting infections to life threatening sepsis and require rapid and aggressive antimicrobial treatment. Again with the emergence of Multi-drug resistant organisms, there is increased risk of death in these patients.

Aim & Objective: The present retrospective study was undertaken to know the profile of organisms causing bacteraemia with their antibiogram from clinically suspected cases of bacteraemia.

Materials and methods: Reports from the blood cultures submitted during the period of one year were analysed. 2994 blood culture samples were received to the microbiology laboratory. The bacterial isolates from positive cultures were identified by standard protocols and antimicrobial sensitivity patterns were determined by CLSI guidelines.

Results: Positive blood cultures were obtained in 10.29 % (308/2994). Of 308 positive cultures, bacterial isolates were obtained in 286/ 308 (92.86%) and 22/308 (7.14%) were Candida spp. Of 286 bacterial isolates, Gram-negative bacteria accounted for 238 (83.22%) cases and Gram-positive were 48(16.78%) with predominance of Klebsiella spp. (22.38%) followed by Pseudomonas spp. (20.98%), Acinetobacter spp. (17.48%) and E.coli 32(11.19%).
Among Enterobacteriaceae, the high % of ESBL production was seen in Klebsiella aeruginosa (55.66%) and in E.coli (29.24%), with highest sensitivity to Imipenem (97.66%), Amikacin (60.16%) and Piperacillin-tazobactum (52.34%). All showed 100% sensitivity to colistin. Among non-fermenters, Imipenem sensitivity was (91.82%) followed by Piperacillin-tazobactum sensitivity (67.27%) & Amikacin sensitivity was (50%). Colistin showed (94.55%) sensitivity. Among Gram-positive isolates (16.78%), Enterococcus spp. were (7.66%) followed by Staphylococcus aureus (6.97%). MRSA was seen in 10% of S.aureus isolates. All Gram-positive isolates showed (100%) sensitivity to Vancomycin and Linezolid.

Conclusion: The present study provides information on the most prevalent pathogens from blood cultures along with their sensitivity towards commonly used antimicrobials. The Majority of bacterial isolates were multidrug resistant. Thus providing useful guide to clinicians in initiating empiric therapy and will also help in formulation of antibiotic policy.

Keywords: Blood stream infections, ESBL, Multidrug resistant bacteria, Antibiotic policy.
INTRODUCTION
Bacteraemia is a state in which bacteria circulate through vascular system whereas Septicaemia is a life threatening condition when bacteria multiply at a rate that exceeds their removal by phagocytes. The symptoms are produced by microbial toxins and cytokines produced by inflammatory cells[1]. Microbial invasion of the blood stream can have serious consequences including shock, multiple organ failure, DIC and death. Thus frequently requiring admissions in ICU for appropriate management[2]. Blood stream infections range from self-limiting infections to life threatening sepsis that requires rapid and aggressive antimicrobial treatment[3]. Bloodstream infections (BSI) are a major cause of morbidity and mortality worldwide. Approximately 200,000 cases of bacteraemia occur annually with mortality rates ranging from 20-50% worldwide[2]. Many bacteria have been reported which cause bacteraemia with variation in distribution from place to place[4]. Organism isolated from blood culture vary according to geographical distribution, and development of multidrug resistant organism is of great concern, as they prolong hospital stay, increase cost of treatment and can be a cause of high mortality [5,6]. In recent years, there has been an increase in the incidence of bacteraemia caused by the members of Enterobacteriaceae and other gram-negative bacilli. Sensitive bacterial strains are now being replaced by multi-drug resistant (MDR) strains of Klebsiella, Pseudomonas, Acinetobacter and Citrobacter species[7,8]. It is therefore necessary to document results obtained from analysis of blood culture for preparing the antibiotic policy for effective management of septicaemia. Timely administration of drugs in patients with septicaemia drastically reduces mortality rate[9]. Thus regular surveillance of blood stream etiology is important in monitoring the spectrum of bacterial pathogens and their sensitivity pattern and providing such data is not only necessary for the clinicians to be aware of the emerging resistant strains of pathogens that are a threat to the community but also provide platform to initiate effective empirical therapy [8,10]. Therefore, this study was done to know the most frequent pathogens from septicaemia patients with their antibiotic susceptibility pattern thus providing useful guide to clinicians in initiating empiric therapy.

MATERIALS AND METHODS
A retrospective analysis was done in the Department of Microbiology Laboratory of Dr. D. Y. Patil Medical College and Hospital on blood samples received for culture over a period of one year from JUNE 2015 to JUNE 2016. A total of 2994 blood culture samples from clinically suspected cases of septicaemia were received and processed. A Data on socioepidemiographic variables such as age, gender, blood culture results and their antibiotic susceptibility pattern were collected manually from laboratory record book for blood culture.

Blood samples were collected aseptically from patients for routine blood culture before taking any antibiotics. The venepuncture site was disinfected with 70% alcohol and 2% tincture of iodine before taking the sample.

From adults 10 ml, and children 3 to 5 ml of blood was collected and immediately inoculated into Adult and Paediatric BACTEC blood culture vials respectively and incubated in BACTEC 9050 blood culture instrument (Becton-Dickenson, USA) at 37°C.

All BACTEC positive samples were subjected to Gram staining followed by inoculation on 5% sheep blood agar, chocolate agar and MacConkey agar plates and were incubated at 37°C FOR 18-24 hours. Identification of isolates were done according to standard bacteriological technique and were identified based on the colony morphology, gram stain and biochemical tests such as Catalase, Coagulase, Bile esculine (BE), Optochin( OP) Bacitacinc(BC) discs for Gram positive isolates and Oxidase, Indole, Citrate, Urease, Triple sugar iron tests for gram negative isolates with serological identification for Salmonella species[11,12,13].

Antibiotic susceptibility testing was performed on Muller-Hinton agar by Kirby-Bauer Disk
Diffusion method according to Clinical Laboratory Standard Institute (CLSI) guidelines\(^ {14}\). For Gram positive bacteria, Ciprofloxacin (CIP) (5 µg), Erythromycin (E) (15 µg), Penicillin (P) (10U), Gentamycin (GM) (10 µg), Vancomycin (VA) (30 µg), Linezolid (LZ) (30 µg), Oxacillin (1 µg), Trimethoprim/Sulphamethoxazole (TMP) (25 µg) were used.

For Gram negative bacteria, Ampicillin (AMP) (10µg), Gentamycin (GM) (10 µg), Ciprofloxacin (CIP) (5 µg), Cefotaxime (30 µg), cefepime (30 µg), Nalidixic acid(30 µg), Ceftriaxone (30 µg), Trimethoprim/Sulphamethoxazole (TMP) (25 µg), Ceftazidime (30 µg), Ceftazidime-Clavulanic acid (30/10 µg), Tobramycin (10 µg), Amikacin (30 µg), Aztreonam (30µg), Colistin (25 µg), Piperacillin-Tazobactum (100/10 µg), Imipenum (30 µg) were used which were procured from Hi-Media, Mumbai.

The susceptibility and resistance were interpreted according to Clinical Laboratory Standard Institute (CLSI) guidelines\(^ {14}\). Escherichia coli (ATCC25922), Staphylococcus aureus (ATCC25923) and Pseudomonas aeruginosa (ATCC27853) were used as reference strains for culture and susceptibility testing.

**DATA ANALYSIS**

Data was analysed using Minitab 17.0 software. The z-test for proportion and Chi-square goodness of fit test was used to test significance. p-values of less than .05 were treated as significant.

**RESULTS**

During the period from June 2015 to June 2016, 2994 blood samples from septicemia suspected patients were received and processed routinely. Out of 2994 blood cultures, 308 (10.29%) were positive for culture growth.

**TABLE 1.** Age and Sex-wise distribution of positive blood cultures (n=308)

| AGE               | MALES | FEMALES | TOTAL |
|-------------------|-------|---------|-------|
| Infant (<1yr)     | 36    | 18      | 54    |
| Children (1-12yrs)| 32    | 16      | 48    |
| Adolescent (13-18yrs) | 17    | 11      | 28    |
| Adult (>18yrs)   | 114   | 64      | 178   |
| **TOTAL**        | 199   | 109     | 308   |

From 308 culture positive samples, 199 (64.61%) were males and 109 (35.39%) were females with age ranging from 1 day to 90 years. Overall proportion of males was found to be significantly higher than the proportion of females (p < .001)

**TABLE 2:** Ward-wise distribution of blood isolates. (n=308)

| Organisms              | NICU | PICU | PAEDS | MICU | FMW | MMW | OTHERS | TOTAL |
|------------------------|------|------|-------|------|-----|-----|--------|-------|
| Klebsiella Spp         | 9    | 5    | 3     | 44   | -   | 1   | 2      | 64    |
| Pseudomonas aeruginosa | 10   | 3    | 2     | 34   | 5   | 4   | 2      | 60    |
| Acinetobacter Spp      | 7    | 6    | 2     | 32   | -   | 1   | 2      | 50    |
| E.coli                 | 4    | -    | 1     | 23   | 3   | 1   | -      | 32    |
| S.typhi                | -    | 2    | 15    | 2    | 2   | 1   | 22     |       |
| Enterobact.sp          | 4    | -    | 2     | 3    | -   | -   | 1      | 10    |
| S.aureus               | 9    | 3    | 5     | 1    | 2   | 2   | 20     |       |
| Enterococcus Spp       | 7    | -    | 4     | 9    | -   | -   | 2      | 22    |
| Streptococcus spp      | 4    | -    | 1     | 1    | -   | -   | -      | 06    |
| Candida spp.           | 6    | 1    | -     | 13   | -   | 1   | 2      | 22    |
| **TOTAL**              | 60   | 20   | 30    | 166  | 9   | 12  | 11     | 308   |

p-values calculated using Chi-square goodness of fit test (p < .0001).

Out of 308 positive blood cultures, medical ICU showed maximum culture positivity 166(53.90%) followed by NICU & PICU combined 81 (26.30%).

Bacteremia was seen in 286(92.86%) of patients whereas fungaemia was seen in 22 (7.14%) cases. All infections were monomicrobial. Among 286 bacterial blood culture isolates, Gram negative and Gram positive bacteria constituted 238(83.22%) and 48(16.78%), respectively.

**Table 3:** Distribution of bacterial isolates obtained from positive blood cultures. (n=286)

| Bacterial isolates           | Number | Percentage (%) |
|-------------------------------|--------|----------------|
| Klebsiella pneumonia          | 64     | 22.38%         |
| Pseudomonas spp.              | 60     | 20.98%         |
| Acinetobacter spp.            | 50     | 17.48%         |
| E.coli                        | 32     | 11.19%         |
| Salmonella typhi              | 22     | 7.70%          |
| Enterococcus spp.             | 22     | 7.70%          |
| S.aureus                      | 20     | 6.99%          |
| Enterobacter spp.             | 10     | 3.50%          |
| Strep.spp                     | 06     | 2.10%          |
| **TOTAL**                     | 286    | 100%           |

p-value calculated using Chi-square goodness of fit test.
The predominant bacterial isolate from blood culture was Klebsiella pneumoniae 64 (22.38%) followed by Pseudomonas aeruginosa 60 (20.98%), Acinetobacter spp. 50 (17.48%), E.coli 32 (11.19%), Salmonella typhi 22 (7.70%), Enterococcus spp. 22 (7.70%), Staphylococcus aureus 20 (6.99%), Enterobacter spp. 10 (3.50%), Streptococcus spp. 6 (2.10%).

| Antibiotic            | Enterococcus spp (n=22) | S.aureus (n=20) | Streptococcus Spp (n=60) |
|-----------------------|-------------------------|-----------------|-------------------------|
| Penicillin            | 0(0%)                   | 0(0)            | 05(83.33%)              |
| Oxacillin             | -                       | 18(90%)         | -                       |
| Erythromycin          | 0(0)                    | 05(25%)         | 05(83.33%)              |
| Gentamicin            | 03(13.64%)              | 10(50%)         | 05(83.33%)              |
| Ciprofloxacin         | 03(13.64%)              | 10(50%)         | 05(83.33%)              |
| Co-trimixazole        | -                       | 06(30%)         | -                       |
| Vancomycin            | 22(100%)                | 20(100%)        | 100%                    |
| Linezolid             | 22(100%)                | 20(100%)        | 100%                    |
| p-value               | <.001**                 | <.001**         | .0139*                  |

*: Sig. at 5% level, **: Sig. at 1% level, NS: Not significant, p-values calculated using Chi-square goodness of fit test

All Gram – positive isolates showed 100% sensitivity to Vancomycin and Linezolid. MRSA was found in 2 isolates of S.aureus 2/20 (10%). The Gram-positive isolates showed high degree of resistance in Penicillin (89.58%) followed by Erythromycin sensitivity in 10 (20.83%) and resistance in 38 (79.17%), Gentamicin sensitivity in 18 (37.5%) and resistance in 30 (62.50%), Ciprofloxacin sensitivity in 18 (37.5%) and resistance in 30 (62.50%), S.aureus showed Co-trimoxazole sensitivity in 6 (30%) and resistance in 14 (70%).

| Antibiotic               | Klebsiella Spp(n=64) | Pseudomonas aeruginosa(n=60) | Acinetobacter.spp (n=50) | E.coli (n=32) | Salmonella (n=22) | Enterobacter Spp(n=10) |
|--------------------------|----------------------|------------------------------|--------------------------|--------------|------------------|------------------------|
| Amikacin                 | 31(48.44%)           | 34(56.67%)                   | 21(42%)                  | 19(59.36%)   | 22(100%)         | 5(50%)                 |
| Ampicillin               | 0                    | NT                           | 0                        | 0(3.12%)     | 0                | 0                      |
| Gentamicin               | 24(37.50%)           | 18(30%)                      | 16(32%)                  | 17(53.12%)   | 22(100%)         | 5(50%)                 |
| Ciprofloxacin            | 23(35.94%)           | 32(53.33%)                   | 20(40%)                  | 4(12.5%)     | 20(90.91%)       | 4(40%)                 |
| Ceftazidime              | 5(7.81%)             | 29(48.33%)                   | 11(22%)                  | 1(3.12%)     | -                | -                      |
| Ceftazidime+Clavulanic acid | 5(7.81%)         | NT                           | 11(22%)                  | 1(3.12%)     | -                | -                      |
| Cefotaxime               | 5(7.81%)             | 13(21.67%)                   | 11(22%)                  | 1(3.12%)     | 20(90.91%)       | 4(40%)                 |
| Ceftriaxone              | 5(7.81%)             | 13(21.67%)                   | 11(22%)                  | 1(3.12%)     | 20(90.91%)       | 4(40%)                 |
| Piperacillin -Tazobactam | 25(39.06%)           | 47(78.33%)                   | 27(54%)                  | 14(43.75%)   | -                | 6(60%)                 |
| Imipenem                 | 61(95.31%)           | 57(95%)                      | 44(88%)                  | 32(100%)     | 22(100%)         | 10(100%)               |
| Colistin                 | 64(100%)             | 58(96.67%)                   | 46(92%)                  | 32(100%)     | 22(100%)         | 10(100%)               |
| CoT                      | 11(17.19%)           | NT                           | 19(38%)                  | 4(12.5%)     | 22(100%)         | 4(40%)                 |
| Nalidixic acid           | NT                   | NT                           | NT                       | NT           | 20(90.91%)       | NT                     |
| Aztreonam                | NT                   | 2(3.33%)                     | NT                       | NT           | NT               | NT                     |
| Tobramycin               | NT                   | 30(50%)                      | NT                       | NT           | NT               | NT                     |
| Cefepime                 | NT                   | 21(35%)                      | NT                       | NT           | NT               | NT                     |
| p-values                 | <.001**              | <.001**                      | <.001**                  | <.001**      | P=.02*           | P=.127, NS              |

*: Sig. at 5% level, **: Sig. at 1% level, NS: Not significant, p-values calculated using Chi-square goodness of fit test
Among Enterobacteriaceae (128), 98 (76.56%) isolates were resistant to 3rd generation Cephalosporins. % of ESBL production (106), in E.coli is (29.24%), Klebsiella aeruginosa (55.66%) and Enterobacter spp. (5.66%). Imipenem sensitivity was seen in 125(97.66%) and resistance in 3(2.34%). All showed 100% sensitivity to colistin. Amikacin sensitivity in 77(60.16%) and resistance in 51(39.84%). Gentamicin sensitivity in 68 (53.13%) and resistance in 36 (32.73%). Ampicillin resistant was seen in 237 (99.58%) of total isolates.

In our study, the clinical suspected septicaemia cases was quiet comparable to the studies by Manjula Et al 2005 [15] showed 9.94% by Usha and Pushpa 2007[16] showed 10% and Mehta Et al 2005[17] showed 9.94% positivity rate and 11.2% Shalini s et al 2010 [18] and 10.8% from Iran,Hamed Ghadhiri et al 2012 [19].

In contrast to the above reports, the studies done in India and other countries have reported high frequency of positive blood culture ranging from 18 to 45% [20-27].

Such differences in prevalence of BSI across countries could be due to the difference in blood culture system, the study design, geographical location, nature of patient population, epidemiological difference of the etiological agents, and differences in the infection control policies between nations [6, 25, 27]. Bacteraemia was seen in 286 (92.86%) of patients whereas fungaemia was seen in 22 (7.14%) cases.

From total septicema cases, 199 (64.61%) were males and 109 (35.39%) were Females. Pathogen isolation rate was found to be high in medical ICUs (53.90%) followed by NICU and PICU (26.30%).

In our study, Gram negative and Gram positive bacteria constituted 83.22% and 16.78% respectively. Similar to the present study, in most of the studies, gram negative bacilli have taken over the gram-positive organisms, especially in hospital settings. This finding was in accordance with other studies [7,8,17,23,28,29,30,31,32,33].

In our study, the predominant isolates were Klebsiella pneumoniae 64 (22.38%) followed by Pseudomonas aeruginosa 60(20.98%), Acinetobacter spp. 50 (17.48%), E.coli 32 (11.19%), which was in accordance with other studies [7,8,23].

The high occurrence of non-lactosefermenters especially Pseudomonas spp. & Acinetobacter spp., which has emerged as important nosocomial pathogens, is of concern; both of these bacteria are associated with a high degree of resistance to antibiotics and are associated with high morbidity and mortality [7, 8, 28, and 32].

In our study, S.typhi was isolated in 7.70% of total cases which was quiet comparable to the findings by Wasihun, Wlekidan 2015 [25] who reported about 5.6% S.typhi. But the other studies reported an increasing incidence of Salmonella species between 12 % and 15 % [7,17,32].

In our study, we found all cases of bacteremia with single microorganism similar to other studies [23, 27, 35].

Surprisingly, in our study Gram-positive isolation from BSI was low 16.78%. Among the Gram-positive isolates from total bacteremic cases, the most predominant isolate was Enterococcus spp. 7.70% followed by S.aureus 6.99% whereas studies by Mathur et al 2014 [28] reported 9% and
Vanitha Et al 2012\textsuperscript{[7]} who isolated 8.7% Enterococcus spp. But their predominant Gram-positive isolate was S.aureus. Such low isolation of Gram-positive pathogens suggest an increasing emergence of Gram-negative isolates with Multi-drug resistance and ESBL production, so a strict antibiotic policy should be implemented with emphasis on local susceptibility findings\textsuperscript{[34]}.

MRSA was found in 2 isolates of S.aureus 2/20 (10%) All Gram – positive isolates showed 100% sensitivity to Vancomycin and Linezolid. With increasing resistance to Penicillin (89.58%) Gentamicin (62.50%), Ciprofloxacin (62.50%) and Erythromycin (79.17%).

In the present study 55.66% of Klebsiella pneumoniae and 29.24% of E.coli & 5.66% of Enterobacter spp. were ESBL producers which was higher than the other studies\textsuperscript{[6, 21]} but such high level of ESBL production was also seen in studies by other authors\textsuperscript{[23, 34]}.

Among Enterobactericeae, Imipenem sensitivity was seen in 97.66% and resistance in 2.34% which was consistent with the other study\textsuperscript{[34]}.

Above observation on possible emergence of Carbapenem-resistant Enterobactereae point towards the possible irrational use of carbapenems occurs\textsuperscript{[36]}. All showed 100% sensitivity to colistin. All Enterobactericeae showed better activity to Amikacin 60.16%, Piperacillin-Tazobactum combination 52.34% and Gentamicin 53.13%. Among non-fermenters, Imipenem sensitivity was seen in 91.82% and resistance in 8.18%. Colistin sensitivity 94.55% and showed better activities to Piperacillin-Tazobactum 67.27% followed by Amikacin 50%. These results were comparable to other studies\textsuperscript{[32, 37]}

S. pneumoniae were isolated. Given this steadily growing danger of MDR and ESBL-producing isolates in South Kivu, as underlined by our findings, a strict antibiotic policy should be implemented urgently in the province with an emphasis on local susceptibility findings.

Another important issue in the province is the lack of regulation regarding prescription of antibiotics, which are widely used, even for minor illnesses such as rhinitis. Whereas no study has assessed this phenomenon, it is worth noting that a recent study documented a high level of irrational prescription of antibiotics by healthcare profession associated with a steady increase of antimicrobial drug resistance.

The study identified Gram negative bacteria as the predominant organisms causing blood stream infections and most of them were found to be multi-drug resistant. Carbapenems, Piperacillin-Tazobactum combination and amikacin were found to be most effective for Gram-negative isolates whereas Vancomycin and Linezolid were found to be most effective for Gram-positive isolates. The increase in the prevalence of Multidrug-resistant bacteria emphasize the immediate need for rational use of antibiotics, formulation of antibiotic policy, and implementation of infection control practices for the effective management and prevention of drug resistance.

Gram-positive bacteria did not play an important role in BSI when compared with other studies in associated with a steady increase of antimicrobial drug resistance.

CONCLUSION

Bacteraemia is an independent risk factor of morbidity and mortality in cases of septicaemia. With the emergence of multi resistant bacteria in many regions worldwide, leaving limited options for treatment. Thus timely detection and knowledge of most likely pathogens causing BSI along with their antibiotic susceptibility pattern will help the clinicians in choosing appropriate antimicrobials for treatment which will reduce the major burden of BSIs in critically ill patients and will also minimize the further emergence of resistance. Therefore, there should be an intensive surveillance, antibiotic policy formulations and preventive efforts for the effective management and prevention of drug resistance.

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