Blood stream infections can lead to life threatening sepsis and require immediate antimicrobial treatment. Blood culture is an essential tool for the investigation of clinically suspected sepsis. The present study has been conducted to describe the profile of bacterial isolates from blood cultures and their antibiotic resistance. This is a prospective study of 273 blood cultures, collected from clinically suspected cases of bacteraemia studied over a period of five months in a tertiary care hospital in Ujjain, M.P. The isolates were identified by standard biochemical tests and antimicrobial resistance patterns were determined by CLSI guidelines. Blood cultures were positive in 70 (25.6%) patients by BacT alert system. Gram positive organism accounted for 51.4% cases; most common being *Staphylococcus aureus* (47.3%) followed by *Enterococci* (4.3%). Of the Gram negative isolates, *Klebsiella spp* (14.3%) was the most common followed by *Pseudomonas spp*. (8.6%). *Candida albicans* was isolated in 2.8% cases. Gram positive isolates showed high resistance to penicillin (81.8%) and least resistance to linezolid (18.2%). Gram negative isolates were found high resistance to amoxy-clav (90%) and least resistance to Imipenem (20%). 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.
needed to detect positive blood cultures as well as decreases the specimen handling (Kim et al., 2010). Published guidelines recommend that the interval between the collection of blood and the entry of the bottles into an automated blood culture system should not be longer than 2 or 4 h; also manufacturer instructions indicate that inoculated vials should be transported to the laboratory as quickly as possible (Clinical Laboratory Standards Institute, 2007; Public Health England, 2014). The changing epidemiology and susceptibility patterns of microorganisms emphasize the necessity of constant surveillance of blood stream infections (Muhammad et al., 2013). The present study was done to analyze various organisms causing bacteremia and their antibiotic resistance pattern. This study wide enable using appropriate antibiotic, may decrease the hospital stay and cost of treatment and reduce Mortality.

The main objectives of this study includes, Isolation and identification of pathogens from blood samples by automated 3D BacT/ALERT blood culture system and their antibiotic resistance patterns in a tertiary care center, Ujjain M.P, India.

Materials and Methods

This study was carried out at the Department of Microbiology, R.D. Gardi Medical College, Ujjain, Central India. A total of 273 blood samples from clinically suspected cases of septicemia were studied during a 5 months period from Oct 2016 to Feb 2017. Blood samples were collected by using strict aseptic precautions and inoculated immediately into BacT/ALERT FA plus and PF plus aerobic blood culture bottles. After collection these bottles were immediately incubated in BacT/ALERT 3D (bioMérieux) – a fully automated blood culture system for detection of growth in blood culture. The negative results were followed up to 7 days and final report was issued. The BacT/ALERT automatically gives a signal alert. The positive bottles were then subculture on blood, MacConkey and chocolate agar. These plates were incubated aerobically at 37 C and examined after 18-24 hours. Final identification was done by colony characteristics, Gram's staining, motility testing (hanging drop preparation) and routine biochemical test (Catalase, coagulase, indole, methyl red, citrate, urease, Triple sugar iron, PPA, and oxidase testing). Fungal isolate was identified by Gram’s staining showing gram positive budding yeast cells and germ tube testing. Antimicrobial susceptibility testing of bacterial isolates was done by the Kirby-Bauer disc diffusion method using Muller Hinton agar media as per CLSI guidelines.

Results and Discussion

During the study period, 273 blood cultures were analyzed of which 70 (25.6%) were positive and 203 (74.4%) were culture negative (Figure 1). Amongst total samples 147 (53.8%) were male and 126 (46.2%) were female patients. The age range varies from 1 month to 87 years. The incidence of blood culture samples was the highest (26.4%) in 1-10 years age group followed by 0-1 yrs (21.3%). Detail of age groups distribution was given in (Table 1 and Table 2). Blood samples were collected from different wards and ICUs in the hospital. Maximum number (37.7%) of blood samples were obtained from pediatrics wards. Details are given in (Table 2). Out of 70 blood culture positive isolates, 36 (51.4%) were Gram positive organisms, 32 (45.7%) were Gram negatives organisms, while 2 (2.8%) isolates were fungi (Candida spp.). We have also isolate 3 gram positive bacilli (bacillus) and 1 coagulase negative staphylococcus (CoNS) were probably skin contaminant excluded from the positive isolates. Detail description of isolates was
shown in (Table 3 and Figure 3). Staph aureus (47.2%) was the predominant organism in all bacterial isolates followed by Klebsiella spp (14.3%). Antibiotic resistance pattern of the gram positive organism are shown in (Table 4). Resistance ranges from 18.2% to 81%. Staph aureus exhibit most resistance to penicillin (81.8%) followed by cefoxitin (MRSA) 63.3% and least resistance to linezolid (18.2%). Among gram negative organisms Klebsiella was the predominant isolate which exhibit most resistance to amoxiclav (90%) followed by Amikacin (80%) and least resistance to imipenem (20%). Pseudomonas exhibit most resistance to Cefepime (66.6%) followed by Ceftazidime (50%) and least resistance to imipenem and Amikacin (16.6%). Detail description of antibiotic resistance pattern of gram negative organisms is shown in (Table 5).

The findings are matching these respected by sepsis is one of the leading causes of death, and rapid identification of blood stream infection is mandatory to perform adequate antibiotic therapy. In the present study a total of 273 blood culture samples were collected and analyzed, of which 70 (25.6%) were positive by BacT/Alert system. which is quite similar to Sahoo et al., (2016) and Alam et al., (2011) but quite lower Kavitha et al., (2010) and Maimoona et al.,(2014). Majority of the patients (26.4%) were in the 1-10 years age group. Maximum number (37.7%) of blood culture came from pediatrics ward. The incidence of Gram-positive organism was (51.4%) while (45.7%) were Gram-negative organisms which was quite similar to Kalpesh et al., (2014) and China et al., (2013), but in other studies like Maimoona et al., and Ayobola et al., (2011) Gram-negative organisms have taken over Gram-positive organisms in hospital settings. This indicates that infections by Gram-positive organisms constitute a significant threat to bacteremia and septicemia in our hospital setup and the spectrum of organisms is subject to geographical alterations. Among Gram positive organism staphylococcus aureus was the commonest (47.2%) isolate followed by Enterococcus (4.3%) which was quite similar to study by Kalpesh et al., (2014) and Anbumani et al., (2008).

While Klebsiella spp was the most predominant (14.3%) isolate among gram negative organism which was in accordance with Panday et al., (2017).

Table 1 Age and gender wise distribution of blood culture of sepsis patients

| Age group     | Total | Male | Female |
|---------------|-------|------|--------|
| 1 month - 1 year | 58    | 28   | 30     |
| >1-10 year    | 72    | 43   | 29     |
| 11-20 year    | 40    | 21   | 19     |
| 21-30 year    | 23    | 14   | 9      |
| 31-40 year    | 18    | 11   | 7      |
| 41-50 year    | 25    | 13   | 12     |
| 51-60 year    | 16    | 9    | 7      |
| >61 year      | 21    | 8    | 13     |
| Total         | 273   | 147  | 126    |
Table 2: Blood samples collected from different wards and ICUs

| Word     | Number of cases | Percentage (%) |
|----------|-----------------|----------------|
| NICU     | 10              | 3.7            |
| MICU     | 19              | 6.9            |
| PICU     | 60              | 21.9           |
| SICU     | 12              | 4.4            |
| P/W      | 103             | 37.7           |
| MEDICINE | 29              | 10.7           |
| SURGERY  | 7               | 2.5            |
| OTHER ICUs | 24           | 8.9            |
| ORTHO    | 9               | 3.3            |

Table 3: Organisms isolated from positive blood samples

| Organism           | Number of positive cases | Percentage |
|--------------------|--------------------------|------------|
| *Staphylococcus*   | 33                       | 47.2%      |
| *Enterococcus*     | 3                        | 4.3%       |
| *Pseudomonas spp.* | 6                        | 8.5%       |
| *E. coli*          | 4                        | 5.7%       |
| *Klebsiella spp.*  | 10                       | 14.3%      |
| *Citrobacter spp.* | 3                        | 4.3%       |
| *Enterobacter*     | 4                        | 5.7%       |
| *Candida*          | 2                        | 2.8%       |
| Non-fermenter GNB  | 5                        | 7.2%       |
| Total              | 70                       | 100%       |

Table 4: Antibiotic resistant pattern of gram positive bacteria

| Antibiotic drugs | Staph. aureus (n=33) | Enterococcus (n=3) |
|------------------|-----------------------|--------------------|
|                  | No of resistance isolates | Percentage (%) | No of resistance isolates | Percentage (%) |
| Penicillin       | 27                     | 81.8              | 2                     | 66.6          |
| Cefoxitin        | 21                     | 63.6              | -                     | -             |
| Cotrimoxazole    | 17                     | 51.5              | -                     | -             |
| Erythromycin     | 19                     | 57.6              | -                     | -             |
| Clindamycin      | 14                     | 42.4              | -                     | -             |
| Tetracycline     | 7                      | 21.2              | -                     | -             |
| Vancomycin       | 12                     | 36.4              | 1                     | 33.3          |
| Linezolid        | 6                      | 18.2              | 0                     | 0             |
| Ampicillin       | -                      | -                 | 2                     | 66.6          |
| High level Gentamycin | -                  | -                 | 1                     | 33.3          |
### Table 5 Antibiotic resistant pattern of gram negative bacteria

| Antibiotic drugs     | E.coli (n=4) Resistance No (%) | Pseudomonas (n=6) Resistance No (%) | Klebsiella (n=10) Resistance No (%) |
|----------------------|--------------------------------|--------------------------------------|-------------------------------------|
| Amikacin             | 1 (25%)                        | 1 (16.6%)                            | 8 (80%)                             |
| Aztronem             | -                              | 2 (33.3%)                            | -                                   |
| Piperacillin         | 3 (75%)                        | 2 (33.3%)                            | 3 (30%)                             |
| Cefepime             | 3 (75%)                        | 4 (66.6%)                            | 7 (70%)                             |
| Ceftazidime          | 2 (50%)                        | 3 (50%)                              | 6 (60%)                             |
| Ciprofloxacin        | 2 (75%)                        | 2 (33.3%)                            | 4 (40%)                             |
| Imipenem             | 1 (25%)                        | 1 (16.6%)                            | 2 (20%)                             |
| Amoxy-clav           | 4 (100%)                       | -                                    | 9 (90%)                             |
| Piperacillin tazobactum | 1 (25%)                      | 2 (33.3%)                            | 5 (50%)                             |

**Fig.1** blood culture results (n=273) by BacT alert system

**Fig.2** Age and gender wise distribution of blood culture cases
Higher prevalence of antimicrobial resistance was noted in this study, especially in gram negative organism. This might be due to indiscriminate use of antibiotics in hospital. Most of the gram negative organisms were multidrug resistance with a very high resistance to beta-lactam antibiotics and least resistance to Imipenem. The overall resistance of gram positive organism was from 0 to 81.8%, and for gram negative organism from 0 to 100% these results are in concordance with Araya et al., (2015) which was 0–83% and 0–100% for gram positive and negative, respectively. Staphylococcus aureus showed high resistance to cefoxitin (MRSA) which is quite similar to Garg et al., (2007). E. coli and Klebsiella was showed high resistance to amoxy-clav which was accordance to Veena mangunath et al., (2015), and least resistance to imipenem accordance to Aziz japoni et al., (2008).

In the current study fungal septicaemia caused by Candida albicans was observed 2.8% which was quite similar with the Panday et al., (2017) they have observed 3% Candida albicans in their study. The rise in antibiotic resistance in blood isolates emphasizes the importance of sound hospital infection control, rational prescribing policies, and the need for awareness to use antimicrobial drugs.

It may be concluded from the study that early diagnosis and appropriate treatment of BSIs should be based on the current knowledge of bacterial profile and antibiotic resistance pattern, which should be provided by microbiology laboratory from time to time. We observed that Staphylococcus aureus and organisms belonging to Enterobacteriaceae family are the leading causes of septicemia. Increasing incidence of drug resistant organisms like MRSA and multidrug resistance gram negative bacilli raises serious concerns and mandates strict antibiotic policy to prevent emergence and spread of antibiotic resistance. We hope that these results could support microbiologists, clinicians and hospital managers in the identification and implementation of strategic targeted actions to coordinate infection control interventions and antimicrobial policies in order to decrease the rate the emergence of resistance and minimize mortality of septicaemic patients.
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