

# Bacteriologic Antibiography Outline of Isolates from Blood Culture at Tertiary Center

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## Abstract

Bacteria found in blood circulation either consistently intermittently are commination to every organs of body. These infections can affect life and death. In India also blood stream infections are major causes of health problem that caused demise of patients in hospital. Timely diagnoses of infection with antimicrobial susceptibility assessment are important for optimization of treatment and best way to reduce hospital stay and improve patient health. In spite of recent advances in clinical diagnostics, blood culture remains the gold standard for the detection of blood stream infections. Studies in different places and regions have indicated the varying microbiological pattern of bloodstream infections which support the need for a continuous examination of the causative organisms. For the diagnosis of septicemia, Blood cultures are the “gold standard” are based on the detection of viable microorganisms in the blood. The main aim of this is to identify the bacteria causing bloodstream infections and to determine and analysis their antibiotic sensitivity pattern in a tertiary center. In this study blood for culture was collected from 940 clinically suspected cases of blood stream infection from the hospital. Collected blood samples were processed in the bacteriology section at microbiology laboratory and standard laboratory methods were used to identified isolates and then antibiotic susceptibility test was performed using CLSI guidelines. Total 940 blood samples were cultured in which 139(14.8%) were found positive. Among isolates, the most predominant organism was *Staphylococcus aureus* (51.8%) followed by *Escherichia coli* (24.5%) and the least was *Salmonella* species (1.4%), *Proteus* species (1.4%) and *Acinetobacter* species (1.4%). Among Gram positive isolates, Penicillin and Erythromycin showed high degree of resistance. Imipenem was particularly susceptible among the isolated. Gentamicin and Amikacin showed high in vitro susceptibility for both Enterobacteriaceae and Nonfermenters. This study provides information on bacteriological profile of septicemic isolates. Therefore continuous monitoring of the susceptibility of organisms towards antibiotics is necessary to prevent and spread of drug resistance.

**Keywords:** Antimicrobial susceptibility, Blood Stream Infections (BSI), Blood Culture

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## INTRODUCTION

Bacteria found in blood circulation either consistently or intermittently are commination to every organs of body. Early diagnosis and rapid relevant treatment of Blood stream infections (BSI) required as it ranges from infection to life threatening and can affect life and death<sup>1,2</sup>. Globally sepsis is one of main causes of morbidity and mortality amongst the usual infection associated with health infections<sup>3</sup>. In cases of bacteremia the mortality rate varies from 20 to 50 percent of cases<sup>4</sup>. The reasons of BSI are described by a wide range of micro-organisms which are subjected to geology alteration<sup>5</sup>. These infections are often classified as primary or secondary when associated with clinical or microbiological confirmation at a defined body site. Furthermore, they have traditionally been classified as either nosocomial or community-acquired infection. In developing countries, increasing cases of septicemia are a great problem of health. For clinicians it produces the huge dispute in the selection of appropriate antimicrobial agents. Further it became complicated due to development of antimicrobial resistance which is the cornerstone of treatment<sup>6,7</sup>. In India BSI is also a major cause of health problem resulting demise of patients in hospital. Timely diagnoses of infection with antimicrobial susceptibility assessment are important for optimization of treatment and best way to reduce hospital stay and improve patient health.

In clinical diagnostics, blood culture, in spite of recent advances, remains as gold standard for the diagnosis of BSI<sup>8</sup> and are based on the detection of viable microorganisms in the blood<sup>1</sup>. Studies in different places and regions have indicated the varying microbiological pattern of BSI which support the need for a continuous examination of the causative organisms. In recent years, incidence of bacteremia has increased by the members of Enterobacteriaceae and other Gram negative bacilli. Nowadays sensitivity of bacterial strains is being replaced by multi drug resistant (MDR) strains of *Klebsiella*, *Pseudomonas*, *Acinetobacter*, and *Citrobacter* species<sup>9</sup>. The increased resistance of grampositive isolates has also been seen in *Staphylococcus aureus* in Methicillin resistance (MRSA) and *Enterococci* in Vacomycin resistance (VRE)<sup>10</sup>. These increasing

resistance in microbial is concern worldwide. MDR-infection is more likely to raise the risk of death, prolong hospital stay and require more costly antibiotic care. Antimicrobial therapy is performed empirically almost in all cases before findings of blood culture are available. Considering the high septicemia-related mortality and morbidity, it is important to make the correct choice of clinical therapy<sup>11</sup>. The development of resistance to bacteria makes important to know the prevalent trend of antibiotic resistance of organism causing septicemia. Antimicrobial resistance levels are growing and there is a change in the distribution of species among important blood stream pathogen in both hospital and community settings. A transition from a prevalence of Gramnegative organism from the time of 70's to present day primacy of gram positive organism has taken place in hospital environment<sup>3</sup>. Therefore, this study was conducted to identify the bacteria causing BSI and to determine and analyses their antibiotic sensitivity pattern in a tertiary center to guide clinicians to initiate antibiotic treatment and formulating antibiotic policy.

## MATERIAL AND METHODS

This study was prospective study conducted in the Department of Microbiology of tertiary care hospital, Wardha in the period of six months. Blood sample for culture was collected from 940 clinically suspected cases of blood stream infection from the hospital. Nevertheless to their age, sex, occupation and religion were also included in this study. From the adult 10-20ml of blood was collected and 2 to 5ml in children in aseptic condition and inoculated into culture bottle containing 70 ml to Brain Heart infusion (BHI) broth with 0.05% Sodium Polyanethol Sulfonate (SPS) as anticoagulant and 20ml of BHI broth with 0.05% SPS with aseptic procedure with maximum precaution. The blood culture bottles (BHI) were incubated aerobically at 37°C. After overnight incubation, subculture was done onto MacConkey agar, Blood agar, Chocolate agar and special media which was suitable for isolation and identification of the species after each 24 hours of incubation. By next day if no growth was observed on plate then subculture was repeated on day 3<sup>rd</sup>, day 4<sup>th</sup> and finally on day 7<sup>th</sup>. The isolation and identification of bacteria were done by conventional standard

procedure such as microbial colony character, gram staining, motility testing, biochemical tests and serological tests.

Antibiotic sensitivity testing was performed by Kirby-Bauer's disc diffusion technique using on Muller Hinton Agar (MHA) as per CLSI guidelines. In this study antibiotic discs used included Erythromycin (5µg), Amikacin (10µg), Amoxycillin (25µg), Cefixime (10µg), Ceftriaxone (10µg), Ceftazidime (10µg), Cotrimoxazole (25µg), Chloramphenicol (10µg), Ciprofloxacin (10µg), Nalidixic acid (30µg), Ofloxacin (30µg) and Cloxacillin (10µg). The variables investigated were age, sex of patients, microbial species and drug sensitivity pattern. Clinical Laboratory Standards (CLSI) interpretive criteria were used for susceptibility results<sup>12</sup>. Using reference strains *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853, quality control was conducted to ensure accuracy of results, procedures and performance.

## RESULT

Total 940 blood samples were cultured in which 139(14.8%) were found as positive (Table 1). The total culture positive males were 92 (66.2%) and females were 47 (33.8%).

The rate of isolation was highest among 0-15 years (46.8%) followed by 16-30 year age group (20.9%). The most common isolate from

infants followed by adults. Out of 139 isolates 86 (61.9%) were Gram positive bacteria and 53 (38.1%) were Gram negative bacteria. Total four different species of Gram positive isolates were identified viz. *Staphylococcus aureus* (51.8%), *Streptococcus species* 6(4.3%), *Enterococcus* 4(2.9%) and Coagulase Negative *Staphylococci* (2.9%). The major Gram negative bacteria were *Escherichia coli* (24.5%), *Pseudomonas aeruginosa*(5.0%), *Klebsiella spp*(4.3%), *Salmonella species* (1.4%), *Proteus species* (1.4%) and *Acinetobacter spp*(1.4%). The overall isolation rate decreased with rising age and the overall positive growth rate was 66.2% higher for males compared to 33.8 percent for females. Among all isolates *Staphylococcus aureus* was the most predominant organism isolated followed by *Escherichia coli* and *Pseudomonas aeruginosa*, however, *Salmonella species*, *Proteus species* and *Acinetobacter species* were the least isolated organism.

The most prevalent organism among the isolates was *Staphylococcus aureus* as 51.8% followed by *Escherichia coli* as 24.5% and the least was *Salmonella species* as 1.4%, *Proteus species* as 1.4% and *Acinetobacter species* as 1.4%.

The isolate among Gram positive, which exhibited no resistance to Linezolid and Vancomycin. MRSA was found to in 48.46% of total *Staphylococcus aureus* isolates whereas MRCoNS was found in 28.52 % of total CoNS isolates. Table 3

**Table 1.** Distribution of positive cases by gender

| Gender | Number of cases | Percentage | Culture positive cases | Percentage |
|--------|-----------------|------------|------------------------|------------|
| Male   | 619             | 65.9       | 92                     | 66.2       |
| Female | 321             | 34.1       | 47                     | 33.8       |
| Total  | 940             | 100.0      | 139                    | 100        |

**Table 2.** Age wise distribution of the isolated organisms

| Age   | <i>E.coli</i> | <i>Stap</i> | <i>Kleb</i> | <i>Sal</i> | <i>Pseudo</i> | <i>Prot</i> | <i>Acineto</i> | <i>Strep</i> | <i>Entero</i> | <i>CoNS</i> | Total |
|-------|---------------|-------------|-------------|------------|---------------|-------------|----------------|--------------|---------------|-------------|-------|
| 0-15  | 21            | 35          | 2           | 1          | 1             | 1           | 1              | 1            | 1             | 1           | 65    |
| 16-30 | 6             | 16          | 1           | 0          | 2             | 1           | 0              | 0            | 2             | 1           | 29    |
| 31-45 | 4             | 12          | 1           | 0          | 3             | 0           | 0              | 2            | 0             | 0           | 22    |
| 46-60 | 3             | 9           | 2           | 1          | 1             | 0           | 1              | 3            | 1             | 2           | 23    |
| Total | 34            | 72          | 6           | 2          | 7             | 2           | 2              | 6            | 4             | 4           | 139   |

Note: *E. coli*- *Escherichia coli*; *Stap*- *Staphylococcus aureus*; *Kleb*- *Klebsiella species*; *Sal*- *Salmonella species*; *Pseudo*- *Pseudomonas aeruginosa*; *Prot*- *Proteus species*; *Acineto*- *Acinetobacter species*; *Strep*- *Streptococcus species*; *Entero*- *Enterobacter species*; *CoNS*- Coagulase Negative *Staphylococci*

**Table 3.** Antibiotic sensitivity pattern of Gram positive organism

| Antibiotics   | <i>Staphylococcus aureus</i> (%) | <i>Enterococcus</i> (%) | <i>Streptococcus</i> species (%) | CONS (%) |
|---------------|----------------------------------|-------------------------|----------------------------------|----------|
| Erythromycin  | 28.77                            | 0                       | 100                              | 31.87    |
| Penicillin    | 15.38                            | 0                       | 96                               | 30.74    |
| Cortimoxazole | 38.46                            | 74.43                   | 89.43                            | 42.58    |
| Linezolid     | 100                              | 100                     | 100                              | 100      |
| Cefoxitin     | 38.46                            | 18.29                   | 89.12                            | 40.52    |
| Vancomycin    | 100                              | 100                     | 100                              | 100      |
| Levofloxacin  | 69.23                            | 16.27                   | 100                              | 86.28    |
| Gentamicin    | 38.46                            | 56.28                   | 97.26                            | 84.54    |
| Doxycycline   | 71.23                            | 36.43                   | 100                              | 84.37    |

**Table 4.** Antibiotic sensitive pattern of Gram negative organisms(53)

| Antibiotics                   | <i>E.coli</i> (%) | <i>Klebsiella</i> spp (%) | <i>Acinetobacter</i> spp (%) | <i>Pseudomonas</i> spp (%) | <i>Proteus mirabilis</i> (%) | <i>Salmonella</i> spp (%) |
|-------------------------------|-------------------|---------------------------|------------------------------|----------------------------|------------------------------|---------------------------|
| Amoxicillin + Clavulanic Acid | 11.2              | 2.75                      | 5.43                         | 50                         | 50                           | 53.37                     |
| Gentamicin                    | 61.52             | 58.35                     | 38.24                        | 50                         | 20                           | 35.29                     |
| Amikacin                      | 37.93             | 52.94                     | 30.59                        | 50                         | 46                           | 50                        |
| Cefuroxime                    | 2.35              | 18.24                     | 28.24                        | 50                         | 54                           | -                         |
| Cefepime                      | 6.55              | 12.35                     | 27.24                        | 50                         | 29.34                        | 65                        |
| Cefotaxime                    | 5.55              | 20.59                     | 6.12                         | 50                         | 34.68                        | -                         |
| Ciprofloxacin                 | 2.64              | 20.59                     | 12.35                        | 100                        | 12.98                        | 70                        |
| Imipenem                      | 100               | 100                       | 35.29                        | 100                        | 100                          | 100                       |
| Cotrimoxazole                 | 6.21              | 22.35                     | 18.24                        | 3.42                       | 29                           | -                         |
| Ceftazidime                   | -                 | -                         | 27.54                        | 15.38                      | -                            | 60                        |
| Piperacillin                  | -                 | -                         | -                            | 28.64                      | -                            | 45                        |
| Piperacillin + Tazobactam     | -                 | -                         | -                            | 74.56                      | -                            | 45                        |

shows Gram positive isolates with their sensitivity pattern toward various antibiotics. Among Gram positive isolates, Penicillin and Erythromycin showed high degree of resistance.

Enterobacteriaceae showed the maximum resistance to Ciprofloxacin followed by Cefuroxime, Cefixime and Ceftriaxone. Among the isolated imipenem showed highly sensitive as shown in table no 4. Gentamicin and Amikacin showed high in vitro susceptibility for both Enterobacteriaceae and Nonfermenters.

Total MDR isolates observed in this study were 43.7%. Among Gram positive organisms isolated MRSA, MRCoNS, and MDR *enterococcus* observed were 48.46%, 28.52%, and 32.42%, respectively. Multidrug resistance was observed in high number among Gram negative isolates,

with 52.8% among Enterobacteriaceae species and 39.46% among nonfermenters.

## DISCUSSION

Among the 940 blood specimen collected from suspected patients who presented persistent fever, 14.8% cases were culture positive which was similar to the study conducted by Qureshi M et al.<sup>13</sup> and Vijaya Devi et al.<sup>14</sup>. This study provides information on the distribution of bacterial isolates causing bloodstream infections along with their antibiotic susceptibility pattern that plays an importance role in proper management of blood stream infection cases. The positivity of the blood culture observed in this study was comparable to levels recorded in various other Indian studies<sup>15</sup>. Previous data showed low culture positivity

ranging from 5.6% to 8.39% while high positivity culture ranged from 33.9% to 52.10% (Sharma M et al.<sup>16</sup>, Anbumani N et al.<sup>17</sup> and Jadhav S et al.<sup>18</sup>). These positive differences in blood culture may be due to the number of samples taken for blood culture and the amount for analysis as explained by Lee et al.<sup>19</sup>. In this study low isolation rate may be explained by the fact that quite a few numbers of patients already undergo some kind of primary treatment at peripheral health centers before reaching a tertiary care hospital. Self drug is also popular due to counter availability of the medicines. Further, Gram positive bacteria were found to be predominant over Gram negative bacteria and men had high culture positivity compared to women as reported earlier by Vanitha RN et al.<sup>9</sup> and Kaur A et al.<sup>20</sup>, respectively. In this study most of the blood culture positive cases were from infant than other age groups which is also similar to the studied conducted by Ayobola et al.<sup>21</sup> and Bichitrananda S et al.<sup>22</sup> who reported culture positivity up to 58.3% and 50% in infants, respectively. The high rate of isolation from infants may be due to their poor immune system relative to adults, as most infants use intravascular devices to take medicine that can easily introduce bacteria into their blood stream.

Coagulase Negative Staphylococci (CoNS) were isolated at 2.9%, which is comparable to the studied of Kante M et al.<sup>23</sup> who had reported as 5.9%. This variability in the incidence of CoNS as a blood pathogen is due to the fact that it is known to be the most common skin commensal and its existence in the blood may result from contamination due to failure to follow proper aseptic blood collection techniques. However there are several studies that say the incidence of CoNS as a true blood pathogen is growing due to increased use of intravascular tools<sup>24,25</sup>. There is always changing in Antibiotic sensitivity pattern of micro-organisms. Penicillin has been effective for Gram positive organism in past but for such organism these days they are usually not effective. In this study, the antibiotics used for testing susceptibility for gram positive organisms, vancomycin (100%) & linezolid (100%) showed highest susceptibility which correlates with the study of Sharma M et al.<sup>16</sup>.

In this study among the Gram negative organism isolated imipenem showed the highest

sensitivity (100%) which correlates with the study of Sanjay D Rathod et al.<sup>26</sup> and Mustafa M et al.<sup>27</sup> who also showed Imipenem as most efficacious drug for Gram negative bacilli.

## CONCLUSION

Globally Blood stream infection is one of the main agent causing morbidity and mortality. This research underscores the complex complexity of trends of antibiotics susceptibility. As the antibiotic resistance rate for blood stream pathogens rises, continuous monitoring of the organism's antibiotic susceptibility has become necessary to prevent improper antibiotic usage. Therefore, continuous evaluation of isolate sensitivity-resistance patterns is advisable in order to make a rational use of antibiotics and compliance with existing guidelines reducing multidrug resistance in pathogenic agents can go a long way.

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## CONFLICT OF INTEREST

The authors declare that there is no conflict of interest.

## AUTHORS' CONTRIBUTION

All authors listed have made a substantial, direct and intellectual contribution to the work, and approved it for publication.

## FUNDING

None.

## DATA AVAILABILITY

All datasets generated or analyzed during this study are included in the manuscript

## ETHICS STATEMENT

Not applicable.

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