

# Bacteriological Profile and Antibiotic Susceptibility Pattern of Blood Culture Isolates from Patients Visiting Tertiary Care Hospital in Kathmandu, Nepal

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## Abstract

Bacterial blood stream infections can lead to life threatening sepsis that requires rapid antimicrobial treatment otherwise may lead to morbidity and mortality of patients. Blood culture is gold standard technique which provides essential information for the diagnosis and appropriate medication to save life of affected patients. Present study was conducted to determine the bacteriological profile of blood stream infections and their antibiotic susceptibility pattern in patients visiting Janamaitri Hospital, Balaju, Kathmandu, Nepal. A total of 838 blood samples were collected from the clinically suspected cases of bacteremia and septicaemia. Isolates were identified by standard biochemical tests, and antibiotic susceptibility test was performed by using CLSI guidelines. Positive blood culture was obtained in 61/838 (7.28)

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**Index terms**— blood stream infections, multi-drug resistant, salmonella paratyphi A, janamaitri hospital.

## 1 I. Introduction

Bacteremia is the presence of viable bacteria in the circulating blood. The detection of bacteria in blood is always abnormal. A minor injury occurring during tooth brushing, tooth extraction, abscesses, infected wound or boils, insertion of intravenous of bladder catheter, surgery and existing B infections like lung infection, Urinary tract infection (UTI), gastrointestinal tract (GTI), burns or bedsores or from areas of localized disease as in pneumococcal pneumonia, meningitis, pyelonephritis, osteomyelitis, cholangitis, peritonitis, enterocolitis and puerperal sepsis are the sources of bacteremia and blood culture is required for the detection of it [1]. Bloodstream infection (BSI) is one of the most important causes of morbidity and mortality globally [2]. Detection of bacteremia by rapid and reliable method is by culturing blood. The blood should be collected aseptically before the administration of antibiotics [3]. Septicaemia is a clinical term used to describe severe life-threatening bacteremia in which multiplying bacteria release toxins into the blood stream and trigger the production of cytokines, causing fever, chills, toxicity, tissue anoxia, reduced blood pressure and collapse. Septic shock is usually a complication of septicemia with Gram-negative bacilli, and less frequently, Gram-positive organisms and prompt treatment is essential [4]. Continuous septicemia occurs primarily in patients with intravascular infections like endocarditis, septic thrombophlebitis, infections associated with intravascular catheter, septic shock whereas intermittent septicemia occurs in patients with localized infections like lung, urinary tract, soft tissues infections [5].

Bloodstream infections are potentially lifethreatening and require rapid identification and antibiotic susceptibility testing of the causative pathogen. Both Gram positive and Gram negative bacteria causes bacteremia and septicemia. Gram negative septicemia, also known as endotoxic shock, which is more severe than Gram positive septicemia [6].

If the infection is caused by multidrug resistant (MDR) bacteria morbidity and mortality will increase which leads to great economic loss encompassing use of more expensive antibiotics to treat infection as well as threat of resistance to them. The infections caused by MDR organisms are more likely to prolong the hospital stay, increase the risk of death and require treatment with more expensive antibiotics [7].

In almost all cases, antimicrobial therapy is initiated empirically before the results of blood culture are available by keeping in mind that high mortality and morbidity are associated with septicemia and right choice of empiric therapy is of importance [6]. The increasing frequency of antimicrobial resistance among microbial pathogens causing nosocomial and community acquired infections is making numerous classes of antimicrobial agents less effective resulting in emergence of antimicrobial resistance [7, ??2]. For the treatment, the isolation of bacterium from blood is valuable, but there is also urgent need of antimicrobial therapy, so sample is taken and treatment is started and after blood culture result, patient is treated as redirected by in vitro antibiotic sensitivity test [8,9]. Therefore, we conducted this study to determine the common bacterial agents associated with bacteremia and their antimicrobial susceptibility patterns in febrile patients visiting in Janamaitri Hospital, Balaju, Kathmandu, Nepal.

## 2 II. Materials and Methods

The study was conducted in Microbiology Laboratory of Janamaitri Hospital, Kathmandu, Nepal from March 2014 to April 2015. Written informed consents were obtained from patients prior to their inclusion in the study. A total of 838 blood samples were processed during the research period. Five ml blood sample was collected from each adult, 2-5ml from each child and 0.5-2ml from infant's aseptically using 70% alcohol and 2% tincture of iodine and inoculated immediately into 50ml Brain Heart Infusion (BHI) Broth with 0.025% of sodium polyanethol sulphonate as anticoagulant. In pediatrics cases, 1-2ml of blood was inoculated in 5-10ml of BHI broth. Negative result was followed by examining the broth daily for the sign of bacterial growth (turbidity, haemolysis, clot formation) and by doing final subculture at the end of seventh day. Bottles that showed sign of growth were further processed by Gram stain, followed by subculture on Blood agar, Mac Conkey agar, Manitol salt agar and examined after 18-24 hrs of incubation. Bacterial isolates were identified by colony morphology, Gram staining, catalase test, coagulase test, oxidase test, methyl red/voges-proskauer test (MR-VP), Triple sugar iron agar test, citrate utilization test, Urease test and Sulfur Indole Motility (SIM) test using standard procedure for bacterial identification [10].

### 3 a) Antibiotic susceptibility test

Antimicrobial susceptibility testing was performed by using Kirby Bauer disc diffusion method following guidelines of Clinical and Laboratory Standard Institute 2012 [11]. The inoculums used for susceptibility testing was prepared in nutrient broth by touching 5/6 colony and matched to 0.5 McFarland standard ( $1.5 \times 10^8$  CFU/ml). Within 15 minutes, a sterile cotton swab was dipped into the inoculums suspension and pressed inside the wall of tube above the fluid level and inoculated at 60°C over the dried surface of Muller-Hilton agar (MHA) plate. After 3-5 minutes of inoculation, the antibiotic discs were applied and gently pressed down to ensure complete contact with agar. Organisms which showed resistance to at least one antibiotic among three or more antimicrobial categories were considered as multidrug resistant (MDR) bacteria [12,13, ??4].

### 4 b) Quality control

Reference strains *E. coli* (ATCC 25922) and *S. aureus* (ATCC 25923) were used as a control reference strains for identifications and drug susceptibility testing [14,15].

### 5 c) Data analysis

The data obtained from the research were analyzed by using statistical tools in SPSS-21 version. The chi-square test was used for statistical analysis of data. A 'P' value less than 0.05 was considered as statistically significant.

## 6 III. Results

A total of 838 clinical blood samples were collected from the patients attending Janamaitri Hospital, Kathmandu to study the prevalence of bacteraemia and septicemia from 9 th August, 2014 to 8 th November, 2015.

### 7 a) Ward wise distribution of positive samples

There were all together 838 blood samples, out of which, 61(7.28%) samples showed growth and rest 777 (92.72%) showed no growth. Again higher percentage of growth was obtained from OPD (Outpatient Department) followed by emergency and wards (Table 1 Out of 838 blood samples, 48 different Gram negative bacteria were isolated. Among the isolates, *Salmonella Paratyphi A* was most predominant followed by *Salmonella Typhi*. Five different bacteria were isolated during study period. Antibiotic susceptibility was performed on them in which imipenem was found most effective followed by amikacin (Table 5). 3 antibiotics and were considered as multidrug resistant. It was found that 16 (33.33%) isolates were resistant to 1 antibiotic and 17(35.42%) isolates were resistant to 2 antibiotics. Among the MDR strains, 43.75% (7 out of 16) of *Salmonella Typhi* were found to be MDR. Similarly

15.38% (4 out of 26) of Salmonella Paratyphi A were found to be MDR. All the isolates (100%) of Escherichia coli, Morganella morganii, and Staphylococcus aureus were found to be MDR (Table ??).

Table ?? : MDR pattern of the bloodstream isolates

## 8 IV. Discussion

Blood culture is a well-established procedure of the standard diagnostic workup for many infectious diseases. In the countries like Bangladesh, where all kinds of drugs including the antibiotics, are sold over the counter, misuse of antibiotics has been found to be responsible for developing pool of resistant bacteria as well as negative results of blood culture [16]. Blood culture is employed for the detection of bacteremia and septicemia in blood. Blood stream infection is one of the main agent causing morbidity and mortality worldwide. Urgent and effective treatment is required to manage blood infections [17].

In the countries like Nepal, the overuse of antibiotics, random use of antibiotics as hit and trial method by clinicians without proper sensitivity test, unawareness of people about emergence of antibiotics resistance, random use of antibiotics without advice of physicians, prolonged intensive care unit (ICU) stay, nursing home residency, severe illness, use of instrumentation or catheterization etc. are the major causes of drug resistance in our country. The less growth percentage may be due to previous exposure of patients to used antibiotics that hindered their growth or dominance of organism's growth [18].

In the present study the isolation rate was (61/838) 7.28% which was comparable to those study conducted by Karki et al where 4.2% were culture positive [19]. Gram negative bacteria were common organisms isolated during this study accounting 48/61 (78.69%). Among Gram negative isolates the most common was Salmonella Paratyphi A 26/48 (54.17%) followed by Salmonella Typhi 16/48 (33.33%). Similar finding was seen from the studies in Kathmandu Model hospital Nepal, where 71% of total isolates from blood were Salmonella Typhi and 16% of the total isolates were Salmonella Paratyphi A [20]. In this study the isolation rate was highest in age group between 16-30 years 33/61 (54.10%) followed by age group 31-45 years 13/61 (21.31%). Similar study conducted in showed that the isolation rate was highest in age group between 21-40 (28%) followed by 41-60 (24%) [21]. In this study, among Gram positive bacteria the most common isolated bacteria was Staphylococcus aureus 5/13 (38.46%) followed by CNS 4/13 (30.77%).

The most effective antibiotic in Gram negative bacteria was imipenem followed by amikacin. The others effective drugs were cefotaxime and gentamycin. Similar study conducted in Nepal in KIST Medical College by Surya et al showed that imipenem and amikacin followed by gentamycin were most effective antibiotics for the treatment of Escherichia coli and Klebsiella pneumoniae [22]. Those organisms which showed resistance to at least one agent in three or more antimicrobial categories were considered as multidrug resistant (MDR) bacteria [23].

In the present study among Gram negative bacteria, 100% of E. coli and Morganella morganii isolates were MDR, followed by Salmonella Typhi (43.75%) and Salmonella Paratyphi A (15.38%). None of the isolates of Salmonella Paratyphi B was found to be MDR. Similar study conducted in Nepal showed that 96.10% of the E. coli isolates were MDR whereas 44.8% of the Salmonella isolates were MDR in Ghana [22, 24].

In this study, among Gram positive bacteria, S. aureus, CNS and Streptococcus pyogenes were found 100% MDR whereas 50% of Enterococci isolates were MDR. Similar study conducted in Ethiopia showed that 100% of the S. aureus and CNS were MDR whereas none of the S. pyogenes isolates were MDR [2]. In the present study vancomycin is the antibiotic of choice for the treatment of Gram positive bacterial isolates followed by imipenem and amikacin. Higher rate of infection by Salmonella species may be due to unhygienic practices, contaminated food and drinking water. The prevalence of typhoid was higher in summer and autumn season which may be due to contaminated water and food [25, 26]. In this study, Imipenem and Amikacin were found most effective for Gram negative isolates whereas vancomycin was found most effective for Gram positive bacteria followed by imipenem and amikacin. These three antibiotics should not be used indiscriminately and kept as reserve drug because if resistance is developed then treatment will be complicated.

## 9 V. Conclusion

Salmonella Paratyphi A and Salmonella Typhi was the major Gram negative organisms causing blood stream infections whereas Staphylococcus aureus and CNS was the major Gram positive organisms. The antibiotic resistance pattern varies according to geographical location, country to country and even institute to institute in the same country and continuously changes over time so determination of antibiotic sensitivity pattern in periodic intervals is mandatory for choosing appropriate antibiotics for the treatment. The antibiotic susceptibility pattern of this study suggested that vancomycin is the drug of choice for Gram positive bacteria while imipenem and amikacin for Gram negative bacteria but the later drugs showed wide coverage for Gram positive organisms too.



Figure 1: C

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Figure 2: Table 1 :

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| Types of patients | Growth<br>Number | %     | Total samples<br>Number | %     |
|-------------------|------------------|-------|-------------------------|-------|
| OPD               | 41               | 67.21 | 476                     | 56.80 |
| Emergency         | 14               | 22.95 | 261                     | 31.15 |
| Wards             | 6                | 9.84  | 101                     | 12.05 |
| Total             | 61               | 100   | 838                     | 100   |

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Figure 3: Table 2 :

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### 3

d) Seasonal variation on the prevalence of bacterial growth  
Out of 48 Gram negative organisms isolated, 20(41.67%) showed growth in summer season and followed by autumn season 20 (41.67%). Growth in winter was 4/48 (8.33) and in spring was 4/48 (8.33%). Out of 13 Gram positive bacterial isolates, 6/13(46.15%) showed growth in summer and followed by spring season 4/13(30.77%). Growth in winter was 2/13(15.38%) and in autumn was 1/13(7.69%). Sample showed highest growth in summer and autumn season (Table 4).

Figure 4: Table 3 :

### 4

Figure 5: Table 4 :

### 5

AMX=Amoxycillin, COT=Cotrimoxazole, CN=Cefalexin, OF=Ofloxacin, CIP=Ciprofloxacin, C=Chloramp  
CFM=Cefixime, NA=Nalidixic acid, CTX=Cefotaxime, GEN=Gentamicin, AK=Amikacin, IPM=Imipenem  
f) Antibiotic resistance pattern of Gram positive

bacteria  
Out of 838 blood samples, only 13 samples showed growth of Gram positive bacteria. Isolated

bacteria were subjected to antibiotic susceptibility test (Table 6).

Figure 6: Table 5 :

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|                             | Season                                         |         |         |            |         |         |            |          |            |         |
|-----------------------------|------------------------------------------------|---------|---------|------------|---------|---------|------------|----------|------------|---------|
|                             | Summer                                         |         |         | Autumn     |         |         | Winter     |          | Spring     |         |
| Gram Negative Bacteria      |                                                |         |         |            |         |         |            |          |            |         |
| Escherichia coli            | 1                                              |         |         | 0          |         |         | 1          |          | 1          |         |
| Morgenella morganii         | 0                                              |         |         | 0          |         |         | 0          |          | 1          |         |
| Salmonella Paratyphi A      | 9                                              |         |         | 14         |         |         | 2          |          | 1          |         |
| Salmonella Paratyphi B      | 0                                              |         |         | 1          |         |         | 1          |          | 0          |         |
| Salmonella Typhi            | 10                                             |         |         | 5          |         |         | 0          |          | 1          |         |
| Total                       | 20 (41.67%)                                    |         |         | 20(41.67%) |         |         | 4 (8.33%)  |          | 4 (8.33%)  |         |
| Gram Positive Bacteria      |                                                |         |         |            |         |         |            |          |            |         |
| Staphylococcus aureus       | 3                                              |         |         | 1          |         |         | 0          |          | 1          |         |
| CNS                         | 1                                              |         |         | 0          |         |         | 1          |          | 2          |         |
| S. pyogenes                 | 1                                              |         |         | 0          |         |         | 0          |          | 1          |         |
| Enterococci                 | 1                                              |         |         | 0          |         |         | 1          |          | 0          |         |
| Total                       | 6 (46.15%)                                     |         |         | 1 (7.69%)  |         |         | 2 (15.38%) |          | 4 (30.77%) |         |
| Gram Negative Bacteria      | Antibiotic susceptibility pattern (%) / number |         |         |            |         |         |            |          |            |         |
|                             | AMX                                            | COT     | CN      | OF         | CIP     | C       | CFM        | NA       | CTX        | GEN     |
| Escherichia coli (3)        | (33.33)                                        | (66.67) | (33.33) | (66.66)    | (33.33) | (33.3)  | (33.3)     | -        | (100)      | (100)   |
|                             | 1                                              | 2       | 1       | 2          | 1       | 1       | 1          |          | 3          | 3       |
| Morgenella morganii (1)     | -                                              | -       | -       | -          | -       | 100     | 100        | -        | 100        | 100     |
|                             |                                                |         |         |            |         | 1       | 1          |          | 1          | 1       |
| Salmonella Paratyphi A (26) | (96.15)                                        | (50)    | (88.46) | (73.08)    | (73.08) | (84.62) | (100)      | -        | (100)      | (96.15) |
|                             | 25                                             | 13      | 23      | 19         | 19      | 22      | 26         |          | 26         | 25      |
| Salmonella Paratyphi B (2)  | (100)                                          | (100)   | (100)   | (100)      | (100)   | (100)   | (100)      | -        | (100)      | (100)   |
|                             | 2                                              | 2       | 2       | 2          | 2       | 2       | 2          |          | 2          | 2       |
| Salmonella Typhi (16)       | (87.50)                                        | (62.50) | (81.25) | (75.00)    | (75.00) | (75.00) | (81.25)    | -(93.75) |            | (87.50) |
|                             | 14                                             | 10      | 13      | 12         | 12      | 12      | 13         |          | 15         | 14      |
| Gram positive bacteria      | Antibiotic susceptibility pattern (%) / number |         |         |            |         |         |            |          |            |         |
|                             | P                                              | AMP     | C       | CIP        | GEN     | MET     | E          | VAN      | PIP        | PIPM    |
| Staphylococcus aureus (5)   | 0                                              | (20)    | (40)    | (40)       | 0       | (60)    | (60)       | (100)    | (100)      | (100)   |
|                             |                                                | 1       | 2       | 2          |         | 3       | 3          | 5        | 5          | 5       |
| CNS (4)                     | 0                                              | (50)    | (25)    | (50)       | (75)    | (50)    | (75)       | (100)    | 0          | (50)    |
|                             |                                                | 2       | 1       | 2          | 3       | 2       | 3          | 4        |            | 2       |
| Streptococcus pyogenes (2)  | 0                                              | (50)    | (50)    |            | (50)    | (100)   | (100)      | (100)    | (50)       | (100)   |
|                             |                                                | 1       | 1       | 0          | 1       | 2       | 2          | 2        | 1          | 2       |
| Enterococci (2)             | 0                                              | 0       | (50)    |            | (50)    | (100)   | (100)      | (100)    | 0          | (100)   |
|                             |                                                |         | 1       | 0          | 1       | 2       | 2          | 2        |            | 2       |

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Figure 7: Table 6 :

| Organisms              |                                           | Number of bacteria |       |       | resistance to |   | MDR   |  |
|------------------------|-------------------------------------------|--------------------|-------|-------|---------------|---|-------|--|
| Gram Negative Bacteria |                                           | 1                  | 2     | 3     | Number        |   | %     |  |
|                        |                                           | antibiotic         | an-   | an-   |               |   |       |  |
|                        |                                           |                    | tibi- | tibi- |               |   |       |  |
|                        |                                           |                    | otic  | otics |               |   |       |  |
| Year                   | Escherichia coli                          | 3                  | 0     | 0     | 3             | 3 | 100   |  |
|                        | Morgenella morganii                       | 1                  | 0     | 0     | 1             | 1 | 100   |  |
|                        | Salmonella Paratyphi A                    | 26                 | 9     | 13    | 4             | 4 | 15.38 |  |
|                        | Salmonella Paratyphi B                    | 2                  | 2     | 0     | 0             | 0 | 0     |  |
|                        | Typhi Gram positive Staphylococcus aureus | 16                 | 5     |       |               | 5 | 43.75 |  |
|                        | CNS                                       | 4                  | 0     | 0     | 4             | 4 | 100   |  |
|                        | Streptococcus pyogenes                    | 2                  | 0     | 0     | 2             | 2 | 100   |  |
|                        | Enterococci                               | 2                  | 0     | 1     | 1             | 2 | 50.00 |  |
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[Note: C]

Figure 8:



## .1 VI. Acknowledgements

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