

Original Research Article

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## Bacteriological Profile and Antibiogram of Blood Culture Isolates from Clinically Suspected Septicemic Cases in a Tertiary Care Hospital Shahjahanpur, UP, India

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### ABSTRACT

#### Keywords

Blood stream infections, Bacteremia, Multidrug resistance, Antibiogram

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Blood stream infections mainly by Bacteremia leading to septicemia were the major cause of life threatening sepsis in hospitalized patients. There should be required rapid diagnosis of pathogenic agents and immediate appropriate therapy. These help to decrease of multidrug resistance cases and also morbidity and mortality. During one year period, total 192 blood samples were processed in microbiology laboratory according to standard protocols. Out of 192 samples, 21 were isolated positive and antibiotic sensitivity patterns were performed according to standard CLSI guidelines. The total 10.94% (21/192) were identified as culture positive of these 66.67% were Gram negative and 28.57% Gram positive and 04.76% Candida species. The gram negative bacteria were predominant isolates. The *Klebsiella pneumoniae* was 100% sensitivity to Ceftazidime, Cefepime, Amikacin, Gentamycin and Aztreonam. All non-fermenter isolates were 100% sensitivity to Ceftazidime, Cefepime, Amikacin, Colistin and Polymyxin-B. Vancomycin sensitivity was seen 100% to all grams positive isolates. Bacteremia was most common causes of septicemia; the most predominant pathogenic agent was *Klebsiella pneumoniae* in our study. Surveillance of pathogenic agents and their antibiotics sensitivity pattern should be required to reduce drug resistance and for selection of appropriate therapy.

### Introduction

Bacteremia is defined as the presence bacteria in bloodstream whereas septicemia indicates the presence of microorganism in blood with their toxins and remove by phagocytes.<sup>1,2</sup> Blood stream infections lead to sepsis is commonly caused by bacteria that are major

cause of morbidity and mortality worldwide. Blood stream infections range from self-limiting bacteremia to life threatening sepsis requires early diagnosis and immediate treatment.<sup>3</sup> Blood culture is gold standard test for diagnosis of septicemia which helps to identify the microorganism. In recent year, Gram negative septicemia take over gram

positive septicemia, among gram negative bacteria the incidence of septicemia are more by the member of Enterobacteriaceae than other gram negative bacilli. Now the sensitivity of bacterial strain of *Klebsiella*, *Pseudomonas* and *Acinetobacter* species are getting multidrug resistant.<sup>4,5</sup> And also some of *Candida* species detected in many hospitals in case of neonatal septicemia and ICU patients with prolonged uses of intravascular devices, multiple antibiotics treatment and neoplasia with persistent neutropenia denoted to be independent risk factors for the addition of nosocomial candidemia.<sup>6</sup>

This study was performed to identify the bacterial isolates and antibiotic sensitivity pattern of the isolated bacteria those are useful to clinicians for start off appropriate treatment.

### **Materials and Methods**

A total 192 blood samples were received for culture of suspected septicemic patients sent by various clinical section at central collection center of Varun Ajun Medical College and Rohilkhand Hospital Shahjahanpur from April 2020 to March 2021.

For adults 7-10 ml and for children 5 ml Blood samples were collected after taking all antiseptic precautions. For this we tied a tourniquet around the arm then cleaned the skin on the prominent vein site by applying 2 % iodine and with 70% Isopropyl alcohol. The blood samples were collected in Brain Heart Infusion (BHI) Broth bottles. For adult's 7-10 ml blood in 70ml broth bottle and for children 5 ml blood in 20 ml broth bottle and separately bottles were slightly shaken to mix the sample properly aseptically and then the bottles were incubated at 37°C before processing.

After 24 hours if turbidity appears, gram stain was done after making smear from broth

bottle directly and communicated the clinician immediately and also subculture was done on the Blood agar, Mac Conkey agar and Saboraud's Dextrose agar. If fungal elements were seen in Gram stain then these growth were inoculated in two plates of Saboraud's Dextrose agar media, one incubated at 37°C for 24 hours and other at 25°C for 24 hours. After 24 hours all inoculated plates colonies were observed.

If no turbidity appears after 24 hours then blind subculture was done on the Blood agar, MacConkey agar and Saboraud's Dextrose agar and observed next day. If no growths appear in culture media then 3 more subculture was done on alternate days up to total 7 days if again no growth appeared on these 3 subculture media then finally declared as culture negative.

Out of 192 samples only 21 were showed growths on culture media. For identification of bacteria firstly colonies character of growth was observed then Gram stain was done from colonies. On the basis of these the gram positive bacteria or gram negative bacteria was identified. For, further identification had chosen various biochemical tests.

The biochemical tests for gram positive isolates such as Catalase, Coagulase, Mannitol salt agar test, Bacitracin (BC), Optochin (OP) and for gram negative isolates such as Catalase, Oxidase, Indole, Citrate, Urease, Methyl red, Voges-Proskauer, Triple sugar iron test, Motility.<sup>7-9</sup>

Antibiotic sensitivity testing was done by Kirby-Bauer Diffusion method on Muller-Hinton agar according to CLSI guidelines.<sup>10</sup> For Gram negative bacteria such as Piperacillin (100µg), moxycillin-clavulanate (20/10µg), Piperacillin-tazobactam (100/10µg), Gentamicin (10µg), Amikacin (30µg), Tobramycin (10µg), Doxycycline (30µg),

Meropenem (10µg), Imipenem (10µg), Ceftazidime (30µg), Cefepime (30µg), Cefotaxime (30µg), Ceftriaxone (30µg), Ofloxacin (5µg), Ciprofloxacin (5µg), Colistin (25µg), Aztreonam (30µg) and Co-trimoxazole (25µg). For Gram positive bacteria the antibiotic were used such as, Penicillin (10U), Erythromycin (15µg), Azithromycin 15µg, Ciprofloxacin (5µg), Ofloxacin (5µg), Levofloxacin (5µg), Gentamicin (10µg), Amikacin (30µg), Tobramycin (10µg), Vancomycin (30µg), Linezolid (30µg), Tetracycline (30µg), Doxycycline (30µg), Co-trimoxazole (25µg) After that next day antibiotic sensitivity and resistant was interpreted according to CLSI guidelines.

## Results and Discussion

A total of 192 blood samples were collected for blood culture from the clinically suspected cases of septicemia patients and performed during one year period. Of the total 192 blood samples cultured, 10.94% (21/192) were noted positive growth and 89.06% (171/192) were noted no growth. Out of the total 21 positive patients, the male patients and female patients were 57.12% (12/21) and 42.87% (09/21) respectively.

Out of the 21 culture positive samples, more culture growth showed positive from Neonatal intensive care unit (NICU) and Intensive care unit (ICU) Patients, of these 66.67% (14/21) were Gram negative bacteria and 28.57% (06/21) Gram positive bacteria and 04.76% (01/21) Candida species. The gram negative were predominant isolates, *Klebsiella pneumonia* isolates were 28.57% (06/21) followed by *Acinetobacter* spp. 19.05% (04/21), *Pseudomonas aeruginosa* 19.05% (04/21), *Staphylococcus aureus* 14.28% (03/21), *Enterococcus* spp. 09.52% (02/21) and *Streptococcus* spp. 04.76% (01/21) (Table 1).

For gram negative isolates sensitivity, the *Klebsiella pneumonia* was 100% sensitive to Ceftazidime, Cefepime, Amikacin, Gentamycin and Aztreonam but Piperacillin 100% resistance. Then Cefotaxime, Tobramycin and Doxycycline were 05 (83.33%) sensitive whereas, Pipracillin-tazobactam, Ceftriaxone, Imipenem and Meropenem were 04(66.67%) sensitive. Co-trimoxazole sensitivity in 03(50%) and Amoxicillin-clavulanic acid, Ciprofloxacin and Ofloxacin were 01(16.67%) sensitive only and 05(83.33%) resistance (Table 2).

For non-fermenters, the all non-fermenter isolates were 100% sensitive to Ceftazidine, Cefepime, Amikacin, Colistin and Polymyxin-B. In these, *Pseudomonas aeruginosa* was 100% resistance to Piperacillin followed by 03(75%) sensitivity to Piperacillin-tazobactam, Gentamycin, Imipenem, Meropenem, Aztreonam. But, sensitivity to Tobramycin was 02(50%) only. *Acinetobacter* spp. was 03(75%) sensitive to Piperacillin-tazobactam, Cefotaxime, Ceftriaxone, Imipenem, Meropenem, Tobramycin and Doxycycline. Then, 02(50%) sensitivity in Amikacin, Co-trimoxazole and 01(25%) sensitive to Ciprofloxacin (Table 2).

For gram positive isolates, all isolates were 100% sensitivity to Vancomycin, and all gram positive isolates showed 100% resistance to Penicillin, Ciprofloxacin, Levofloxacin followed by resistance of Ofloxacin, Azithromycin, Linezolid and Doxycycline were 03(50%) and Erythromycin, Gentamycin, Amikacin, Tetracycline were 04 (66.67%) resistance respectively. Then Co-trimoxazole was 05 (83.33%) resistance (Table 3).

In this study, blood culture positivity of blood stream infection was 10.94% which are similar to developing countries that is 13.3%<sup>11</sup> but in developed countries blood culture

positive rate is high that is 13.9%-29.4%.<sup>12,13</sup> This present study is comparable to 9.94% by Manjula *et al.*, 2005<sup>14</sup>, 10% by Usha and Pushpa 2007<sup>17</sup>, 11.2% by Shalini *et al.*, 2010(18) and 10.8% by Hamed Ghadhiri *et al.*, 2012<sup>19</sup> at Iran. The differences in developing countries and developed countries to blood culture positivity is due to geographical location, climatic variation, population race, epidemiological variation of etiological agent and variation in infection control policies.<sup>20-22</sup>

In the present study, gram negative isolates were 66.67% and gram positive isolates were 28.57%. Out of these gram negative isolates, the predominant was *Klebsiella pneumoniae* 28.57% followed by *Acinetobacter* spp. 19.05%, *Pseudomonas aeruginosa* 19.05%. This finding was in accordance with other studies.<sup>23-25</sup>

The *Pseudomonas aeruginosa* and *Acinetobacter* spp. are common pathogen causing nosocomial infection, these are highly resistance to antibiotics led to high morbidity and mortality.<sup>23,24,26,27</sup>

In Enterobacteriaceae, Imipenem and Meropenem were noted 66.67% sensitive and 33.33% resistance. This finding was in accordance with the other study.<sup>28</sup> Above study showed possible appearance of Carbapenem resistance Enterobacteriaceae which indicate towards unreasonable use of carbapenemes.<sup>29</sup> The Enterobacteriaceae were reported 100% sensitivity to Amikacin, Gentamycin, Cefepime, Ceftazidime and Aztreonam with better interest towards Cefotazidime, Tobramycin and Doxycyclin were 83.33% sensitivity. All non-fermenter isolates were 100% sensitive to Ceftazidime, Cefepime, Amikacin, Colistin and Polymyxin-B. Then, for *Pseudomonas aeruginosa* 100% resistance to Piperacillin followed by

Piperacillin-tazobactam, Imipenem and Meropenem were 75% sensitivity, Gentamycin 62.50% sensitivity and Tobramycin 62.50% sensitivity. These finding were similar with other studies.<sup>27,30</sup> In our study, Gram positive isolates were only 28.57%. Out of total isolates the most predominant gram positive isolates was *Staphylococcus aureus* 14-28%, next one *Enterococcus* spp. 09.52% and last one *Streptococcus* spp. 04.76% which were comparable with study by Mathur *et al.*, 2014,<sup>26</sup> who was isolated 14.5% *Staphylococcus aureus* followed by 9% *Enterococcus* spp. whereas the study by Radha Rani *et al.*, 2017<sup>31</sup> isolates 14.40% *Staphylococcus aureus* and 10.14% *Enterococcus* spp.

These low isolates of Gram positive bacteria led to increasing of Gram negative pathogens with increasing multidrug resistance and ESBL production, so, there need to apply strict antibiotic policy with importance on antibiotic sensitivity findings (28). In our study, All Gram positive isolates were 100% sensitivity to Vancomycin and all gram positive isolates showed 100% resistance to Penicillin, Ciprofloxacin, and Levofloxacin. But, Ofloxacin, Azithromycin, Linezolid and Doxycycline were 03(50%) resistance only followed by Erythromycin, Gentamycin, Amikacin and Tetracycline were 04(66.67%) resistance. Then Co-trimoxazole was 05(83.33%) resistance.

The present study reported Gram negative isolates were predominant organisms causing septicemia. The Amikacin, Cefepime, Ceftazidime, Gentamycin, Piperacilline-tazobactam and Carbapenems were established most successful antibiotics for Gram negative isolates. Although Vancomycin, Azithromycin, Linezolid and Doxycycline were established most successful antibiotics for Gram positive isolates.

**Table.1** Table showing various positive isolates from blood cultures (n-21)

Microorganism	Isolated microorganism	Number of isolates	Percentage (%)
Gram negative bacteria	<i>Klebsiella pneumoniae</i>	06	28.57%
	<i>Acinetobacter</i> spp.	04	19.05%
	<i>Pseudomonas aeruginosa</i>	04	19.05%
Gram positive bacteria	<i>Staphylococcus aureus</i>	03	14.28%
	<i>Enterococcus</i> spp.	02	09.52%
	<i>Streptococcus</i> spp.	01	04.76%
Fungus	<i>Candida</i> spp.	01	04.76%

**Table.2** Antibiotics sensitivity of isolated gram negative bacteria from blood culture (n-14)

Antibiotics	<i>Klebsiella pneumoniae</i> (06) (Enterobacteriaceae)	<i>Acinetobacter</i> spp. (04) (Non-fermenter)	<i>Pseudomonas aeruginosa</i> (04) (Non-fermenter)
Piperacillin	00(00%)	-	00(00%)
Amoxicillin clavulanate	01(16.67%)	-	-
Piperacillin-tazobactam	04(66.67%)	03(75%)	03(75%)
Ceftazidime	06(100%)	04(100%)	04(100%)
Cefotaxime	05(83.33%)	03(75%)	-
Ceftriaxone	04(66.67%)	03(75%)	-
Cefepime	06(100%)	04(100%)	04(100%)
Amikacin	06(100%)	04(100%)	04(100%)
Gentamycin	06(100%)	02(50%)	03(75%)
Tobramycin	05(83.33%)	03(75%)	02(50%)
Imipenem	04(66.67%)	03(75%)	03(75%)
Meropenem	04(66.67%)	03(75%)	03(75%)
Colistin	-	04(100%)	04(100%)
Polymixin-B	-	04(100%)	04(100%)
Aztreonam	06(100%)	-	02(50%)
Ciprofloxacin	01(16.67%)	01(25%)	01(25%)
Ofloxacin	01(16.67%)	-	00(00%)
Co-trimoxazole	03(50%)	02(50%)	-
Doxycyclin	05(83.33%)	03(75%)	-

**Table.3** Antibiotics sensitivity of isolated gram positive bacteria from blood culture (n-06)

Antibiotics	<i>Staphylococcus aureus</i> (03)	<i>Enterococcus spp.</i> (02)	<i>Streptococcus spp.</i> (01)
Penicillin	00(00%)	00(00%)	00(00%)
Azithromycin	02(66.67%)	-	01(100%)
Erythromycin	01(33.33%)	01(50%)	00(00%)
Gentamycin	02(66.67%)	-	-
Amikacin	02(66.67%)	-	-
Tetracycline	01(33.33%)	00(00%)	01(100%)
Doxycycline	01(33.33%)	01(50%)	01(100%)
Vancomycin	03(100%)	02(100%)	01(100%)
Linezolid	01(33.33%)	01(50%)	01(100%)
Co-trimoxazole	00(00%)	-	01(100%)
Ciprofloxacin	00(00%)	00(00%)	00(00%)
Levofloxacin	00(00%)	00(00%)	00(00%)
Ofloxacin	02(66.67%)	01(50%)	00(00%)

But the increasing rate of multidrug resistance is due to lack of regulation of prescribed antibiotics, which are commonly used in minor illnesses such as sore throat and running nose. These alarm the clinicians and microbiologists for reasonable use of antibiotics, identify appropriate alternate antibiotics and use that alternate antibiotic to prevent the drug resistance.

Bacteremia was the most common causes of septicemia in hospitalized patients. The gram negative bacteria were commonly found in blood stream infection in our study. Among these *Klebsiella pneumoniae* was the most predominant bacteria causing septicemia. The most common successful antibiotics for *Klebsiella pneumoniae* were Amikacin, Cefepime, Ceftazidime, Gentamycin, Aztreonam but for non-fermenters Ceftazidime, Cefepime, Amikacin, Colistin and Polymyxin-B were most commonest successful antibiotics. For all Gram positive isolates Vancomycin was most successful antibiotics. This study indicates to increase multidrug resistance septicemia that is more challenging for clinicians and microbiologists.

So, there need early detection of causative agents and their antibiotic sensitivity pattern with regular treatment by appropriate antibiotics without interruption prescribed by clinicians. That will minimize the load of septicemia and also decrease the appearance of more resistance. So, there should be required periodic surveillance of pathogenic agents and their antibiotic sensitivity pattern that direct the selection of appropriate antibiotic for treatment.

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