Current Trends of Gram-negative Bacteremia in a State of North India, the Forthcoming Challenges
IGMC Shimla

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Authors’ contributions

This work was carried out in collaboration between both authors. Both authors read and approved the final manuscript.

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ABSTRACT

Background: Vascular endothelial dysfunction plays a critical role in the evolution of sepsis there is an increase in vascular permeability leading to coagulation abnormalities and subsequent dysfunction of major organs. Bloodstream infections are a cause of life-threatening complications in all age groups of the population especially the extreme age groups who are very much prone to the risk. Antibiotic usage is associated with bacteremia, septicemia, and mortality through many factors. We are aware that Gram-negative bacteria due to many causes are becoming increasingly resistant to the currently used antimicrobial therapy. Here, in this study, we report the incidence and etiology and of the Gram-Negative bacterial isolates and their clinical significance from our tertiary care institute located in the Northern part of India.

Materials and Methods: Prospective Observational Study conducted in the Department of Microbiology IGMC Shimla for a tenure of one year to determine the incidence and etiology of Gram-negative bacterial isolates and their antimicrobial resistance profile. All the blood culture samples received in the Department of Microbiology IGMC Shimla for culture by Bactec BD fx from July 2015 to June 2016 were included in the study except for falling in the exclusion criteria. The
blood culture was observed in the Bactec bd fx system for at least 5 days before they are reported as sterile.

**Results:** Among the total 1275 cultures which were positive for bacteria, 931 (73.02%) were positive for Gram-negative bacteria. Among the total of 931 cultures that were positive for Gram-negative bacteria, The Non-fermenter group of organisms was isolated in 292 (31.36%) cultures, followed by *E. coli* 266 (28.57%). Among the total of 931 cultures positive for GNB, a maximum of 335 (35.98%) belonged to 0-1 year. Among them, 574 (61.65%) were males while 357 (38.35%) were females.

**Conclusion:** Nonfermenter group was the commonest organism among Gram-negative isolates. Infants were more commonly positive as compared to another age group. Males were more commonly culture positive as compared to females among gram-negative isolates.

**Keywords:** Blood stream infections; culture, bactec; gram-negative bacteria.

### 1. INTRODUCTION

Blood cultures are one of the most reliable diagnostic tools for the diagnosis of bloodstream infections (BSI diagnosis) and are frequently used in the clinical microbiology laboratory. Blood cultures are indicated whenever there is clinical evidence of sepsis or an unknown systemic infection [1]. Blood culture is also indicated when there is clinical suspicion of infection that may be associated with bacteremia, such as meningitis, endocarditis, and osteomyelitis. In addition, blood cultures should also be done in clinical conditions like pneumonia, pyelonephritis, septic arthritis, epiglottitis, omphalitis (infants and neonates), abscesses, and deep-seated or extended skin and soft tissue infections. Typhoid fever, brucellosis, or leptospirosis can also be diagnosed by blood cultures. It is also indicated in the diagnostic evaluation of fever of unknown origin (FUO), the metastatic focus of BSI or abscesses associated with intermittent BSI [2-3].

Increasing overuse and misuse of antibiotics and their resistance in the population has lead us to a stage where colonization or the emergence of resistant isolates is leading us to a poor outcome worldwide [4]. Not implemented standard empirical therapy protocols and increasing prescription of broad-spectrum antibiotics is leading to toxic side effects harmful for the patients and subsequently leading to failure of treatment. The most threatening is the detection of bloodstream infections in patients undergoing treatment in critical care units as it is mostly nosocomial in origin, these infections are very difficult to treat due to the presence of Extended Spectrum Beta Lactamases in Gram-Negative bacteria and are associated with increasing failure of therapy and subsequent mortality but can be prevented by strict aseptic precautions.

The increasing carbapenem resistance is starting to leave infectious disease clinicians with lesser alternatives. Blood culture isolates vary from one critical care setting to another, and there is geographical variation in sensitivity patterns. Hence the need to periodically review the resistance profile and implement the empirical therapy as per the sensitivity pattern of prevailing pathogens.

BSIs have also been associated with increased rates of hospitalization, increased length of stay, and increased hospital costs and are a financial burden on the patient and their families. The earliest possible identification of BSIs allows for prompt initialization of antibiotics therapy and less need for additional invasive or diagnostic studies, which may help to decrease hospitalization stay and cost. Factors that have a clinical impact to initiate antimicrobial therapy immediately are an increase in the body temperature, respiratory rate, and an increase in the number or percentage of immature neutrophils.

Indira Gandhi Medical College(IGMC) Shimla is a tertiary care hospital and patients with above mentioned clinical conditions are treated both in the outpatient department(OPD) and inpatient department (IPD) and usually require blood culture to establish the etiological diagnosis.

Previously no study has been done in IGMC Shimla using an automated Bactec BD FX machine for blood culture. The present study attempts to identify Gram-negative isolates and their clinical relevance. Hence, the present study is designed to determine the incidence and etiological profile of gram-negative bacterial positivity in Invasive bloodstream infections in all age groups using automated Bactec systems.
1.1 Aims and Objectives

To determine the incidence and etiological profile of gram-negative bacterial positivity in Invasive bloodstream infections in all age groups using automated Bactec systems.

2. MATERIALS AND METHODS

Study Design: Prospective Observational Study

Study Setting: Department of Microbiology, Indira Gandhi Medical College And Hospital, Shimla

Study Period: one year from July 2015 to June 2016

2.1 Inclusion Criteria

1. All the blood culture samples received in the department of microbiology for blood culture by bactec bd fx system.
2. Patients willing to study or their parents consented to study.
3. Blood cultures from all age groups

2.2 Exclusion Criteria

1. Patient not willing to study.
2. Blood cultures showing mixed growth that is more than 3 different types of growth.

3. METHODOLOGY

All the blood culture samples received in the department of microbiology for culture by Bactec Bd fx were included in the study. The blood culture was observed in the Bactec Bsd fx system for at least 5 days before they are reported as sterile. Blood culture samples were collected by aseptic techniques.

The sample to be tested is inoculated into the Bactec™ plus aerobic/f culture bottle for adults and Bactec™ peds plus/f for children which is then inserted into the Bd bactec fluorescent series instrument for incubation. Each bottle has a sensor that can detect an increase in CO2 produced by the growth of microorganisms. The sensor monitors every 10 minutes for an increase in its fluorescence, which is proportional to the amount of CO2 present. A positive reading indicates the presumptive presence of viable microorganisms in the bottle. The Positive bottle will be sub-cultured on Blood agar and Macconkey™ agar plates. Following the subculture on solid media from each positive bottle a smear will be prepared for gram staining from that blood culture bottle. The Gram-stained smear will be examined for the presence of microorganisms and presumptive report conveyed to respective departments. The Blood agar and Mac Conkey agar plates will be incubated aerobically at 37°C for 24 to 48 hrs and then observed for the growth of bacteria. All bacterial isolates will be identified using standard biochemical identification methods. All the positive isolates were stocked.

3.1 Statistical Analysis

The data was analyzed using statistical analysis-epi info7. the data collected was entered into a spreadsheet. The data was checked for any missing values and completed. analysis in terms of demographic variables, positivity in the processed samples, type of species prevalent, was done using statistical software epi-info version 7(7.1.1.0).

4. RESULTS

In the current study, among the total of 5473 samples suspected of BSI's received in the department of microbiology, IGMc, Shimla 1441 were positive. Among the total positive culture, 1275(88.48%) were positive for Bacteria while 166(11.52%) were positive for Fungi. Among the total 1275 cultures which were positive for bacteria, 931(73.02%) were positive for gram negative bacteria while the rest 344(26.98%) were of gram-positive bacteria.

Among the total of 931 culture which were positive for gram negative bacteria , Non fermenter group of organisms was isolated in 292(31.36%) cultures, followed by E. coli 266(28.57%), Klebsiella pneumoniae 122 (13.10%), Pseudomonas aeruginosa 120(12.89%), Salmonella Typhi 44 (4.73%), Enterobacter aerogenes 27 (2.90%), Citrobacter freundii 23 (2.47%), Salmonella Paratyphi A 20 (2.15%), Acinetobacter baumannii 11 (1.18%), Burkholderia cepacia complex 3 (0.32%) and Proteus mirabilis 3 (0.32%) (Table 1, Fig. 1). Among the total of 931 culture positive for gram-negative bacteria, 335 (35.98%) belonged to 0-1 year, 45 (4.83%) were 2-5 years, 79 (8.49%) were 6-18 years, 232 (24.92%) were 19-45 years, 159 (17.08%) were 46-65 years, 81(8.70%) were ≥66 years. (Fig. 2)
Table 1. Distribution of gram-negative bacterial positivity in culture

<table>
<thead>
<tr>
<th>ORGANISM</th>
<th>Frequency</th>
<th>Percent</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>120</td>
<td>12.89%</td>
</tr>
<tr>
<td>Acinetobacter baumannii</td>
<td>11</td>
<td>1.18%</td>
</tr>
<tr>
<td>Burkholderia cepacia complex</td>
<td>3</td>
<td>0.32%</td>
</tr>
<tr>
<td>Non fermenter group of organisms</td>
<td>292</td>
<td>31.36%</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>266</td>
<td>28.57%</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>122</td>
<td>13.10%</td>
</tr>
<tr>
<td>Proteus mirabilis</td>
<td>3</td>
<td>0.32%</td>
</tr>
<tr>
<td>Enterobacter aerogenes</td>
<td>27</td>
<td>2.90%</td>
</tr>
<tr>
<td>Salmonella Typhi</td>
<td>44</td>
<td>4.73%</td>
</tr>
<tr>
<td>Salmonella Paratyphi A</td>
<td>20</td>
<td>2.15%</td>
</tr>
<tr>
<td>Citrobacter freundii</td>
<td>23</td>
<td>2.47%</td>
</tr>
<tr>
<td>Total</td>
<td>931</td>
<td>100.00%</td>
</tr>
</tbody>
</table>

Fig. 1. Distribution of gram-negative bacterial positivity in culture

Fig. 2. Age Group distribution of Gram-Negative bacterial positivity in culture
Among the total of 931 culture positive for gram-negative bacteria, 574 (61.65%) were male while 357 (38.35%) were female. (Fig. 3)

5. DISCUSSION

In the present study, we attempted to determine the incidence and etiological profile of gram-negative bacterial positivity in bloodstream infections in all age groups using automated Bactec systems and their clinical significance. The results obtained in the present study were analyzed and compared with other studies. The global burden of sepsis is increasing but data on the subject is scarce due to less availability of verified data from underdeveloped or low-income developing countries. The intact vascular endothelium protects the body by preventing pathogen entry into the tissues thereby preventing a cascade of coagulation. Extreme age groups like infants are also more prone to sepsis due to less developed immune status, low birth weight, premature rupture of membranes, or hypoxia during the birth process. The aging people due to comorbidities are also more prone to sepsis due to vascular endothelial dysfunction. Sepsis in the elderly can be attributed to increasing levels of inflammatory processes and oxidative stress which in turn can lead to hypotension, multiple organ failure, and subsequent shock. Studies conducted by Zador et al.explain the multimorbidity and specific subgroups associated with increased risk of septicemia and mortality. The groups which had a higher association with sepsis-related mortality include the hepatic/addiction subgroup, cardiac, cardiopulmonary, and complicated diabetes subgroups [5].

Among the total 1275 cultures which were positive for bacteria, 931(73.02%) were positive for gram-negative bacteria while the rest 344(26.98%) were of gram-positive bacteria.

In the current study, Among the total of 931 culture which were positive for Gram-negative bacteria , Non fermenter group was isolated in 292(31.36%) cultures, followed by E. coli 266(28.57%), Klebsiella pneumonia 122 (13.10%), Pseudomonas aeruginosa 120(12.89%), Salmonella Typhii 44 (4.73%), Enterobacter aerogenes 27 (2.90%), Citrobacter freundii 23 (2.47%), Salmonella Paratyphi A 20 (2.15%) , Acinetobacter baumannii 11 (1.18%), Burkholderia cepacia complex 3 (0.32%) and Proteus Mirabilis 3 (0.32%)

In the present study, Among the total of 931 culture positive for Gram-negative bacteria, 335 (35.98%) belonged to 0-1 year, 45 (4.83%) were 2-5 years, 79 (8.49%) were 6-18 years, 232 (24.92%) were 19-45 years, 159 (17.08%) were 46-65 years, 81(8.70%) were ≥66 years

In our study, among the total of 931 culture-positive Gram-negative bacteria, 574 (61.65%) were male while 357 (38.35%) were female.

In a study conducted by [6] in Nigeria, they found that Escherichia coli in 23.4% was the frequent isolates, in our study, it was also a significant isolate accounting for. (28.57%), corroborating
the findings of our study [7] also reported *Escherichia coli* 12.1% as the most pathogenic bacteria recovered from the blood samples. In certain another study done by Karlowsky JA et al. [8], among the GNB isolated, *Escherichia coli* was the commonest. Which also augments our findings [9-10]. Showed that *Pseudomonas aeruginosa* was a more commonly isolated organism, our study also reported it as a significant isolate.

However, in other studies, Gram-negative bacteria predominated, like *K.pneumoniae* [11] and *P. aeruginosa* [12].

Gram-negative nonfermenting (NFGNB) aerobic bacteria are being increasingly implicated in human disease. The complex physicochemical properties of these organisms necessitate a battery of tests for their precise identification. In addition, there is still much confusion regarding the taxonomic status of many of these nonfermenters. Identification of these nonfermenters has often being neglected. They are believed to play a significant opportunistic role in patients whose immune system is debilitated due to sepsis or extremes of age.

S.Joshi et al [13] described enteric fever as a common cause of morbidity in India and *S.Typhi* account for the majority of infections [14]. *S.Paratyphi A* is less common but has shown a rising trend over the past five years whereas *S.Paratyphi B* is rare.

6. CONCLUSION

The non-fermenter group of organisms was the commonest organism among Gram-negative isolates. Infants were more commonly positive as compared to another age group. Males were more commonly culture positive as compared to females among gram-negative isolates.

ETHICAL APPROVAL

As per international standard or university standard written ethical approval has been collected and preserved by the author(s).

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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