Original Article

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# Bacteriological Profile and Antibiotic Susceptibility Pattern of Isolates from Suspected Neonatal Septicemia Patients in Different Hospitals of Chattogram

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**ABSTRACT**

***Background:*** *Bacterial blood stream infection is an important cause of morbidity and mortality in neonates. This study was undertaken to identify the bacterial isolates from suspected neonatal septicemia patients and to understand their antimicrobial susceptibility pattern in Chattogram.*

***Materials and methods:*** *A cross-sectional descriptive type of study was done for a period of two years from January 2022 to December 2023. Blood samples from 340 neonates with clinically suspected septicemia were taken for automated culture and susceptibility test was conducted in Bacteriology Laboratory of IbneSina, Chattogram. The samples were collected and processed following standard microbiological techniques and an antibiotic susceptibility test was done on pure culture isolates using disc-diffusion method for the commonly used antibiotics. The data were analyzed by using SPSS version 20 and the results were summarized by using tables and graphs.*

***Results:*** *Out of 340 blood culture done, 62 (18.24%) were positive. Among them 61.3% were of early onset sepsis. The predominant bacteria isolated was Klebsiella spp. 27 (43.6%) followed by Coagulase negative staphylococci 12 (19.4%), Salmonella typhi 10 (16.1%), E. coli 6 (9.6%) and Pseudomonas 5 (8.0%). Antibiotic susceptibility tests showed that the most sensitive antibiotics to Gram-negative organisms wereImipenem (81-100%), Tazobactam-Piperacillin (74-100%), Amikacin (67-90%), Ceftriaxone (60-100%) and Levofloxacin (63-80%). Maximum resistance among Gram-negative organisms were seen in Ampicillin (60-100%), Cotrimoxazole (60-100%) and Cefixime (30-80%). Among Gram-positive organisms, all strains (100%) were sensitive to Linezolid and Vancomycin. Other alternates with good sensitivity were Amikacin (92%), Imipenem (83%), Ceftriaxone (83%) and Levofloxacin (75%). While maximum resistance was seen to Ampicillin (83%), Amoxiclav (75%), Cefixime (75%) and Ciprofloxacin (75%).*

***Conclusions:*** *In the present study, most of the pathogens isolated from blood cultures were Gram negative and they showed high resistance to commonly used antibiotics. Therefore, rational use of antibiotics after sensitivity testing should be practiced.*

**Key words:** Antibiotic susceptibility pattern; Bacterial blood stream isolates; Neonatal septicemia.

## Introduction

Neonatal sepsis is defined as a clinical syndrome in an infant within the first month of life, manifested by systemic symptoms and signs of infection and isolation of a bacterial pathogen from the bloodstream. [1] Bacterial blood stream infection is an important cause of morbidity and mortality in neonates. It is responsible for about 30-50% of the total neonatal deaths in developing countries. It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of sepsis related causes.[2] The incidence of neonatal sepsis in the developed countries is 1-10/1000 live births, whereas it is roughly three times more in developing countries. According to National Neonatal Perinatal Database in India, the incidence of neonatal septicemia is 30 per 1000 live births and sepsis is one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths.[3] In Bangladesh, about 20.2% of death of newborns are due to sepsis.[2] One study from NICU of the concerned public hospitals in Dhaka, the prevalence of sepsis was 69.35%.[4] In Chittagong prevalence of neonatal sepsis found to be 32%.[5]

Neonatal sepsis can be classified into two major categories depending on the onset of symptoms, Sepsis occurring in the first week of life is called as Early Onset Sepsis (EOS) and one occurring in the rest three weeks of neonatal period is Late Onset Sepsis (LOS).[6]

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Identification of etiological agents causing neonatal septicemia is important, since it can induce a change in the management policy.[4] Effective management of neonatal septicemia with appropriate antibiotics can minimize the risk of severe morbidity and mortality. Rational antibiotic use following the bacteriological profile and the antibiotic sensitivity pattern play a significant role in reducing the emergence of multi-drug resistant organisms.[5,6]

This study was carried out to determine the bacteriological profile and antibiotic sensitivity pattern of neonatal sepsis, so that the antibiotics which were used in empirical treatment could be appropriate to tackle the organism causing septicemia in Chattogram region.

**Materials and methods**

A cross-sectional descriptive type of study was done for a period of two years from January 2022 to December 2023. A total of 340 blood culture samples received at Ibne Sina Diagnostic Center, Chattogram from clinically suspected septicemia cases of neonates aged between one to thirty days of birth. All the samples were collected from different hospitals of Chattogram city.

From every patient using strict aseptic precautions, I-2 ml blood was collected and inoculated immediately into pediatric version of aerobic blood culture bottles containing 30 ml of complex medium of BD BACTEC FX 40 automated blood culture system. Inoculated blood culture bottles were loaded into the system and incubated at 37°C, monitored for up to 5 days when there was no signal. In case of growth, the automated system gave an alert. Positive bottles were then sub-cultured on Blood agar and MacConkey's agar medium. Isolate was identified by their characteristic appearance on their respective media, Gram staining and confirmed by the pattern of biochemical reactions using the standard method.[7]

Gram negative bacilli were identified by using TSI, MIU medium, Citrate utilization and oxidase tests. For Gram positive bacteria, coagulase, catalase, bacitracin and optochin susceptibility tests were used where needed. Antimicrobial susceptibility testing was performed for all blood culture isolates in Mueller-Hinton agar medium by Kirby–Bauer disc diffusion method as recommended in the National Committee for Clinical Laboratory Standards (NCCLS) guidelines.[8] The media and antibiotic discs were obtained from HI media (India) Laboratories.

The data were analyzed by using SPSS version 20 and the results were summarized by using tables and graphs. Percentages were used in this study to analyze epidemiological variables and statistics. The significance among percentages was calculated with the Chi square test and p value of < 0.05 was considered statistically significant.

**Results**

During the study period, 340 blood cultures of suspected septicemia cases of neonates were analyzed of which 62 were positive (18.24%). Figure 1 Among the culture positive cases, 25 (40.32%) were male and 37 (59.68%)werefemale neonates and the male female ratio was 1:1.48 (Table I). Considering the age of onset of clinical symptoms of sepsis, it was found that early onset sepsis cases were higher than late onset sepsis with 38 (61.3%) cases had early onset sepsis and 24 (38.7%) had late onset sepsis (Table II).

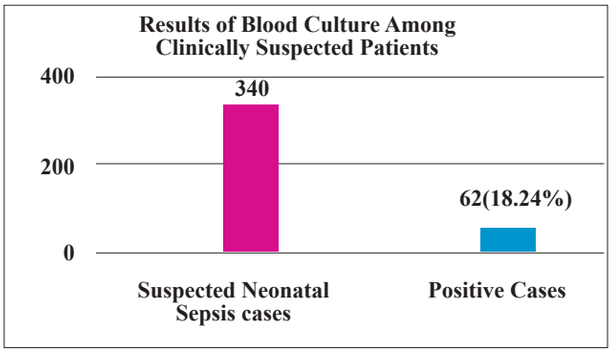


**Figure 1** Results of Blood Culture among clinically suspected septicemia cases of neonates (n=340)

**Table I** Frequency distribution of culture positive cases by sex (n=62)

| Sex | Number | Percentage (%) |
|---|---|---|
| Male | 25 | 40.32 |
| Female | 37 | 59.68 |

Male Female Ratio: 1: 1.48.

**Table II** Distribution of cases by onset of sepsis (n=62)

| Days of onset of Sepsis | Number | Percentage (%) | p value |
|---|---|---|---|
| Early onset Sepsis (≤ 7 days) | 38 | 61.3 | >0.05 |
| Late onset Sepsis (8 -30 days) | 24 | 38.7 | |

p- value = 0.9670. p > 0.05, statistically no significant difference.

**Table III** Distribution of isolated bacteria among blood culture positive case (n=62)

| Name of Bacteria | Number | Percentage (%) |
|---|---|---|
| Klebsiella spp. | 27 | 43.6 |
| Coagulase Negative Staphylococcus | 12 | 19.4 |
| *Salmonella typhi* | 10 | 16.1 |
| *Esch. Coli* | 06 | 09.6 |
| *Pseudomonas spp.* | 05 | 08.1 |
| *Staphylococcus aureus* | 02 | 03.2 |
| Total | 62 | 100.0 |

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The distribution and percentage of various bacterial isolates are shown in Table III. Out of total 62 isolates 48 (77.4%) were gram negative and 14 (22.6%) were gram positive. During the study period, no multiple positive blood cultures were observed from any patient. Predominant organism was Klebsiella spp. 27(43.6%) followed by Coagulase Negative Staphylococcus 12(19.4%), Salmonella typhi 10 (16.1%), Esch. coli 06 (9.6%), Pseudomonas spp. 05 (8.1%) and the least was Staphylococcus aureus 02 (3.2%).

Antibacterial resistance patterns of the Gram-negative and Gram-positive blood stream isolates are shown in Tables IV and V, respectively.

**Table V** Drug resistance pattern of Gram-negative isolates (n=48)

| Antibiotics | Klebsiella spp. (n=27) | | Salmonella typhi (n=10) | | Escherichia coli (n=06) | | Pseudomonas spp. (n=05) | |
|---|---|---|---|---|---|---|---|---|
| | S (%) | R | S | R | S | R | S | R |
| Amikacin | 18(66.7) | 09(33.3) | 09(90) | 01(10) | 05(83.3) | 01(16.7) | 04(80.0) | 01(20.0) |
| Amoxiclav | 14(51.8) | 13(48.2) | 10(100) | 00(00) | 04(66.7) | 02(33.3) | 02(40.0) | 03(60.0) |
| Ampicillin | 05(18.5) | 22(81.5) | 04(40) | 06(60) | 00 (00) | 06(100) | 01(20) | 04(80) |
| Azithromycin | 14(51.8) | 13(48.2) | 06(60) | 04(40) | 02(33.3) | 04(66.7) | 03(60.0) | 02(40.0) |
| Cefixime | 06(22.2) | 21(77.8) | 07(70) | 03(30) | 02(33.3) | 04(66.7) | 01(20) | 04(80) |
| Ceftriaxone | 17(63.0) | 10(27.0) | 10(100) | 00(00) | 04(66.7) | 02(33.3) | 03(60.0) | 02(40) |
| Ciprofloxacin | 13(48.2) | 14(51.8) | 07(70) | 03(30) | 04(66.7) | 02(33.3) | 03(60.0) | 02(40) |
| Cotrimoxazole | 10(37.0) | 17(63.0) | 06(60.0) | 04(40) | 02(33.3) | 04(66.7) | 00 (00) | 05(100) |
| Gentamycin | 13(48.2) | 14(51.8) | 08(80) | 02(20) | 04(66.7) | 02(33.3) | 02(40.0) | 03(60.0) |
| Imipenem | 22(81.5) | 05(18.5) | 10(100) | 00(00) | 05(83.3) | 01(16.7) | 05(100) | 00(00) |
| Levofloxacin | 17(63.0) | 10(37.0) | 07(70) | 03(30) | 04(66.7) | 02(33.3) | 04(80.0) | 01(20.0) |
| Tazobactam-Piperacillin | 20(74.0) | 07(26.0) | 09(90) | 01(20) | 05(83.3) | 01(16.7) | 05(100) | 00(00) |
| Chloramphenicol* | | | 08(80) | 02(20) | | | | |

*Chloramphenicol was used only for Salmonella species.

Among the Gram-negative isolates, *Klebsiella spp.* showed least resistance to Imipenem (18.5%), Tazobactam-Piperacillin (26%) and Amikacin (33.3%) and high resistance to Ampicillin (81.5%), Cefixime (77.8%) and Cotrimoxazole (63%). *Salmonella typhi* isolates were excellently sensitive to most of the antibiotics and showed 100% sensitivity to Imipenem, Ceftriaxone and Amoxycillin-Clavulanic acid, 90% to Amikacin and Tazobactam-Piperacillin, 80% to both Gentamycin and Chloramphenicol. Salmonella showed 60% resistance to Ampicillin, 40% to Cotrimoxazole and Azithromycin, 30% to Ciprofloxacin and Levofloxacin. *Esch. coli* showed least resistance (16.7%) to Imipenem, Amikacin and Tazobactam-Piperacillin. 33.3% resistance to Amoxiclav, Levofloxacin, Gentamycin, Ceftriaxone and very high resistance (100%) to Ampicillin and 66.7% resistance to Azithromycin, Cefixime and Cotrimoxazole. *Pseudomonas spp.* showed no resistance to Tazobactam-Piperacillin and Imipenem, 20% resistant to Amikacin and Levofloxacin. It was highly resistant (100%) to Cotrimoxazole, 80% to Ampicillin and Cefixime, 60% resistant to Amoxiclav and Gentamycin.

**Table V** Drug resistance pattern of Gram-positive isolates (n=14)

| Antibiotics | CoNS (n=12) | | Staphylococcus aureus (n=02) | |
|---|---|---|---|---|
| | Sensitive (%) | Resistant (%) | Sensitive (%) | Resistant (%) |
| Amikacin | 11 (92) | 01 (08) | 02 (100) | 00 (00) |
| Amoxyclav | 03 (25) | 09 (75) | 02 (100) | 00 (00) |
| Ampicillin | 02 (17) | 10 (83) | 00 (00) | 02 (100) |
| Azithromycin | 06 (50) | 06 (50) | 01 (50) | 01 (50) |
| Cefixime | 03 (25) | 09 (75) | 02 (100) | 00 (00) |
| Ceftriaxone | 10 (83) | 02 (17) | 01 (50) | 01 (50) |
| Ciprofloxacin | 03 (25) | 09 (75) | 00 (00) | 02 (100) |
| Cotrimoxazole | 08 (66.7) | 04 (33.3) | 00 (00) | 02 (100) |
| Gentamycin | 08 (66.7) | 04 (33.3) | 01 (50) | 01 (50) |
| Imipenem | 11 (92) | 01(08) | 02 (100) | 00 (00) |
| Levofloxacin | 10 (83) | 02 (17) | 01 (50) | 01 (50) |
| Linezolid | 12 (100) | 00 (00) | 02 (100) | 00 (00) |
| Vancomycin | 12 (100) | 00 (00) | 02 (100) | 00 (00) |

Among the Gram-positive isolates, Coagulase Negative Staphylococcal Strain (CoNS) was most frequent and showed no resistance to Linezolid and Vancomycin (00% each), 08% resistant to Amikacin and Imipenem, 17% to Levofloxacin and Ceftriaxone, 33% resistant to Gentamicin and Co-trimoxazole.CoNS is highly resistant (83%) to Ampicillin, 75% resistant to Amoxiclav, Ciprofloxacin and Cefixime each and 50% resistant to Azithromycin (Table V). The number of Staphylococcus aureus isolates are very few, so sensitivity pattern is not considered.

## Discussion

In the present paper, blood culture positivity was seen in 62 out of 340 (18.24%) cases which is quite similar to Kaistha et al. who found (13.17%) and Wuni FK et al. found 13.4%, but other studies have higher positivity like El-Din et al. found 45.7%, Kavitha et al. 44% and Roy et al. from northern India 47.5%.[9-13]

In this study Early Onset Sepsis (EOS) was present in 61.3% and Late Onset Sepsis (LOS) in 38.7%. Similar result was also observed by Mamta L et al. who found 61.41% (EOS) and 38.59% (LOS) cases, but El Din et al. in Egypt found higher late onset sepsis 55.8%.[14,11]

The incidence of Gram-negative organisms was 48/62(77.4%) while 14/62 (22.6%) were Gram-positive isolates in this study. It is in accordance with the studies of Mamta L et al. Gram-negative septicemia (60.67%)

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were encountered more than Gram-positive (32.01%). According to study of Chinna and Gupta, Gram positive organisms were 49.3% and gram negative 50.6%. Kamga et al. Ashwini M et al. and Karlowsky et al. also reported similar incidences. But according to Hoque et al. in Chattogram, incidence of Gram-negative septicemia was 98.25% which was more than this study.[14-19]

*Klebsiella spp.* was isolated in 27 (43.6%) cases and CoNS in 12 (19.4%) of cases in the present study. The isolation of Klebsiella as predominant pathogen is consistent with the study of Arora and Devi, Roy et al. and Karlowsky et al. where the reported isolation of the organism was 27.3%, 14% and 16.5%, respectively.[20,13,18] However, prevalence of CoNS was 20.16%, 16.5% and 42%, respectively, in these studies quite higher than isolation of CoNS was seen.[20,13,18] But in accordance with Ashwini M et al. *Staphylococcus aureus* was reported 36.4% and CoNS 1.12%. CoNS isolated from blood are often skin contaminants which may be clinically insignificant.[17] Current study revealed, *Esch. coli* was 9.6% (n=6) of isolated cases and *Pseudomonas spp.* 8.1%(n=5), a nearlysimilar report seen in the study of Crowe M et al. who found *Esch. coli* and *Pseudomonas spp.* in 6% and 5.1 % cases respectively.[17,21]

Interestingly in this study 10 (16.1%) isolates were Salmonella typhi among the neonatal sepsis cases. Generally, Salmonella is a community-acquired infection in the general population which gains entry via faeco-oral route.[22] Vertical intrauterine transmission from a typhoid-infected mother is implicated in neonatal typhoid fever.[23,24,25]According to Chin KC et al. three infants of Pakistani immigrant mothers developed typhoid fever in the neonatal period.[26] According to Mohanty S et al. Salmonella Typhi and Salmonella Paratyphi A strains were isolated from five neonatal septicemia cases.[27] Reed, R. P and Klugman, K. P. found 10 cases of neonatal typhoid fever at a rural African hospital.[28] There were reports of vertical transmission from mother to fetus and horizontal transmission from exogenous routes either by fecal contamination of lower birth canal or aspiration or ingestion of contaminated food as top feed or reports of oral suction in nursery leading to sepsis.[28]

In present study Klebsiella spp. showed high resistance to Ampicillin 22 (81.5%), Cefixime 21 (77.8%) and Cotrimoxazole 17 (63%). Similar reports of high resistance to Ampicillin (71%) were reported by Bhat et al. and 64.28% by Prabhu k et al.[29,30] Nearly similar findings were reported by Dagnew et al. where resistance to Ampicillin was 75%, Cotrimoxazole 50% and ceftriaxone 62.5%.[31] In the current study *Klebsiella spp.* showed least resistance to Imipenem 05 (18.5%), Another similar study by Jyothi, et al. showed most sensitivity to Imipenem (93%).[32]

Current study showed *Salmonella typhi* isolates were excellently sensitive to most of the antibiotics. But it showed 40% resistance to Ampicillin and 60% to Cotrimoxazole. Dagnew et al.[31] in 2013, showed *Salmonella spp.* were 100% resistant to Ampicillin and 66.7% to Cotrimoxazole which was quite higher than this study. This may be due to less frequent use of these antibiotics for enteric fever now a days.

The study revealed, *Esch. coli* was highly (100%) resistant to Ampicillin, 66.7% resistance to Cefixime and Cotrimoxazole, 33.3% resistance to Amoxycillin-Clavulanic acid and Ceftriaxone. Similarly reported by Dagnew et al. found resistance to Ampicillin 100%, followed by 60% to Tetracycline and 40% to Chloramphenicol.[31]

In the case of Pseudomonas spp.no resistance was revealed to Tazobactam-Piperacillin and Imipenem, but it was 100% resistant to Cotrimoxazole, 80% to Ampicillin and Cefixime. Similarly, Dalal P et al. found Pseudomonas was most sensitive to Piperacillin-Tazobactam (93%) and Carbapenem (96%).[33]

Current study revealed Coagulase Negative Staphylococcal strains (CoNS) were most frequent Gram positive isolate and showed no resistance to Linezolid and Vancomycin and highly resistant (83%) to Ampicillin, similar findings reported by El Din et al. where all isolates were sensitive to Vancomycin and high resistance to Ampicillin (95.9%).[11] So, in all Gram-negative and positive isolates Ampicillin was the least effective drug. Same report also given by Guha et al.[34]

**Conclusion**

Organisms belonging to Enterobacteriaceae family are the leading causes, CoNS and Staphylococcus aureus also contribute significantly to causing neonatal septicemia. They are highly resistant to commonly used antibiotics and the most sensitive drugs for Gram-negative isolates were Imipenem, Tazobactam-Piperacillin, Amikacin, Ceftriaxone and Levofloxacin. Most sensitive drugs for Gram-positive bacteria were Vancomycin, Linezolid, Amikacin, Imipenem, Ceftriaxone and Levofloxacin. As the practice of prescribing antibiotics is completely unregulated, usage of all kinds of antibiotics for even minor illnesses are widespread, it is foreseen that antibiotic resistance will go up and crisis of antibiotics will develop in near future. Therefore, rational use of antibiotics after sensitivity testing should be practiced.

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