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Etiology of Neonatal Sepsis and Antimicrobial Resistance Pattern of Gram-Negative Bacteria in a Tertiary Care Hospital of Bangladesh

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Abstract

Background: Neonatal sepsis is one of the most common causes of infection and mortality in neonates specially in developing countries. **Objective:** This study was undertaken to find out the etiology and antimicrobial resistance patterns of Gram-negative bacteria isolated from neonates suspected of having sepsis. **Methodology:** This cross-sectional study was conducted at department of Microbiology, Mymensingh medical college, Mymensingh over a period from March 2023 to February 2024. Blood samples were collected from clinically suspected septicemic neonates. Isolation and identification of organisms were done by automated blood culture method and modified Kirby Bauer disc diffusion method was used to test for antimicrobial susceptibility, as recommended by the Clinical and Laboratory Standards Institute, 2023. **Results:** Out of 207 cases, 102(49.27%) showed positive blood culture. Among them, Gram positive isolates were 13(12.75%), Gram negative isolates were 48(47.06%) and *Candida* 41(40.19%). Most of the bacterial isolates were Gram negative predominantly *Klebsiella spp* (n=26, 42.62%) cases followed by Acinetobacter (n=13, 21.31%). Among cultures with gram positive species, Coagulase negative *Staphylococci* (CoNS) were 8(13.1%). Third generation cephalosporins, gentamicin, meropenem were almost resistance to all Gram negative organisms, however, all isolates were sensitive to tigecycline. **Conclusion:** *Klebsiella spp* was emerged as the predominant etiological agent of neonatal sepsis in the present study. A considerable proportion of the isolates exhibited multi-drug resistance.

Keywords: Neonatal sepsis; Automated blood culture; Multi-drug resistant (MDR); *Coagulase Negative Staphylococci (CoNS)*; Neonatal Intensive Care Unit (NICU).

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Introduction

Neonatal sepsis is defined as systemic infections occurring in infants in the first 28 days of life[1]. It remains a critical global health concern, particularly in low and middle-income countries. The World Health Organization (WHO) estimates that neonatal infections are responsible for about 1.6 million deaths worldwide each year, with nearly 40.0% of all neonatal deaths occurring in developing country[2]. In Bangladesh, neonatal sepsis ranks as the second leading cause of newborn deaths and the neonatal mortality rate is 20 per 1000 live births[3,4]. In rural areas of Bangladesh, sepsis accounts for 12.0% of direct neonatal deaths[5], whereas in the slums of Dhaka, it is responsible for 20.0% of such deaths[6]. It is estimated that around 20.0% of newborns develop sepsis and about 1.0% die from complications related to it[7].

Neonatal sepsis is mainly attributed to a wide range of bacterial, viral and fungal (Candida) pathogens[8]. The pattern of microorganisms responsible for neonatal sepsis changes over time and differs across various regions,[9,10,11]. Epidemiological data reveal notable variations in the distribution of causative bacterial agents and their antimicrobial susceptibility patterns[10,12]. In developed countries, Group B *Streptococcus* (GBS), *Escherichia coli* and *Listeria monocytogenes* are the leading causes of neonatal

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sepsis, whereas in developing nations, Gram negative bacteria and coagulase-negative *Staphylococci* (CoNS) are more commonly responsible for neonatal sepsis[9].

The clinical manifestations of neonatal sepsis are often nonspecific, making diagnosis challenging. Therefore, rapid identification of the causative pathogens and timely initiation of appropriate therapy are essential for effective management[10]. Blood culture remains the gold standard for confirming the diagnosis of sepsis[13,14]. Blood culture techniques are classified into three main types: conventional/manual blood culture systems, lysis centrifugation systems and continuous monitoring blood culture systems. In recent years, automated and computerized continuous monitoring systems have become widely adopted for detecting bloodstream infections. The three main commercially available systems are: BacT/ALERT 3D blood culture system, BACTEC 9000 series, VersaTREK system[13].

Microorganisms implicated in sepsis have developed resistance to commonly used antibiotics over the past few decades, making effective treatment increasingly challenging[15,16]. There is an alarming increase in resistance to the commonly used antibiotics. The World Health Organization (WHO) has recently emphasized the significance of this growing problem and urged coordinated global efforts to limit the spread of antibiotic resistance[17]. Neonatal Gram-negative sepsis is increasingly reported worldwide, accompanied by a worrying surge in multidrug-resistant infections[18]. The rise of multidrug-resistant bacteria highlights the critical need for well-defined antibiotic policies in neonatal intensive care units[19].

It is now essential to carry out regular surveillance to monitor the evolving patterns of bacterial isolates responsible for neonatal sepsis. Hence, understanding the causative agents and their antimicrobial susceptibility profiles is essential for initiating timely and effective patient treatment. In Bangladesh, a limited number of studies have documented the prevalence of neonatal sepsis across various regions of the country[20-21]. This study was designed to investigate the etiology of sepsis in neonates and susceptibility patterns of Gram-negative bacteria related to sepsis.

Methodology

Study Settings: This cross-sectional study was conducted in the Department of Microbiology, Mymensingh Medical College Hospital (MMCH), Mymensingh, Bangladesh during the period from March 2023 to February 2024.

Study Population: The patients who were suspected of neonatal septicemia and admitted in the Neonatology Department of MMCH, were included in this study. Clinically suspected neonates aged group (0-28 days) with one or more clinical features like hyperthermia/hypothermia, respiratory distress, apnea, tachycardia/bradycardia, lethargy, abdominal distension, vomiting, poor feeding or inability to feed, hypo or hyperglycemia, irritability, high pitched cry, seizure, hypotonia or absent neonatal reflexes, umbilical redness or discharge. cyanosis, petechiae, purpura, jaundice, bulging fontanelle, poor perfusion were included. Neonates who did not have above features of sepsis, any critically ill neonates, known case of major congenital anomalies and when guardians refused to give informed written consent were excluded.

Study Procedure: Informed written consent from legal guardians were taken. All the relevant information detailed maternal obstetrical history, neonatal birth history, clinical findings and laboratory findings of every subject were systemically recorded in a pre-designed case record form.

Isolation and Identification of Organisms: Venous blood was collected aseptically following universal safety precaution from peripheral vein (antecubital vein). 1- 2 ml of blood according to body weight was drawn by using a sterile disposable syringe with butterfly needle (JMS 27G). After removing the cap, the top of the BacT/ALERT® PF plus bottles (bioMérieux, Marcy l'Etoile, France) was disinfected by 10% povidone iodine followed by 70% alcohol was allowed to dry. Blood specimens were inoculated into BacT/ALERT® PF Plus bottles. Then the bottles were agitated gently to mix the blood with the media. The bottles were labelled with the appropriate patient information- sample number, patient name, date and the time of collection, registration number and taken to the Microbiology laboratory of Mymensingh Medical College. The culture bottles were incubated at 37°C in the BacT/ALERT 3D automated blood culture system (bioMérieux, Marcy l'Etoile, France) for up to 7 days. Bottles that showed no growth signal after 7 days of incubation were considered culture-negative and were discarded. Bottles indicating positive growth were removed from the system, gently mixed, and 1 mL of broth was aseptically withdrawn using a sterile syringe and needle. The broth was sub-cultured into blood agar and MacConkey agar plates, which were incubated aerobically at 37°C for 18–24 hours. Culture-positive isolates were identified based on colony morphology,

Gram-staining characteristics, pigment production, motility, and relevant biochemical tests following standard microbiological procedures.

Antibiotic Disks, Culture Media and Control Strain: All the bacterial isolates were tested for antimicrobial susceptibility according to the CLSI guideline 2023[22]. The antimicrobial disks (Himedia, Ltd, India) were used according to a standard antibiotic panel for isolated organisms. For Gram negative organisms, ceftazidime (30ug/disk), cefepime (30ug/disk), ceftriaxone (30ug/disk), gentamicin (10ug/disk), amikacin (30ug/disk), ciprofloxacin (5ug/disk), meropenem (10ug/disk), imipenem (10ug/disk), piperacillin-tazobactam (100/10ug/disk), trimethoprim- sulfamethoxazole (25/5ug/disk) antibiotics were used. Susceptibility of the Gram-negative organism to tigecycline was determined by 15ug tigecycline disk, and the criteria of the United States Food and Drug Administration (FDA)[23] were used for interpretation. Mueller-Hinton agar media was used for antimicrobial susceptibility testing. *Escherichia coli* ATCC 25922 was used as a control strain to assess the performance of the method.

Statistical analysis: Microsoft Excel program was used for statistical analysis and figure generation.

Ethical approval: The research protocol was approved by the Institutional Review Board (IRB) of Mymensingh Medical College and Hospital, Memo no MMC/ IRB/2023/585.

Results

Among 207 septicemic neonates, 102 (49.27%) were found blood culture positive and 105 (50.73%) were culture negative (Figure I).

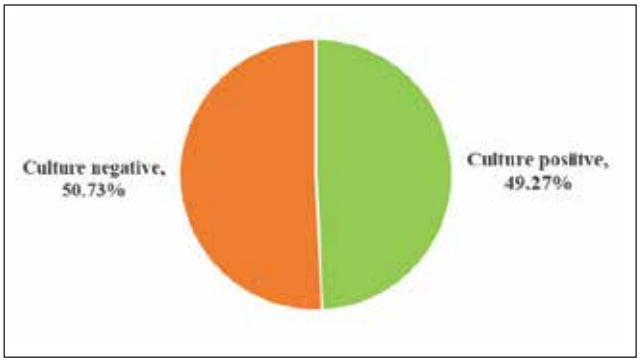

Figure I: The results of blood culture among suspected septicemic neonates(N=207)

Out of total 102 culture positive cases, bacteria were isolated from 61 (59.81%) cases and candida spp were isolated 41 (40.19%) cases (Table 1).

Table 1:Distribution of Pathogenic Isolates by Automated blood culture method (N=102)

Isolated organisms	Frequency	Percent
Bacteria	61	59.81
Candida	41	40.19

Out of 61 bacterial isolates, Gram negative bacteria were 48 (78.69%) while Gram positive bacteria were 13 (21.31%) (Figure II).

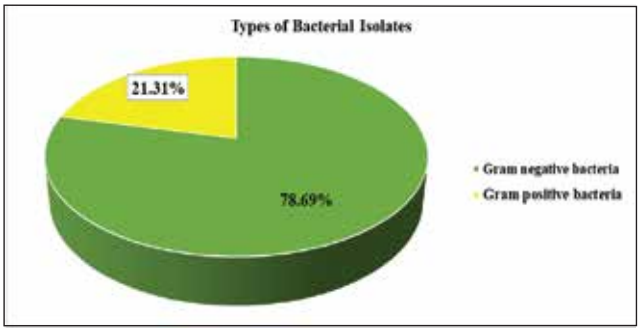

Figure II: Types of bacterial isolates from blood culture (N= 61)

Klebsiella spp was the predominant isolate 26 (42.62%) followed by Acinetobacter 13 (21.31%), Coagulase negative Staphylococci (CoNS) 8 (13.1%) (Table 2). Some Escherichia coli, Pseudomonas, Staphylococcus aureus and Enterobacter species were also isolated. These findings suggest that Klebsiella and Acinetobacter spp are mostly responsible for neonatal sepsis.

Table 2: Distribution of bacterial isolates from automated blood culture method (N=61)

Bacterial pathogens	Frequency	Percentage
Gram Positive Bacteria		
Coagulase negative *staphylococci*	8	13.1
Staphylococcus aureus	5	8.2
Gram Negative bacteria		
Klebsiella spp	26	42.62
Acinetobacter	13	21.31
Escherichia coli	5	8.19
Pseudomonas	3	4.92
Enterobacter	1	1.64
Total	**61**	**100.00**

Among the Gram-negative organisms, the commonest organism, Klebsiella isolates showed complete resistance to ceftriaxone, ceftazidime, and cefepime (100.0% each). High resistance was also observed to gentamicin (76.9%), amikacin (73.1%), and ciprofloxacin (73.1%). Carbapenem resistance was notable, with 73.1% resistance to both imipenem and

meropenem. Additionally, 80.8% of isolates were resistant to trimethoprim-sulfamethoxazole and piperacillin-tazobactam. No resistance to tigecycline was detected (0.0%). Acinetobacter isolates demonstrated high resistance to ceftriaxone, ceftazidime, and cefepime (92.31% each). Resistance to gentamicin and amikacin was 76.92%, while ciprofloxacin resistance was 53.85%. Carbapenem resistance was also high (76.92% for both imipenem and meropenem). Resistance to piperacillin-tazobactam and trimethoprim-sulfamethoxazole was 76.9% and 66.7%, respectively. Tigecycline was not tested. Escherichia coli showed complete resistance to ceftriaxone and ceftazidime (100.0%), with lower resistance to cefepime (80.0%). Resistance to aminoglycosides and ciprofloxacin was 60.0% each. Carbapenem resistance was comparatively lower at 40.0%. Resistance to trimethoprim-sulfamethoxazole and piperacillin-tazobactam was 60.0%, with no resistance to tigecycline (0.0%) (Table 3).

Table 3: Antibiotic Resistance Profile among Major Gram-Negative Isolates (N=44)

Antibiotics	*Klebsiella spp* **(n=26)**	*Acinetobacter* **(n=13)**	*Escherichia coli* **(n=5)**
Ceftriaxone	26 (100.0%)	12(92.31)	5(100.0%)
Ceftazidime	26 (100.0%)	12(92.3%)	5(100.0%)
Cefepime	26 (100.0%)	12(92.3%)	4(80.0%)
Amikacin	19 (73.1%)	10(76.9%)	3(60.0%)
Gentamicin	20(76.9%)	10(76.9%)	3(60.0%)
Ciprofloxacin	19(73.1%)	7(53.8%)	3(60.0%)
Imipenem	19(73.1%)	10(76.9%)	2(40.0%)
Meropenem	19(73.1%)	10(76.9%)	2(40.0%)
Trimethoprim-Sulfamethoxazole	21(80.8%)	9(66.7%)	3(60.0%)
Piperacillin-tazobactam	21(80.8%)	10(76.9%)	3(60.0%)
Tigecycline	0 (0.0%)	-	0(0.0%)

Discussion

Neonatal sepsis remains a diagnostic and treatment challenge for the neonatal health care providers. The spectrum of organisms responsible for neonatal sepsis changes over time and also varies from region to region. The study of bacteriological profile plays a very important role in effective management of neonatal sepsis.

In the present study, 102(49.3%) cases were blood culture positive and 105 (50.73%) were culture negative out of 207 septicemic neonates. Almost a similar higher proportion of blood culture-positive neonatal sepsis was also reported by Rahman[24] (71.69%) in NICU of DMCH.

The prevalence of neonatal sepsis observed in earlier studies conducted in Bangladesh was relatively lower, with Hafsa et al[20] reporting (7.45%) and Islam QR et al[10] reporting (31%). Higher isolation rate in this study might be due to the use of an automated blood culture method. Furthermore, overcrowding in NICU of MMCH along with a high patient-to-healthcare provider ratio may also contribute to high isolation rate. However, the incidence of neonatal sepsis may vary from country to country as well as, within the same country from one institution to another and even year to year in the same institution[24].

In this study, bacteria accounted for (59.8%) and candida (40.2%). This finding agrees with the study conducted by Singh[25] who found 65.95% bacteria and 34.1% Candida. On the other hand, Rahman et al.[24] reported that bacteria were 84.2% and candida 15.79% cases, which is dissimilar to this study. This discrepancy may be due to the differences in patient population, antibiotic exposure, infection control practices, and diagnostic purposes.

The present study documented that Gram-negative bacteria (78.69%) are more responsible than Gram-positive bacteria (21.3%). Similar findings were reported by other investigators[10,26,27]. But the current study disagrees with the findings of other researchers who found predominance of Gram-positive bacteria in their study[1,3,28]. The majority of Gram-negative organisms isolated in this study and other studies reviewed suggest that these infections may be acquired from the hospital or community environment, due to poor hygienic practices during delivery and postnatal care, rather than reflecting vertical transmission to the infant from exposure to vaginal tract flora[29].

The most predominant bacterial species isolated was *Klebsiella species*, which was present in (42.62%) organisms, followed by *Acinetobacter* (21.31%) and CONS (13.1%). In Bangladesh, Islam et al[10] reported 41.9% *Klebsiella species* as the most common pathogen, followed by 19.4% *Escherichia coli* and in India, Mulley et al30 found *Klebsiella pneumoniae* (35.4%) as the predominant pathogen, and in Nepal, Pokhrel et al[31] reported *Klebsiella species* (33.3%) were predominant isolates. On the other hand, *Escherichia coli* was found as the predominant bacteria in neonatal sepsis by others[32-33]. The high prevalence of *Klebsiella species* in neonatal sepsis is a major concern. The organism's resistance may be

attributed to several factors, including the presence of a protective capsule, multidrug resistance efflux pumps, its ability to spread easily, and its strong pathogenic nature with an enhanced capacity to acquire and transfer resistance plasmids[16].

A high proportion of organisms were resistant to commonly used antibiotics, which is alarming in our study. All the Gram-negative isolates showed 92.31% to 100% resistance to third-generation cephalosporins. This is almost similar to the study conducted by Rahman et al[34] who reported 88.89% to 100% resistance to third-generation cephalosporins. In Nepal, Pokhrel et al.[31] reported that Gram-negative bacteria had 80.5% resistance to cefotaxime. The reported high resistance rates to one of the first-line antibiotics may be due to the misuse and overuse of those antibiotics in our country.

This study observed that all the isolated *Klebsiella species* showed 76.92% resistance to gentamicin, 73.8% resistance to amikacin, imipenem, meropenem, and ciprofloxacin respectively. These findings of the present study correlate with the findings of the study by Rahman et al[34] who found 88.89% resistance to both amikacin and gentamicin; Siddiqui et al[35] also reported *Klebsiella species* showed 70.6% resistance to amikacin, 82.4% to ciprofloxacin, 58.8% to both meropenem and imipenem. In contrast to this study, some other researchers documented moderate to higher sensitivity to *Klebsiella species*[3,28,36].

In the current study, *Acinetobacter species* showed 76.9% resistance to amikacin, gentamicin, imipenem, meropenem and piperacillin-tazobactam, 66.7% to SXT, 53.8% to ciprofloxacin. This was supported by Rahman et al[34] from Bangladesh who reported 100.0% resistance to amikacin, gentamicin, piperacillin-tazobactam, 75.0% to ciprofloxacin and meropenem, 100.0% sensitivity to tigecycline for *Acinetobacter spp*. In India, Siddiqui et al[35] also reported 100% resistance to amikacin, ciprofloxacin, imipenem, meropenem. This finding disagrees with Amin et al[37] who reported *Acinetobacter species* had 100.0% sensitivity to netilmicin, colistin and imipenem.

This study documented that 60.0% resistance to amikacin, gentamicin, ciprofloxacin, PIT, SXT and 40% to imipenem and meropenem was observed in Escherichia coli. All the isolated *E.coli* spp were sensitive to tigecycline. This result is consistent with the results submitted by Monjur et al.[38] who reported that 78.6% resistance to gentamicin, 64.3% to ciprofloxacin in Bangladesh. In contrast to this, Afrin et al[3] and Chharra et al[39] showed good susceptibility towards commonly used antibiotics. This indicates the emergence of highly resistant strains of *Escherichia coli* in our setting.

Resistance to meropenem among different Gram-negative isolates ranged from 40.0% to 76.9%. This is almost similar to the study done by Rahman et al[34] who reported that all of the isolated Gram-negative organisms were 50.0% to 83.3% resistant to meropenem. Previously, Hafsa et al.20 from Bangladesh reported that carbapenem was the effective drug (100.0%) for all the isolates. This study clearly shows that meropenem, which is used as a last-resort drug in the NICU, is developing resistance at an alarming rate. However, almost all the Gram-negative isolates were sensitive to tigecycline.

Limitation

Due to the limited blood volume obtainable from neonates, collection of two separate blood culture sets was not feasible.

Conclusion

The findings indicate that the predominance of Gram-negative organisms, particularly *Klebsiella species, Acinetobacter,* and *Escherichia coli* which exhibited high levels of resistance to commonly used antibiotics. The increasing prevalence of multidrug-resistant isolates poses a serious challenge to effective treatment and underscores the urgent need for continuous surveillance, rational antibiotic use and strict infection control measures. Strengthening antimicrobial stewardship programs and updating empirical therapy guidelines based on local resistance trends are essential steps to improve neonatal outcomes and combat the growing threat of antimicrobial resistance in hospital settings. To prevent further resistance, it may be recommended to avoid irrational use of antibiotics and to update empirical treatment regimen in every institution periodically. NICU infection control protocol is recommended to implement, follow and monitor strictly.

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Conflict of Interest
All authors declared no conflict of interests.

Financial Disclosure
No funds were received for this study.

Authors' contributions
Uhtsa Khisa conceived and designed the study, analyzed the data, interpreted the results and wrote up the draft manuscript; Nazia Haque, Pompy Dey and Sumya Mahbuba contributed to the analysis of the data, interpretation of the results and critically reviewing the manuscript; Kallins Chakma and Mahfuz Islam were involved in the manuscript review and editing; All authors read and approved the final manuscript.

Data Availability
Any inquiries regarding supporting data availability of this study should be directed to the corresponding author and are available from the corresponding author on reasonable request.

Ethics Approval and Consent to Participate
Ethical approval for the study was obtained from the Institutional Review Board. All methods were performed in accordance with the relevant guidelines and regulations.

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