Original Article

Alarming Pattern of Antimicrobial Resistance in Neonatal Sepsis Observed in Neonatal Intensive Care Unit of a Tertiary Care Hospital in Bangladesh

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Abstract

Background: *Resistance of micro-organisms to multiple broad-spectrum antimicrobial agents is a major problem in treating neonatal sepsis. It is a matter of utmost importance to have knowledge of trends in changing pattern of antimicrobial resistance.* ***Objective:*** *This study was done to observe antimicrobial resistance of gram-positive and gram-negative bacteria isolated from cases of neonatal sepsis.* ***Material and Methods:*** *This cross sectional descriptive study was conducted in Department of Microbiology in collaboration with Department of Neonatology, Dhaka Medical College Hospital, Dhaka. Antimicrobial resistance of all the isolated bacteria was performed by Modified Kirby-bauer disk diffusion method following standard guideline after isolation and identification of bacteria from blood samples of suspected septicemic neonates by automated blood culture and standard microbiological protocol.* ***Results:*** *All of the isolated Staphylococcus aureus, Coagulase negative Staphylococcus, Group-B Streptococcus and Micrococcus showed 100% resistance to ceftriaxone, cefotaxime and ceftazidime. Among the isolated gram-negative bacteria, all of Enterobacter spp., Pseudomonas aeruginosa and Citrobacter spp. showed 100% resistance to amoxiclav, amikacin, ceftriaxone, cefixime, ceftazidime.* ***Conclusion:*** *Majority of the gram-positive and gram-negative bacteria are developing resistance to multiple antimicrobial agents and surveillance is necessary to tackle this alarming situation.*

Key words: *Neonatal Sepsis, Antimicrobial Resistance, Neonatal Intensive Care Unit (NICU)*

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Introduction

Neonatal sepsis is the term that has been used to describe the systemic response to severe infection in the first month of life[1] and is considered a prime health problem around the world.[2] Neonatal mortality rate is a crucial indicator to assess the health status of a nation and sepsis remains to be one of major causes of neonatal mortality and morbidity worldwide.[3] Every year 1 to 10 per 1000 live births occur globally due to neonatal sepsis caused by bacteria[4] with a much higher incidence in developing countries than in developed countries.[3] According to World Health Organization (WHO) estimates, about 5 million neonatal deaths occur each year globally, of which 98% in developing countries.[5] Antibiotic resistance is a global problem that can cross international boundaries and spread between countries very easily and speedily. World health leaders have described antibiotic resistant organisms as "nightmare bacteria" that pose a catastrophic threat to people in every country in the world. The extensive and inappropriate use of antimicrobial agents has invariably resulted in the development of antibiotic resistance which, in recent years, has become a great problem worldwide.[6]

Reports of multi-drug resistant bacteria causing neonatal sepsis in developing countries are increasing, particularly in intensive care units. The antimicrobial sensitivity pattern differs in different studies which may be due to emergence of resistant strains as a result of indiscriminate use of antibiotics for both prophylactic and therapeutic treatment of hospitalized newborns.[7] Methicillin resistant Staphylococcus aureus (MRSA), Extended spectrum β-lactamase (ESBL) producing

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and multi drug-resistant gram-negative bacteria represent the principal concern for clinicians who have to fight against infection.[8] It is reported that gram-negative bacteria and in particular Klebsiellaand Enterobacter spp. are the leading causes of neonatal sepsis in Bangladesh and almost all are resistant to ampicillin, gentamicin and third generation cephalosporin.[9] One of the serious problems associated with antibiotic therapy is resistance to newer drugs like carbapenems. About 89% Metalo beta-lactamases (MBLs) producing isolates are reported in imipenem resistant gram-negative bacteria in Bangladesh[10] and it imperils control of infection in our country. Therefore, in this study, the investigators aimed to determine the antibiotic sensitivity patterns of aerobic isolates from blood cultures of neonates in a tertiary care hospital in Dhaka, Bangladesh.

Materials and Methods

This cross-sectional descriptive study was carried out in Department of Microbiology, in collaboration with Department of Neonatology at Dhaka Medical College Hospital (DMCH), during the period of January 2016 to December 2016. A total of 106 neonates of either sex of age 0-28 days, both inborn and out-born, admitted in Neonatal Intensive Care Unit (NICU) of DMCH with suspected clinical features of sepsis at admission or developed such features afterwards when admitted for other indication, irrespective of antibiotic intake were included. Neonates with congenital anomalies, acute bilirubin encephalopathy, perinatal asphyxia, meconium aspiration syndrome, history of prolonged rupture of membrane and prolonged labor of mother were excluded from this study. A predesigned questionnaire including information regarding baby's illness and mother's antenatal period was provided to mother or concerned attendants of neonates and were asked to fillup for selection of suspected cases. The study was approved by Research Review Committee of the department of Microbiology and Ethical Review Committee of Dhaka Medical College. After proper explanation about the nature of the study, written consent was taken from legal guardian of each neonate.

Isolation and identification of organisms

Blood samples were collected with all aseptic precaution and were inoculated into BacT/ALERT PF Plus bottle (bioMerieux, Inc, Durham, North Carolina) and these bottles were processed for automated blood culture using BacT/ALERT 3D 60 Microbial Detection System (bioMerieux, Inc, Durham, North Carolina). Subcultures were done on Blood agar, MacConkey agar and Chocolate agar media. Isolates were identified by colony morphology, hemolytic criteria, pigment production, different biochemical reactions and staining character as per standard techniques.[11]

Antibiotic disks, culture media and control strain

All bacterial isolates were tested for antimicrobial susceptibility according to the CLSI, 2015.[12] The antimicrobial disks (Oxoid Ltd, UK) were used according to the standard antibiotic panel for isolated organisms. For gram positive organisms Cefoxitin (30µg/disk), ceftazidime (30µg/disk), ceftriaxone (30µg/disk), ciprofloxacin (5µg/disk), amikacin (30µg/disk), gentamicin (10µg/disk), levofloxacin (5µg/disk), linezolid (3 µg/disk), teicoplanin (30µg/disk), vancomycin (30µg/disk), cefotaxime (30µg/disk) antibiotics were used. For gram negative organisms Amoxiclav (amoxicillin 20µg + clavulanic acid 10µg/disk), cefixime (30µg/disk), ceftazidime (30µg/disk), ceftriaxone (30µg/disk), ciprofloxacin (5µg/disk), amikacin (30µg/disk), gentamicin (10µg/disk), meropenem (10µg/disk), colistinsulphate (10µg/disk), piperacillin/ tazobactum (100/10µg/disk) antimicrobials were used. Susceptibility of the gram-negative organism to tigecycline was determined using 15µg tigecycline disk and the criteria of the United States Food and Drug Administration was used for interpretation.[13] Mueller-Hinton agar media was used for antimicrobial susceptibility testing and Esch. coli ATCC 25922 was used as control strain to assess the performance of the method.

Preparation of inoculum

Using a sterile wire-loop, 3-5 well isolated colonies of test organism were emulsified in 3 ml of sterile normal saline. The turbidity of the suspension was compared with McFarland turbidity standard 0.5 by adding normal saline and placing a printed card behind the test inoculum and McFarland turbidity standard under proper light.[11]

Inoculation of test organism, disk placement and incubation

After standardization of inoculums a sterile swab stick was immersed into the bacterial suspension. The excess suspension was removed by rotating the swab with firm pressure against the inner side of the tube above the fluid level. The swab was streaked evenly on the surface of the Mueller-Hinton agar plate in 3 different directions by rotating the plate approximately 600 angle each time to get uniform distribution of inoculums. Inoculated plate was allowed to dry for 3-5 minutes. Antibiotic disks were placed on inoculated plate 15 mm away from the edge of the plate and 25 mm apart from one disc to another from center to center. Within 30 minutes of placement of antibiotic disks inoculated plates were incubated aerobically at 370C overnight.

Interpretation of zone of inhibition diameter

After overnight incubation each plate was examined to ensure the confluence growth of organism. The zone of inhibition was measured in mm using a ruler on the undersurface of the plate and was evaluated with standard chart of CLSI, 2015 guideline.[12] The presence or absence of clear zone of inhibition around the disk was interpreted as sensitive and resistant respectively.

Results

Twelve Gram-positive bacteria were isolated from 106 blood samples of suspected septicemic neonates. Among those, *Staphylococcus aureus* were 4, Coagulase negative *Staphylococcus* (CONS) were 4, Group B *Streptococcus* (GBS) were 2 and *Micrococcus* were 2 in number (Figure 1).

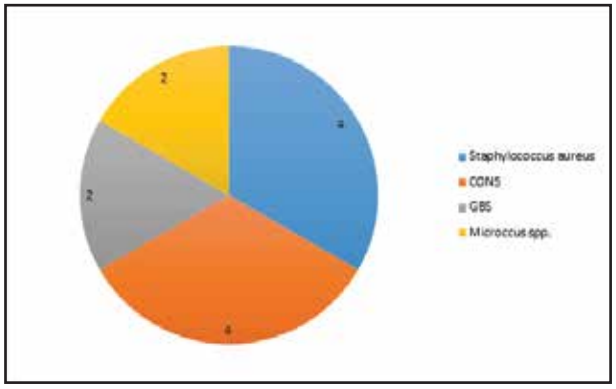

Figure 1: Distribution of isolated Gram-positive bacteria (N=12).

Fifty-two gram-negative bacteria were isolated from 106 blood samples of suspected septicemic cases. Among those *Enterobacter* spp. were 20, *Klebsiella pneumoniae* were 18, *Pseudomonas aeruginosa* were 8, *Acinetobacter* spp. were 4 and *Citrobacter* spp. were 2 in number (Figure 2).

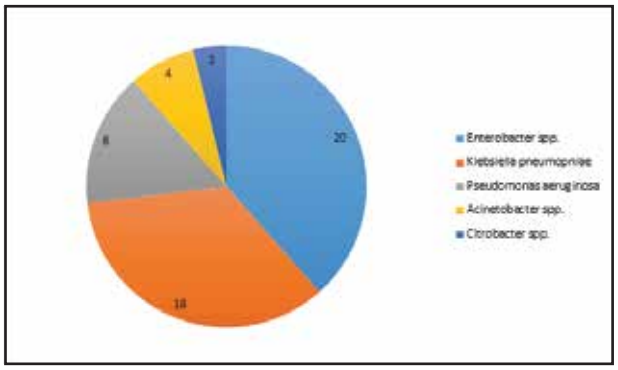

Figure 2: Distribution of isolated Gram-negative bacteria (N=52).

All (100%) the isolated gram-positive bacteria of this study were resistant to ceftriaxone, ceftazidime and cefotaxime. None was resistant to cefoxitin, vancomycin, linezolid and teicoplanin. Among the isolated *Staphylococcus aureus*, 50% were resistant to amikacin, 75% to gentamicin, ciprofloxacin and 25% to levofloxacin. Among the isolated CONS, 75% were resistant to amikacin and gentamicin, 50% to ciprofloxacin and none was resistant to levofloxacin. Among the isolated GBS, 50% were resistant to amikacin, levofloxacin and all (100%) were resistant to gentamicin and ciprofloxacin. Among the isolated *Micrococcus* spp., 50% were resistant to amikacin, ciprofloxacin, 100% to gentamicin and none was resistant to levofloxacin (Table I).

Table I: Antimicrobial resistance pattern of isolated gram positive bacteria (N = 12).

Antimicrobial drugs	***Staphylococcus aureus*** **(N = 4)** **n (%)**	**CONS N = 4)** **(n (%)**	**GBS (N = 2)** **n (%)**	***Micrococcus spp*** **(N = 2)** **n (%)**
Amikacin	2 (50.00)	3 (75.00)	1 (50.00)	1 (50.00)
Gentamicin	3 (75.00)	3 (75.00)	2 (100.00)	2 (100.00)
Cefoxitin	0 (000)	0 (0.00)	0 (0.00)	0 (0.00)
Levofloxacin	1 (25.00)	0 (0.00)	1 (50.00)	0 (0.00)
Linezolid	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Vancomycin	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Teicoplanin	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Ceftriaxone	4 (100.00)	4 (100.00)	2 (100.00)	2 (100.00)
Cefotaxime	4 (100.00)	4 (100.00)	2 (100.00)	2 (100.00)
Ceftazidime	4 (100.00)	4 (100.00)	2 (100.00)	2 (100.00)
Ciprofloxacine	3 (75.00)	2 (50.00)	2 (100.00)	1 (50.00)

CONS = Coagulase Negative *Staphylococcus*
GBS = Group B *Streptococcus*
N = Total number
n = Number of resistant bacteria

All (100%) the isolated gram-negative bacteria of the present study were sensitive to colistin and tigecycline. All (100%) the isolated *Enterobacter* spp., *Pseudomonas aeruginosa, Acinetobacter baumannii* and *Citrobacter* spp. were resistant to amoxiclav, amikacin, ceftriaxone, cefixime and ceftazidime. Among the isolated Enterobacter spp., 90% were resistant to gentamicin, 30% to ciprofloxacin, 75% to meropenem and 80% to piperacillin with tazobactam. Among the isolated *Klebsiella pneumoniae,* 88.89% were resistant to amoxiclav, amikacin, gentamicin, ceftriaxone, cefixime and ceftazidime, 16.67% to ciprofloxacin, 83.33% to meropenem and 77.75% to piperacillin with tazobactam. Among the isolated *Pseudomonas aeruginosa*, 87.5% were resistant to gentamicin, 50% to ciprofloxacin, 62.5% to meropenem and 75% to piperacillin with tazobactam. Among the isolated *Acinetobacter baumannii*, 75% were resistant to ciprofloxacin, meropenem and all (100%) were resistant to gentamicin, piperacillin with tazobactam. Among the isolated *Citrobacter* spp., 50% were resistant to ciprofloxacin, meropenem, piperacillin with tazobactam and all (100%) were resistant to gentamicin (Table II).

Table II: Antimicrobial resistance pattern of isolated gram negative bacteria (N = 52).

Anti microbial drugs	*Entero bacter* spp. (N = 20) n (%)	*Klebsiella pneumoniae* (N = 18) n (%)	*Pseudomonas aeruginosa* (N = 8) n (%)	*Acinetobacter* spp. (N = 4) n (%)	*Citrobacter* spp. (N = 2) n (%)
Amoxiclav	20 (100.00)	16 (88.89)	8 (100.00)	4 (100.00)	2 (100.00)
Amikacin	20 (100.00)	16 (88.89)	8 (100.00)	4 (100.00)	2 (100.00)
Gentamicin	18 (90.00)	16 (88.89)	7 (87.5)	4 (100.00)	2 (100.00)
Ceftriaxone	20 (100.00)	16 (88.89)	8 (100.00)	4 (100.00)	2 (100.00)
Cefixime	20 (100.00)	16 (88.89)	8 (100.00)	4 (100.00)	2 (100.00)
Ceftazidime	20(100.00)	16 (88.89)	8 (100.00)	4 (100.00)	2 (100.00)
Ciprofloxacin	6 (30.00)	3 (16.67)	4 (50.00)	3 (75.00)	1 (50.00)
Meropenem	15 (75.00)	15 (83.33)	5 (62.5)	3 (75.00)	1 (50.00)
Piperacillin-Tazobactam	16 (80.00)	14 (77.78)	6 (75.00)	4 (100.00)	1 (50.00)
Colistin	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)
Tigecycline	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)	0 (0.00)

N = Total number
n = Number of resistant bacteria

Discussion

The time that is required to diagnose neonatal sepsis by gold-standard blood culture and assessing antimicrobial sensitivity of the isolated organism is very much crucial for saving lives of neonates. Immediate empirical antimicrobial therapy plays most important role in such condition. So, regular surveillance of resistance pattern of routinely used antimicrobial agents is necessary for a proper and effective treatment plan of neonatal sepsis.

In this study, all the isolated gram-positive cocci were found sensitive to cefoxitin, linezolid, vancomycin and teicoplanin. This finding was in agreement with the study conducted by Sarwar[14] from DMCH where 39.40% *Staph. aureus*, 15% *Micrococci* and none of the CONS were resistant to cefoxitin. In another study from DMCH in NICU, Rahman[15] reported that 66.67% of *Staph. aureus* and 33.33% of CONS were resistant to cefoxitin. The significantly lower resistance to cefoxitin might be due to the rational and cautious use of penicillins and maintaining contact precautions in NICU of DMCH. Sarwar[14] also reported that among the isolated gram-positive cocci, all were sensitive to linezolid, 10% of *Staph. epidermidis* were resistant to vancomycin and 6.06% were resistant to teicoplanin, which was almost similar to the present findings. It was evident from this study that 50% to 75% of the different species of gram-positive cocci were resistant to amikacin and 75% to 100% were resistant to gentamicin. This corresponds to the study conducted by Rahman[15] who reported 54.17% to 83.33% of the isolated gram-positive cocci were resistant to amikacin and 79.17% to 83.33% were resistant to gentamicin. The reported high resistance rates to one of the first line antibiotics may be due to the misuse and overuse of these antibiotics in DMCH as well as in our country.

In the present study, isolated gram-negative organisms showed 88.89% to 100% resistance to third-generation cephalosporins such as, ceftriaxone, cefixime and ceftazidime. This is almost similar to the study conducted by Rahman[15] who reported that 96.55% of the isolated gram-negative organisms were resistant to ceftriaxone and 89.65% to ceftazidime. In Nepal, Shrestha et al.[16] reported that 87.34% and 86.7% gram-negative bacteria were resistant to ceftriaxone and ceftazidime respectively.

In the present study, all the *Pseudomonas aeruginosa* were sensitive to colistin and 75% were resistant to piperacillin-tazobactam. This is contradictory to the previous study conducted by Rahman[15] who reported that all the Pseudomonas aeruginisa were resistanct to colistin and all were sensitive to piperacillin-tazobactam. Fifty to 83.33% of the isolated different species of gram-negative organisms were found resistant to meropenem in this study. Rahman[15] reported that 53.45% of the isolated gram-negative organisms were resistanct to meropenem. Previously, Begum[17] and Hafsa et al.[18] from Bangladesh reported that carbapenem was the most effective drug (100%) for all the isolates. So, it is obvious from this study that meropenem, which is used as last resort drugs in NICU is developing resistance at an alarming rate day by day. However, all the gram-negative isolates were sensitive to both colistin and tigecycline.

Conclusion

It is apparent from this study that all of the isolated bacteria were resistant to multiple antimicrobial agents. Cefoxitin, vancomycin, linezolid in case of gram-positive bacteria and colistin, tigecycline in case of gram-negative bacteria are left for the empiric antibiotic therapy of neonatal sepsis. Therefore, policy makers should focus on formulation, implementation and monitoring of guideline for rational use of antimicrobial agents. Furthermore, public-awareness should be raised on dangers of indiscriminate use of antibiotics as this has already created ineffectiveness of major broad-spectrum antibiotics.

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