Original Article

Bacteriological Profile and Antimicrobial Sensitivity Pattern of Neonatal Sepsis

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Abstract

Introduction: Neonatal sepsis (NS) is one of the important causesof neonatal morbidity anddeath in neonatal ward and neonatal medical care unit,especiallyin emergent nations. Frequent monitoring on pathogens with recent updates and their antimicrobial sensitivity pattern is compulsory for more satisfactory management.

Methods: This cross sectionalstudy was outlined to evaluate the bacteriological profile and antimicrobialsensitivity pattern of NS in neonatal ward and neonatal intensive care unit (NICU)of Sylhet Women's Medical College and Hospital, Sylhet, Bangladesh from October 2019 to December 2020. Blood samples from 90 clinically suspected neonatal sepsis cases were accumulated and processed in the microbiological protocol and their antimicrobial sensitivity pattern were determined.

Results: Among the90 cases, two third (61, 67.78%) showed positive blood culture. Among the positive blood culture, 38(62.29%) were gram positive isolates and gram negative isolates were 23(37.7%). This study showed Staphylococcus aureuswas the most common organism (59.01%) followed by Escherichia coli (E. Coli)(27.87%), Klebsiella pneumonia (9.87%) and Streptococcus pneumonia (3.28%) among the isolates. Gram positive isolates were highly reactive to Amikacin and Amoxicillin and Clavulenic acid whereas gram negative isolates were highly reactive to Gentamicin and Meropenem.

Conclusion: Gram positive organism are the predominant source of NS which was highlighted in our study,though gram negative organism also plays an important role in the mortality cause by neonatal sepsis.

Keywords: Neonatal sepsis, Coagulase negative bacteria, Antimicrobial susceptibility.

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Introduction

Neonatal sepsis is a clinical condition caused by invasion and multiplicationof bacteria inblood stream within the first 28 days of life and is more common in developing countries.

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[1]Infections are the single largest cause of death of neonates worldwide. According to World Health Organization (WHO) estimates, there are 5 million neonatal deaths in a year.[2]It is estimated that,NS is responsible for about 25% of the neonatal deaths in the world and frequently in developing countries. In Asia, the reported incidents of NS vary from 7 to 38 for each 1000 live birth.[3]According to United Nations Children's Fund(UNICEF) report, neonatal mortality rate (NMR) is 27/1000 live birth and NS contributes to 36% of total demises in Bangladesh[3]. The incidence of NS in India according to National Perinatal Database (2002-2003) was 30 for each 1000 live births and two common isolated organisms were Klebsiella pneumonia and Staphylococcus aureus.[4]

There are some maternal risk factors might be responsible forNS likematernal pyrexia within 2 weeks before delivery, premature rupture of

membrane (PROM), foul smelling liquor or instrumental delivery and nutritional deficiency. Low birth weight, birth asphyxia and congenital anomalies are the foetal risk factors responsible for NS..[5]According to the onset of clinical sign and symptoms, NS canbe classified as early onset sepsis (EOS), occurring within the 1st 72 hours of birth (up to 7 days of life) and late onset sepsis (LOS) which occurs after passing 72 hours(8 to 28 days). EOS may result from vertical transmission at the time of labourwhich includes sepsis, meningitis and pneumonia and LOS may be caused by vertical, and nosocomial infections.[6]

The microorganisms most commonly associated with EOS include Group - B Streptococcus, E. coli, coagulase negative Staphylococcus species (CONS), Hemophilusinfluenzae and Listeria monocytogen and late onset septicemia is caused by CONS, Staphylococcus aureus,E.coli,Klebsiella, Pseudomonus, Enterobactor, Candida. The recent trends shown increase in infections due to CONS.[7]

More than last few decades, it had been seen that, microorganism responsible for NS developed resistance to antimicrobials which was making treatment difficult. Use of antibiotic is a guess work and mostly irrational in developing countries. As there is no fixed universal diagnostic markerand due to poor investigation facilities, most of the physicians prescribe antibiotic on the basis of clinical doubt. As the organism and sensitivity pattern are mostly unknown, maximum prescribed antibiotics are broad spectrum.[8]

Various studies in bacteriological profile of NS hadpreviously been done in different hospital like Dhaka Shishu Hospital and Bangladesh Institute of Research and Rehabilitation in Diabetes, Endocrine and Metabolic Disorders(BIRDEM) hospital. Butit was important to remember that, bacterial flora was dynamic, different from one place to another and it changes in the same place over a period of time. So, it was necessary to run periodic surveillance to access the changing pattern of organisms causing NS. Therefore updated data regarding the pattern of bacterial isolates and their antimicrobial susceptibility pattern will come in handy for prompt treatment of the patients. With a view to finding out the bacteriological profile of NS and their antimicrobialsensitivity pattern this study was conducted. This will help in formulating the usage of antibiotics locally in order to decide about using the best possible antibiotics while treating neonatal sepsis.[9]

Materialsand Methods

This cross sectional study was done in the neonatal ward and NICU of Sylhet Women's Medical College and Hospital, Sylhet, Bangladeshfrom October 2019 to December 2020. Blood samples from 90 clinically suspected neonatal sepsis cases were accumulated.A sample, 1-2 ml of blood was aseptically obtained from the peripheral vein of each neonate and dispensed into a sterilized universal bottle containing 20 ml of Tryptone Soy Broth (TSB) and brain heart infusion broth to make a 1:10 dilution. Before collection of blood the site was cleaned with povidoniodine and chlorhexidine before blood. Blood culture samples were then sent to the laboratory and incubated at 37^0Cfor 24 hours using thermo scientific detection system. After detection of presence of organism, subculture were done on solid media (bloodagar, McConkey agar and chocolate agar media) on appearance of turbidity on day 1, 2, 3 and 7. Organisms were identified according to the standard microbial procedures including gram stain, colonymorphology, motilityandbiochemical reactions. All the records of this study were carefully revised and data including birth weight, preterm or term and clinical features consistent with sepsis, results of culture, antimicrobial sensitivity were recorded into a data collection

sheet. Cerebro spinal (CSF) fluid was collected by lumber puncture when meningitis was suspected. Antimicrobialsensitivity testing was carried out for all blood culture isolates, according to the criteria of the national laboratory standard by disk diffusion method.Babies born in the hospital and admission from outside the hospital were included in the study. Neonates presented with one or more features like presence of fever (>_38^{0}C) or hypothermia(<_36^{0}C),reluctant to feed, lethargy, abdominal distension, vomiting, diarrhea, jaundice, convulsion, bulging fontanels, respiratory distress, hypoglycemia and umbilical discharge were included in the study.Mother of those neonates who delivered by either cesarean section or vaginal delivery with history of two or more risk factors like PROM >12 hours, prolonged labour, foul smelling liquor, meconium stained amniotic fluid were also included in this study.Neonates who were suspected as sepsis but not confirmed by haematological investigation were excluded from this study. Neonates with congenital anomalies were also excluded from this study. A rigorousphysical examination was carried out aftertaking detailed and careful history of each case. Patient's caregivers were interviewed and only those babies were enrolled where the guardians gave permission for collection of bloodsamples in neonatal ward during the time of study.Data on the isolated pathogens and their antimicrobial sensitivity pattern were collected after laboratory tests were completed. Data was collected for the variables like demographic profile, blood culture result andantimicrobial sensitivitypattern.Data input was done into microsoft excel sheetand cleaned by cross checking for missing data, duplicates and outliers. Pathogens isolated from the laboratory investigation and antibiotic susceptibility of the isolates were presented in frequencies and proportions.

Results

During the study period, data was collected from 90 neonates with sepsis admitted in NICU and neonatal ward. Among them, two thirds (61, 67.78%) were blood culture positive and one third (29, 32.22%)were culture negative (Figure1). Among culture positive cases, EOS were 45, male were predominant 35 and LOS was 16 in which male were14,totalnumbers of affected male neonates were (49, 80.33 %) (Table I) .The medium gestationalweek was 35 weeks (34-38 weeks) and approximate birth weight was2kg (1.5-2.5)kg (Table II).The commonest risk factors weremothers havingPROM and had history of prolong labour (Table III).

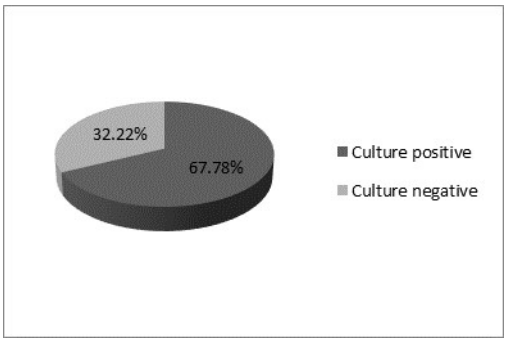

Figure 1: Pie chart depicting the result of culture (n=90)

Table-I: Gender distribution of positive neonates (n=61)

Sex	Number		Total (%)
	EONS	LONS	
Male	35	14	49 (80.33%)
Female	10	2	12 (19.67%)

EOS: Early onset neonatal sepsis; LOS: Late onset neonatal sepsis

Table-II: Demographic characteristics of the study population

Variables		Frequency	Percentage
Age in days	0-7 days	49	80.33
	8-28 days	12	19.67
Weight in kilogram	1.5 - 2.4 kg	44	72.13
	2.5 - 3.9 kg	17	27.87

Table III: Maternal factors of the study population

Variable		Frequency	Percentage
Gestational age inweeks	≤ 35 weeks	36	59.01
	36 - 38 weeks	25	40.99
Maternal factors	History of PROM	15	24.59
	Foul smelling liquor	12	19.67
	PV exam without gloves	10	16.39
	Prolong labour	10	16.39
	Antibiotic prophylaxis	14	22.95
Mode of delivery	NVD	34	55.74
	CS	27	44.26
Place of delivery	In hospital	35	57.38
	Outside hospital	26	42.62

More common organisms were gram positive bacteria. Staphylococcus aureus were the most isolated prevalent gram positive bacteria (59.01%).The second commonest organism was E.coli which was found in 17 neonates (27.87%). That was 27.87% of the total positives. Klebsiellapneumonia was 9.84% and Streptococcuspneumoniawas seen in about 3.28% cases ((Figure 2 and Table IV). Death was low in this study (2 in number, 3.27%).

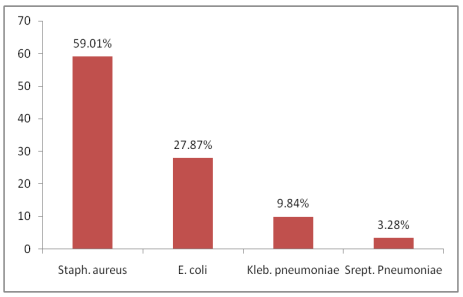

Figure-2: Bar chart showing the organisms isolates in culture

Table-IV:Types of organism isolates

Organism	EONS		LONS	
	Frequency	Percentage	Frequency	Percentage
Staphylococcusaureus	26	42.62%	10	16.39%
Escherichia coli	13	21.31%	4	6.57%
Klebsiella pneumonia	4	6.56%	2	3.28%
Streptococcus pneumonia	2	3.28%	0	0

EOS: Early onset neonatal sepsis; LOS: Late onset neonatal sepsis

In this study,organisms showed completely resistant to Ampicillin.In Staphylococcusaureus, common sensitive drugs were Amoxicillin and Clavulanic acid,Amikacin,Colistin,Doxycycline,Vancomycin,Ciprofloxacin,Levofloxacin and linezolid which were 32%, 32%, 16%, 16%, 8.2%, 8.2%, 8.2%, 8.2% respectivelyin case of sensitivity (Table V). In E.coli, the most common sensitive ones were Gentamicin, Meropenum, Levofloxacin, Linezolid, Colistin which were 32%, 16%, 16%, 16%, 16% respectively (Table V). In Klebsiella pneumonia, most common sensitive drugswere Meropenum(32%)in preterm and low birth weight babies (Table V).

Table-V: Antimicrobial sensitivitypattern

Name of antibiotic	Staphylococcus aureus	Escherichia coli	Klebsiella pneumonia
	Number (%)	Number(%)	Number (%)
Amoxicillin and Clavulanic	20 (32%)	5 (8.2%)	0 (0%)
Azithromycin	0 (0%)	5(8.2%)	5 (8.2%)
Amikacin	20 (32%)	5(8.2%)	10 (16%)
Ceftriaxone	5 (8.2%)	5(8.2%)	5 (8.2%)
Ciprofloxacin	5 (8.2%)	5(8.2%)	5 (8.2%)
Colistin	10 (16.0%)	10(16%)	10 16%
Doxicycline	10(16.0%)	5(8.2%)	5 (8.2%)
Gentamicin	5 (8.2%)	20(32%)	10(16%)
Meropenum	5 (8.2%)	10(16%)	20 (32%)
Imipenum	5 (8.2%)	5(8.2%)	10(16%)
Levofloxacin	5 (8.2%)	10(16%)	5(8.2%)
Linezolid	5 (8.2%)	10(16%)	5(8.2%)
Vancomycin	5(8.2%)	5 (8.2%)	10(16%)

Discussion:

Neonatal sepsis is one of the leading health problems in developing countries including Bangladesh and India. Primarily based on clinical evaluation clinic-pathological and microbioligicalassociation requires early diagnosis of NS.[10] Diagnosis of NS is among the most difficult tasks for physicians because there is no single reliable test for early discovery of sepsis. Currently blood culture is the most reliable procedure remains the gold standard in detecting bacterial infections for neonatal sepsis though it's sensitivity is 50-80%.Quick laboratory test with high specificity for neonatal sepsis help in making a therapeutic decision preventing unneeded, long term usage of antibiotics when treating patients with clinicalasx6 traits of sepsis but negative blood cultures.[11]

EOS was found more common than LOS which was in agreement with the reports from other developing countries including Bangladesh (70.7%) and Cameroon (7-7.5%) but in contrast with other reports from Pakistan (42%) and Libiya (31%) where as LOS was more common.[11]In our study,it was found that, out of 90 clinical neonatal septicemia cases , 67.78% had growth on blood culture and among the infected newborn, EOS (45) were prominent than LOS and male were predominant (80.33%). It might be due to the factors regulating the synthesis of globulin situated on the x-chromosome. Male had only one x-chromosome. So, female was immunologically more protected than male.[12] This study showedamong the maternal risk factors,PROM were found (24.59%),fouel smelling liquor(19.67%),repeated per vaginal

examination without gloves(16.39%),prolong labour (16.39%) and intrapartum antibiotic prophylaxis (22.95%) also resulted in a substantial change in the bacterial flora responsible for EOS. Another study showed among patients with weak immunity including hospitalized patients and newborn that need resuscitation is a major risk factor of neonatal sepsis.[13]During resuscitation it could be related with poor practices and non adherenceto guidelines by health professionals and maternal infections like febrile illness two weeks before delivery,meconium stained liquor,prolonged rupture of membranes >24 hours,multiple per vaginal examinations in labour,prolonged and difficult delivery may predispose the neonate with a higher risk of developing sepsis. Study conducted in India was also similar to this study.[14]In a Bangladeshi study E.coli was shown as the leading organism (30%) however Klebsiellawas the dominant organism (55%) in Indian study.[8,11] Another study showed that,E.coli and Klebsiella were the dominant pathogens of neonatal sepsis in Riyadh, Saudi Arabia and group B beta haemolytic was an infrequent cases. In another study in the pediatric hospital, Tabriz,Iran reported that, coagulase negative Staphylococci were the most common gram positive pathogen and most common gram negative organism was Klebsiella while second most common organism was E.coli.[15]In this particular study, Staphylococcus aureus was the commonestgram positive isolate, accounting about 59.01% of NS and E.coli was the leading most gram negative pathogens whereas study report from Dhaka Shishu Hospital showed that,main organisms were gram negative bacteria and they were Klebsiella (41.7%) and E. coli and gram positive organisms were Staphylococcus aureus and Streptococci (equal in number,5.9%).[16]The greater prevalence of resistance to commonly used antibiotics has too been stated in recent studies.[17] The development of multi drug resistance pathogens to commonly used antibiotic was one of the most alarming findings in our study in neonatal ward and NICU. In this study most of the strains showed a low sensitivity to Azithromycin, Cefixim and Ceftazidim. Low sensitivity of Gentamicin to gram positive pathogens were found in our study whereas high degree sensitivity of gram negative pathogen,E.coli and Klebsiella to Gentamicin observed in reports. Very low sensitivity of Gentamicin to these pathogens were found in other studies[17]whereas other observer showed their reports higher degree of sensitivity to gram negative pathogens.[18] The analysis of drug resistance pattern in NICU, Dhaka ShishuHospital showed that, among gram negative isolates, maximum numbers were resistant to Ampicillin and lowest to Ceftriaxone.[19] In this study Staphyloccus were more sensitive to Amoxicillin and Clavulenicacid and Amikacin. Frequently sensitive drug for E.coli was Gentamicin and forKlebsiella pneumoniae was Meropenum in preterm and low birth weight neonates.

For clinical management of NS antimicrobial resistance was a major challenge in our study, the entire isolated organisms were also multidrug resistant. Commonly available antibiotic like Cephalosporins, Aminoglycosides, Ampicillin and Quinolones were highly resistant in both gram positive and gram negative organisms. Same pattern of a very high incidence of multidrug resistant organism also reported in other studies from Bangladesh. And also other different countries of theworld like India, Nepal, and Ethiopia.[20]Amikacin had shown to both gram positive and gram negative organisms. This guide also confirmed in data from Lahore, Peshwar and Multan.[21]Bacterial pathogen and organisms responsible for NS and antimicrobial sensitivity pattern might vary from one country to another country and within a country from one region to another region.There might even vary at different times within the

same place, in different studies at different time in the same medical institution.[22]

Conclusion

This study with 90 hospitalized patients of NShighlighted that, in SWMCH, the main causative gram positive organism were Staphylococcus aureus andmost of these pathogens were sensitive to Amoxicillin and Clavulenicacid as well as Amikacin. Gramnegative organisms wereE.coli andKlebsiella pneumoniae. Neonatal sepsis commonly occured during early period of life and male were predominant. E.coli showed higher sensitivity rate to Gentamicin and Klebsiella pneumoniae showed to Meropenem. Incidents of antimicrobial resistance among different organismoccur fromtime to time. Continuous evaluation at different levels of local pattern of bacteriological profile and antimicrobial sensitivity of pathogen should be reviewed to develop empirical therapy.This result also indicated that there was an increasing rate of antibiotic resistance to the commonly used antibiotics.

Key message

1. Staphylococcus aureuswas the most common gram positive and E.coliwas leading gram negative organism responsible for NS in a tertiary level hospital, SWMCH, Sylhet, Bangladesh.
2. Most of the gram positive isolates were sensitive to Amikacin, Amoxicillin and Clavulanic acid and gram negative pathogens were sensitive to Gentamicin and Meropenum.

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Conflict of interest

There is no conflict of interest.

Abbreviation:

CONS:Coagulage negative Staphylococcusspecies.
EOS: Early onset neonatal sepsis.
LOS: Late onset neonatal sepsis.
NICU: Neonatal Intensive Care Unit/
NS: Neonatal Sepsis
PROM: Pre mature rupture of membrane.

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