Original Article

Outcome of Bacterial Sepsis in Neonate with Determination of Pathogens and Their Antimicrobial Susceptibility

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Abstract:

Background: *Sepsis in neonate remains a significant cause of mortality and morbidity in developing countries. Neonatal sepsis requires accurate and timely clinical and laboratory diagnosis and proper management for better outcome. In this study an attempt has been made to know the positivity rate of neonatal sepsis, identify the bacterial isolates responsible for EOS and LOS and determine their sensitivity pattern to various antimicrobial agents, and outcome of neonatal sepsis.*

Methods: *A cross sectional descriptive single-centre study was conducted on neonates , over a period of 12 months. Essential investigations were sent by collecting samples under aseptic precautions. Empirical antimicrobial therapy was started according to antimicrobial guidelines in this NICU.*

Results: *Out of 1423 screened blood cultures 13.49% reported as positive and EOS and LOS accounted for 33.85% and 66.15% of cases respectively. Over half (52.60%) of neonates had sepsis with gram negative bacteria and 47.40% with gram positive bacteria and ratio was 1.1:1. Common organisms were Coagulase-Negative Staphylococci-CONS (42.19%), Acinetobacter (22.40%),Enterobacter (18.75%).Total mortality rate was 6.96% & death rate was 23.96% in culture proven sepsis & 04.31% in culture negative isolates (p-value: <0.00001). In culture-proven sepsis the mortality rate 30.71% & 10.77% (P-value: 0.00219) in LOS & EOS; and 30.69% &16.48% (p-value: 0.02126) in gram positive & gram negative sepsis respectively. Acinetobacter (44.19%) showed higher death rate followed by MRSA (33.33%), E.Coli (33.33%). In EOS, gram positive & gram negative bacteria accounted for 55.38% & 44.62%; and in LOS, 43.31% & 56.69% respectively. CONS (50.77%), the commonest followed by Acinetobacter (18.46%) and Enterobacter (13.85%) in EOS. In LOS, CONS (37.80%) was the most isolated prevalent organism followed by Acinetobacter (24.41%) and Enterobacter (21.26%). Gram positive organisms showed higher level of sensitivity to Vancomycin (91%), Linezolid (90%), Netilmicin (65%). Among the Staphylococci (Coagulase negative & positive), around 86% were resistant to Cloxacillin/Methicillin. Gram negative bacteria had best susceptibilities to Colistin (74%) & Polymyxin-B (70%) than Cotrimoxazole (51%), Netilmicin (32%), Ciprofloxacin (32%), Piperacillin+ Tazobactem (27%), and Levofloxacin (24%). Among the commonly used antibiotics, the susceptibilities were remarkably low to Ampicillin (5.73%), Gentamicin (26.04%), Amikacin (11.46%), Ceftazidime (4.69%), Cefepime (2.08%) in comparison to Ciprofloxacin (31.25%), Imipenem/Meropenem (35.42%) & Netilmicin (47.92%) for both gram positive & negative isolates.*

Conclusion: *Present study indicated that more mortality observed in culture-proven sepsis especially in gram negative sepsis. CONS continue to be the predominant causative organism in both EOS and LOS followed by Acinetobacter, Enterobacter. The antibiotic susceptibility profile suggested that for a given cohort empiric Netilmicin- Imipenem / Vacomycin or Netilmicin- Ciprofloxacin / Vancomycin initial choice where patient can not improve with initial empiric antimicrobial therapy (Ampicillin and Gentamicin) in EOS before blood culture reports are available or can considered be for initial empiric therapy in LOS. Early intervention with evidence based unit specific empiric chemotherapy before availability of culture reports that will improve outcome of neonate with sepsis and can reduce the morbidity and mortality as well.*

Keywords: *Early Onset Sepsis (EOS), Late Onset Sepsis (LOS), culture isolates, antimicrobial sensitivity.*

Introduction:

Neonatal sepsis is a systemic infection occurring in infants at ≤ 28 days of life and is an important cause of morbidity and mortality of newborns[1]. Infections in newborn are the commonest cause of neonatal mortality along with Perinatal asphyxia and consequence of Prematurity & Low birth weight (LBW) in Bangladesh[2]. Mortality related to neonatal sepsis is more common in developing countries compared with developed countries[2]. Infant and child mortality rates are basic indicators of a country's socioeconomic situation and quality of life. The first 28 days of life the neonatal period are the most vulnerable time for a child's survival. Children face the highest risk of dying in their first month of life, at a global rate of 19 deaths per 1,000 live births and it accounts for 46% of all deaths among children under- 5 deaths[3]. Globally 2.6 million children die in the first month of life approximately 7,000 newborn deaths occur every day. The majority (75%) occurs during the first week of life, and about 1 million newborns die within the first 24 hours. The main causes of newborn deaths are preterm birth related complications (35%), intrapartum related events (24%), and serious infections (21%, sepsis or meningitis and pneumonia). These causes account for nearly 80% of deaths in this age group and almost all of these deaths occur in developing countries[3]. In Bangladesh neonatal death (30 deaths per 1,000 live births) is still significantly higher in

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comparison to global neonatal death (19 deaths per 1,000 live births). It is also notable that deaths in the neonatal period account for 67% of all under-5 deaths (45 deaths per 1,000 live births) [4]. During infancy (38 deaths per 1000 live births), the risk of dying in the first month of life (30 deaths per 1,000 live births) is nearly four times greater than in the subsequent 11 months (8 deaths per 1,000 live births) [4]. An estimated 62,000 newborns die every year in Bangladesh and 50% of them die on 1st day of life[3]. The main causes of neonatal deaths are prematurity (29.7 percent), birth asphyxia and trauma (22.9 percent) & sepsis (19.9 percent) and accounts for around 75% on total death[5]. Neonatal sepsis remains as an important cause of morbidity and mortality among infants in developing countries accounting for 30-50% of total deaths per year[6]. The incidence of neonatal sepsis depends on geographic area and may vary from country to country as well as within the same country. In developing countries, neonatal mortality resulting from all causes of neonatal sepsis is about 34 per 1000 live births**,** occurring mainly in the first week of life· whilst it is 5 per 1000 live birth in developed country[7]. Neonatal sepsis has been classified as either early onset (day of life 0-3) or late onset sepsis (day of life 4 or later) i.e. infections occurring before and after 72 hours of life[8]. The reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live birth in Asia[9], from 6.5 to 23 per 1000 live birth in Africa[10] and from 3.5 to 8.9 per 1000 live birth in South America and the Caribbean[11, 12.] By comparison, rates reported in the United States and Australia range from 6-9 per 1000 live birth[13, 14] and in Europe 0.3-3% per 1000 live birth[15]. Early onset bacterial infection places the neonate at risk of death and long term morbidity[16, 17.18]. Improvement in outcome and successful treatment depends on early initiation of appropriate antibiotic therapy. The pattern of causative organisms has been constantly changing[19] and the frequent emergence of resistant bacteria[13] compounds the problem further. This highlights the need for surveillance of sepsis for optimum therapy. Knowledge of likely causative organisms and their antimicrobial sensitivity pattern could aid in choosing prompt and appropriate therapy for early- onset sepsis (EOS). Of newborns with EOS, 85% present within 24 hours (median age of onset 6 hours), 5% present at 24-48 hours, and a smaller percentage present within 48-72 hours. Onset is most rapid in premature neonates. EOS is associated with acquisition of microorganisms from the mother. Infection can occur via hematogenous, transplacental spread from an infected mother or, more commonly, via ascending infection from the cervix. Organisms that colonize the mother's genitourinary (GU) tract may be acquired by the neonate as it passes through the colonized birth canal at delivery. The epidemiology of EOS in the developed and developing countries shows some important differences in the pattern of etiological bacteria & their antibiotic susceptibility[16,17,18,20,21]. In developed countries, Group B Streptococcus (GBS) was the common etiological agent for EOS[20, 21]. Following adoption of preventive strategies for GBS, Escherichia coli (E.coli) was identified as predominant pathogen[21, 22]. Trends in late-onset sepsis (LOS) show an increase in Coagulase-negative Staphylococcus (CONS),[23] & the infant's skin, respiratory tract, conjunctivae, gastrointestinal tract, and umbilicus may become colonized via contact with the environment or caregivers. Developing nations reported an entirely a different bacterial spectrum[18,21,23,24,25]. In most developing countries, gram negative bacteria remain the major cause of neonatal sepsis[26,27]. These organisms developed increased drug resistance over the last two decades[28] Since the spectrum of organisms that cause neonatal sepsis changes over time and varies from region to region and hospital to hospital even in the same city or the country, it is necessary to have periodic surveillance to understand the changing pattern of organism causing neonatal sepsis. In addition rapidly changing antibiotic sensitivity pattern of bacterial agent causing neonatal sepsis, making its management more difficult for the health care providers[29]. Therefore knowledge of the pattern of bacterial isolates and their antimicrobial susceptibility is useful for treating patients with appropriate empiric antibiotics. Although an extensive research is available worldwide[28, 30] but a few reports are available on neonatal sepsis in Bangladesh.

Aims and objectives:

The present study was undertaken to find out the positivity rate of neonatal sepsis, identify the bacterial isolates responsible for EOS and LOS & determine their susceptibility pattern to various antimicrobial agents that were investigated for rule out sepsis and outcome of neonatal sepsis on basis of culture status, onset, gram staining and bacterial pathogens admitted in tertiary care NICU at Ad-din Women's Medical college Hospital (AWMCH), Dhaka, from January to December 2019.

Materials and Methods:

This was a cross- sectional descriptive single centre study conducted on neonates (0-28days) in the Level-III Neonatal Intensive Care Unit (NICU) of AWMCH, Dhaka, over a period of 12 months from January to December 2019. All neonates were investigated to rule out sepsis admitted here within 28 days of birth with fulfilling the following admission criteria: 1. Gestational age less than 34 weeks (GA <34 wks)**,** 2. Birth weight less than 1.8 kg (BW <1800 gm)**,** 3.Unwell/sick Neonates e.g. respiratory distress, suspected

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sepsis, requires oxygen supplementation, convulsion, etc, 4. After prolonged resuscitation**,** 5. Neonates need mechanical ventilation**,** 6. Severe (Non lethal) congenital anomalies, 7. Any Neonates requiring surgery, 8. Neonates with cord pH less than 7.0 and metabolic acidosis in early neonatal arterial blood sample (pH < 7.20 and base deficit ≥ 12mmol/L) within first hour of birth irrespective of gestation, 9. Hypoglycemia (if persistent despite oral feeds or if <1.1mmol/L). Exclusion criteria included: 1. Newborns with lethal congenital anomalies, 2. Hyperbilirubinemia requiring intensive phototherapy, and 3.Neonates held in a place of safety as a result of child protection proceeding. Written informed consent was obtained from their parents and was investigated for bacterial etiologic agents. Clinical and other relevant data were obtained by attending pediatrician and were transferred to the questionnaire prepared for this study. Studied neonates were limited to timing as early onset (from birth to 72hours old) and late onset (day of life 4 or later) sepsis. Blood culture (aerobic), chest x-ray and laboratory tests including complete blood count (CBC), CRP, blood sugar (BS) and electrolytes were performed for all subjects. An area of approximately 5 cm over the venipuncture site was disinfected with 70% alcohol, rubbing vigorously and allowed to dry. This was followed by application of povidine Iodine in concentric circles over the site and allowed to dry for at least 1 minute. About 1-2 ml venous blood was drawn from the peripheral vein for each culture and then the blood was inoculated into a BD BACTEC Peds plus culture vials (40ml). The specimens were transported immediately to microbiological laboratory and the test were carried out by BD BACTEC automated blood culture system & incubated for 120 hours in 37^0C and were daily checked for evidence of bacterial growth. For positive cultures, subcultures were made solid media (Blood agar and McConkey agar) and were incubated in 37^0C for 24 to 48 hours. The grown bacteria were identified by colony morphology, gram stain and biochemical tests. Diagnostic microbiology cultures which did not yield any growth following subcultures were reported negative at the end of 5 days. Antimicrobial susceptibility testing was done for all blood culture isolates according to the criteria of the National Committee for Clinical Laboratory Standards by disk diffusion method. Those positive reports had suspicion of contamination were discarded. Outcome was noted on the basis of onset, gram staining and bacterial isolates. Analyses were performed using the Statistical Package for Social Science (SPSS) updated version. P value less than 0.5 is considered statistically significant.

Results:

During the study period a total 1423 neonates were investigated to rule out sepsis and 192 neonates were found positive blood cultures and the positivity rate was 13.49%. Early and late onset sepsis were found in 33.85% (n=65) and 66.15 % (n=127) of cases respectively (Table 1).

In this study, total mortality rate was 6.96% (n=99) among the 1423 cases screened for blood cultures & the death rate was 23.96% (n=46) in culture proven sepsis and 04.31% (n=53) in culture negative isolates (p-value: <0.00001) (Table-5). In culture- proven sepsis the mortality rate was higher in LOS (30.71%) in comparison to EOS (10.77%) (P-value: 0.00219); and in Gram negative (30.69%) sepsis in relation of gram positive (16.48%) sepsis (p-value: 0.02126) (Table-6). Acinetobacter (44.19%) showed higher death rate than that of other microbes; MRSA (33.33%), E.Coli (33.33%), Enterobacter (22.22%), Klebsiella (20%), CONS (16.05%) and Proteus (14.29%) (Table-7).

Table- 1. Number of bacterial isolates based on sepsis onset

Sepsis onset	Total culture positive	Positivity rate (%)
EOS	65	33.85%
LOS	127	66.15%
Total	192	100%

Table- 2. Organisms isolated with respect to classification of sepsis.

Bacterial isolates	**Type of sepsis**		**TOTAL (%) (n=192)**
	EOS (n=65)	**LOS (n=127)**	
Gram positive isolates	**36(55.38%)**	**55 (43.31%)**	**91 (47.40%)**
CONS	33 (50.77%)	48 (37.80%)	81 (42.19%)
Staphylococcus aureus (MRSA)	01 (01.54%)	05 (03.93%)	06 (03.13%)
Staphylococcus aureus (MSSA)	02 (03.07%)	01 (00.79%)	03(01.56%)
Enterococci	00	01(00.79%)	01(00.52%)
Gram negative isolates	**29 (44.62%)**	**72 (56.69%)**	**101 (52.60%)**
Acinetobacter	12 (18.46%)	31(24.41%)	43 (22.40%)

Enterobacter	09 (13.85%)	27(21.26%)	36 (18.75%)
Klebsiella	01(01.54%)	09 (07.09%)	10 (05.20%)
Proteus	05(07.69%)	02 (01.57%)	07 (03.65%)
E.Coli	01(01.54%)	02(01.57%)	03 (01.56%)
Salmonella paratyphi	01(01.54%)	00	01(00.52%)
Citrobacter	00	01(00.79%)	01(00.52%)
Total	**65 (100%)**	**127 (100%)**	**192 (100%)**

Table-3. Antimicrobial sensitivity of Gram positive isolates.

Gm +ve organism no (%)	Amp	**GM**	Cot	Amxc	Clox	**Vn**	**Lz**	Cft	Cp	Lev	Mp/Imip	Netil	Teico	Clar
CONS (81) 89.01%	9 (11%)	25 (31%)	34 (42%)	34 (42%)	10 (12%)	75 (93%)	74 (91%)	14 (17%)	24 (30%)	26 (32%)	42 (52%)	52 (64%)	22 (27%)	7 (9%)
MRSA (6) 6.59%		1 (17%)	1 (17%)			5 (83%)	5 (83%)	1 (17%)	1 (17%)	1 (17%)	2 (33%)	5 (83%)	2 (33%)	1 (17%)
MSSA (3) 3.3%		3 (100%)	1 (33%)	2 (67%)	3 (100%)	2 (67%)	2 (67%)	1 (33%)	2 (67%)	2 (67%)	2 (67%)	2 (67%)		1 (33%)
Enterococci (1) 1.1%							1 (100%)	1 (100%)					1 (100%)	
Total (91) 100%	9 (10%)	29 (32%)	36 (39%)	36 (39%)	13 (14%)	83 (91%)	82 (90%)	16 (18%)	27 (29%)	29 (32%)	46 (50%)	59 (65%)	25 (27%)	9 (10%)

CONS: Coagulase negative staphylococcus, MRSA: Methicillin resistant staphylococcus aureus, MSSA: Methicillin sensitive staphylococcus aureus. Amp: Ampicillin, GM: Gentamicin, Cot: Cotrimoxaxole, Mp: Meropenem,, Imip: Imipenem, Lz: Linezolid, Vn: Vancomycin, Amxc: Amoxyclave, Clox:Cloxacillin,Netil: Netilmicin, Teico: Teicoplanin, Cp: Ciprofloxacin, Lev: Levofloxacin ,Cft: Cefotaxime, Clar: Clarithromycin

Table-4. Antimicrobial sensitivity of Gram negative organisms.

Gm-ve org No (%)	Amp	GM	Amk	Im/Mp	Cp	Lev	Cft	Czdm	Pip	Neti	Amxc	Coli	Poly	Cefpm	Cot
Acinetobacter (43) 42.58%	1 (2%)	9 (21%)	10 (23%)	8 (19%)	16 (37%)	15 (35%)	2 (5 %)	3 (7%)	16 (37%)	19 (44%)	4 (9%)	24 (56%)	31 (72%)	2 (5%)	22 (51%)
Enterobacter (36)35.65%	00	8 (22%)	7 (19%)	8 (22%)	14 (39%)	5 (14%)	4 (11%)	3 (8%)	6 (17%)	8 (22%)		31 (86%)	25 (69%)	00	17 (47%)
Klebsiella (10) 9.9%	00 (10%)	00 (10%)	1 (10%)	1 (30%)	1 (10%)	3 (20%)	1 (20%)		2 (10%)	2 (100%)	1 (70%)	10 (10%)	7 (60%)	1	6
Proteus (7) 6.92%	00	1 (14%)	1 (14%)	3 (43%)			2 (29%)	2 (29%)	2 (29%)	3 (43%)	2 (29%)	6 (86%)	5 (71%)		5 (71%)
E. Coli (3) 2.97%		1 (33%)	1 (33%)	1 (33%)	1 (33%)	1 (33%)	1 (33%)	1 (33%)	1 (33.3%)	1 (33%)		3 (100%)	3 (100%)	1 (33%)	2 (67%)
S. Paratyphi (1) 0.99%	1 (100)	1 (100%)	1 (100%)	1 (100%)					1 (100%)		1 (100%)	1 (100%)			
Citrobacter (1)0.99%		1 (100%)	1 (100%)		1 (100%)	1 (100%)									
Total (101), 100%	2 (2%)	21 (20%)	22 (21%)	22 (21%)	33 (32%)	25 (24%)	10 (10%)	9 (9%)	28 (27%)	33 (32%)	8 (8%)	75 (74%)	71 (70%)	4 (4%)	52 (51%)

S. Paratyphi: Salmonella Paratyphi. Amp: Ampicillin,GM: Gentamicin, Amk: Amikacin, Mp: Meropenem, Imip:Imipenem, Amxc: Amoxyclave, Cp: Ciprofloxacin, Lev: Levofloxacin, Cft: Cefotaxime. Cefpm: Cefepime, Czdm: Ceftazidime, Netil: Netilmicin, Cot: Cotrimoxazole, Pip: Piperacillin, Coli: Colistin, Poly: Polymyxin-B.

Table-5. Outcome of neonates based on Culture status.

Culture status	Survived (%)	Expired (%)	Total (%)	P – value
Culture- proven sepsis	146 (76.04)	46 (23.96)	192 (100)	< 0.00001.
Culture negative	1178 (95.69)	53 (04.31)	1231 (100)	
Total	1324(93.04)	99 (06.96)	1423 (100)	

Table-6. Outcome of neonates in culture- proven sepsis based on onset and Gram staining.

Variables	Survived (%)	Expired (%)	Total (%)	P – value
EOS	58 (82.23)	07 (10.77)	65 (100)	.00219
LOS	88 (69.29)	39 (30.71)	127 (100)	
Total	146 (76.04)	46 (23.96)	192 (100)	
Gram positive	76 (83.52)	15 (16.48)	91 (100)	.02126.
Gram negative	70 (69.31)	31 (30.69)	101 (100)	
Total	146 (76.04)	46 (23.96)	192 (100)	

Table-7. Outcome of neonates based on bacterial isolates.

Name of bacteria	Survived (%)	Expired (%)	Total (%)
CONS	68 (83.95)	13(16.05)	81 (100)
MRSA	04 (66.67)	02 (33.33)	06(100)
MSSA	03 (100)	00	03(100)
Enterococci	01 (100)	00	01(100)
Acinetobacter	24(55.81)	19 (44.19)	43(100)
Enterobacter	28 (77.78)	08 (22.22)	36(100)
Klebsiella	08 (80.00)	02 (20.00)	10(100)
Proteus	06 (85.71)	01(14.29)	07(100)
E.Coli	02 (66.67)	01(33.33)	03(100)
S. Paratyphi	01(100)	00	01(100)
Citrobacter	01(100)	00	01(100)
Total	146 (76.04)	46 (23.96)	192 (100)

Discussions:

Sepsis is an important cause of neonatal morbidity and mortality. The incidence and the causative organisms of sepsis vary from place to place. It is one of the leading (3rd) cause of death among neonates in Bangladesh along with prematurity and its related complications, birth asphyxia and trauma, and congenital anomalies.

In this study, prevalence of culture-proven neonatal sepsis was 13.49%.This is low compared to about 20% yield reported by Baltimore et al[22] and Gladstone et al[31] and 26% by Ahmed et al[25] but higher to Mannan (5.43%) et al[32] and Haque (8.7%) et al[33] study. In the present investigation 33.85% and 66.15% of neonates presented with EOS and LOS respectively. We found that EOS was less common than LOS, which is in agreement with the reports from Saudi Arabia (39% vs 61%)[34] and Pakistan (42% vs 58%)[35], but in contrast with reports from other developing countries; in Iran, Moniri et al[36] (77.5% vs.

22.5%) and in study of Bangladesh, Haque (74.86% vs 25.14%) et al[33] and in Pakistan Rasul (70.7 vs 29.3%)[37], where early onset sepsis is more common. The possible explanation for a higher frequency of LOS in the study might be the delayed referral of more sick newborns from other centre or outside of Dhaka city to our centre with history of poor antenatal care (ANC) and delivery by unskilled birth attendant.

Over half (52.60%) neonates had sepsis with gram negative bacteria and 47.40% with gram positive bacteria and ratio was 1.1:1. This study finding is similar to that of other studies which shows that gram negative bacteria were the commonest cause of neonatal sepsis[25,33,36,38,]. This was opposite to other studies which shows gram positive bacteria are the common cause of neonatal sepsis[12,26,32,39] , while Umran et al[34] study shows the frequency of isolation of gram positive & gram negative bacteria were equal. Among the gram negative isolates Acinetobacter (22.40%) was the commonest isolated pathogen followed by Enterobacter (18.75%), Klebsiella (5.20%), Proteus (3.65%) and E.Coli (1.56%) causing neonatal sepsis in this study. Generally the spectrum of gram negative organism causing neonatal sepsis in this study is similar to that reported from developing countries, with gram negative bacteria being responsible in most cases. But the pattern of pathogen in our study slightly differs from the finding of Haque[33] and Jahan[40] where Acinetobacter (34.4%) was the leading cause followed by Pseudomonas spp. (21.8%), Klebsiella spp. (6.9%), Enterobacter (3.4%) and E.Coli (2.2%) and Monir[36] (Iran) where Pseudomonas spp. was the most common cause followed by Klebsiella spp. and E.Coli. In similar studies from Bangladesh and Pakistan, E.Coli was leading cause of neonatal sepsis followed by Klebsiella spp. [25,35].

In EOS, isolation of gram positive bacteria (55.38%) was higher than gram negative (44.62%) bacteria in this study which is near confirmatory to finding of Mannan (71%vs29%) et al[32] and Jahan (75.76%vs24.24%) et al[40]. And in LOS, gram negative bacteria contributed 56.69% & gram positive 43.31% of cases which is near confirmatory to Jahan et al[40] study where shows the frequency of isolation of gram negative isolates higher than gram positive isolates (24%vs.76%).

CONS (42.19%) was the most common observed pathogen among all organisms causing neonatal sepsis followed by Acinetobacter (22.40%), Enterobacter (18.75%) and Klebsiella (5.02%). This study is similar to the study which shows gram positive bacteria such as CONS, Staphylococcus Aureus and Group B Streptococcus (GBS) were found to be the most common causes of neonatal sepsis Robillard [12], Mannan[32], Mugalu[39] and Ramesh[41]. But this study result is opposite to studies of most developing countries[,32,33,38,], where showed gram negative (Acinetobacter, Haque[33], Acinetobacter, Jahan[40] and Klebsiella, Rana[42]) organisms were the common cause of neonatal sepsis. Gram positive bacteria- CONS was major culprit for both early (50.77%) and late (42.19%) onset sepsis. This observation is comparable to the study of Mannan[32] which shows CONS (68.4%) is prime culprit in EOS. Studies from different countries reported CONS as predominant organisms in LOS[23,43,44].

In the present study, CONS, S. Aureus and other gram positive organism showed higher level of susceptibilities to Vancomycin (91%), and Linezolid (90%); and moderate level to Netilmicin (65%) and Meropenem/Imipenem (50%) in comparison of Amoxyclave (39%), Cotrimoxazole (39%), Gentamicin (32%), Levofloxacin (32%), Ciprofloxacin (29%) & Teicoplanin (27%). And remarkably lower level of sensitivity to Cefotaxime (18%), Cloxacillin (14%), Ampicillin (10%) & Clarithromycin (10%). These findings almost compatible to study of Mannan[32] & Haque[33], but different in Gentamicin and Levofloxacin sensitivity where it is 70% & 55% and 71.43% &95.23% sensitive respectively.

All gram negative bacteria were best susceptible to Colistin (74%) & Polymyxin-B (70%) than Cotrimoxazole (51%), Netilmicin (32%), Ciprofloxacin (32%), Piperacillin+ Tazobactem (27%), Levofloxacin (24%), Amikacin (21%), Meropenem/Imipenem (21%) and Gentamicin (20%). Marginal level (near resistant) of sensitivity to Cefotaxime (10%), Ceftazidime (9%), Amoxyclave (8%), Cefepime (4%), and Ampicillin (2%). Higher susceptibility to colistin was reported by Mannan[32] (91%) and Haque[33] (96.9%). Minimal level of sensitivity to Ampicillin, Cefotaxime, and Ceftazidime is reported in many earlier studies[13,36,45,46]. The most common gram negative organism was Acinetobacter (42.58%) and most sensitive to Polymyxin-B (72%) followed by Colistin (56%) and Cotrimoxazole (51%). Less sensitive to Netimicin (44%), Piperacillin+tazobactem (37%), Ciprofloxacin (37%), and Levofloxacin (35%). Remarkably lower sensitive to Amikacin (23%), Gentamicin (21%) and Imipenem/Meropenem (19%); and almost resistant to Ampicillin, Amoxyclave, Cefotaxime, Ceftazidime, & Cefepime. Different earlier studies are reported Polymyxin/Colistin higher rate of sensitivity and complete to near resistant to Ampicillin, Cefotaxime, Ceftazidime, and Cefepime[32, 33].

In culture-proven sepsis the mortality rate was 23.96% out of 192 culture positive case. The mortality rate was higher in LOS (30.71%) in comparison to EOS (10.77%); and in Gram negative (30.69%) sepsis in relation of Gram positive (16.48%) sepsis. Acinetobacter (44.19%) showed higher death rate than of other microbes; MRSA (33.33%), E.Coli (33.33%), Enterobacter (22.22%), Klebsiella (20%), CONS (16.05%) and Proteus (14.29%). This finding is comparable with present neonatal mortality rate (19.9%) of Bangladesh due to sepsis[2,5], other main causes of neonatal deaths were prematurity (29.7 percent), birth asphyxia and trauma (22.9 percent) and Congenital anomalies (12.7percent) [2,5], but higher than that findings of Jahan N et al (5.7%)[40]. We found more mortality rate in LOS than EOS, this finding opposite to findings of Haque ZSM[,33] and Jahan N[,40] , they observed more deaths in EOS. Gram negative sepsis contributed majority of death in this study and main organism causing mortality was Acinetobacter, this findings are similar to the other earlier studies[33,40], but disparity is that Pseudomonas spp. was the main organism causing mortality.

Our results have demonstrated that in general both gram positive and gram negative bacterial pathogens showed lower resistance rate to Netilmicin, Cotrimoxazole, Ciprofloxacin, and Imipenem. Gram positive organisms had high level of resistance to Ampicillin, Cloxacillin, Clarithromycin and Cefotaxime and Gram negative bacteria showed high level of resistance to Ampicillin, Gentamicin, Amikacin, Cefotaxime, Cetazidime, and Cefepime. This observation is comparable to that of other researchers[26,27,28, 36,38].

In the present study 74% - 83% organisms were sensitive to Netilmicn - Imipenem / Vacomycin or Netilmicin - Ciprofloxacin / Vancomycin. So these can be initial choice of combination in EOS where patient does not improve with initial empiric therapy (Ampicillin & Gentamicin) before preliminary blood culture reports are available or can consider for initial empiric therapy for LOS. Once culture and sensitivity results are available antibiotics should be adjusted accordingly. However these results are limited to study cohorts and every center should have idea about their own bacterial sensitivity pattern. Different neonatal intensive care unit (NICU) shows different epidemiological data for neonatal sepsis. So collection of up-to-date & site specific data is mandatory for appropriate use of antibiotic.

Conclusion and Recommendation:

Present study indicated more mortality observed in culture-proven sepsis especially in gram negative sepsis. The gram positive bacteria, CONS continue to be the predominant causative organism in both EOS and LOS followed by Acinetobacter, Enterobacter, Klebsiella, & Proteus in gram negative species. The antibiotic susceptibility profile suggested that for a given cohort empiric Netilmicin - Imipenem / Vacomycin or Netilmicin – Ciprofloxacin / Vancomycin is the initial choice where patient can not improve with initial empiric antimicrobial therapy (Ampicillin and Gentamicin) in EOS before preliminary blood culture reports are available or can be considered for initial empiric therapy in LOS is the most rational. Early intervention with evidence based unit specific empiric chemotherapy before availability of culture reports will improve outcome of neonate with sepsis and can reduce the morbidity and mortality as well.

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