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Bacteriological study of neonatal septicemia in neonatal intensive care unit

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Abstract
Neonatal sepsis is a clinical syndrome of bacteraemia characterized by systemic signs and symptoms in first 28 days of life. It is the leading cause of neonatal mortality and morbidity in Bangladesh. The objectives of this study is to investigate the neonatal sepsis condition in the Chittagong area of Bangladesh and to suggest some ways to reduce un-necessary use of antibiotics,its cost and hazard also neonatal mortality and morbidity rate. The study of 1 year included clinically suspected cases of neonatal septicemia admitted in NICU. Blood samples were cultured using tryptone soya broth (TSB) according to standard method. 1095 blood samples were collected, processed and isolates were identified .Blood culture was positive in 87 (7.96%) cases. Among the culture positive cases, 38 (43.67%) were males and 49 (56.33%) females, 25(28.73%) were born in this hospital and 62(71.26%) were outborn. EONS (Early Onset Neonatal Sepsis) was present in 48(55.18 %) and LONS (Late Onset Neonatal Sepsis) in 39(44.12%) cases. Among the isolated organisms *Klebsiellapneumoniae* accounted for 34(39.08%), *Acinetobactor*25 (28.73%), *Staphylococcus aureus*15 (17.24%) and *Serratiamarcescens*13 (14.94%). Mortality rate was 19.54%. Neonatal septicemia is a life-threatening emergency.Early diagnosis, specific treatment and strict infection control practices in neonatal intensive care unitscan reduce neonatal mortality and morbidity.

Keywords: Neonatal, Blood culture, Septicemia.

1. Introduction

Neonatal sepsis is a clinical syndrome of systemic illness accompanied by bacteremia occurring in the first 28 days of life [1]. The immaturity of immune system in the neonates makes them especially susceptible to infections during the neonatal and perinatal period [2]. It is the commonest causes of neonatal mortality in the developing world accounting for 30-50% of 5 million neonatal deaths per year [3]. For epidemiological and therapeutic purposes, neonatal septicemia is categorized into early onset neonatal septicemia (EONS) which presents within the first 72 hours of life and late onset neonatal septicemia (LONS) presenting after 72 hours of life [1].This distinction has clinical relevance, as EONS is generally acquired from pathogens of maternal genital tract, whereas LONS has its origin either from community or from hospital [4].

In Bangladesh, very few studies have reported on the prevalence of neonatal sepsis in the different area of the country and their causative organisms antibiotic resistant pattern.But there is still lack of information about the actual neonatal sepsis condition in the different area of Bangladesh.

Early diagnosis is a key to reduce morbidity and mortality of neonatal septicemia. The gold standard for diagnosis of septicemia is the isolation of bacterial agents from the blood culture. But definitive culture results take at least 48-72 hours resulting in treatment delays[5].Both gram-negative and gram-positive bacteria have been isolated from blood. Organism causing sepsis and their susceptibility to different antibiotics vary from place to place. Therefore, the objective of the study is to investigate the organisms causing neonatal septicemia and to study mortality rate in neonatal sepsis.

2. Materials and Methods

2.1 Study Design

The observational study of 1 year was carried out in the Department of Paediatrics, Chattagram Maa Shishu-O-General Hospital (Neonatal Intensive Care Unit) from January, 2017 to December, 2017.

2.2 Data Collection

Clinically suspected cases of neonatal septicemia admitted in NICU were included in the study.Neonatal sepsis with other co-morbities (eg. PNA, Neonatal jaundice, Muconium Aspiration Syndrome) were excluded from this study. Data collection was done during this period by using a standard record abstraction form.Detailed history of each neonate along with history of maternal risk factors, neonatal risk factors and mode of delivery etc was recorded. A semi structured questionnaire was prepared which include name, date of birth,age, sex,weight,address, presenting complaints, socio-economic condition, examination findings on admission etc.Before enrollment a parent of the neonate was given a detailed explanation of the study.Written consent was taken from the parents. The data were compiled, analyzed and then tabulated according to key variables.

2.3 Blood Culture

The skin of venepuncture site was disinfected with 70% alcohol and 1% iodine for at least 1 minute and allowed to dry. With precaution to avoid touching and contaminating venepuncture site, 2 ml of blood was withdrawn with disposable needle and syringe and inoculated into blood culture bottle containing 20 ml of tryptica soy broth. The blood and broth mixed gently; the bottle were transported immediately to laboratory and incubated at 35 to 37^{C} up to 7 days.Blood culture bottles were examined at 14 to 17 h and then everyday for up to 7 days. Turbidity or lysis of the erythrocytes was monitored as an indicative of growth which was sub-cultured immediately.

After bacterial growth was detected, the micro-organisms were transferred to culture media for isolation of bacteria.Before sub-culturing the bottle was swirled to mix the contents.Using a sterile needle and small syringe about 1 ml of the broth culture was withdrawn from the positive blood broth and inoculated into Blood agar, Chocolate agar and MacConkey agar. All of the plates were incubated at $37^{o}C$ for 24 to 48 hrs. Blood agar and MacConkey agar plates were incubated aerobically whereas Chocolate agar plates incubated in Carbon-di-oxide (5 to 10%) incubator. If there was no bacterial growth after 7 days of incubation, the culture was reported to be negative.

Identification of isolates obtained in pure culture was based on Gram staining, biochemical characteristics and growth pattern on selective and deferential media and according to the procedure recommended in the Bergey's Manual of Determinative Bacteriology.Antimicrobial susceptibility of all bacterial isolates was done by Kirby-Bauer disk diffusion technique as per CLSI 2014 guidelines [6, 7, 8].

3. Results and Discussion

Out of total 1095 blood samples subjected for culture, 87 (7.96%) were culture positive and 1008 (92.04%) were culture negative. The culture positivity rate was 7.96% (Figure 1). Among the infected child, 25 (28.73%) were born in this hospital and 62 (71.26%) were outborn. The birth status of the infected child is shown in Figure 2.

Out of total 87 culture positive cases 48 (55.18%) were of age less than 3 days belonging to early onset septicemia, while 39 (44.82%) cases were between the age of 3 days to 28 days belonging to late onset septicemia. Among them 38 (43.67%) were males and 49 (56.32%) were females with male to female ratio 1:1.2 (Table 1).

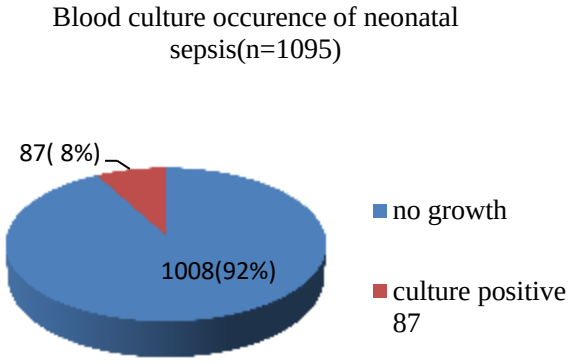

Figure 1. Occurrence of neonatal sepsis in the study period.

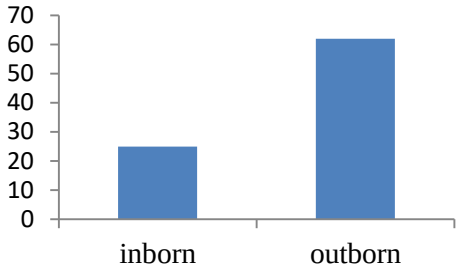

Figure 2. Birth status of infected child.

Table 1. Age and sex wise distribution of culture positive cases (n=87).

Blood CulturePositive	No. of pt	In percentage
Total	87	7.96%
< 3 days	48	55.18%
>3 days	39	44.82%
Sex- Male	38	43.67%
Sex- Female	49	56.32%

Table 2 shows birth weight wise distribution of the cases, out of the total 87 culture positive neonates, maximum neonates 53 (60.91%) were low birth weight babies.Of this 87 mothers 46 (52.87%) delivered their infants prematurely (<37 weeks) and 41 (47.12%) delivered their infant maturely (>37 weeks).

Table 2. Birth weight and gestational age wise distribution of culture positive cases (n=87).

Neonatal birth wt	No.of culture positive	Gestational age	No. of culture positive
Normal birth weight	34 (39.09%)	Term(>37 wks)	41 (47.12%)
Low birth weight	53 (60.91%)	Preterm(<37 weeks)	46(52.87%)

Table 3 shows the isolates from blood culture of neonatal septicaemiacases, Gram negative bacilli72 (82.75%) (*Klebsiella, Acinetobactor, Serratia*) were common etiological agents as compared to gram

positive cocci15 (17.24%). The most common gram negative organism causing sepsis was *Klebsiellapneumoniae34* (39.08%), and gram-positive organism was *Staphylococcus aureus*15 (17.24%). Out of total 48 EONS cases, Gram negative bacilli were 40(83.33%) and Gram positive cocci were 8 (16.66%). Of them *Klebsiellapneumoniae*21 (43.75%) was the commonest isolate. In LONS cases (39), Gram negative bacilli 32 (82.05%) predominated as compared to gram positive cocci 7 (17.95%) and the most common pathogen was Acinetobactor14 (35.89%) as shown in Figure 3.

Table 3. Distribution of organism as per the onset of septicemia.

Organism	No. of pt	EONS	LONS
Staphylococcus	15	8	7
Klebsiella	34	21	13
Acinetobacter	25	11	14
Serratia	13	8	5

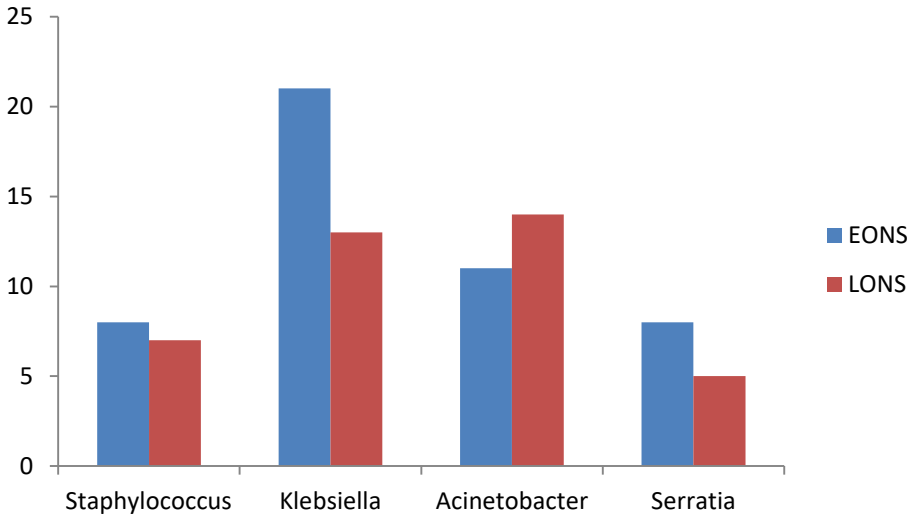

Figure 3. Blood Culture of Neonatal Septicemia cases.

Table 4 shows the mortality of neonates according to onset of septicemia. Out of 48 EONS cases 11(22.91%) and 39 LONS cases 6(15.38%) died of septicemia. Overall mortality rate was 19.54%. The statistical difference in the mortality rates between two types of septicemia was not significant (p= > 0.05).

Table 4. Mortality of culture positive cases as per the onset.

Outcome	Total	Mortality	Outborn	Inborn
EONS	48	11	8	3
LONS	39	6	6	0

3.1 Blood culture results

Blood culture has been regarded as the gold standard for the confirmation of sepsis. In the present study, blood culture positivity in neonatal septicemia cases was 7.96%.There is wide variation in the culture positivity worldwide ranging from 5% to 60.4% [9, 10]. These variations may be due to

differences in predisposing factors and infection control practices in the different centers. Our findings were similar to the prevalence rate of 7.45% reported by Chittagong Medical College from Bangladesh [15].This may be indicative of relatively similar management practices, hospital facilities and service provided. However Sharma CM et al and Gandhi S et al reported higher prevalence of 37.69% and 32% respectively [16, 17]. Low blood culture isolation rate could be due to administration of antibiotic before blood collection from the primary centers or the possibility of infection with anaerobes. A negative blood culture does not exclude sepsis due to anaerobes [14].Bukhari and Alrabiaah reported a much lower prevalence of 5% in Saudi Arabia which was postulated to be due to the very aggressive clinical management of infants presenting with apparent sepsis [9].

3.2 Age and sex wise distribution of cases

In the present study, 48 (55.18 %)neonates were of age less than 3 days belonging to EONS(Early Onset Neonatal Sepsis), while 39 (44.12%) were between the age of 3 days to 28 days belonging to LONS (Late Onset Neonatal Sepsis). Among them, 38 (43.67%) were males and 49 (56.33%) were females with male to female ratio 1:1.2 (Table 1).The EONS(Early Onset Neonatal Sepsis) was more common than LONS (Late Onset Neonatal Sepsis) which is compatible with the reports from the other developing countries [11, 12], but in contrast with a previous report from Bangladesh that LONS (Late Onset Neonatal Sepsis) was more common within the rural population of Bangladesh without absence of specialized neonatal care facilities [13]. Among the originating causes of neonatal sepsis prematurity or low birth-weight (defined as delivary before 37 weeks of gestation or small-for-gestational-age) was the leading cause of neonatal sepsis.

3.3 Birth weight

In the present study, 53 (60.91%) neonates were having low birth weight. In recent years, there has been a lot of improvement in medical facilities and as a result, the survival rate of the preterm and LBW babies has improved. But at the same time, these neonates with immature immune defences are exposed to NICU flora for a longer duration. Most of the neonatal septicemia cases are either LBW or preterm [18].

Bacteriological profile of neonatal septicemia

The detection of microorganism in a patient's blood has great diagnostic and prognostic significance, particularly, in neonates with suspected sepsis. Many infections in neonatal and paediatric age group can only be established on the basis of etiological agent recovered from the blood.The causative organisms is different in different hospitals and even in the same hospital at different time.

In the present study, gram-negative organisms predominated being responsible for 82.75% of cases of septicaemia. Similar findings were made by Muley VA et al and Tankhiwale et al [21, 22]. The probable reasons being newborns most probably acquire these gram-negative rods from the vaginal and faecal flora of the mother and the environment where the delivery occurs[28]. The increased susceptibility of neonates to gram negative bacteria may be explained by the fact that antibodies against these organisms are primarily IgM type which do not transfer passively through placenta and are at very low level in blood at birth (about 5% of adult value) and reaches the adult level by 2 years of age.This is in contrast with IgG type, which are passively transferred to placenta and are almost at adult level at birth and falls gradually reaching lowest level around 3 to 4 months of age after which they start to rise again gradually. Adequate IgG levels at term (except IgG 2 subtype) afford protection against several gram-positive bacteria [2].

In present study, the most frequently isolated organism was *Klebsiellapneumoniae*34 (39.08%) followed by *Acinetobactor*25 (28.73%).Our findings were similar to Muley VA et al [21] .*K. pneumonia*was reported as a predominant pathogen in NNPD Report 2002-2003 and also by Sharma CM et al , Iregbu et al, Chelliah A et al and Tankhiwale SS et al [3, 10, 22-24]. The next common organism was *Staphylococcus aureus*followed by *Serratiamarcescens*isolated in 15 (17.24%) and 13 (14.94%) respectively. *K. pneumoniae*and*S.aureus*can survive in the environment for a relatively long time and fairly widely distributed in the hospital environment and therefore have the potential for being transmitted

from the environment to the patients through practices that breach infection control measures [25]. The bacteriological profile differs in EONS and LONS and it also differs in developing and developed countries. In western countries , *group B streptococci (GBS)* and E. coli are the most common G +ve and G –vebacteria isolated respectively (19). In Middle East 43% of neonatal sepsis responsible for *pseudomonas spp*, while some other group showed 6% predominance of *Klebsiellaspp* [20].

3.4 Mortality among blood culture positive neonates

Mortality rate is higher in EONS compared to LONS(26).In the present study, the difference between the mortality rate of EONS (22.91%)and LONS (15.38%) was not significant (P =>0.05).Our result matches with the results of Movahedian et al [26]**.**The greater incidence of mortality in EONS may be due to lower host resistance, under weight babies, associated birth trauma and anoxia .The level of compliment in blood of newborn is less so also the immunoglobulins like IgM and Ig G [27]. Further EONS is usually a fulminent and multisystemic infection and hence has higher case fatality rate than late onset sepsis [28].

4. Recommendations

Neonatal septicemia is important cause of morbidity and mortality among neonates. Early diagnosis, specific treatment and strict infection control practices in neonatal intensive care units can reduce neonatal mortality and morbidity. Regular hand washing, defumigation and strict visitor control is one of the important aspects for the precaution of sepsis in neonates. Empirical treatment should always be guided by proper antibiotic protocol. Isolation of organism and sepsis markers before initiation of antibiotics is a gold standard for the management and prevention of sepsis and drug resistance.

5. Conclusion

Gram negative bacilli were found to be commonest cause of neonatal septicemia in our setup. Female neonates were more prone to infection.Incidence of EONS was common as compared to LONS. The prematurity and low birth weight neonates were at an increased risk of developing sepsis.The most common pathogen isolated in EONS cases was *K. pneumonia* and in LONS cases was *Acinetobactor.*

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