Original article

Neonatal bacteremia in a neonatal intensive care unit: analysis of causative organisms and antimicrobial susceptibility

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Abstract

Background: Bangladesh has a neonatal death rate that is substantially high and demands urgent attention. ***Objective:*** This retrospective study was performed to determine the incidence of bacterial neonatal sepsis condition in the Chittagong area of Bangladesh with focus on various demographic characteristics of neonates, causative organisms and their antibiotic susceptibility. ***Methodology:*** Blood culture was performed on all neonates with risk factors or signs of suggestive sepsis. Blood samples were cultured using tryptone soya broth (TSB- blood broth) according to standard method. ***Results:*** From the 1400 neonates 104 had positive blood culture for neonatal sepsis infection. Among the infected children 40 (38.46%) were born in the hospital and 64 (61.54%) were born at home. The EONS (Early Onset Neonatal Sepsis) accounted for 68 (65.38%) and LONS (Late Onset Neonatal Sepsis) accounted for 36 (34.62%). Among the isolated organism *Klebsiella pneumoniae* accounted for 79 (75.96%), *Serratia marcescens* 19 (18.27%), *Pseudomonas aeruginosa* 04 (3.85%) and *Staphylococcus aureus* accounted for 02 (1.92%). Among the isolated species 102 were attributed to G (-ve) bacteria and 02 were attributed to G (+ve) bacteria. Most of the G (-ve) bacteria showed resistance to commonly used antibiotics such as ampicillin, ceftriaxon and gentamicin. In this study all isolates showed sensitivity to the imipenem. ***Conclusion:*** Collection of up-to-date data is mandatory for appropriate use of antibiotics.

Key words: Neonatal sepsis, Interleukin-6, antibiotics, susceptibility.

Introduction

Neonatal sepsis is one of the commonest causes of neonatal mortality in the developing world accounting for 30 -50 percent of 5 million neonatal deaths per year [1]. The neonatal intensive care units (NICUs) today face one common problem of tackling sepsis. A strictly endorsed aseptic measures policy is the best hope for the solution to the latter. Nevertheless, neonatologists remain constantly baffled by the changing patterns of microbial flora and their sensitivity patterns, making neonatal septicemia a difficult problem to tackle [2]. A number of organisms is associated with neonatal sepsis such as *Klebsiella pneumoniae, Staphylococcus aureus, Escherichia coli, Pseudomonas aeruginosa, Acinetobacter* spp, *Enterobacter* spp etc.[2].

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

To fill the knowledge gap, the objective of this study was to investigate the neonatal sepsis condition in the Chittagong area of Bangladesh and to suggest some ways to reduce the un-necessary use of antibiotics, its cost & hazard and also the neonatal mortality and morbidity rate. In order to understand the epidemiology of neonatal sepsis in the NICU, we retrospectively collected data on bacteremia to analyze its microbiology and determine the antibiotic susceptibilities of the causative organisms.

Materials and methods

Study Design

The study was carried out in the Department of Paediatrics, Chittagong Medical College Hospital (Neonatal Unit) from September'2008 to August'2009.

Data Collection

Data has been collected from January'2009 to July'2009. The data was collected from an infant

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whose blood culture found to be positive. Data collection was done during this period by using a standard record abstraction form. Data was collected on suspected material risk factors for neonatal sepsis, including prenatal infections, obstetrical history and delivery. A semi structured questionnaire was prepared which include name, age, sex, weight, residence, socio-economic, socio-demographic, & psychological history, etc. Isolates were defined as sensitive, intermediate and resistant to various antimicrobial agents on the basis of local laboratory routine testing. Before enrollment a parent of the neonate was given a detailed explanation of the study. Written consent was taken from the parents. All the collected data was checked & verified for its constancy. The data was compiled, analyzed & then tabulated according to key variables.

Blood Sample Collection
Whenever possible blood was collected before antimicrobial treatment has been started. Blood was collected as soon as possible after receiving the doctor's request. Blood was collected aseptically using standard method. To reduce the risk of contamination, blood from neonates was collected from a peripheral vein not from the umbilical vein. Using a sterile syringe and needle about 2 ml of blood was collected from the young child.

Blood Sample Processing
The top of each culture bottle was wiped using a fresh ethanol-ether swab and the tape or protective cover replaced. Each bottle was clearly labeled with the patient ID, date and time of collection. Two to three ml of blood was cultured in Trypticase Soy Broth (TSB) with growth supplement Iso-VitaleX (1%) to support growth of fastidious organism. Normal bactericidal properties of blood and potential antimicrobial agents were neutralized by adding 0.025% Sodium Polyanetholesulfonate (SPS) to the culture media. The media were incubated at 35 to 37°C up to 7 days. Blood culture bottles were examined at 14 to 17 h and then every day for up to 7 days. Turbidity or lyses of the erythrocytes was monitored as an indicative of growth which was sub-cultured immediately.

Isolation of Bacteria
After bacterial growth was detected, the microorganisms were transferred to culture media for isolation of bacteria. Subculture were performed after 14 to 17 h of incubation, again at 48 h and at day 7, regardless of the appearance of the blood culture bottles since the absence of turbidity did not always correlate with the absence of bacterial growth. 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 onto Blood agar, Chocolate agar and MacConkey agar. All of the plates were incubated at 37°C for 24 to 48 hrs. Blood agar and MacConkey agar plates were incubated aerobically whereas Chocolate agar plates incubated in Cabon-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 Bacteria
Primary identification was based on selectivity of media, Biochemical characteristics, morphological characteristics of the colonies and gram stain. Biochemical tests were performed using Kliger's Iron Agar (KIA), Simmon's Citrate agar, Motility Indole Urea (MIU), Lysine Iron agar (LIA), Urea broth, Peptone water, Methyl Red-Voges Proskauer (MR-VP) broth, Nutrient Nitrate Broth (NB), carbohydrate fermentation broth with added lactose, sucrose, glucose as sole carbon source, Starch uitilization agar, Oxidase and Catalase tests. Identification of isolates obtained in pure culture was based on Gram staining , biochemical characterisitcs and growth pattern on selective and differential media and; according to the procedures recommended in the Bergey's Manual of Determinative Bacteriology [3,4].

Antibiotic Susceptibility
Antibiogram of various isolates were determined by Kirby-Bauer method. Mueller- Hinton Agra medium was used with discs (Oxoid., Basingstoke, UK) containing Ampicillin, AMP (10 µg); Ceftriaxone, CRO (30 µg); Gentamycin, GEN (10 µg); Ciprofloxacin, CIP (5 µg); Amikacin, AK (30 µg); Imipenem, IPM (10 µg); Piperacillin, PIPE (l0 µg); Penicillin, PEN (10 µg); Erythromycin, ERY (15 µg). A control strain of E. coli ATCC 25922 was included in each plate. Antimicrobial breakpoints and interpretation were taken from the CLSI standards [5].

Results and discussion

Patient population
Total 104 cases of neonatal sepsis infection identified during the surveillance period (05.01.2009 to 06.07.2009) in the Chittagong

Medical College Hospital. The overall sepsis rate is about 7.45% of all neonates (1400) get admitted in the hospital during the survey period which is shown in figure 1. Among the infected child 64 (61.54%) were born in the home and 40 (38.46%) were born in the hospital. The birth status of the infected child is shown in figure 2.
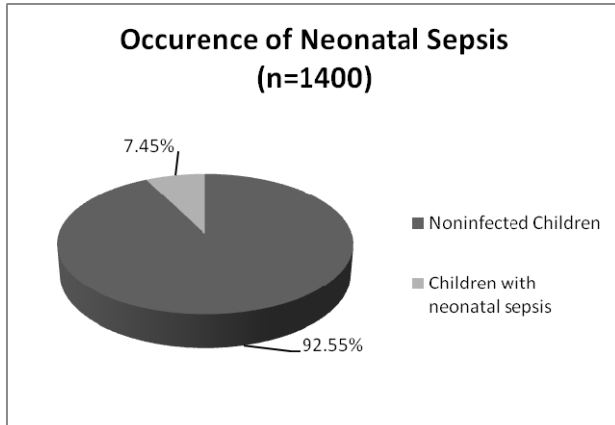

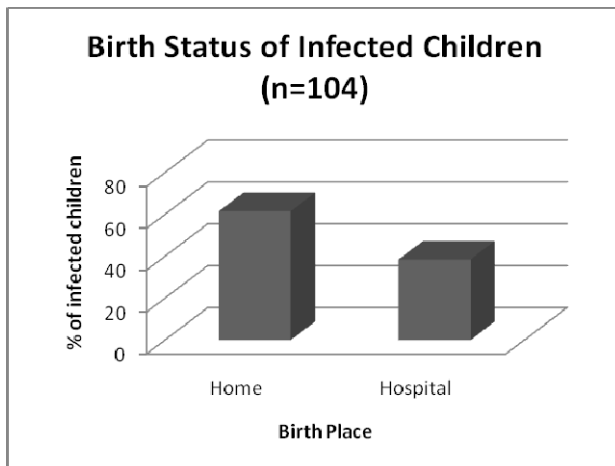**Figure 1:** Occurrence of neonatal sepsis in the study period
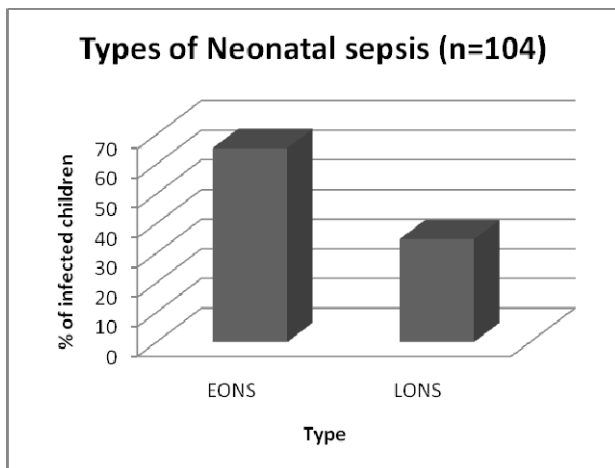

Figure 2: Birth status of infected child

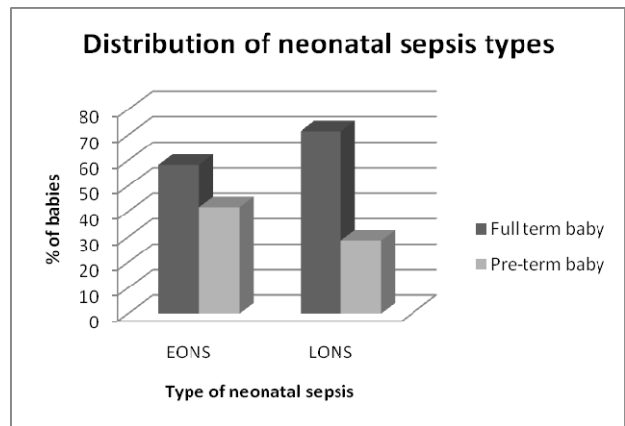**Figure 3:** Types of Neonatal Sepsis found

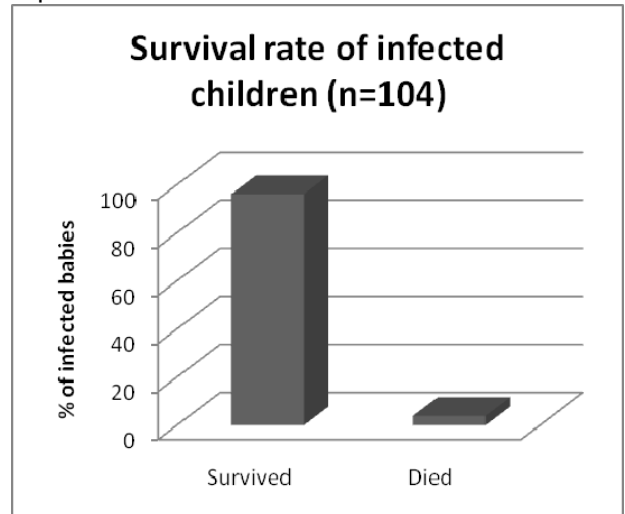**Figure 4:** Distribution of different types of neonatal sepsis

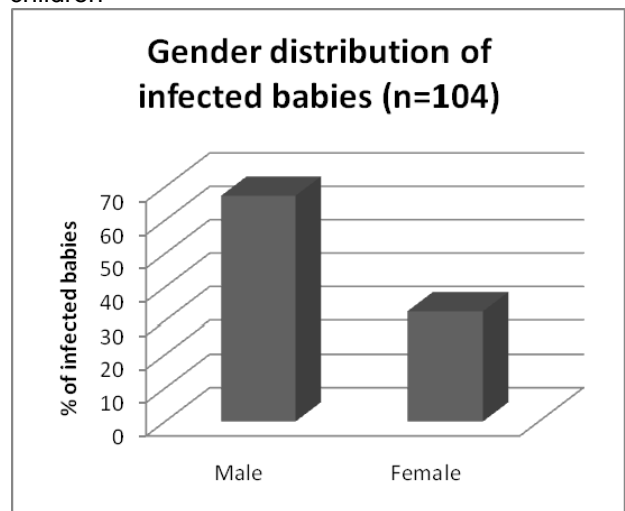**Figure 5:** Survival rate of neonatal sepsis infected children

Figure 6: Gender distribution of neonatal sepsis infected children

Types of Neonatal Sepsis:
Date of disease onset was available for all 104 cases. Onset of disease was the day of birth (day 0) in 30 (28.85%) of cases.

Table 1: Categorization of Neonatal Sepsis cases

Category	Total no.(%) of neonates presenting	Full term babies			Pre term babies		
		No. of neonates	No. of females	No. of males	No. of neonates	No. of females	No. of males
EONS (Early Onset Neonatal Sepsis)	68 (65.38%)	28	10	18	40	15	**25**
LONS (Late Onset Neonatal Sepsis)	36 (34.62%)	20	08	12	16	6	**10**
Total	104	48	18	30	56	21	35

Among the 104 cases of infection, 68 (65.38%) were identified as EONS (Early-onset Neonatal Sepsis) (infection occurring from 0 to 6 days) and 36 (34.62%) were identified as LONS (Late-onset Neonatal Sepsis) (infection occurring after 6days). Distribution of EONS (Early Onset Neonatal Sepsis) and LONS (Late Onset Neonatal Sepsis) and their characteristics is given in figure 3, table 1 and figure 4.

Clinical characteristic
The clinical and demographic data was assessed for all of the 104 cases. Among them 74 (71.15%) had symptomatic neonatal sepsis and 20 (19.23%) had asymptomatic neonatal sepsis. The remaining cases have various focal findings including 3 (2.88%) with pneumonia, 2 (1.92%) with meningitis and 5 (4.81%) with respiratory distress syndrome. Among the 104 cases male accounted for 70 (67.31%) cases and female accounted for 34 (32.69%) cases. Among the 104 infants 100 (96.15%) survived and 4 (3.85%) died. The clinical characteristics observed in this study are summarized in figure 5, figure 6 and figure 7.
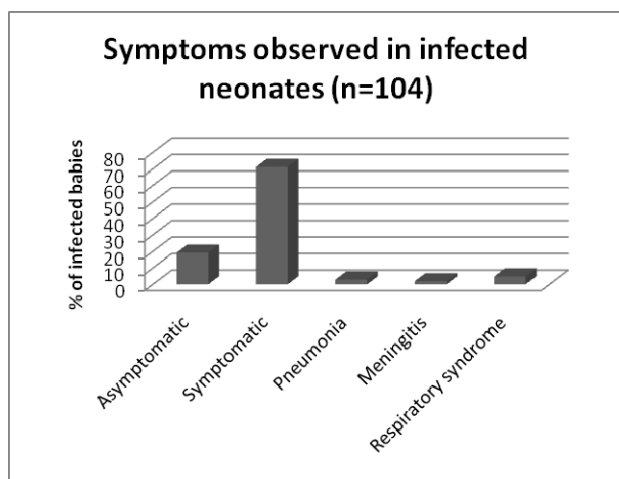

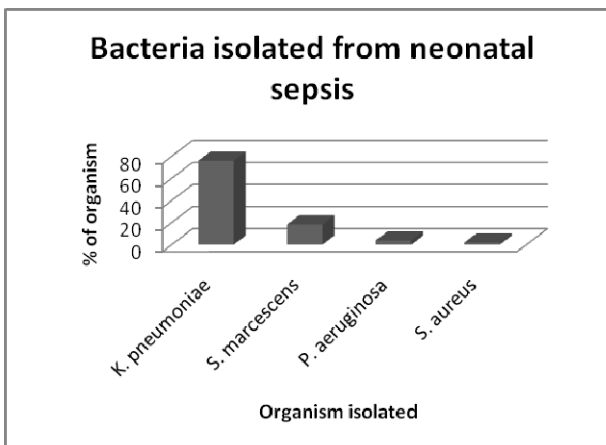**Figure 7:** Symptoms observed in neonatal sepsis infected children

Figure 8: Bacteria isolated from neonatal sepsis in neonates
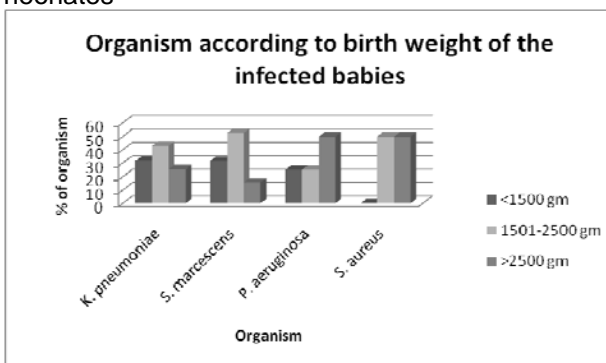

Figure 9: Organism isolated according to birth weight of the infected neonates

Maternal Features
The maternal charts were reviewed for all of the cases. Among them 30 (28.85%) had received intrapartum antibiotics. The reason for intrapartum antibiotics were summarized in table 2.

Of these 104 mothers 54 (51.92%) delivered their infants prematurely (<37 weeks) and 50 (48.08%) delivered their infants maturely (>37 weeks) but they also suffering in the neonatal sepsis.

Microbiology of Neonatal Sepsis
The percentage of species isolated from the blood during 05.01.2009 to 06.07.2009 is shown in figure 8. Only one isolate of a particular species per infant

was counted. When more than one sepsis was isolated during the first 6 days of life each was counted as a separate infection. Among the 104 isolates 102 (98.08%) were attributable to G-ve bacteria and 2 (1.92%) were attributable to G+ve bacteria. *Klebsiella pneumoniae* was the predominant pathogen in this study and accounted for 79 (75.96%) & *Serratia marcescens* accounted 19 (18.27%) of the cases detected. The other common pathogens isolated in this study include- *Pseudomonas aeruginosa* 04 (3.85%) and *Staphylococcus aureus* 02 (1.92%).

Distribution of isolated organisms according to birth weight of the infected neonates is listed in table 3 and figure 9. Healthy neonates (>2500 gm) found to be less prone to sepsis than neonates with less weight during birth.

Characteristics of sepsis type, gestational age & sex in neonatal sepsis according to causative organisms is listed in table 4 and figure 10. *Klebsiella pneumoniae* is predominant in all types of neonatal sepsis.

Antibiotic Susceptibility Pattern of the Isolated Organisms

Various types of antibiotics were used against the isolated organism. In case of G-ve bacteria Ampicillin, Ceftriaxon, Ciprofloxacin, Gentanjicin, Amikacin, Imipenem, Piperacillin, Penicillin and Ceftazidime were used. In case of G+ve bacteria Erythromycin in combination with Amikacin, Imipenem, Ciprofloxacin, Gentamycin and Penicillin were used. The sensitivity patterns of the isolated organism against the various antibiotics are summarized below.

Antibiogram of Klebsiella pneumoniae

79 cases of *Klebsiella pneumoniae* infection were identified during the survey period. All of the isolates showing resistance to ampicillin (100% resistant) on the other hand all of the isolates showing sensitive to imipenem (100% sensitive). In case of ciprofloxacin 59 (74.68%) isolates showing sensitivity against the antibiotics, 15 (18.90%) are intermediate and 5(6.33%) showing resistance. 72 (91.14%) isolates showing sensitive to the amikacin and 5 (6.33%) are intermediate and 2 (2.53%) are resistant. 77 (97.47%) isolates showing resistance to the ceftriaxon and 2 (2.53%) are resistant, there are no isolates found which showing sensitivity to the

ceftriaxon. The antibiotic susceptibility pattern of isolated *Klebsiella pneumoniae* is shown in figure 11.

Antibiogram of Serratia marcescens

19 cases of *Serratia marcescens* infection were identified during the survey period. All of the isolates showing resistance to ampicillin (100% resistant) on the other hand all of the isolates showing sensitive to imipenem (100% sensitive).
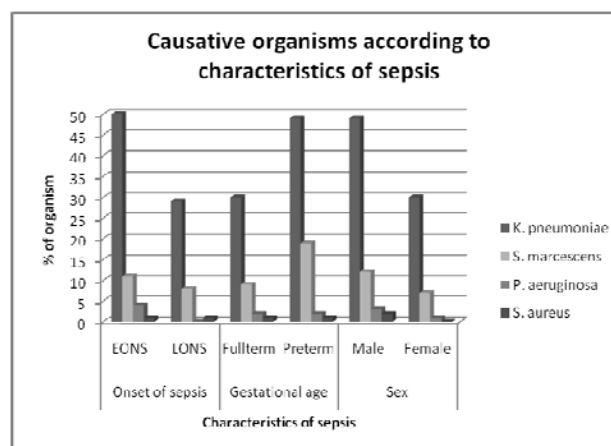

Figure 10: Causative organisms according to characteristics of sepsis (type, gestational age & sex) in neonatal sepsis

Table 2: Percentage of mothers receiving intrapartum antibiotic with reasons

Reason for intrapartum antibiotic	% of Mother receiving antibiotic
Premature rupture of membrane	08 (26.67%)
Prematurely	15 (50%)
Prolonged rupture of membrane	03 (10%)
Fever	04 (13.33%)

Table 3: Number of isolated organisms according to birth weight

Microorganism	<1500 gm	1501-2500 gm	>2500 gm	Total
Klebsiella pneumoniae	25	34	20	79
Serratia marcescens	06	10	03	19
Pseudomonas aeruginosa	01	01	02	04
Staphylococcus aureus	00	01	01	02
Total	32	46	26	104

Table 4: Characteristics of sepsis type, gestational age & sex in neonatal sepsis according to causative organisms

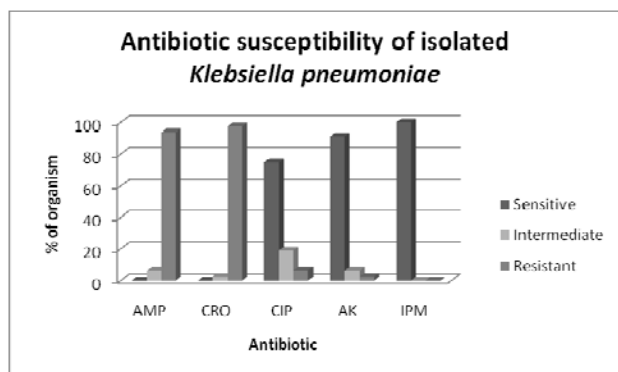| Features	Klebsiella pneumoniae	Serratia marcescens	Pseudomonas aeruginosa	Staphylococcus aureus
Onset of sepsis				
EONS (Early Onset Neonatal Sepsis)	50	11	04	01
LONS (Late Onset Neonatal Sepsis)	29	08	00	01
Gestational Age				
Term	30	09	02	01
Preterm	49	10	02	01
Sex				
Male	49	12	03	02
Female	30	07	01	00

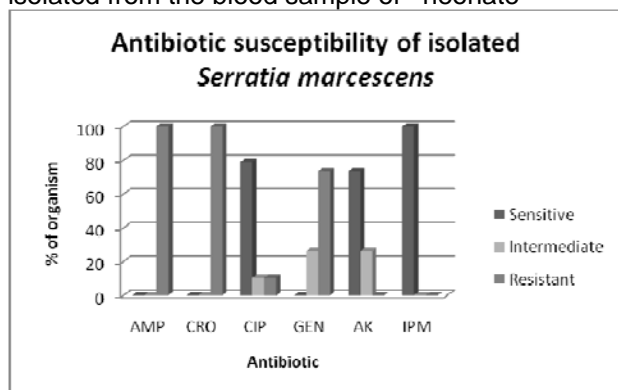**Figure 11:** Antibiogram of Klebsiella pneumoniae isolated from the blood sample of neonate

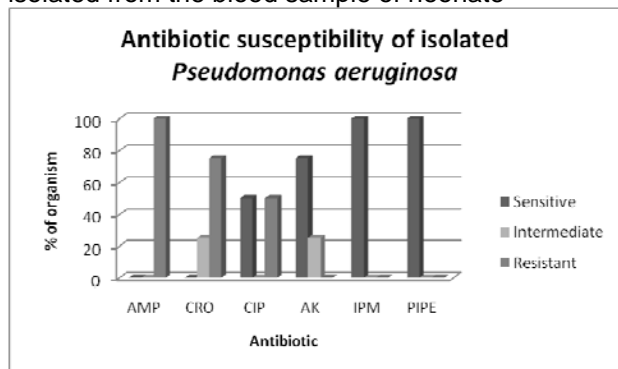**Figure 12:** Antibiogram of Serratia marcescens isolated from the blood sample of neonate

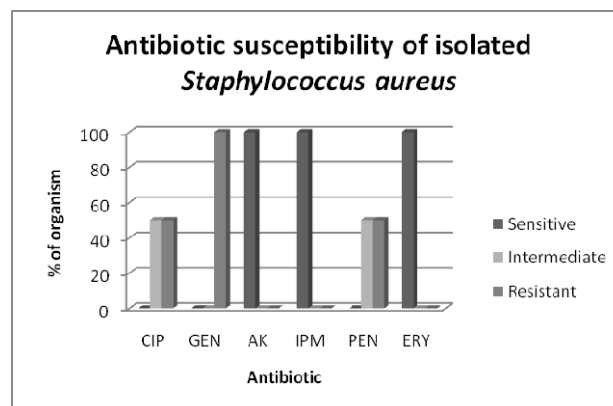**Figure 13:** Antibiogram of Pseudomonas aeruginosa isolated from the blood sample of neonate

Figure 14: Antibiogram of Staphylococus aureus isolated from the blood sample of neonate

In case of ciprofloxacin 15 (78.95%) isolates showing sensitivity against the antibiotics, 02 (10.53%) are intermediate and 02 (10.53%) showing resistance. 14 (73.68%) isolates showing sensitivity to the amikacin and 5 (26.32%) are intermediate. 100%. of the isolates showing sensitivity against the ceftriaxon. The antibiotic susceptibility pattern of isolated *Serratia marcescens* is shown in figure 12.

Antibiogram of Pseudomonas aeruginosa

Total 04 cases of *Pseudomonas aeruginosa* infection were identified during the survey period. All of the isolates showing resistance to ampiciflin (100% resistant) on the other hand all of the isolates showing sensitive to imipenem (100%Sensitive). In case of ciprofloxacin 02 (50%) isolates showing sensitivity against the antibiotics, 02 (50%) are resistant. 03 (75%) isolates showing sensitivity to the amikacin and 01 (25%) are intermediate to the antibiotics. 100% of the isolates showing sensitive against the Piperacillin. The antibiotic susceptibility pattern of isolated *Pseudomonas aeruginosa* is shown in figure 13.

Antibiogram of Staphylococcus aureus

Total 02 cases of *Staphylococcus aureus* infection were identified during the survey period. All of the

isolates showing resistance to gentamycin (100% resistant) on the other hand all of the isolates showing sensitive to amikacin, imipenem and erythromycin (100% sensitive). In case of ciprofloxacin and penicillin 01 (50%) isolates showing intermediate, resistance and 01 (50%) isolates showing resistance against the antibiotics. The antibiotic susceptibility pattern of isolated *Staphylococcus aureus* is shown in figure 14.

Discussion

The overall sepsis rate was 7.45% of all neonates (1400) admitted in this study period and by some researchers who gave a figure of 7.4% neonatal intensive care unit admissions [6]. There were 68 (65.38%) cases of EONS (Early Onset Neonatal Sepsis) and 36 (34.62%) of LONS (Late Onset Neonatal Sepsis). 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 [7,8], 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[9]. Among the originating causes of neonatal sepsis prematurity or low birth-weight (defined as delivery before 37 weeks of gestation or small-for-gestational- age) was the leading cause of neonatal sepsis contributing about two-third of EONS (Early Onset Neonatal Sepsis) in this study.

In this study among the infected child's 74 (71.15%) had symptomatic neonatal sepsis and 20 (19.23%) had asymptomatic neonatal sepsis The remaining 9.62% have various focal findings including pneumonia, meningitis and respiratory distress syndrome. In our study mortality rate in EONS (Early Onset Neonatal Sepsis) was significantly higher than LONS (Late Onset Neonatal Sepsis).

The maternal charts were reviewed for all of the 104 cases among them 30 (28.85%) had received intrapartum antibiotics which are close together the reports of Bangladesh maternal health services and maternal mortality survey 2001[10].

The causative organisms in this study were- *Klebsiella pneumoniae*, *Seirratia marcescens*, *Pseudomonas aeruginosa* and *Staphylococcus aureus*. Another study from Bangladesh revealed that *Klebsiella spp* is the most common causative

agent of neonatal sepsis[9]. In the present study *Klebsiella pneumoniae* accounted for 79 (75.96%), *Serratia marcescens* 19 (18.27%), *Pseudomonas aeruginosa* 04 (3.85%) and *Staphylococcus aureus* 02 (1.92%). But in western countries, group B Sfreptococci (GBS) and *E. coli* are the most common G+ve and G-ve bacteria isolated respectively[11]. In Middle East 43% of neonatal sepsis responsible for *Pseudomonas spp*, while some other group showed 6% predominance of *Klebsiella spp*[12].

Drug resistance in causative organisms of sepsis is a rapidly emerging issue. This study revealed a very high degree of resistance of isolated microorganisms not only to commonly used antibiotics, but also predominantly to broad spectrum antibiotics. On the contrary studies from Sydney Neonatal Infection Surveillance have mentioned that all the G-ve organisms were susceptible to gentamycin and third generation cephalosporin. Data from the present study gave us an idea about the resistance pattern of isolated organisms against the first, second and third generation of drug. *Klebsiella pneumoniae* were ampicillin (AMP) resistant which was similar with the data from Malakar Rad *et al*[13] study where they found most of the *Klebsiella pneumoniae* were ampicilhn resistant. In this study most G-ve bacteria are resistant to ampicillin, ceftriaxon and gentamycin. Ciprofloxacin and amikacin are mostly sensitive to G-ve bacteria but Ciprofloxacin and amikacin resistant strain also found. In the present study all the isolates both G-ve and G+ve show sensitivity against the antibiotic imipenem (100% sensitive).

Although positive blood culture is the gold standard in the diagnosis of neonatal sepsis, but in the absence of proof of sepsis many clinicians and even some neonatologist felt obliged to continue antibiotics for a full of 10 day course. There is also danger of removing useful or susceptible organisms and encouraging resistant ones. If this occurs we shall reach a stage to go on to use more expensive antibiotics. Strict infection control in neonatal units, hand washing combined with judicious policy for antibiotic therapy may be a solution to this problem.

To control infections, prolonged use of broad-spectrum antibiotics is often encountered, which leads to the resurgence of multidrug-resistant organisms.[14] Therefore, preventive antibiotics should be used as little as possible, while

therapeutic antibiotics should be specific and used as short period of time as possible. The combined use of various antibiotics should likewise be judicious. In conditions wherein the use of antibiotics is necessary, rotating antibiotic regimens has been suggested and may be a way to solve this problem.

Conclusion

In conclusion, different neonatal intensive care units (NICUs) have different epidemiologies of nosocomial infections. Collection of up-to-date data is mandatory for appropriate use of antibiotics. To select antibiotics conscientiously according to susceptibility tests is very important, and strategies to avoid the resurgence of multidrug-resistant strains should be established.

Acknowledgement

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