Evaluation of bacterial pathogens in neonatal sepsis and their susceptibility pattern: A Hospital Based Study

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Abstract

Neonatal sepsis is one of the major causes of neonatal morbidity and mortality, particularly in developing countries. Epidemiology and surveillance of neonatal sepsis helps in implementation of rational empirical antibiotic strategy. A cross-sectional prospective study was conducted in the special care baby unit under department of Paediatrics and Neonatology, BIRDEM General Hospital during the period of November 2008 to September 2009 to determine the pattern of bacterial agents causing neonatal sepsis and their susceptibility pattern to various antimicrobial agents. Blood cultures were performed on admitted newborn babies (0-28 days) to rule out sepsis. 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. Out of 720 screened blood cultures, 64 (8.9%) reported as positive and the gram positive and gram negative bacteria accounted for 6 (9.4%) and 58 (90.6%) respectively. The most common gram positive organisms were Staphylococcus aureus (6.3%) and Enterococci (3.1%) & gram negative organisms were Klebsiella pneumoniae (37.5%), Serratia (25%), Pseudomonas aeruginosa (10.9%), Citrobacter (10.9%) and Acinetobacter (6.3%). The susceptibilities were remarkably low to Ampicillin (3.12 %) & Cefotaxim (10.9%) for both gram positive & gram negative isolates. Gram positive group had susceptibilities of 66.7% to Ciprofloxacin and Imipenem, 83.3% to Gentamicin, & 100% to Amikacin & Vancomycin. Gram negative isolates showed higher sensitivities to Imipenem (94.8%), Ciprofloxacin (89.7%), Amikacin (72.4%) respectively. Gram-negative bacteria showed high level of resistance to commonly used antibiotics (Ampicillin, Ceftazidim and Cefotaxim). Gentamicin, Amikacin, Imipenem and Ciprofloxacin were the most effective drugs compared to others. Routine bacterial surveillance and their sensitivity patterns must be an essential component of neonatal care.

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Introduction

Neonatal sepsis is considered to be an important cause of neonatal mortality (deaths in the first 28 days of life)[1, 2]. The World Health Organization estimated that there are approximately four million neonatal deaths occur worldwide every year, 98% of which occur in developing countries, particularly Asia and Africa[3,4]. These neonatal deaths are attributed principally to infection, birth asphyxia and consequences of prematurity and low birth weight. Neonatal sepsis remains as an important cause of morbidity and mortality among infants in developing countries accounting for 30-50% of total deaths each year [5]. 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 birth, occurring mainly in the first week of life, whilst it is 5 per 1000 live birth in developed country[6]. Infant mortality rate (IMR) in

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Bangladesh is 38/1000 live births[7] out of them about 70% of death occur in neonatal period (<28 days) and Neonatal mortality rate (NMR) in our country 27/1000 live birth[7]. The reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live birth in Asia[8], from 6.5 to 23 per 1000 live birth in Africa[9] and from 3.5 to 8.9 per 1000 live births in South America and the Caribbean[10, 11].

By comparison, rates reported in the United States and Australia range from 6–9 per 1000 live birth[12, 13] and in Europe 0.3-3 per 1000 live birth[14].

In most developing countries, gram-negative bacteria remains the major cause of neonatal sepsis[15, 16]. These organisms have developed increased drug resistance over the last two decades[17].

On the other hand Group B Streptococcus (GBS) has been the most frequent etiological agent of neonatal sepsis in developed countries, being responsible for high morbidity and mortality rates[18]. 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/country, it is necessary to have periodic surveillance to understand the changing pattern of organisms causing neonatal sepsis. In addition, rapidly changing antibiotic sensitivity pattern of bacterial agents causing neonatal sepsis, making its management more challenging for the health care providers [19]. Therefore knowledge of the pattern of bacterial isolates and their antimicrobial susceptibility is useful for treating patients with appropriate antibiotics. Although an extensive research is available worldwide[17, 20] very few reports are available on neonatal sepsis in Bangladesh. The present study was undertaken to identify the bacterial pathogens and their antibiotic sensitivity pattern in neonates with clinical diagnosis of septicemia, admitted in SCABU, at BIRDEM General Hospital, Dhaka, Bangladesh from November 2008 to September 2009.

Methods

In a cross- sectional prospective study, a total of 720 neonates (0 to 28 days of age) were investigated who were admitted to rule out sepsis in SCABU, at BIRDEM General Hospital over a period of 11 months (November 2008 to September 2009). Both premature and full terms were included in the study. Written informed consent was obtained from their parents/guardians and was investigated for bacterial etiologic agents. Demographic, clinical and other relevant data were obtained by attending pediatrician/s and were transferred to the questionnaire prepared for this study. Studied neonates were divided into two groups according to timing of clinical signs as early onset (clinical signs of sepsis from birth to 7 days old) and late onset (clinical signs of sepsis from 8 to 28 day old) sepsis. Neonates were also classified into normal birth weight (birth weight >2500gm), and low birth weight (birth weight <2500gm) and also into those with term (gestational age >37 weeks) and preterm (gestational age<37 weeks) according to gestational age. Blood culture, chest X-ray and laboratory tests including complete blood count (CBC), blood sugar and serum electrolytes were performed for all subjects.

Using aseptic technique by applying Povidone iodine and 70% alcohol at the site of vein puncture, 2 ml venous blood was drawn from the peripheral vein by the attending nurse and then the blood was inoculated into a blood culture bottle containing Tryptone Soy Broth (TSB) and Brain Heart infusion Broth. The specimens were transported immediately to microbiological laboratory and incubated for one week in 37^0C and were daily checked for evidence of bacterial growth. For positive broth cultures, subcultures were made on 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. Antimicrobial susceptibility testing was performed for all blood culture isolates according to the criteria of the National Committee for Clinical Laboratory Standards by disk diffusion method.

Result

Positive blood cultures were obtained for 64 neonates (8.9%). Among neonates with sepsis, 45 (70.3%) had early onset and 19 (29.7%) had late-onset neonatal sepsis. Among 64 newborns with sepsis, 45 (70.3%) were preterm and 19 (29.7%) were term. There were 47(73.4%) neonates with low birth weight and 17 newborns (26.6%) with normal birth weight. Considering sex preponderance there were more cases of sepsis in male neonates in comparison to female (40 male and 24 female with 1.7:1 ratio). The mortality rate was 6 (9.4 %) in this study (Table- 1).

Among these, 58(90.6%) had sepsis with gram negative bacteria and 6(9.4%) with gram positive bacteria. The most common isolated gram negative bacteria was Klebsiella pneumoniae (37.5%) , other gram negative agents were Serratia (25%), Pseudomonas aeruginosa (10.9%), Citrobacter (10.9%), Acinetobacter (6.3%). Staphylococci aureus (S. aureus) was the most isolated prevalent gram positive bacteria 4(6.3%) (Table- 2). Based on the results from susceptibility testing, all of the gram negative isolates were resistant to Ampicillin. Klebsiella pneumonia and Serratia isolates were also resistant to Cefotaxim and ceftazidime. K. pneumoniae had low sensitivities to Gentamicin (33.3%). However this species showed 100% sensitivity to Imipenem and 66.7% sensitivity to Amikacin (Table-3). Serratia showed lower sensitivity to Gentamycin (50%) in comparison to Amikacin(62.5%), Ciprofloxacin(100%), Imepenem(100%). All of the isolated gram positive bacteria poorly sensitive to Ampicillin (33.3%) and resistant to Cefotaxim and highly sensitive to Vancomycin (100%) and Amikacin (100%).

Table-1: Treatment outcome according to sex, gestational age, birth weight and type of sepsis

	Male/ Female	Early onset	Late onset	Wt ≥500 gm	Wt <2500 gm	Term >37 weeks	Pre Term <37 weeks	In born	Out born
Recovery	36/22 = 58	45 (70.3)	19 (29.7)	17 (26.6)	47 (73.4)	19 (29.7)	45 (70.3)	36 (56.3)	28 (43.7)
Death	4/2 = 6	6 (9.4)	0	0	6 (9.4)	0	6 (9.4)	2 (3.1)	4 (6.3)
Total	40/24 = 64	51 (79.7)	19 (29.7)	17 (26.6)	53 (82.8)	19 (29.7)	51 (79.7)	38 (59.4)	32 (50.0)

Table: 2. Antimicrobial susceptibility of gram -positive isolates:

Gram positive Organism no (%)	Antibiotics no.(%)								
	AM	GM	AMK	VAN	NETIL	CLP	COTRIM	IMP	CP
Staph. Aureus =4(66.7)	0	3 (75)	4 (100)	4 (100)	4 (100)	2 (50)	2 (50)	2 (50)	2 (50)
Enterococci =2(33.3)	2 (100)	2 (100)	2 (100)	2 (100)	0	0	0	2 (100)	2 (100)
Total =6(100)	2 (33.3)	5 (83.3)	6 (100)	6 (100)	4 (66.7)	2 (33.3)	2 (33.3)	4 (66.7)	4 (66.7)

AM: Ampicillin, GM: Gentamicin, AMK: Amikacin, VAN: Vancomycin, NETIL : Netilmycin, CLP: Chloramphenicol, COTRIM: Cotrimoxazole, IMP: Imipenam, CP: ciprofloxacin

Table: 3. Antimicrobial susceptibility of gram -negative isolates:

Gram Negative Organism no (%)	Antibiotics no (%)									
	NETIL	GEN	AMK	CP	CTX	IMI	CFD	COTRIM	PI (%)	CLP
KL.Pneumoniae N=24 (41.4)	4 (16.7)	8 (33.3)	16 (66.7)	20 (83.3)	0	24 (100)	0	3 (12.5)	16 (66.7)	11 (45.8)
Serratia N=16 (27.6)	3 (18.8)	8 (50)	10 (62.5)	16 (100)	0	16 (100)	0	2 (12.5)	3 (18.8)	2 (12.5)
Pseudomonas aeruginosa N =7(12.0)	2 (28.6)	4 (57.1)	6 (85.7)	7 (100)	3 (42.9)	6 (85.7)	7 (100)	3 (42.9)	7 (100)	3 (42.9)
Citrobacter N =7(12.0)	3 (42.9)	3 (42.9)	6 (85.7)	7 (100)	3 (42.9)	5 (71.4)	0	2 (28.6)	0	3 (42.9)
Acinetobacter N =4(7.0)	1 (25)	3 (75)	4 (100)	2 (50)	1 (25)	4 (100)	0	0	2 (50)	0
Total N=58(100)	13 (22.4)	26 (44.8)	42 (72.4)	52 (89.7)	7 (12.1)	55 (94.8)	7 (12.1)	10 (17.2)	28 (48.3)	19 (32.8)

Key: NETIL: Netilmycin, GM: Gentamicin, AMK: Amikacin, CP: Ciprofloxacin, CTX: Cefotaxime, IMP: Imipenem, CFD: Ceftazidim, COTRIM: Cotrimoxazole, PI: Piperacillin, CLP: Chloramphenicol

Discussion

In this study, prevalence of documented neonatal sepsis with positive culture was 8.9% which is similar to another study[21]. This is low compared to about 20% yield reported by Haque[22], Baltimore[23] and Gladstone [24]. In the present study, 70.3 % and 29.7% neonates presented with early onset sepsis (EOS)and late onset sepsis (LOS) respectively. We found that EOS was more common than LOS, which is in agreement with the reports from other developing countries e.g. in Iran[2] (77.5% vs.22.5%) and in a study of Bangladesh[25] (70.7 vs. 29.3%), but in contrast with reports from Saudi Arabia (39% vs. 61%)[26] and Pakistan (42% vs.58%)[27], where late onset sepsis is more common. The possible explanation for a higher frequency of EOS in this study might be the more referral of preterm labors and preterm newborns to our center. Isolation of gram positive and gram

negative bacteria in this study was 9.4% and 90.6% respectively. This finding is similar to that of other studies which showed that gram negative bacteria were responsible in most cases of neonatal sepsis[2, 28, 29]. This was in contrast to other studies where gram positive bacteria were the commonest cause of neonatal sepsis[11,15,30], while another study showed, the frequency of isolation of both gram positive and gram negative bacteria were equal[26]. Klebsiella pneumoniae was the most common isolates (37.5%) causing neonatal sepsis and serratia was the second most common organism isolated in this study. Studies from different countries report Coagulase negative staphylococcus (CONS) as the predominant organisms in LOS[31,32]. Generally the spectrum of organisms causing neonatal sepsis in this study is similar to that reported from developing countries, with gram negative bacteria being responsible in most cases . Klebsiella pneumoniae is emerging as a common bacteria in hospital settings[21,28,33] But the pattern of isolated organisms in our study slightly differs from the findings in Iran[2] where Pseudomonas Aeruginosa was the most common cause of neonatal sepsis followed by Klebsiella pneumoniae and Escherichia coli (E. coli).In a similar study from Bangladesh, Nepal and Pakistan, E. coli was the leading cause of neonatal sepsis followed by Klebsiella pneumoniae [27,28]. In other studies gram positive bacteria such as S. aureus and Group B streptococcus (GBS) were found to be the most common isolates in neonatal septicaemia[11, 30].

In the present study, Klebsiella pneumoniae was best susceptible to Imipenem (100%), Ciprofloxacin (83.3%), Amikacin(66.7%), Piperacillin (66.7%) and less susceptible to Gentamicin (33.3%), Netilmycin (16.7%) which was similar to that of other studies[34,35] and completely resistant to Ampicillin which is similar to another study[36]. Serratia were susceptible to Imepenem (100%), Ciprofloxacin (100%), Amikacin (62.5%), Gentamicin (50%) and completely resistant to Ampicillin. Low sensitivity to Ampicillin is similar to many other studies [12,37,38]. S. Aureus and Enterococci in our study were better susceptible to Imipenem (66.7%), Gentamicin (83.3%), Amikacin (100%), Vancomycin (100%). Our results have demonstrated that in general both gram positive and Gram negative bacterial isolates showed higher sensitivity rates to Amikacin, Ciprofloxacin, Imepenem. Gram-negative bacteria showed high-level resistance to Ampicillin, Ceftazidime and Cefotaxime. This observation is comparable to that of other researchers[2,15,16,17,29]. However, these results are limited to study cohorts and every center should have idea about their own bacterial sensitivity pattern. Ampicillin and Gentamicin are the first line treatment for Neonatal sepsis in many centers. These antibiotics seem to be less useful according to our study. Use of Amikacin or Imipenem plus Ciprofloxacin could be a more effective combination in our center. Once culture and sensitivity results are available, antibiotics can be adjusted accordingly.

Conclusion

Present study indicated that gram- negative species continue to be the predominant causative organism among the study cohorts. Klebsiella pneumoniae and Serratia played a major role and Pseudomonas aeruginosa, Citrobacter, Acinetobacter, Staphylococcus aureus, and Enterococci contributed to the rest. A low Susceptibility to commonly used antibiotics like Ampicillin is a cause for concern. The antibiotic susceptibility profiles suggested that for a given cohort, initial choice of Amikacin or Imipenem in combination with Ciprofloxacin is the most rational choice of empiric therapy. In our country where cost is an issue, we can use Amikacin and Ciprofloxacin combination as the initial choice of antibiotics for treating neonatal sepsis.

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