

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 year⁵. 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 country⁶. Infant mortality rate (IMR) in

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Bangladesh is 38/1000 live births⁷ out of them about 70% of death occur in neonatal period (<28 days) and Neonatal mortality rate (NMR) in our country 27/1000 live birth⁷. The reported incidence of neonatal sepsis varies from 7 to 38 per 1000 live birth in Asia⁸, from 6.5 to 23 per 1000 live birth in Africa⁹ 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 birth¹⁴.

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 decades¹⁷.

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 rates¹⁸. 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 providers¹⁹. 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°C 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°C 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 pneumoniae* 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 week s	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	COTRI M	IMP
Staph. Aureus =4(66.7)	0	3 (75)	4 (100)	4 (100)	4 (100)	2 (50)	2 (50)	2 (50)
Enterococci =2(33.3)	2 (100)	2 (100)	2 (100)	2 (100)	0	0	0	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)

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

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

Gram Negative Organism no (%)	Antibiotics no (%)									
	NETI L	GEN	AMK	CP	CTX	IMI	CFD	COTR IM	PI (%)	CL P
KL.Pneumonia e 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 study²¹. This is low compared to about 20% yield reported by Haque²², Baltimore²³ and Gladstone²⁴. 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 Iran² (77.5% vs.22.5%) and in a study of Bangladesh²⁵ (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 equal²⁶. *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 Iran² 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%), Netilmicin (16.7%) which was similar to that of other studies^{34,35} and completely resistant to Ampicillin which is similar to another study³⁶. *Serratia* were susceptible to Imipenem (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, Imipenem. 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.

References

1. Dawodu A, Al Umran K, Danso K. A case study of neonatal sepsis in very low birth weight infants. *N Engl J Med* 2002;347: 240-7.
2. Movahedian AH, Moniri R, Mosayebi Z. Bacterial Culture of Neonatal Sepsis. *Iranian J Publ Health* 2006;35:84-89.
3. Zupan J, Aahman E. Perinatal mortality for the year 2000: estimates developed by WHO. Geneva: World Health Organization. 2005.
4. Stoll BJ. The global impact of neonatal infection. *Clin Perinatol* 1997;24:1-21.
5. Bang AT, Reddy HM, Deshmukh MD, Baitule SB, Bang RA. Neonatal and infant mortality in the ten years (1993 to 2003) of the Gadchiroli field trial: effect of home based neonatal care. *J Perinatol* 2005;25:S92-107.
6. Costello A, Francis V, Byrne A. The state of the world's newborns. Washington: Save the Children Fund. 2001.
7. UNICEF. The State of World's Children 2012.87 – 107.
8. Lim NL, Wong YH, Boo NY, Kasim MS, Chor CY. Bacteraemic infections in a neonatal intensive care unit: a nine months survey. *Med J Malaysia* 1995; 50:59 –63.
9. Airede AI. Neonatal septicaemia in an African city of high altitude. *J Trop Pediatr* 1992;38:189 –91.
10. Moreno MT, Vargas S, Poveda R, Sáez-Llorens X. Neonatal sepsis and Meningitis in developing Latin American. *Paediatr Infect Dis.* 1994; J 13:516-20.
11. Robillard PY, Nabeth P, Hulsey TC, Sergeant MP, Périain J, Janky E. Neonatal bacterial septicaemia in a tropical area. Four-year experiences in Guadeloupe (French West Indies). *Acta Paediatr* 1993; 82:687 –9.
12. Hyde TB, Hilger TM, Reingold A, Farley MM, O'Brien KL, Schuchat A. Active Bacterial Core surveillance (ABCs) of the Emerging Infections Program Network. Trends in incidence and antimicrobial resistance of early-onset sepsis: population-based surveillance in San Francisco and Atlanta. *Pediatrics* 2002;110:690 –95.
13. Heath PT, Nik Yusoff NK, Baker CJ. Neonatal meningitis. *Arch Dis Child Fetal Neonatal Ed.* 2003;88:F173 –78.
14. Vesikari T, Janas M, Grönroos P, Tuppurainen N, Renlund M, Kero P, et al. Neonatal septicaemia. *Arch Dis Child.* 1985; 60:542-46.
15. Anwer SK, Mustafa S, Pariyani S, Ashraf S, Taufiq KM. Neonatal sepsis: an etiologic study. *J Pak Med Assoc* 2000;50:91-94.
16. Joshi SG, Ghole VS, Niphadkar KB. Neonatal gram negative bacteremia. *Indian J Pediatr* 2000;67:27-32.
17. Bhutta ZA. Neonatal bacterial infections in developing countries: strategies for prevention. *Semin Neonatol.* 1999;4:159-71.
18. Freedman RM, Ingram DL, Gross I, Ehrenkranz RA, Warshaw JB, Baltimore RS. A half century of neonatal sepsis at Yale. *Am J Dis Child* 1981;135:140-44.
19. Motara F, Ballot DE, Perovic O. Epidemiology of neonatal sepsis at Johannesburg Hospital. *Southern Afr. J. Epidemiol. Infect* 2005; 20: 90-93.
20. Klein JO. From harmless commensal to invasive pathogen coagulase-negative staphylococci. *N Engl J Med* 1990;323:339-40.
21. Hossain MM, Afroza S, Shirin M, Chowdhury NA, Saha SK. Bacterial aetiology of neonatal sepsis in a tertiary care hospital in Bangladesh. *Bangladesh J Child Health* 2004;28(3):81-85.
22. MF Haque, SM Safiquezaman, AFM Salim, F Monzur, SH Banu. Bacteriological profile of neonatal septicemia in a neonatal unit (SCANU). *DS (Child) H J* 2008;24(1&2):4-8.
23. Baltimore RS, Huie SM, Meek JI, Schuchat A, O'Brein KL. Early-onset neonatal sepsis in the era of group B Streptococcal prevention. *Pediatrics* 2001;108:1094-98.
24. Gladstone IM, Ehrenkranz RA, Edberg SC, Baltimore RS. A ten-year review of neonatal Sepsis and comparison with the previous fifty-year experience. *Pediatr Infect Dis J* 1990;9:819-25.
25. Rasul CH, Hassan MA, Habibullah M. Neonatal sepsis and use of antibiotic in tertiary care hospital. *Pak J Med Sci* 2007;23:78- 81.
26. Umran K, Twum-Danso K. A case control study of neonatal sepsis: Experience from Saudi Arabia. *J Trop Pediatr* 1997;43:84-88.
27. Aftab R, Iqbal I. Bacteriological agents of neonatal sepsis in NICU at Nishtar Hospital Multan. *J Coll Physicians Surg Pak* 2006;16:216-19.
28. Ahmed AS, Chowdhury MA, Hoque M, Darmstadt GL. Clinical and bacteriological profile of neonatal septicemia in a tertiary level pediatric hospital in Bangladesh. *Indian Pediatr* 2002;39:1034-39.
29. Rahman S, Hameed A, Roghani MT, Ullah Z. Multi-drug resistant neonatal sepsis in Peshawar, Pakistan. *Arch Dis Child Fetal Neonatal* 2002;87: F52-F54.

30. Mugalu J, Nakakeeto MK, Kiguli S, Kaddu-Mulindwa DH. Aetiology, Risk factors and immediate outcome of bacteriologically confirmed neonatal septicaemia in Mulago hospital, Uganda. *African Health Science* 2006;6:120-26.
31. Munson DP, Thompson TR, Johnson DE, Rhame FS, VanDrunen N, Ferrieri P. Coagulase-negative staphylococcal septicemia: experience in a newborn intensive care unit. *J Pediatr*. 1982;101:602-15.
32. Baumgart S, Hall SE, Campos JM, Polin RA. Sepsis with coagulase-negative staphylococci in critically ill newborns. *Am J Dis Child* 1983;137:461-63.
33. Mannan MA, Shahidullah M, Noor MK, Dey AC, Nasrin N, Marma U. Nosocomial infections in a newborn intensive care unit of a tertiary care health. *Bangladesh J Child Health* 2008;32(3):92-96.
34. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath P. Neonatal sepsis: an international perspective. *Arch Dis Child Fetal Neonatal Ed* 2005; 90:F220-24
35. Ramesh Bhat Y, Leslie ES Lewis, Vandana KE. Bacterial isolates of early-onset neonatal sepsis and their antibiotic susceptibility pattern between 1998 and 2004: an audit from a center in India. *Indian Journal of Pediatrics* 2011; 37:32.
36. Rad EM and Momtazmanesh N. Neonatal Sepsis due to *Klebsiella* : Frequency , Outcome and Antibiotic Sensitivity. *Iranian J Publ Health*2004; 33(2):43-48.
37. Tallur SS, Kasturi AV, Nadgir SD, Krishna BVS: Clinico- bacteriological study of neonatal septicemia in Hubli. *Indian J Pediatr* 2000; 67:169-74.
38. Bizzaro MJ, Raskind C, Baltimore RS, Gallagher PG: Seventy- five years of neonatal sepsis at Yale: 1928-2003. *Pediatrics* 2005;116:595-602.