

Study of Bacteriological pathogen causing neonatal sepsis at NICU in Ad-din medical college hospital

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Abstract

Objective : To determine the common causative bacterial agents of neonatal sepsis and their antimicrobial susceptibility in NICU

Methods : Newborns with clinical signs of septicemia and positive blood culture during twelve months were prospectively studied. Samples for blood cultures, complete blood count, C-reactive protein, sugar, electrolytes were obtained ; ampicillin and gentamycin were started empirically.

Result : Seven hundred and three neonates were investigated to rule out of sepsis. The male, female ratio was 1.3:1 and 56(63.63%) of patients were preterm and 32(36.36%) were term neonates. There were 68 (77.27%) cases of Early onset neonatal sepsis (EONS) and 20(22.64%) of Late onset neonatal sepsis (LONS). Coagulase negative staphylococcus (CONS) was the most common 37(42.04%) Gm+ve pathogen causing early onset neonatal sepsis and Acinetobacter was common Gm-ve pathogen in both early onset 16(18%) and late-onset 8 (9%) sepsis. CONS sepsis showed high degree of resistance to commonly used antibiotics; ampicillin (86.5%), oxacillin (60%), cefotaxime (67%) but comparatively low resistance to vancomycin (0%), gentamicin (25%), Levofloxacin(36%) & Imipenem (49%). Acinetobacter were highly resistant to ampicillin (100), gentamicin(75%), cefotaxime(100) and sensitive to Amikacin (58.33%), ciprofloxacin (54.16%), Levofloxacin and Imipenem (62.5%)and Colistin (95.83%).

Conclusion : Neonatal sepsis in our NICU is mainly caused by gram-positive organisms, which are developing resistance to commonly used antibiotics. The initial empirical choice of Ampicillin and Gentamicin appears to be unreasonable for our environment.

Key Words : Septicemia; EONS- Early onset neonatal sepsis, LONS- Late onset neonatal sepsis, CONS. Coagulase negative staphylococcus, Antibiotic sensitivity.

Introduction

Sepsis neonatorum is the term used to describe any systemic bacterial infection documented by a positive blood culture in the first month of life¹. Sepsis appearing from birth to seven days is called Early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) were defined as illnesses appearing from eight to twenty-eight days postnatal period. Neonatal septicemia remains one of the important causes of mortality and morbidity despite considerable progress in hygiene, introduction of new and potent

antimicrobial agents and advanced measures for diagnosis and treatment². Up to 10% of infants have infections in the first month of life³ which are responsible for 30-50% of total neonatal deaths in developing countries⁴. 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^{5,6}. A number of organism is associated with neonatal sepsis and bacterial pathogens may vary from one country to another and within a country from one hospital or region to another⁷. These organisms may even vary at different times within the same place^{8,9}. In developed countries Group B streptococcus (GBS), E.coli and Listeria monocytogenes are the most common causes of neonatal sepsis, however, in developing countries, these bacteria are replaced by gram-negative bacilli, Coagulase negative staphylococcus (CONS) and others¹⁰. Over the last two decades most of the organisms have developed increased drug resistance and management of the patients is becoming a major problem¹¹. Identification of the etiology is important since it can induce a change in management policy⁷. For effective management of neonatal septicemia with appropriate antibiotics that would minimize the risk of severe morbidity and mortality & to reduce the emergence of multi-drug resistant organisms by rational antibiotic use, study of bacteriological profile and their antibiotic sensitivity pattern plays a significant role^{1,12}. The present study was carried out to determine the bacteriological profile with antibiotic sensitivity pattern of neonatal sepsis in NICU Ad-din Medical college & hospital.

Subjects & Methods

In a cross sectional descriptive study, a total of 703 neonates (0 to 28 days of age) were investigated who were admitted to rule out sepsis in NICU at Ad-din Medical College Hospital over a period of 13 months (January 2012 to January 2013). Both premature and full terms, inborn and out born 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 questionnaire

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prepared for this study. Early onset neonatal sepsis (EONS) and late onset neonatal sepsis (LONS) were defined as illnesses appearing from birth to seven days and from eight to twenty-eight days postnatal respectively. Blood culture, chest X-ray and laboratory tests including complete blood count (CBC), C-reactive protein (CRP), blood sugar (BS) and 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

Results : Positive blood cultures were obtained for 88 neonates (13%). Among them 45 (51.12%) with gram positive bacteria and 43 (48.84%) had sepsis with gram negative bacteria. The most common gram positive bacteria was Coagulase Negative Staphylococci (CONS) 37 (42.07%), other were Staphylo coccus 5 (5.68%), Enterococcus 3 (3.40%) and gram negative bacteria were Acinetobacter 24 (27.27%), Pseudomonas spp. 8 (9.09%), Klebsiella Pneumoniae 5 (5.68%), Moraxella 3 (3.40%) and Enterobacter 3 (3.40%) (Table 1).

Table I: Types of micro organism in early and late onset neonatal sepsis

Sepsis organism	Early onset		Late onset		Total
	Preterm	Term	Preterm	Term	
CONS	18 (20.04%)	15(17.04%)	3 (3.40%)	1 (1.13%)	37 (42.04%)
Staphylo coccus	2 (2.27%)	3 (3.40%)	0	0	5 (5.68%)
Entero coccus	2 (2.27%)	1 (1.13%)	0	0	3(3.40%)
Acinetobacter	11 (12.50%)	5 (5.68%)	6 (6.81%)	2(2.27%)	24 (27.27%)
Pseudomonas	5(5.68%)	2 (2.27%)	1 (1.13%)	0	8 (9.09%)
Klebsiella	0	1(1.13%)	2 (2.27%)	2 (2.27%)	5 (5.68%)
Enterobacter	1 (1.13%)	0	2 (2.27%)	0	3 (3.40%)
Morexiella	2 (2.27%)	0	1 (1.13%)	0	3 (3.40%)
Total	41 (46.59%)	27 (30.68%)	15 (17.04%)	5 (5.602%)	88 (100%)

68 patients (77.27%) had early onset and 20 (22.64%) had late-onset neonatal sepsis. Coagulase Negative Staphylococci (CONS) was the most isolated prevalent gram positive bacteria 37 (42.04%) and common in early onset neonatal sepsis (48.52%). Acinetobacter was common pathogen in both early onset 16 (65%) and late-onset 8 (35 %) sepsis. Among 88 newborns with sepsis, 56 (63.63%) were preterm and 32(36.28%) were term.

Table II: Distribution of neonates according to neonatal characteristics(n=88):

Neonatal characteristics		Total No(%)	Early onset	Late onset	Expired
Sex	Male	51(57.95%)	39(44.31%)	12(13.63%)	3(3.40%)
	Female	37(42.04%)	26(29.54%)	10(11.36%)	2(2.27%)
Place of delivery	In born	63 (71.59%)	46 (52.27%)	17 (19.31%)	2(2.27%)
	Out born	25(28.40%)	19(21.59%)	6 (6.81%)	3(3.40%)
Mode of delivery	LUCS	56(63.63%)	40 (45.45%)	16(18.18%)	4(4.54%)
	NVD	32(36.36%)	25 (28.40%)	7 (7.95%)	1(1.13%)
Birth weight	>2500gm	27 (30.68%)	24 (27.27%)	3 (3.40%)	1(1.13%)
	<2500gm	61(69.31%)	41 (46.59%)	20 (22.72%)	4(4.54%)

There were 61(69.31 %) neonates with low birth weight and 27 newborns (30.68%) with normal birth weight. Considering sex preponderance there were more cases of sepsis in male neonates in comparison to female (51 male and 37 female with 1.3:1 ratio). The mortality rate was 5 (5.68 %) in this study (Table 2). Death were comparatively higher in out born (3.40%) then in born (2.27%) neonates. Most of the neonates were delivered by LUCS and motility was also higher in this group (4.5%).

Table III : Sensitivity pattern of common micro-organism

Drug	CoNS (n=37)	Staphyl c. aureous (n=5)	Acinetobacter (n=24)	Pseudomonus (n= 8)	Klebsiella (n=5)	Enterobacter (n=3)
Ampicillin	5(13.5%)	0	0	0	0	0
Gentamicin	28(75.67%)	5(100%)	6(25%)	3(37.50%)	0	0
Amikacin	NT	NT	14(58.33%)	6(75.0%)	3(60%)	0
Oxacillin	15(40.54%)	2(40%)	NT	NT	NT	NT
Vancomycin	37(100%)	5(100%)	NT	NT	NT	NT
Ciprofloxacin	16(43.24%)	2(40%)	13(54.16%)	4(50%)	2(40%)	3(100%)
Levofloxacin	24(64.86%)	3(60%)	15(62.5%)	5(62.5%)	3(60%)	3(100%)
Imepenem	19(51.35%)	5(100%)	15(62.5%)	6(75 %)	4(80%)	3(100%)
Cefotaxime-	5(13.5%)	2(40%)	0	0	0	0
Colistin	NT	NT	23(95.83%)	8(100)	5(100%)	3(100%)
Piperacillin	NT	NT	10(41.66%)	6(75.0%)	3(60%)	3(100%)

Based on the results from susceptibility testing, all of the isolated gram positive bacteria poorly sensitive to Ampicillin(13.5%) and Cefotaxime (13.5%) and highly sensitive to Vancomycin (100%) and Gentamicin (75.67%). All of the gram negative isolates were resistant to Ampicillin and Cefotaxim. Acinatobacter had low sensitivities to Gentamicin (25%). Sensitivity to Amikacin (58.33%), Imepenem and Levofloxacin (62.5%), and Colistin (95.65%). Pseudomonas had sensitivity 75% to Amikacin, Imepenem and Piperacillin. Colistin had highly sensitive to Pseudomonus, Klebsiella pneumoniae, Enterobacter.

Discussion

Neonatal Sepsis is the commonest cause of neonatal morbidity and mortality. Low birth weight is an important risk factor contributing to sepsis. Among 88 babies who developed neonatal sepsis during the study period 69.31 % (61 cases) neonates with low birth weight, this is

similar with other studies where LBW was found to be important risk factor for sepsis.^{13,14} LBW babies are mostly premature are predispose to sepsis due to multiple reasons like immune incompetence at various level of defense, and needed to invasive intervention etc. Considering sex preponderance there were more cases of sepsis in male neonates in comparison to female (51 male and 37 female with 1.3:1 ratio). Other study also shows male female ratio nearly 2:1², this could be gender biasness in presentation to the hospital for care. Population based studies would be needed to address this important question.

In this study documented neonatal sepsis with positive culture was 13%. This is low compared to about 20% yield reported by Baltimore¹⁵ and Gladstone¹⁶. Earlier, culture positive rate of 26% by Ahmed² and much higher rates of 51% by Karthikeyan¹⁷ and 64% by Tallur¹⁸ was described. In the present investigation, 77.27 % and 22.64 % neonates presented with EONS and LONS respectively. We found that early onset sepsis (EONS) was more common than late onset sepsis (LONS), which is in agreement with the reports from other developing countries e.g. in Iran¹¹ (77.5% vs.22.5%) and in a study of Bangladesh¹⁹ (70.7 vs. 29.3%), but in contrast with reports from Saudi Arabia (39% vs. 61%)²⁰ and Pakistan (42% vs.58%)²¹, where late onset sepsis is more common. The possible explanation for a higher frequency of EONS 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 were 51.13% and 48.86%, respectively. This finding is similar to that of other studies where gram positive bacteria were the commonest cause of neonatal sepsis^{22,23} This was in contrast to other studies which showed that gram negative bacteria were responsible in most cases of neonatal sepsis^{11,21}. In the present study in case of EONS, gram positive and gram negative bacteria accounted for 60.29% and 39.71% respectively.

Gram-positive organisms constituted the major group of isolates (51.13%) from neonatal septicemia cases. In general, Coagulase Negative Staphylococci was the most common isolates (42.04%) causing neonatal sepsis in this study and it was the commonest cause of early onset neonatal sepsis (48.52%) and the rest included staphylococcus aureus (5.68%) and Enterococcus (3.40%). The predominance of gram positives correlated the findings of other workers.^{24,25} Other authors^{9,24} have reported increasing trend of CONS in neonatal sepsis. Studies from different countries report CONS as the predominant organisms in LONS²⁶. 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 septicemia²². Pro-inflammatory responses to *S. epidermidis* are dependent on gestational age in preterm infants²⁷, thus the predominance of neonatal sepsis in preterm newborns in our study may be one reason to this finding. The variance of bacteriologic growth is not clear but it may be the population difference with regard to the maternal colonization rate, nosocomial acquisition of bacteria by the mother and the neonate, genetics of the immune response in selected population groups⁸.

Acinetobacter (18%) and *Pseudomonas*(7.95%) spp. were the most common gram negative organisms in early onset neonatal sepsis. In late onset sepsis, *Acinetobacter* (9.08%) was the predominant organism. In general, *Acinetobacter* was the 2nd most common isolates (27.27%) causing neonatal sepsis in this study. *Pseudomonas* was the

3rd most common organism isolated in this study. The pattern of isolated organisms in our study slightly differs from the findings in Iran¹¹ where *Pseudomonas Aeruginosa* was the most common cause of neonatal sepsis followed by *Klebsiella* spp. and *E. coli*. In a similar study from Bangladesh, Nepal and Pakistan, *E. coli* was the leading cause of neonatal sepsis followed by *Klebsiella* spp^{2,21}.

Coagulase negative staphylococcus (CONS), *S. Aureus* and other gram positive organisms in our study were better susceptible to Gentamicin (75.67%), Vancomycin (100%), Imipenem (51.35%). Described in the literature *Staphylococcus* was resistant to Ampicillin²⁸ in 79.3% of the cases and low sensitivity to all commonly used antibiotics¹⁷. *Acinetobacter* was best susceptible to Imipenem (62.5%), Amikacin (58.33%), Ciprofloxacin (54.18%), Levofloxacin (62.5%), Colistin (95.83%) and less susceptible to Gentamicin (25%) and completely resistant to Ampicillin¹⁷, Cefotaxime. Higher susceptibility to Amikacin was reported by Ramesh²⁹⁽³⁴⁾. *Pseudomonas* species were susceptible to Amikacin (75%), Gentamicin (37.50%), Imepenem (75%), Levofloxacin (62.5%), Piperacillin (75.%), Colistin (100%) and completely resistant to Ampicillin and cefotaxime. Low sensitivity to ampicillin is similar to many earlier study.^{23,18} Lower susceptibility to Amikacin (45%) was found by Agarwal.³⁰

The antimicrobial sensitivity pattern differs in different studies as well as at different times in the same hospital³¹. This may be due to emergence of resistant strains as a result of indiscriminate use of antibiotics. The high resistance rates found in this study may be associated with the frequent use of antimicrobial drugs for both prophylactic and therapeutic treatment of hospitalized newborns³². In view of the above facts the strategy of antibiotic usage in neonates should be reviewed periodically even in the same hospital

Conclusion

CONS and *Acinetobacter* together with *Pseudomonas* are the leading causative agents of neonatal sepsis in our unit. They were resistant to commonly used antibiotics, thus due to antibiotic sensitivity results, appropriate initial empirical antibiotic therapy for neonatal sepsis should be replaced. Every unit should carefully follow the bacterial spectrum and resistance patterns of microorganism responsible for neonatal infections to design a specific empirical antibiotic regimen for unit.

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