

Original Research Article

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Study of Microorganisms Causing Neonatal Sepsis in a Tertiary Care Hospital and their Antimicrobial Susceptibility Pattern

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ABSTRACT

Neonatal sepsis is a clinical syndrome characterized by signs and symptoms of infection with or without accompanying bacteremia in the first month of life. It encompasses various systemic infections of the newborn such as septicemia, meningitis, pneumonia, arthritis, osteomyelitis and urinary tract infections. A total of 50 suspected cases of Neonatal sepsis in a Tertiary care hospital were studied for 2 months (August 2011-September 2011). Blood samples obtained from cases of neonatal sepsis were cultured to analyse for bacteriological profile and antibiotic sensitivity pattern. Gram negative bacterial isolates obtained were analysed for extended spectrum beta lactamase (ESBL) production and *Staphylococcus aureus* and Coagulase negative staphylococci (CONS) isolates obtained were analysed for methicillin resistance by phenotypic method. Out of the 50 blood samples collected blood culture was positive in 31(62%) and 31 bacterial isolates were obtained. Out of the 31 bacterial isolates 26(83.88%) were gram negative bacteria and 5(16.12%) were gram positive bacteria. Twelve (46.15%) of the 26 gram negative bacteria were ESBL producers. All the ESBL producers (100%) were sensitive to imipenem. One (50%) of 2 *Staphylococcus aureus* and 2(66.67%) of the 3 CONS were methicillin resistant. All the methicillin resistant *Staphylococcus aureus* and CONS were sensitive to vancomycin. This study emphasizes the need for routine bacterial surveillance and study of their resistance pattern in neonatal care for implementation of a rational empirical treatment strategy.

Keywords

Neonatal sepsis,
Blood culture,
MRSA, ESBL,
Phenotypic
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Introduction

Neonatal septicemia continues to be one of the commonest causes of neonatal mortality and morbidity despite advances in healthcare. Neonatal sepsis may be categorized as early onset sepsis (EOS) or late onset (LOS) according to time of onset of the disease. Presence of risk factors such as low birth weight or preterm baby, febrile illness in the mother 2 weeks prior to delivery, foul smelling or meconium stained liquor,

prolonged rupture of membrane, prolonged labor /difficult labor, perinatal asphyxia have been associated with increased risk of early onset sepsis (Reeta Rasaily January-March 2009). Organism that colonize in the mother's genitourinary tract may cause transplacental infection or an ascending infection from the cervix. The infant may also acquire microbe by passage through a colonized birth canal at delivery. The infant's skin, respiratory tract,

conjunctiva, gastrointestinal tract and umbilicus may become colonized from environment leading to the possibility of late onset sepsis from invasive microorganism (Reeta Rasaily, January-March 2009).

The reported incidence of neonatal sepsis varies from 7.1 to 38 per 1000 live births in Asia (Shrestha *et al.*, 2011). It is estimated that up to 20% of neonates develop sepsis and approximately 1% die of serious systemic infections. In developing countries, sepsis including meningitis, respiratory infections, diarrhea, and neonatal tetanus is the commonest cause of mortality responsible for 30-50 per cent of 5 million total neonatal deaths each year. The present study was done to identify the bacteria causing Neonatal sepsis and their antimicrobial susceptibility and resistance pattern to institute early and effective antibiotic therapy.

Materials and Methods

Study design and duration of study: This cross sectional study was done over a period of two months.

Sample size: 50

Inclusion criteria: Neonates suspected to have sepsis, temperature 99°F, respiratory rate more than 60 /min, change in behaviour, abnormal cry, not accepting feed, drowsy or unconscious, septic focus on skin or umbilicus, diarrhea, seizures were included

Exclusion criteria: Clinically not suspected septic neonates, no characteristics indicating probable sepsis and prior antibiotic administration were excluded.

A total of 50 suspected cases of Neonatal sepsis were included in the study. One to two millilitre of blood was collected from each patient under strict aseptic precautions and inoculated immediately into 5 ml of brain

heart infusion broth aseptically with utmost care.

The broths were sub-cultured after overnight incubation at 37°C on chocolate agar, MacConkey agar and 5% sheep blood agar along with direct gram smear from the specimen. A negative result was followed up by examining the broth daily and doing a final subculture at the end of 7 days along with a gram smear or at appearance of turbidity, whichever was earlier. Any growth was identified based on colony morphology, Gram staining, motility and standard biochemical tests (Bailey and Scott's Diagnostic Microbiology 12th edition). The Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method in the Mueller Hinton agar medium (MHA).

For gram negative bacilli belonging to enterobacteriaceae the antimicrobial susceptibility testing included ampicillin 10µg, gentamicin 10µg, amikacin 30µg, ciprofloxacin 5µg, cefotaxime 30µg, ceftazidime 30µg and for non-fermenters it included gentamicin 10µg, amikacin 30µg, ciprofloxacin 5µg and ceftazidime 30µg discs. All the gram negative isolates that showed resistance to third generation cephalosporins were evaluated for ESBL production by using phenotypic confirmatory test.

Phenotypic confirmatory test

Antibiotic sensitivity testing was done on Mueller Hinton agar with 0.5 Mc Farland's standard of the test isolate. The drugs used were cefotaxime and ceftazidime each 30µg alone and in combination with clavulanic acid 10µg. Organisms with 5mm increase in zone of inhibition with third generation cephalosporin and clavulanic acid, were confirmed as ESBL's.

Quality control strains- non ESBL producing organism (*Escherichia coli* ATCC 25922) and

an ESBL producing organism (*Klebsiella pneumoniae* ATCC 700603) were used as controls.

The antimicrobial susceptibility testing for *Staphylococcus aureus* and Coagulase negative staphylococci included cefazolin 30µg, cefotaxime 30µg, gentamicin 10µg, amikacin 30µg, ciprofloxacin 5µg and ceftazidime 30µg discs.

Cefoxitin disc diffusion test

Cefoxitin disc diffusion testing is now recommended by CLSI as a preferred method of detection of oxacillin resistance in staphylococci. However it is important to report the findings from cefoxitin disc diffusion test as indicative of either oxacillin susceptibility/resistance; cefoxitin report should not be reported. All the isolates were subjected to cefoxitin disc diffusion test using a 30µg disc. A 0.5 Mc Farland standard suspension of the isolate was made and lawn culture done on MHA plate. Plates were incubated at 37°C for 18-24 hrs and zone diameters measured. An inhibition zone diameter of ≤ 21 mm for *Staphylococcus aureus* and ≤ 24 mm for CONS was reported as oxacillin resistant.

Quality control strains-Methicillin sensitive *S. aureus* (MSSA) ATCC 25923 and methicillin resistant *S. aureus* (MRSA) ATCC43300 were used as negative and positive controls respectively.

C Reactive Protein (CRP) estimated for the study cases were noted to correlate with culture positivity.

Results and Discussion

The total number of cases was 50 of which 31(62%) were males and 19(38%) were females. Of the total 50 cases, 29(58%) cases were in the age of 1-7 days, 14(28%) in the

age of 8-14 days and 7(14%) in the age of 15-28 days. In this study 32(64%) were term neonates and 18(36%) were preterm; 32(64%) neonates belonged to low birth weight category <2500 grams and 18(36%) neonates birth weight was >2500 grams. The presenting symptoms in most of the neonates were respiratory distress 34(68%), refusal of feeds 17(34%), lethargy 13(26%), fever 12(24%), abdominal distension 9(18%), jaundice 6(12%) and convulsion 4(8%). Blood culture yielded growth of bacteria in 31(62%) cases and it was negative in 19(38%) cases.

Organisms isolated by blood culture in 31 neonates with sepsis are given in Table 1. Gram negative bacteria (83.88%) were predominantly isolated. The antibiotic sensitivity pattern of enterobacteriaceae, non-fermenters and gram positive isolates are shown in Tables 2, 3 and 4, respectively. Table 5 shows ESBL production by gram negative bacteria. All ESBL producers were sensitive to imipenem.

Out of the 2 *Staphylococcus aureus* obtained 1(50%) was found to be MRSA and out of 3 CONS obtained 2(66.67%) was found to be MRCONS. All the MRSA and MRCONS were sensitive to vancomycin.

Correlation of CRP positivity with culture positivity is shown in Table 6. The p value for the same is 0.0003 which is statistically significant.

In our study 62% were males and 38% were females (male: female ratio- 1.6:1). DO Awoniyi *et al.*, (2009) also showed that prevalence of bacterial infection was higher in samples from male (65%) neonates. Jain *et al.*, (2003) have reported a male: female ratio of 2:1. Bhat *et al.*, (2011) reported a male to female ratio of 1.6:1 which is similar to our result.

The culture positivity rate in our study was 62%. Tallur *et al.*, (2000) reported a culture positivity rate of 64%. Roy *et al.*, (2002) reported a culture positivity rate of 47.5%. Similar report (62.8%) was reported by Rahman *et al.*, (2002). However, culture positivity was only 26.9% in a study by Mane *et al.*, (2010). This may be because of the larger sample size.

In our study neonatal sepsis was common in low birth neonates. Khatua *et al.*, (1986) reported a higher incidence of sepsis in low birth weight infants. Prematurity and low birth weight was identified as a significant neonatal risk factor by Shah *et al.*, (2006). Premature and low birth weight babies are relatively immune deficient, which predispose them to infections. Moreover, these babies are likely to be subjected to different interventional procedures leading to nosocomial infections.

An area based knowledge of the bacteriological spectrum is essential because the first antibiotic administered will not wait for the culture results and keeping in mind the high morbidity and mortality associated with neonatal sepsis, a right choice of such empiric therapy is of utmost importance. In our study gram negative organisms (83.8%) were predominantly isolated. In a study by (Bhat *et al.*, 2011) 90.2% of isolates were gram

negative bacteria. Predominance of gram negative isolates (67.2%-92.5%) has been reported by developing countries. Mane *et al.*, (2010) also reported that Gram negative bacteria (61.3%) are predominant organisms causing neonatal sepsis. In a study by (Rushda Aftab *et al.*, March 2009) gram negative organisms were isolated in 67% of the total isolates.

The pathogens most often implicated in neonatal sepsis in developing countries differ from those seen in developed countries. Group B *Streptococci* is extremely rare in developing countries but is frequently an important pathogen in developed countries. In our study Group B *Streptococci* was not isolated from any of the cases. The spectrum of bacteria causing neonatal sepsis in our study is comparable to the national neonatal perinatal database report (Jeeva Sankar *et al.*, 2008).

In our study *Klebsiella pneumoniae* (35.48%) was the most common isolate which is similar to the other Indian studies (Roy *et al.*, 2002; Mane *et al.*, 2010; Bhat *et al.*, 2011). We obtained 16.12% of *Pseudomonas aeruginosa*. Bhat *et al.*, (2011) reported *Pseudomonas* (33.2%) to be the most common pathogen causing neonatal sepsis. *Pseudomonas* isolation rate varies from 8.9%-38.3%.

Table.1 Organisms isolated by blood culture in neonates with sepsis (n=31)

Name of the organism isolated in blood culture	Number	Percentage
<i>Klebsiella pneumoniae</i>	11	35.48%
<i>Pseudomonas aeruginosa</i>	5	16.12%
Coagulase negative <i>Staphylococci</i>	3	9.68%
<i>Acinetobacter</i> species	3	9.68%
<i>Proteus mirabilis</i>	3	9.68%
<i>Proteus vulgaris</i>	1	3.23%
<i>Staphylococcus aureus</i>	2	6.45%
<i>Escherichia coli</i>	1	3.23%
<i>Klebsiella oxytoca</i>	1	3.23%
<i>Citrobacter freundii</i>	1	3.23%

Table.2 Antibiotic Sensitivity Pattern of Enterobacteriaceae Isolates (n=18)

Antibiotics	<i>Klebsiella pneumoniae</i> (n=11)		<i>Proteus mirabilis</i> (n=3)		<i>Proteus vulgaris</i> (n=1)		<i>Klebsiella oxytoca</i> (n=1)		<i>Escherichia coli</i> (n=1)		<i>Citrobacter freundii</i> (n=1)	
	S	R	S	R	S	R	S	R	S	R	S	R
Ampicillin	0	11 100%	0	3 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%
Gentamicin	0	11 100%	1 33.3%	2 66.7%	0	1 100%	0	1 100%	1 100%	0	0	1 100%
Amikacin	5 45.5%	6 54.5%	2	1 33.3%	0	1 100%	1 100%	0	0	1 100%	0	1 100%
Ciprofloxacin	5 45.5%	6 54.5%	1 33.3%	2 66.7%	1 100%	0	0	1 100%	1 100%	0	1 100%	0
Cefotaxime	4 36.3%	8 72.7%	3 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0
Ceftazidime	4 36.4%	8 72.7%	3 100%	0	1 100%	0	1 100%	0	1 100%	0	1 100%	0

Table.3 Antibiotic sensitivity of *Pseudomonas aeruginosa* And *Acinetobacter* Species

Antibiotics	<i>Pseudomonas aeruginosa</i> (n=5)		<i>Acinetobacter</i> species (n=3)	
	Sensitive	Resistant	Sensitive	Resistant
Ciprofloxacin	4(80%)	1(20%)	1(33.3%)	2(66.7%)
Amikacin	3(60%)	2(40%)	1(33.3%)	2(66.7%)
Gentamicin	0	5(100%)	0	3(100%)
Ceftazidime	2(40%)	3(60%)	1(33.3%)	2(66.7%)

Table.4 Antibiotic sensitivity of *Staphylococcus aureus* and Cons

Antibiotics	CONS (n=3)		STAPHYLOCOCCUS AUREUS (n=2)	
	Sensitive	Resistant	Sensitive	Resistant
Cefazolin	1(33.3%)	2(66.7%)	1(50%)	1(50%)
Cefotaxime	1(33.3%)	2(66.7%)	1(50%)	1(50%)
Cefoxitin	1(33.3%)	2(66.7%)	1(50%)	1(50%)
Gentamicin	0	3(100%)	0	2(100%)
Amikacin	3(100%)	0	1(50%)	1(50%)
Ciprofloxacin	2(66.7%)	1(33.3%)	2(100%)	0

Table.5 ESBL production by gram negative bacteria

Isolate	Total no.of isolates (26)	ESBL producers (12)
<i>Klebsiella pneumoniae</i>	11	8(72.72%)
<i>Pseudomonas aeruginosa</i>	5	2(40%)
<i>Acinetobacter</i> species	3	2(66.67%)
<i>Proteus mirabilis</i>	3	0
<i>Proteus vulgaris</i>	1	0
<i>Escherichia coli</i>	1	0
<i>Klebsiella oxytoca</i>	1	0
<i>Citrobacter freundii</i>	1	0

Table.6 Correlation of CRP positivity with culture positivity

CRP Test	Blood culture	
	Positive	Negative
Positive(26)	22	4
Negative(24)	9	15
Total	31	19

In our study, *Acinetobacter* spp accounted for 9.68% of the cases.14.3% of isolates were *Acinetobacter* in a study by Mane *et al.*, (2010). Similar results (10.4%) were reported by Agnihotri *et al.*, (2004).

9.6% Coagulase negative staphylococci (CONS) was obtained in this study. This bacterium is often regarded as a contaminant, possibly from the skin, but Leon *et al.*, (1984) opined that the presence of this bacterium in

the blood can no longer be taken as contamination especially in patients in critical care units. This view is also shared by Favre *et al.*, (2005) who concluded their study reporting that CONS bacteremia harbor a significant mortality and a single positive blood culture in the presence of signs of sepsis should be considered as clinically relevant.

We obtained 6.45% of *Staphylococcus aureus*. The rates of *Staphylococcus aureus* infection in a study by Bhat *et al.*, (2011) was 9.2%. Similar reports with rate of infection varying from 3.7%-7% have been found previously. However, Karthikeyan *et al.*, (2001) in their analysis identified *Staphylococcus aureus* as a predominant pathogen (61.5%).

Multidrug resistance of the causative organisms of sepsis is a rapidly emerging, potentially disastrous problem. The situation is worst in developing countries because of the lack of control of the use of antibiotics, the non-existence of legislation on antibiotic prescription, over the counter sale of antibiotics, poor sanitary conditions, lack of basic facilities and practices such as hand washing, lack of surveillance of the standards of maternity homes, and the practices of traditional birth attendants.

Our study shows a very high degree of resistance of Gram negative organisms to first line antibiotics. All *Klebsiella* isolates were resistant to ampicillin and gentamicin. This suggests that the WHO recommended ampicillin and gentamicin combination for treatment of neonatal sepsis may no longer be effective in treating many newborns with sepsis.

Klebsiella pneumoniae isolates were maximally sensitive to imipenem (100%), ciprofloxacin (54.54%) and amikacin

(54.54%). High degree of resistance was seen to cefotaxime and ceftazidime.

In our study *Pseudomonas aeruginosa* was resistant to gentamicin and ceftazidime. They were sensitive to ciprofloxacin (80%) and amikacin (60%).

CONS and *Staphylococcus aureus* isolates in our study were sensitive to ciprofloxacin and amikacin. They were resistant to gentamicin, ceftazidime and cefotaxime.

Increasing resistance to third generation cephalosporins among *Klebsiella* and *Escherichia coli* is also notable. *Klebsiella* and *Escherichia coli* resistance is usually acquired via plasmid-mediated extended spectrum beta-lactamase (ESBL) production.

Owing to the presence of other resistance-conferring genes on these transferable plasmids, such organisms are also often resistant to other drugs, including aminoglycoside antibiotics.

Risk factors for acquiring ESBL organisms include heavy antibiotic use, including use of third generation cephalosporins.

In our study ESBL production was shown by 72.72% of *K. pneumoniae* isolates, followed by 66.67% of *Acinetobacter* isolates and 40% of *Pseudomonas aeruginosa* isolates.

Various authors have given different observations of ESBL production in their studies on neonatal septicemia. Kim *et al.*, (2002) reported ESBL production by 52.9% of *K. pneumoniae* and 17.9% of *Escherichia coli* isolates.

Jain *et al.*, (2003) have reported a high ESBL production by *Klebsiella* spp (87.2%), *Enterobacter* spp (72.5%), *Escherichia coli* (65.3%) and *Acinetobacter* (33.3%).

The high percentage of ESBL producing *Klebsiella* species may be due to the selective pressure imposed by extensive use of antimicrobials in intensive care unit.

According to the latest report from national nosocomial infection surveillance system approximately 60% of all *Staphylococcus aureus* nosocomial infections in intensive care units were methicillin resistant. Karthikeyan *et al.*, (2001) reported that 66% of *Staphylococcus aureus* isolated from cases of neonatal sepsis was methicillin resistant.

The degree of resistance or sensitivity of *Staphylococcus aureus* towards commonly used antibiotics is diverse from region to region, so it is inevitable to look for MRSA in every *Staphylococcus aureus* isolated.

In our study 50% of *Staphylococcus aureus* and 66.67% of CONS were methicillin resistant. MRSA detection was done by cefoxitin disc diffusion method.

In this study CRP was elevated in 26 cases of which 22 were confirmed bacteriologically.

Sabel *et al.*, (1974) in their study of 14 cases of neonatal sepsis observed increased CRP in 85.7% of cases with positive blood cultures. In a study by Lakshmi *et al.*, (1992) CRP was found to be raised in 89.47% cases of neonatal sepsis whereas blood culture was positive only in 63.15% cases. They have concluded that CRP positivity highly correlates with infection positivity.

Neonatal sepsis is a leading cause of mortality and morbidity in neonates in our country.

Bacterial spectrum causing neonatal sepsis is different in developed and developing countries Routine bacterial surveillance and study of their resistance patterns must be an essential component of neonatal care.

Periodic evaluations not only show the trend of increasing resistance to commonly used antibiotics but also help in implementation of a rational empirical treatment strategy. Present study indicated that Gram-negative bacteria continue to be the predominant causative organisms of neonatal sepsis. Gram negative bacteria showed high resistance to first line drugs like gentamicin, ampicillin and to drugs like cefotaxime. Most of them had good sensitivity to ciprofloxacin and amikacin. Imipenem is best for multidrug resistant gram negative bacilli. Increased incidence of MRSA has been found.

CRP estimation can help in the early diagnosis of neonatal sepsis.

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