RESEARCH ARTICLE

INCIDENCE OF NEONATAL SEPSIS IN TERTIARY CARE HOSPITAL: AN OVERVIEW

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DOI: 10.5455/ijmsph.2013.090320131 **Received Date: 08.01.2013** **Accepted Date: 09.03.2013**

ABSTRACT

Background: Neonatal sepsis is one of the commonest causes of neonatal mortality in the developing world.
Aims & Objective: To determine the bacteriological profile of neonatal septicaemia, their antibacterial susceptibility pattern (AST) and production of extended spectrun β-lactamase (ESBL) by gram negative bacteria.
Material and Methods: Blood culture specimens were collected from 238 neonates. Identification of organisms, their AST, methicillin resistant *Staphylococcus aureus* (MRSA) and ESBL detection was done.
Results: Gram negative bacteria were more frequently isolated than gram positive bacteria. The gram positive bacteria were highly resistant to penicillin. Vancomycin and amoxyclav exhibited good activity against both *Staphylococcus aureus* and coagulase negative *Staphylococcus*. Gram negative bacteria also exhibited high resistance to the commonly prescribed group of drugs such as penicillins, cephalosporins and aminoglycosides. Out of the total 16 isolates of *S. aureus*, 31.25% were MRSA. ESBL production was seen in 52.9% of Escherichia coli and 50% of *Klebsiella pneumoniae*.
Conclusion: *E. coli* and *S. aureus* were the most common bacteria associated with neonatal sepsis. Gram negative bacteria were isolated predominantly and many of them were resistant to several groups of drugs. Also high resistance was seen to third generation cephalosporins in case of E. coli and *K. pneumoniae* due to ESBL production.

KEY-WORDS: Antibiotic Susceptibility Pattern; Extended Spectrum β-Lactamases (ESBL); Methicillin Resistant Staphylococcus Aureus (MRSA); Neonatal Sepsis; Premature Rupture of Membrane (PROM)

Introduction

Neonatal sepsis is an important cause of morbidity and mortality among neonates. It is responsible for 30- 50% of the total neonatal deaths in developing countries.[1,2] It is estimated that up to 20% of the neonates develop sepsis and approximately 1% die of sepsis related causes.[2] The incidence of neonatal sepsis according to the data from National Neonatal Perinatal Database (NNPD, 2002-03) is 30 per 1000 live births. The database comprising 18 tertiary care neonatal units across India found sepsis to be one of the commonest causes of neonatal mortality contributing to 19% of all neonatal deaths.[3] Early onset (within first week of life) neonatal sepsis is generally acquired from pathogens of maternal genital tract, whereas late onset sepsis (after first week till 28 days of life) has its origin either from the community or from hospital.[4]

Multiple factors have been associated with increased risk of infections in neonatal life. Various maternal, foetal and environmental factors contribute towards sepsis in neonates. One of the maternal factors is premature rupture of membrane (PROM) which is defined as spontaneous rupture of membranes at any time after 37 completed weeks of pregnancy but before onset of labour.[5] PROM is considered as a major risk factor for sepsis due to danger of ascending infection. Other maternal risk factors are infection and fever of mother during labour, foul smell of amniotic fluid, meconeal amniotic fluid, multiple gestations and caesarean section. Neonatal risk factors which have been shown to contribute to neonatal sepsis are prematurity, low birth weight, asphyxia, congenital anomaly and long stay in neonatal intensive care unit (NICU).[6]

The gold standard for diagnosis of septicaemia is the isolation of bacterial agents from the blood culture.[7] Both gram negative and gram positive bacteria have been isolated from blood and

predominance of one type over the other varies from place to place and even in the same place over time.[8] In most of the developing countries gram negative bacilli remain the major cause of neonatal septicaemia. Bacteria commonly isolated in the sample included *Klebsiella pneumoniae, Escherichia coli, Enterobacter spp, Pseudomonas aeruginosa, Staphylococcus aureus, Streptococcus spp, Citrobacter spp,* and coagulase negative *Staphylococcus (CONS).*[9,10]

The susceptibility of the isolates to different antibiotics varies and also there is an increasing concern of isolation of highly antibiotic resistant organisms.[11] β-lactam antibiotics are commonly used among the neonatal septicaemia cases. The emergence of resistance to these agents in the past decades has resulted in major clinical crisis.[12] Resistance to β-lactam antimicrobial agents especially to cephalosporins due to production of extended spectrum β-lactamases (ESBL) by gram negative bacteria is on rise worldwide.[13,14] Thus a rational protocol for sepsis management must be based on adequate knowledge of the causative organisms and their antibiotic susceptibility patterns. Hence, this study was undertaken to determine the bacteriological profile of neonatal septicaemia, their antibacterial susceptibility pattern and production of ESBL by gram negative bacteria. We also correlated the incidence of neonatal sepsis with two important maternal risk factors i.e. PROM and mode of delivery.

Materials and Methods

A prospective study was conducted in the department of Microbiology, MG Medical College, Indore, India from February 2007 to August 2007. All neonates of either sex admitted in NICU with suspected clinical sepsis were included in the study. Two hundred thirty eight neonates were studied during the period. Fully informed and voluntary consents were obtained from the parents or attendants. Detailed history and complete physical examinations of each neonate was carried out and recorded along with any history of PROM and mode of delivery. Blood sample was collected for culture with proper aseptic precautions before initiating antibiotic therapy, whenever possible. Approximately 1-3 ml of blood was collected in sterile bottle containing 1% glucose broth and incubated at 37°C. Blind subculture were made on blood agar, and Mac Conkey agar after 24 hours, 48 hours, 72 hours and 7 days, which were further incubated at 37°C for 18–24 hours. The plates were observed the following day but extended to 48 hours if there was no bacterial growth within 24 hours. If no growth was observed on plates after 7th day, the sample was reported as negative. Isolated colonies were subjected to gram staining and biochemical tests for identification. Identification was carried out according to the standard biochemical tests.[15] Second blood culture was also performed in few cases which were not showing improvement after initial treatment.

Antimicrobial susceptibility test was carried out on isolated and identified colonies using commercially prepared antibiotic disk (HiMedia) on Mueller Hinton agar plates by the disk diffusion method, according to the Central Laboratory Standards Institute (CLSI) guidelines.[16] Methicillin resistant *Staphylococcus aureus* (MRSA) detection was done using oxacillin disc (1 µg) and Mueller Hinton agar with 2% NaCl. The plates were incubated for 24 hours at 35˚C and zone diameter was measured. If zone diameter was ≥13mm, it was considered as Methicillin sensitive *Staphylococcus aureus* (MSSA) and ≤10 mm then it was considered as MRSA.[16] The ESBL detection in case of E. coli and *K. pneumoniae* isolates was done using phenotypic confirmatory method as per CLSI guidelines.[16] An isolate was considered as ESBL producer when zone diameter of ceftazidime/clavulanic acid disc (30/10 µg) was ≥5 mm than the diameter of ceftazidime (30 µg) alone.

Statistical Analysis

The statistical analysis was performed using standard tests. Fisher's exact test was applied when two or more set of variables were compared. P value less than 0.05 was considered statistically significant.

Results

During the study period of six months, 238 blood samples of clinically suspected cases of neonatal

septicaemia were processed. Out of these 238 samples, blood culture was positive in 76 cases (32%). Blood culture showed no growth in 154 cases (65%) and 8 samples (3%) showed growth of skin contaminants. Among the 76 cases of neonatal septicaemia, EOS was found in 59% cases whereas the rest 41% cases were of LOS. The total number of pathogenic isolates was 82, out of which 2 isolates were of *Candida albicans* and rest 80 were bacterial isolates. Polymicrobial infection was seen in 6 cases. Gram negative bacteria (58%) were more frequently isolated than the gram positive bacteria with *E. coli* accounting for a maximum of 21.25 % of all bacterial isolates. *S. aureus* was the second most common isolated pathogen. Other common isolates were *K. pneumoniae, CONS, β-haemolytic Streptococci, Acinetobacter spp, P. aeruginosa* and *Citrobacter spp.* Their distribution in association with EOS and LOS is shown in table.1. *E. coli, S. aureus*, and *β-haemolytic Streptococci* were frequently isolated from EOS cases while *CONS* and *P. aeruginosa* were isolated pre dominantly from LOS. When correlating the incidence of neonatal sepsis with PROM, it was observed that out of 26 cases of PROM, 77% neonates developed sepsis while only 26.4% neonates developed sepsis in the absence of PROM (p value 0.002, statistically significant). The most frequently isolated organism in PROM cases were *E. coli, β-hemolytic Streptococci* and *K. pneumoniae*. When considering the mode of delivery, out of the total 238 cases 167 neonates were delivered by normal vaginal delivery (NVD) while 71 by lower segment caesarean section (LSCS). Blood culture positivity was seen in 27.54% (46/167) NVD cases as compared to 42.25% (30/71) cases of LSCS (p value 0.123, not statistically significant). The distribution of organisms in relation to mode of delivery is shown in table 2. The susceptibility pattern to various antimicrobial agents was different for different organisms and is shown in table 3. Out of the total 16 isolates of S. aureus 31.25% were MRSA. ESBL production was seen in 52.9% of *E. coli* and 50% of *K. pneumoniae*.

Table-1: Distribution of Various Bacteria causing Early and Late Onset Neonatal Sepsis

Organism	EOS	LOS
Escherichia coli	12	5
Staphylococcus aureus	11	5
Klebsiella pneumoniae	10	6
Coagulase negative Staphylococcus aureus (CONS)	2	7
β-Hemolytic Streptococci	6	2
Pseudomonas aeruginosa	2	4
Acinetobacter spp	2	3
Citrobacter spp.	1	2
Candida albicans	0	2
Total	46	36

Table-2: Distribution of Various Bacteria in Relation with Mode of Delivery

Organism	NVD	LSCS
Escherichia coli	11	6
Staphylococcus aureus	9	7
Klebsiella pneumoniae	10	6
Coagulase negative Staphylococcus aureus (CONS)	2	7
β-Hemolytic Streptococci	7	1
Pseudomonas aeruginosa	2	4
Acinetobacter spp	2	3
Citrobacter spp.	3	0
Candida albicans	2	0
Total	48	34

Table-3: Antibiotic Susceptibility Pattern of the Organism Isolated from Neonatal Sepsis

Antibiotics	Causative Bacteria (%)							
	Escherichia coli (17)	*Klebsiella spp* (16)	*Pseudomonas aeruginosa* (6)	*Acinetobacter spp* (5)	*Citrobacter spp.* (3)	*Staphyloccus aureus* (16)	*CONS** (9)	*β-Haemolytic Streptococci* (8)
Ampicillin	2 (11.76)	3 (18.75)	0	1 (20)	1 (33.33)	2 (12.5)	4 (44.44)	7 (87.5)
Amikacin	5 (29.41)	6 (37.5)	3 (50)	3 (60)	2 (66.66)	-	-	-
Cotrimaxazole	6 (35.29)	6 (37.5)	2 (33.33)	2 (40)	2 (66.66)	-	-	-
Cefotaxime	6 (35.29)	6 (37.5)	3 (50)	2 (40)	3 (100)	-	-	-
Ceftrioxone	6 (35.29)	6 (37.5)	3 (50)	2 (40)	2 (66.66)	-	-	-
Ciprofloxacin	10 (58.82)	10 (62.5)	4 (66.66)	3 (60)	2 (66.66)	-	-	-
Gentamicin	7 (41.170	6 (37.5)	3 (50)	2 (40)	2 (66.66)	-	-	-
Netilmicin	4 (23.52)	7 (43.75)	4 (66.66)	3 (60)	2 (66.66)	-	-	-
Meropenem	15 (88.23)	14 (87.5)	5 (83.33)	4 (80)	3 (100)	-	-	-
Erythromycin	-	-	-	-	-	14 (87.5)	7 (77.77)	8 (100)
Amoxyclav	-	-	-	-	-	15 (93.75)	8 (88.88)	8 (100)
Vancomycin	-	-	-	-	-	16 (100)	9 (100)	8 (100)
Cephalexin	-	-	-	-	-	14 (87.5)	9 (100)	8 (100)
Penicillin	-	-	-	-	-	2 (12.5)	4 (44.44)	7 (87.5)

** Coagulase negative Staphylococcus aureus*

Discussion

The incidence and microbiology of neonatal sepsis varies worldwide. Blood culture has been regarded as the gold standard for the confirmation of sepsis. Reports from all over world show the isolation rates on blood culture to vary from 6.7% to 55.4%.[3,17] In the present study, blood culture positivity for infecting pathogens was 32%. Early onset sepsis was seen in 59% cases while remaining 41% cases were of LOS. These findings are similar to a study done by Chugh et al in which they have reported EOS cases to be more in number than LOS.[18] They reported *K. pneumoniae* as the commonest isolate in both EOS and LOS while in the present study *E. coli, S. aureus* and *β-haemolytic Streptococci* were the common isolates in *EOS* and *CONS*, *K. pneumoniae* and *P. aeruginosa* in case of LOS. Mane et al also reported S. aureus and CONS as common causes of EOS and LOS respectively.[19]

Overall the isolation of gram negative bacteria was higher than gram positive bacteria. These results were consistent with the findings of many previous studies which also reported gram negative bacteria to be more common in neonatal sepsis.[9,10] Most of the studies carried out in developing countries have shown *K. pneumoniae* as the most implicated gram negative bacteria for neonatal sepsis.[10,20] However in the present study, *K. pneumoniae* (accounting for 20% of the cases) was closely superseded by *E. coli* which was responsible for 21.25% cases. *E. coli* was also found to predominant in other study done by Mondal et al.[20] Among the gram positive bacteria, *S. aureus* was the most common to be associated with neonatal septicaemia which is similar to studies conducted elsewhere.[19,21]

The two risk factors of neonatal sepsis evaluated were PROM and caesarean section. In the present study, 77% neonates of mothers who had PROM developed sepsis and this association was found to be statistically significant. Organisms which were associated with PROM were *E. coli, β-haemolytic Streptococci* and *K. Pneumonia*. Prolonged leaking and premature rapture of membrane is considered as a major risk factor for sepsis because of danger of ascending infection from perineum and vagina. Similar findings were also reported by other authors.[22] Regarding mode of delivery, it was observed that the incidence of sepsis was more in cases of babies born from caesarean section as compared to vaginal delivery. This finding is similar with a previous study which showed that baby born from caesarean section have a 1.89 times higher risk than non caesarean to develop sepsis.[23]

The antibiotic susceptibility patterns of all the isolated organisms were also studied. The gram positive bacteria were highly resistant to penicillin except for *β-hemolytic Streptococci* which exhibited nominal resistance to the various drugs tested. Vancomycin and amoxyclav exhibited good activity against both *S. aureus* and *CONS*. About 31.25% of *S. aureus* were found to be *MRSA* which is similar to a study conducted by Kayange et al.[22] In case of gram negative bacteria most common isolated organism were *E. coli* and *K. pneumoniae*. Both these organisms were more frequently isolated in early onset cases and were highly resistant to penicillin (88.24%) and cephalosporin (64.71%). The high resistance of these organisms to third generation cephalosporins can be attributed to the frequent production of ESBL by these organisms. Other gram negative bacteria such as *P. aeruginosa, Acinetobacter spp,* and *Citrobacter spp* also exhibited high resistance to the commonly prescribed group of drugs such as penicillins, cephalosporins and aminoglycosides. Shaw et al also observed in their study that 68% of *K. pneumoniae* and *E. coli* isolated from neonatal sepsis cases were resistant to gentamicin and 90% resistant to ampicillin which is similar to the present study.[21]

ESBL production was tested in all isolates of *E. coli* and *K. pneumoniae* which were resistant to third generation cephalosporins. Out of 17 isolates of *E. coli* 52.9% were ESBL producer while 50% of total *K. pneumoniae* isolates were positive for ESBL production. Different rates of ESBL production have been reported by various authors Ananthkrishnan et al reported an incidence of 58.06% for ESBL producing *E. coli*.[24] In a study conducted in Nagpur, India ESBL production was shown by 58.3% of *K. pneumoniae* and 50% of E. coli which is quite similar to the present study.[19] Most of the gram negative bacteria were sensitive

to carbapenem group. Similar findings have been reported by Bloomberg et al.[25] However because of worldwide rising resistance, carbapenems should be kept as reserve drugs for multidrug resistant isolates especially ESBL producers.

Conclusion

In conclusion, *E.coli* and *S. aureus* were the most common gram negative and gram positive bacteria causing neonatal sepsis in our NICU. Gram negative bacteria were isolated predominantly and many of them were resistant to several groups of drugs. Also high resistance was seen to third generation cephalosporins in case of *E. coli* and *K. Pneumoniae* due to ESBL production which poses a significant challenge in the use of these drugs as the first line therapy in management of neonatal sepsis.

Development of sepsis in a neonate is a medical emergency and generally the clinicians do not wait for microbiology report and start treatment empirically. Local microbiological databases should be prepared including information regarding the commonly isolated organisms and their drug resistance patterns. These databases should be monitored and reviewed regularly to provide updated information to guide clinicians in forming an effective empirical therapy for management of neonatal sepsis.

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Cite this article as: Gandhi S, Ranjan KP, Ranjan N, Sapre N, Masani M. Incidence of neonatal sepsis in tertiary care hospital: an overview. Int J Med Sci Public Health 2013; 2:548-552.
Source of Support: Nil
Conflict of interest: None declared