



## Original Research Article

# A Study of Neonatal Septicaemia at a Tertiary Care Institute

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## ABSTRACT

### Keywords

Antibiotic Susceptibility Pattern; Bacteriological Profile, Neonatal Sepsis; Resistance, Extended Spectrum  $\beta$ -Lactamases (ESBL)

Neonatal sepsis is one of the commonest causes of neonatal mortality in the developing world. To determine the bacteriological profile of neonatal septicaemia, their antibacterial susceptibility pattern (AST) Blood culture specimens were collected from 100 neonates. Identification of organisms, their AST was done. Gram negative bacteria were more frequently isolated than gram positive bacteria. The gram positive bacteria were highly resistant to penicillin. Gram negative bacteria also exhibited high resistance to the commonly prescribed group of drugs such as penicillins, cephalosporins and aminoglycosides. ESBL production was seen in 52.9% of *Klebsiella* and 50% of *E.coli*, *Klebsiella.pneumoniae* was 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 *Klebsiella pneumoniae* and *E.coli* due to ESBL production.

## 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, meconium 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)  $\beta$ -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  $\beta$ -lactam antimicrobial agents especially to cephalosporins due to production of extended spectrum  $\beta$ -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.

## **Materials and Methods**

A prospective study was conducted in the department of Paediatric & Microbiology. All neonates of either sex admitted in NICU with altered body temperature, tachypnoea/apnoea, lack of activity, poor feeding, abdominal distension, jaundice, irritability and convulsion etc were clinically diagnosed to have septicaemia were included in the study. A total of 100 neonates were studied during the period of six months. 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. 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 (Hi-

media) on Mueller Hinton agar plates by the disk diffusion method, according to the Central Laboratory Standards Institute (CLSI-2015) guidelines.(16) Methicillin resistant *Staphylococcus aureus* (MRSA) detection was done using cefoxitin disc (30 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  $\geq 22$ mm, it was considered as Methicillin sensitive *Staphylococcus aureus* (MSSA) and  $\leq 21$  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  $\geq 5$  mm than the diameter of ceftazidime (30 µg)

## Results and Discussion

During the study period of six year, 100 blood samples of clinically suspected cases of neonatal septicaemia were processed. Out of these 100 samples, blood culture was positive in 56 cases. Blood culture showed no growth in 44 cases. Among the 56 cases of neonatal septicaemia, Early onset sepsis(EOS) was found in 34 (60%) cases whereas the rest 22 (40%) cases were of Late onset sepsis (LOS). The total number of pathogenic isolates was 56, out of which 2 isolates were of *Candida albicans* and rest 54 were bacterial isolates. Gram negative bacteria 31(58%) were more frequently isolated than the gram positive bacteria with *Klebsiella pneumoniae* accounting for a maximum of 26 % of all bacterial isolates (14 cases). *Staphylococcus aureus* was the second most common isolated pathogen isolated in 12 cases( 22%) . Other common isolates were. *E.coli* (5 cases), *CONS*(8cases),  *$\beta$ -haemolytic Streptococci*(3

cases), *Acinetobacter spp*(4 cases), *P. aeruginosa* (6 cases) and *Citrobacter spp.* (2 cases). *E. coli*, *Staphylococcus aureus* were isolated from EOS cases while *CONS* and *P. aeruginosa* were isolated pre dominantly from LOS. Out of the total 12 isolates of *Staphylococcus aureus* 33.3% were MRSA(Methicillin resistant *Staphylococcus aureus*). ESBL production was seen in 57.1% of *Klebsiella pneumoniae* and 60% of *E.coli*

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 31.2.%. 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 *Klebsiella pneumoniae* as the commonest isolate in both EOS and LOS while in present study *Klebsiella*, *Staphylococcus aureus* and  *$\beta$ -haemolytic Streptococci* were the common isolates in EOS and CONS, *E.coli* and *P. aeruginosa* in case of LOS.

Mane et al also reported *Staphylococcus 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 *Klebsiella pneumoniae* as the most implicated gram negative bacteria for neonatal sepsis.(10,20)

**Table.1** Blood Culture → Organism Isolate

Sr no	Organism Isolated	Numbers of Isolate
1	<i>Klebsiella pneumoniae</i>	14
2	<i>E.coli</i>	5
3	<i>Acinetobacter</i>	4
4	<i>Pseudomonas</i>	6
5	<i>Citrobacter</i>	2

Drugs	<i>Klebsiella</i>	<i>E.coli</i>	<i>Acinetobacter</i>	<i>Pseudomonas</i>
Amikacin (AMK)	50%	48%	44%	35%
Ciprofloxacin(CIP)	66%	63%	55%	33%
Ceftriaxone (CTR)	10%	15%	10%	10%
Ceftazidime (CAZ)	15%	13%	8%	8%
Ceftazidime+Clavulanic Acid(CAC)	20%	14%	13%	8%
Meropenem(MERO)	76%	74%	60%	60%
Ampicillin(AMP)	10%	20%	15%	30%

However in the present study, *Klebsiella pneumoniae* (accounting for 31.25% of the cases) was closely followed by *E. coli* which was responsible for 11% cases. *E. coli* was found to predominant in other study done by Mondal et al.(20). The antibiotic susceptibility patterns of all the isolated organisms were also studied. In case of gram negative bacteria most common isolated organism were *Klebsiella pneumoniae* and *E. coli*. Both these organisms were more frequently isolated in early onset cases and were highly resistant to cephalosporin (64.71%).

The high resistance of these organisms to third generation cephalosporins can be attributed to the frequent 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 *Klebsiella pneumoniae* and *E. coli* which were resistant to third generation cephalosporins. Out of 17 isolates of *Klebsiella* 52.9% were ESBL producer while 50% of total *E.coli* 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, *Klebsiella* was the most common gram negative organism 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 *Klebsiella pneumoniae* and *E.coli* 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. 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.

In conclusion, *Klebsiella pneumoniae* was the most common gram negative 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 *K. pneumoniae* and *E.coli* 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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