

# Bacteriology and Antibiotic Resistance Pattern in Neonatal Septicemia

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## ABSTRACT

**Introduction:** Neonatal septicemia is a major cause of mortality and morbidity among neonates around the world, even after advancements in antibacterial therapy. However rationale antibiotic treatment depends upon the hospital antibiotic policy which in addition to neonatal life support measures and the early detection of risk factors may be useful in treating these patients with a better outcome. Hence the present study was conducted to look for spectrum of bacterial isolates in cases of neonatal septicemia, and their antimicrobial resistance pattern.

**Material and methods:** In this prospective study in central India, neonates suspected to be having septicemia were enrolled. Those who were on antimicrobial treatment were excluded. Detailed history and clinical findings were noted. One to two millilitre of blood was collected and inoculated in brain heart infusion broth. Blood Culture and Antibiotic susceptibility testing were performed as per standard protocols. Detailed history and clinical findings were recorded. Data was analysed using SPSS software.

**Results:** Out of 80 cases studied, bacterial growth was obtained in 41(51.25%) blood samples. Klebsiella (46.3%), followed by S.aureus (29.2%) were the most common isolates. E.coli (9.7%), CONS (4.8%) and others like Pseudomonas aeruginosa, Enterobacter cloacae, Proteus vulgaris, Citrobacterfreundii were less frequent isolates. Among all isolates, resistance to penicillin and ampicillin was frequent. Case fatality rate (CFR) was significantly higher in the culture positive group. Sepsis with MODS was the most common causes of death in clinically suspected culture negative group, followed by sepsis with pneumonia.

**Conclusion:** Here it is recommended that Choice of antibiotics should be based on routine surveillance of sensitivity pattern of particular organism.

**Keywords:** Neonatal Septicaemia, Antimicrobial Resistance, Gram Negative Septicaemia,

in neonatal wards cannot be overemphasized, so that local antibiotic administration policy can be formulated. Appropriate antibiotic treatment as per hospital antibiotic policy, in addition to neonatal life support measures and the early detection of risk factors may be useful in treating these patients with a better outcome.<sup>1,2</sup> Hence the present study was conducted to look for spectrum of bacterial isolates in cases of neonatal septicemia, and their antimicrobial resistance pattern.

## MATERIAL AND METHODS

80 clinically suspected cases of neonatal sepsis admitted in neonatology ward were enrolled over a period of one year. One to two mililitre of blood samples were collected aseptically and sent to the microbiology department of the same college for processing. Detailed history and clinical findings were recorded. Study was Approved by institutional ethics committee.

Neonates having one or more perinatal or clinical risk factors were included in the study. Risk factors considered for inclusion were Low birth weight, Prematurity, Birth asphyxia, history of premature rupture of membranes, maternal pyrexia, use of instruments during delivery, inability to take feed, delivery at home, lethargy, poor activity, fever/hypothermia, jaundice, difficulty in breathing or fast breathing, abdominal distension associated with vomiting and Diarrhea, skin mottling, bleeding tendencies, seizures. However Neonates who had already been treated with antibiotics before admission or with major congenital malformations were excluded.

All neonates were categorized into early onset sepsis (within 72 hours of birth) or late onset sepsis (after 72 hours of birth). Detailed history and clinical findings were recorded. One to two millilitre of blood was collected from each patient with due aseptic precautions and inoculated into 20 mL of brain heart infusion broth with 0.025% Sodium polyanetholsulfonate as anticoagulant (HiMedia Laboratories). Similarly, a second sample was drawn from a different site after few hours to rule out contamination with commensal flora. After overnight incubation, the broths

## INTRODUCTION

Neonatal septicemia is a major cause of mortality and morbidity among neonates around the world, even after advancements in antibacterial therapy. Neonates are particularly susceptible to all infections as they are immunologically weak. Moreover there are various identifiable risk factors both in mother and neonates which make them more susceptible to infections. Blood stream infections or septicemia is one of the major infections in this age group. Also, the organisms isolated are often resistant to multiple antimicrobials which make the treatment difficult and grave sequel ensue. Thus, the need for bacteriological monitoring

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were sub cultured on chocolate agar, MacConkey agar and 5% sheep blood agar. If culture results were negative, then they were followed up by daily examination of broth and doing a final subculture at appearance of turbidity or at the end of 7 days, whichever was earlier. Bacterial isolates were identified by colony morphology and biochemical tests by standard methods.<sup>2</sup> Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method following the CLSI guidelines.<sup>3</sup>

### STATISTICAL ANALYSIS

The statistical analysis was done using the results of the study. Data analysis was carried out using SPSS software for windows package.

### RESULTS

Out of 80 cases studied, cultures were positive 51.25% blood samples. More cultures were positive among late onset sepsis (66.66%), as compared to early onset sepsis (44.66%), which was not statistically significant. Males (67.5%) to female (32.5%) ratio was almost 2:1, But this was not significant with respect to the rates of culture positivity. 78% of the culture positives were spontaneously delivered however 22% were assisted deliveries.

Of the bacterial isolates, Klebsiella(46.3%), followed by Staphylococcus aureus (29.2%) were the most common

isolates. Escherichia coli(9.7%),Coagulase Negative Staphylococci (4.8%) and others like Pseudomonas aeruginosa, Enterobacter cloacae, Proteus vulgaris, Citrobacter freundii were less commonly isolated. This frequency was similar in both EOS and LOS cases (table-1). On antimicrobial susceptibility testing among gram positive isolates, resistance to penicillin and ampicillin was frequent in S. aureus (91.7%) and CONS(100%). Resistance to third generation cephalosporins was from 33.3% to 50%. However proportion of Methicillin resistance was 16.7%. All the isolates were susceptible to Vancomycin, teicoplanin and Ofloxacin. Resistance to other antibiotics was variable (Table 2).

Among Gram negative isolates, resistance to penicillin and ampicillin was almost 100%. Resistance to cephalosporins ranged from 21.1% to 79% and ESBL production could be demonstrated in 4 (14.8%) isolates which included Klebsiella pneumoniae (2), Escherichia coli (1) and Enterobacter cloacae (1). Resistance to other group of antibiotics like aminoglycosides and fluoroquinolones were variable. Carbapenem resistance was very low and could be seen only in Pseudomonas aeruginosa and Proteus vulgaris, one isolate each (Table 3).

Case fatality was highest due to sepsis with gram negative organisms, Klebsiella being the most common and alone contributing to 54.4% of mortality. Sepsis with MODS was

Bacterial isolates	Culture positive cases		Total n=41
	EOS n=25	LOS n=16	
Gram positive isolates 14(34.14%)			
Staphylococcus aureus	28%	31.25%	29.26%
Coagulase negative staphylococcus	4%	6.25%	4.8%
Gram negative isolates 27(65.85%)			
Klebsiella pneumoniae	44%	50%	46.3%
Escherichia coli	8%	12.5%	9.7%
Pseudomonas aeruginosa	4%	0	2.4%
Enterobacter cloacae	4%	0	2.4%
Proteus vulgaris	4%	0	2.4%
Citrobacter freundii	4%	0	2.4%

**Table-1:** Distribution of bacterial isolates with respect to early onset and late onset sepsis

Antibiotics	Staphylococcus aureus n=12	Coagulase Negative Staphylococcus n=2
Amoxycylav (AmoxC)	41.7%	50%
Ampicillin (Amp)	91.7%	100%
Pencillin (P)	91.7%	100%
Cefotaxime (Ctx)	33.3%	50%
Amikacin (Amk)	33.3%	50%
Vancomycin (Va)	0	0
Oxacillin (Ox)	16.7%	50%
Cefadroxil (Cfr)	41.7%	50%
Cefazolin (Cfz)	33.3%	50%
Gentamycin (Gm)	50%	100%
Ciprofloxacin (Cip)	41.7%	100%
Ofloxacin (Ofx)	0	0
Erythromycin (E)	75%	100%
Cotrimoxazole (Cot)	75%	100%
Tetracycline (T)	66.6%	100%

**Table-2:** Antibiotic resistance patterns of gram positive bacteria

Isolate (n)→	Klebsiella(18)	E. coli(4)	Pseudomonas (2)	Enterobacter (1)	Proteus(1)	Citrobacter(1)
Ampicillin (Amp)	5.2%	100%	100%	0	0	0
Amikacin (Amk)	21%	25%	50%	0	0	100%
Gentamycin (Gen)	10.4%	100%	50%	0	0	100%
Ofloxacin (Ofx)	78.9%	75%	100%	100%	0	100%
Cefotaxime (Ctx)	21%	25%	50%	0	0	0
Ceftriaxone (Ctr)	21%	50%	50%	0	0	0
Ceftriaxone (Caz)	26.3%	50%	100%	0	0	100%
Ceftizoxime (Czx)	78.9%	50%	50%	0	0	100%
Carbenicillin (Cb)	5.2%	100%	100%	0	0	100%
ESBL	10.4%	25%	0	100%	0	0
Imipenem (Imp)	0	0	50%	0	100%	0

ESBL – Extended spectrum β-lactamase production using double disc diffusion method,

**Table-3:** Antibiotic resistance pattern of gram negative bacterial isolates

the most common causes of death in clinically suspected culture negative group, followed by sepsis with pneumonia.

## DISCUSSION

Our basic aim was to study neonates with suspected septicemia to describe the spectrum of isolates, and their antimicrobial resistance pattern so that appropriate treatment may be started early for better treatment outcome. It was seen that few simple measures could suffice for the problems of many neonates enrolled in the study, like proper positioning of the baby during breast feed for those complaining of inability to take feed, Kangaroo mother care for babies complaining of hypothermia. Also many babies did better by proper counselling and explanation of danger signs as per IMNCI guidelines of the UNICEF.

Highest culture positive rates were observed in neonates with early onset septicemia (EOS), preterm babies and low birth weight babies. Similar observations were seen in previous studies.<sup>4,5,6</sup> Higher proportions of culture positive cases were inborn admission. This probably could be because hospital, being a tertiary referral centre for both Obstetrics and Pediatrics cases, has maximum late referral and previously treated cases with higher proportion of babies born with adverse intrapartum risk factors for neonatal sepsis.

The culture positive neonatal septicemia cases were higher among the males than the females showing a ratio of 1.4: 1. EOS may follow rupture of membranes or trial of labour such as at home or in PHCs/ CHCs, leading to ascending infection. Many of these cases are referred to the Medical College from distant locations in emergency. The higher proportion of EOS cases may be due to the neonates exposed to risk factors in the first week of life. They are also supposed to have immature immune function so that they are more vulnerable to infection in this period.<sup>7</sup>

In the present study, 41 out of the 80 cases studied were culture positive, giving a positivity rate of 51.25%, comparable with other Indian studies.<sup>6,8,9</sup> Since there are varying microbiological pattern of neonatal septicemia in various studies, that indicates towards the need for time to time review of the bacteriological spectrum and their antibiotic sensitivity pattern. Some national and international studies show the incidence of neonatal septicemia to vary

between 36% to 55%<sup>10,11,12</sup> while studies conducted by Joshi et al, Madhu Sharma et al and NNPD<sup>7,13,14</sup> showed a very low culture positivity. In our study, incidence of neonatal septicemia confirmed by culture was 51.25%. The culture positivity depends on time of sampling, extent of bacteremia in neonate and prior antibiotic treatment in the neonate. A hospital based data regarding bacteriological isolates and their antibiotic resistance pattern is the need of hour because the first antibiotic administered cannot be delayed till the culture results are available and keeping in mind the high morbidity and mortality associated with neonatal sepsis, an appropriate empiric therapy is very important.

In tropical areas, early onset neonatal infections may be caused by multiresistant hospital acquired bacteria, which are transmitted during delivery by lack of hygiene. These organisms are usually resistant genera of *Enterobacteriaceae* family, *Pseudomonas* spp. and *Staphylococcus*.<sup>4</sup> In our study, *Klebsiella pneumoniae* (46.3%) was the most common isolate, followed by *Staphylococcus aureus* (29.2%). Gram negative isolates (65.8%) were more common than Gram positive isolates (34.1%). These results were comparable with studies conducted by others. The present study is compared with NNPD (2002-03) data from 18 centers of various institutions throughout India with respect to the trend in antibiotic sensitivity pattern with *Klebsiella*, *S.aureus* and *E.coli*. Group B *Streptococcus* is not common in our study and this finding was in accordance with NNPD data. Nearly 80% of *Klebsiella*, 100% of *E.coli* and 45% of *S.aureus* are resistant to gentamycin; 70% of all *Klebsiella*, 75% of *E.coli* and 20% of *S.aureus* are resistant to amikacin. Resistance to cefotaxime and ceftazidime is very common among gram negative isolates. Ceftazidime seems to be the only useful antibiotic for *Pseudomonas*. Fluoroquinolones are best alternatives as all isolates are susceptible to it. Most of the organisms were resistant to gentamycin and amikacin. This is comparable with NNPD data except that fluoroquinolones resistance is already a menace and vancomycin resistance is increasing.

Culture positive cases had high death rates, more frequently associated with gram negative organisms. This finding is comparable with other studies. This may probably be due to lack of specific IgM antibodies and complement deficiency

in newborn which are required for protection against gram negative organism. This indicates that higher proportion of babies had infection with gram negative organisms which were resistant to commonly used antibiotics i.e. cephalosporins and aminoglycosides. Probably reason behind this is free supply of these drugs in government setups. The mode of death may be endotoxin mediated multiple organ dysfunction. The death rate is also high because of confounding factors like preterm birth and low birth weight, which may lead to the inefficient immunity to combat infection.

Most of the cases detected by positive blood culture occurred in the first week of life (71.3%). This indicates towards need for close follow up of the neonates especially those in high risk categories as soon as they are born. Administration of empiric antimicrobial therapy for gram negative bacteria is suggested in suspected cases of neonatal septicaemia. The major gram positive isolates i.e. *Staphylococcus aureus* and Coagulase Negative Staphylococci were mostly penicillin resistant. Resistance rates for other antimicrobials like erythromycin, gentamicin, tetracycline and ciprofloxacin were also very high i.e. 40%. High frequency of resistance against these  $\beta$  lactam and non  $\beta$  lactam antibiotics have been seen in Methicillin resistant *Staphylococcus aureus* and Methicillin resistant Coagulase Negative Staphylococci.<sup>15</sup> None of our strains showed resistance against vancomycin or teicoplanin and these drugs therefore can be effectively used if methicillin resistance is suspected during treatment. Gram negative isolates of *Enterobacteriaceae* family offered resistance to anti gram negative penicillins as well as to extended spectrum cephalosporins in quite large numbers, making it clear that the use of these drugs alone may be ineffective. It was however interesting to note that ofloxacin resistance was less frequent among these bacteria. This fact was further supported by *in vivo* results of the drug as could be learnt from the clinical side. The high frequency of resistance to  $\beta$  lactam group of antibiotics may be due to their inappropriate use at first encounter. This can be reduced by using drugs to which most isolates show sensitivity. Regarding gram negative isolates, which were major pathogens, fluoroquinolones and aminoglycosides like ciprofloxacin and amikacin are good options and they will also be more economical.

## CONCLUSION

Here it is recommended that choice of antibiotics should be based on routine surveillance of sensitivity pattern of particular organism

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