

Original Research Article

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## Neonatal Septicemia: Bacteriology and Risk Factors in a Tertiary Care Hospital of Central India

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### ABSTRACT

Neonatal septicemia continues to be a major cause of mortality and morbidity among neonates around the world, in spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection of risk factors. The present study was undertaken to describe the spectrum of isolates in cases of neonatal septicaemia, and their antimicrobial susceptibility pattern and to look for their association with various risk factors. In this prospective study in central India we prospectively enrolled the suspected cases of neonatal septicaemia, which were not on antimicrobials. One to two millilitre of blood from these neonates was cultured 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 analysis was carried out using Statistical Package for Social Sciences for windows package. Out of 80 cases studied, bacterial growth was obtained in 41(51.25%) blood samples. The most frequent risk factor among neonates was low birth weight in both EOS and LOS groups. Septicemia was the most common cause of clinical presentation, followed by pneumonia and meningitis. Of the bacterial isolates, 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* and *Citrobacter freundii* 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. Here it is recommended that Perinatal risk score involving intrapartum risk factors should be used for triage of high risk babies and Choice of antibiotics should be based on routine surveillance of sensitivity pattern of particular organism.

#### Keywords

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### Introduction

Neonatal septicemia continues to be a major cause of mortality and morbidity among neonates around the world, In spite of great advances in antimicrobial therapy, neonatal life support measures and the early detection

of risk factors (Rajiv Aggarwal *et al.*, 2001). Neonates are particularly vulnerable to infections because of weak immune barrier. Moreover several risk factors have been identified both in the neonates and in the mother, which make them susceptible to infections. Blood stream infections have been

quoted as the most common 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 in neonatal wards cannot be overemphasized, so that local antibiotic administration policy can be formulated. The present study was undertaken to describe the spectrum of isolates in cases of neonatal septicaemia, and their antimicrobial susceptibility pattern and to look for their association with various risk factors.

### **Materials and Methods**

A total of 148 clinically suspected cases of neonatal sepsis admitted in neonatology ward were enrolled over a period of one year. The blood samples were collected and sent to the pathology and microbiology department of the same college for evaluation. But of these all the required investigations of only 80 patients could be traced.

The remaining 68 had to be excluded on account of some missing investigations or due to non-compliance of relatives and other inevitable circumstances like dropouts. Detailed history and clinical findings were recorded. Study was approved by institutional ethics committee.

Neonates presenting with Perinatal or clinical risk factors like Low birth weight, Prematurity, Birth asphyxia, Home delivery, History of PROM in mother for more than 24 hours, Maternal fever, Instrumentation, Poor feeding, lethargy, reduced activity, Sclerema, Hypothermia/fever, Jaundice, Apnea, tachypnea, Abdominal distension and vomiting, Diarrhea, Skin mottling, Bleeding tendencies, Seizures were enrolled for the study. However Neonates who received antibiotics before admission or having major congenital malformations were excluded.

All neonates were categorized into early onset sepsis EOS (0-72 hours) or late onset sepsis LOS (>3 days) based on the day of presentation in the postnatal life. Detailed history and clinical findings were recorded. Among early onset sepsis, perinatal risk factors were noted and each baby was given a score (Lawn *et al.*, 2005; Ved Parkash Takkar *et al.*, 1974) based on these risk factors (given in parenthesis for each factor) i.e. Foul smelling liquor (2), Unclean vaginal examination done before delivery (2), Duration of labour exceeding 24 hours (2), Birth asphyxia (Apgar < 6 at 1 min) (2), Birth weight 2.5 Kgs or less and/ or gestation age <37 weeks (3), Duration of rupture of membrane before delivery > 24 hours (1), and Maternal pyrexia (1) thus making a total score of 13. Babies with Score 0-3 were observed clinically while with Score >4 were Investigated. All neonates who were clinically symptomatic or having a septic score > 4, One to two millilitre of blood was collected from each patient using proper aseptic precautions and inoculated immediately into 10 mL of brain heart infusion broth with 0.025% Sodium polyanethol sulfonate as anticoagulant (HiMedia Laboratories, Mumbai). A second similar sample was obtained on the same day from a different site after few hours to rule out contamination with skin flora. The broths were subcultured after overnight incubation on chocolate agar, MacConkey agar and 5% sheep blood agar. A negative result was followed up by examining the broth daily and doing a final subculture at the end of 7 days or at appearance of turbidity, whichever was earlier. Any growth was identified by colonial characteristics and standard biochemical tests (Collee *et al.*, 14 edn). Antimicrobial susceptibility testing was performed by the Kirby-Bauer disc diffusion method as per the CLSI guidelines (CLSI, 2010).

The statistical analysis was done using the results of the present study. Sepsis score and

sepsis screen test results were compared with the blood culture results as the gold standard. Data analysis was carried out using Statistical Package for Social Sciences for windows package.

## Results and Discussion

Out of 80 cases studied, bacterial growth was obtained in 41(51.25%) blood samples. culture positivity was higher among late onset sepsis (66.66%), as compared to early onset sepsis (44.66%), which was not statistically significant. Males (67.5%) were affected more as compared with female (32.5%), but this is not significant with respect to the rates of culture positivity.

The most frequent risk factor among neonates was low birth weight in both EOS and LOS groups. However among maternal factors preterm labour was most common. Other common intra partum risk factors were Labour duration >24hours (mostly outside/ emergency cases), Unclean/ repeated vaginal examinations, Foul smelling liquor and they were significantly associated with culture proven sepsis. PROM>24 hours and maternal fever had no significant association.

Higher proportion of babies with culture proven sepsis had >2 risk factors. Among LOS babies, history of hospitalization in prior one week was also a risk factor. More often they were due to faulty feeding practices, prelacteal feeds and some focus of infection as risk factors for sepsis. Predominant clinical findings in these patients were Refusal of feeds (72.5%), Lethargy and weak reflexes (81.5%), Hypothermia (26.2%), Hyperthermia (13.75%), Cyanosis (22.5%), Dehydration (6.25%), Tachypnoea (37.5%), Apnea (16.25%), Chest retraction (31.25%), Tachycardia (48.75%), and Shock (CRT>3 sec), Loose stools, Abdominal distension, Jaundice, Vomiting, High pitched cry,

Convulsion, Bulging AF, Bleeding manifestation, Sclerema in varying proportions. More than one finding could be seen in one patient. Higher proportion of septic babies had vague symptoms like variation in established feeding pattern with failure to suck and lethargy.

Lethargy is also a danger sign of returning back immediately in the hospital, which was explained to all the mothers whose babies were discharged from the hospital and hence was an important sign with babies with LOS.

78% of the spontaneously delivered infants were positive for culture. 22% of the culture positives were assisted deliveries.

Septicemia was the most common cause of clinical presentation, constituting about 48.75% of cases, followed by pneumonia and meningitis. Among early onset sepsis, septicemia (60.3%) followed by pneumonia (36%) was the most common presentation. Among LOS, pneumonia (37.5%) followed by meningitis (25%) and infective diarrhoea were the most common modes of presentation (Table 1).

Of the bacterial isolates, *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*, *Citrobacter freundii* were less frequent isolates. This frequency was similar in both EOS and LOS cases.

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%. None of the isolates were resistant to Vancomycin,

teicoplanin and Ofloxacin. Resistance to other antibiotics was variable. Among Gram negative isolates, almost all were resistant to penicillin and ampicillin. 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 2).

Overall mortality rate in the 80 cases studied was 33.75% with no statistical difference among the early and the late onset cases. Case fatality rate (CFR) was higher in the culture positive group as compared to the culture negative group and this was statistically significant ( $\chi^2 = 3.88$ ,  $p < 0.05$ ). No statistical significance was attributed to the differences seen in distribution with respect to place of delivery or sex of the neonate. Death rates were higher in LBW/VLBW babies (24 out of 58 i.e. 41.38%) as compared with normal birth weight babies (3 out of 22 i.e. 5.17%) and it was statistically significant ( $\chi^2 = 4.10$ ,  $p < 0.05$ ).

Total case fatality was more amongst the preterm babies i.e. 77.7% and it is statistically significant. ( $\chi^2 = 4.96$ ,  $p < 0.05$ ). CFR among culture positive preterm babies was 44.68%, while it was 18.18% among term babies which was again statistically significant. ( $\chi^2 = 4.18$ ,  $p < 0.05$ )

Septicemia with multiple organ dysfunction (shock, cardio-respiratory failure, DIC) was the most common mode of death (38.8%) with high risk of mortality in culture proven cases. Pneumonia accounted for 38.8%, while meningitis accounted for ~11.1% of mortality. Septicemia with MODS and NEC carries

100% risk of mortality, although its presentation was not significant. 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 the most common causes of death in clinically suspected culture negative group, followed by sepsis with pneumonia.

Our basic aim was to study neonates with suspected septicemia to describe the spectrum of isolates, and their antimicrobial resistance pattern and to look for their association with various risk factors so that appropriate treatment may be started early to ensure better survival and prevent morbidity.

It was seen that many neonates who were enrolled in the study, could be easily excluded by simple measures like proper positioning of the baby during breast feed (for those complaining of feed refusal), Kangaroo mother care (for those with complaints of hypothermia).

Also many babies were excluded, as they did better by thorough counselling and explanation of danger signs as per IMNCI guidelines of the UNICEF.

Maximum culture positivity was seen in neonates with early onset septicemia (EOS), preterm babies and low birth weight babies. Similar observations were seen in previous studies (Varsha *et al.*, 2003; National Neonatal Perinatal Database, 2002-03; Rekha Sriram, 2011).

Higher proportions of culture positive cases were inborn admission. This probably reflects that hospital, being a tertiary referral hospital for both Obstetrics and Pediatrics cases, has maximum late referral and intervened cases with higher proportion of babies born with adverse intrapartum risk factors for neonatal sepsis (Table 2–4).

**Table.1** Distribution of organisms with respect to early onset and late onset sepsis

Bacterial isolates	Culture positive cases		Total n=41(%)
	EOS n=25	LOS n=16	
Gram positive isolates 14(34.14%)			
<i>Staphylococcus aureus</i>	7(28%)	5(31.25%)	12(29.26%)
Coagulase negative <i>Staphylococcus</i>	1(4%)	1(6.25%)	2(4.8%)
Gram negative isolates 27(65.85%)			
<i>Klebsiella pneumoniae</i>	11(44%)	8(50%)	19(46.3%)
<i>Escherichia coli</i>	2(8%)	2(12.5%)	4(9.7%)
<i>Pseudomonas aeruginosa</i>	1(4%)	0	1(2.4%)
<i>Enterobacter cloacae</i>	1(4%)	0	1(2.4%)
<i>Proteus vulgaris</i>	1(4%)	0	1(2.4%)
<i>Citrobacter freundii</i>	1(4%)	0	1(2.4%)

**Table.2** Shows the antibiotic resistance patterns of gram positive bacteria

Antibiotics	<i>Staphylococcus aureus</i> n=12	Coagulase Negative <i>Staphylococcus</i> n=2
Amoxyclav(AmoxC)	5(41.7%)	1(50%)
Ampicillin (Amp)	11(91.7%)	2(100%)
Pencillin (P)	11(91.7%)	2(100%)
Cefotaxime (Ctx)	4(33.3%)	1(50%)
Amikacin (Amk)	4(33.3%)	1(50%)
Vancomycin (Va)	0	0
Oxacillin (Ox)	2(16.7%)	2(100%)
Cefadroxil (Cfr)	5(41.7%)	1(50%)
Cefazolin (Cfz)	4(33.3%)	1(50%)
Gentamycin (Gm)	6(50%)	2(100%)
Ciprofloxacin (Cip)	5(41.7%)	2(100%)
Ofloxacin(Ofx)	0	0
Erythromycin (E)	9(75%)	2(100%)
Cotrimoxazole(Cot)	9(75%)	2(100%)
Tetracycline(T)	8(66.6%)	2(100%)

**Table.3** Shows the antibiotic resistance pattern of gram negative bacterial isolates

ISOLATE→ ANTIBIOTIC ↓	<i>Klebsiella</i> (19)	<i>E. coli</i> (4)	<i>Pseudomonas</i> (1)	<i>Enterobacter</i> (1)	<i>Proteus</i> (1)	<i>Citrobacter</i> (1)
Ampicillin (Amp)	18(5.2%)	4	0	0	0	0
Amikacin (Amk)	15(21%)	3(25%)	1(50%)	0	0	1(100%)
Gentamycin (Gen)	17(10.4%)	4	1(50%)	0	0	1(100%)
Ofloxacin (Ofx)	4(78.9%)	1(75%)	2(100%)	1(100%)	0	1(100%)
Cefotaxime (Ctx)	15(21%)	3(25%)	1(50%)	0	0	0
Ceftriaxone (Ctr)	15(21%)	2(50%)	1(50%)	0	0	0
Ceftriaxone (Caz)	14(26.3%)	2(50%)	2(100%)	0	0	1(100%)
Ceftizoxime (Czx)	4(78.9%)	2(50%)	1(50%)	0	0	1(100%)
Carbenicillin (Cb)	18(5.2%)	4	2(100%)	0	0	1(100%)
ESBL	2	1	0	1	0	0
Imipenem (Imp)	0	0	1	0	1	0

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

**Table.4** Correlation of clinical diagnosis with mortality in culture positive cases

CLINICAL DIAGNOSIS	Culture positive EOS n=25		Culture positive LOS n=16		Mortality Rate n=18
	Cases	Mortality	Cases	Mortality	
Septicemia	7	1(14.2%)	1	0	1(5.5%)
Septicemia with MODS	6	6(100%)	1	1(100%)	7(38.8%)
Pneumonia	9	4(44.4%)	6	3(50%)	7(38.8%)
Meningitis	1	1(100%)	2	1(50%)	2(11.1%)
Infective diarrhea	0	-	2	0	-
Umbilical sepsis	0	-	2	0	-
NEC	0	-	1	1(100%)	2(11.1%)
UTI	1	0	0	-	-
Septic arthritis	0	-	1	0	-



The culture positive neonatal septicemia cases were higher among the males than the females showing a ratio of 1.4: 1. The male preponderance in neonatal septicemia may be linked to the X- linked immunoregulatory gene resulting in the host's susceptibility to infections in the males. The present study clearly shows a greater proportion of cases having higher number of attempted per vaginal examinations before delivery, prolonged rupture of membranes for 24 hrs and prolonged labour for >24 hrs in developing definitive septicemia, comparable with other studies. (Yancey *et al.*, 1996; Joseph *et al.*, 1984) EOS could be due to ascending infection following rupture of membranes or during trial of labour such as at home or in PHCs/ CHCs.

Many of these cases are referred to the Medical College from far off destination on an emergent basis. The higher proportion of EOS cases may be due to the immature immunological responses of the neonates exposed to risk factors in the first week of life, making them more susceptible to infection in this period (Stoll *et al.*, 1996).

Nearly an equal proportion of cases had birth weight < 2.5 kgs and Gestational age < 37 weeks as risk factors for developing septicemia comparable with past studies. (Rekha Sriram, 2011; Tallur *et al.*, 2000; Roy *et al.*, 2002; Dawodu *et al.*, 1997) Other major risk factors associated with sepsis in present study were Perinatal asphyxia associated with unattended or outside deliveries with an Apgar score <6 and Clinical chorioamnionitis (31.3% of the culture proven sepsis) diagnosed by the presence of foul smelling liquor and maternal fever. The variations in the occurrence of perinatal risk factors probably reflect differences in the rates of occurrence of the predisposing risk factors in the various studies (Rekha Sriram, 2011; Yancey *et al.*, 1996;

Joseph *et al.*, 1984). 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 conducted by Tallur *et al.*, and Roy *et al.*, Rekha Sriram *et al.*. The varying microbiological pattern of neonatal septicaemia warrants the need for an ongoing review of the causative organisms and their antibiotic sensitivity pattern. Some reports from home and abroad show the incidence of neonatal septicaemia to vary between 36% to 55% (Datta *et al.*, 2006; Gerdes, 2004; Ashok K Deorari, 2006) while studies conducted by Joshi *et al.*, Madhu Sharma *et al.*, and NNPD showed a very low culture positivity. In our study, incidence of neonatal septicaemia 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. 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 for such empiric therapy is of utmost importance.

In western countries, antibiotics of choice are directed towards group B *Streptococcus* and *E. coli*. But 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*. (Varsha *et al.*, 2003) In our study, *Klebsiella pneumoniae* (46.3%) was the predominant isolate, followed by *S. aureus* (29.2%). Gram negative organisms formed the majority of the isolates as compared to Gram positive organisms (65.8% vs 34.1% respectively), 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*, as is evident from the same report, is not common in our country and we also did not isolate group B *Streptococcus* from our cases. 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.

Higher proportion of mortality was associated with early onset culture proven sepsis and most of them were preterm and or low birth weight. This was statistically significant (p value<0.05) and is comparable with other studies. Mortality rate was high among culture positive cases (isolates mostly being gram negative organisms). Gram negative sepsis is associated with high mortality in present study which is comparable with other studies. This is probably related to lack of specific IgM antibodies and complement deficiency in newborn which are required for protection against gram negative organism.

There was high case fatality rate among babies presenting with difficulty in breathing, hypothermia, blood in stools, abdominal distension, lethargy/excessive irritability or refusal of feeds and those who developed refractory shock, DIC, respiratory failure, meningitis and necrotizing enterocolitis during the course of illness and it was

comparable with other studies. This probably reflects that higher proportion of babies had infection with highly virulent gram negative organisms which predominate as the causative agents and were resistant to commonly used cephalosporins and aminoglycosides, which are freely supplied in the government setups, as already reported. The mode of death is probably due to endotoxin mediated multiple organ dysfunction. The death rate is also high because a higher proportion of babies are preterm and low birth weight, which lacked the inherent immunity to combat infection.

Most of the cases detected by blood culture occurred in the first week of life (71.3%). This calls for close monitoring of the newborns especially those in high risk categories as soon as they are born. Administration of empiric antimicrobial therapy aimed at gram negative bacteremia in suspected cases of neonatal septicaemia is suggested. The major gram positive isolates viz. *S. aureus* and CONS were frequently found to be penicillin resistant. Resistance percentage to other antimicrobials like erythromycin, gentamicin, tetracycline and ciprofloxacin were above 40%. High frequency of resistance against these  $\beta$  lactam and non  $\beta$  lactam antibiotics have been seen in MRSA and MRCNS. (Joseph *et al.*, 1984) 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 antibiotics can well be due to their indiscriminate use as first line drugs. This can be avoided by using drugs to which most organisms were susceptible. In case of gram negative isolates, which turned out to be the major pathogens, ciprofloxacin and amikacin are good alternatives and they will also provide some economical relief to the patient.

Here it is recommended that Perinatal risk score involving intrapartum risk factors should be used for triage of high risk babies and Choice of antibiotics should be based on routine surveillance of sensitivity pattern of particular organism.

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