



## Original Research Article

# A STUDY ON THE BACTERIOLOGICAL PROFILE, ANTIBIOTIC SUSCEPTIBILITY PATTERN AND ASSOCIATED RISK FACTORS IN NEONATAL SEPSIS IN A TERTIARY CARE HOSPITAL

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Received : 03/01/2025  
Received in revised form : 24/02/2025  
Accepted : 11/03/2025

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DOI: 10.70034/ijmedph.2025.1.221

Source of Support: Nil,  
Conflict of Interest: None declared

Int J Med Pub Health  
2025; 15 (1); 1179-1184

### ABSTRACT

**Background:** Neonatal sepsis is one of the leading cause of neonatal mortality and morbidity worldwide. A wide spectrum of organisms cause neonatal sepsis and they are often resistant to multiple antimicrobials which make the treatment very difficult and grave sequelae ensue.

**Materials and Methods:** This descriptive cross-sectional study was conducted in the Department of Microbiology and Department of Paediatrics at Government Medical College Hospital, Thiruvallur, over a period of 6 months from April 2023 to September 2023. Blood samples collected for culture and sensitivity from 200 neonates suspected to have clinical sepsis, were processed as per standard procedures. Antibiotic susceptibility pattern of the isolates were determined according to CLSI guidelines. Neonatal and maternal risk factors were analyzed

**Results:** Blood cultures were positive in 17(8.5%) newborns, out of which 13(76.5%) were EOS and 4(23.5%) were LOS. Of the 17 bacterial isolates, 12(70.6%) were Gram-negative and 5(29.4%) were Gram-positive. Klebsiella pneumoniae and Acinetobacter species were the common organisms isolated. Gram negative isolates were highly sensitive to meropenem. Gram positive isolates were highly sensitive to Vancomycin and linezolid. Prematurity and low birth weight were the most common neonatal risk factors observed. Maternal risk factors observed were Premature Rupture Of Membrane (PROM) >18 hrs, followed by maternal fever and maternal urinary tract Infection.

**Conclusion:** The bacteriological profile of neonatal sepsis keeps changing from time to time and region to region. From this study we were able to determine the common causative bacterial pathogens, their antibiotic susceptibility pattern and the associated risk factors of neonatal sepsis in our hospital.

**Keywords:** neonatal sepsis, bacteriological profile, antibiotic susceptibility, early onset sepsis, late onset sepsis.

## INTRODUCTION

Neonatal Sepsis is defined as a systemic condition of bacterial, viral or fungal origin that is associated with hemodynamic changes which shows clinical manifestations and results in significant morbidity

and mortality.<sup>[1]</sup> It is the second leading cause of mortality among neonates, killing more than one million neonates annually.<sup>[2]</sup> In India nearly one-third of neonatal mortality is due to sepsis and death occurs in 30% of culture positive neonates.<sup>[3]</sup> Sepsis remains as one of the most important cause of

mortality in neonate especially in very low birth weight preterm infants and its incidence increases in the presence of maternal and neonatal risk factors.<sup>[4]</sup> Neonatal sepsis is divided into two groups based on the time of presentation after birth: early-onset sepsis (EOS) and late-onset sepsis (LOS). EOS refers to sepsis in neonates at or before 72 hours of life and LOS is defined as sepsis occurring at or after 72 hours of life.<sup>[5]</sup>

EOS presents where the maternal genital tract is the source of ascending infection. Maternal risk factors like premature rupture of membrane (PROM), Chorio amnionitis, Puerperal fever, Urinary tract infection within 2 weeks prior to the delivery and prolonged rupture of membranes >18 hours, multiple gestations, caesarean sections are associated with EOS. LOS occurs as a result of postnatal nosocomial infections or community acquired infections and the risk factors associated with LOS are prematurity, prolonged invasive interventions like mechanical ventilations, intravascular catheterization, failure of early enteral feeding with breast milk, long duration of parenteral nutrition, hospitalization, surgery and underlying respiratory and cardiovascular disease.<sup>[6]</sup>

The organisms commonly associated with EOS are group B Streptococcus, Escherichia coli, Coagulase negative Staphylococcus, Haemophilus influenzae and Listeria monocytogenes. The bacterial agents implicated in LOS are Coagulase negative staphylococci (CONS), Staphylococcus aureus, Klebsiella pneumoniae, Escherichia coli, Enterobacter species, Pseudomonas aeruginosa and Acinetobacter species.<sup>[7]</sup> The causative organisms of neonatal sepsis vary significantly between developed and developing countries. In developing countries Klebsiella pneumoniae is the most common bacterial agent causing neonatal sepsis whereas in developed countries group B Streptococcus and CONS are the common agents.<sup>[7]</sup> The clinical signs and symptoms of neonatal sepsis are indistinct and non-specific, which makes its early diagnosis difficult. The difficulties in diagnosing sepsis have led to the erratic use of antibiotics which has given rise to the emergence of multi drug resistance pathogens (MDR). A remarkable percentage of the deaths are due to MDR pathogens, further complicating sepsis management. Hence, understanding the risk factors, clinical features, organisms involved, their antibiotic sensitivity pattern becomes crucial and guides management and promotes antibiotic stewardship.

#### **Aim and objectives**

This study was undertaken to determine the common bacterial agents, their antibiotic susceptibility pattern and to analyze the maternal

and neonatal risk factors associated with neonatal sepsis in a tertiary care hospital in Tamil Nadu.

## **MATERIALS AND METHODS**

This descriptive cross-sectional study was conducted in the Department of Microbiology and Department of Paediatrics at Government Medical College & Hospital, Tiruvallur, Tamilnadu over a period of 6 months from April 2023 to September 2023. This study was approved by the Institutional ethical committee and informed consent was obtained from newborn parents/attendants.

**Inclusion Criteria:** All the neonates having symptoms and signs suggestive of sepsis were included in the study.

**Exclusion Criteria:** Neonates not suspected to have sepsis and on prior antibiotic administration were excluded.

A detailed antenatal, natal and post-natal history was taken. The birth weight, sex and day of onset of sepsis were noted. Details regarding risk factors such as ventilator support, CPAP, central line and exchange transfusion prior to the onset of sepsis were noted. Blood culture was done for all the neonates. Blood was collected under strict aseptic precautions before starting antibiotics and inoculated into brain heart infusion broth. Blood culture bottles were transported immediately to the Microbiology Laboratory and the bottles were incubated aerobically at 37°C for 7 days and subcultured onto blood agar and MacConkey agar at 48 hours and 7 days or at an in-between period when visible turbidity appeared. The isolates were processed as per standard microbiological techniques and the isolates were identified.<sup>[8]</sup> Antibiotic sensitivity testing was performed on Mueller-Hinton agar plates by Kirby-Bauer disk diffusion method as per Clinical Laboratory Standard Institute guidelines. Screening for MRSA was done using a cefoxitin (30 µg) disc. ESBL production is tested by phenotypic confirmatory disc diffusion test using cefotaxime with and without clavulanic acid.<sup>[9]</sup> The organisms were classified based on the time-point at which the blood was collected for culture: Up to 72 hrs after birth as causing early onset sepsis and >72 hrs as causing late onset sepsis.<sup>[10]</sup>

#### **Statistical Analysis**

The collected study data was entered in Microsoft Office Excel 2013 and analyzed using SPSS software version 21. Categorical variables were expressed in frequency and percentage. Chi square test was used to compare two categorical variables. All the tests were two tailed and the results were considered statistically significant if the p-value was less than 0.05.

## RESULTS

**Table 1: Gender distribution of cases of neonatal sepsis**

Gender	Culture positive No. of cases (%)	Culture negative No. of cases (%)	Total No (%)	P value
Male	11 (64.7)	107 (58.5)	118 (59)	0.617
Female	6 (35.3)	76 (41.5)	82 (41)	
Total	17 (8.5%)	183 (91.5%)	200 (100%)	

In the study, out of 200 newborns with clinical signs of sepsis, 118 (59%) were male and 82(41%) were female. Blood cultures were positive in 17(8.5%) newborns and 183(91.5%) were culture negative. Of

the culture positive neonates, 11(64.7%) were male whereas 6(35.3%) were female. The male female ratio in culture positive cases was found to be 1.83:1.

**Table 2: Distribution of bacterial isolates according to onset of neonatal sepsis**

Organisms	EOS No (%)	LOS No (%)	Total No
<i>Klebsiella pneumoniae</i>	4 (30.8)	2 (50)	6
<i>Acinetobacter species</i>	3 (23)	1 (25)	4
<i>Escherichia coli</i>	1 (7.7)	0	1
<i>Pseudomonas aeruginosa</i>	1 (7.7)	0	1
MS <i>Staphylococcus aureus</i>	1 (7.7)	0	1
MR <i>Staphylococcus aureus</i>	0	1 (25)	1
MR CoNS	2 (15.4)	0	2
MS CoNS	1 (7.7)	0	1
Total	13	4	17

Of the 17 isolates, 12(70.6%) were Gram-negative bacteria and 5(29.4%) were Gram-positive bacteria. Of the total 12(70.6 %) Gram negative isolates, 6 (50%) were *Klebsiella pneumoniae*, 4 (33.3%) were *Acinetobacter species*, 1 (8.3%) was *Pseudomonas*

*aeruginosa* and 1 (8.3%) was *Escherichia coli*. Among Gram positive isolates of total 5(29.4%) , 3(60%) were Coagulase negative *Staphylococcus* and 2 (40%) were *Staphylococcus aureus*

**Table 3: Antibiotic sensitivity pattern of Gram-positive bacteria**

Antibiotic	<i>Staphylococcus aureus</i> (n=2) (%)	CONS (n=3) (%)
Penicillin	0	0
Gentamicin	2 (100)	1 (33.3)
Cotrimoxazole	1 (50)	1 (33.3)
Clindamycin	2 (100)	2 (66.7)
Linezolid	2 (100)	3 (100)
Vancomycin	2 (100)	3 (100)

All the isolates were sensitive to Linezolid and vancomycin (100%). Out of 2 *Staphylococcus aureus* isolated, 1 (50%) isolate was methicillin resistant and out of 3 Coagulase negative

*Staphylococcus* isolated, 2 (66.7%) isolates were methicillin resistant.

**Table 4: Antibiotic sensitivity pattern of Gram-negative bacteria**

Antibiotic	<i>Klebsiella Pneumoniae</i> (n=6), (%)	<i>Escherichia coli</i> (n=1), (%)	<i>Acinetobacter species</i> (n=4), (%)	<i>Pseudomonasaeruginosa</i> (n=1), (%)
Ampicillin	NT	0	NT	NT
Gentamicin	4 (66.7)	1 (100)	3 (75)	NT
Cotrimoxazole	4 (66.7)	0	3 (75)	NT
Amikacin	4 (66.7)	1 (100)	3 (75)	NT
Ciprofloxacin	2 (33)	1 (100)	2 (50)	1 (100)
Cefotaxime	3 (50)	0	2 (50)	NT
Ceftazidime	NT	NT	0	0
Piperacillin Tazobactam	4 (66.7)	1 (100)	3 (75)	1 (100)
Meropenem	6 (100)	1 (100)	4 (100)	1 (100)

(NT - Not tested)

All the Gram negative isolates were found to be sensitive to Meropenem (100 %). Among the 7

enterobacterales, 4 isolates (57%) were found to be ESBL producers

**Table 5: Distribution of maternal risk factors in culture positive neonatal sepsis**

Maternal risk factors	Culture positive No (%)	Culture negative No (%)	Total No (%)	P value
<i>PROM &gt; 18 hours</i>	11 (64.7)	87 (47.5)	98 (49)	0.292
<i>Maternal fever</i>	5 (29.4)	62 (33.9)	67 (33.5)	
<i>Maternal UTI</i>	1 (5.9)	34 (18.6)	35 (17.5)	
<i>Total</i>	17	183	200	

The maternal risk factors observed in the culture positive neonatal sepsis were Premature Rupture of Membrane (PROM) > 18hrs in 64.7%, followed by

maternal fever 29.4% and maternal urinary tract Infections 5.9 %.

**Table 6: Distribution of neonatal risk factors in culture positive neonatal sepsis**

Neonatal Risk factors	Culture positive, No (%)	Culture negative No (%)	Total No (%)	P value
Prematurity	13 (76.5)	111 (60.6)	98 (49.0)	0.199
Low birth weight	12 (70.6)	114 (62.3)	126 (63)	0.498
Birth asphyxia	6 (35.3)	51 (27.9)	45 (22.5)	0.516
Meconium stained liquor	4 (23.5)	31 (17)	35 (17.5)	0.494

Prematurity (76.5%) was the most common neonatal risk factor observed in the culture positive neonatal sepsis, followed by low birth weight (70.6%), birth asphyxia(35.3%) and meconium stained liquor (23.5%). Culture-positive neonatal sepsis is

observed more among neonates with birth weight <2.5 kgs (70.6%) and among neonates with birth weight of 2.5 -3.5 kgs,it was noted in 4(23.5%) and in >3.5 kgs it was 1(5.9%) .

## DISCUSSIONS

Neonatal sepsis is one of the public health issues of global concern. It is also one of the main problems and challenges faced by NICU.<sup>[9]</sup> The correct and timely identification of infectious agents and their antimicrobial susceptibility patterns are essential to guide the clinicians regarding both the empirical and definitive treatment.<sup>[8]</sup> There has been a wide variation in the culture-positivity rate for aerobic organisms in neonates in India; ranging from 16% to 54%.<sup>[11]</sup> Our blood culture positivity rate was 8.5% while positivity rates reported by Gupta and Kashyap was 16.5% ,Ansari et al was 12.6% and Anjana et al was 41.9%.<sup>[12,13,14]</sup> The variation could be due to inadequate or improper sampling of blood and also sepsis due to other infectious agents like fungal, viral or anaerobic pathogens.<sup>[15]</sup>

Higher incidence of culture positive sepsis was observed among male neonates 11(64.7%) when compared to female neonates 6(35.3%) and in our study, the male female ratio in culture positive cases was found to be 1.83:1 which corresponds to the male to female ratio of 1.3:1 reported by Eman et al.<sup>[16]</sup> Similarly in a study done at Bangladesh male neonates were affected more 42 (55.26%) than female 34(44.74%).<sup>[17]</sup> Male predominance was found in almost all the studies of neonatal sepsis.<sup>[14,18,19]</sup> This sex difference may be due to a gene located on the X chromosome that is involved with the function of the thymus or with the synthesis of immunoglobulins in the males thus conferring

less immunological protection compared to females.<sup>[20]</sup>

In our study EOS was noticed in 138 (69%) neonates, whereas 62(31%) occurred after 72 hours (LOS). Among the culture positive neonates, 13(76.5%) were EOS and 4 (23.5%) were LOS similar to a study done by Jimba Jatsho et al where culture-positive EOS (54.5%) was higher than LOS (45.5%).<sup>[21]</sup> In contrast, in another study done in Indonesian tertiary neonatal care unit it was noted that of all culture positive neonates 13 (25%) in EOS and 39 (75%) in LOS.<sup>[22]</sup> More incidence of EOS in our study may be due to more preterm neonate being affected than term neonate and EOS is observed more in preterm. Infant factors associated with EOS includes prematurity.<sup>[23]</sup>

Gram-negative organisms were predominately isolated 12(70.6%) and Gram positive organisms accounted for 5(29.4%) in our study and this corroborated with findings by Shrestha et al and kumaravel et al.<sup>[24,25]</sup> In contrast, Rashmi et al reported Gram-positive isolates to be more common.<sup>[4]</sup> In developing countries GBS is reported to be rare, similarly, in our study also we did not isolate any GBS.

Among Gram negative isolates of total 12(70.6%), 6(50%) were Klebsiella pneumoniae. 4 (33.3%) isolates were Acinetobacter species, 1 (8.3%) was Pseudomonas aeruginosa and 1 (8.3%) was Escherichia coli. (%). Klebsiella pneumoniae was the most common cause of Gram-negative sepsis followed by Acinetobacter spp. similar to other studies.<sup>[14,21]</sup> Among Gram positive isolates of total 5(29.4%), the most common isolate was Coagulase



negative *Staphylococcus* 3(60%), followed by *Staphylococcus aureus* 2 (40%).

All the Gram negative isolates were found to be sensitive to Meropenem (100 %). Our study also revealed a large number of organisms exhibiting resistance to many of the antibiotics similar to those reported in recent studies.<sup>[14,21,4]</sup> Among the 7 enterobacteriales, 4 isolates (57%) were found to be ESBL producers. Gram-positive bacteria isolated in our study showed highest sensitivity to linezolid and vancomycin (100%), consistent with other findings (26). Out of 2 *Staphylococcus aureus* isolated, 1(50%) isolate was methicillin resistant and out of 3 Coagulase negative *Staphylococcus* isolated, 2 (66.7%) isolates were methicillin resistant, consistent with the findings of Rashmi P et al where methicillin resistance was seen in 97.36% of CONS and 66.66% of *Staphylococcus aureus*.<sup>[4]</sup>

In this study, 98 (49.0%) mothers had PROM lasting more than 18 hours before labour and 67 (33.5%) had maternal fever and 35 (17.5%) had maternal UTI as risk factors. Of the 17 culture-positive neonatal sepsis, 11 (64.7%) had PROM as a maternal risk factor which is similar in a study done by Pokhrel et al.<sup>[26,27]</sup> PROM is an important risk factor for both EOS and preterm births. This may be due to increased risk of the chance of ascending infection from the birth canal into the amniotic fluid.

In the present study, Prematurity was the most common neonatal risk factor observed in 76.5% of the culture positive neonatal sepsis, followed by low birth weight in 70.6%, birth asphyxia in 35.3% and meconium stained liquor in 23.5%. It is observed that the incidence of culture-positive neonatal sepsis is more among low birth weight neonates (70.6%) and the incidence is inversely proportional to the birth weight as seen in Table 6. Prematurity predispose to high risk of neonatal sepsis which is in agreement with Manadhar et al,<sup>[28]</sup> results, that may be due to underdeveloped immune responses as well as a lack of maternally produced passively acquired antibodies.

## CONCLUSION

The aetiological agents of neonatal sepsis are different in different circumstances. Hence, the antibiotics used should be specific based on culture and sensitivity. Gram negative organisms were most commonly isolated in neonatal sepsis and they were most sensitive to carbapenem and piperacillin-tazobactam whereas linezolid and vancomycin were most effective against the Gram-positive cocci isolated in the study. Every hospital should monitor its antibiotic sensitivity pattern against the common isolates that can serve as a basis for empirical therapy in emergency situations. Sepsis related mortality may be reduced by maintenance of a pathogen-limited neonatal environment during and after delivery and identifying high risk neonates and targeting them for intensive care and therapy. To

supplement the management of sepsis in neonates, surveillance of the NICUs should be done and periodic guidelines for empirical treatment should be formulated.

**Conflict of Interest:** None declared

**Source of support:** Partially by TNSRC, Chennai.

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