



## Bacteriological Profile and Antibiotic Susceptibility and Outcome of Neonatal Sepsis in Special Newborn Care Unit of a Tertiary Care Hospital in Central India

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### ABSTRACT

Neonatal sepsis is a prominent contributor to neonatal mortality on a global scale, particularly in developing nations such as India. Sepsis, encompassing pneumonia, was found to be accountable for 17.4% of neonatal fatalities and ranks third among the primary causes of neonatal mortality in India. The objective of this study is to ascertain the profile, antibiotic susceptibility pattern and clinical outcome of neonatal sepsis cases that have been confirmed. Study Design Prospective observational studies collect data on a group of people in the future. The study was conducted in the Department of Paediatrics, MGM Medical College and associated Chacha Nehru Balchikitsalaya and Maharaja Yashwant Rao Hospital, a tertiary care hospital, over a period of 1 year. About 394 babies blood samples were collected, processed and analysed from clinically suspected neonates who were admitted to a special newborn care unit. They were enrolled and followed until the outcome. The variables that were documented in this study included microorganisms that were isolated from blood cultures, antibiotic susceptibility and follow-up until the final outcome. Among the 394 blood samples analysed, the rate of culture positivity was found to be 19.8%. The study revealed that 71.1% of the subjects were male while 28.9% were female. In terms of birthplace, 64.2% were born outside the hospital while 35.8% were born inside. Furthermore, 53.8% of the subjects were born prematurely while 46.2% were born at full term. Additionally, 65.4% of the subjects had low birth weight while 34.6% had appropriate weight at birth. The prevalence of gram-negative microorganisms was 70.5%, while gram-positive microorganisms accounted for 28.2% of the sample. Candida species were detected in 1.3% of the specimens. The prevalent isolates were *Klebsiella*, *E. coli* and *Staphylococcus*, accounting for 32.1, 17.9 and 12.8% of the total, respectively. The study revealed that half of the gram-negative isolates exhibited resistance towards ampicillin, piperacillin tazobactam and cephalosporin, while all of them demonstrated sensitivity towards colistin. Half of the Gram-positive bacteria exhibited methicillin-resistant *Staphylococcus aureus* (MRSA) characteristics, while all of them demonstrated susceptibility to vancomycin. Following the conclusion of the treatment regimen, a majority of 87.2% of patients were discharged, while a minority of 9.9% chose to leave the medical facility against medical advice. Additionally, a small percentage of 3.8% of patients expired. Neonatal sepsis is predominantly caused by Gram-negative microorganisms. The majority of these microorganisms exhibit resistance to first-line antibiotics that are commonly employed, thereby rendering empirical treatment strategies ineffective. The implementation of regular monitoring could prove to be a valuable strategy in the fight against antibiotic resistance. The present study aims to investigate the bacterial profile and antibiotic susceptibility patterns in relation to multidrug resistance. Additionally, the study seeks to evaluate the effectiveness of the current antibiotic policy in addressing the issue of multidrug resistance.

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#### Key Words

Antibiotic, newborn, India, neonatal, *Klebsiella* spp.

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## INTRODUCTION

Neonatal sepsis is a prominent contributor to neonatal mortality on a global scale, particularly in developing nations such as India. As per the findings of the Delhi Neonatal Infection Study (DeNIS) collaboration in 2016, the occurrence of neonatal sepsis was observed to be 14.3% in this particular cohort study. Moreover, the rate of culture-positive sepsis was recorded to be 6.2%. According to a study, sepsis was responsible for approximately 25% of neonatal mortality<sup>[1]</sup>. According to recent data, infections such as neonatal pneumonia and sepsis have been identified as the third most significant cause of neonatal mortality in India, accounting for 17.4% of such deaths. The management of sepsis has become a growing global concern due to the rise in antimicrobial resistance. The management of neonatal sepsis has become a significant challenge due to the emergence of antimicrobial resistance, which has resulted in a limited reserve of antibiotics. Comprehending the prevailing bacterial isolates and their susceptibility patterns to antibiotics holds significant importance in selecting the suitable empirical antibiotic therapy to mitigate the morbidity and mortality linked with sepsis. The objective of this study is to ascertain the bacterial isolate profile, antibiotic sensitivity pattern and confirmed neonatal sepsis outcome in a tertiary care hospital located in the central region of India, in light of the emergence of antimicrobial resistance.

## MATERIALS AND METHODS

The aforementioned study was an observational investigation carried out in the Department of Paediatrics at MGM Medical College Indore, along with the affiliated Maharaja Yashwant Rao Hospital (M.Y.H.) and Chacha Nehru BalChikitsalaya (CNBC). The research was carried out over a duration of 12 months, spanning from January 2019 to January 2020.

Inclusion criteria A neonate who is suspected to have a clinical condition based on the presence of one or more of the following signs and symptoms:

- The clinical manifestations observed in the patient include refusal to feed, poor crying and lethargy
- The patient's condition is characterised by the presence of fever, defined as a body temperature greater than 37.5°C, or hypothermia, defined as a body temperature below 36.5°C
- The simultaneous occurrence of diarrhoea and vomiting
- The manifestation of respiratory distress, apnea and gasping respiration
- The presence of tachycardia, defined as a heart rate greater than 160 beats per minute and hypotension, defined as a blood pressure below the 10th percentile

## Exclusion criteria:

- Infant over 28 days old
- A neonate who is receiving antibiotics
- Neonates exhibiting indications of septicemia subsequent to hospitalisation

A cohort of 394 neonates was identified based on the established inclusion and exclusion criteria. The variables that were documented encompassed neonatal history, which comprised sex, gestational age, birth weight, term or preterm status, as well as outborn or inborn status. The study collected data on maternal risk factors for neonatal sepsis, which included the duration of labour, mode of delivery, maternal fever, chorioamnionitis characterised by foul-smelling liquor, maternal urinary tract infection (UTI) and duration of rupture of membrane. The study utilised a structured proforma to gather data, which was subsequently classified, analysed and evaluated using SPSS version 21 for Windows in accordance with the research aims and objectives.

A percutaneous venous puncture was performed to obtain approximately 1-2 mL of venous blood, with adherence to strict aseptic precautions utilising the 3 swab technique. The obtained blood was then aseptically introduced into blood culture bottles containing 5-10 mL of brain and heart infusion broth. The blood culture bottles were subjected to aerobic incubation at 37°C for a period of one night. The primary subcultures were obtained subsequent to a 24 hrs incubation period on both blood agar and Mac-Conkey agar. In cases where no growth was observed on the plates following overnight incubation, the bottles were subjected to additional incubation. The broth was subsequently monitored on a daily basis and a final subculture was performed either on the seventh day or upon the emergence of indications of growth, whichever event occurred earlier. The identification of positive growth was carried out using conventional techniques in accordance with established laboratory protocols. These methods included the assessment of colony morphology, Gramme staining and biochemical reactions. Following the identification of bacteria, antimicrobial susceptibility testing was conducted using the Kirby-Bauer disc diffusion method on Muller Hinton agar in accordance with the guidelines established by the Clinical and Laboratory Standards Institute (CLSI). The antibiotics employed in our study were in accordance with the hospital's customary protocol and departmental guidelines, as specified by the Clinical and Laboratory Standards Institute (CLSI).

## RESULTS

This study aims to provide an overview of the general characteristics and clinical profile of the subjects under investigation. Throughout the

designated research time frame, a total of 10,508 live births were recorded, with 2558 admissions to the neonatal unit, encompassing both inborn and outborn cases. A cohort of 394 infants were registered, meeting the established inclusion criteria. Blood cultures were conducted in 394 neonates. Among the 394 blood cultures collected from infants, 22.6% (n = 89) exhibited the presence of one or more microorganisms, while 54.1% (n = 213) were deemed bacteriologically sterile. Additionally, 3.6% (n = 14) of the cultures yielded contaminants.

Among the 78 sepsis episodes that were confirmed bacteriologically, 64.2% (50/78) were found to occur in outborn infants, while 35.8% (28/78) were observed in inborn infants. A majority of the infants, specifically 53.8%, were born preterm, while 65.4% of them had low birth weight. The incidence of sepsis was comparatively greater in neonates with very low birth weight and premature birth, albeit lacking statistical significance. 50% of the infants experienced early onset neonatal sepsis, while the other 50% experienced late-onset neonatal sepsis. The intramural birth cohort exhibited an incidence rate of 7.4 per 1000 live births for confirmed neonatal sepsis, encompassing both early and late onset cases. During the study period, the unit exhibited a crude infection rate of 15.4% (394/2558), with a confirmed sepsis rate of 19.7% (78/394). Table 1 presents the fundamental features of neonates with confirmed neonatal sepsis based on culture results. The most frequent clinical manifestations observed in the study population were refusal to feed, which was reported in 25 cases (32.1%), followed by respiratory distress in 17 cases (21.8%), diarrhoea, regurgitation/vomiting in 14 cases (17.9%), lethargy in 13 cases (16.7%) and fever in 9 cases (11.5%). The majority of infants exhibited multiple co-morbidities. Severe co-morbidities were frequently observed to include perinatal asphyxia, respiratory distress syndrome, meconium aspiration syndrome, patent ductus arteriosus and shock. Three infants, accounting for 3.8% of the total, experienced mortality, while seven infants, representing 9% of the total, departed from medical advice, with unknown outcomes. Among the maternal risk factors associated with neonatal sepsis, the most prevalent was the rupture of membranes lasting over 24 hrs, accounting for 5 cases (11.55%). This was followed by maternal urinary tract infection in 7 cases (8.9%), maternal fever in 5 cases (6.4%) and foul-smelling amniotic fluid in 3 cases (3.8%).

**Bacterial profile:** Table 2 presents a comprehensive examination of the 78 culture isolates. The observed microorganisms comprised of gram-negative bacilli (55/78, 70.5%), gram-positive cocci (22/78, 28.2%) and *Candida* species (1/78, 1.3%). The microorganisms

Table 1: Baseline characteristic

Parameters	Values	Percentage
Age of onset of sepsis (days) median (range)	2	1-29
<b>Sex</b>		
Males	56	71.7
Females	22	28.3
<b>Birth weight (g)</b>		
>2500 g	27	34.6
2499-1500 g	30	38.4
1499-1000 g	16	20.6
<1000 g	5	6.4
<b>Gestational age (weeks)</b>		
≥37 weeks	36	46.2
<37 weeks	42	53.8
<b>Type of delivery</b>		
Spontaneous vaginal delivery	57	73.1
Caesarean section	21	26.9
<b>Place of delivery</b>		
Inborn	28	35.8
Outborn	50	64.2
<b>Maternal risk factor</b>		
Maternal fever	5	6.4
Foul smelling liquor	3	3.8
Maternal UTI	7	8.9
Rupture of membrane >24 hrs	9	11.5
<b>Onset of sepsis</b>		
Early onset	39	50
Late onset	39	50
<b>Duration of hospitalization (days)</b>		
Median (range)	15 (1-27)	
<b>Outcome</b>		
Discharge	68	87.2
Died	3	3.8
Leave against medical advice	7	9

Table 2: Distribution (frequency) of bacterial isolates (n = 78)

Bacterial isolates	Number	Percentage
Gram-negatives	55	70.5
<i>Klebsiella</i> species	25	32.1
<i>Escherichia coli</i>	14	17.9
<i>Pseudomonas</i>	11	14.1
<i>Citrobacter</i>	05	6.4
Gram-positives	22	28.2
<i>Staphylococcus aureus</i>	10	12.8
<i>Staphylococcus haemolyticus</i>	04	5.2
CONS	03	3.8
<i>Non-hemolytic streptococcus</i>	03	3.8
<i>Enterococcus</i>	02	2.6
<b>Candida</b>		
Albicans	1	1.3
Total	78	100.0

CONS: Coagulase negative staphylococci

*Klebsiella*, *E. coli* and *Staphylococcus* were identified as the primary causative agents of infections, with respective proportions of 32.1% (25/78), 17.9% (14/78) and 12.8% (10/78) of the isolates. Several Gram-negative isolates were identified, including *Pseudomonas* (11/78, 14.1%), *Citrobacter* (5/78, 6.4%) and *Staphylococcus hemolyticus* (04/78, 5.2%), CONS (03/78, 3.8%), non-hemolytic streptococcus (03/78, 3.8%) and *Enterococcus* (02/78, 2.6%). There was no discernible disparity noted in the causation of sepsis between the early and late onset forms. *Candida* species were identified from a single blood culture, treated with voriconazole and discharged with favourable outcomes.

**Pattern of susceptibility to antibiotics:** Table 3 and 4 display the antibiotic sensitivity pattern of Gram-positive and Gram-negative organisms, respectively. Multidrug resistance was observed to be more

Table 3: Antimicrobial pattern of gram-positive organism

Antimicrobials	<i>Staphylococcus aureus</i> (n = 10)	<i>Staphylococcus hemolyticus</i> (n = 04)	CONS (n = 03)	<i>Streptococci</i> (n = 03)	<i>Enterococcus</i> (n = 02)	Total
Cefoxitin	5/10	2/4	2/3	0/1	0	9/18 (50%)
Cefotaxime	0/1	1/1	1/3	0/1	0	2/6 (33.3%)
Ciprofloxacin	1/5	0/4	3/3	0/1	0/2	4/15 (26.7%)
Clindamycin	3/10	2/4	3/3	1/3	0	9/20 (45%)
Doxycycline	8/10	3/4	3/3	3/3	1/1	18/21 (85.7%)
Erythromycin	3/10	4/4	2/3	1/1	0/2	10/20 (50%)
Gentamycin	6/10	4/4	3/3	2/3	0/2	15/22 (68.2%)
Linezolid	9/10	4/4	3/3	3/3	1/2	20/22 (90.9%)
Vancomycin	10/10	4/4	3/3	3/3	2/2	22/22 (100%)
Cotrimoxazole	2/4	2/4	1/3	1/1	0	6/12 (50%)
Chlormphenicol	0/0	0/0	0/0	0/0	2/2	2/2 (100%)

Table 4:Antimicrobial sensitivity pattern of Gram negative Organism

Antimicrobials	<i>Klebsiella</i> (n = 25)	<i>Escherichia coli</i> (n=14)	<i>Pseudomonas</i> (n = 11)	<i>Citrobacter</i> (n = 5)	Total
Cefepime	2/8	1/6	2/2	0	5/16 (31.3%)
Gentamycin	3/4	3/8	2/2	4/4	12/18 (66.7%)
Cotrimoxazole	6/9	0/1	0	0	6/9 (66.7%)
Ampicillin	5/9	1/4	0/1	0	6/14 (42.9%)
Ceftriaxone	3/14	2/5	1/3	0	6/22 (27.3%)
Pip-tazo	6/14	3/7	3/3	0	12/24 (50%)
Amikacin	11/18	6/11	7/7	5/5	29/41 (70.7%)
Imipenam	9/18	6/9	3/3	5/5	23/35 (65.7%)
Levofloxacin	10/18	5/8	2/2	2/5	19/33 (57.8%)
Colistin	3/3	3/3	0/3	0	6/9 (66.7%)
Meropenam	5/7	0/1	0	0	5/8 (62.5%)
Ceftazidime	1/6	1/5	0	0	2/11 (18.2%)

prevalent among Gram-negative bacteria, with approximately 50% of the isolates exhibiting resistance to ampicillin, piperacillin tazobactam, ceftazidime, cefepime and ceftriaxone. All isolates, with the exception of pseudomonas, exhibited sensitivity to colistin. A sensitivity rate of over 70% was observed for gentamycin, amikacin, imipenam and levofloxacin among the isolates.

Within the cohort of gram-positive isolates, it was observed that half of the staphylococcal isolates were identified as methicillin-resistant *Staphylococcus aureus* (MRSA), while 60% of the isolates exhibited sensitivity to aminoglycosides. Additionally, 70% of the isolates demonstrated susceptibility to linezolid and all isolates were found to be susceptible to vancomycin.

**Limitations:** This study is a short-term, single-center investigation lasting for a duration of 12 months. Conducting a multicentric study would provide a more comprehensive understanding of the broad range of neonatal sepsis and antibiotic sensitivity patterns, thereby facilitating the formulation of an appropriate antibiotic policy for neonates.

## DISCUSSIONS

Neonatal septicemia is a significant contributor to neonatal morbidity and mortality in developing nations such as India. Bacterial isolates exhibit variability across distinct geographic regions and within the same hospital over varying time periods<sup>[2,3]</sup>. The findings of our investigation indicate that the rate of culture positivity was 19.8%, a figure that is consistent with the majority of previously documented studies, which have reported rates ranging from 13-22%<sup>[4-6]</sup>. The study

found that the confirmed occurrence of neonatal sepsis in the birth cohort was 7.4 per 1000 live births. This result is consistent with a previous study conducted in 17 level 3 neonatal units in Asia, which reported a sepsis incidence ranging from 3-15 per 1000 live births<sup>[7]</sup>. Similar findings have also been reported in other studies (reference 10). Among the 78 culture-confirmed infants, a higher proportion of males (71.7%) than females (28.3%), low birth weight (65.4%) than normal weight (34.6%) infants, preterm (53.8%) than term (46.2%) infants and outborn (64.2%) than inborn (35.8%) infants were found to be infected. Zakariya *et al.*<sup>[4]</sup> and Bhat *et al.*<sup>[8]</sup> have reported comparable outcomes of sepsis among male, preterm and low birth weight infants<sup>[4,8]</sup>. The most frequent clinical manifestation observed in 25 cases (32.1%) was the act of declining to consume food, followed by respiratory distress in 17 cases (21.8%), diarrhoea, regurgitation/vomiting in 14 cases (17.9%), lethargy in 13 cases (16.7%) and fever in 9 cases (11.5%). These findings align with the results reported by and Garg and Agrawal<sup>[9]</sup> and Roy *et al.*<sup>[10]</sup>. Among the maternal risk factors associated with neonatal sepsis, the most prevalent factor was the rupture of membranes for a duration exceeding 24 hrs, accounting for 11.55% of cases. This was followed by maternal urinary tract infection in 8.9% of cases, maternal fever in 6.4% of cases and foul-smelling amniotic fluid in 3.8% of cases. Comparable outcomes were identified in the investigation conducted by Roy *et al.*<sup>[10]</sup>.

The predominant isolates were gramme negative bacilli (55/78, 70.5%), gramme positive cocci (22/78, 28.2%) and *Candida* species (1/78, 1.3%). The earlier studies conducted by DeNIS<sup>[1]</sup>, Vishwanathan *et al.*<sup>[2]</sup>,

Shrestha *et al.*<sup>[3]</sup>, Zakariya *et al.*<sup>[4]</sup> and Iregbu *et al.*<sup>[6]</sup> yielded comparable findings. The predominant bacterial strains identified in our investigation were *Klebsiella* (32.1%), *E. coli* (17.9%) and *Staphylococcus* (12.8%). The obtained outcomes were consistent with those of prior investigations<sup>[1,2,4,6,8]</sup>. Multidrug resistance was observed to be prevalent among Gram-negative bacteria, with approximately 50% of the isolates exhibiting resistance to ampicillin, piperacillin tazobactam, ceftazidime, cefepime and ceftriaxone. Previous study has yielded comparable outcomes<sup>[1,11]</sup>. All isolates, with the exception of *Pseudomonas*, exhibited sensitivity to colistin. The sensitivity rates for gentamycin, amikacin, imipenam and levofloxacin were over 70%, which is consistent with findings reported by Madavi *et al.*<sup>[12]</sup> and Basavara *et al.*<sup>[13]</sup>. Previous studies have reported the presence of multidrug resistant *Klebsiella*, *Acinetobacter* and *E. coli*.<sup>[2,4,6,8,14]</sup> Within the group of gram-positive isolates, it was observed that half of the staphylococcal strains were identified as methicillin-resistant *Staphylococcus aureus* (MRSA). Previous research has reported a comparable level of initial antibiotic resistance, as evidenced by studies<sup>[2,4]</sup>. Aminoglycosides exhibit sensitivity in over 60% of gram-positive isolates, while linezolid displays sensitivity in 70% and vancomycin in 100%. These findings are consistent with the results reported in previous studies conducted by Shah *et al.*<sup>[11]</sup>, Jagoo *et al.*<sup>[15]</sup> and Sheth *et al.*<sup>[16]</sup>. Among the 78 neonates whose cultures were confirmed, three expired due to significant co-morbidities necessitating ionotropic support and ventilatory care. Additionally, seven neonates were discharged against medical advice and their outcomes remain unknown. The research conducted revealed that neonatal sepsis is predominantly caused by gram-negative organisms. The emergence of multidrug-resistant isolates that exhibit a high level of resistance to commonly used antibiotics poses a significant challenge in the management of neonatal sepsis and continence. To address this issue, it is imperative to implement antibiotic stewardship measures.

### CONCLUSION

The findings of the current investigation demonstrate that neonatal sepsis is primarily caused by gram negative organisms, with *Klebsiella* spp. being the predominant pathogen. Gramme positive organisms, specifically Staphylococci, were also identified as a common cause of neonatal sepsis. The majority of the isolates exhibit resistance to the commonly prescribed first-line antibiotics. Antibiotic resistance is a widespread issue that results in the

inefficacy of empirical therapy. Regular monitoring and surveillance in accordance with established protocols is imperative to ascertain the prevailing trends in causative microorganisms and their antibiotic sensitivity patterns. Such information would be invaluable in determining an appropriate antibiotic policy. In order to address the issue of multidrug-resistant Gram-negative and Gram-positive isolates, it is necessary to administer higher doses of antibiotics such as carbapenems, colistin and vancomycin. Additionally, the use of linezolid should be reserved and employed with discretion. It is recommended that healthcare facilities conduct regular microbiological investigations to determine the prevailing microbial trends and their corresponding susceptibility patterns. The formulation and implementation of antibiotic policies would be highly beneficial.

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