



BACTERIOLOGICAL PROFILE AND ANTIBIOGRAM OF NEONATAL SEPSIS IN A TERTIARY NEONATAL INTENSIVE CARE UNIT OF CENTRAL INDIA: A CROSS-SECTIONAL OBSERVATIONAL STUDY

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ABSTRACT

Background: Neonatal sepsis is a leading cause of NICU mortality in India. Empirical antibiotic therapy guided by local bacteriological surveillance is essential given the rising prevalence of multidrug-resistant organisms. This study aimed to describe the bacteriological profile and antibiogram of neonatal sepsis at a tertiary NICU in central India. **Methods:** A single-centre, hospital-based, cross-sectional observational study was conducted over 18 months in the NICU of Amaltas Institute of Medical Sciences, Dewas. A total of 250 neonates aged 0–28 days with clinical suspicion of sepsis were enrolled consecutively. Blood culture, sepsis screen, and antibiotic susceptibility testing (CLSI disc diffusion) were performed for all. Sepsis was classified as proven (positive culture), probable (positive screen, negative culture), or no sepsis. **Results:** Blood culture was positive in 70 (28%) neonates. Proven sepsis was seen in 28%, probable sepsis in 48.40%, and no sepsis in 23.60%. Late-onset sepsis was slightly more common (54% vs 46%). *Acinetobacter* spp. was the most frequent isolate (25.71%), followed by *Klebsiella pneumoniae* (24.29%), CoNS (21.43%), *E. coli* (17.14%), and *S. aureus* (11.43%). Ampicillin showed the lowest sensitivity across all isolates. Polymyxin B, colistin, amikacin, and vancomycin/linezolid retained the highest activity. Maternal fever (39.2%), PROM (34.8%), and meconium-stained liquor (34.4%) were the most common risk factors. Mortality was 9.60%. **Conclusion:** Gram-negative organisms, particularly *Acinetobacter* and *Klebsiella*, dominate neonatal sepsis bacteriology at this NICU, with high ampicillin resistance. Colistin, polymyxin B, amikacin, and vancomycin/linezolid retain the best activity and should inform local empirical therapy guidelines. Continuous bacteriological surveillance and antimicrobial stewardship are essential for improving neonatal outcomes.

KEYWORDS

Neonatal Sepsis; Bacteriological Profile; Antibiogram; NICU; *Acinetobacter*; *Klebsiella*; Antimicrobial Resistance; Central India

INTRODUCTION

Neonatal sepsis remains one of the foremost causes of morbidity and mortality in neonatal intensive care units worldwide, particularly in low- and middle-income countries. The World Health Organization estimates that infections account for approximately 26% of all neonatal deaths globally, with sepsis and meningitis together responsible for a substantial fraction.^[1] In India, neonatal infections are disproportionately lethal, contributing significantly to the country's neonatal mortality rate of approximately 20 per 1000 live births.^[2] The burden is especially pronounced in tertiary NICUs that receive high-risk outborn neonates, where the combination of delayed referral, pre-hospital antibiotic exposure, invasive procedures, and cross-contamination creates a milieu favouring resistant and nosocomial pathogens. Neonatal sepsis is clinically classified into early-onset sepsis (EOS), occurring within 72 hours of birth and typically linked to vertical transmission from mother to neonate, and late-onset sepsis (LOS), arising after 72 hours and more commonly associated with environmental or nosocomial exposure.^[3] The bacteriological profile differs between these two subtypes and varies considerably across regions, institutions, and time periods, making local epidemiological data essential for guiding empirical antibiotic therapy.

Across Indian NICUs, Gram-negative organisms — particularly *Klebsiella pneumoniae*, *Acinetobacter*, and *E. coli* — have emerged as the predominant pathogens responsible for neonatal sepsis, often carrying extensive drug resistance including carbapenem resistance.^[4,5] The rising prevalence of multidrug-resistant (MDR) organisms has narrowed therapeutic options, making last-line agents such as polymyxins and tigecycline increasingly relevant in neonatal practice. Simultaneously, Gram-positive organisms including CoNS and *Staphylococcus aureus* continue to cause both community-acquired and device-associated sepsis in many settings.

Despite growing awareness of the problem, region-specific bacteriological data from central India, and from Madhya Pradesh in particular, remain limited. Empirical antibiotic policies at many institutions continue to be extrapolated from data collected in other geographic contexts, which may not reflect local resistance patterns. The present study was designed to address this gap by systematically describing the bacteriological profile, risk factors, clinical features, and antibiotic susceptibility patterns of neonatal sepsis at a tertiary NICU in Dewas, central India.

Aim

To identify the bacteriological profile and antibiogram of neonatal sepsis at a tertiary Neonatal Intensive Care Unit in central India.

MATERIAL AND METHODS

This was a single-centre, hospital-based, cross-sectional observational study. The study was conducted in the Neonatal Intensive Care Unit of the Department of Paediatrics, Amaltas Institute of Medical Sciences, Dewas, Madhya Pradesh — a tertiary care teaching hospital serving both inborn and outborn neonates from a broad catchment area in central India. The study was conducted over 18 months, with 12 months dedicated to participant recruitment and data collection. Ethical clearance was granted by the Institutional Ethics Committee of Amaltas Institute of Medical Sciences prior to participant recruitment. The study was conducted in accordance with the Declaration of Helsinki. Written informed consent was obtained from parents or legal guardians of all enrolled neonates in their preferred language before enrolment.

The primary outcome was identification of bacterial isolates from blood cultures of neonates with suspected sepsis, including culture positivity rate, species distribution, and early-onset versus late-onset classification. The secondary outcome was the antibiotic susceptibility pattern of each isolate. Outcome data including survival and mortality were also recorded. Neonatal sepsis was defined as a systemic infection in a neonate aged 0–28 days with clinical features including temperature instability, poor feeding, lethargy, respiratory distress, abnormal perfusion, or altered activity. Early-onset sepsis was defined as infection within the first 72 hours of life, linked to maternal or perinatal factors. Late-onset sepsis was infection developing after 72 hours. Proven sepsis required a positive blood culture. Probable or clinical sepsis was defined by positive sepsis screen with negative culture and suggestive clinical features. Antibiotic susceptibility was assessed by disc diffusion per CLSI standards.

Inclusion Criteria

1. Neonates born in or outside the institute who presented with signs or symptoms of sepsis on arrival or during their NICU stay.
2. Neonates with recognised maternal or perinatal risk factors: febrile illness in mother within two weeks before delivery, meconium-stained or foul-smelling liquor, prolonged rupture of membranes (≥ 18 hours), preterm pre-labour PROM, unclear or multiple vaginal examinations, and home or instrumental delivery.

3. Neonates with clinical manifestations of sepsis: hypothermia or fever, lethargy, poor suck, poor cry, abnormal perfusion, prolonged capillary refill time, absent reflexes, hypotonia, bradycardia or tachycardia, respiratory distress, apnoea or gasping, and abnormal glucose levels.
4. Neonates with system-specific features: feed intolerance, abdominal distension, seizures, bulging fontanelle, hepatomegaly, renal dysfunction, bleeding tendencies, or skin pustules.

Exclusion Criteria

1. Neonates with major congenital anomalies, particularly thoracic or cardiac defects.
2. Neonates who had received antibiotics during the two weeks preceding data collection.
3. Neonates whose parents or legal guardians were unavailable for interview.
4. Neonates with nasopharyngeal pathology including choanal atresia, cleft lip or palate.
5. Neonates who died within thirty days following surgery.
6. Moribund neonates expected to die within twenty-four hours after cardiopulmonary resuscitation.
7. Healthy neonates with no suggestive symptoms.

Sample Size and Sampling Methodology

Sample size was calculated using the formula $n = (Z\alpha/2)^2 \times p(1-p) / d^2$, with $Z = 1.96$ (95% CI), P based on anticipated prevalence from prior studies, and d representing acceptable margin of error. The calculated requirement was 250 neonates. A non-random, consecutive convenience sampling method was used; eligible neonates admitted to the NICU during the 12-month recruitment period were enrolled as they presented.

Data Collection Procedure

Following consent, a structured proforma was completed capturing maternal details, perinatal risk factors, gestational age, birth weight, mode of delivery, clinical signs and symptoms, and sepsis classification. Blood culture was collected under strict aseptic precautions. Samples were inoculated onto 5% sheep blood agar, MacConkey agar, and chocolate agar. Gram staining and standard biochemical tests (catalase, oxidase, indole, methyl red, citrate, urease, triple sugar iron) were used for organism identification. Antibiotic susceptibility testing was performed using the Mueller–Hinton agar disc diffusion method per CLSI guidelines, using commercial discs from HiMedia Laboratories, Mumbai. Sepsis screen (CRP, total leucocyte count, band cells, micro-ESR, platelet count) was performed for all enrolled neonates.

Statistical Analysis

Data were entered into Microsoft Excel and analysed using Stata version 17.0. Categorical variables are presented as frequencies and percentages. No inferential testing was pre-specified for the descriptive primary outcomes; all percentages are reported as proportions of the relevant denominator.

RESULTS

A total of 250 neonates aged 0–28 days were enrolled and analysed. All participants had complete clinical assessment, sepsis screen, and blood culture results. The detailed findings are presented below.

Table 1: Characteristics of Participants

Category	Frequency (n)	Percentage (%)
Age at Presentation		
0–7 days	164	65.60
8–14 days	50	20.00
15–21 days	18	7.20
22–28 days	18	7.20
Gender		
Male	141	56.40
Female	109	43.60
Birth Weight		
Normal (2500–3999 g)	186	74.40
Low birth weight (1500–2499 g)	64	25.60
Mode of Delivery		
LSCS	149	59.60
Normal vaginal delivery	101	40.40

Table 1 shows the baseline characteristics of the 250 neonates. The 0–7 day age group was the largest (65.60%), consistent with the

predominance of early and peri-natal presentations. Male neonates constituted 56.40% of the cohort. Normal birth weight neonates represented 74.40%, with 25.60% in the low birth weight category. LSCS was the most common mode of delivery (59.60%).

Table 2: Type of Sepsis, Classification, Blood Culture and Outcome

Parameter	Frequency (n)	Percentage (%)
Type of Sepsis		
Early-onset sepsis (≤ 72 h)	115	46.00
Late-onset sepsis (> 72 h)	135	54.00
Sepsis Classification		
Proven sepsis (culture positive)	70	28.00
Probable sepsis (screen positive, culture negative)	121	48.40
No sepsis (both negative)	59	23.60
Blood Culture Result		
No growth	180	72.00
Culture positive	70	28.00
Sepsis Screen Result		
Positive	121	48.40
Negative	129	51.60
Outcome		
Survived	226	90.40
Died	24	9.60

Table 2 shows that late-onset sepsis slightly predominated (54% vs 46%). Proven sepsis was confirmed by positive blood culture in 70 (28%) neonates; probable sepsis (positive screen, negative culture) in 121 (48.40%); and no sepsis in 59 (23.60%). The sepsis screen was positive in 48.40% of all participants. Mortality was 9.60%, with 226 (90.40%) neonates surviving to discharge.

Table 3: Maternal Risk Factors for Neonatal Sepsis

Maternal Risk Factor	Frequency (n)	Percentage (%)
Maternal fever	98	39.2
Prolonged rupture of membranes (≥ 18 h)	87	34.8
Meconium-stained amniotic fluid	86	34.4
Maternal urinary tract infection	76	30.4
Foul-smelling amniotic fluid	51	20.4
Chorioamnionitis	47	18.8

Table 3 presents maternal risk factors. Maternal fever was the most common (39.2%), followed by prolonged rupture of membranes (34.8%), meconium-stained amniotic fluid (34.4%), maternal UTI (30.4%), foul-smelling amniotic fluid (20.4%), and chorioamnionitis (18.8%). Multiple risk factors coexisted in many mothers.

Table 4: Clinical Signs and Symptoms at Presentation

Sign / Symptom	Frequency (n)	Percentage (%)
Lethargy	173	69.2
Respiratory distress	150	60.0
Poor feeding	134	53.6
Poor or excessive cry	118	47.2
Hypotonia	81	32.4
Abdominal distension	78	31.2
Seizures	47	18.8
Bulging fontanelle	44	17.6

Table 4 shows the clinical presentation. Lethargy was the most common feature (69.2%), followed by respiratory distress (60%), poor feeding (53.6%), poor or excessive cry (47.2%), hypotonia (32.4%), and abdominal distension (31.2%). Seizures were present in 18.8% and bulging fontanelle in 17.6%.

Table 5: Bacterial Isolates from Culture-Positive Neonates (n = 70)

Organism (n = 70 culture-positive)	Frequency (n)	Percentage (%)
Acinetobacter spp.	18	25.71
Klebsiella pneumoniae	17	24.29
Coagulase-negative Staphylococci (CoNS)	15	21.43
Escherichia coli	12	17.14
Staphylococcus aureus	8	11.43

Table 5 shows the bacteriological profile of the 70 culture-positive neonates. Acinetobacter spp. was the most frequent isolate (25.71%), followed closely by Klebsiella pneumoniae (24.29%), CoNS

(21.43%), *E. coli* (17.14%), and *Staphylococcus aureus* (11.43%). Gram-negative organisms collectively accounted for 67.14% of all isolates.

Table 6: Antibiotic Sensitivity Profile of Isolated Organisms (% Sensitive)

Antibiotic	Kleb n=17 %S	CoNS n=15 %S	Acin n=18 %S	S.aur n=8 %S	E.coli n=12 %S
Ampicillin	5.9	13.3	0	50.0	25.0
Amikacin	94.1	73.3	94.4	75.0	58.3
Levofloxacin	82.4	73.3	94.4	87.5	58.3
Meropenem	70.6	100	88.9	75.0	75.0
Vancomycin	100	80.0	72.2	87.5	100
Linezolid	64.7	60.0	72.2	87.5	83.3
Polymyxin B	100	66.7	94.4	62.5	66.7
Colistin	88.2	80.0	88.9	87.5	91.7
Tigecycline	76.5	53.3	72.2	50.0	66.7

%S = percentage of isolates sensitive. Kleb = *Klebsiella pneumoniae*; Acin = *Acinetobacter* spp.; S.aur = *S. aureus*

Table 6 presents the consolidated antibiogram for all five isolates. Ampicillin showed the lowest sensitivity across all Gram-negative organisms (*Acinetobacter* 0%, *Klebsiella* 5.9%, *E. coli* 25%), confirming near-universal resistance. Polymyxin B and amikacin retained high activity against *Klebsiella* and *Acinetobacter* (100% and 94.1–94.4% respectively). Colistin showed consistently high sensitivity across all organisms (88.2–91.7%). Meropenem retained good activity, particularly against CoNS (100%), *Acinetobacter* (88.9%), and *E. coli* (75%). Vancomycin showed 100% sensitivity for *Klebsiella* and *E. coli* and remained effective against Gram-positive organisms. Linezolid showed broad-spectrum activity (60–87.5%) across all organisms.

DISCUSSION

This cross-sectional study enrolled 250 neonates with clinical suspicion of sepsis at a tertiary NICU in Dewas, central India, and systematically characterised their bacteriological profile, risk factors, clinical presentation, antibiogram, and outcomes. Blood culture was positive in 28%, with *Acinetobacter* spp. being the most common pathogen. Ampicillin resistance was near-universal among Gram-negative organisms, while colistin, polymyxin B, amikacin, and vancomycin retained the best activity. Mortality was 9.60%.

Blood Culture Positivity and Sepsis Classification

The blood culture positivity rate of 28% in the present study is higher than the range reported in many Indian studies. Thapa S et al. (2019) reported positivity in only 10.8% of 516 suspected neonates,^[9] and Yadav NS et al. (2018) found 16.9% of 350 suspected cases to be culture-positive.^[10] Mahesh Kumar T et al. (2024) reported a similar rate of 10.8%.^[11] The higher rate in the present study may reflect a more severely ill referral population at this NICU, or differences in culture technique and blood volume inoculated. Pandit BR et al. (2020) reported 30.25% positivity among 1200 suspected neonates,^[12] closer to the present finding, and noted that the majority of cases were managed as probable sepsis — a pattern mirrored in the present study where probable sepsis (48.40%) exceeded proven sepsis (28%).

Early-Onset Versus Late-Onset Sepsis

Late-onset sepsis was slightly more prevalent (54% vs 46%) in the present cohort. This near-equal distribution likely reflects the combined burden of vertical transmission from maternal risk factors and horizontal nosocomial acquisition within the NICU. Thapa S et al. (2019) similarly reported LOS in 62.5% of cases,^[9] and Pokhrel B et al. (2018) found LOS in 78.3% of their predominantly preterm cohort.^[13] In contrast, Pandit BR et al. (2020) observed a predominance of EOS (79.89%),^[12] possibly reflecting differences in antenatal care quality and the proportion of outborn referrals. The higher EOS rate in some studies may also be explained by the preponderance of 0–7 day age group neonates (65.60%) in the present cohort, who predominantly present with early infections.

Maternal Risk Factors

Maternal fever (39.2%), PROM (34.8%), and meconium-stained liquor (34.4%) were the most common risk factors. This pattern is consistent with established pathophysiological pathways: maternal fever facilitates ascending bacterial colonisation of the amniotic cavity; PROM breaches the mechanical barrier protecting the fetus from vaginal flora; and meconium-stained liquor is associated with

fetal distress and aspiration risk.^[13] Maternal UTI, documented in 30.4%, is also a recognised risk factor for vertical transmission of uropathogens. Chorioamnionitis, present in 18.8%, directly seeds the amniotic cavity and fetal surfaces with pathogenic organisms. Similar patterns of maternal risk factors have been reported by Pandit BR et al. (2020)^[12] and Mahesh Kumar T et al. (2024),^[11] confirming the consistency of these associations across Indian NICU settings.

Bacteriological Profile

Gram-negative organisms dominated, accounting for 67.14% of all isolates. *Acinetobacter* spp. was the most common (25.71%), followed by *Klebsiella pneumoniae* (24.29%), *E. coli* (17.14%), CoNS (21.43%), and *S. aureus* (11.43%). The dominance of *Acinetobacter* in the present study is consistent with its emergence as a major nosocomial pathogen in Indian NICUs, driven by its capacity for environmental survival, biofilm formation, and rapid acquisition of resistance determinants.^[4] Thapa S et al. (2019) reported CoNS (35.7%) and *Klebsiella* (28.6%) as the dominant organisms,^[9] while Yadav NS et al. (2018) identified *Klebsiella* as the leading pathogen (39.0%).^[10] Pokhrel B et al. (2018) also found *Klebsiella* predominant, with *E. coli* and *Acinetobacter* as subsequent isolates.^[13] Mahesh Kumar T et al. (2024) reported *Klebsiella* as the most common (41.7%), followed by *E. coli* (25.0%).^[11] The variation in species distribution across studies reflects temporal trends, geographic differences, and institutional infection control practices. The high proportion of *Acinetobacter* in the present study suggests significant nosocomial transmission within the NICU environment and highlights the need for enhanced surface decontamination, hand hygiene protocols, and rational antibiotic stewardship.

Antibiogram and Antimicrobial Resistance

Ampicillin showed near-universal resistance in Gram-negative organisms (*Acinetobacter* 0%, *Klebsiella* 5.9%, *E. coli* 25%), confirming that this antibiotic is no longer suitable as empirical monotherapy for neonatal sepsis at this centre. This mirrors findings across Indian NICU settings, where ampicillin resistance has been documented in 80–100% of Gram-negative isolates in recent series.^[5,10] Polymyxin B and colistin retained the best Gram-negative activity: *Klebsiella* 100% and 88.2%; *Acinetobacter* 94.4% and 88.9%; *E. coli* 66.7% and 91.7% respectively. Amikacin also retained strong activity against *Klebsiella* (94.1%) and *Acinetobacter* (94.4%), making it a valuable component of empirical Gram-negative coverage. Meropenem retained good activity, particularly against CoNS (100%), supporting its role as a broad-spectrum backbone for severe neonatal sepsis. For Gram-positive organisms, vancomycin (*Klebsiella* 100%, *S. aureus* 87.5%), linezolid (CoNS 60%, *S. aureus* 87.5%), and colistin (*S. aureus* 87.5%, CoNS 80%) showed consistent activity. Tigecycline retained moderate activity (50–76.5%), consistent with its role as a reserve agent. These findings collectively suggest that empirical neonatal sepsis therapy at this centre should combine amikacin or a carbapenem for Gram-negative coverage with vancomycin or linezolid for Gram-positive cover, while reserving polymyxins and colistin for confirmed MDR infections. Pandit BR et al. (2020)^[12] and Yadav NS et al. (2018)^[10] similarly reported high resistance to penicillins and early-generation cephalosporins, with aminoglycosides and carbapenems retaining better activity.

Mortality

Mortality was 9.60% (24/250). This compares favourably with rates of 10–25% reported from other Indian NICUs managing similarly complex neonatal populations.^[6,7] The majority (90.40%) survived to discharge, likely reflecting the relatively structured supportive care protocols and the availability of microbiological guidance for antibiotic rationalisation at this centre. Higher mortality is expected in settings with a greater proportion of preterm or VLBW neonates or with more severely resistant organisms.

CONCLUSION

Neonatal sepsis at this tertiary NICU in central India is predominantly caused by Gram-negative organisms, with *Acinetobacter* spp. (25.71%) and *Klebsiella pneumoniae* (24.29%) being the most common isolates. Ampicillin resistance is near-universal among Gram-negative bacteria, rendering it unsuitable as empirical monotherapy. Polymyxin B, colistin, and amikacin retain the best Gram-negative activity; vancomycin and linezolid remain reliable for Gram-positive sepsis. Maternal fever, PROM, and meconium-stained liquor are the most common risk factors. Overall mortality was 9.60%. These findings support the urgent need for local antibiogram-guided

empirical therapy guidelines, enhanced infection control practices, and a sustained antimicrobial stewardship programme to preserve the efficacy of last-line agents in this resource-constrained NICU setting.

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