Bacteriological Profile and Antibiotic Susceptibility Pattern of Blood Culture and Other Sterile Fluid Culture Isolates in Preterm and Term Neonates with Neonatal Sepsis

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ABSTRACT

Background: Neonatal sepsis continues to be a major contributor to neonatal morbidity and mortality, particularly among preterm and low birth weight infants. The evolving spectrum of causative organisms and increasing antimicrobial resistance necessitate regular surveillance of local bacteriological profiles and antibiotic susceptibility patterns to optimize empirical treatment strategies.

Aim: To evaluate the bacteriological profile and antimicrobial susceptibility pattern among culture-positive preterm and term neonates with sepsis.

Materials and Methods: A prospective analytical observational study was conducted in the Neonatal Intensive Care Unit (NICU), Department of Paediatrics, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Haryana, from October 2024 to December 2025. Neonates with clinical suspicion of sepsis were enrolled after obtaining informed consent. Blood cultures and, where indicated, cultures of sterile body fluids were obtained under aseptic conditions prior to initiation of antibiotic therapy. Isolates were identified using standard microbiological techniques, and antimicrobial susceptibility testing was performed according to established laboratory guidelines. Data were analyzed using SPSS version 21.

Results: Of the 220 neonates evaluated, 107 (48.6%) had culture-confirmed sepsis. Late-onset neonatal sepsis was more frequent than early-onset sepsis. Candida species constituted the largest group of isolates, with *Candida krusei* being the predominant fungal pathogen. Among bacterial isolates, *Acinetobacter baumannii* was the most common organism, followed by *Klebsiella pneumoniae* and *Serratia marcescens*. Gram-negative organisms demonstrated substantial resistance to several routinely used antibiotics, including piperacillin-tazobactam and carbapenems. Gram-positive isolates remained uniformly sensitive to vancomycin, linezolid, and teicoplanin, while all fungal isolates were sensitive to amphotericin B.

Conclusion: Neonatal sepsis remains a significant challenge in the NICU, with an increasing burden of late-onset infections, fungal sepsis, and multidrug-resistant gram-negative pathogens. Continuous local antibiogram surveillance, judicious antimicrobial use, and stringent infection control measures are essential to improve neonatal outcomes and limit the emergence of antimicrobial resistance.

Keywords: Neonatal sepsis, Blood culture, Antimicrobial susceptibility, Antimicrobial resistance, *Acinetobacter baumannii*, *Candida krusei*, Preterm neonates, Late-onset neonatal sepsis.

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Introduction:

Neonatal sepsis is a dysregulated host response to infection resulting in life-threatening organ dysfunction and remains a major cause of neonatal morbidity and mortality worldwide. Despite significant advances in neonatal care, neonatal mortality continues to contribute substantially to

infant deaths, particularly in low- and middle-income countries (LMICs), which account for nearly 99% of global neonatal mortality. Neonatal sepsis, along with prematurity and birth asphyxia, remains one of the leading causes of neonatal deaths worldwide. [1,2]

Neonates are particularly susceptible to severe infections because of their immature immune system, characterized by reduced complement activity, impaired cell-mediated immunity, and inadequate opsonization.[3] Delayed diagnosis, limited access to healthcare facilities, inadequate infection control practices, and uneven distribution of laboratory resources further contribute to adverse outcomes in developing countries.

The epidemiology of neonatal sepsis varies considerably across regions. In developed countries, Group B Streptococcus and coagulase-negative staphylococci are commonly implicated pathogens, whereas in developing countries, Gram-negative organisms such as *Klebsiella pneumoniae*, *Escherichia coli*, *Pseudomonas aeruginosa*, and *Acinetobacter* species, along with *Staphylococcus aureus*, predominate. Recent reports from South Asia have highlighted an increasing burden of neonatal sepsis caused by *Staphylococcus aureus* and *Acinetobacter baumannii*. [4,5]

The emergence of antimicrobial resistance (AMR) has further complicated the management of neonatal sepsis. The increasing prevalence of extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria, methicillin-resistant *Staphylococcus aureus* (MRSA), and carbapenem-resistant *Acinetobacter baumannii* poses a significant therapeutic challenge. [6,7]. Inadequate antimicrobial stewardship and infection control measures have contributed substantially to the spread of these resistant pathogens.[8]

Clinical manifestations of neonatal sepsis are often nonspecific and include temperature instability, poor feeding, lethargy, respiratory distress, jaundice, vomiting, abdominal distension, and seizures. [9] Blood culture remains the gold standard for diagnosis; however, prior antibiotic exposure and low bacterial yield may limit its diagnostic utility. Therefore, continuous surveillance of causative organisms and their antimicrobial susceptibility patterns is essential for timely diagnosis and appropriate empirical therapy.

Several maternal and neonatal factors increase the risk of neonatal sepsis. Maternal risk factors include prolonged rupture of membranes, chorioamnionitis, intrapartum fever, urinary tract infection, and inadequate antenatal care.[10,11] Neonatal risk factors include prematurity, low birth weight, birth asphyxia, prolonged hospitalization, invasive procedures, mechanical ventilation, and total parenteral nutrition.[12]

The microbial profile and antibiotic susceptibility patterns of neonatal sepsis vary across geographical regions and healthcare settings, necessitating periodic local surveillance. Understanding the bacteriological spectrum and resistance patterns is crucial for guiding empirical antibiotic therapy, optimizing antimicrobial stewardship, and improving neonatal outcomes. Therefore, the present study was undertaken to evaluate the bacteriological profile and antimicrobial susceptibility patterns of blood culture isolates in neonatal sepsis, with special emphasis on preterm neonates, who represent a particularly vulnerable population.

Review of Literature:

Neonatal sepsis remains a major contributor to neonatal morbidity and mortality, particularly in low- and middle-income countries. A systematic review and meta-analysis by Fleischmann et al. reported a pooled global incidence of 2,824 neonatal sepsis cases per 100,000 live births with an overall mortality rate of 17.6%, while the burden was significantly higher in LMICs.[13] Similarly, the Global Burden of Disease Study 2019 estimated 6.31 million incident cases of neonatal sepsis worldwide, with the highest burden concentrated in South Asia and sub-Saharan Africa.[14] In India, neonatal sepsis continues to account for a substantial proportion of neonatal deaths despite improvements in neonatal care. [4,8]

The bacteriological profile of neonatal sepsis varies across geographical regions and healthcare settings. In high-income countries, Group B Streptococcus and *Escherichia coli* are the predominant pathogens associated with early-onset sepsis (EOS). [15,16] In contrast, studies from India and other developing countries consistently report Gram-negative organisms as the leading pathogens. Sundaram et al.[5], Dalal et al. [17], Siddiqui et al.[12] and Mahich et al. [6] identified *Klebsiella pneumoniae* as the most common isolate in neonatal sepsis, accounting for a substantial proportion of culture-positive cases. Siddiqui et al. reported that Gram-negative organisms constituted nearly 70% of isolates, with *Klebsiella pneumoniae*, *Acinetobacter baumannii* and *E. coli* being the predominant pathogens.[12]

Prematurity and low birth weight are recognized as important risk factors for neonatal sepsis. Collins et al. highlighted the increased susceptibility of preterm neonates due to immature innate and adaptive immune responses.[10] Flannery et al. demonstrated that late-onset sepsis remains particularly common among very preterm infants requiring prolonged NICU care and invasive

procedures.[18] Mabunda et al. observed that 67% of neonates with Gram-negative bacteraemia were preterm and reported multidrug resistance in 88% of isolates.[19] These findings emphasize the increased vulnerability of preterm neonates to severe infections and antimicrobial resistance.

Antimicrobial resistance has emerged as one of the greatest challenges in the management of neonatal sepsis. The widespread occurrence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae, methicillin-resistant *Staphylococcus aureus* (MRSA), and carbapenem-resistant Gram-negative organisms has significantly reduced the effectiveness of conventional empirical antibiotic regimens.[7,20] Multicentric Indian studies have documented high resistance rates to ampicillin, gentamicin and third-generation cephalosporins, while amikacin and carbapenems generally retain better sensitivity.[12,21]Mahich et al. reported that amikacin and piperacillin-tazobactam remained relatively effective against Gram-negative isolates, whereas vancomycin retained excellent activity against Gram-positive pathogens.[6]

The bacteriological spectrum and antibiotic susceptibility patterns of neonatal sepsis continue to evolve over time because of changing antimicrobial practices, infection control measures and the emergence of multidrug-resistant organisms.[12] Several studies have highlighted significant temporal changes in pathogen distribution and resistance patterns, emphasizing the need for periodic local surveillance and antibiogram-based empirical therapy.[8] Therefore, continuous monitoring of culture isolates and their antimicrobial susceptibility patterns remains essential for guiding appropriate antibiotic policies, strengthening antimicrobial stewardship and improving neonatal outcomes.

Study Rationale

Given the dynamic nature of neonatal sepsis epidemiology and increasing antimicrobial resistance, periodic evaluation of local bacteriological profiles and antibiotic susceptibility patterns is necessary. Furthermore, limited data are available comparing contemporary trends with historical institutional data. Therefore, the present study was undertaken to evaluate the bacteriological profile and antimicrobial susceptibility patterns among preterm and term neonates with culture-proven sepsis and to compare the findings with data from a similar study conducted at the same institution approximately a decade earlier

Materials and Methods

Study Design and Setting

This hospital-based prospective analytical observational study was conducted in the Neonatal Intensive Care Unit (NICU), Department of Paediatrics, Maharishi Markandeshwar Institute of Medical Sciences and Research (MMIMSR), Mullana, Haryana, a tertiary care teaching hospital catering to both inborn and out born neonates.

Study Population

The study included neonates admitted to the NICU with clinically suspected sepsis and subsequently confirmed culture-positive sepsis. Both preterm and term neonates with positive blood culture and/or sterile body fluid culture were enrolled after obtaining informed consent from parents or guardians.

Neonates with culture-negative sepsis, congenital malformations, known immunodeficiency disorders, or lack of parental consent were excluded from the study.

Study Duration and Sampling

The study was conducted over a period of 15 months, from October 2024 to December 2025. Consecutive sampling was employed, and all eligible neonates fulfilling the inclusion criteria during the study period were included.

Data Collection

Detailed demographic and clinical data, including gestational age, birth weight, sex, maternal risk factors, and clinical manifestations of sepsis, were recorded using a structured proforma. Blood samples were collected under strict aseptic precautions before initiation of antibiotic therapy and processed using an automated blood culture system (BACTEC). In selected cases, other sterile body fluids were also subjected to microbiological evaluation.

Organisms isolated from cultures were identified using standard microbiological techniques. Antimicrobial susceptibility testing was performed according to standard laboratory guidelines, and the bacteriological profile along with antibiotic sensitivity patterns was documented. All enrolled neonates were followed throughout their hospital stay, and relevant clinical outcomes were recorded.

Statistical Analysis

Data were entered into Microsoft Excel and analyzed using SPSS version 21. Continuous variables were expressed as mean ± standard deviation, whereas categorical variables were presented as frequencies and percentages. Comparisons between preterm and term neonates were performed using the independent t-test for

continuous variables and Chi-square test or Fisher's exact test for categorical variables, as appropriate. Antimicrobial susceptibility patterns were analyzed as percentages of sensitivity and resistance. A p-value of <0.05 was considered statistically significant.

Result:

In our study of 220 neonates male neonates constituted 55.9% of subjects.

Preterm births accounted for 60.0% of cases, while low birth weight neonates represented the largest birth weight category (47.7%). Most neonates were appropriate for gestational age (75.5%), and maternal PPROM was present in 20.5% of cases.

LONS was more common than EONS, accounting for 66.4% (n=146) and 33.6% (n=74) of cases, respectively. Of the 220 blood culture specimens in this study, the growth was documented in 107 subjects (48.6%) confirming neonatal sepsisas shown in figure 1. No growth was documented in 113 subjects (51.4%).

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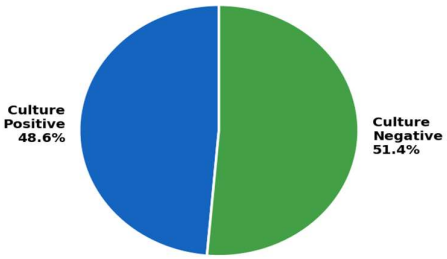
Fig. 1: Culture Outcome Summary (N=220)



The clinical manifestations among the 220 study subjects revealed lethargy as the most frequently reported symptom (24.5%), followed by vomiting (20.9%). Seizures and apnoea were observed in 12.7% and 10.0% of neonates, respectively. Among the clinical signs, hypoglycaemia was the most common finding (20.0%), followed by respiratory distress syndrome (RDS) (19.1%) and transient tachypnoea of the newborn (TTNB) (16.8%). Jaundice was the least frequently observed clinical feature, occurring in 2.3% of subjects as shown in Table 1.

Table 1: Clinical feature Presentation among Study participants (N-220)

Clinical Feature	Number of Subjects (n)	Percentage (%)
RDS (Respiratory Distress Syndrome)	42	19.1
TTNB (Transient Tachypnoea of Newborn)	37	16.8
Lethargy	54	24.5
Vomiting	46	20.9
Hypoglycaemia	44	20.0
Hypothermia	42	19.0
Abdominal distension	39	17.7
Poor feeding	40	18.2
Seizures	28	12.7
Apnea	22	10.0
Jaundice	5	2.3

Of all the samples collected Candida species constituted the largest proportion of isolates (31.8%), with *Candida krusei* being the predominant fungal pathogen (16.8%). Among bacterial isolates, *Acinetobacter baumannii* was the most frequently identified organism (28.0%), followed by *Klebsiella pneumoniae* (15.9%) and *Serratia marcescens* (8.4%). Gram-positive organisms were infrequently isolated, with methicillin-resistant *Staphylococcus aureus* (MRSA) and *Staphylococcus epidermidis* each accounting for 1.9% of isolates as shown in Table 2.

Table 2: **Blood Culture Isolates (Culture-Positive Subjects, n=107)**

Organism Isolated	Subjects (n)	Percentage (%)
Candida species (combined)	34	31.8

Organism Isolated	Subjects (n)	Percentage (%)
Candida krusei	18	16.8
Candida albicans	6	5.6
Candida pelliculosa	4	3.7
Candida tropicalis	4	3.7
Other Candida species	2	1.9
Acinetobacter baumannii	30	28.0
Klebsiella pneumoniae	17	15.9
Serratia marcescens	9	8.4
Escherichia coli	5	4.7
Enterobacter cloacae	3	2.8
Enterococcus faecium	3	2.8
MRSA	2	1.9
Staphylococcus epidermidis	2	1.9
Total	**107**	**100.0**

Our study also revealed that colistin demonstrated the highest activity against Gram-negative isolates, with a susceptibility rate of 45.8%. High resistance to carbapenems was observed, while all Gram-negative isolates were resistant to piperacillin-tazobactam. Amphotericin B showed 100% sensitivity among fungal isolates, whereas all Gram-positive isolates remained fully susceptible to vancomycin, linezolid, and teicoplanin as shown in table 3.

Table 3: **Antibiogram-Sensitivity and Resistance Profile of Isolates (Culture-Positive, n=107)**

Antimicrobial Agent for gram negative isolates (total-64)	**S (n)**	**S (%)**	**R (n)**	**R (%)**	**Remarks**
Colistin	49	76.6	58	54.2	
Cotrimoxazole	27	43.5	35	56.5	
Gentamicin	19	29.7	45	70.3	
Meropenem	16	25.0	48	75.0	
Imipenem	15	23.4	49	75.8	
Tigecycline	14	21.9	50	78.1	
Amikacin	10	15.6	54	84.4	
Cefepime	10	15.6	54	84.4	
Ciprofloxacin	8	12.5	56	87.5	
Levofloxacin	5	7.8	59	92.2	
Piperacillin-Tazobactam	0	0.0	64	100.0	Pan-resistant
Antimicrobial Agent for gram positive isolates (total-7)	**S (n)**	**S (%)**	**R (n)**	**R (%)**	**Remarks**
Cotrimoxazole	7	100.0	0	0.0	
Vancomycin	7	100.0	0	0.0	
Linezolid	7	100.	0	0.0	

		0			
Teicoplanin	5	100.0	0	0.0	
Tetracycline	5	71.4	2	28.6	
Antimicrobial Agent for fungal isolates (total-30)	**S (n)**	**S (%)**	**R (n)**	**R (%)**	**Remarks**
Amphotericin B	30	100.0	0	0.0	Universal activity
Voriconazole	27	79.4	7	20.6	
Caspofungin	21	72.4	8	27.6	
Micafungin	20	69.0	9	31.0	
Fluconazole	5	21.7	18	78.3	C. krusei intrinsic R
Flucytosine	5	33.3	10	66.7	

Ous study also revealed Gram-negative organisms predominated in both EONS and LONS, while Candida species were more frequently isolated in EONS. Gram-positive bacteria constituted only a small proportion of isolates in both groups. The distribution of pathogens did not differ significantly between EONS and LONS ($p>0.05$).

Elevated CRP was the most common abnormal sepsis screen parameter (20.0%), followed by low ANC (14.5%). Leukopenia was uncommon (2.3%), and 8.2% of subjects demonstrated two or more abnormal screening parameters. The I:T ratio was below 0.2 in all neonates as depicted in Table 4.

Table 4: **Table 10: Findings of Sepsis Screen Parameters (N=220)**

Sepsis Screen Parameter	Number Positive (n)	Percentage (%)
CRP (>10 mg/L)	44	20.0
Leukopenia (TLC <5000/mm3)	5	2.3
Low ANC count (ANC <1800/mm3)	32	14.5
Immature: Total (I:T) ratio >=0.2	0	0.0
Two or more screen parameters positive	18	8.2

CRP (>10 mg/L) was the only sepsis screening parameter significantly associated with culture-proven sepsis ($p<0.05$), demonstrating a sensitivity of 25.2%, specificity of 72.6%, and PPV of 94.4%. Although leukopenia, low ANC, and the presence of two or more abnormal parameters showed excellent specificity (97–98%) and PPV (100%), their low sensitivity limited their diagnostic performance as shown in table 5.

Table 5: **Diagnostic Performance of Sepsis Screen Parameters Against Blood Culture**

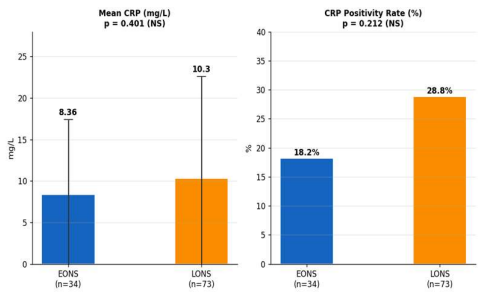
Parameter	Sensitivity (%)	Specificity (%)	PPV (%)	p-value
CRP (>10 mg/L)	25.2	72.6	94.4	<0.05
Leukopenia (TLC <5000/mm3)	4.7	98.2	100.0	>0.05
Low ANC count (ANC <1800/mm3)	15.9	97.3	100.0	>0.05
Two or more parameters positive	8.4	98.2	100.0	>0.05

While comparing the inflammatory markers between the early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS) groups among the 107 culture-positive neonates. Mean CRP levels were higher in the LONS group (10.30 ± 12.33 mg/L) than in the EONS group (8.36 ± 9.07 mg/L). Similarly, the proportion of CRP-positive cases (>10 mg/L) was greater in LONS (28.8%) compared to EONS (18.2%). However, these differences were not statistically significant (p=0.212). Mean total leukocyte count, frequency of leukopenia, low absolute neutrophil count (ANC), and mean birth weight were comparable between the two groups, with no statistically significant differences observed as depicted in table 6 and figure 2.

Table 6: Comparison of Inflammatory Markers between EONS and LONS (Culture-Positive Subjects, n=107)

Parameter	EONS (n=34)	LONS (n=73)	p-value
Mean CRP (mg/L) +/- SD	8.36 +/- 9.07	10.30 +/- 12.33	0.401
CRP positive (>10 mg/L), n (%)	6 (18.2%)	21 (28.8%)	0.212
Mean TLC (/mm3) +/- SD	8782 +/- 2807	8686 +/- 2461	0.860
Leukopenia, n (%)	2 (5.9%)	3 (4.1%)	0.652
Low ANC count, n (%)	6 (17.6%)	12 (16.4%)	1.000
Mean birth weight (g) +/- SD	2269 +/- 781	2332 +/- 792	0.702

Figure 2: **Mean CRP and CRP Positivity Rate — EONS vs LONS (Culture-Positive, n=107)**



While comparing our study with those of a similar study conducted at the same institution approximately a decade earlier. A significant epidemiological shift was observed, with the proportion of early-onset neonatal sepsis (EONS) declining from 90.0% to 33.6% (p<0.001), while late-onset neonatal sepsis (LONS) increased from 10.0% to 66.4% (p<0.001). Another notable finding was the marked rise in fungal sepsis, with Candida species accounting for 31.8% of isolates compared with 7.0% in the previous study as shown in Table 7. In addition, *Klebsiella pneumoniae* and *Serratia marcescens* emerged as important pathogens in the current study, reflecting a changing microbiological profile within the NICU.

The antimicrobial susceptibility pattern also demonstrated a concerning increase in resistance. All Gram-negative isolates were resistant to piperacillin-tazobactam and ampicillin, while substantial resistance was observed to meropenem (75.0%), colistin (54.2%), and tigecycline (78.0%). These findings highlight the evolving burden of antimicrobial resistance and underscore the need for strengthened infection control measures, regular antibiogram surveillance, and robust antimicrobial stewardship programs in NICU settings.

Table 7: **Comparison of Present Study (n=220) with Previous Hospital-Based Study (n=50, ~10 Years Ago)**

Parameter	Previous Study (n=50)	Present Study (n=220)	Test Used	p-value
EONS	45 (90.0%)	74 (33.6%)	Chi-square	<0.001
LONS	5 (10.0%)	146 (66.4%)	Chi-square	<0.001
RDS	22 (44.0%)	42 (19.1%)	Chi-square	<0.001
TTNB	--	37 (16.8%)	--	--
Lethargy	4 (8.0%)	54 (24.5%	Chi-square	<0.001

Parameter	Previous Study (n=50)	Present Study (n=220)	Test Used	p-value
		)		
Poor feeding	26 (52.0%)	40 (18.2%)	Chi-square	<0.001
Apnea	20 (40.0%)	22 (10.0%)	Chi-square	<0.001
Hypothermia	19 (38.0%)	42 (19.0%)	Chi-square	<0.001
Vomiting	2 (4.0%)	46 (20.9%)	Chi-square	<0.001
Abdominal distention	2 (4.0%)	39 (17.7%)	Chi-square	<0.001
Convulsions	4 (8.0%)	28 (12.7%)	Chi-square	<0.001
Culture positivity	27/50 (54.0%)	107/220 (48.6%)	Chi-square	0.512
Acinetobacter spp.	20 (40.0%)	30 (28.0%)	Chi-square	0.176
Citrobacter spp.	8 (15.0%)	0 (0.0%)	Fisher's exact	<0.001
Candida spp.	2 (7.0%)	34 (31.8%)	Chi-square	<0.001
Gram positive isolates	8 (16.0%)	7 (6.5%)	Chi-square	<0.001

Discussion:

In the present study, microbial growth was detected in 48.6% of clinically suspected neonatal sepsis cases. This culture positivity rate is comparable to that reported by Rajyaguru et al. (48.5%) [22], higher than those observed by Siddiqui et al. (40%) [12] and Sundaram et al. (35.4%) [5], but slightly lower than the 54.0% positivity reported from the same institution approximately a decade earlier. The reduction in culture positivity may reflect improvements in infection prevention and control practices over time.

Late-onset neonatal sepsis (LONS) constituted the majority of cases (66.4%), whereas early-onset neonatal sepsis (EONS) accounted for 33.6%. Similar predominance of LONS has been reported by Siddiqui et al. (62%) [12] and Rajyaguru et al. (54.4%) [22], and was also observed by Rubio-Mora et al.[23]In contrast, an earlier study from the same institution reported a predominance of EONS, suggesting a changing epidemiological pattern of neonatal sepsis over time. Flannery et al. documented a substantially lower incidence of LONS (8.9%) among very preterm infants in developed healthcare settings, highlighting regional differences in neonatal care and infection control practices.[18]

Male neonates constituted 55.9% of culture-positive sepsis cases, which is consistent with previous reports by Siddiqui et al. [12] Dalal et al. [17] Rajyaguru et al. [22] and Das et al. [24]The observed male predominance has been attributed to sex-related immunological differences, including the influence of immune-regulatory genes located on the X chromosome and variations in complement activity and immunoglobulin responses. [9,15]

Prematurity and low birth weight emerged as major risk factors in the present study, with 60% of affected neonates being preterm and 66.4% belonging to the low birth weight category. Similar findings have been reported by Rajyaguru et al. [22] Mabunda et al. [19] and Sahu et al. [25]who documented a high burden of sepsis among preterm and low birth weight neonates. The increased susceptibility of these neonates is primarily related to immature immune function, reduced complement activity, impaired neutrophil function, and decreased transplacental transfer of maternal antibodies.[10,26] Furthermore, very low birth weight infants are at increased risk of late-onset sepsis because of prolonged hospitalization and greater exposure to invasive procedures.[18,25] These findings underscore the importance of targeted infection prevention strategies, including strict infection control measures, early

breastfeeding, and kangaroo mother care, particularly among preterm and low birth weight neonates.

The bacteriological profile observed in the present study differed considerably from most published Indian NICU studies. Fungal isolates constituted the largest group of pathogens, with Candida species accounting for 31.8% of all culture-positive cases, compared to only 7% reported in a previous study from the same institution. Among bacterial pathogens, *Acinetobacter baumannii* (28.0%) was the predominant isolate, followed by *Klebsiella pneumoniae* (15.9%), *Serratia marcescens* (8.4%), and *Escherichia coli* (4.7%). Gram-positive organisms were relatively uncommon, with MRSA and *Staphylococcus epidermidis* accounting for 1.9% each.

These findings contrast with reports from North Indian NICUs, where *Klebsiella pneumoniae* has consistently been identified as the predominant pathogen. Siddiqui et al.[12] Mahichet al.[6] Dalal et al. [17] and Sundaram et al. [5] reported *Klebsiella* as the leading isolate among culture-positive neonatal sepsis cases. The predominance of *Acinetobacter baumannii* in the present study may reflect changing microbial epidemiology within NICUs. Its ability to survive in the hospital environment, form biofilms, and rapidly acquire multidrug resistance makes it an important nosocomial pathogen.[7,20]

A notable finding was the high prevalence of Candida species, particularly *Candida krusei* (16.8%). The emergence of fungal sepsis is clinically significant because *C. krusei* exhibits intrinsic resistance to fluconazole, limiting empirical treatment options. Flannery et al. identified fungal infections as an important cause of late-onset sepsis among very low birth weight neonates and reported substantial associated mortality. [18] The disappearance of *Citrobacter* species, which constituted 15% of isolates in the previous institutional study but were absent in the present study, further highlights the evolving microbiological profile within the NICU.

Gram-negative organisms predominated in both early-onset and late-onset sepsis, a finding consistent with previous reports by Mabunda et al. [19] and Das et al. [24] Although Candida isolates were more frequently observed in early-onset sepsis than late-onset sepsis, the difference was not statistically significant. Similar observations have been attributed to the vulnerability of extremely preterm neonates and exposure to antenatal corticosteroids.[18]

The antibiogram revealed an alarming level of antimicrobial resistance among gram-negative isolates. Although colistin demonstrated the highest sensitivity, more than half of the isolates were resistant. High resistance rates were also observed against carbapenems, aminoglycosides, cephalosporins, and piperacillin-tazobactam. Compared with reports from Siddiqui et al. [12] Mahich et al. [6] Jaybhayeet al.[27] and Mabunda et al. [19] the present study demonstrated substantially lower susceptibility to amikacin, carbapenems, and colistin. These findings reflect the growing burden of multidrug-resistant organisms and are consistent with global trends reported by the Antimicrobial Resistance Collaborators.The findings underscore the urgent need for strengthened antimicrobial stewardship and infection control practices.

Among fungal isolates, amphotericin B demonstrated 100% sensitivity, consistent with previous reports by Opare-Asamoah et al. [28] and Mukherjee et al.[7] Voriconazole showed good activity, whereas fluconazole susceptibility was markedly low, largely because of the predominance of intrinsically resistant *Candida krusei*.This finding highlights the importance of species-level identification and antifungal stewardship in NICU settings.

All gram-positive isolates remained sensitive to vancomycin, linezolid, and teicoplanin, which is consistent with findings reported by Siddiqui et al. [12],Mahich et al. [6]Jaybhaye et al. [27]

The clinical presentation of neonatal sepsis in the present study was largely nonspecific, with lethargy, vomiting, hypoglycaemia, poor feeding, and abdominal distension being the most frequent manifestations. Similar findings have been reported by Siddiqui et al. [12] and Das et al. [24] reinforcing the fact that no single clinical feature is diagnostic of neonatal sepsis. Compared with the earlier institutional study, lower frequencies of poor feeding and respiratory distress were observed, whereas seizures were reported more frequently, suggesting possible changes in disease presentation over time.

Among the sepsis screening parameters, CRP demonstrated a significant association with culture-proven sepsis and showed high positive predictive value but relatively low sensitivity. Similar studies by Celik et al. [29]Rajyaguru et al. [22] and Saxena et al. [30] have reported higher CRP sensitivities than observed in the present study. The lower sensitivity may partly be explained by the substantial proportion of fungal sepsis in the study population. In contrast, leukopenia, low absolute neutrophil count, and combinations of abnormal sepsis screen parameters showed high specificity but limited diagnostic sensitivity. The immature-to-total neutrophil ratio was not useful in the present cohort. These findings support recent

recommendations advocating the incorporation of newer biomarkers such as procalcitonin and interleukin-6 into neonatal sepsis diagnostic algorithms.[29]

Prematurity and low birth weight were the predominant neonatal risk factors identified in the study population, while maternal PPROM was the most common maternal risk factor. Although these factors did not differ significantly between early- and late-onset sepsis groups, their importance has been consistently demonstrated in previous studies. Das et al. [24] reported maternal PROM, maternal urinary tract infection, and advanced maternal age as significant predictors of early-onset sepsis, while Sahu et al. [25] identified extreme prematurity and extremely low birth weight as major predictors of adverse outcomes.Similar associations between prematurity and gram-negative sepsis have also been documented by Mabunda et al. [19]

Comparison with institutional data from a decade earlier revealed a marked epidemiological shift, with the EONS ratio changing from 90:10 to 33:66. This observation is consistent with contemporary reports from India and other regions demonstrating an increasing burden of late-onset sepsis.The microbiological profile has also changed substantially, with Candida species emerging as the leading pathogen and Citrobacter species no longer being isolated. Concurrently, high resistance rates to carbapenems and piperacillin-tazobactam among gram-negative organisms reflect the growing challenge of antimicrobial resistance. These findings parallel national and global reports highlighting the rapid emergence of multidrug-resistant neonatal pathogens and emphasize the need for robust antimicrobial stewardship and continuous microbiological surveillance. [7,8,20]

Overall, the study highlights a changing epidemiology of neonatal sepsis characterized by increasing late-onset infections, emergence of fungal pathogens, and rising antimicrobial resistance, underscoring the need for strengthened infection control measures and evidence-based antimicrobial policies in NICU settings.

Conclusion:

Neonatal sepsis remains a significant cause of morbidity among NICU admissions, particularly in preterm and low birth weight neonates, with late-onset sepsis being more common than early-onset sepsis. The study demonstrated a predominance of Candida species and multidrug-resistant gram-negative organisms, particularly *Acinetobacter baumannii* and *Klebsiella pneumoniae*. High levels of antimicrobial resistance among bacterial isolates underscore the importance of culture-guided therapy and periodic local antibiogram surveillance. Strengthening infection control practices, promoting rational antimicrobial use, and ensuring timely microbiological diagnosis are essential to improve neonatal outcomes and curb the emergence of antimicrobial resistance.

Limitation:

This was a single-center, hospital-based study conducted in a tertiary care NICU; therefore, the findings may not be generalizable to other healthcare settings or the wider community. Prior antibiotic exposure, particularly among out born neonates, may have influenced culture positivity and organism isolation. Anaerobic and viral pathogens were not evaluated, and long-term clinical outcomes were not assessed. Additionally, antimicrobial resistance patterns are dynamic and may change over time, highlighting the need for periodic local surveillance and larger multicentric studies to validate the findings.

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