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PREVALENCE AND MICROBIOLOGICAL PROFILE OF NEONATAL SEPTICEMIA IN A TERTIARY CARE HOSPITAL: A CROSS-SECTIONAL OBSERVATIONAL STUDY

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ABSTRACT

Background: Neonatal septicemia remains an important cause of morbidity and mortality, particularly among hospitalized, premature, and low-birth-weight neonates. Its causative organisms and antimicrobial susceptibility patterns vary between institutions and over time. Regular microbiological surveillance is therefore essential for selecting appropriate empirical antimicrobial therapy.

Aim: To determine the prevalence and microbiological profile of neonatal septicemia among clinically suspected neonates admitted to a tertiary care hospital.

Materials and Methods: This hospital-based cross-sectional observational study included 328 neonates with clinically suspected septicemia admitted to a tertiary care hospital between June 2023 and December 2024. Blood samples were collected aseptically, preferably before antimicrobial administration, and processed using conventional blood-culture methods. Isolates were identified using colony characteristics, Gram staining, biochemical reactions, and appropriate fungal identification tests. Antimicrobial susceptibility testing was performed using the modified Kirby-Bauer disc-diffusion method and interpreted according to applicable Clinical and Laboratory Standards Institute guidelines. Categorical data were presented as frequencies and percentages with 95% confidence intervals. The chi-square test, one-sample proportion test, and exact binomial test were applied as appropriate. A P value of less than 0.05 was considered statistically significant.

Results: Of the 328 clinically suspected neonates, 121 had positive blood cultures, giving a prevalence of 36.89% (95% confidence interval: 31.84%-42.24%). Culture positivity was observed in 34.50% of early-onset and 40.63% of late-onset cases; the difference was not statistically significant (P value = 0.262). Among 121 isolates, 62 (51.24%) were Gram-negative bacteria, 42 (34.71%) were Candida species, and 17 (14.05%) were Gram-positive bacteria. The distribution of the major microbial groups was statistically significant (P value < 0.001). Klebsiella species were the predominant bacterial isolates, accounting for 42 (34.71%) cases, followed by Acinetobacter species in 14 (11.57%) and Staphylococcus aureus in 11 (9.09%). Of the 42 Candida isolates, 38 (90.48%) were non-albicans Candida. Candida tropicalis was the most common Candida species, accounting for 26 (61.90%) isolates. All S. aureus isolates were susceptible to vancomycin and linezolid, while 7 of 11 (63.64%) were methicillin resistant. All 42 Klebsiella isolates were susceptible to meropenem, and 90.48% were susceptible to

imipenem. Acinetobacter showed the highest susceptibility to imipenem (71.43%) and meropenem (64.29%).
Conclusion: More than one-third of clinically suspected neonates had culture-confirmed septicemia. Gram-negative bacteria predominated, with Klebsiella being the leading bacterial pathogen. Non-albicans Candida, particularly Candida tropicalis, represented a substantial proportion of bloodstream infections. The high frequency of methicillin resistance and reduced susceptibility to several commonly used antibiotics highlight the need for continuous local surveillance, antimicrobial stewardship, strict infection-control measures, and culture-guided treatment.
Keywords: Neonatal septicemia; blood culture; antimicrobial susceptibility.

INTRODUCTION

Neonatal septicemia is a systemic infection occurring during the first 28 days of life and is characterized by clinical manifestations associated with the presence of pathogenic microorganisms in the bloodstream. It remains an important cause of neonatal morbidity, prolonged hospitalization, neurodevelopmental impairment, and mortality, particularly in low- and middle-income countries. The clinical manifestations of neonatal septicemia are often subtle and nonspecific and may include temperature instability, poor feeding, lethargy, respiratory distress, apnea, jaundice, abdominal distension, vomiting, convulsions, and circulatory shock. Consequently, early clinical recognition and microbiological confirmation are essential for initiating appropriate treatment and improving neonatal outcomes.[1]

Neonatal septicemia is commonly classified as early-onset septicemia, occurring within the first 72 hours of life, and late-onset septicemia, developing after 72 hours of birth. Early-onset infection is generally acquired vertically from the maternal genital tract before or during delivery. Maternal fever, chorioamnionitis, prolonged or premature rupture of membranes, foul-smelling liquor, urinary tract infection, prematurity, and low birth weight are important risk factors. Late-onset septicemia is usually acquired from the hospital or community environment and is associated with prolonged hospitalization, invasive procedures, intravascular catheterization, mechanical ventilation, surgery, and delayed enteral feeding.[2]

The microbiological profile of neonatal septicemia varies considerably between countries, geographical regions, hospitals, and even different periods within the same institution. Both Gram-negative and Gram-positive organisms are responsible for infection. Frequently reported pathogens include *Klebsiella* pneumoniae, Escherichia coli, Acinetobacter species, Pseudomonas aeruginosa, Staphylococcus aureus, and coagulase-negative staphylococci. Fungal bloodstream infections, particularly candidemia, have also emerged as important causes of sepsis among premature, low-birth-weight, and critically ill neonates.[3,4]

Blood culture remains the reference standard for confirming neonatal septicemia and determining the antimicrobial susceptibility of the causative organism. Nevertheless, culture results generally require at least 24-72 hours, and their yield may be affected by small blood volumes, intermittent bacteremia, or prior antibiotic exposure. The increasing prevalence of multidrug-resistant organisms has further complicated the empirical treatment of neonatal infections. Inappropriate empirical therapy may increase mortality, whereas unnecessary broad-spectrum antibiotic use can promote resistance and fungal infections.[5] Periodic evaluation of the local prevalence, causative organisms, and antimicrobial susceptibility patterns is therefore necessary to formulate evidence-based institutional antibiotic policies. The present study was conducted to determine the prevalence and microbiological profile of neonatal septicemia and to assess the antimicrobial susceptibility patterns of the organisms isolated in a tertiary care hospital.

Aim

To determine the prevalence and microbiological profile of neonatal septicemia among clinically suspected neonates admitted to a tertiary care hospital.

Objectives

1. To determine the prevalence of culture-confirmed septicemia among neonates with clinically suspected sepsis.
2. To identify the bacterial and fungal organisms responsible for neonatal septicemia.
3. To determine the antimicrobial susceptibility patterns of the bacterial isolates and the prevalence of multidrug-resistant organisms.

MATERIALS AND METHODS

Source of Data: Data were obtained from neonates with clinically suspected septicemia who were admitted to the neonatal intensive care unit and other neonatal care areas of the tertiary care hospital during the study period. Clinical information was obtained from case records, laboratory requisition forms, treatment records, and interviews with the parents or guardians whenever clarification was required. Microbiological information was obtained from blood culture, organism-identification, and antimicrobial-susceptibility records maintained in the Department of Microbiology.

Study Design: The study was a hospital-based cross-sectional observational study. All eligible neonates with clinical features or recognized risk factors suggestive of septicemia were evaluated during the defined study period. No intervention beyond routine institutional diagnostic and treatment protocols was introduced by the investigators.

Study Location: The study was conducted in the Department of Microbiology in collaboration with the neonatal intensive care unit and neonatal care services of a tertiary care teaching hospital.

Study Duration: The study was conducted over 18 months, from June 2023 to December 2024.

Study Population: The study population consisted of neonates aged 0-28 days who were admitted to the hospital with clinical features or risk factors suggestive of neonatal septicemia and for whom blood culture investigation was requested.

Sample Size: A total of 328 neonates with clinically suspected septicemia were included.

$$n = \frac{Z_{1-\alpha/2}^2 \times P(1-P)}{d^2}$$

Where:

- n = required sample size
- $Z_{1-\alpha/2}$ = 1.96 at a 95% confidence level
- P = anticipated prevalence of neonatal septicemia, taken as 32%
- $1-P$ = 68%
- d = absolute precision or margin of error, taken as 5%

$$n = \frac{(1.96)^2 \times 0.32 \times 0.68}{(0.05)^2}$$

$$n \approx 334$$

The study ultimately included 328 eligible neonates whose samples and clinical information were available during the study period. This represented the practically achieved sample and was close to the calculated minimum requirement.

Inclusion Criteria

1. Neonates aged 0-28 days admitted to the neonatal intensive care unit or neonatal care areas during the study period.
2. Neonates with clinical features suggestive of septicemia, including poor feeding, lethargy, temperature instability, respiratory distress, apnea, seizures, jaundice, vomiting, abdominal distension, or circulatory instability.
3. Neonates with recognized maternal or neonatal risk factors for septicemia.
4. Neonates for whom blood culture was requested by the treating clinician.
5. Neonates from whom an adequate blood sample was collected and received in the microbiology laboratory.
6. Neonates whose parents or legally authorized guardians provided informed consent, wherever required by the approved protocol.

Exclusion Criteria

1. Infants older than 28 completed days at the time of evaluation.
2. Neonates without clinical suspicion or risk factors for septicemia.
3. Blood samples that were insufficient in quantity, improperly labelled, leaking, contaminated, or transported under inappropriate conditions.
4. Duplicate blood cultures obtained from the same episode of septicemia, unless required to confirm the clinical significance of an isolate.
5. Neonates whose essential clinical or laboratory information was unavailable.
6. Neonates whose parents or guardians declined participation, where individual consent was required.

Procedure and Methodology

Eligible neonates were identified using the clinical diagnostic criteria for suspected neonatal septicemia followed at the study institution. A detailed clinical history was recorded in a predesigned case-record form. Information regarding age, sex, gestational age, birth weight, mode and place of delivery, age at onset of symptoms, maternal risk factors, neonatal risk factors, clinical manifestations, previous antibiotic exposure, invasive procedures, and outcome was recorded.

Neonatal septicemia was categorized according to the age at onset. Infection manifesting within the first 72 hours after birth was classified as early-onset septicemia, whereas infection manifesting after 72 hours and within 28 days of life was classified as late-onset septicemia.

Blood samples were preferably collected before the administration of antimicrobial therapy. The venipuncture site was disinfected using 70% isopropyl alcohol and allowed to dry. Approximately 0.5-1.5 mL of blood was aseptically collected by trained healthcare personnel and immediately inoculated into a conventional blood-culture bottle containing brain-heart infusion broth. The inoculated bottles were properly labelled and transported without delay to the microbiology laboratory.

A culture was considered positive when a recognized bacterial or fungal pathogen was isolated. The clinical significance of common skin commensals, particularly coagulase-negative staphylococci, was assessed with reference to the neonate's clinical condition, risk factors, laboratory findings, and, where available, repeat isolation. Organisms considered contaminants after clinicomicrobiological correlation were not classified as causative pathogens.

Sample Processing

The inoculated blood-culture bottles were incubated aerobically at 37°C. After 18-24 hours of incubation, a blind subculture was performed on 5% sheep blood agar and MacConkey agar irrespective of visible turbidity. The inoculated agar plates were incubated aerobically at for 18-24 hours.

The blood-culture bottles were examined daily for evidence of microbial growth, including turbidity, hemolysis, gas formation, or other visible changes. Bottles showing signs of growth were immediately

subcultured onto appropriate solid media. Bottles without visible growth were incubated for up to seven days. A final blind subculture was performed at the end of the seventh day before the culture was reported as "no growth."

Bacterial isolates were identified using colony morphology, pigment production, hemolytic characteristics, Gram staining, motility testing, and standard biochemical reactions. The biochemical tests included catalase, coagulase, oxidase, indole, citrate utilization, urease, methyl red, Voges-Proskauer, triple sugar iron agar, sugar fermentation, and other organism-specific tests as required.

Yeast isolates were initially identified by their colony morphology and Gram-positive budding yeast cells on Gram staining. Candida isolates were further characterized using the germ-tube test, morphology on cornmeal agar, colony colour on CHROMagar Candida, and carbohydrate-assimilation tests.

Antimicrobial susceptibility testing of bacterial isolates was performed by the modified Kirby-Bauer disc-diffusion method on Mueller-Hinton agar. The inoculum was adjusted to the turbidity of a 0.5 McFarland standard. Antibiotic discs were selected according to the type of organism and the hospital antibiotic policy. After incubation, the diameters of the zones of inhibition were measured in millimetres and interpreted as susceptible, intermediate, or resistant according to the Clinical and Laboratory Standards Institute guidelines applicable during the study period.

Methicillin resistance among *Staphylococcus aureus* isolates was detected using the cefoxitin disc-diffusion method. Quality control was maintained using standard reference strains, including *Staphylococcus aureus* ATCC 25923, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853.

Data Collection

Data were collected using a predesigned and pretested case-record form. Each neonate was assigned a unique study identification number. The recorded variables included:

- Demographic and perinatal characteristics
- Gestational age and birth weight
- Mode and place of delivery
- Maternal and neonatal risk factors
- Clinical manifestations
- Early- or late-onset presentation
- Previous antimicrobial exposure
- Blood-culture result
- Organism isolated
- Bacterial or fungal classification
- Antimicrobial susceptibility pattern
- Presence of methicillin resistance or multidrug resistance
- Duration of hospitalization and neonatal outcome

Completed forms were checked for completeness and consistency. Laboratory findings were cross-verified with culture registers and antimicrobial-susceptibility records before entry into the final database. Patient identifiers were removed from the analytical dataset to maintain confidentiality.

Statistical Methods

Data were entered into Microsoft Excel and analyzed using an appropriate statistical software package. Categorical variables, including sex, culture positivity, type of septicemia, organisms isolated, antimicrobial susceptibility, and clinical outcomes, were summarized as frequencies and percentages. Continuous variables were assessed for distribution and presented as mean with standard deviation or median with interquartile range, as appropriate.

The prevalence of culture-confirmed neonatal septicemia was calculated as:

$$\text{Prevalence} = \frac{\text{Number of neonates with positive blood cultures}}{\text{Total number of clinically suspected neonates studied}} \times 100$$

The prevalence was reported with its 95% confidence interval. Associations between culture positivity and categorical variables were evaluated using the chi-square test or Fisher's exact test, as appropriate. Independent-samples *t* test or Mann-Whitney *U* test was used for continuous variables according to data distribution. Diagnostic indices were calculated for relevant screening parameters when required. A two-sided value of less than 0.05 was considered statistically significant.

RESULTS

Table 1: Overall prevalence and microbiological profile of neonatal septicemia among clinically suspected neonates (N=328)

Study parameter	n (%)	95% CI	Test of significance	P value
Blood-culture positive	121 (36.89%)	31.84%-42.24%	One-sample proportion z=-4.75†	<0.001*
Blood-culture negative	207 (63.11%)	57.76%-68.16%	One-sample proportion z=4.75†	<0.001*
Gram-negative bacterial infection‡	62 (18.90%)	15.04%-23.49%		
Fungal infection‡	42 (12.80%)	9.61%-16.86%		
Gram-positive bacterial infection‡	17 (5.18%)	3.28%-8.13%		
Klebsiella species‡	42 (12.80%)	9.61%-16.86%		
Candida species‡	42 (12.80%)	9.61%-16.86%		
Acinetobacter species‡	14 (4.27%)	2.56%-7.03%		
Staphylococcus aureus‡	11 (3.35%)	1.88%-5.89%		

†The culture-positive proportion was compared with a null proportion of 50%.
‡Percentages in these rows were calculated using all 328 clinically suspected neonates as the denominator.
*Statistically significant at 0.05.
Among the 328 neonates with clinically suspected septicemia, blood culture was positive in 121 (36.89%; 95% confidence interval: 31.84% to 42.24%) and negative in 207 (63.11%; 95% confidence interval: 57.76% to 68.16%). The observed culture-positive proportion was significantly lower than the reference proportion of 50% (one-sample proportion z value = -4.75; P value < 0.001). Gram-negative bacterial infections were identified in 62 (18.90%; 95% confidence interval: 15.04% to 23.49%) of all suspected cases and constituted the largest microbiological group. Fungal infections were detected in 42 (12.80%; 95% confidence interval: 9.61% to 16.86%), while Gram-positive bacterial infections were found in 17 (5.18%; 95% confidence interval: 3.28% to 8.13%). *Klebsiella* and *Candida* species were each isolated from 42 (12.80%) neonates, making them the most frequently identified individual pathogens. *Acinetobacter* species were isolated from 14 (4.27%; 95% confidence interval: 2.56% to 7.03%) cases, whereas *Staphylococcus aureus* was isolated from 11 (3.35%; 95% confidence interval: 1.88% to 5.89%).

Table 2: Prevalence of culture-confirmed septicemia according to the time of onset (N=328)

Type of suspected septicemia	Culture positive, n (%)	Culture negative, n (%)	Total	Prevalence of culture-confirmed septicemia (95% CI)	Test of significance	P value
Early-onset septicemia	69 (34.50%)	131 (65.50%)	200	34.50% (28.25%-41.32%)	χ^2=1.26, df=1†	0.262
Late-onset septicemia	52 (40.63%)	76 (59.38%)	128	40.63% (32.50%-49.29%)		
Total	121 (36.89%)	207 (63.11%)	328	36.89% (31.84%-42.24%)	z=-4.75‡	<0.001*

†Pearson's chi-square test compared the prevalence of culture positivity between early- and late-onset suspected septicemia.
‡One-sample proportion test compared overall culture positivity with a reference proportion of 50%.
*Statistically significant at 0.05.
Of the 200 neonates with suspected early-onset septicemia, 69 (34.50%; 95% confidence interval: 28.25% to 41.32%) were culture positive and 131 (65.50%) were culture negative. Among the 128 neonates with suspected late-onset septicemia, 52 (40.63%; 95% confidence interval: 32.50% to 49.29%) had positive cultures, while 76 (59.38%) had negative cultures. Thus, culture positivity was 6.13 percentage points higher in late-onset than in early-onset septicemia. However, this difference was not statistically significant (chi-square value = 1.26; degrees of freedom = 1; P value = 0.262), indicating that the time of onset was not significantly associated with blood-culture positivity. Overall, 121 of the 328 suspected cases were culture confirmed, corresponding to a prevalence of 36.89% (95% confidence interval: 31.84% to 42.24%). The overall culture-positive proportion was significantly lower than the reference proportion of 50% (z value = -4.75; P value < 0.001).

Table 3: Bacterial and fungal organisms isolated from neonates with culture-confirmed septicemia (N=121)

Organism isolated	n (%)	95% CI	Test of significance†	P value
Major microbial group				
Gram-negative bacteria	62 (51.24%)	42.44%-59.96%	χ^2=25.21, df=2	<0.001*
Fungi (Candida species)	42 (34.71%)	26.83%-43.54%		
Gram-positive bacteria	17 (14.05%)	9.00%-21.35%		
Gram-negative isolates				
Klebsiella species	42 (34.71%)	26.83%-43.54%	χ^2=110.26, df=4‡	<0.001*
Acinetobacter species	14 (11.57%)	7.03%-18.49%		
Escherichia coli	2 (1.65%)	0.45%-5.82%		
Citrobacter species	2 (1.65%)	0.45%-5.82%		
Pseudomonas aeruginosa	2 (1.65%)	0.45%-5.82%		
Gram-positive isolates				
Staphylococcus aureus	11 (9.09%)	5.16%-15.54%	χ^2=8.94, df=2§	0.011*
Coagulase-negative staphylococci	5 (4.13%)	1.77%-9.28%		
Enterococcus species	1 (0.83%)	0.15%-4.52%		
Fungal isolates				
Non-albicans Candida	38 (31.40%)	23.81%-40.14%	Exact binomial test¶	<0.001*
Candida albicans	4 (3.31%)	1.29%-8.18%		

†The distribution of Gram-negative, fungal and Gram-positive isolates was tested against equal expected proportions.
‡Goodness-of-fit chi-square test among the five Gram-negative species.
§Goodness-of-fit chi-square test among the three Gram-positive species.
¶Comparison of non-*albicans Candida* and *C. albicans* among 42 Candida isolates.
*Statistically significant at 0.05.

Detailed Candida species distribution

Candida species	n (%) among Candida isolates (n=42)	95% CI	Test of significance†	P value
Candida tropicalis	26 (61.90%)	46.81%-74.99%	χ^2=39.90, df=4	<0.001 *
Candida parapsilosis	7 (16.67%)	8.34%-30.57%		
Candida albicans	4 (9.52%)	3.77%-22.06%		
Candida krusei	3 (7.14%)	2.46%-19.01%		
Candida glabrata	2 (4.76%)	1.32%-15.79%		

†Goodness-of-fit chi-square test against equal expected proportions across the five Candida species.

Among the 121 neonates with culture-confirmed septicemia, Gram-negative bacteria were the predominant microbial group, accounting for 62 (51.24%; 95% confidence interval: 42.44% to 59.96%) isolates. Fungi belonging to the genus *Candida* accounted for 42 (34.71%; 95% confidence interval: 26.83% to 43.54%), whereas Gram-positive bacteria accounted for 17 (14.05%; 95% confidence interval: 9.00% to 21.35%). The distribution across the three major microbial groups was statistically significant (chi-square value = 25.21; degrees of freedom = 2; P value < 0.001), confirming the predominance of Gram-negative organisms.

Among the Gram-negative isolates, *Klebsiella* species were the most common and were identified in 42 (34.71%) culture-positive neonates, followed by *Acinetobacter* species in 14 (11.57%). *Escherichia coli*, *Citrobacter* species, and *Pseudomonas aeruginosa* were each isolated from 2 (1.65%) neonates. The distribution of the five Gram-negative organisms differed significantly (chi-square value = 110.26; degrees of freedom = 4; P value < 0.001), principally because of the predominance of *Klebsiella* species.

Among the Gram-positive organisms, *Staphylococcus aureus* was the most frequent isolate, accounting for 11 (9.09%; 95% confidence interval: 5.16% to 15.54%) cases. Coagulase-negative staphylococci were isolated from 5 (4.13%) cases, while *Enterococcus* species were isolated from 1 (0.83%) case. The distribution of Gram-positive isolates was statistically significant (chi-square value = 8.94; degrees of freedom = 2; P value = 0.011), demonstrating that *Staphylococcus aureus* was the predominant Gram-positive pathogen.

Of the 42 Candida isolates, 38 (90.48%) were non-*albicans Candida*, whereas only 4 (9.52%) were *Candida albicans*. Non-*albicans Candida* isolates therefore significantly predominated over *Candida albicans* (exact binomial test; P value < 0.001). When all culture-positive cases were considered, non-*albicans Candida* accounted for 31.40%, while *Candida albicans* accounted for 3.31%.

Within the Candida group, *Candida tropicalis* was the predominant species, accounting for 26 of the 42 isolates (61.90%; 95% confidence interval: 46.81% to 74.99%). It was followed by *Candida parapsilosis* with 7 (16.67%), *Candida albicans* with 4 (9.52%), *Candida krusei* with 3 (7.14%), and *Candida glabrata* with 2 (4.76%) isolates. The Candida species were not equally distributed (chi-square value = 39.90; degrees of freedom = 4; P value < 0.001), establishing *Candida tropicalis* as the principal cause of candidemia in this population.

Table 4. Antimicrobial susceptibility patterns of the principal bacterial isolates

Table 4A. Antimicrobial susceptibility of Gram-positive isolates

Organism and antimicrobial	Sensitive isolates, n/N (%)	95% CI	Exact binomial test†	P value
Staphylococcus aureus (n=11)				
Vancomycin	11/11 (100.00%)	74.12%-100.00%	Binomial	0.001*
Linezolid	11/11 (100.00%)	74.12%-100.00%	Binomial	0.001*
Clindamycin	8/11 (72.73%)	43.44%-90.25%	Binomial	0.227
Erythromycin	7/11 (63.64%)	35.38%-84.83%	Binomial	0.549
Cotrimoxazole	6/11 (54.55%)	28.01%-78.73%	Binomial	1.000
Gentamicin	5/11 (45.45%)	21.27%-71.99%	Binomial	1.000
Oxacillin	4/11 (36.36%)	15.17%-64.62%	Binomial	0.549
Tetracycline	3/11 (27.27%)	9.75%-56.56%	Binomial	0.227
Coagulase-negative staphylococci (n=5)				
Vancomycin	5/5 (100.00%)	56.55%-100.00%	Binomial	0.063
Linezolid	5/5 (100.00%)	56.55%-100.00%	Binomial	0.063
Clindamycin	4/5 (80.00%)	37.55%-96.38%	Binomial	0.375
Erythromycin	4/5 (80.00%)	37.55%-96.38%	Binomial	0.375
Cotrimoxazole	4/5 (80.00%)	37.55%-96.38%	Binomial	0.375

†Exact binomial test against a reference sensitivity proportion of 50%.
*Statistically significant at 0.05.

All 11 Staphylococcus aureus isolates were susceptible to vancomycin and linezolid, producing sensitivity rates of 100% (95% confidence interval: 74.12% to 100.00%) for both antimicrobial agents. These sensitivity proportions were significantly greater than the reference level of 50% (exact binomial test; P value = 0.001 for each antimicrobial). Clindamycin demonstrated activity against 8 (72.73%) isolates, followed by erythromycin against 7 (63.64%), cotrimoxazole

against 6 (54.55%), and gentamicin against 5 (45.45%). Lower sensitivity was observed for oxacillin, with only 4 (36.36%) susceptible isolates, and tetracycline, with 3 (27.27%) susceptible isolates. Apart from vancomycin and linezolid, none of the observed sensitivity proportions differed significantly from the reference proportion of 50%, partly because of the small number of Staphylococcus aureus isolates. All five coagulase-negative staphylococcal isolates were susceptible to vancomycin and linezolid. Clindamycin, erythromycin, and cotrimoxazole were each active against 4 of the 5 isolates, corresponding to a sensitivity of 80.00% (95% confidence interval: 37.55% to 96.38%). Nevertheless, these findings were not statistically significant because of the very small number of isolate.

Table 4B. Antimicrobial susceptibility of *Klebsiella* and *Acinetobacter* isolates

Organism and antimicrobial	Sensitive isolates, n/N (%)	95% CI	Exact binomial test†	P value
Klebsiella species (n=42)				
Meropenem	42/42 (100.00%)	91.62%-100.00%	Binomial	<0.001*
Imipenem	38/42 (90.48%)	77.92%-96.20%	Binomial	<0.001*
Gentamicin	29/42 (69.05%)	53.96%-80.92%	Binomial	0.020*
Piperacillin-tazobactam	28/42 (66.67%)	51.55%-78.98%	Binomial	0.044*
Amoxicillin-clavulanate	26/42 (61.90%)	46.81%-74.99%	Binomial	0.164
Tetracycline	25/42 (59.52%)	44.49%-72.99%	Binomial	0.280
Ciprofloxacin	21/42 (50.00%)	35.53%-64.47%	Binomial	1.000
Cotrimoxazole	13/42 (30.95%)	19.08%-46.04%	Binomial	0.020*
Amikacin	11/42 (26.19%)	15.28%-41.07%	Binomial	0.003*
Cefotaxime	5/42 (11.90%)	5.20%-25.00%	Binomial	<0.001*
Acinetobacter species (n=14)				
Imipenem	10/14 (71.43%)	45.35%-88.28%	Binomial	0.180
Meropenem	9/14 (64.29%)	38.76%-83.66%	Binomial	0.424
Ceftazidime	7/14 (50.00%)	26.80%-73.20%	Binomial	1.000
Piperacillin-tazobactam	7/14 (50.00%)	26.80%-73.20%	Binomial	1.000
Gentamicin	6/14 (42.86%)	21.38%-67.41%	Binomial	0.791
Ciprofloxacin	4/14 (28.57%)	11.72%-54.65%	Binomial	0.180
Amikacin	4/14 (28.57%)	11.72%-54.65%	Binomial	0.180
Cotrimoxazole	2/14 (14.29%)	4.01%-39.94%	Binomial	0.013*

†Exact binomial test against a reference sensitivity proportion of 50%.
*Statistically significant at 0.05.

All 42 *Klebsiella* isolates were susceptible to meropenem, giving a sensitivity of 100% (95% confidence interval: 91.62% to 100.00%; P value < 0.001). Imipenem also demonstrated high activity, with 38 (90.48%; 95% confidence interval: 77.92% to 96.20%) susceptible isolates (P value < 0.001). Gentamicin and piperacillin-tazobactam were active against 29 (69.05%) and 28 (66.67%) isolates, respectively. Both sensitivity rates were significantly greater than the reference proportion of 50% (P value = 0.020 for gentamicin and P value = 0.044 for piperacillin-tazobactam). Intermediate levels of sensitivity were observed for amoxicillin-clavulanate at 61.90%, tetracycline at 59.52%, and ciprofloxacin at 50.00%.

In contrast, *Klebsiella* demonstrated low sensitivity to cotrimoxazole, amikacin, and cefotaxime. Only 13 (30.95%) isolates were susceptible to cotrimoxazole (P value = 0.020), 11 (26.19%) were susceptible to amikacin (P value = 0.003), and 5 (11.90%) were susceptible to cefotaxime (P value < 0.001). These sensitivity proportions were significantly below the reference level of 50%, suggesting substantial resistance to these commonly used antimicrobial agents. Overall, carbapenems demonstrated the greatest activity against *Klebsiella* isolates.

Among the 14 *Acinetobacter* isolates, imipenem showed the highest sensitivity, with 10 (71.43%) susceptible isolates, followed by meropenem with 9 (64.29%). Ceftazidime and piperacillin-tazobactam were each active against 7 (50.00%) isolates, while gentamicin was active against 6 (42.86%). Relatively low sensitivity was observed for ciprofloxacin and amikacin, with only 4 (28.57%) susceptible isolates for each antimicrobial. Cotrimoxazole demonstrated the lowest activity, with only 2 (14.29%; 95% confidence interval: 4.01% to 39.94%) susceptible isolates. Cotrimoxazole sensitivity was significantly lower than the reference proportion of 50% (P value = 0.013). Although imipenem and meropenem exhibited comparatively greater activity, their findings did not reach statistical significance because only 14 *Acinetobacter* isolates were evaluated.

Table 4C. Selected resistant phenotype among bacterial isolates

Resistant phenotype	n/N (%)	95% CI	Test of significance†	P value
Methicillin-resistant S. aureus among S. aureus isolates	7/11 (63.64%)	35.38%-84.83%	Exact binomial test	0.549
Oxacillin-susceptible S. aureus	4/11 (36.36%)	15.17%-64.62%		
Multidrug-resistant bacterial isolates	Not determinable‡			

†The MRSA proportion was compared with a reference proportion of 50%.
‡The attached dataset provides only aggregated susceptibility counts for each antimicrobial.
Of the 11 Staphylococcus aureus isolates, 7 (63.64%; 95% confidence interval: 35.38% to 84.83%) were methicillin resistant, whereas 4 (36.36%) were oxacillin susceptible. Although methicillin-resistant Staphylococcus aureus represented almost two-thirds of the Staphylococcus aureus isolates, its prevalence did not differ significantly from the reference proportion of 50% (exact binomial test; P value = 0.549). The wide confidence interval reflected the small number of isolates and indicated uncertainty surrounding the estimated prevalence of methicillin resistance. Nevertheless, the observed proportion suggested a clinically important burden of methicillin resistance.
The prevalence of multidrug-resistant bacterial isolates could not be calculated from the available results because antimicrobial susceptibility was presented as aggregated totals for each antibiotic. Classification of multidrug resistance requires the susceptibility profile of each individual isolate across at least three antimicrobial categories.

DISCUSSION

Table 1: Overall prevalence and microbiological profile

In the present study, 121 of the 328 neonates with clinically suspected septicemia had positive blood cultures, giving a culture-confirmed prevalence of 36.89% (95% confidence interval: 31.84%-42.24%). The culture-positive proportion was significantly lower than the selected reference proportion of 50% (z value = -4.75; P value < 0.001). Nevertheless, a positivity rate of more than one-third represents a substantial burden of microbiologically confirmed neonatal infection. Blood-culture positivity varies widely between studies because of differences in patient selection, illness severity, blood volume, timing of sample collection, previous antibiotic exposure, culture technique, and definitions of clinically suspected sepsis. Klingenberg et al. (2018),[1] emphasized that many neonates treated for suspected sepsis remain culture negative because the clinical manifestations are nonspecific and blood-culture sensitivity may be reduced by inadequate sample volume or prior antibiotic exposure.
The culture positivity of 36.89% in the present study was higher than the 20.5% reported by Pokhrel et al. (2018),[2] among 336 neonates in Nepal and the 14% reported by Jatsho et al. (2020).[3] It was also higher than the 16.9% culture positivity reported by Yadav et al. (2018),[4] among 350 suspected cases. Conversely, it was lower than the higher culture yields reported in some hospital-based studies, reflecting differences in referral patterns, laboratory systems, inclusion criteria, and antimicrobial exposure before blood collection. In a multicentre cohort from Delhi, the Delhi Neonatal Infection Study collaboration (2016),[5] reported culture-positive sepsis in 6.2% of enrolled neonates, although that denominator included a much broader hospital-born neonatal population and is therefore not directly comparable with a cohort restricted to clinically suspected cases.
Gram-negative bacterial infections constituted the largest microbial group in the present study. Among the 121 positive cultures, Gram-negative bacteria accounted for 51.24%, followed by Candida species at 34.71% and Gram-positive bacteria at 14.05%. The predominance of Gram-negative bacteria was consistent with the Delhi Neonatal Infection Study collaboration (2016),[5] in which 64% of 1,005 isolates were Gram negative. Pokhrel et al. (2018),[2] similarly reported that most isolates were Gram negative, with Klebsiella species forming the largest group group. Gamit et al. (2022),[6] also documented an important contribution of Gram-negative organisms to neonatal septicemia in Gujarat. These findings support the continuing importance of Gram-negative hospital-associated pathogens in neonatal intensive care settings.
Among all clinically suspected neonates, Klebsiella and Candida species were each detected in 12.80%, followed by Acinetobacter species in 4.27% and Staphylococcus aureus in 3.35%. The equal contribution of Klebsiella and Candida is clinically important because both organisms are associated with outbreaks, prolonged hospitalization, invasive procedures, and substantial mortality. The high fungal contribution may indicate a population containing premature and low-birth-weight neonates exposed to broad-spectrum antibiotics, vascular catheters, mechanical ventilation, or prolonged neonatal intensive care.

Table 2: Culture positivity according to the time of onset

Culture-confirmed septicemia was observed in 69 of 200 suspected early-onset cases, giving a prevalence of 34.50%, and in 52 of 128 late-onset cases, giving a prevalence of 40.63%. Although late-onset cases showed a 6.13 percentage-point higher positivity rate, the difference was not statistically significant (chi-square value = 1.26; P value = 0.262). Thus, the study did not establish a significant association between the time of onset and culture positivity.
The relatively comparable culture positivity in early- and late-onset disease suggests that the burden of microbiologically confirmed infection extended across the entire neonatal period. Jatsho et al. (2020),[3] reported that 54.5% of culture-positive cases were early onset, whereas Pokhrel et al. (2018),[2] reported a more marked predominance of early-onset sepsis, accounting for 78.3% of positive cases. The Delhi Neonatal Infection Study collaboration (2016),[5] found that approximately two-thirds of sepsis episodes occurred within the first 72 hours of life. Importantly, the pathogen

composition did not differ substantially between early- and late-onset cases in that multicentre study, suggesting that hospital-associated Gram-negative organisms may contribute even to infections presenting early after birth.

By contrast, the slightly higher late-onset culture positivity in the present study may reflect longer hospitalization, exposure to invasive devices, mechanical ventilation, cross-transmission within the neonatal unit, and prolonged antibiotic administration. Mohakud et al. (2022),[7] also demonstrated that the distribution of culture-positive infections may differ by hospital setting and reported S. aureus as the predominant pathogen in both early- and late-onset sepsis. These variations indicate that the early-onset versus late-onset classification alone may not reliably predict microbial etiology, particularly in tertiary referral hospitals. Local surveillance should therefore guide empirical treatment in both groups.

Table 3: Distribution of bacterial and fungal isolates

Gram-negative organisms accounted for 62 of the 121 isolates (51.24%), Candida species for 42 (34.71%), and Gram-positive organisms for 17 (14.05%). The difference in distribution was statistically significant (chi-square value = 25.21; P value < 0.001), confirming that the microbial groups were not equally represented. This pattern agrees with Dalal et al. (2017),[8] who reported a predominance of Gram-negative organisms in neonatal sepsis and found high sensitivity of these organisms to carbapenems. The predominance of Gram-negative organisms is also consistent with the multicentre findings of the Delhi Neonatal Infection Study collaboration (2016).[5]

Klebsiella species were the predominant bacterial pathogens, accounting for 42 isolates or 34.71% of all positive cultures. Acinetobacter species were the next most common Gram-negative organisms, accounting for 11.57%, whereas E. coli, Citrobacter species, and P. aeruginosa each accounted for 1.65%. The distribution of Gram-negative species differed significantly (chi-square value = 110.26; P value < 0.001). Pokhrel et al. (2018),[2] similarly identified Klebsiella as the leading pathogen, accounting for 33.3% of culture-positive sepsis. Jatsho et al. (2020),[3] also identified Klebsiella pneumoniae as an important neonatal pathogen. In the Delhi multicentre study, however, Acinetobacter species were the most frequent Gram-negative isolates at 22%, followed by Klebsiella at 17% and E. coli at 14%.[5] The differences between hospitals reinforce the importance of unit-specific microbiological surveillance.

Among Gram-positive isolates, S. aureus was the most frequent organism, accounting for 11 isolates or 9.09% of all positive cultures. Coagulase-negative staphylococci accounted for 4.13%, while Enterococcus species accounted for 0.83%. Their distribution was statistically significant (chi-square value = 8.94; P value = 0.011). This finding resembles that of Mohakud et al. (2022),[7] who reported S. aureus as the most common isolate, accounting for 35.6% of culture-positive cases. In contrast, Pokhrel et al. (2018),[2] found coagulase-negative staphylococci to be the predominant Gram-positive organisms. Such differences may be related to catheter use, skin preparation, infection-control practices, and criteria used to distinguish true coagulase-negative staphylococcal infection from blood-culture contamination.

The high frequency of candidemia was a notable finding. Of the 42 Candida isolates, 38 (90.48%) were non-albicans Candida, while only 4 (9.52%) were C. albicans. This difference was statistically significant (P value < 0.001). Shettigar et al. (2018),[9] similarly found a predominance of non-albicans Candida, which accounted for 64.81% of neonatal candidemia, although their percentage was lower than the 90.48% observed in the present study. Biswas et al. (2023),[10] reported non-albicans Candida in 82.14% of neonatal Candida infections, further supporting the epidemiological shift away from C. albicans.

Among individual Candida species, C. tropicalis was predominant, accounting for 61.90%, followed by C. parapsilosis at 16.67%, C. albicans at 9.52%, C. krusei at 7.14%, and C. glabrata at 4.76%. The species distribution differed significantly (chi-square value = 39.90; P value < 0.001). Basu et al. (2017),[11] also reported C. tropicalis among the most frequently isolated Candida species in neonatal bloodstream infections. Biswas et al. (2023),[10] found C. tropicalis to be the leading species at 46.42%, which was lower than the 61.90% observed in the present study. Conversely, Shettigar et al. (2018),[9] found C. krusei to be the most frequent non-albicans species. These variations may reflect local antifungal exposure, catheter practices, environmental colonization, infection-control performance, and differences in the gestational age and birth weight of admitted neonates.

Table 4A: Antimicrobial susceptibility of Gram-positive isolates

All S. aureus isolates were susceptible to vancomycin and linezolid. These sensitivity rates were significantly greater than the 50% reference proportion (P value = 0.001 for each agent). Clindamycin and erythromycin retained activity against 72.73% and 63.64% of isolates, respectively, while susceptibility to gentamicin, oxacillin, and tetracycline was lower. The complete susceptibility to vancomycin and linezolid was consistent with Pokhrel et al. (2018),[2] who also reported 100% susceptibility of coagulase-negative staphylococci to these two drugs. Mohakud et al. (2022),[7] nevertheless documented extensive resistance to commonly used penicillins and cephalosporins, illustrating the diminishing usefulness of several conventional empirical agents.

All five coagulase-negative staphylococcal isolates were susceptible to vancomycin and linezolid, while 80% were susceptible to clindamycin, erythromycin,

and cotrimoxazole. However, the small number of isolates produced wide confidence intervals, and these percentages should be interpreted cautiously. The findings nevertheless suggest that glycopeptides and oxazolidinones retained strong in-vitro activity against the Gram-positive isolates in this setting.

Table 4B: Antimicrobial susceptibility of Gram-negative isolates

All 42 Klebsiella isolates were susceptible to meropenem, and 90.48% were susceptible to imipenem. Gentamicin and piperacillin-tazobactam were active against 69.05% and 66.67%, respectively. In contrast, susceptibility to cotrimoxazole, amikacin, and cefotaxime was only 30.95%, 26.19%, and 11.90%, respectively. Pokhrel et al. (2018),[2] reported a closely comparable pattern: Klebsiella showed 90.5% resistance to cefotaxime but 100% susceptibility to carbapenems. Dalal et al. (2017),[8] likewise found high carbapenem activity against Gram-negative isolates. Sarangi et al. (2015),[12] reported substantial resistance to commonly used antibiotics among neonatal blood-culture isolates, supporting the need for local antibiograms.

Although the present carbapenem susceptibility is reassuring, the extensive use of meropenem or imipenem as empirical therapy could accelerate the emergence of carbapenem-resistant organisms. The Delhi Neonatal Infection Study collaboration (2016),[5] found multidrug resistance in 82% of Acinetobacter, 54% of Klebsiella, and 38% of E. coli isolates. Therefore, carbapenems should ideally be reserved for neonates with severe infection, documented resistance, or strong epidemiological risk factors.

Among Acinetobacter isolates, susceptibility was highest to imipenem at 71.43% and meropenem at 64.29%. Only 28.57% were susceptible to ciprofloxacin and amikacin, while 14.29% were susceptible to cotrimoxazole. This low cotrimoxazole susceptibility was statistically significant (P value = 0.013). The pattern agrees with the broader concern raised by Yusef et al. (2018),[13] regarding the increasing contribution of multidrug-resistant organisms to neonatal intensive care unit sepsis. The Delhi multicentre findings also identified Acinetobacter as the organism with the highest MDR prevalence.[5]

Table 4C: Methicillin resistance and multidrug resistance

Seven of the 11 S. aureus isolates were methicillin resistant, giving an MRSA prevalence of 63.64%. Although the proportion did not differ significantly from 50% (P value = 0.549), the lack of significance was likely influenced by the small number of S. aureus isolates and the resulting wide confidence interval. The observed prevalence was higher than the 38% methicillin resistance among S. aureus reported by the Delhi Neonatal Infection Study collaboration (2016).[5] The high MRSA proportion, together with preserved vancomycin and linezolid activity, emphasizes the importance of resistance-directed therapy and careful antimicrobial stewardship.

A valid overall MDR prevalence could not be calculated because the data were available only as aggregated susceptibility totals for individual antibiotics. MDR status must be assigned to each isolate based on nonsusceptibility to at least one agent in three or more antimicrobial categories. Pokhrel et al. (2018),[2] using isolate-level information, reported an MDR prevalence of 73.9%, while the Delhi multicentre study found particularly high MDR levels among Acinetobacter and Klebsiella isolates.[5]

CONCLUSION

The present study demonstrated a substantial burden of neonatal septicemia in the tertiary care hospital, with blood-culture-confirmed infection identified in 121 of 328 clinically suspected neonates, giving a prevalence of 36.89%. Culture positivity was slightly higher among neonates with late-onset septicemia than among those with early-onset septicemia; however, the difference was not statistically significant.

Gram-negative bacteria constituted the predominant microbial group, followed by *Candida* species and Gram-positive bacteria. *Klebsiella* species were the most frequently isolated bacterial pathogens, while *Staphylococcus aureus* predominated among Gram-positive organisms. The high proportion of candidemia was noteworthy, with non-*albicans* *Candida* accounting for most Candida isolates and *Candida tropicalis* being the leading fungal species.

Vancomycin and linezolid demonstrated complete in-vitro activity against *S. aureus* and coagulase-negative staphylococci. Meropenem and imipenem showed the highest activity against *Klebsiella* and *Acinetobacter* species. Conversely, low susceptibility to cefotaxime, amikacin, and cotrimoxazole was observed among the major Gram-negative isolates. Nearly two-thirds of the *S. aureus* isolates were methicillin resistant, indicating a clinically important burden of antimicrobial resistance.

These findings emphasize the need for timely blood-culture testing, species-level identification of Candida isolates, continuous surveillance of antimicrobial resistance, strict infection-prevention practices, and periodic revision of the institutional empirical antibiotic policy. Antimicrobial treatment should be modified according to culture and susceptibility results to improve neonatal outcomes and limit the further emergence of drug-resistant organisms.

Limitations of Study

1. The study was conducted at a single tertiary care hospital; therefore, its findings may not be generalizable to primary-care facilities, community settings, or hospitals in other geographical regions.

2. The cross-sectional observational design permitted estimation of prevalence and microbial distribution but could not establish temporal or causal relationships between potential risk factors and neonatal septicemia.
3. Only neonates with clinically suspected septicemia for whom blood culture was requested were included. Consequently, selection or referral bias may have influenced the observed prevalence.
4. Conventional blood-culture techniques were used. Their sensitivity may have been lower than that of automated blood-culture systems, particularly in neonates with low-level or intermittent bacteremia.
5. The small volume of blood that could be safely obtained from neonates may have reduced the diagnostic yield of blood cultures.
6. Some neonates might have received antibiotics before sample collection, which could have produced false-negative blood-culture results.
7. Anaerobic bacterial cultures, viral investigations, and molecular diagnostic methods were not routinely performed. Therefore, some potential causes of culture-negative neonatal sepsis may have remained unidentified.
8. The clinical significance of coagulase-negative staphylococci can be difficult to distinguish from blood-culture contamination, particularly when repeat cultures are unavailable.
9. Antifungal susceptibility testing was not comprehensively reported for all Candida isolates despite the high prevalence of non-albicans Candida species.
10. The numbers of some bacterial species, particularly E. coli, Citrobacter, Pseudomonas, and Enterococcus, were small, resulting in imprecise species-specific estimates.
11. The onset-wise organism totals in the source records were not fully consistent with the early- and late-onset culture-positivity totals, limiting reliable comparison of individual pathogens according to the time of onset.
12. Antimicrobial susceptibility results were available as aggregated totals for each antibiotic rather than as isolate-level susceptibility profiles. Therefore, the prevalence of multidrug-resistant, extensively drug-resistant, and pan-drug-resistant organisms could not be calculated reliably.
13. The study did not undertake multivariable analysis to identify independent maternal, perinatal, clinical, or treatment-related predictors of culture-positive septicemia.
14. Long-term outcomes, including neurodevelopmental impairment, recurrent infection, and post-discharge mortality, were not assessed.

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