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Bacteriological and Hematological Profile of Neonatal Sepsis: Rural-Urban Comparison in a Tertiary Care Setting

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ABSTRACT

Objectives: To determine the frequency of blood culture-proven neonatal sepsis (NNS) and to compare the bacteriological and hematological profiles of culture-positive cases between rural and urban neonates.

Methodology: This descriptive, cross-sectional study was conducted at the Department of Pediatric Unit 1, Bahawal Victoria Hospital, Bahawalpur, over a period of seven months from January 2023 to July 2023 and enrolled 111 neonates with suspected sepsis. Preterm neonates, as well as those with a ruptured meningomyelocele or a history of prior hospitalization, were excluded from the study. For each participant, demographic and clinical details were recorded, and hematological parameters, including white blood cell count, hemoglobin, and platelet count, were assessed. Blood cultures were also performed to identify Gram-positive and Gram-negative organisms, and the resulting findings were compared between rural and urban cases.

Results: Blood culture-proven sepsis was found in 27 neonates (24.32%). Gram-negative organisms predominated, accounting for 58.82% of rural and 70% of urban cases. Gram-positive organisms accounted for 41.18% of rural and 30% of urban cases. Thrombocytopenia and leukopenia were significantly more common in Gram-negative infections and among rural neonates ($p < 0.05$). Low birth weight and early-onset sepsis were significantly associated with sepsis ($p<0.05$).

Conclusion: Blood culture-proven NNS was found in nearly one-fourth of suspected cases. Gram-negative organisms predominated. Hematological abnormalities were common. Thrombocytopenia and leukopenia were more frequent in Gram-negative and rural cases, suggesting severe disease. Early pathogen identification, local bacteriological surveillance, and monitoring hematological markers are key to guide therapy and improve outcomes.

Keywords: Blood culture, Gram-positive bacteria, Gram-negative bacteria, Leukopenia, NICU Neonatal infections, Neonatal sepsis, Rural-urban differences, Thrombocytopenia.

Authors' Contribution:
[1,2]Conception; Literature research; manuscript design and drafting; [3,4]Critical analysis and manuscript review; [1,4]Data analysis; Manuscript Editing.

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Introduction

NNS remains a major cause of illness and death worldwide. It affects nearly three million newborns each year. Around 570,000 neonatal deaths are reported annually.[1] Low- and middle-income countries carries the highest burden. Early and accurate diagnosis is crucial because clinical signs are often nonspecific.[2] In Pakistan, neonatal mortality is high, with rates reported around 42 per

1,000 live births; NNS contributes substantially to this figure.[3]

Blood culture proven NNS allows definitive identification of causative organisms and guides targeted therapy. However, its sensitivity may be suboptimal in settings with limited laboratory resources, delays, or prior antibiotic exposure.[4] In parallel, hematological parameters including total leukocyte count (TLC), neutrophil count, hemoglobin levels, and platelet count are often used as adjunctive markers for diagnosis of NNS, prognosis, and monitoring response. Alterations in these parameters (such as leukocytosis, leukopenia, neutropenia or neutrophilia, thrombocytopenia, and anemia) have been associated with severity of illness and worse outcomes in neonates with culture-proven sepsis.[5,6] Thrombocytopenia, in particular, has been reported in 78.4% of neonates with culture-positive sepsis in a tertiary hospital study from Karachi, where it was strongly linked to Gram-negative pathogens such as *Klebsiella pneumoniae* and *Burkholderia cepacian.*[7] Similarly, a recent neonatal intensive care unit (NICU) based study in Turkey found coagulase-negative staphylococci as the leading isolates overall, while *Klebsiella* spp. and *E. coli* predominated among Gram-negatives, with pronounced hematological derangements including thrombocytopenia commonly observed in affected infants.[8]

Differences in epidemiological factors, including geographical setting (urban vs rural), affect both risk factors and the spectrum of bacterial pathogens. For example, studies have shown significant variation in prevalence of Gram-negative vs Gram-positive organisms, as well as varying antibiotic resistance patterns, between urban tertiary care centres and rural health facilities[9]. Nevertheless, there is a paucity of studies comparing both bacteriological and hematological profiles of blood culture proven NNS in rural versus urban neonates in Pakistan.

Therefore, this study aims to compare the bacteriological profile (i.e., type of pathogens, Gram stain distribution) and hematological profile (including WBC count, hemoglobin, platelet count) among neonates with culture proven sepsis in rural versus urban neonates. The goal is to identify whether there are distinguishable differences that could help in tailoring empirical therapy and prognostic assessment, especially in resource-limited settings.

Methodology

This descriptive, cross-sectional study was conducted in the department of Pediatrics, Unit 1, Bahawal Victoria Hospital, Bahawalpur, from January 2023 to July 2023. A sample of 111 neonates was calculated using the WHO sample size calculator with a 95% confidence level, a 7% margin of error, and an expected prevalence of blood culture proven neonatal septicemia of 16.9%[10]. A non-probability, consecutive sampling technique was used.

All neonates admitted in NICU with clinical suspicion of sepsis, of either gender, and belonging to both rural and urban areas were included. Clinical suspicion of NNS was based on feeding intolerance, lethargy, hypotonia, irritability, fever, tachypnea, tachycardia, poor perfusion, and mottled/cold skin. Exclusion criteria were preterm neonates (delivered before 37 weeks as per history), neonates with ruptured meningocele/meningomyelocele, and those with a past history of hospitalization. For each neonate, demographic and clinical details including age, gender, gestational age, weight at presentation, duration of illness, place of residence (rural or urban), monthly household income (<20,000; 20,001–40,000; >40,000 PKR), maternal education (illiterate, primary, middle, matric, graduate), and type of housing (kacha or pakka) were recorded on a structured proforma. Blood cultures were performed to identify the causative organisms (Gram-positive or Gram-negative bacteria). In

addition, hematological parameters such as TLC, hemoglobin, and platelet count were measured at admission and documented. For analysis, specific hematological cutoffs were used. Leukopenia was defined as a WBC count below 5,000/mm³. Leukocytosis was defined as a WBC count above 27,000/mm³. Anemia was defined as hemoglobin below 12 g/dL. Thrombocytopenia was defined as a platelet count below 150,000/mm³.

Data were analyzed using Statistical Package for the Social Sciences (SPSS) version 25. Normality of quantitative variables was checked using the Shapiro–Wilk test. Quantitative data were summarized appropriately. Mean and standard deviation were used for normally distributed variables. Median and interquartile range were used for skewed data. Variables analyzed included age and weight at presentation. Gestational age and duration of illness were recorded. Hematological parameters were also assessed. These included total leukocyte count, hemoglobin, and platelet count. Frequencies and percentages were calculated for categorical variables. These included gender and place of residence. Maternal education and type of housing were recorded. Monthly income was also noted. The type of neonatal sepsis was classified. This included early-onset and late-onset sepsis. Bacterial growth was categorized as Gram-positive or Gram-negative. Stratification was done to control for effect modifiers. These included age, weight, and gestational age. Duration of illness and income were also considered. Maternal education, residence, and type of organism were included. Post-stratification analysis was performed. The Chi-square test or Fisher's exact test was used where appropriate. A p-value of ≤0.05 was considered statistically significant.

Ethical approval was obtained from the Institutional Ethical Review Committee (BVH-26/12/2022-IRB-1044), and informed verbal consent was taken from parents or guardians

Results

Out of 111 neonates with clinically suspected sepsis, 27 (24.3%) had blood culture-proven sepsis. The mean age of neonates was 15.08 ± 4.90 days, and males predominated with a male-to-female ratio of 2:1. Mean gestational age was 38.77 ± 1.41 weeks, mean duration of illness 3.44 ± 1.36 days, and mean weight 2.6 ± 0.30 kg, mentioned in table I. Low weight (≤2.5 kg) was significantly associated with culture positivity (p = 0.0003), as 45% of culture-positive neonates had low weight compared to 10.3% of culture-negative cases. Early-onset NNS was also significantly more frequent among culture-positive neonates as compared to neonates with culture negative NNS (p < 0.001). No statistically significant associations were found for age group, gender, duration of illness, gestational age, income, maternal education, place of living, or home structure.

Table I. Baseline characteristics of study population (n=111)

Variable	Category	n (%) / Mean ± SD
Age (days)	≤14	64 (57.64)
	15–28	47 (42.34)
Mean age		15.08 ± 4.90
Gender	Male	74 (66.67)
	Female	37 (33.33)
Gestational age (weeks)	37–39	75 (67.57)
	40–42	36 (32.43)
Mean GA		38.77 ± 1.41
Duration of illness (days)	≤3	54 (48.65)
	>3	57 (51.35)
Mean duration		3.44 ± 1.36
Weight (kg)	≤2.5	21 (18.92)
	>2.5	90 (81.08)
Mean weight		2.9 ± 0.70

The table I reflects a generally healthy baseline profile, though nearly one-fifth had low weight, which is a recognized risk for sepsis.

Table II. Socio-demographic profile (n=111)		
Variable	**Category**	**n (%)**
Monthly income	<20,000	43 (38.74)
	20,001–40,000	53(47.75)
	>40,000	15 (13.51)
Mother education	Illiterate	18 (16.22)
	Primary	16 (14.41)
	Middle	32 (28.83
	Matric	28 (25.23)
	Graduate	17 (15.32)
Place of living	Rural	68 (61.26)
	Urban	43 (38.74)
Home structure	Kacha	41 (36.94)
	Pakka	70 (63.06)
Type of NNS	Early-onset	7 (6.31)
	Late-onset	104 (93.69)

Among the 27 culture-positive cases, Gram-negative bacteria (17/27, 62.96%) were more common than gram-positive organisms (40.7%). In rural neonates, Klebsiella spp. and E. coli predominated, whereas in urban cases, Coagulase-negative staphylococci (CoNS) and Staphylococcus aureus were more frequent.

Hematological abnormalities, particularly thrombocytopenia and leukopenia, were more frequent in Gram-negative infections and among neonates from rural areas (both $p<0.05$). Low hemoglobin (<12 g/dL) was common in both groups but showed no significant association.

The majority of neonates belonged to middle or low-income families, with mothers largely having only basic education. Rural residence was more common, and most families lived in permanent (pakka) houses. Late-onset sepsis was the predominant type.

Table III. Blood culture proven NNS and stratified analysis				
Variable	**Category**	**Culture proven Sepsis=Yes n=27 (%)**	**Culture proven Sepsis = No n=84 (%)**	**p value**
Age (days)	≤14	17 (26.56)	47 (73.44)	0.68
	15–28	10 (21.28)	37 (78.72)	
Gender	Male	17 (22.97)	57 (77.03)	0.81
	Female	10 (27.03)	27 (72.97)	
Duration (days)	≤3	15 (27.78)	39 (72.22)	0.55
	>3	12 (21.05)	45 (78.95)	
GA (weeks)	37–39	17 (22.67)	58 (77.33)	0.73
	40–42	10 (27.78)	26 (72.22)	
Weight (kg)	≤2.5	14 (66.67)	7 (33.33)	0.0003
	>2.5	13 (14.44)	77 (85.56)	
Type of NNS	Early	7 (100.0)	0 (0.0)	0.001
	Late	20 (19.23)	84 (80.77)	
Income	<20,000	6 (13.95)	37 (86.05)	0.12
	20,001–40,000	16 (30.19)	37 (69.81)	
	>40,000	5 (33.33)	10 (66.67)	
Maternal education	Illiterate	6 (33.33)	12 (66.67)	0.28
	Primary	5 (31.25)	11 (68.75)	
	Middle	6 (18.75)	26 (81.25)	
	Matric	6 (21.43)	22 (78.57)	
	Graduate	4 (23.53)	13 (76.47)	
Place of living	Rural	17 (25.00)	51 (75.00)	0.17
	Urban	10 (23.20)	33 (76.80)	
Home structure	Kacha	11 (26.83)	30 (73.17)	0.81
	Pakka	16 (22.86)	54 (77.14)	
Bacterial profile (culture +ve)	Gram positive	10 (37.03)	-	0.68
	Gram negative	17 (62.96)	-	

Gram-negative isolates predominated in both rural and urban settings, though the difference was not statistically significant.

Table IV: Hematological profile in neonates with culture proven sepsis according to pathogen type and residence (n=27)									
Group	**n**	**Mean WBC ($\times10^3$/mm^3)**	**Leukopenia <5,000 n (%)**	**Leukocytosis >27,000 n (%)**	**Mean Hb (g/dL)**	**Low Hb <12 n (%)**	**Mean Platelets ($\times10^3$/mm^3)**	**Thrombocytopenia <150×10^3 n (%)**	**p value**
Gram-positive	10	17.5	2 (20.0)	1 (10.0)	11.8	5 (50.0)	176	3 (30.0)	0.02
Gram-negative	17	9.6	8 (47.1)	3 (17.6)	11.4	11 (64.7)	88	13 (76.5)	0.02
Rural	13	10.2	6 (46.2)	2 (15.4)	11.4	9 (69.2)	92	9 (69.2)	0.01
Urban	14	16.3	4 (28.6)	2 (14.3)	11.9	8 (57.1)	170	5 (35.7)	0.01

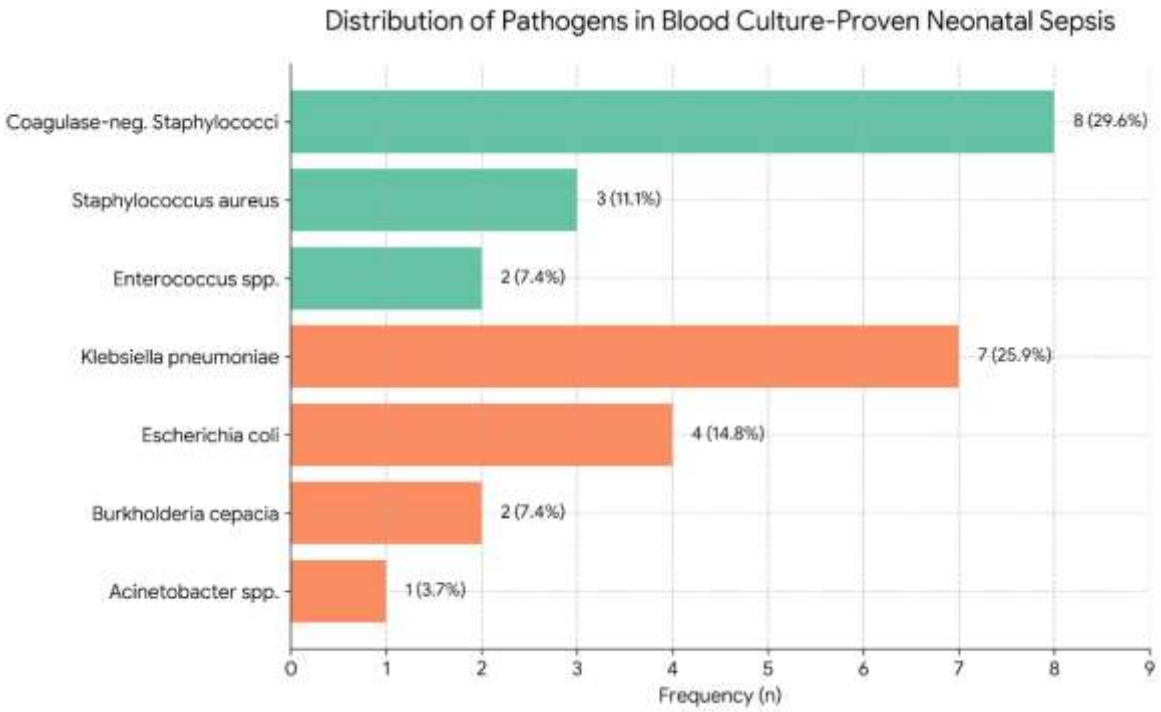

Figure 1: Distribution of Pathogens in Blood Culture-Proven Neonatal Sepsis

Leukopenia and thrombocytopenia were significantly more frequent in Gram-negative sepsis and among rural neonates, suggesting a more severe inflammatory and bone marrow suppressive effect in these groups. Low hemoglobin was common across groups but did not show statistical significance, indicating that anemia may be a nonspecific finding in neonatal sepsis.

This horizontal bar chart displaying the frequency and percentage of seven different bacterial pathogens isolated in cases of neonatal sepsis. Coagulase-negative Staphylococci among Gram +ve cases (29.6%) and Klebsiella pneumoniae among Gram -ve sepsis (25.9%) being the primary organisms responsible for NNS in this cohort.

Discussion

This cross-sectional study of 111 neonates with clinically suspected sepsis identified blood culture-proven sepsis in 27 (24.3%) cases. This culture positivity rate aligns with findings from other South Asian and low- and middle-income countries settings, which report rates ranging from 16% to 30%.[1] Conversely, Yadav et al. reported 16.9%

culture positivity of NNS in a tertiary centre, which is low as compared to our study.[10] These differences likely due to variation in pre-sampling antibiotic exposure, blood volume collected, culture methods, and case-selection criteria.

A male predominance (male: female ≈ 2:1) was observed, consistent with regional reports indicating higher male representation among suspected and culture-positive sepsis cases.[11]

The study found a significant association between culture-positive sepsis and low weight of the neonates, with 14 (51.9%) of the 27 culture-positive cases being with low weight at presentation. This finding is consistent with previous studies highlighting low birth weight (LBW) as a major risk factor for NNS.[12] Additionally, late-onset sepsis was more prevalent among culture-positive cases, aligning with literature indicating that late-onset sepsis is more common in LBW and preterm infants.[13]

Microbiologically, the study identified both Gram-positive and Gram-negative organisms, with CoNS and Klebsiella species being the most common isolates. This is in line with findings from other NICU and tertiary-centre studies.[8,14] A study from Karachi highlights the predominance of Gram-negative bacteria with significant antibiotic resistance in NNS.[15] In contrast with some studies reporting Gram-positive predominance, such as the Islamabad/Mansehra series where 76.8% of culture-positives were Gram-positive.[16]

Hematological parameters were significantly associated with culture-positive sepsis. Thrombocytopenia and leukopenia were more frequent in Gram-negative infections and among neonates from rural areas. These findings corroborate previous studies linking thrombocytopenia to culture-proven sepsis and to Gram-negative organisms in particular.[7,17,18] Similarly, other NICU studies have documented that Gram-negative sepsis is frequently associated with more pronounced hematological derangements (including thrombocytopenia and leukopenia) and worse clinical courses.[8,19] Taken together, these data suggest that thrombocytopenia and leukopenia remain useful adjunctive indicators of more severe or Gram-negative sepsis in resource-limited settings, though they are not specific.[20]

Overall, our study underscores the importance of early identification and appropriate management of NNS, particularly in resource-limited settings. The findings highlight the need for continuous surveillance, adherence to infection control practices, and the development of local antibiograms to guide empirical therapy. Further multicentered studies are warranted to validate these findings and inform national guidelines for the management of neonatal sepsis.

The study has a few limitations including, a single-centre study, small number of culture-positive cases, hematological data recorded only at admission, lack of systematic data on prior antibiotic exposure, and possible CoNS contamination

Conclusion

This study demonstrated that nearly one-quarter of neonates with clinically suspected sepsis had blood culture-proven infection, with Gram-negative organisms predominating. Low birth weight and early-onset presentation were significantly associated with culture positivity. Hematological abnormalities, particularly thrombocytopenia and leukopenia, were more frequently observed in Gram-negative infections and among neonates from rural areas, suggesting a potentially more severe disease profile in this subgroup.

The rural-urban comparison provides important clinical and public health insight. The higher frequency of hematological derangements among rural neonates may reflect delayed presentation, limited access to antenatal and perinatal care, suboptimal infection prevention practices, or referral bias from peripheral facilities. These findings highlight disparities in neonatal health and

emphasize the need for strengthened primary healthcare systems in rural areas.

Recommendations: Based on these observations, targeted recommendations include:

1. Enhanced antenatal screening and intrapartum infection control in rural settings.
2. Early referral protocols and improved transport systems for high-risk neonates from peripheral centers.
3. Routine hematological screening (including platelet count and total leukocyte count) at admission for early risk stratification, particularly in rural neonates.
4. Development of region-specific antibiograms to guide empirical antibiotic therapy.
5. Strengthening surveillance systems to monitor bacteriological trends across rural and urban populations.

In resource-limited settings, integrating microbiological surveillance with simple hematological markers may facilitate earlier diagnosis and risk stratification, ultimately improving neonatal outcomes and reducing sepsis-related morbidity and mortality.

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