Original Article

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Bacteriological profile in blood culture proven neonatal sepsis

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ABSTRACT

Background: Neonatal sepsis is a systemic infection caused by bacteria, viruses, fungi, and parasites. It results in significant morbidity and mortality, accounting for up to half of all newborn deaths in developing countries. **Aim**: Hence, this study aimed to determine the bacteriological profile of neonatal sepsis and assess its relationships with other demographic variables. **Methods**: A prospective, cross-sectional study was carried out on 87 neonates with blood culture-proven neonatal sepsis. They were categorized according to the age of onset of symptoms as having early-onset sepsis and late-onset sepsis. The bacterial profile that resulted from their blood cultures was analyzed. **Results**: The findings revealed that the most common isolated bacterial was *Staphylococcus aureus*, reported in 34.48% of the patients. Collectively, Gram-positive bacteria represent 67.82% of bacterial profiles versus 32.18% for Gram-negative bacteria. Significantly more *Acinetobacter spp*. were isolated from newborns with EOS than those with LOS (23.08% vs 6.56%). Contrarily, *Streptococcus viridans* were significantly more common among neonates with late-onset sepsis than those with early-onset sepsis. Interestingly, Gram-negative bacteria were present more frequently (53.85% vs. 22.95%) in infants with EOS than LOS, indicating a significant difference. In addition, Gram-positive bacteria accounted for 77.59% of sepsis in full-term neonates and 48.28%% of preterm neonates, with a significant difference. **Conclusions**: Early-onset sepsis and premature newborns were more likely to have gram-negative bacterial sepsis, whereas late-onset sepsis and full-term neonates were more expected to have gram-positive bacteria.

Keywords: neonatal sepsis, blood culture, early-onset sepsis, late-onset sepsis

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INTRODUCTION

Neonatal sepsis is a systemic infection that typically affects sterile body fluids and is accompanied by hemodynamic abnormalities and other clinical symptoms. It results in significant morbidity and mortality and is primarily caused by bacteria and viruses; though, it can also be triggered by fungi and parasites.[1,2] Because of the increasing number of premature babies, who are more vulnerable to neonatal sepsis because of their weakened innate immune function and the necessity for invasive procedures to maintain their life support, the incidence of neonatal sepsis is anticipated to rise internationally.[3]

Neonatal sepsis is more common in developing countries, accounting for half of all neonatal deaths;

however, the incidence may differ from one country to another.[4] The lack of knowledge of the warning signs of sepsis, inadequate training of medical staff, and an inadequate number of dependable laboratories contribute to the high number of neonatal sepsis patients in developing countries. Consequently, care is compromised, outdated antibiotic guidelines are followed, and resistance to certain antibiotics develops.[5,6]

Neonatal sepsis is classified based on when it first manifests: early-onset sepsis (EOS), which occurs within the first week of life, and late-onset sepsis (LOS), which occurs between days 8 and 28 of life. [6-8] However, other authors have classified it into EOS, in which clinical symptoms manifest within the first 72 hours of life, and LOS, which occurs within the next 4 to 28 days of life.[9-11]

The gold standard for the diagnosis of sepsis is blood culture. White blood cell (WBC) count and C-reactive protein are two additional biomarkers that can be used as diagnostic indicators.[4,12] A systemic inflammatory response considerably changes WBC counts, resulting in neutrophilia and/or relative lymphopenia.[13]

Hence, neonatal sepsis is an emergency medical situation that requires immediate care and appropriate antibiotic treatment.[14] In addition, the recent increase in antimicrobial resistance poses a great concern. One longitudinal analysis of newborn infections in developing nations found that *group B streptococcus* was uncommon in South Asia, gram-positive organisms were almost as common as gram-negative ones in African countries, and *Staphylococcus aureus* was less common in Latin America and East Asia than in the other regions.[15] As a result, the bacteriological profile varies considerably, depending on the geographic region, and occasionally fluctuates in the same environment. Therefore, examining the local epidemiology to develop preventative measures and improve empirical antibiotic use is vital.[1,16,17] This study aimed to determine the bacteriological profile of neonatal sepsis and to assess its relationships with other demographic variables.

MATERIALS AND METHODS

A prospective, cross-sectional study was carried out on newborns diagnosed with neonatal sepsis. All neonates admitted to the neonatal care unit with signs and symptoms of sepsis proven by positive blood cultures were included in the study, which was conducted at Central Child's Teaching Hospital in Baghdad, Iraq. Eight-seven cases were included over 5 months from the 1st of February, 2023 to the end of June, 2023. According to a standardized questionnaire, a thorough history and physical examination were performed on all patients. Age at admission, sex, birth weight [normal birth weight (NBW) vs. low birth weight (LBW)], gestational age [term vs. preterm], and delivery method [vaginal vs. cesarean section] were considered and recorded as "neonatal information." All patients were sent for laboratory investigations, which included WBC counts with differential cell counts and blood cultures. Cases were then divided into two groups according to the age of onset of their symptoms, where EOS was used if symptoms started in the first week of life, and LOS was used if symptoms started from 8 to 28 days after birth, [6-8] then demographic and laboratory results were compared between the two groups. Parents' informed consent was obtained to include the neonates in the study. Mustansiriyah University's local ethics committee approved the study. (IRB 3 in Jan 2023).

SPSS software version 25.0 (SPSS, Chicago) was used to conduct all statistical analyses. The Shapiro-Wilk test was utilized to assess the continuous data. A Student t-test was used to examine data with a normal distribution and was expressed as mean and standard deviation. The Mann-Whitney U test assessed the non-normally distributed data, which was reported as median and range. The Chi-square test was used to assess categorical variables, presented as numbers and percentages. The predictive value of the N/L ratio in predicting the type of sepsis was evaluated using the receiver operating characteristic (ROC) curve. A statistically significant difference was determined to exist when the p-value was less than 0.05.

RESULTS

Eighty-seven cases were included in the study. Table 1 details the demographic and clinical characteristics, while Table 2 lists the leucocyte counts.

Table 3 enumerates the types of bacteria causing sepsis. The Gram-positive bacteria represent 67.82% of bacterial profiles vs. 32.18% for Gram-negative bacteria. Out of 87 neonates, 26 (29.89%) had EOS, while 61 neonates (70.11%) had LOS (Fig. 1).

More females had EOS than LOS (61.54% vs. 39.34%). Other variables were equivalent and did not significantly differ between the two groups (Table 4).

When newborns with EOS were compared to those with LOS, the median neutrophil count was higher, while the median lymphocyte count was lower. However, the

differences were non-significant. In contrast, the median NLR in neonates with EOS was 2.51, much higher than that of neonates with LOS (1.77), with a significant difference (Table 5).

The ROC curve was used to assess the predictive value of NLR in differentiating EOS and LOS. The area under the curve (AUC) was 0.659, 95% CI = 0.527-0.791, with p = 0.019. The sensitivity and specificity of the test at NLR = 1.93 were 66% and 54%, respectively (Fig. 2).

Significantly more *Acinetobacter spp*. were isolated from newborns with EOS than those with LOS (23.08% vs. 6.56%). On the other hand, *Street. viridans* were more common among neonates with LOS (27.87%) than those with EOS (3.85%), demonstrating a significant difference. Interestingly, Gram-negative bacteria were present more frequently (53.85% vs. 22.95%) in infants with EOS than LOS, presenting a significant difference, as shown in Table 6.

Staph aureus was isolated from 43.1% and 17.24% of full-term and preterm neonates, respectively, with a significant difference. On the other hand, *Klebsiella spp.* and *E. coli* were isolated from 27.59% and 13.79% of preterm neonates, respectively, versus 10.34% and none of full-term neonates, respectively, with significant differences. Collectively, Gram-positive bacteria accounted for 77.59% of sepsis in full-term neonates and 48.28%% of preterm neonates, suggesting a significant difference (Table 7).

Table 1: Demographic and clinical characteristics of the neonates (n = 87)

Variables	Values
Age, days	
Mean±SD	13.63±7.46
Range	1.0-25.0
Sex	
Male	47 (54.02%)
Female	40 (45.98%)
Gestational age	
Term	58 (66.67%)
Preterm	29 (33.33%)
Mode of delivery	
Vaginal	44 (50.57%)
Cesarean section	43 (49.43%)
Birth weight	
Normal	50 (57.47%)
Low	37 (42.53%)

Table 2: Leukocyte counts and differential WBC counts in the studied neonates (n = 87)

Variables	Values
WBC count ×10^9/L	
Mean±SD	17.52±6.58
Median	16.5
Range	5.5-42.0
Neutrophil ×10^9/L	
Mean±SD	9.64±5.68
Median	8.77
Range	2.48-27.3
Lymphocyte×10^9/L	
Mean±SD	5.6±2.4
Median	5.18
Range	2.05-11.58
N/L ratio	
Mean±SD	2.26±1.84
Median	2.0
Range	0.33-7.24

Table 3: Types of bacteria that cause sepsis (n = 87)

Type of Bacteria	Frequency and Percentage
Staph. aureus	30 (34.48%)
Acinetobacter spp.	10 (11.49%)
Klebsiella spp.	14 (16.09%)
Strept. viridans	18 (20.69%)
Enterococcus spp.	4 (4.6%)
Staph. epidermidis	5 (5.75%)
E. coli	4 (4.6%)
Listeria spp.	2 (2.3%)
Gram-positive	59 (67.82%)
Gram-negative	28 (32.18%)

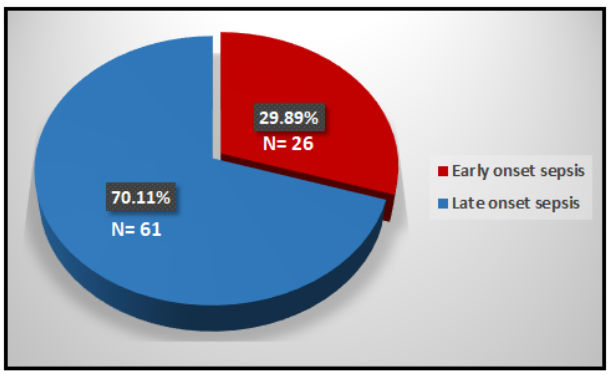

Figure 1: The incidence of early and late-onset sepsis (n = 87)

Table 4: Association of demographic and clinical characteristics with the type of sepsis

Variables	EOS (n = 26)	LOS (n = 61)	p-value
Sex			
Male	10 (38.46%)	37 (60.66%)	0.057
Female	16 (61.54%)	24 (39.34%)	
Birth weight			
Normal	18 (69.23%)	32 (52.46%)	0.147
Low	8 (30.77%)	29 (47.54%)	
Gestational age			
Term	20 (76.92%)	38 (62.3%)	0.185
Preterm	6 (23.08%)	23 (37.7%)	
Mode of delivery			
Vaginal	15 (57.69%)	29 (47.54%)	0.386
Cesarean section	11 (42.31%)	32 (52.46%)	

Table 5: Correlation of leukocyte counts with the type of sepsis

Variables	EOS (n = 26)	LOS (n = 61)	p-value
WBC count×10^9/L			
Median	16.5	16.3	0.101
Range	12.16-27.0	5.5-42.0	
Neutrophil ×10^9/L			
Median	12.28	8.67	0.140
Range	2.98-18.7	2.48-27.3	
Lymphocyte×10^9/L			
Median	4.8	5.18	0.147
Range	2.51-9.15	2.05-11.58	
N/L ratio			
Median	2.51	1.77	**0.019**
Range	0.51-7.24	0.33-7.24	

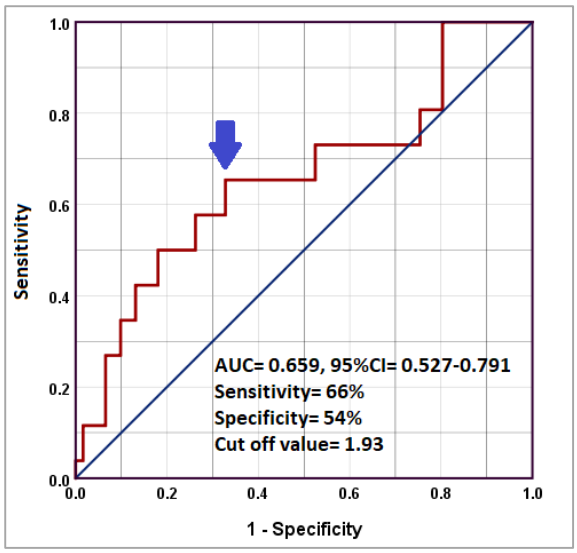

Figure 2: Predictive value of the N/L ratio

Table 6: Association of bacterial profile with sepsis pattern

Type of Bacteria	EOS (n=26)	LOS (n=61)	p-value
Staph. aureus	8 (30.76%)	22(36.06%)	0.634
Acinetobacter spp.	6 (23.08%)	4 (6.56%)	**0.037**
Klebsiella spp.	6 (23.08%)	8 (13.11%)	0.274
Strept. viridans	1 (3.85%)	17 (27.87%)	**0.011**
Enterococcus spp.	1 (3.85%)	3 (4.92%)	1.00
Staph. epidermidis	0(0%)	5 (8.2%)	0.316
E. coli	2 (7.69%)	2 (3.28%)	0.580
Listeria spp.	2 (7.69%)	0(0%)	0.087
Gram stain			
Gram-positive	12 (46.15%)	47 (77.05%)	**0.005**
Gram-negative	14 (53.85%)	14 (22.95%)	

Table 7: Association of bacterial profile with gestational age

Type of Bacteria	Full term (n = 58)	Preterm (n = 29)	p-value
Staph. aureus	25 (43.1%)	5 (17.24%)	**0.017**
Acinetobacter spp.	7 (12.07%)	3 (10.34%)	0.812
Klebsiella spp.	6 (10.34%)	8 (27.59%)	**0.039**
Strept. viridans	13 (22.41%)	5 (17.24%)	0.574
Enterococcus spp.	3 (5.17%)	1 (3.45%)	1.0
Staph. epidermidis	3 (5.17%)	2 (6.9%)	1.0
E. coli	0 (0%)	4 (13.79%)	**0.011**
Listeria spp.	1 (1.72%)	1 (3.45%)	0.55
Gram stain			
Gram-positive	45 (77.59%)	14 (48.28%)	**0.008**
Gram-negative	13 (22.41%)	15 (51.72%)	

DISCUSSION

The mean WBC count was 17.52 ± 6.58 × 109/L, which is slightly higher than the result found by Adane et al.[13] in Ethiopia (mean WBC = 11.8 ± 6.88 × 109/L), while Abdul-Rahman et al.[4] in Erbil showed that elevated WBC counts more than 10 × 109/L were found in most cases (90%). This study also demonstrated that the absolute neutrophil and lymphocyte counts were 9.64 ± 5.68 × 109/L and 5.6 ± 2.4 × 109/L, respectively, which were higher than the results of Adane et al.[13]

Regarding the gram stain of bacteria isolated from the blood cultures, gram-positive bacteria were found in the majority of cases (67.8%), while gram-negative bacteria were isolated from 32.2% of them. These results are consistent with Ferreira et al.[1], Sorsa et al.[7], Mohakud et al.,[11] Adane et al.,[13] Acheampong et al.,[18] and Lim et al.[19] However, Almohammady et al.,[9] Uwe et al.,[14] Boulos et al.,[15] Das et al.,[20] and Ehsan et al.[17] found that gram-

negative bacteria were predominant. These variations could be attributed to the differences in sample size, age of the onset of sepsis, and general characteristics between different populations.

In terms of the type of isolated bacteria, *Staphylococcus aureus* was most commonly encountered in the current study (34.48%), followed by *Strept. viridans* (20.69*%), Klebsiella spp*. (16.09%), and *Acinetobacter spp*. (11.49%). This finding is approximately similar to those found by Mohakud et al.[11] in India and Uwe et al.[14] in Nigeria. On the other hand, Karmila et al.[21] in Indonesia and Hammoud et al.[3] in the Arabian Gulf area found that coagulase-negative *staphylococcus* was the most common pathogen, followed by *Klebsiella* and *Acinetobacter species* (30.9%, 18.1%, 10.7%, and 34.65%, 22.8%, 4.84% respectively).

The majority of neonates in this study were diagnosed with late-onset sepsis (70.11%), which was supported by Ferreira et al.[1] (82.2%), Almohammady et al.[9] (58.6%), Mohakud et al.[11] (72.2%), Das et al.[20] (68.6%), Yusef et al.[22] (72%), and Siddiqui et al.[23] (69.8%). All these papers have chosen the same neonatal characteristics as used in the current study. On the other hand, Abdul-Rahman et al.,[4] Uwe et al.,[14], Acheampong et al.,[18] and Ehsan et al.[17] found that early-onset sepsis was predominant (77.77%, 76.7%, 67.6%, 71.8%, 70.3%, and respectively).

Furthermore, there were non-significant differences between EOS and LOS groups regarding birth weight, gestational age, and mode of delivery, which is in line with Almohammady et al.[9] and Karmila et al.[21] but different from Das et al.[20] study, which found that low birth weight was significantly associated with LOS, and premature neonates were predominantly seen in LOS. These differences could result from the higher percentage of premature neonates taken by Das et al.[20] compared to the present study.

Regarding sex, the present study found that more females had EOS while males were more common in LOS. These outcomes are similar to Ferreira et al.'s findings.[1]

In addition, total WBC counts and the absolute number of neutrophils were higher in EOS than in LOS but did not reach statistical significance, but the N/L ratio was significantly higher in EOS than in LOS. Normal N/R ratio values in healthy neonatal or pediatric populations have been reported with an average of 0.52–0.91.[24] All data are consistent with the fact that a systemic inflammatory response causes neutrophilia and/or relative neutropenia.[13] Lim et al.,[19] for instance, investigated very low birth weight neonates in Taiwan and revealed that patients with EOS had significantly higher percentages of neutropenia than those with LOS. This result could be explained by the very low birth weight of their cases compared to our study, in which the majority had normal birth weights.

Staphylococcus aureus was the most commonly encountered microorganism in EOS and LOS (30.76% and 36.06%, respectively) but with non-significant differences between both groups. The second most common pathogens were *Acinetobacter app*. in EOS and *strept. viridian* in LOS with significant differences. *Klebsiella species* were also common among early- and late-onset sepsis but did not result in non-significant association. Additionally, the current study supported Sorsa et al.[7] and Almohammady et al.[9] by demonstrating that gram-positive bacteria were much more prevalent in LOS, and gram-negative bacteria were significantly more prevalent in EOS. These findings could be explained by maternal birth canal colonization with gram-negative organisms responsible for EOS, while LOS is commonly a community-acquired infection. In contrast, Acheampong et al.[18] found that EOS and LOS were mostly caused by gram-positive and gram-negative bacteria, respectively. This variation could be explained by a higher number of EOS cases than LOS in Acheampong et al.[18] compared to the present study.

Regarding the correlation of bacterial profile with the gestational age, the current study exhibited that gram-positive bacteria were predominantly seen in full-term neonates, while gram-negative bacteria were more commonly encountered in preterms, which is supported by Ba-alwi et al.[25] and Uwe et al.[14] studies, in which gram-negative sepsis predominated in the preterm groups (37.8% and 72.7%, respectively). In addition, Staphylococcus aureus was the most common microorganism in full-term neonates and significantly more in full-term than in preterm infants. Furthermore, *Klebsiella spp*. were mostly encountered in preterm neonates, with statistical significance, while *E. coli* was found in full-term neonates only. These results reflected that the impaired immune response of preterm neonates puts them at a higher risk of gram-negative bacterial sepsis. These findings are similar to those found by Ferreira et al.[1] and Ba-alwi et al.[25]

CONCLUSIONS

Staphylococcus aureus was the most commonly isolated bacteria, followed by *streptococcus viridans* and *Klebsiella species*. Early-onset sepsis and premature newborns were more likely to have gram-negative bacterial sepsis, whereas late-onset sepsis and full-term neonates were expected to have more gram-positive bacteria.

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