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Comparison of risk factors, clinical characteristics, laboratory findings and bacterial etiology between early-onset and late-onset neonatal sepsis in Sana'a City, Yemen

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Abstract

Background Sepsis is a major cause of neonatal mortality worldwide. However, its risk factors, clinical characteristics, laboratory findings, and bacterial etiology vary across countries. Therefore, this study compared these factors between early-onset sepsis (EOS) and late-onset sepsis (LOS) in Sana'a city, Yemen.

Methods A prospective, cross-sectional study was conducted among 156 neonates with suspected sepsis in Sana'a. Data about risk factors, clinical characteristics, and laboratory findings were collected using a data collection sheet, and blood samples were collected for culture according to established procedures. The data were then analyzed at a significance level of < 0.05.

Results Of neonates with suspected sepsis, 65.4% had LOS and 34.6% had EOS. However, sepsis was confirmed in 62.2% of cases. Compared to EOS, preterm birth (OR = 4.1, 95% CI: 1.27–13.02; $P = 0.013$), and exteremely low birthweight (ELBW) and very low birthweight (VLBW) (OR = 4.7, 95% CI: 1.02–22.19; $P = 0.033$) were significantly associated with a higher risk of LOS, while premature rupture of membranes (PROM) was significantly associated with a lower risk of LOS (OR = 0.2, 95% CI: 0.03–0.99; $P = 0.043$). Jaundice was significantly more common in neonates with EOS, while apnea was significantly more common in those with LOS. However, no significant differences were found between EOS and LOS in terms of other clinical characteristics, leukocyte and platelet counts, or C-reactive protein (CRP). Gram-positive cocci were the most frequent bacterial isolates (63.9%), with coagulase-negative staphylococci (CoNS) being the predominant species (63.6% in EOS and 42.2% in LOS). Gram-negative bacilli were isolated from 36.1% of cases and were more common in LOS (77.1%) than in EOS (22.9%), with *Klebsiella* species being the most predominant (19.6%).

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Conclusion Most neonatal sepsis cases in Sana'a are LOS, mostly commonly caused by Gram-positive cocci and associated with preterm birth and ELBW and VLBW. Differences in risk factors associated with EOS and LOS highlight the need for targeted preventive measures in neonatal care settings.

Keywords Neonatal sepsis, Early-onset sepsis, Late-onset sepsis, Risk factors, Bacteria, Yemen

Introduction

The World Health Organization (WHO) estimated that approximately 5 million children under the age of five died in 2020, with around half of these deaths occurring within the first 28 days of life [1]. Furthermore, over half of neonatal deaths may occur during the first three days of life [2]. With about 420,000 deaths per year, neonatal sepsis ranks as the third most common cause of death in newborns [3].

Based on the age at which it manifests in the neonatal intensive care unit (NICU), neonatal sepsis is categorized as either early-onset sepsis (EOS) or late-onset sepsis (LOS) [4]. EOS is identified by the isolation of bacteria in the blood or cerebrospinal fluid within 72 h of birth [4, 5]. However, LOS typically occurs between days 3 and 28 of life [4, 5]. The epidemiology, clinical presentation, causative agents, and outcomes vary between the two types of sepsis [6]. Newborns at risk of EOS can be identified using various indicators, such as their general appearance of illness, clinical signs suggestive of maternal intraamniotic infection, premature rupture of membranes (PROM) lasting longer than 18 h, colonization of Group B *Streptococcus* (GBS) in mothers not receiving adequate intrapartum antibiotic prophylaxis (IAP), and preterm birth [7].

Diagnosis of neonatal sepsis is primarily made by performing blood cultures, but this method has low sensitivity due to the small amount of blood that can be collected for culture or administration of empirical antibiotics prior to sample collection [8]. GBS have been identified as the most common causative agent of EOS [7]. However, a recent systematic review conducted in middle-income countries in the Middle East has revealed that Gram-negative bacteria are the leading cause of EOS, second only to coagulase-negative staphylococci (CoNS) [9]. In the Arabian Gulf states, CoNS and *Klebsiella* species are the most predominant bacteria isolated in cases of LOS [10]. In Yemen, about three-quarters of bacteria identified in both EOS and LOS were Gram-negative, most commonly with *Burkholderia cepacia* and *Klebsiella* species [11]. Distinguishing the disparities between EOS and LOS regarding risk factors, clinical presentation, laboratory findings and bacterial etiology can help healthcare providers make informed decisions regarding diagnosis, treatment, and prevention strategies for neonatal sepsis. Given the dearth of studies comparing the two types of neonatal sepsis in Yemen, this study compared the risk factors, clinical characteristics, laboratory findings, and bacterial etiology between EOS and LOS among neonates in a tertiary care hospital in Sana'a city, Yemen.

Methods

Study design and setting

A prospective, cross-sectional study was conducted in the NICU of the University of Science and Technology Hospital (USTH) from May 2020 to June 2021. The USTH is a private hospital that serves as a referral hospital in Sana'a city. It is equipped with a 17-bed NICU and has an infection control committee.

Study population

During the study period, 156 neonates (up to 28 days old), who were either born or admitted to the NICU of the USTH with clinical symptoms and/or signs of sepsis, were included in this study. Among them, those with positive blood cultures were confirmed to have sepsis, which was further classified into EOS or LOS based on the timing of onset. Neonates were excluded from the study if their parents or legally authorized guardians refused to provide informed consent.

Data and sample collection

Data about the potential risk factors, clinical manifestations, and laboratory findings of sepsis were collected using a structured data collection sheet. Neonatal risk factors included gestational age, age at onset of symptoms, sex, and weight, while maternal risk factors included the mode and place of delivery, gravidity, and PROM. Clinical data included general, respiratory, neurological and gastrointestinal manifestations. The laboratory findings included the bacterial species isolated, C-reactive protein (CRP) levels, platelet counts, as well as total leukocyte and neutrophil counts. Blood samples were collected from neonates by trained nurses using aseptic procedures. At least one milliliter of venous blood was inoculated into pre-labeled blood culture bottles (Bact-Alert Ped, BioMerieux, France). These bottles were then sent to the Laboratory Department of the USTH for microbiological investigations.

Microbiological investigations

Microbiological investigations were performed according to standard procedures. Briefly, the Bact Alert 3D system (BioMerieux, France) was used incubate blood culture bottles at 37 ˚C until the system signaled them either as positive or negative. In cases of positive blood

cultures, sub-culturing was performed on blood, MacConkey, and chocolate agars, which were subsequently incubated at 37 ˚C for 48 h. Morphological characteristics of bacterial colonies were observed, and Gram staining was used to distinguish between Gram-positive and Gram-negative bacteria. In a test tube, 3 ml of sterile saline was used to suspend pure bacterial colonies. Then, the VITEK 2 Compact system (BioMerieux, France) was used to identify the bacterial species in the suspension, according to the manufacturer's instructions. The VITEK GN and VITEK GP ID identification cards were used to characterize Gram-negative and Gram-positive bacteria, respectively.

Data analysis

Data were analyzed using IBM SPSS Statistics, version 23 (IBM Corp., Armonk, NY, USA). Continuous variavles with normally distributed data were described using the mean and standard deviation. Univariate analysis, using the Pearson's chi-square or Fisher's exact test, was performed to test the difference in risk factors, clinical characteristics, and laboratory findings between EOS and LOS. The odds ratios (ORs) and their 95% confidence intervals (CIs) were reported to compare the likelihood of clinical manifestations and laboratory findings in LOS vs. EOS and to quantify the association between risk factors and LOS vs. EOS. On the other hand, the independent samples *t*-test was used to compare the mean counts of leukocytes and platelets between neonates with EOS and LOS, which were confirmed to be normally distributed using the Kolmogorov-Smirnov test. P values < 0.05 were considered statistically significance.

Table 1 Characteristics of neonates included in the study*

Characteristics	*n*	(%)
Sex		
Male	90	(57.7)
Female	66	(42.3)
Gestational age		
Pre-term	50	(32.1)
Full-term	106	(67.9)
Weight at diagnosis		
ELBW (< 1000 gm)	6	(3.9)
VLBW (< 1500 gm)	23	(14.7)
LBW (1500– <2500 gm)	47	(30.1)
Normal birthweight (≥ 2500 gm)	80	(51.3)
Delivery location		
Inborn	73	(46.8)
Outborn	83	(53.2)
Mode of delivery		
Vaginal birth	79	(50.6)
Cesarean section	77	(49.4)

* Total number of neonates with suspected sepsis enrolled in the study was 156. *ELBW* extremely low birthweight; *VLBW* very low birthweight; *LBW* low birthweight

Results

Characteristics of enrolled neonates

Most neonates were males (57.7%) and full-term (67.9%). The mean gestational age was 36 ± 3.3 weeks, with the majority of neonates were full-term (67.9%). The mean weight of neonates at diagnosis was 2382 ± 782 gm, with 18.6% having exteremely low birthweight (ELBW) and very low birthweight (VLBW). More than half of neonates were born outside health facilities. Equal proportions of neonates were delivered vaginally (50.6%) and by Cesarean Sect. (49.4%) (Table 1).

Neonatal sepsis categories

Of 156 neonates with suspected sepsis, 102 (65.4%) were diagnosed with LOS and 54 (34.6%) with EOS. However, sepsis was confirmed by blood culture in 97 (62.2%) neonates. Of these, 64 (66%) had LOS and 33 (34%) had EOS.

Comparison between risk factors associated with culture-proven EOS and LOS

Preterm birth (OR = 4.1, 95% CI: 1.27–13.02; $P = 0.013$), ELBW and VLBW (OR = 4.7, 95% CI: 1.02–22.19; $P = 0.033$) were significantly associated with a higher risk of LOS compared to EOS, while PROM was significantly associated with a lower risk of LOS compared to EOS (OR = 0.2, 95% CI: 0.03–0.99; $P = 0.043$). In contrast, there was no significant association between either type of sepsis and male sex, multigravidity, vaginal delivery, or birth outside health facilities (Table 2).

Comparison of clinical and laboratory findings between neonates with culture-proven EOS and LOS

Jaundice was significantly more common in neonates with EOS (45.5%) than in those with LOS (18.8%), with LOS neonates having one-third of the likelihood of experiencing jaundice compared to those with EOS (OR = 0.3, 95% CI: 0.11–0.70; $P = 0.005$). On the other hand, apnea was significantly more common in neonates with LOS (21.9%) than in those with EOS (6.1%), with LOS neonates being over four times more likely to experience apnea compared to those with EOS (OR = 4.3, 95% CI: 1.05–20.40; $P = 0.047$). Tachypnea (50% vs. 30.3%), cyanosis (29.7% vs. 12.1%), retraction (28.1% vs. 12.1%), vomiting (15.6% vs. 3%), seizures (14.1% vs. 9.1%), and edema (4.7% vs. 3%) were observed more commonly in neonates with LOS than in those with EOS, with no statistically significant differences. However, fever (33.3% vs. 23.4%), lethargy (48.5% vs. 43.8%), poor feeding (39.4% vs. 32.8%), and diarrhea (3% vs. 1.6%) were observed more commonly in neonates with EOS than in those with LOS, with no statistically significant differences. On the other hand, there were no statistically significant differences in terms of leukocytosis, leukopenia, neutropenia, or thrombocytopenia between neonates with EOS and

Table 2 Comparison of neonatal and maternal risk factors associated with culture-proven EOS and LOS among neonates in a tertiary care hospital in Sana'a City, Yemen (2020–2021)

Risk factors	EOS (*N* = 33)		LOS (*N* = 64)		OR for LOS (95% CI)	*P*-value
	n	(%)	*n*	(%)		
Preterm birth	4	(12.1)	23	(35.9)	4.1 (1.27–13.02)	0.013
Male sex	19	(57.6)	33	(51.6)	0.8 (0.34–1.83)	0.574
ELBW and VLBW	2	(6.1)	15	(23.4)	4.7 (1.02–22.19)	0.033
PROM	5	(15.2)	2	(3.1)	0.2 (0.03–0.99)	0.043
Multigravidity	24	(72.7)	48	(75.0)	1.1 (0.43–2.91)	0.808
Vaginal delivery	16	(48.5)	35	(54.7)	1.28 (0.55–2.98)	0.562
Outborn birth	19	(57.6)	34	(53.1)	0.8 (0.36–1.95)	0.677

EOS early-onset sepsis, *LOS* late-onset sepsis, *ELBW* extremely low birthweight, *VLBW* very low birth weight, *PROM* premature rupture of membranes, *OR* odds ratio, *CI* confidence interval

Table 3 Comparison of clinical and laboratory findings in neonates with culture-proven EOS and LOS in a tertiary care hospital in Sana'a City, Yemen (2020–2021)

Findings	EOS (*N* = 33)		LOS (*N* = 64)		OR for LOS (95% CI)	*P*-value
	n	(%)	*n*	(%)		
Fever	11	(33.3)	15	(23.4)	0.6 (0.24–1.55)	0.297
Tachypnea	10	(30.3)	32	(50.0)	2.3 (0.95–5.60)	0.064
Grunting	2	(6.1)	4	(6.3)	1.0 (0.17–5.58)	1.000
Retraction	4	(12.1)	18	(28.1)	2.8 (0.87–9.22)	0.075
Cyanosis	4	(12.1)	19	(29.7)	3.1 (0.95–9.91)	0.054
Apnea	2	(6.1)	14	(21.9)	4.3 (1.05–20.40)	0.047
Poor feeding	15	(45.5)	23	(35.9)	0.7 (0.29–1.58)	0.363
Lethargy	16	(48.5)	30	(46.9)	0.9 (0.40–2.17)	0.880
Seizures	3	(9.1)	9	(14.1)	1.6 (0.41–6.51)	0.746
Vomiting	1	(3.0)	10	(15.6)	5.9 (0.72–48.47)	0.092
Diarrheal	1	(3.0)	1	(1.6)	0.5 (0.03–8.39)	1.000
Jaundice	15	(45.5)	12	(18.8)	0.3 (0.11–0.70)	0.005
Edema	1	(3.0)	3	(4.7)	1.6 (0.16–15.75)	1.000
Leukocytosis (> 20 × 10^3/μl)	6	(18.2)	14	(21.9)	1.3 (0.43–3.65)	0.670
Leukopenia (< 5 × 10^3/μl)	2	(6.1)	2	(3.1)	0.5 (0.07–3.72)	0.603
Neutropenia (< 2 × 10^3 /μl)	4	(12.1)	6	(9.4)	0.8 (0.20–2.87)	0.731
Thrombocytopenia (< 150 × 10^3/μl)	11	(33.3)	21	(32.8)	1.0 (0.40–2.28)	0.959
Elevated CRP level (> 5 ng/ml)	18	(54.5)	46	(71.9)	2.1 (0.89–5.11)	0.088

EOS early-onset sepsis, *LOS* late-onset sepsis, *OR* odds ratio, *CI* confidence interval, *CRP* C-reactive protein

Table 4 Comparison of the mean leukocyte and platelet counts among neonates with culture-proven EOS and LOS in a tertiary care hospital in Sana'a City, Yemen (2020–2021)

Parameters	EOS (*N* = 33)	LOS (*N* = 64)	*P*-value
	Mean ± SD		
Total leukocyte count (× 10^3/μl)	13.0 ± 7.2	14.6 ± 8.2	0.360
Neutrophil count (× 10^3/μl)	7.4 ± 6.0	7.9 ± 6.7	0.747
Platelet count (× 10^3/μl)	244.0 ± 167.7	262.0 ± 179.8	0.634

EOS early-onset sepsis, *LOS* late-onset sepsis, *SD* standard deviation

LOS. Although elevated CRP levels were observed more commonly in neonates with EOS (71.9%) than those with LOS (54.5%), the difference was not statistically significant (Table 3).

Comparison of mean leukocyte and platelet counts between neonates with culture-proven EOS and LOS

There were no statistically significant differences between neonates with EOS and LOS in the mean counts (×10^3/μl) of total leukocytes (13.0 ± 7.2 vs. 14.6 ± 8.2), neutrophils (7.4 ± 6.0 vs. 7.9 ± 6.7), or platelets (244.0 ± 167.7 vs. 262.0 ± 179.8) (Table 4).

Comparison of bacterial profiles between culture-proven EOS and LOS

Gram-positive cocci were the most frequently isolated bacterial species (63.9%) in neonates with culture-proven sepsis, being more common in LOS (59.7%) than in EOS (40.3%). CoNS were isolated from approximately half of neonates with culture-proven sepsis, being more common in EOS (63.6%) than in LOS (42.2%). Enterococci were the second most common Gram-positive cocci

Table 5 Bacterial species isolated from neonates with culture-proven sepsis in a tertiary care hospital in Sana'a City, Yemen (2020–2021)

Bacterial species	EOS (*N* = 33)		LOS (*N* = 64)		Total (*N* = 97)	
	n	(%)	*n*	(%)	*n*	(%)
Gram-positive cocci	25	(40.3)	37	(59.7)	62	(63.9)
Coagulase-negative staphylococci	21	(63.6)	27	(42.2)	48	(49.5)
Enterococci	0	(0.0)	5	(7.8)	5	(5.2)
Staphylococcus lentus	0	(0.0)	1	(1.6)	1	(1.0)
Staphylococcus aureus	2	(6.1)	0	(0.0)	2	(2.1)
Staphylococcus hominis	1	(3.0)	3	(4.7)	4	(4.1)
Streptococcus species	1	(3.0)	1	(1.6)	2	(2.1)
Gram-negative bacilli	**8**	**(22.9)**	**27**	**(77.1)**	**35**	**(36.1)**
Escherichia coli	0	(0.0)	4	(6.3)	4	(4.1)
Klebsiella species	4	(12.2)	15	(23.4)	19	(19.6)
Pseudomonas aeruginosa	2	(6.1)	3	(4.7)	5	(5.2)
Enterobacter species	1	(3.0)	2	(3.1)	3	(3.1)
Burkholderia species	0	(0.0)	1	(1.6)	1	(1.0)
Acinetobacter species	1	(3.0)	2	(3.1)	3	(3.1)

EOS early-onset sepsis, *LOS* late-onset sepsis

and were isolated from 5.2% of neonates with LOS. On the other hand, Gram-negative bacilli were isolated from 36.1% of neonates with sepsis and were more common in LOS (77.1%) than in EOS (22.9%). In neonates with both types of sepsis, *Klebsiella* species were the most frequently isolated Gram-negative bacilli (19.6%), followed by *Pseudomonas aeruginosa* (5.2%). *Escherichia coli* was isolated from 4.1% of neonates with LOS. *Burkholderia* species (1%) and *Acinetobacter* species (3.1%) were the least frequently isolated Gram-negative bacilli (Table 5).

Discussion

This study found that LOS accounted for two-thirds of suspected neonatal sepsis, possibly attributed to the study being conducted in a tertiary care hospital where regular antenatal care and routine IAP reduce EOS incidence. In contrast, a study in a public referral hospital in Sana'a city found a higher prevalence of EOS (84.2%) compared to LOS (15.8%) [12], but the latter study defined EOS as occurring within the first week of life. Our finding also contradicts a recent study in public and private hospitals in Sana'a city [11], which found a higher prevalence of culture-proven EOS (50.3%) compared to LOS (27.1%), as well as those in Pakistan [13], India [14, 15], Nigeria [16, 17], Egypt [18], Nepal [19], and Saudi Arabia [20]. However, a higher prevalence of LOS was reported in Saudi Arabia (88.9%), Bosnia (71.3%), and Iran (68.6%) [21–23]. EOS and LOS prevalence may vary due to differences delivery places, healthcare settings, patient populations, infection control measures, and neonatal care practices.

The present study found that 62.2% of suspected cases were proven by blood culture, being comparable to that (57%) in a public referral hospital in Sana'a [12] but lower than that (77.4%) recently reported in six referral hospitals in the city [11]. A similar proportion of culture-proven sepsis was reported among Pakistani neonates (62.8%) [24], while less than half of neonatal cases were confirmed by blood culture elsewhere [18, 19, 25, 26]. The disparities in confirmation rates between countries highlight the need for standardized diagnostic protocols and further investigation into factors contributing to these variations, especially when detecting bacteria in blood at low densities [27]. Blood culture sensitivity is further limited by fastidious or non-culturable organisms and antibiotic use by mothers [28]. Meanwhile, false-positive cultures due to blood contamination by skin flora or the neonate's environment present another challenge [29], potentially leading to inflated proportions of culture-proven neonatal sepsis.

Published studies on neonatal sepsis in Yemen, as well as most studies published elsewhere, have mainly focused on identifying the risk factors associated with neonatal sepsis without specifically comparing them between EOS and LOS. On the contrary, the approach of the present study was to compare risk factors between the distinct types of sepsis to provide a clearer understanding of the associated risks. Specifically, this study found that preterm birth as well as ELBW and VLBW were significantly associated with a higher risk of culture-proven LOS than EOS, possibly due to underdeveloped immune systems of preterm neonates that make them more susceptible to vertical and horizontal infections [30]. Yemen's humanitarian crises have led to high malnutrition rates over the past decade, affecting approximately 1.5 million pregnant and breastfeeding mothers [31], potentially contributing to intrauterine growth restriction (IUGR) and increasing the incidence of LBW [32] and vulnerability to neonatal sepsis. Addressing the increased risk of sepsis in preterm

and LBW neonates, especially in malnutrition-affected regions, requires providing adequate nutritional support to pregnant women. Further research is needed to understand the complex interplay between maternal malnutrition, LBW, and LOS. Consistent with the findings of this study, preterm birth and ELBW and VLBW were identified as risk factors for neonatal sepsis in Hajjah city, north of Yemen [33]. However, a recent study conducted in Sana'a found no association between neonatal sepsis and gestational age or birthweight [11]. In Nigeria, LBW was found to be significantly associated with LOS [17]. Generally, VLBW/LBW and/or preterm birth have been consistently identified as risk factors for neonatal sepsis in several countries [21, 34–39], but without a comparison of risk between EOS and LOS. The present study found that PROM was significantly associated with a lower risk of LOS compared to EOS, contradicting previous findings in Yemen and Nigeria [11, 17]. However, it is consistent with several other studies [14, 35, 40–43]. PROM increases the risk of maternal and, therefore, neonatal infections [44] and was identified as an independent birth-related risk factor for EOS in a recent international Delphi study [45].

Culture-proven LOS was more common than EOS among male neonates in the present study, but the difference did not reach statistical significance. Similarly, studies in Yemen and Nigeria found no significant association between neonatal sex and sepsis [11, 17]. The present study also found that most neonates with LOS were born vaginally and outside health facilities, compared to neonates with EOS. It is noteworthy that approximately 5.5 million women of childbearing age face reproductive care service limitations [46]. Consequently, pregnant women may resort to home delivery, even if an emergency cesarean section is required. Neither vaginal delivery nor delivery outside health facilities was significantly associated with an increased risk of LOS when compared to EOS. In contrast, vaginal delivery was significantly associated with neonatal sepsis in Yemen and elsewhere [11, 15, 18, 33], and delivery outside health facilities was significantly associated with LOS among Nigerian neonates [17].

Distinguishing between EOS and LOS is rather challenging due to the non-specific clinical characteristics of neonatal sepsis [47]. However, certain clinical symptoms may occur more commonly in one type of sepsis than the other. In the present study, jaundice, lethargy, poor feeding, and diarrhea were more common in neonates with EOS. On the other hand, apnea, tachypnea, cyanosis, retraction, vomiting, seizures, and edema were more common among neonates with LOS. However, of these symptoms, jaundice was significantly more common in neonates with EOS, and apnea was significantly more common in neonates with LOS. In contrast, a recent study in Sana'a did not find a significant association between jaundice and neonatal sepsis [11], but without comparing the risk between EOS and LOS. Jaundice was not also found to be associated with a significantly higher risk of EOS compared to LOS among Nigerian neonates [17]. In accordance with the present study, apnea was found to be significantly associated with culture-proven sepsis among neonates in a tertiary care hospital in Sana'a [12], but a more recent study did not find a significant association between respiratory rate and sepsis among neonates in the city [11]. In contrast to the present study, respiratory distress was found to be a significant risk factor for EOS rather than LOS among Nigerian neonates [17].

The lack of a significant difference between EOS and LOS in terms of leukopenia, neutropenia, or thrombocytopenia in the present study aligns with that previously reported in Sana'a city [11]. Likewise, neither neutropenia nor thrombocytopenia differed significantly between Nigerian neonates with EOS and LOS [17]. The present study also found non-significant differences in mean leukocyte and platelet counts between neonates with EOS and LOS. In contrast, the mean platelet count was significantly lower among Nigerian neonates with EOS compared to those with LOS [17]. On the other hand, in the present study, elevated CRP levels were more common among neonates with EOS compared to those with LOS. However, this difference did not reach statistical significance. In contrast, a previous study in Sana'a city reported a significant association between elevated CRP levels and neonatal sepsis [11], even though that study did not specifically compare EOS and LOS.

Gram-positive bacteria were the predominant bacterial isolates from septic neonates in the present study, accounting for approximately 64% of infections, as CoNS predominated in both EOS and LOS. This finding contrasts with the traditionally known dominance of GBS in EOS [7]. The decline in infections due to GBS may be attributed to the widespread use of IAP [48]. Of concern, the finding of the present study differs from that recently reported in Sana'a, where most neonatal sepsis cases (74%) were attributed to Gram-negative bacilli, particularly *Burkholderia cepacia* and *Klebsiella* species [11]. Gram-negative bacteria have also been found to be the leading cause of neonatal sepsis in neighboring Saudi Arabia and elsewhere [14, 21, 22, 49]. These contrasting findings highlight that the bacterial etiology of neonatal sepsis may vary even within healthcare facilities within the same country, in addition to variations in pathogen epidemiology over time. Differences in the incidence and etiology of neonatal sepsis within the same region, even within different healthcare facilities, emphasize the importance of continuous surveillance to monitor changes in the epidemiology of neonatal sepsis in order

to effectively manage it by guiding appropriate antimicrobial strategies and interventions.

The predominance of CoNS among neonates with EOS and LOS in the present study is consistent with that reported in Egypt, Ghana, and Mexico [18, 37, 50]. However, the criteria for defining CoNS sepsis in neonates remain controversial, possibly leading to an overrepresentation of CoNS as a true pathogen in neonatal sepsis. To address this controversy, it has been suggested that true bacteremia in neonates be considered based on at least two positive blood cultures [51]. Numerous diagnostic algorithms have been proposed to minimize false-positive diagnoses. These algorithms include time-to-positivity of blood cultures, quantitative cultures and colony counts, consideration of the presence of indwelling central venous catheters [52, 53]. In contrast to the present study, in the Jazan region of Saudi Arabia, CoNS accounted for only 11% of the bacterial species isolated from neonates with EOS, being ranked as the third most frequently isolated bacteria after *Escherichia coli* (29%) and GBS (17%) [49]. In Nigeria, *Staphylococcus aureus* was the predominant bacterial isolate in both EOS (69%) and LOS (76.5%) [17]. *Klebsiella* species were the second most frequent isolates in both EOS and LOS among neonates in the present study, which is consistent with findings from Yemen and Nigeria [11, 17]. However, in Indonesia, *Klebsiella* species were the most frequent isolates from neonates with EOS and LOS (20.3% and 18.7%, respectively) [54]. Differences in the bacterial etiology of neonatal sepsis have important implications for clinical management, particularly with regard to the selection of appropriate empirical antibiotic therapy. Therefore, it is important to understand the local epidemiology of neonatal sepsis to guide treatment decisions and infection control measures.

This study has several limitations and strengths that should be considered when interpreting its findings. First, the study was conducted in a single health facility, which may raise questions regarding the generalizability of its findings to the broader population of septic neonates in the community. However, it is rather challenging to conduct such studies at the community level due to various logistical and ethical constraints. Furthermore, unlike other published studies in the country, this study provides valuable insights by prospectively comparing the risk factors and various aspects of EOS and LOS, thereby contributing to the existing literature in a country where such comparisons are lacking. Second, the small sample size might not have the sufficient statistical power to detect significant differences in certain characteristics or risk factors. Nevertheless, as a preliminary study, it still serves to reveal important differences between EOS and LOS in the country's neonatal population. Moreover, its findings can serve as a guide for the design and implementation of large-scale studies that extensively investigate the epidemiology, risk factors, and consequences of neonatal sepsis types in Yemeni neonates. Third, the absence of a distinction between hospital-acquired and community-acquired infections in LOS is a major limitation of this study, as the causative agents and their antibiotic sensitivity patterns differ largely in both categories. Finally, this study did not include an analysis of the antibiotic susceptibility patterns of the pathogens causing neonatal sepsis, as its focus was on the comparison between EOS and LOS rather than investigating the antibiotic susceptibility of individual bacterial species that can cause both types of sepsis. Therefore, it is recommended that antibiotic susceptibility analysis be included in future similar studies.

Conclusion

Most neonatal sepsis cases in Sana'a are LOS, mostly commonly caused by Gram-positive cocci and associated with preterm birth, and ELBW and VLBW. Differences in risk factors associated with EOS and LOS highlight the need for targeted preventive measures in neonatal care settings.

Abbreviations

AOR	Adjusted Odds Ratio
CI	Confidence Interval
CoNS	Coagulase-Negative Staphylococci
CRP	C-Reactive Protein
ELBW	Extremely Low Birthweight
EOS	Early-Onset Sepsis
GBS	Group B Streptococcus
IAP	Intrapartum Antibiotic Prophylaxis
IUGR	Intrauterine Growth Restriction
LBW	Low Birth Weight
LOS	Late-Onset Sepsis
NICU	Neonatal Intensive Care Unit
OR	Odds Ratio
PROM	Premature Rupture of Membranes
SD	Standard Deviation
SPSS	Statistical Package for the Social Sciences
USTH	University of Science and Technology Hospital
WHO	World Health Organization

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Author contributions

MA, WF, and GA developed the study idea and collected data. MA, RA, and WF designed the study. MA and RA drafted the manuscript. MA, RA, and FA analyzed the study data. ME contributed to data interpretation and revised the manuscript draft. YA collected data and supervised data collection. All authors approved the final manuscript.

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Data availability

Data are available from the corresponding author upon reasonable request.

Declarations

Ethics approval and consent to participate

This study was conducted in accordance with the Declaration of Helsinki for studies on human subjects. Ethical approval was obtained from the Research Ethics Committee of the Faculty of Medicine and Health Sciences, University of Science and Technology, Sana'a, Yemen. Informed consent was obtained from parents or legally authorized guardians for the voluntary inclusion of their neonates in the study after informing them of its purpose and their right to exclude their neonates from the study at any time without giving reasons. In situations where parents or guardians were illiterate or had given verbal consent but expressed concerns about signing consent forms or providing fingerprints, additional informed consent was obtained from literate legally authorized guardians who agreed to sign the consent forms. The confidentiality of participants and the privacy of their data were guaranteed.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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