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Trends of neonatal sepsis and its etiology at Hawassa, Ethiopia: a five year retrospective cross-sectional study

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Abstract

Background Neonatal sepsis is a significant cause of morbidity and mortality in low- income countries. Neonatal sepsis is classified as early-onset neonatal sepsis (EONS) and late-onset neonatal sepsis (LONS). Etiologies responsible for EONS are mostly acquired vertically from the mother during or before birth with the possibility of prevention. The burden and etiology of neonatal sepsis is not uniform across the globe with huge disparities based on the income level of the countries. This study aimed to determine neonatal sepsis trends, prevalence, and etiologies at Hawassa University Comprehensive Specialised Hospital (HUCSH).

Methods A hospital-based retrospective cross-sectional study was conducted among newborns aged 0 to 90 days who were admitted to the HUCSH from January 2019 to July 2023. Patient-related information and the culture results were obtained from HUCSH microbiology laboratory registration book. Data analysis was performed using SPSS version 25 software.

Results Out of 2364 newborns suspected of having sepsis, 56% (95% CI: 54–58%) had culture-confirmed sepsis. When excluding Coagulase Negative Staphylococcus (CONS), the prevalence of culture-confirmed neonatal sepsis was 36.9%. The highest numbers of culture-confirmed cases was observed in 2021. The predominant bacteria identified were Coagulase Negative Staphylococcus (CONS) (34.1%), Klebsiella pneumoniae (12.9%), and Enterococcus (10.6%). Among culture-confirmed neonatal sepsis, 59.9% and 40.1% of cases were EONS and LONS, respectively. Coagulase Negative Staphylococcus and Enterococcus were the major bacteria found in both EONS and LONS while. Klebsiella pneumoniae was the second most common bacteria among newborns with EONS following CONS.

Conclusions The prevalence of culture-confirmed neonatal sepsis was relatively high in the study area. Early-onset neonatal sepsis was consistently more prevalent than LONS. The predominant etiologies of neonatal sepsis excluding CONS were K. pneumoniae, Enterococcus, Enterobacter agglomerans, Acinetobacter species, and Staphylococcus aureus. Among newborns with EONS, the predominant bacteria were K. pneumoniae, Enterococcus, Enterobacter agglomerans, and Acinetobacter species.

Keywords Burden, Etiology, Neonatal sepsis, Early-onset, Late-onset, Neonates, Newborns, Hawassa, Ethiopia

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Background

Neonatal sepsis is a clinical condition characterized by multiple non-particular signs and symptoms [1]. Sepsis is a major cause of morbidity and mortality among neonates, especially in resource-limited countries [2]. Sepsis can be classified as early-onset neonatal sepsis (EONS) or late-onset neonatal sepsis (LONS) based on the age it occurs.

Important methods to tackle neonatal sepsis include the immediate use of broad-spectrum antibiotics, as sepsis can be very life-threatening. Diagnostic methods have advanced from traditional blood cultures to quicker molecular techniques that identify bacteria, allowing for prompt antibiotic treatment. Clinical guidelines and risk assessment tools assist healthcare providers in identifying newborns at higher risk for sepsis, enabling more focused care. Preventive measures, like improving maternal health and infection control in neonatal intensive care units, are also crucial for lowering sepsis rates. Combining these strategies is essential to improve outcomes for newborns at risk of sepsis [1, 3].

Early-onset sepsis occurs within the first week of life, often manifesting within the first 48 h. Etiologies of EONS are acquired vertically during or before birth from mothers harboring the pathogen in the recto-vaginal compartment [4, 5]. early-onset neonatal sepsis can be prevented with the implementation of appropriate prevention strategies similar to those used for group B Streptococcus (GBS) [6]. late-onset neonatal sepsis occur after the first week to three months of life. Unlike EONS, LONS is acquired after birth from hospital setting, from family members, or anyone who comes in the contact with the newborns [6, 7]. Intrapartum Antibiotic Prophylaxis, while effective for preventing EONS, does not prevent LONS. However, vaccines have the potential to be effective for both EONS and LONS [8].

Globally, neonatal sepsis is the third major cause of mortality among neonates [9]. Fifteen per cent of all neonatal deaths globally in 2018 were due to EONS and it is almost three times that of LONS. About 70% of the causative agents of early-onset sepsis are mainly GBS and *Escherichia coli* [10]. Late-onset sepsis is often linked to hospital and community-acquired bacterial infections, including those associated with intravenous catheterization and the most common etiologies are *Staphylococcus aureus*, Coagulase-negative Staphylococcus (CONS), *Escherichia coli* and *Klebsiella pneumoniae* [5, 11].

In the Sub-Saharan Africa region, neonatal mortality rates are highest with 27 deaths per 1000 live births reported [12]. In Ethiopia, sepsis is a major contributor to neonatal mortality, accounting for 33% of all neonatal deaths [13]. According to a report of the 2019 Ethiopian Demographic and Health Survey (EDHS) there were 30 deaths per 1000 live births [14]. A systematic review and meta-analysis in Ethiopia reported 45% of neonatal sepsis with a range of 17–78% [15]. The emergence of multidrug-resistant bacteria for instance *K. pneumoniae* further complicates management of sepsis [16].

Despite neonatal sepsis being a significant cause of morbidity and mortality in low-income countries, there are only few studies from Ethiopia that report burden and etiologies of neonatal sepsis [17]. The etiology of EONS in low-income countries is controversial which could be due to either variation laboratory methods used or presence of pre-existing antibodies [18, 19]. Evidence from high-income countries indicates that the GBS was the leading cause of EONS until the implementation of Intrapartum Antibiotic Prophylaxis (IAP) [6]; however, situation might be different in Ethiopia where there is no robust study that determined the burden of EONS due to GBS. In this context, our study aimed to determine the trend, prevalence, and etiologies of neonatal sepsis among newborns aged 0 to 90 days attending Hawassa University Comprehensive Hospital (HUCSH), Sidama, Ethiopia.

Operational definition

EONS: Culture-confirmed sepsis that occur among newborns between 0 – 7 days [6].

LONS: Culture-confirmed sepsis that occur among newborns between 8 – 90 days [6].

Newborns suspected of sepsis

Neonates presented with any one of the systemic manifestations of danger signs: not feeding well, convulsions, drowsy or unconscious, movement only when stimulated or no movement at all, fast breathing (60 breaths per min), grunting severe chest in-drawing, raised temperature > 38 °C, hypothermia < 35.5 °C, central cyanosis or could be severe jaundice, severe abdominal distension or localizing signs of infection were diagnosed as having sepsis [20].

Methods

Study area

A hospital-based retrospective cross-sectional study was conducted at HUCSH. The data collection period was from 14/10/2023 to 14/11/2023. The hospital is located in Hawassa city, which is the capital of the newly formed Sidama Regional State. The hospital provides services for more than 15 million people residing in the Sidama Regional State and other adjacent regions.

Study population

All newborns between the ages of 0 and 90 days who were suspected of having sepsis and sought treatment at the HUCSH hospital between January 2019 and July 2023

were part of the study. The HUCSH microbiology maintained records of the bacteriological tests conducted on these newborns. The decision to collect blood samples for microbiological culture was made by the physicians at HUCSH. For newborns with suspected sepsis, 2 ml of venous blood was collected and placed in tryptone soy broth for initial bacterial cultivation. Sub-culturing and identification of bacteria were performed using blood agar, Chocolate agar, MacConkey agar, Mannitol salt agar, biochemical tests. Since some bacteria, such as Coagulase Negative Staphylococcus (CONS), are considered contaminants [21], the results were presented both with and without including CONS. The patient-related information and bacteriological culture results were obtained from the microbiology laboratory registration book using a semi-structured data collection format. The study included reports on bacterial pathogens isolated from all newborns less than three months of age who attended the hospital between January 2019 and July 2023. All documented data during this study period were included in the anlaysis, while incomplete data were excluded.

Data analysis and interpretation

Data was entered in to Microsoft Excel spreadsheet and analyzed using SPSS version 25 software. Data entered into SPSS was checked for completeness and coded as follows: sex (male/female), age (recorded in days/hours), and year of isolation (initially recorded using the Ethiopian calendar and later converted to the Gregorian calendar). Blood culture results were categorized as negative or positive, and the type of bacteria was documented by its name. To determine the 95% confidence interval, we conducted bootstrapping with 1,000 iterations. Data was analyzed by cross-tabulation and frequency counts and the results were presented using texts, percentages, tables, and figures.

Results

Socio-demographic characteristics

From 2019 to 2023, 7790 admissions were recordedwith 2454 underwent blood culture testing. Of 2454 newborns aged 0 – 90 days suspected of sepsis, 90 participants were excluded because of incomplete data. Of 2364 participants included, 58.7% were males and 41.3% were females. Among the newborns 62.5% were aged between 0 – 7 days. The highest number of suspected neonatal sepsis cases was recorded in 2019, while the highest number of culture-confirmed cases occurred in 2021. (Table 1).

Prevalence of culture-confirmed neonatal sepsis

Culture-confirmed prevalence of neonatal sepsis was 1324[(56%) 95% CI: 54–58%)]. Excluding CONS, the prevalence of culture-confirmed neonatal sepsis was 873(36.9%). The proportion of culture-positive newborns in 0 to 7 and 8 to 90 days were 793 (59.9%) and 531 (40.1%), respectively. From January 2019 to July 2022, both suspected and culture-confirmed cases of neonatal sepsis increased, peaking in 2021. (Figs. 1 and Supplement Fig. 2).

Distribution of bacteria based on age

The most common bacteria recovered was CONS (451/1324; 34.1%) followed by *Klebsiella pneumoniae* (171/1324; 12.9%), *Enterococcus* (140/1324; 10.6%), and *Enterobacter agglomerans* (77/1324; 5.8%) (Table 2 and Supplement Table 1). When considering all Klebsiella species, they were responsible for 18.4% (244/1324) of neonatal sepsis cases. If only When considering all *Klebsiella* species, they responsible for 18.4% (244/1324) of neonatal sepsis cases. The common bacterial etiology of EONS were CONS ($n = 221/793$; 27.9%), *Klebsiella pneumoniae* ($n = 111/793$; 13.9%), *Enterococcus* ($n = 76/793$; 9.6%), *Enterobacter agglomerans* ($n = 68/793$; 8.6%), and *Acinetobacter* species ($n = 45/793$; 5.7%). When considering all *Klebsiella* species, they were responsible for 21.1% (167/793) of EONS.

Table 1 Socio-demographic characteristics of newborns attending HUCSH, Hawassa, Sidama regional State, Ethiopia ($N = 2364$)

Variables	Category	Number of admission	Frequency (%)	Negative *n*(%)	Positive *n*(%)	X^2	*p*-value
Sex	Male		1387(58.7)	610 (44)	777 (56)	--	--
	Female	--	977(41.3)	430 (44)	547 (56)		
Age group in days	0 – 7days		1478 (62.5)	685 (46.3)	793 (53.7)	8.863	0.03
	8 – 90 days	--	886 (37.5)	355 (40.1)	531 (59.9)		
Year	2019	2010	125 (5.3)	67 (53.6)	58 (46.4)	42.23	< 0.0001
	2020	1490	288 (12.2)	108 (53.6)	180 (62.5)		
	2021	1350	420 (17.8)	164 (39)	256 (61)		
	2022	1468	866 (36.6)	347 (40.1)	519 (59.9)		
	2023	1472	665 (28.1)	354 (53.2)	311 (46.8)		

The denominator for 'Frequency' column is the total study participants ($n = 2364$) whereas the denominator for 'Negative' and 'Positive' column is the value in preceding 'Frequency' column

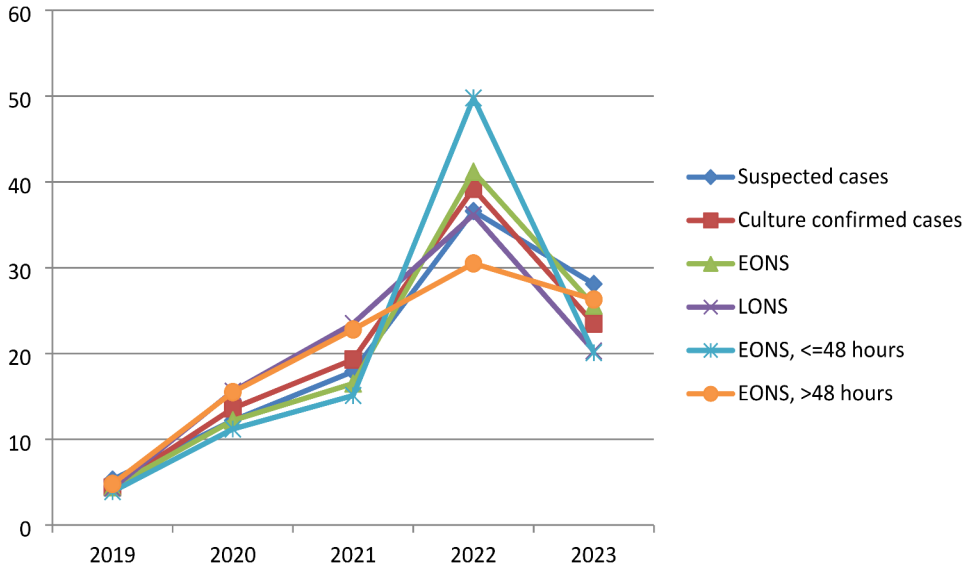

Fig. 1 Trends of suspected and culture-confirmed neonatal sepsis at Hawassa University Comprehensive Specialized Hospital (x axis is for year y axis for percentages)

To increase the likelihood of detecting bacteria acquired from the mother before birth (vertically transmitted), we have further investigated the etiologies based on the age of onset: age < 48 h and > 48 h. The common etiologies among newborns aged < 48 h were CONS ($n = 110$; 14.1%), *Klebsiella pneumoniae* ($n = 39$; 5%), *Enterobacter agglomerans* ($n = 37$; 4.7%), *Enterococcus* ($n = 28$; 3.6%), *Acinetobacter* species ($n = 19$; 2.4%), GPR ($n = 18$; 2.3%), and *Pseudomonas* species ($n = 16$; 2%) (Table 3, Supplement Table 1, Supplement Fig. 1).

Distribution of bacteria based on year isolated

The distribution of bacteria varied by year. The highest proportions of *S. aureus*, *K. pneumoniae*, *Enterococcus*, and CONS were observed in 2022, 2020, 2022, and 2022, respectively. There was an increasing trend in the prevalence of *Acinetobacter* species and CONS from 2019 to 2022. The proportion of CONS was higher than 10% in all years except 2019 (Table 3).

Discussion

Neonatal sepsis is the leading cause of admissions to neonatal intensive care units and a major cause of mortality in developing countries [22]. In this retrospective cross-sectional study, the overall prevalence of culture-confirmed neonatal sepsis was 56% (95% CI: 54–58%) which is lower than the rates reported in studies from,. The Gondar, Ethiopia (63.69%) [23], Shashamane, Ethiopia (77.9%) [24], Dessie, Ethiopia (79.4%) [25], Arbaminch, Ethiopia (78.3%) [26], Gondar, Ethiopia (64.8%) [27], and Sudan (61.3%) [28]. The burden of neonatal sepsis varies significantly based on the age at onset ($p = 0.03$) and the year of admission ($p < 0.0001$).

In this study, a significant increase in the number of culture-confirmed sepsis cases, including both EONS and LONS, was observed over five years. This upward trend could be attributed to several factors. One contributing factor may be the improvement of laboratory capabilities, which have likely led to better identification and diagnosis of neonatal sepsis. Additionally, another possible explanation for this increase could be a rise in the number of referral cases to the hospital, indicating that more healthcare providers are recognizing the signs of sepsis and directing patients to appropriate care.

Our finding indicates a higher prevalence of neonatal sepsis compared to two pooled prevalence reported from Ethiopia (45%) [15] and 49.98% [29]; and several other studies: Arsi University Teaching and Referral Hospital (34%) [28], Hawassa (36.5%) [20], Gondar (46.6%) [30], and Wolaita Sodo (33.8%) [31]. This variation could be due factors such as the time of blood collection, the definition of sepsis, and prior antibiotic administration.

The prevalence of sepsis may be relatively reduced if the criteria for identifying newborns with suspected sepsis are not strictly followed or if blood specimens are collected after starting antibiotic treatment. Additionally, the rate of culture positivity may increase due to contamination if there is insufficient decontamination during blood specimen collection. In our specific context, ensuring strict adherence to the criteria such as the timing of blood specimen collection, transportation of specimens, and proper disinfection is challenging until a system is implemented to identify and rectify any errors that may occur.

We have observed an increase in neonatal sepsis cases from 2019 to 2022 with a decline in 2023 which could

Table 2 Distribution of bacteria among newborns suspected of neonatal sepsis at HUCSH, Hawassa, Sidama regional State, Ethiopia (*N* = 1324)

Types of bacteria	Frequency *n*(%) d = 1324	Etiology, EONS, LONS *n*(%)		Etiology: >48, ≤ 48 h	
		EONS d = 793	LONS d = 531	> 48 h d = 965	≤ 48 h d = 359
Staphylococcus aureus	70 (5.3)	38 (4.8)	32 (6)	55 (5.7)	15 (4.2)
Streptococcus viridans	4 (0.3)	1 (0.1)	3 (0.6)	3 (0.3)	1 (0.3)
Escherichia coli	35 (2.6)	21 (2.7)	14 (2.6)	24 (2.5)	11 (3.1)
Acinetobacter species	70 (5.3)	45 (5.7)	25 (4.7)	51 (5.3)	19 (5.4)
Pseudomonas species	34 (2.6)	20 (2.5)	14 (2.6)	18 (1.9)	16 (4.5)
GPR	38 (2.9)	27 (3.4)	11 (2.1)	20 (2.1)	18 (2.3)
Klebsiella species	2 (0.2)	--	2 (0.4)	2 (0.2)	--
Bacillus species	2 (0.2)	2 (0.3)	--	2 (0.2)	--
Yeast cell	14 (1.1)	5 (0.6)	9 (1.7)	13 (1.3)	1 (0.3)
Citrobacter	5 (0.4)	3 (0.4)	2 (0.4)	3 (0.3)	2 (0.7)
Enterobacter agglomerans	77 (5.8)	68 (8.6)	9 (1.7)	40 (4.1)	37 (10.4)
Klebsiella oxytoca	24 (1.8)	18 (2.3)	6 (1.1)	17 (1.7)	7 (2)
Staphylococcus lugdunensis	38 (2.9)	25 (3.2)	13 (2.4)	28 (2.9)	10 (2.8)
Klebsiella ozaenae	47 (3.6)	37 (4.7)	10 (1.9)	35 (3.6)	12 (3.4)
Klebsiella rhinoscleromatis	4 (0.3)	2 (0.3)	2 (0.4)	4 (0.4)	--
Streptococcus pyogenes & *Enterococcus* species	2 (0.2)	2 (0.3)	--	1 (0.1)	1 (0.3)
Enterococcus & *Staphylococcus aureus*	2 (0.2)	--	2 (0.4)	2 (0.2)	--
Enterobacter agglomerans & *Klebsiella pneumoniae*	1 (0.1)	1 (0.1)	--	1 (0.1)	--
Serratia species & *Enterobacter agglomerans*	1 (0.1)	--	1 (0.2)	1 (0.1)	--
Enterobacter cloacae	14 (1.1)	13 (1.6)	1 (0.2)	8 (0.8)	6 (1.7)
Stenotrophomonas	2 (0.2)	1 (0.1)	1 (0.2)	1 (0.1)	1 (0.3)
Listeria monocytogenes	1 (0.1)	1 (0.1)	-	--	1 (0.3)
CONS	451 (34.1)	221 (27.9)	230 (43.3)	341 (35.2)	110 (31)
Klebsiella pneumoniae & *Enterococcus*	4 (0.3)	1 (0.1)	3 (0.6)	4 (0.4)	--
Klebsiella pneumoniae & *Klebsiella ozaenae*	1 (0.1)	1 (0.1)	--	--	1 (0.3)
Klebsiella pneumoniae & *Pseudomonas* species	1 (0.1)	--	1 (0.2)	1 (0.1)	--
Enterobacter agglomerans & *Staphylococcus lugdunensis*	1 (0.1)	1 (0.1)	--	1 (0.1)	--
Staphylococcus lugdunensis& Enterococcus	1 (0.1)	--	1 (0.2)	1 (0.1)	--
Klebsiella pneumoniae & *Klebsiella oxytoca*	1 (0.1)	--	1 (0.2)	1 (0.1)	--
Klebsiella ozaenae& Enterococcus	1 (0.1)	1 (0.1)	--	1 (0.1)	--
Providencia rettgeri	2 (0.2)	1 (0.1)	1 (0.2)	2 (0.2)	--
Enterococcus	140 (10.6)	76 (9.6)	64 (12.1)	112 (11.6)	28 (7.9)
Beta hemolytic streptococci	2 (0.2)	1 (0.1)	1 (0.2)	1 (0.1)	1 (0.3)
Enterobacter aerogenes	4 (0.3)	4 (0.5)	--	2 (0.2)	2 (0.6)
Acinetobacter species & Beta hemolytic streptococci	1 (0.1)	--	1 (0.2)	1 (0.1)	--
Providencia stuartii	2 (0.2)	2 (0.3)	--	2 (0.2)	--
Candida albicans	1 (0.1)	1 (0.1)	--	1 (0.1)	--
Enterococcus & *E. coli*	2 (0.2)	1 (0.1)	1 (0.2)	2 (0.2)	--
Enterobacter species	2 (0.2)	1 (0.1)	1 (0.2)	2 (0.2)	--
Morganella morganii	2 (0.2)	--	2 (0.4)	2 (0.2)	--
Micrococcus	3 (0.2)	3 (0.4)	--	1 (0.1)	2 (0.6)
Klebsiella pneumoniae	171 (12.9)	111 (14)	60 (11.3)	132 (13.6)	39 (11)
GNR	9 (0.7)	8 (1)	1 (0.2)	5 (0.5)	4 (1.1)
Klebsiella pneumoniae & *Staphylococcus aureus*	1 (0.1)	1 (0.1)	--	1 (0.1)	--
Serratia species	17 (1.3)	16 (2)	1 (0.2)	14 (1.5)	3 (0.9)
Enteric bacteria	1 (0.1)	--	1 (0.2)	1 (0.1)	--
Providencia stuartii	1 (0.1)	--	1 (0.2)	1 (0.1)	--
Others	15 (1.1)	12 (1.5)	3 (0.6)	8 (0.8)	7 (2)
Total	**1324**	**793**	**531**	**969**	**355**

GNR stands fir other Gram negative rod, CONS: Coagulase negative *S. aureus*, GPR stands forother Gram positive rod, d = denominator

Table 3 Distribution of bacteria based on the year of isolation

Bacterial type	Year isolated, Frequency (%)				
	2019	**2020**	**2021**	**2022**	**2023**
Staphylococcus aureus	17 (24.3)	10 (14.3)	7 (10)	22 (31.4)	14 (20)
Streptococcus viridans	1 (25)	2 (50)	–	1 (25)	–
Escherichia coli	1 (2.9)	5 (14.3)	7 (20)	12 (34.3)	10 (28.6)
Acinetobacter species	1 (1.4)	10 (14.3)	18 (25.7)	33 (47.1)	8 (11.4)
Pseudomonas species	1 (2.9)	5 (14.7)	6 (17.6)	15 (44.1)	7 (20.6)
GPR	2 (5.3)	4 (10.5)	9 (23.7)	10 (26.3)	13 (34.2)
Klebsiella species	2(100)	–	–	–	–
Bacillus species	–	2(100)	–	–	–
Yeast cell	–	5 (35.7)	2 (14.3)	2 (14.3)	5 (35.7)
Citrobacter	–	1 (20)	1 (20)	3 (60)	–
Enterobacter agglomerans	–	1 (1.3)	4 (5.2)	61 (79.2)	11 (14.3)
Klebsiella oxytoca	2 (8.3)	1 (4.2)	5 (20.8)	6 (25)	10 (41.7)
Staphylococcus lugdunensis	–	11 (28.9)	–	6 (15.8)	21 (55.3)
Klebsiella ozaenae	–	2 (4.3)	12 (25.5)	23 (48.9)	10 (21.3)
Klebsiella rhinoscleromatis	–	–	2 (50)	1 (25)	1 (25)
Streptococcus pyogenes & *Enterococcus* species	–	–	1 (50)	1 (50)	–
Enterococcus & *Staphylococcus aureus*	–	–	1 (50)	1 (50)	–
Enterobacter agglomerans & *Klebsiella pneumoniae*	–	–	–	1 (100)	–
Serratia species & *Enterobacter agglomerans*	–	–	–	1 (100)	–
Enterobacter cloacae	–	–	–	8 (57.1)	6 (42.9)
Stenotrophomonas	–	–	–	2 (100)	–
Listeria monocytogenes	–	–	–	1 (100)	–
CONS	7 (1.6)	55 (12.2)	100 (22.2)	187 (41.5)	102 (22.6)
Klebsiella pneumoniae & *Enterococcus*	–	–	–	3 (75)	1 (25)
Klebsiella pneumoniae & *Klebsiella ozaenae*	–	–	–	–	1 (100)
Klebsiella pneumoniae & *Pseudomonas* species	–	–	–	1 (100)	–
Enterobacter agglomerans & *Staphylococcus lugdunensis*	–	–	–	1 (100)	–
Staphylococcus lugdunensis & *Enterococcus*	–	–	–	1 (100)	–
Klebsiella pneumoniae & *Klebsiella oxytoca*	–	–	–	1 (100)	–
Klebsiella ozaenae & *Enterococcus*	–	–	–	–	1 (100)
Providencia rettgeri	–	–	–	2 (100)	–
Enterococcus	1 (0.7)	12 (8.6)	44 (31.4)	59 (42.1)	24 (17.1)
Beta hemolytic streptococci	–	–	–	–	2 (100)
Enterobacter aerogenes	–	–	–	–	4 (100)
Acinetobacter species & *Beta hemolytic streptococci*	–	–	–	–	1 (100)
Providencia stuartii	–	–	–	–	2 (100)
Candida albicans	–	–	–	–	1 (100)
Enterococcus & *E. coli*	–	–	–	1 (50)	1 (50)
Enterobacter species	–	–	–	–	1
Morganella morganii	–	1	1	–	–
Micrococcus	–	–	–	3	–
Klebsiella pneumoniae	16 (9.4)	52 (30.4)	32 (18.7)	30 (17.5)	41 (24)
GNR	–	–	–	9 (100)	–
Klebsiella pneumoniae & *Staphylococcus aureus*	–	–	–	–	1 (100)
Serratia species	5 (29.4)	–	3 (17.6)	8 (47.1)	1 (5.9)
Providencia stuartii	1 (100)	–	–	–	–

GNR: Gram negative rod, CONS: Coagulase negative *S. aureus*, GPR: Gram positive rod

be due incomplete data capture for 2023. We observed similar trend for overall culture-confirmed sepsis, EONS, and LONS cases. The increase in EONS from 2019 to 2022 is higher than LONS with high disparity in 2022. The rise in suspected neonatal sepsis cases between 2020 and 2023 may be attributed to an increase in the number of referrals, the presence of more qualified medical personnel, and advancements in the Microbiology

laboratory infrastructure.The etiology of neonatal sepsis differ by geographic locations. In our study, the predominant bacteria were Gram-positive bacteria with CONS accounting for 34.1%, *S. aureus* (5.3%), and *Enterococcus* (10.6%), followed by Gram-negative bacteria such as *Klebsiella pneumoniae* (12.9%), *Enterobacter agglomerans* (5.8%), and *Acinetobacter* species (5.3%). There was a similar report from Ethiopia where *Klebsiella* species (33.9%), CONS (18.2%), and *S. aureus* (16.9%) were the most common bacteria recovered from newborns with sepsis [32]. A study from Sudan identified *Klebsiella* species (71.1%), *S. aureus* (15.8%), *E. coli* (5.3%), a Gram-positive cocci (2.6%), and *Serratia marcescens* (5.3%) from newborns with sepsis [28]. In contrast to our study, a high pooled prevalence of 38% for *Klebsiella* species has been reported in low and lower-middle-income countries [33]. Similarly, a prevalence of 42% for *Enterococcus* was observed in neonates suspected of sepsis in Colorado, which is notably higher than 10.6% observed in our study [34].

Excluding CONS, the prevalent bacteria among EONS were *Klebsiella pneumoniae* (13.9%) followed by *Enterococcus* (9.6%), *Enterobacter agglomerans* (8.6%), and *Acinetobacter* species (5.7%). Whereas the prevalent bacteria among LONS excluding CONS were *Klebsiella pneumoniae* (11.3%) followed by *Enterococcus* (12.1%) and *Acinetobacter* species (4.7%).

In this study, we attempted to determine whether the bacteria causing neonatal sepsis were acquired vertically (from the mother) or horizontally (from the environment). Our observations revealed that certain bacteria, such as *Acinetobacter* species (64.3% vs. 35.5%), *Enterobacter agglomerans* (88.3% vs. 11.7%), and *Klebsiella pneumoniae* (64.9% vs. 35.1%), were predominant in the younger age group, indicating that these pathogens are likely acquired from mothers during delivery.

Moreover, to increase the chance of picking bacteria that could be transferred vertically we have reduced the age of early-onset neonatal sepsis to less than or equal to 48 h. Some bacteria with a prevalence of > 20 among newborns aged less than or equal to 48 h include *S. aureus*, *E. coli*, *Acinetobacter* species, *Enterobacter agglomerans*, *Klebsiella oxytoca*, and *K. pneumoniae*. In our previous study, we have found that most of these bacteria are among colonizers of the vaginal compartment of pregnant women [34]. This may indicate that there are bacteria that can be transferred vertically and cause sepsis among newborns like that of Group B Streptococcus known to cause early onset neonatal sepsis.

In this study, the overall prevalence of CONS among all age groups was unexpectedly high. Since CONS are commensal of skin, this high prevalence could be due to contamination during blood collection, necessitating further investigation to confirm its role in sepsis in the study area. Additionally, in our previous study, the most common colonizer of the vaginal compartments was CONS (46.4%) which could lead to contamination of newborns skin during birth [35]. As this study is retrospective study, we were unable to control the quality of blood specimen collection and the laboratory method used for identification of bacteria. However, the study's strength lies in its incorporation of a five-year dataset, encompassing a substantial cohort of newborns suspected of having sepsis.

Limitation of the study

Since this study employed a cross-sectional retrospective design, we were unable to control the quality of the data collected. This type of study design relies on previously gathered data, which means we could not ensure the accuracy, consistency, or completeness of the information available. Consequently, factors such as data collection methods, timing, and potential biases inherent in past records could influence the reliability of our findings. Therefore, the limitations associated with this design must be acknowledged when interpreting the study's results.

Conclusion

The prevalence of culture-confirmed neonatal sepsis was high. The prevalence of EONS was higher than LONS. The common etiologies of neonatal sepsis excluding CONS were *Klebsiella pneumoniae*, *Enterococcus*, *Enterobacter agglomerans*, *Acinetobacter* species, and *Staphylococcus aureus*. The predominant bacteria among newborns with EONS were *Klebsiella pneumoniae*, *Enterococcus*, *Enterobacter agglomerans*, *and Acinetobacter* Species.

Recommendations

It is essential to conduct prospective studies to determine the prevalence and causes of neonatal sepsis in the study area, including an examination of vertical transmission of pathogens from pregnant women to newborns. Adhering to specific guidelines for blood sample collection prior to initiating antibiotic treatment is crucial to improve culture positivity rates and ensure proper disinfection during the process. Additionally, investing in microbiology laboratory infrastructure and equipment will facilitate prompt and accurate diagnoses. Promoting collaboration among pediatricians, microbiologists, and infection control experts to improve diagnosis and management strategies for neonatal sepsis. Increasing awareness of neonatal sepsis through educational initiatives targeted at healthcare professionals and pregnant women will support early detection and intervention. Finally, implementing a quality assurance program will help maintain compliance with established protocols and identify

opportunities for improvement in blood sample collection and laboratory procedures.

Supplementary Information

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Supplementary Material 1

Author contributions

M.M.A. Design the study, data analysis, prepare map, and prepare the original and revised manuscript G.k. Conception, Supervision, Revision of the manuscript M.M. Conception, Data collection, Data entry, Data analysis, Original manuscript preparation B.K.Data analysis, manuscript preparation T.H. Data collection, retrieving laboratory dataE.W. Data collection, retrieving laboratory data D.A.F. Data collection, retrieving laboratory data T.L. Data collection, retrieving laboratory data, review of the manuscript T.A. Data collection, retrieving laboratory data, review of the manuscript D.Y.R. Revise manuscript, data collection, data entry.

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Data availability

Dataset used for analysis can be obtained from corresponding author.

Declarations

Ethical approval and consent to participate

The study was approved by the Institutional Review Board of Hawassa University College of Medicine and Health Sciences (reference number: IRB/395/15) with the issued date of 03/10/2023. As this study is retrospective, consent was waived by the Institutional Review Board of Hawassa University. The confidentiality of the study participants was maintained at each stage by using codes, and the information gathered was used only to achieve the stated objective of the study. All methods were conducted in compliance with the relevant guidelines and regulations outlined in the Declaration of Helsinki.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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