

Bacterial Etiology, Antimicrobial Susceptibility Patterns and Associated Factors of Bacterial Sepsis among Sepsis suspected Neonates Admitted to Jigjiga University-Sheik Hassan Yabare Specialized Comprehensive Hospital, Jigjiga, Eastern Ethiopia

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Abstract

Background: Neonatal sepsis is a systemic infection that affects newborns younger than 28 days and is associated with significant morbidity and mortality, especially in the developing world. The emergence of drug resistance is also a major public health threat. Hence, continuous monitoring of pathogens and their antibiotic resistance patterns is crucial for managing neonatal sepsis.

Objectives: To determine the prevalence, causative bacterial agents, drug susceptibility patterns and associated factors of neonatal sepsis among neonates admitted to Jigjiga University Sheik Hassan Yabare Specialized Comprehensive Hospital, Jigjiga Eastern Ethiopia.

Methods: An institution-based cross-sectional study was conducted on 330 Neonates Suspected of having sepsis at Jigjiga University Sheik Hassan Yabare Specialized Comprehensive Hospital from April 1 –September 30, 2023. A structured questionnaire was used to collect sociodemographic information, clinical parameters and potential risk factors from the study participants. Approximately 2 ml of blood was drawn aseptically and inoculated into Tryptone Soya Broth at the patient's bedside. Bacterial identification was performed via standard microbiological techniques. The disk diffusion method was used to determine the antibiotic susceptibility patterns. Data entry was performed via Epi-data version 4.6, and the data were analyzed via SPSS version 25. Bivariate and multivariate logistic regression analyses were carried out to assess the risk factors associated with neonatal sepsis. A p value < 0.05 at the 95% confidence interval was considered statistically significant.

Results: The overall prevalence of neonatal sepsis was 23% (95%CI: 18.5 – 27.5%). Among isolated strains, 39 (51.3%) were gram-negative. *E. coli* (23, 30.3%), *S. aureus* (20, 26.3%) and *CoNS* (14, 18.4%) were the leading isolates. Ciprofloxacin and amikacin were the most effective antibiotics for gram-negative bacteria. Multidrug resistance was observed in 78.9% of the bacterial isolates. Gestational age ≤ 37 weeks (AOR: 1.9 (1.1–3.4), $p=0.02$) and breastfeeding initiation (AOR: 0.36 (0.16–0.81), $p=0.01$) were found significantly associated with neonatal sepsis.

Conclusion: *E. coli* and *S. aureus* were the dominant bacterial isolates. The prevalence of multidrug resistance was considerably high (78.9%). Amikacin and ciprofloxacin were the most effective agents against most of the isolates. Therefore, a regular review of antibiotic resistance profiles is highly recommended. Furthermore, large-scale studies are necessary to describe each bacterial strain and to study more risk factors.

Keywords: Bacterial Etiologies, Antimicrobial susceptibility, Neonatal sepsis, Jigjiga

List of Abbreviations/Acronyms

ANC	Antenatal Care
AOR	Adjusted odds ratio
ATCC	American type culture collection
CI	Confidence interval

CLSI	Clinical and Laboratory Standard Institute
CONS	Coagulase negative Staphylococcus
COR	Crude Odds ratio
EOS	Early Onset Sepsis
GBS	Group B Streptococci
JJU-SHYCSH	Jigjiga University Sheik Hassan Yabare Comprehensive Specialized Hospital
LOS	Late Onset Sepsis
MDR	Multi-Drug Resistance
SPSS	Statistical Package for Social Science

1. Introduction

Bloodstream infections are very common in pediatric patients, leading to morbidity and mortality in neonates and children [1]. Neonatal sepsis is a systemic infection that affects newborns younger than 28 days [2]. It is a serious, life-threatening infection that can worsen very quickly because of the spread of microorganisms and their toxins in the blood [2]. It is the leading cause of neonatal mortality and morbidity, particularly in the developing world, including Ethiopia [2]. Globally, neonatal sepsis is the leading cause of morbidity and mortality in the first 4 weeks of life, with an estimated annual death rate of 400,000–700,000, accounting for 15% of all neonatal deaths. Among these deaths, approximately 42% of neonatal deaths occur in the first week of life [3]. In developing countries, nearly 50% of neonatal deaths are due to sepsis, and the highest neonatal death rate has been reported in sub-Saharan Africa, with 27 deaths/1000 live births [4]. In Ethiopia, neonatal sepsis is a major newborn health problem that accounts for 33% of all neonatal deaths [5]. According to a systematic review in Ethiopia, the incidence of neonatal sepsis was estimated to be 45%, with a range of 17–78% [6]. Neonatal sepsis is categorized into early-onset sepsis and late-onset sepsis according to the symptoms that occur before or after 72 hours of life [2,7,8]. Early-onset sepsis (EOS) is a type of sepsis that occurs at least three days after birth and is caused by microorganisms in the maternal genital tract before or at the time of birth [9]. Many contributing risk factors for EOS have been reported, such as lower birth weight, premature birth, infection during pregnancy and lack of antenatal care [10]. The major bacterial causative agents are group B Streptococci (GBS) and E. coli; however, intrapartum antibiotic prophylaxis can reduce significantly the incidence of GBS in newborns [11]. Late-onset sepsis (LOS) starts after 72 hours of life; its main source is commonly considered to be hospital-acquired bacteria or from society at the time of delivery [12]. The most common bacterial causative agents are Staphylococcus aureus, coagulase-negative Staphylococcus (CoNS), E. coli and Klebsiella pneumoniae; however, there is continuous variation in these etiological agents from country to country and even within countries [13,14]. Blood culture remains the mainstay of investigation for the definitive diagnosis and management of blood stream infections in infants and children [15]. The emergence of drug resistance has also posed a global health threat, and approximately 30% of neonatal deaths are linked to anti-microbial resistance [16]. There is limited microbiological identification and antimicrobial susceptibility patterns of bacterial profiles in

resource-limited settings, including Ethiopia. In addition, there are no published data on this problem in the study area. For this reason, the present study aimed to determine bacterial etiologic agents, antimicrobial susceptibility patterns and associated risk factors among of Bacterial sepsis among sepsis suspected neonates admitted to Jigjiga University Sultan Hassan Yabare Comprehensive Specialized Hospital, Jigjiga, Ethiopia.

2. Methods and Materials

2.1. Study Area and Period

The study was conducted at Jigjiga University Sultan Hassan Yabare Comprehensive Specialized Hospital (JJU-SHYCSH), which is the only specialized hospital in the region found in Jigjiga city, the capital of the Somali region. It is located 636 km east of Addis Ababa; the capital city of Ethiopia. The study was conducted from April 1 to September 30, 2023.

2.2. Study Design and Population

An institution-based cross-sectional study design was used. All neonates suspected of having sepsis admitted to JJU-SHYCSH composed the source population. All neonates suspected of having sepsis and admitted to JJU-SHYCSH during the study period composed the study population. Neonates suspected of having sepsis who were admitted to JJU-SHYCSH and willing to participate in the study were included. Neonates that received antibiotic treatment 5 days before blood sample collection were excluded.

2.3. Sample Size Determination and Sampling Technique

The sample size for bacterial agents was calculated by taking the prevalence of culture-proven neonatal sepsis in a previous Ethiopian study in Dire-Dawa ($p=32.1\%$) and using a single population proportion formula, with a margin of error (d) of 0.05 and a confidence interval ($Z\alpha/2$) of 95%. The final sample size was 334. The sample size was also calculated for the second objectives and compared. Eligible study participants were enrolled using convenience sampling technique.

2.4. Data and Sample Collection Methods

Data collectors (experienced nurses and laboratory technologists) were recruited and trained to collect data using semi-structured questionnaires. The purpose of the study as well as any related harm or benefits were explained to the study participants accordingly. Demographic data and other information, including the presence of chronic disease, indwelling medical device, birth weight and pre-sample

antibiotic history data, were collected by reviewing different medical records and interviews. Data on the nutritional status of the mothers and neonates were collected on the basis of their clinical diagnosis. Since antibiotic drug treatment before blood sample collection could compromise the culture results, those who were taking antibiotics for more than 5 days were excluded from the study. Using aseptic techniques, two sets of blood with volume of 2 ml in each were collected from different hands after the venous site was cleaned with 70% alcohol, inoculated immediately into 5 ml of tryptone soya broth and transported to the microbiology laboratory within 10 minutes at room temperature. The bottles were labeled with unique sample number, date and time of collection.

2.5. Bacteriological Analysis

After the samples were collected aseptically, the blood culture bottles were incubated at 35–37°C for up to 7 days or until growth was detected. Bottles were observed macroscopically daily for visible evidence of bacterial growth, such as hemolysis, turbidity, gas production, or formation of discrete colonies. Regardless of the state of bacterial growth, subcultures were made after 24 hrs, 48 hrs, and 72 hrs and finally, at 7 days on sheep blood agar and MacConkey agar; then, the cultures were incubated aerobically at 37°C for 24 hrs, and chocolate agar was incubated at 37°C for 48 hrs at 5–10% CO₂. Gram staining was performed for macroscopically positive blood samples. For those that had grown on the subculture media, biochemical tests involving the isolation of pure colonies were performed first. On the basis of the colonial morphological characteristics and biochemical test results, the etiologic agent was identified. For gram-positive bacteria, catalase, coagulase, novobiocin, bacitracin and mannitol salt agar were used. For gram-negative indole test, citrate utilization, triple sugar iron agar, urea, lysine decarboxylase, and motility tests were performed, and the organisms were identified according to standard procedures. Coagulase negative staphylococci were considered potential pathogen when isolated from both sets of the blood to minimize the skin contamination.

2.6. Antibiotic Susceptibility Testing

Drug susceptibility testing of the isolates was performed on Mueller–Hinton agar. The standard disk diffusion technique of the modified Kirby–Bauer method was used as recommended by the Clinical and Laboratory Standard Institute (CLSI) guideline 2022 [17]. Approximately three to five pure colonies of the test organism were taken by using a sterile wire loop and emulsified in 2 ml of normal saline. McFarland (0.5) was used as a standard to check the 5% turbidity of the bacterial suspensions. Then, a sterile cotton swab was dipped into the suspension and squeezed from the excess fluid against the inside wall of the test tube. The test organisms were uniformly seeded on the surface of Muller–Hinton agar. Drugs, including penicillin (10 µg), tetracycline (30 µg), ampicillin (10 µg), ceftriaxone (30 µg), amikacin (30 µg), ciprofloxacin (5 µg), gentamicin (10 µg), trimethoprim-sulphamethoxazole (1.25/23.75

µg), norfloxacin (5 µg), erythromycin (30 µg), cefepime (30 µg), and meropenem (10 µg), were selected on the basis of local drug use and CLSI 2022 guidelines [17]. The selected antibiotic disks were applied to the plates and incubated overnight at 35–37°C. The diameter of the zone of bacterial growth inhibition was measured in millimeters (mm) via a clipper, and the isolates were classified as sensitive, intermediate or resistant according to CLSI 2022. Bacteria that were resistant to 3 or more drugs of different structural classes were considered Multidrug resistant (MDR).

2.7. Data Quality Control

During laboratory analysis, pre-analytical, analytical and post-analytical standard operating procedures of the medical microbiology laboratory were followed to ensure quality. The sterilization of the culture media was ensured by incubating 5% of each batch of the prepared media at 37°C for 24 hr. The culture media were prepared and sterilized according to the manufacturer's instructions. Instructions, the sterility and performance of the culture media were checked by incubating 3–5% of the batch at 35–37°C. The principal investigator oriented the data and blood collectors and supervised them daily. The collected data were checked for completeness and double entered to ensure accuracy. The standard reference strains of the American Type Culture Collection *S. aureus* ATCC-25923 and *E. coli* ATCC-25922 were used as controls.

2.8. Data Analysis Methods

The data were entered into Epi-data version 4.6 software and exported to SPSS 25 for cleaning and data analysis. Descriptive statistics are presented as frequencies/percentages for all variables. Bivariate and multivariate logistic regression analyses were performed to assess potential risk factors associated with neonatal sepsis. Only variables with $P < 0.25$ at the 95% confidence interval in the bivariate analysis were considered for inclusion in the multivariable analysis. Variables with $P < 0.05$ in the multivariable analysis were considered statistically significant.

3. Results

3.1. Socio-Demographics and Health Related Characteristics of the Study Population

This study enrolled 330 participants making 98% response rate. More than half (60.9%) of the neonates were female. More than half (62.1%) of study participants were born at more than 37 weeks. Among the mothers of the study participants, 62.7% were urban residents, and 57.6% of the mothers were under 31–45 years. Almost half 49.7% of the mothers had reached primary to secondary education. In addition, 62.4% of the mothers were multigravida (Table 1). Half (51.2%) of the mothers received 1–3 ANC visits. About 57% of the neonates were breastfed after 1–2 hours of delivery. Moreover, more than half (59.4%) of the mothers gave birth in public health facilities, 57.3% of them delivered via spontaneous vaginal delivery and 73% of them delivered via spontaneous labor (Table 2).

Table 1: Neonatal and Maternal Socio-Demographics of Neonates Suspected of Having Sepsis at JJU-SHYCSH from April 1 to September 30, 2023 (n=330).

Neonatal socio-demographics			
Variable	Category	Frequency (n)	Percent (%)
Age of neonate (in days)	0 – 3	142	43
	3 – 28	188	57
Sex of neonates	Female	129	39.1
	Male	201	60.9
Birth weight	2.5 kg	141	42.7
	>2.5 kg	189	57.3
Gestational age (in weeks)	≤37	125	37.9
	> 37	205	62.1
Maternal socio-demographics			
Age of mother (in years)	18 – 30	94	28.5
	31 – 45	190	57.6
	>45	46	13.9
Residence	Urban	207	62.7
	Rural	123	37.3
Educational status of mother	Illiterate	110	33.3
	primary –secondary	164	49.7
	Tertiary	56	17
Gravidity	Primigravida	124	37.6
	Multigravida	206	62.4
Parity	Primiparous	164	49.7
	Multiparous	166	50.3

Table 2: Health Related Characteristics of Neonates Suspected of Having Sepsis at JJU-SHYCSH from April 1 to September 30, 2023 (n = 330)

Variable	Category	Frequency (n)	Percent (%)
ANC visits	Not at all	131	39.7
	1-3 visits	169	51.2
	4 visits	30	9.1
Place of delivery	Public HFs	196	59.4
	Private HFs	105	31.8
	Home	29	8.8
Delivery Mode	SVD	189	57.3
	AVD	116	35.2
	CS	25	7.6
Resuscitation at birth	No	167	50.6
	Yes	163	49.4
Breast feeding initiation (in hours)	<1	75	22.7
	1-2	188	57
	>2	67	20.3
onset of labor	Spontaneous	241	73
	Induced	89	27
Duration of admission before sampling (in hours)	<24	138	41.8
	24-48	155	47
	>48	37	11.2

Abbreviation: HF = health facilities, SVD = spontaneous vaginal delivery, AVD = assisted vaginal delivery, CS = cesarean section

3.2. Magnitude of Culture Confirmed Neonatal Sepsis and the Bacterial Profile

Among the study participants, 23% (95% CI 18.5–27.5%)

had bacteriologically confirmed neonatal sepsis. Among the neonatal positive study participants, 51.3% had early-onset sepsis. Approximately 51.3% of the isolates were gram-negative bacteria, followed by 48.7% that were gram-positive. The most predominant isolates were *E. coli* (30.3%) and *S. aureus* (26.3%), followed by *Coagulase-negative staphylococcal* species (CoNS) (18.4%) (Table 3).

Table 3: Patterns of Bacterial Species Isolated from of Bacterial Sepsis Among Sepsis Suspected Neonates at JJU-SHYCSH from April 1 to September 30, 2023 (n=330)

Bacterial isolates	Frequency(n)	Percentage(%)
gram-negative		
<i>E.coli</i>	23	30.3
<i>K.pneumonia</i>	11	14.5
<i>Acinetobacter</i>	5	6.6
gram-positive		
<i>S.aureus</i>	20	26.3
CoNS	14	18.4
Group B streptococci	3	3.9

According to the onset of neonatal sepsis, more than half (51.3%, 39) of the isolated bacterial organisms were recovered from neonates aged less than 3 days; in other words, early-onset sepsis (EOS) was detected. Of these, 53.8% (21/39) of the bacterial isolates were gram-negative.

E. coli dominated among the bacterial isolates in EOS. Among neonates aged 4–28 days, 51.4% (19/37) were gram-positive; in other words, late-onset sepsis (LOS) predominated. Among these bacteria, *S. aureus* was the most common isolated bacteria, accounting for 52.6% (10/19) (Table 4).

Table 4: The Distribution of Bacterial Isolates According to the Time of Infection from Suspected Septic Neonates at JJU-SHYCSH from April 1 to September 30, 2023 (n=330)

Bacterial isolates in EOS and LOS n(%)								
Variable	Category	<i>E. coli</i> (23)	<i>K. pneumonia</i> (11)	<i>Acinetobacter</i> (5)	<i>S. aureus</i> (20)	CoNS(14)	GBS(3)	Total n(%)
Neonate Age (days)	0 – 3	12(52.2)	6(54.5)	3(60)	10(50)	6(42.9)	2(66.7)	39(51.3)
	4 – 28	11(47.8)	5(45.6)	2(40)	10(50)	8(57.1)	1(33.3)	37(48.7)

3.3. Antimicrobial Susceptibility Testing of Bacterial Isolates

Antimicrobial sensitivity testing was performed against 14 antimicrobial agents. The isolated gram-negative bacteria were highly resistant to commonly used empiric antibiotics. *E. coli*, and *K. pneumoniae* was extremely resistant to ampicillin in 18 (78%) and 11 (100%) patients, respectively. In addition, *K. pneumoniae*, *E. coli* and *Acinetobacter* bacteria were highly resistant to gentamicin [10 (91%), 12 (52%), and 5 (100%), respectively]. In addition, most gram-negative isolates were completely resistant to tetracycline, ranging from 80–100%. *K. pneumoniae* has a relatively high rate of resistance

to almost all antimicrobial agents, with the exception of amikacin. Ciprofloxacin and amikacin showed significant effectiveness against most identified gram-negative bacteria. The isolated gram-positive bacteria presented increased resistance to penicillin, erythromycin, and norfloxacin. *S. aureus*, CoNS and GBS were highly resistant to penicillin (16 (80%), 12 (86%), and 2 (67%) strains, respectively). *S. aureus* and CoNS were also highly resistant to erythromycin (17 (85%) and 10 (71%) strains, respectively). Erythromycin, ceftriaxone and Cefepime were more susceptible to group B streptococci. Ciprofloxacin was moderately effective against the gram-positive isolates (Table 5).

Table 5: Antimicrobial Susceptibility Patterns of Bacterial Isolates from Suspected Septic Neonates at JJU-SHYCSH from April 1 to September 30, 2023 (n=330).

Bacterial species	Resistance to antimicrobial agents; n(%)												
	AMP	PEN	ERY	GEN	MER	CTR	CFP	CPR	AMK	CRX	NOR	TET	SXT
Gram Negative													
<i>E. coli</i> (23)	18(78%)	NA	NA	12(52)	9(39)	11(48)	14(61)	7(30)	5(22)	15(65)	14(61)	18(78)	12(52)
<i>K. pneumoniae</i> (11)	11(100%)	NA	NA	10(91)	11(100)	11(100)	11(100)	10(91)	4(36)	11(100)	9(82)	11(100)	10(91)
<i>Acinetobacter</i> (5)	5(100)	NA	NA	5(100)	2(40)	3(60)	4(80)	5(100)	1(20)	4(80)	5(100)	4(80)	4(80)
Total(n=39)													
Gram Positive													
<i>S. aureus</i> (20)	NA	16(80)	17(85)	12(60)	NA	NA	NA	11(55)	NA	NA	12(60)	15(75)	14(70)
CoNS (14)	NA	12(86)	10(71)	6(43)	NA	NA	NA	8(57)	NA	NA	10(71)	8(57)	9(64)
GBS(3)	2(67)	2(67)	1(33)	2(67)	NA	1(33%)	0(0)	NA	NA	3(100)	3(100)	1(33)	1(33)
Total(n=37)													

Abbreviation: AMP=Ampicillin, PEN=Penicillin, ERY=Erythromycin, MER= Meropenem, CTR= Ceftriaxone, CPR= Ciprofloxacin, AMK= Amikacin, CRX= Cefuroxime, NOR= Norfloxacin, TET= Tetracycline, SXT= Trimethoprim/sulfamethoxazole

3.4. Multidrug Resistant (MDR) Bacterial Isolates

The majority (60, 78.9%) of the bacterial isolates from neonatal blood culture were found to be multidrug resistant (MDR), with the most common antibiotic used being MDR. Among the bacterial isolates, MDR bacteria were found in 10 (90.9%) *K. pneumoniae* strains, 17 (73.9%) *E. coli* strains, 16 (80%) *S. aureus* strains and 5 (100%) *Acinetobacter* spp (Table 6).

Table 6: MDR levels of Bacterial Isolates from Suspected Septic Neonates at JJU-SHYCSH from April 1 to September 30, 2023 (n=330)

Bacterial isolates	Degree of resistance n(%)					Total MDR isolates ≥R3)
	R0	R1	R2	R3	R4	
<i>E. coli</i> (23)	0(0)	2(8.7)	4(17.4)	14(60.9)	3(13)	17(73.9)
<i>K. pneumoniae</i> (11)	0(0)	0(0)	1(9.1)	8(72.7)	2(18.2)	10(90.9)
<i>Acinetobacter</i> spp(5)	0(0)	0(0)	0(0)	4(80)	1(20)	5(100)
<i>S. aureus</i> (20)	0(0)	1(5)	3(15)	13(65)	3(15)	16(80)
CoNS(14)	0(0)	2(14.3)	3(21.4)	8(57)	1(7.1)	9(64.3)
GBS(3)	0(0)	0(0)	0(0)	2(66.7)	1(33.3)	3(100)
Total(n = 76)	0(0)	5(6.6)	11(14.5)	49(64.5)	11(14.5)	60(78.9)

R0: No antibiotic resistance, **R1:** resistance to one category of antimicrobial agent, **R2** - resistance to two different categories of antimicrobial agents, **R3** - resistance to three different categories of antimicrobial agents, **R4:** resistance to four different categories of antimicrobial agents.

3.5. Bivariate and Multivariate Logistic Analyses for Associated Factors

3.5.1. Bivariate Regression Analysis

Bivariate and multivariate logistic regression were computed to assess the association between the independent variables and the dependent variable. In bivariate logistic regression, residence, maternal age, educational status of the mother, labor onset mode and breast feeding onset were candidates for multivariate logistic regression ($p \leq 0.25$) (Table 7).

Table 7: Bivariate Logistic Regression Analysis of Suspected Septic Neonates at JJU-SHYCSH from April 1 to September 30, 2023 (n=330).

Variable	Category	Neonatal sepsis		COR(95%CI)	P value
		NoN(%)	YesN (%)		
Sex	Female	97(75.2)	32(24.8)	1.2(0.7-1.98)	0.54
	Male	157(78.1)	44(21.9)	Ref	
neonate Age(days)	0 – 3	103(72.5)	39(24.5)	1.55(0.9-2.6)	0.98
	4 – 28	151(80.3)	37(19.7)	Ref	
Residence	Urban	155(74.9)	52(25.1)	1.4(0.8– 2.4)	0.24*
	Rural	99(80.5)	24(19.5)	Ref	
Birthweight(kg)	2.5	102(72.3)	39(27.7)	0.64(0.4-1.06)	0.86
	>2.5	152(80.4)	37(19.6)	Ref	
Gestational age(weeks)	≤37	89(71.2)	36(28.8)	1.7(0.99-2.8)	0.053*
	>37	165(80.5)	40(19.5)	Ref	
maternal age(year)	18-30	80(85.1)	14(14.9)	0.5(0.21-1.2)	0.11*
	31 – 45	140(73.7)	50(26.3)	1.01(0.5-2.1)	0.98
	>45	34(73.9)	12(26.1)	Ref	
Education	Illiterate	89(80.9)	21(19.1)	0.54(0.26-1.14)	0.105*
	primary – secondary	126(76.8)	38(23.1)	0.7(0.4-1.4)	0.29
	Tertiary	39(69.6)	17(30.4)	Ref	
Gravidity	primigravida	92(74.2)	32(25.8)	1.3(0.8– 2.2)	0.35
	Multigravida	162(78.6)	44(23.4)	Ref	
ANC visits	not at all	109(83.2)	22(16.8)	0.4(0.17– 0.98)	0.45
	1-3 visits	125(74)	44(26)	0.7(0.31– 1.62)	0.41
	4 visits	20(66.7)	10(33.3)	Ref	

delivery mode	SVD	147(77.8)	42(22.2)	0.9(0.3-2.4)	0.84
	AVD	88(75.9)	28(24.9)	1.01(0.37– 2.8)	0.98
	CS	19(76)	6(24)	Ref	
place of delivery	publicHFs	159(81.1)	37(18.9)	0.6(0.25-1.5)	0.28
	privateHFs	74(70.5)	31(29.5)	1.1(0.44– 2.74)	0.84
	Home	21(72.4)	8(27.6)	Ref	
resuscitation at birth	No	136(81.4)	31(18.6)	0.6(0.36– 1.0)	0.52
	Yes	118(72.4)	45(27.6)	Ref	
labor onset	Induced	63(70.8)	26(29.2)	1.6(0.91– 2.74)	0.11*
	Spontaneous	191(79.3)	50(20.7)	Ref	
breastfeeding onset	<1 hr	62(82.7)	13(17.3)	0.33(0.15– 0.72)	0.005*
	1 – 2 hr	151(80.3)	37(19.7)	0.39(0.21– 0.71)	0.002*
	>2 hrs	41(61.2)	26((38.8)	Ref	
Duration of admission before sampling	<24 hrs	103(74.6)	35(25.4)	1.06(0.46– 2.5)	0.89
	24 – 48 hrs	123(79.4)	32(20.6)	0.81(0.35– 1.9)	0.62
	>48 hrs	28(75.7)	9(24.3)	Ref	
Parity	Primiparous	128(78)	36(22)	0.89(0.53– 1.5)	0.64
	Multiparous	126(75.9)	40(24.1)	Ref	

Abbreviation: N = number, CI= confidence interval, COR= crude odds ratio, hrs = hours *pvalue< 0.25, Ref: reference category

3.6. Multivariate Regression Analysis

In the multivariate logistic regression analysis, gestational age in weeks (AOR: 1.9 [95% CI 1.1–3.4] and breastfeeding initiation (AOR: 0.36 [95% CI 0.16–0.81] were found to be

statistically significant at $p \leq 0.05$. Neonates who were born preterm (≤ 37 weeks) were 1.9 times more likely to develop bacterial neonatal sepsis than were those who were born at fullterm (>37 weeks). In addition, neonates who were breastfed in the first hour of their life were less likely to develop neonatal sepsis than those who were breastfed after 2 hours (**Table 8**).

Table 8: Multivariate Logistic Regression Analysis of Neonates Suspected of having Sepsis at JJU-SHYCSH from April 1 to September 30, 2023 (n=330)

Variable	Category	Neonatal sepsis		AOR(95%CI)	P value
		No N(%)	Yes N (%)		
Residence	Urban	155(74.9)	52(25.1)	1.1(0.6– 2.0)	0.76
	Rural	99(80.5)	24(19.5)	Ref	
Gestational age(weeks)	≤ 37	89(71.2)	36(28.8)	1.9(1.1– 3.4)	0.02**
	>37	165(80.5)	40(19.5)	Ref	
maternal	18-30	80(85.1)	14(14.9)	0.49(0.19– 1.26)	0.14

age(year)	31 –45	140(73.7)	50(26.3)	1.1(0.5– 2.45)	0.79
	>45	34(73.9)	12(26.1)	Ref	
Education	Illiterate	89(80.9)	21(19.1)	0.55(0.24– 1.26)	0.16
	primary – secondary	126(76.8)	38(23.1)	0.7(0.34– 1.44)	0.33
	Tertiary	39(69.6)	17(30.4)	Ref	
Laboronset	Induced	63(70.8)	26(29.2)	1.45(0.81– 2.6)	0.21
	spontaneous	191(79.3)	50(20.7)	Ref	
breastfeeding onset	<1 hr	62(82.7)	13(17.3)	0.36(0.16– 0.81)	0.013**
	1 – 2 hr	151(80.3)	37(19.7)	0.4(0.21– 0.76)	0.005**
	>2 hrs	41(61.2)	26((38.8)	Ref	

Abbreviation: AOR: Adjusted odds ratio, N=number, CI=confidence interval, **p≤0.05, Ref: Reference category

4. Discussion

In Ethiopia, 1/3 of neonatal deaths are attributed to sepsis. The range of bacteria that cause neonatal sepsis changes over time, necessitating an ongoing assessment and monitoring of bacterial isolates [18,19]. Neonatal sepsis is considered the leading cause of infant mortality and morbidity [4]. In this study, the overall prevalence of neonatal bacterial sepsis was 23% (76/330, 95% CI 18.5–27.5%). This finding was in line with studies performed in Addis-Ababa, 21%, and studies performed in Nepal, 20.5% [20,21]. In addition, similar findings were reported from Dire-Dawa 23%, Nigeria, 22.4%, Ghana 21%, Addis Ababa 27%, and India 26.2% [23–25]. However, the findings of the current study are lower than those of studies performed in Tigray (36.6%), Gondar (46.6%), and other countries, such as Zambia (33%), Uganda (59%) and Egypt (40.7%) [26–29]. The lower prevalence in the current study might be due to similar signs and symptoms of septicemia with non-bacterial infections, such as viruses and fungi, which can cause negative blood culture results and differences in diagnostic methods or sample sizes [30]. In the present study, approximately 51.3% of the isolates were gram-negative bacteria, followed by 48.7% (37 of gram-positive isolates). The most predominant isolates were *E. coli* (23, 30.3%) and *S. aureus* (20, 26.3%), followed by coagulase-negative staphylococcal species (CoNS) (14, 18.4%). This finding was in agreement with study done in Peshawar, Pakistan [31,39]. This was also in line with studies performed in Nepal [32], Uganda Addis-Ababa, and Dire-Dawa, Ethiopia where gram-negative bacteria were common pathogens [22,28,33]. However, in terms of the bacterial species isolated, these studies reported that *Klebsiella* spp. were the most isolated pathogens [32,33]. In the present study, EOS (51.3%) was more prevalent than LOS was, which is in agreement with studies performed in Wolega, Ethiopia and Nepal [32,34]. This higher prevalence of EOS could be attributed to prematurity, low birth weight and poor hygien-

ic conditions during delivery [35,36]. In the present study, *E. coli* was the predominant bacteria in EOS, which is consistent with studies performed in Atlanta, Asella, Ethiopia in Nepal and in Addis-Ababa, Ethiopia reporting among the gram-negative isolates *E. coli* was the leading pathogen [20,21,33,37]. Emerging antibiotic resistance is posing a global health threat to infectious disease prevention and control. The trend of antimicrobial resistance is currently changing over time and may vary among countries worldwide [38,39]. In our study, most of the isolated bacterial organisms, particularly the gram-negative bacteria, were highly resistant to ampicillin and gentamicin, ranging from 78–100% and 52–100%, respectively. This was consistent with previous studies in Ethiopia, which reported high resistance rates of bacteria against ampicillin and gentamicin (90–100%) [24,37,40]. Similarly, high resistance towards ampicillin was also reported from Egypt, Ghana India and in Nepal [21,25,41–45]. In the present study, amikacin and ciprofloxacin were effective against most gram-negative isolates. This finding is comparable with those of studies performed in Asella, Southeast Ethiopia, Addis-Ababa, Ethiopia and in Nepal [21,33,37]. In the present study, the proportion of MDR among the bacterial isolates was 78.9% (60/76, 95% CI; 74.5–83.3%). This finding was in agreement with studies performed in Bahir Dar (78.2%), Addis-Ababa, Ethiopia (78.94%), and Nepal (73.9%) [32,33,44]. However, it was higher than that reported in other studies conducted in Gondar, Odisha, India, Nepal, and India [21,43,45,46]. In the present study, the proportion of MDR isolates was greater in gram-negative bacteria (53.3%, 32/60), which is in line with studies performed in Bahir Dar, Ethiopia, and Nepal [21,44]. In the present study, we also assessed potential associated risk factors. Neonates who were born preterm (gestational age ≤ 37 weeks) were 1.9 times more likely to develop bacterial sepsis than those who were born at term (>37 weeks) (AOR: 1.9 (95% CI 1.1–3.4), p=0.02). This finding is similar to those of studies performed in Gondar, Addis-Ababa Ethiopia, Nepal, Mexico and Indonesia [45,47–50]. This association could be due to the weaker immune system of preterm infants, which can easily lead

to systemic infection. In addition, in the present study, neonates who were breastfed in the first hour of their life were less likely to develop neonatal sepsis than those who were breastfed after 2 hours (AOR: 95% CI 0.36 (0.16–0.81)). This is supported by a study conducted in Zimbabwe reporting that delayed initiation of breast feeding increases the risk of neonatal sepsis and about 33 % neonatal deaths can be averted if breastfeeding is initiated within an hour of birth [51].

5. Conclusion and Recommendations

EOS was caused primarily by *E. coli*, whereas LOS was caused by *S. aureus*. Gram-negative bacteria are highly resistant to most antibiotics, such as ampicillin, gentamicin, cefepime and tetracycline. Similarly, gram-positive isolates were also highly resistant to penicillin and erythromycin. Ciprofloxacin and amikacin are the most effective agents against most identified gram-negative bacteria. The proportion of multi-drug resistance was 78.9%, indicating great concern. In the multivariable logistic regression analysis, gestational age in weeks (AOR: 1.9 (95% CI 1.1–3.4), $p=0.02$) and breast feeding initiation (AOR: 0.36 (95% CI 0.16–0.81), $p=0.01$) were found to be statistically significant ($p \leq 0.05$ with 95% CI). Therefore, a regular review of antibiotic resistance profiles is highly recommended. Furthermore, large-scale studies are necessary to describe each bacterial strain and to study more risk factors. Limitations of the study As conventional blood culturing has been used, due to the lack of use of nutritionally enriching substances, inability to neutralize the inherent antimicrobial components in blood and/or in patients receiving antimicrobial treatment, the study may suffer from poor sensitivity. In addition, anaerobic culturing was not feasible due to the laboratory setting and fastidious organisms were expected but not captured.

Availability of data and material

The datasets used and/or analyzed during the current study are available from the corresponding author on reasonable request.

Competing Interest

Authors declare no competing interest

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