Original Article

Bacterial profile and antimicrobial susceptibility pattern of neonatal sepsis in Felege-Hiwot Referral Hospital, Bahir Dar, northwest Ethiopia: A cross-sectional study design

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Abstract

Background: Neonatal sepsis is a life-threatening medical condition that occurs when host and pathogen interaction leads to organ/tissue damage. Determining the bacterial profile and the antimicrobial susceptibility pattern, and associated factors, in certain geographic regions is vital for rapid empirical medical decisions.

Objective: To assess the bacterial profile, antimicrobial susceptibility pattern, and factors associated with neonatal sepsis, in Felege-Hiwot Referral Hospital, Ethiopia.

Methods: A facility-based cross-sectional study was conducted from April 2018 to July 2018. A total of 412 neonates were included in the study. Socio-demographic and clinical data were collected using a structured and pre-tested questionnaire. About 2ml of blood sample was withdrawn from each participant, and processed for bacterial identification and susceptibility testing, following 2017 Clinical and Laboratory Standards Institute guidelines. Data were analysed using Statistical Package for the Social Sciences version 23. Logistic regressions were used to determine the association between independent variables and dependent variables in relation to bacterial profiles and antimicrobial susceptibility patterns. Odds ratios, and their 95% confidence intervals, were calculated, and the results were considered statistically significant at a p-value less than 0.05.

Results: Of the 412 neonates who were enrolled, 41.3% (170/412) were positive for blood culture. *Klebsiella pneumoniae*, 28.2% (48/170) was the predominant isolate, followed by *Staphylococcus aureus*, 24.7% (42/170). The majority of the isolates developed resistance to ampicillin and penicillin. The overall proportion of multidrug resistance was 78.2% (133/170). Preterm (<37 weeks) [AOR = 2.049; 95% CI: 1.151, 3.647], low birth weight (<2,500gm) [AOR = 2.357; 95% CI: 1.352, 4.109], prolonged rupture of membrane (≥18 hours) [AOR = 4.282; 95% CIL: 1.615, 11.354], and caesarean section modes of delivery [AOR = 2.826; 95% CI: 1.618, 4.936] showed statistical association with bacteriologically confirmed neonatal sepsis.

Conclusions: The majority (78.2%) of presumptive neonatal sepsis cases tested positive for blood culture. *Klebsiella pneumoniae* and *Staphylococcus aureus* were the leading isolates recovered from neonatal sepsis cases. Most of the bacterial isolates from NS cases were resistant to multiple classes of antibiotic. Auspiciously, majority of these isolates were susceptible to ciprofloxacin; as such this replication inhibitor antibiotic could be a choice of physicians for empirical treatment decision. Since it is a single facility based study, further study is recommended. [*Ethiop. J. Health Dev.* 2021; 35(1):18-28]

Key words: Bacteria; neonate; early-onset neonatal sepsis; late-onset neonatal sepsis

Introduction

Neonatal sepsis (NS) is a blood infection that occurs in an infant less than 90 days old. NS is the cause of substantial mortality and morbidity (1). Based on the onset time of symptoms, NS is classified as either early-onset neonatal sepsis (EONS) or late-onset neonatal sepsis (LONS) (2,3). The EONS is defined as sepsis that developed within 72 hours of life, and is mainly associated with organisms acquired before and during delivery (3,4). Whereas, LONS occurs after the first three days of life and the organisms are mostly acquired after delivery (5,6).

The burden of neonatal death is unevenly distributed across the globe, with the highest magnitude in developing countries (7). According to a UNICEF global report in 2016, neonatal death rates globally and in Africa were 1.9% and 2.7%, respectively (8). Majority of the newborn deaths occurred in the two regions, at which 39% and 38% accounted in southern Asia and sub-Saharan Africa, respectively. Moreover, India (24%), Pakistan (10%), Nigeria (9%), Democratic Republic of Congo (4%) and Ethiopia (3%) were the five countries accounted half of all the newborn deaths (8). In Ethiopia, the neonatal mortality rate is 29 per 1,000 live births, which is higher than the average global death rate (19 per 1,000 live births) (9).

Different studies have shown that prolonged rupture of membrane (PROM) (≥18 hours), urinary tract infection (UTI), invasive procedures, and colonization by Group B *Streptococcus* (GBS) are maternal factors associated with NS (6). Additionally, low birth weight (LBW), low appearance, pulse, grimace, activity and respiration (APGAR) scores, poor hygiene and poor cord care (6,10) showed associations with NS, confirming the multiple factors responsible for NS. The APGAR score is used to assess the health status of the new born within a few minutes of birth. Any score below seven is an indicator of some pathology (10). It is measured within one- and five-minute intervals, immediately after the mother has delivered. One of the authors of this paper, a

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paediatrician, reviewed the literature, and recommended the classification stated in the 'Results' section.

Evidence confirms the link between drug-resistant pathogens and the mortality of neonates (11). As such, ampicillin/penicillin with gentamicin combination therapy is considered as the first line of treatment choice (12). Such empirical combination therapy comes from periodic surveillance of pathogens and associated antimicrobials. Hence, the aim of the current study was to assess the bacterial profile, antimicrobial susceptibility pattern and factors associated with NS in Felege-Hiwot Referral Hospital (FHRH).

Materials and methods

Study design, period and setting: A hospital-based cross-sectional study was conducted from April to July 2018 at Felege-Hiwot Referral Hospital (FHRH). FHRH is located in Bahir Dar, Amhara National Regional State, Ethiopia, about 565km from Addis Ababa, the capital city of Ethiopia. Nearly 1,350 NS cases are admitted and diagnosed annually in the hospital (Unpublished data of FHRH).

Study population: The study population comprised all neonates presumptive to sepsis and who attended FHRH during the study period. All neonates with clinical signs and symptoms of sepsis who had not been administered antibiotics during the data collection period were eligible. The sample size (N) was calculated using a single population proportion formula and taking 46.6% prevalence from a previous study done at Gondar University Hospital, Ethiopia (14); 95% certainty and 5% degree of freedom. Hence, the minimum sample size was set at 412, and participants were conveniently recruited until the intended sample size was complete.

Data collection – Socio-demographic and clinical data: Socio-demographic features, pre and/or postnatal history and clinical factors (with the potential for explaining NS) were collected from medical records and from mothers/caregivers in face-to-face interviews.

Data collection – Laboratory methods: Two milliliters (2ml) of venous blood was collected twice from each neonate by an experienced nurse using two test tubes (1ml for each). Each collected blood sample was inoculated directly into a tryptone soya broth (1:10 ratio) (Oxoid Ltd, UK) and sent to FHRH Medical Microbiology Laboratory for culture and antimicrobial susceptibility test (AST). All culture broths were incubated aerobically at 37°C and observed daily for the sign or presence of visible microbial growth up to seven days. Sub-culturing was done on blood agar, chocolate agar, and MacConkey agar (all Oxoid Ltd, UK). Blood and chocolate agar plates were incubated in a candle jar, which can generate about 5% CO_2. All of the sub-cultured agar plates were incubated at 37°C and examined for visible bacterial growth after 24 hours. For samples showing growth on the tryptone soya broth and/or sub-culture media, species identification was done using biochemical methods (13).

Antimicrobial susceptibility patterns of isolates were determined using the Kirby-Bauer disc diffusion method. Using a sterile wire loop, loop-full pure colonies were suspended in a 5ml sterile normal saline (0.85% NaCl) and mixed gently until it formed a homogenous suspension. The turbidity of the suspension was adjusted to the turbidity of 0.5 McFarland standards. The test organisms were uniformly inoculated on the surface of Mueller Hinton Agar (MHA) (Oxoid Ltd, UK) for non-fastidious group and MHA with 5% defibrinated sterile sheep blood for fastidious pathogens. The antimicrobial impregnated discs were placed, based on the Clinical and Laboratory Standards Institute (CLSI) guidelines to the seeded plate with sterile forceps and incubated at 37°C for 18 to 24 hours. The antibiotics were selected based on the national drug list, frequently used in the study area and CLSI recommendation.

The following AST discs were used: gentamicin (GEN: 10µg), tetracycline (TET: 30µg), trimethoprim/sulfamethoxazole (SXT: 25µg), erythromycin (ERY: 15µg), penicillin (PEP: 10 unit), clindamycin (CLN: 2µg), ampicillin (AMP: 10µg), ceftriaxone (CTR: 30µg), ciprofloxacin (CIP: 5µg), ceftazidime (CAZ: 30µg), cefoxitin (CXT: 30µg) and amoxicillin-clavulanic acid (AMC) (30µg) (14).

Data processing and analysis: The collected data were checked for completeness and cleaned manually, then entered and analyzed using Statistical Package for the Social Sciences (SPSS) version 23. Descriptive analysis was performed to describe the proportions of NS and pathogens. The pattern of AST result was depicted using figures and tables. Both bivariate and multivariate logistic regressions were computed to identify factors associated with NS. The dependent variables were bacterial profile and antimicrobial susceptibility pattern. The analyzed independent factors were age, sex, residence of mother, weight at birth, gestational week, place of delivery, mode of delivery, PROM and APGAR score. A p-value of <0.05 in the multivariate logistic regression was considered as a statistically significant factor for NS.

Quality Control: The questionnaire was pretested on 5% of study participants and subsequently checked for its completeness. All types of laboratory activities were done as per the standard operating procedure. Daily and weekly quality control was done to ensure the sterility and performance of the media. To ensure sterility, 5% of biochemical test and AST discs, American Type culture Collection (ATCC) strains were used and run as clinical samples. More specifically, *Staphylococcus aureus* (ATCC-25923) was used as a control representing Gram-positive isolates. Similarly, *Escherichia coli* (ATCC-25922) and *Pseudomonas aeruginosa* (ATCC-27853) controls delegating Gram-negatives, and that of *Haemophilus influenzae* (ATCC-49247) for fastidious bacteria (14), were also used.

Ethical considerations

Ethical clearance was obtained from Bahir Dar University, College of Medicine and Health Sciences ethical review board. An official letter of co-operation was provided to FHRH prior to data collection. Written informed consent was obtained from parents/guardians of neonates who participated in the study. The purpose

of the study, the procedure, the importance of their contribution in the study, risk, and their rights were thoroughly explained. All information was coded and participants were anonymized. Confidentiality of the results was maintained and all results were communicated with responsible physicians.

Results

Socio-demographic and clinical characteristics of the participants: Of the total of 412 recruited presumptive NS cases in the study, 218 (52.9%) neonates presented with EONS. Of the 412 neonates, 219 (53.2%) were males. Nearly, 386 (93.7%) of the mothers had sought antenatal care (ANC) service and 390 (94.7%) delivered in institutions. Of the neonates studied, 130 (31.6%) were preterm (<37 weeks of gestation), 146 (35.4%) had LBW (<2,500g), 150 (36.4%) were delivered by cesarean section (CS), and 31 (8.5%) of the mothers had PROM ≥18 hours. The detailed information is summarized in Table 1.

Table 1: Socio-demographic characteristics of presumptive neonatal sepsis and mothers at FHRH, Bahir Dar, Ethiopia, 2018

Variable	Frequency	Percentage (%)
Socio-demographic data		
Age of neonates (days)		
0-3	218	52.9
>3-28	194	47.1
Sex		
Male	219	53.2
Female	193	46.8
Maternal data		
History of UTI during pregnancy		
Yes	38	9.23
No	374	90.8
ANC follow-up		
Yes	386	93.7
No	26	6.3
Type of delivery		
Vaginal delivery/SVD	233	56.6
CS	150	36.4
Vacuum/Instrumental	29	7.0
Place of birth		
Home	22	5.3
Institutional	390	94.7
PROM (≥18hrs)		
Yes	31	8.5
No	381	91.5
Neonatal data		
Birth weight		
<2,500g (LBW)	146	35.4
≥2,500g	266	64.6
Gestational age (weeks)		
<37 weeks	130	31.6
37-42 weeks	280	67.9
>42 weeks	2	0.5
APGAR score (n=281)		
<7/1st minute	138	49.1
≥7/1st minute	143	50.9
APGAR score (n=281)		
<7/ 5th minute	140	49.8
≥7/5th minute	141	50.2

Key: LBW = low birth weight; CS = caesarean section; SVD = spontaneous vaginal delivery; APGAR: activity, pulse, grimace, appearance and respiration; UTI = urinary tract infection; ANC = antenatal care; PROM = prolonged rupture of membrane

Bacterial profile of neonatal sepsis: Of the 412 blood samples analyzed, 41.3% ((170/412) at 95% CI: 36-48%) were positive for pathogenic bacteria, of which 60% (102/170) were Gram-negative. *K. pneumoniae* and *S. aureus* were the predominant isolates and gauged at 28.2% and 24.7%, respectively (see Table 2).

Table 2: Profile of bacterial isolates from culture positive neonates in FHRH, Bahir Dar, Ethiopia, 2018 (N=170)

Bacteria Isolated	EONS (0-3 days) N (%)	LONS (>3-28) N (%)	Total isolates N (%)
Gram-positive	**42 (61.8)**	**26 (38.2)**	**68 (40)**
S. aureus	26 (28.9)	16 (20)	42 (24.7)
CoNS	14 (15.6)	9 (11.3)	23 (13.5)
Gram-positive	**42 (61.8)**	**26 (38.2)**	**68 (40)**
S. aureus	26 (28.9)	16 (20)	42 (24.7)
CoNS	14 (15.6)	9 (11.3)	23 (13.5)
S. pyogenes	2 (2.2)	1 (1.3)	3 (1.8)
Gram-negative	48 (47.1)	54 (53.9)	102 (60)
K. pneumoniae	26 (54.2)	22 (45.8)	48 (28.2)
E. coli	15 (62.5)	9 (37.5)	24 (14.1)
K. ozaenae	2 (22.2)	7 (77.8)	9 (5.3)
Citrobacter spp.	3 (37.5)	5 (62.5)	8 (4.7)
Total	**90 (52.9)**	**80 (47.1)**	**170 (100)**

Key: CoNS = coagulase-negative *Staphylococcus*; EONS = early-onset neonatal sepsis; LONS = late-onset neonatal sepsis; spp. = species

Antimicrobial susceptibility pattern of bacterial isolates: Overall, the resistance pattern of Gram-negative bacteria was 61% (62/102), and 39% (27/68) for Gram-positive bacteria. The resistance rates of S. aureus and coagulase-negative Staphylococcus (CoNS) to penicillin were 88% and 83%, respectively. Moreover, 38.1% of S. aureus and 60.9% of CoNS isolates were resistant to cefoxitin. On the other hand, bacterial isolates from NS cases were relatively susceptible for ciprofloxacin (82.4%) and clindamycin (75%) compared to other antibiotics (see Table 3).

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Table 3: **Antimicrobial susceptibility pattern of Gram-positive bacteria isolated from neonates' blood culture in FHRH, Bahir Dar, Ethiopia, 2018 (N=68**)

Organisms		Antimicrobial agents N (%)									Total N (%)
		GEN	CIP	CTR	SXT	TET	CLN	ERY	PEP	CXT	
***S.aureus* (n=42)**	S	27 (64.2)	34 (81.0)	26 (61.9)	26 (62.0)	22 (52.4)	31 (74.0)	14 (33.3)	5 (12.0)	24 (57.1)	23 (54.8)
	I	2 (4.8)	2 (5.0)	3 (7.1)	1 (2.4)	5 (11.9)	3 (7.0)	0 (0.0)	0 (0.0)	2 (4.8)	2 (4.7)
	R	13 (31.0)	6 (14.0)	13 (31.0)	15 (35.6)	15 (35.7)	8 (19.0)	28 (66.7)	37 (88.0)	16 (38.1)	17 (40.5)
CoNS (n=23)	S	16 (69.5)	19 (82.6)	11 (47.8)	14 (61.9)	10 (43.5)	17 (74.0)	9 (39.2)	4 (17.0)	8 (34.8)	12 (52.2)
	I	0 (0.0)	1 (4.4)	2 (8.7)	0 (0.0)	2 (8.7)	1 (4.3)	1 (4.3)	0 (0.0)	1 (4.3)	1 (4.3)
	R	7 (31.5)	3 (13.0)	10 (43.5)	9 (39.1)	11 (47.8)	5 (21.7)	13 (56.5)	19 (83.0)	14 (60.9)	10 (43.5)
***S.pyogenes* (n=3)**	S	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)	3 (100.0)
	I	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
	R	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Total (n=68)	S	46 (67.7)	56 (82.4)	40 (58.8)	43 (63.2	35 (51.5)	51 (75.0)	26 (38.2)	12 (17.5)	35 (51.5)	38 (55.9)
	I	2 (2.9)	3 (4.4)	5 (7.4)	1 (1.5)	7 (10.3)	4 (5.9)	1 (1.5)	0 (0.0)	3 (4.4)	3 (4.4)
	R	20 (29.4)	9 (13.2)	23 (33.8)	24 (35.3)	26 (38.2)	13 (19.1)	41 (60.3)	56 (82.5)	30 (44.1)	27 (39.7)

Key: S = sensitive; I = intermediate; R = resistant; CoNS = coagulase-negative *Staphylococcus*; GEN = gentamicin; CIP = ciprofloxacin; CTR = ceftriaxone; SXT = trimethoprim/sulfamethoxazole; TET = tetracycline; CLN = clindamycin; ERY = erythromycin; PEP = penicillin; CXT = cefoxitin

Regarding Gram-negative isolates, *K. pneumoniae* was 100% and 81% resistant to ampicillin and amoxicillin-clavulanate, respectively. For *E. coli* isolates, 92% developed resistance to ampicillin and 71% developed resistance to both tetracycline and amoxicillin-clavulanate. *P. aeruginosa* was highly resistant to the majority of antibiotics; 67% of *P. aeruginosa* isolates were sensitive to ceftazidime (see Table 4).

Table 4: **Antimicrobial susceptibility pattern of Gram-negative isolates from NS in FHRH, Bahir Dar, Ethiopia, 2018 (N=102)**

Organisms		**Antimicrobial agents N (%)**								Total N (%)
		AMP	GEN	CIP	CTR	SXT	TET	AMC	CAZ	
K. pneumoniae (n=48)	S	0 (0)	16 (33)	35 (73)	17 (35)	11 (23)	16 (33)	7 (14)	22 (45)	16 (33)
	I	0 (0)	2 (4)	2 (4)	3 (6)	3 (6)	5 (11)	2 (4)	5 (11)	3 (6)
	R	48 (100)	30 (63)	11 (23)	28 (59)	34 (71)	27 (56)	39 (81)	21 (44)	30 (61)
E. coli (n=24)	S	2 (8)	19 (79)	21 (87)	12 (50)	7 (29)	7 (29)	6 (25)	13 (54)	11 (46)
	I	0 (0)	0 (0)	0 (0)	3 (13)	1 (4)	0 (0)	1 (4)	2 (8)	1 (4)
	R	22 (92)	5 (21)	3 (13)	9 (37)	16 (67)	17 (71)	17 (71)	9 (38)	12 (50)
K. ozaenae (n=9)	S	0 (0)	2 (22)	6 (67)	3 (33)	2 (22)	1 (11)	4 (45)	5 (56)	2 (22)
	I	0 (0)	1 (11)	0 (0)	1 (11)	0 (0)	0 (0)	2 (22)	2 (22)	1 (11)
	R	9 (100)	6 (67)	3 (33)	5 (56)	7 (78)	8 (89)	3 (33)	2 (22)	6 (67)
Citrobacter spp. (n=8)	S	0 (0)	4 (50)	6 (75)	1 (13)	4 (50)	3 (38)	4 (50)	5 (63)	3 (37)
	I	1 (12)	0 (0)	0 (0)	1 (12)	1 (12)	2 (25)	0 (0)	0 (0)	1 (13)
	R	7 (88)	4 (50)	2 (25)	6 (75)	3 (38)	3 (38)	4 (50)	3 (38)	4 (50)
Acinetobacter spp. (n=5)	S	0 (0)	1 (20)	3 (33)	0 (0)	0 (0)	1 (20)	1 (20)	1 (20)	1 (20)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	5 (100)	4 (80)	2 (67)	5 (100)	5 (100)	4 (80)	4 (80)	4 (80)	4 (80)
Enterobacter spp. (n=5)	S	0 (0)	1 (20)	2 (40)	0 (0)	1 (20)	3 (60)	1 (20)	2 (40)	1 (20)
	I	0 (0)	0 (0)	0 (0)	2 (40)	0 (0)	0 (0)	1 (20)	0 (0)	0 (0)
	R	5 (100)	4 (80)	3 (60)	3 (60)	4 (80)	2 (40)	3 (60)	3 (60)	4 (80)
P. aeruginosa (n=3)	S	0 (0)	0 (0)	1 (33)	1 (33)	0 (0)	0 (0)	0 (0)	2 (67)	0.5 (10)
	I	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
	R	3 (100)	3 (100)	2 (67)	2 (67)	3 (100)	3 (100)	3 (100)	1 (33)	2.5 (90)
Total isolates (n=102)	S	2 (2)	43 (42)	74 (73)	34 (33)	25 (25)	31 (30)	23 (23)	50 (49)	35 (34)
	I	1 (1)	3 (3)	2 (2)	10 (10)	5 (5)	7 (7)	6 (6)	9 (9)	5 (5)
	R	99 (97)	56 (55)	26 (25)	58 (57)	71 (70)	64 (63)	73 (72)	43 (42)	62 (61)

Key: S = sensitive; I = intermediate; R = resistant; spp. = species; AMP = ampicillin; GEN = gentamicin; CIP = ciprofloxacin; CTR = ceftriaxone; SXT = trimethoprim/sulfamethoxazole; TET = tetracycline; AMC = amoxicillin-clavulanate; CAZ = ceftazidime

The overall multidrug resistance (MDR) rate of isolates from NS was 78.2% (133/170). Of these, 67.7% (90/133) of the MDR isolates were Gram-negative bacteria and 32.3% (43/133) were Gram-positive (see Table 5).

Table 5: **Multidrug-resistance pattern of bacterial isolates among isolates from NS cases in FHRH, Bahir Dar, Ethiopia, 2018 (n=170)**

Organism isolated	**Degree of resistance (R0 to ≥R6) N (%)**							**MDR ≥R3**
	R0	R1	R2	R3	R4	R5	≥R6	Total (%)
Gram-positive (n=68)	4 (5.9)	1 (1.5)	19 (27.9)	7 (10.3)	20 (29.4)	4 (5.9)	12 (17.6)	43 (63.2)
S. aureus (n=42)	1 (2.4)	1 (2.4)	12 (28.6)	3 (7.1)	16 (38)	3 (7.1)	5 (11.9)	27 (64.3)
CoNS (n=23)	0 (0.0)	0 (0.0)	7 (30.4)	4 (17.4)	4 (17.4)	1 (4.3)	7 (30.4)	16 (69.6)
S. pyogenes (n=3)	3 (100)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)
Gram-negative (n=102)	0 (0.0)	3 (2.9)	9 (8.8)	5 (4.9)	26 (25.5)	24 (23.5)	35 (34.3)	90 (88.2)
K. pneumoniae (n=48)	0 (0.0)	0 (0.0)	3 (6.3)	4 (8.3)	13 (27.1)	9 (18.8)	19 (39.6)	45 (93.8)
E. coli (n=24)	0 (0.0)	2 (8.3)	4 (16.7)	1 (4.2)	5 (20.8)	8 (33.3)	4 (16.7)	18 (75.0)
K. ozaenae (n=9)	0 (0.0)	0 (0.0)	1 (11)	0 (0.0)	3 (33.0)	3 (33.3)	2 (22.2)	8 (88.9)
Citrobacter spp. (n= 8)	0 (0.0)	1(12.5)	1 (12.5)	1 (12.5)	4 (50.0)	1 (12.5)	1 (12.5)	7 (87.5)
Acinetobacter spp. (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	5 (100)	5 (100)
Enterobacter spp. (n=5)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	1 (20)	3 (60)	1 (20.0)	5 (100)
P. aeruginosa (n=3)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	0 (0.0)	3 (100)	3 (100)
Total (n=170)	4 (2.3)	4 (2.3)	28 (16.5)	12 (7)	46 (27)	28 (17)	47 (28)	133 (78.2)

Key: CoNS = coagulase-negative *Staphylococcus*; spp. = species; R0 = susceptible to all antibiotics; R1 = resistant to one antibiotic class; R2 = resistant to two antibiotic classes; R3 = resistant to three antibiotic classes; R4 = resistant to four antibiotic classes; R5 = resistant to five antibiotic classes; ≥R6 = resistant to six or more antibiotic classes; MDR = multidrug resistance

Factors associated with neonatal sepsis: In the bivariate analysis, variables with a p-value of <0.2 were included in the multivariate analysis. In the final multivariate analysis, delivery by CS [AOR = 2.826; 95% CI: 1.618, 4.936], gestational age <37 weeks [AOR = 2.049; 95% CI: 1.151, 3.647], LBW [AOR = 2.357; 95% CI: 1.352, 4.109] and PROM (≥18 hours) [AOR = 4.282; 95% CI: 1.615, 11.354] demonstrated significant association with NS (see Table 6).

Table 6: **Logistic regression analysis for assessing factors associated with NS in FHRH, Bahir Dar, Ethiopia, 2018**

Variables	Bacteria isolated		Bivariate analysis	Multivariate analysis
	Yes (%)	No (%)	COR (95% CI)	AOR (95% CI)
Mode of delivery				
SVD	76 (32.6)	157 (67.4)	1	1
CS	80 (53.3)	70 (46.7)	2.361 (1.548, 3.600)	2.826 (1.618, 4.936)*
Instrumental	14 (48.3)	15 (51.7)	1.928 (0.885, 4.198)	2.480 (0.945, 6.508)
Gestational age				
37-42 weeks	102 (36.2)	180 (63.8)	1	1
<37 weeks	68 (58.5)	62 (41.5)	1.935 (1.270, 2.949)	2.049 (1.151, 3.647)*
Birth weight				
≥2,500 gram	87 (32.7)	179 (67.3)	1	1
<2,500 gram (LBW)	83 (56.8)	63 (43.2)	2.711 (1.789, 4.108)	2.357 (1.352, 4.109)*
History of UTI				
No	150 (40.1)	224 (59.9)	1	1
Yes	20 (52.6)	18 (47.4)	1.659 (0.849, 3.241)	1.811 (0.793, 4.135)
PROM (≥ 18 hours)				
No	147 (38.6)	234 (61.4)	1	1
Yes	23 (74.2)	8 (25.8)	4.577 (1.995, 10.501)	4.282 (1.615, 11.354)*
APGAR score (n=281)				
≥ 7/minute 5				
< 7/minute 5	50 (34.9)	93 (66.1)	1	1
APGAR score (n=281)	67 (48.5)	71 (51.5)	1.755 (1.087, 2.834)	1.093 (0.435, 2.745)
≥ 7/minute 1				
< 7/minute 1				
	49 (34.7)	92 (66.3)	1	1
	68 (48.5)	72 (51.5)	1.773 (1.098, 2.864)	1.441 (0.578, 3.593)

Key: LBW = low birth weight; PROM = prolonged rupture of membrane; APGAR = activity, pulse, grimace, appearance and respiration; COR = crude odds ratio; AOR = adjusted odds ratio; CI = confidence interval; *statistically associated

Discussion

The proportion of NS was 41.3% (95% CI: 36-48%), which is a very high rate. Similar findings have been documented in Addis Ababa (44.7%) (15) and Gondar (46.6%) (16), in Ethiopia, and in Egypt (40.7%) (17) and India (44%) (18). On the other hand, the proportion of NS in our study (41.3%) was higher than studies conducted in Gondar (32.1%) (19), Tanzania 19.2% (20), Nigeria (22.4%) (21) and Pakistan (7.2%) (22) and (21.1%) (23).The proportion of NS in the current study was lower compared to studies in Sudan (61.3%) (24) and India (54.1%) (25). The discrepancy in the findings could be explained by several factors, including differences in the habits of infection prevention practices, living conditions and variability in the management of antibiotics.

Gram-negative bacteria were responsible for 60% of NS. This could be due to the low level of maternal immunoglobulin G ability towards opsonization against Gram-negative bacteria than Gram-positive bacteria (26,27). Moreover, the Enterobacteriaceae families are the abundant normal gastrointestinal microbiota, but are a potential pathogen outside of that site. This finding is consistent with studies reported by Gebrehiwot *et al.* (62%) in Gondar (19), Ali *et al.* (60%) in Iraq (28), and Jayasimha *et al.* (62.5%) in India (20). *Klebsiella pneumoniae* was the predominant isolate, 48 (28.2%), which is similar to other studies (16, 24, 25, 29, 30). This could be due to its high capsulated nature and its ability to form a thick layer of biofilm on medical devices and surrounding areas (31). The proportion of *S. aureus* was 24.7% (42/170) and that of CoNS was 13.5% (23/170) among Gram-positive isolates. These bacteria are often skin colonizers that probably enter the bloodstream during medical procedures, thereby causing NS (32). This finding is in agreement with studies conducted elsewhere (16,24,25,29,30).

From this study, antimicrobial resistance patterns were assessed. *S. aureus* and CoNS were resistant to penicillin, whereas *K. pneumoniae* was highly resistant to ampicillin and amoxicillin-clavulanate. The overuse of antibiotics, and/or use of drugs without laboratory confirmation could be the explanatory factor (33). Overall, Gram-negative isolates showed a higher MDR rate. Similarly, a high proportion of Gram-negative

MDR bacteria were reported by Gebrehiwot *et al*. (19). This might be due to the fact that Gram-negative bacteria have a high ability of gene transfer mechanism, intrinsic antibiotic resistance and beta-lactamase production (34). Particularly, extreme MDR (100%) was found in *P. aeruginosa* and *Acinetobacter* species. This might be due to a combination of beta-lactamase production, an increased efflux pump activity, and outer membrane modifications of the bacteria (35).

The probability of developing sepsis among neonates delivered by CS was 2.8 times greater than normal delivery [AOR = 2.826; 95% CI: 1.618, 4.936]. Previous studies corroborate this result (17,16,36). This could be due to the fact that CS is medically indicated for mothers who have a sign of labor complication, such as long duration of labor, PROM ≥18 hours, obstructed labor, foetal distress and mal-presentation (37,38).

In line with the present study, several lines of evidence show that neonates with a gestational age less than 37 weeks are more likely to develop NS in Ethiopia (15,16), and in other studies worldwide (3,28,36,39,40). The possible reason for this could be a decreased production of neutrophils and low concentrations of immunoglobulin occurring in preterm neonates (26,38,41,42). Another probable reason might be the absence of trans-placental passage of complement and other immunoglobulin (such as IgA) (26). Moreover, preterm neonates have less activation of complement components and are unable to prevent the colonization of pathogenic bacteria on the mucosal membrane (26).

In our study, LBW neonates were 2.5 times more likely to develop sepsis than normal birth weight neonates [AOR = 2.546; 95% CI: 1.409, 4.600]. This finding is supported by previous studies in Ethiopia (15,16) and elsewhere (17,28,36). The LBW neonatal skin is functionally still in development, and the incomplete maturation may lead to the entrance of bacteria to cause systemic infections (42). Immaturity of the immune system, frequent resuscitation, longer hospital stay and invasive procedures (nutritional or breathing support) are also common in LBW babies, compared to the normal birth weight counterparts (26).

Mothers with PROM ≥18 hours were four times more likely to develop sepsis compared to neonates born from mothers who had PROM <18hrs. This is in line with studies done in Ethiopia (44) and Tanzania (39). In most cases, the foetus is not exposed to pathogenic bacteria until the membranes rupture. However, pathogens might infect the foetus through ascending via the amniotic fluid, placenta, cervix, or vaginal canal (26,45,46). This finding is also found in related studies (21,47). Mothers with a history of UTI and APGAR <7 did not show any association with NS.

Conclusions

High proportions of NS presumptive cases were positive for pathogenic bacteria. *K. pneumoniae* and *S. aureus* were the leading recovered pathogens. Gram-negative isolates demonstrated a high rate of resistance to ampicillin, and the Gram-positive isolates were more resistant to penicillin. The majority of recovered pathogens in NS were susceptible to ciprofloxacin. The overall prevalence of MDR was high. Preterm, CS, LBW and PROM ≥18 hours showed statistical association with NS.

Taken together, the results of this study provide a glimpse into NS epidemiology in one of the biggest referral hospitals in Ethiopia. The study could be used as baseline information for further studies. Additionally, in the absence of other high-quality evidence, the study can be a base for empirical treatment decisions. However, bias may have been introduced given that this was a single facility-based study, with a low sample size, design effect, and a convenient sampling technique. The quality of the evidence is low and caution must be taken in interpreting and extrapolating from the findings. As such, further research is highly desirable.

Abbreviations

APGAR: appearance, pulse, grimace, activity and respiration
CoNS: coagulase negative *Staphylococcus* species
EONS: early-onset neonatal sepsis
LBW: low birth weight
LONS: late-onset neonatal sepsis
MDR: multidrug resistance
PROM: prolonged rupture of membrane
UTI: urinary tract infection

Availability of data and materials

The findings of this study were generated from the original data collected during the study period and analyzed based on the stated methods. The original data is available upon reasonable request from the principal author, Tazeb Molla.

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Authors' contributions

TM conceived the project idea, participated in the data collection, analysis and interpretation, and wrote the initial draft of the manuscript. YZ, DM, LB participated in sample collection, data analysis and interpretation of the results. All authors read and approved the final version of the manuscript.

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