Research Square

Preprints are preliminary reports that have not undergone peer review.
They should not be considered conclusive, used to inform clinical practice,
or referenced by the media as validated information.

Bacterial Profile and Antimicrobial Susceptibility Patterns among Neonates with Suspected Sepsis at a Tertiary Hospital (ACSH) in Tigray, Northern Ethiopia: A Prospective Observational Study

Kiros Weldegerima Gezehagne
kiros.gezehagne@mu.edu.et
Mekelle University
Tedros Hailu Abay
Mekelle University
Atsede Gebrekidan Gebremicael
Mekelle University
Hailemariam Gebrearegay Haileeyesus
Mekelle University
Teklit Gebremedhin Teklu
Mekelle University
Abraha Gebregziabher
Mekelle University
Hiwot Araya
Mekelle University
Rovel Kinfe Berhe
Mekelle University
Gidey Guesh Weleabezgi
Mekelle University
Kibra Hailu Desta
Mekelle Universities
Mulu Gebretsadik Gebremedhin
Mekelle Universities
Aregawi Gebreyesus Belay
Mekelle University

Research Article

Keywords: Neonatal sepsis, Antimicrobial resistance/susceptibility patterns, Bacterial profile, Gram-negative bacteria, Neonatal intensive care unit, Post-conflict health, Tigray, Ethiopia

Posted Date: August 15th, 2026

DOI: https://doi.org/10.21203/rs.3.rs-10539102/v1

License: This work is licensed under a Creative Commons Attribution 4.0 International License. Read Full License

Additional Declarations: No competing interests reported.

Abstract

Background

Neonatal sepsis remains a major cause of morbidity and mortality worldwide, particularly in low-resource and post-conflict settings. The increasing burden of antimicrobial resistance (AMR) has substantially reduced the effectiveness of empirical antibiotic therapy. Local evidence on bacterial pathogens and antimicrobial susceptibility patterns is essential for optimizing clinical management and guiding institutional treatment protocols.

Objective

To determine the bacterial profile and antimicrobial susceptibility patterns among neonates with suspected sepsis admitted to the Neonatal Intensive Care Unit (NICU) of Ayder Comprehensive Specialized Hospital (ACSH), Tigray Region, Northern Ethiopia.

Methods

A hospital-based prospective observational study was conducted in the NICU of ACSH from January 1 to December 31, 2025. Consecutive neonates with clinically suspected sepsis were enrolled. Blood cultures were obtained under aseptic conditions before initiation of antibiotic therapy. Bacterial isolates were identified using standard microbiological methods, and antimicrobial susceptibility testing was performed using the Kirby–Bauer disk diffusion technique according to Clinical and Laboratory Standards Institute (CLSI) guidelines. Data were analyzed using IBM SPSS Statistics version 27.

Results

Among 526 neonates with suspected sepsis, 132 (25.1%) had culture-confirmed sepsis, yielding 137 bacterial isolates. Gram-negative bacteria predominated (69.3%), with Klebsiella species (26.3%) and Enterobacter cloacae (19.0%) being the most frequently isolated pathogens. Coagulase-negative Staphylococci (CoNS) (16.0%) were the leading Gram-positive isolates. High resistance rates were observed against commonly used empirical antibiotics, including ampicillin (96.2%), ciprofloxacin (63.2%), and Ceftazidime (74.7%). The highest susceptibility was demonstrated to Amikacin, followed by Meropenem and levofloxacin. Multidrug-resistant organisms accounted for 43.3% of isolates. Early-onset neonatal sepsis represented 60.6% of confirmed cases, while the overall mortality rate was 13.5%.

Conclusion

Gram-negative organisms were the predominant cause of neonatal sepsis and exhibited extensive resistance to commonly prescribed first-line antibiotics. These findings indicate that current empirical treatment regimens require urgent review. Strengthening antimicrobial stewardship, infection prevention strategies, and continuous microbiological surveillance is essential to improve neonatal outcomes in this post-conflict setting health care provision center.

Introduction

Neonatal sepsis is a life-threatening systemic infection occurring within the first 28 days of life and remains one of the leading causes of neonatal morbidity and mortality worldwide, particularly in low- and middle-income countries (LMICs)(1, 2, 3). It encompasses bacterial, viral, or fungal infections that trigger systemic inflammatory responses and may present

as septicemia, pneumonia, meningitis, or other invasive infections. Because its clinical manifestations are often nonspecific—including poor feeding, temperature instability, respiratory distress, lethargy, apnea, and circulatory compromise—early diagnosis remains a major clinical challenge(4). Other signs are thermal disturbances (hypothermia or fever), lethargy, incessant crying, hypotonia, peripheral perfusion disorder (prolonged hair recolouration time), blunt neonatal archaic reflexes, cardiac arrhythmias (bradycardia or tachycardia), metabolic disorders such as hypoglycemia or hyperglycemia, and metabolic acidosis (5, 6).

Based on the timing of symptom onset, neonatal sepsis is classified as early-onset sepsis (EOS), occurring within the first 72 hours of life and usually acquired through vertical transmission from the mother, and late-onset sepsis (LOS), which develops after 72 hours and is primarily acquired from hospital or community sources. This distinction is clinically important because the causative organisms, risk factors, and preventive strategies differ between the two forms (7, 8).

Despite substantial global progress in reducing under-five mortality over the past three decades, neonatal deaths continue to account for nearly half of all deaths among children younger than five years. Infection remains one of the leading causes of these deaths. Ethiopia has achieved remarkable improvements in child survival; however, the decline in neonatal mortality has been considerably slower than that observed for older children, highlighting the need for targeted interventions to improve neonatal outcomes. (9–15).

In Northern Ethiopia, the prolonged armed conflict in Tigray severely disrupted the healthcare system, resulting in shortages of essential medicines, laboratory supplies, trained healthcare personnel, and diagnostic services. These disruptions have compromised neonatal care and may have contributed to increased neonatal morbidity and mortality. In addition, prolonged dependence on empirical antibiotic therapy during periods of limited microbiological diagnostic capacity may have accelerated the emergence and spread of antimicrobial-resistant pathogens within neonatal intensive care units (NICUs). In our context, neonatal infection was observed complicating almost all neonates admitted with different health conditions; e.g. A 2 years institutional perinatal audit report demonstrated that neonatal sepsis (both early and late onset) was the direct cause of 29.3% deaths. It has also complicated 31% deaths due to prematurity, 26.4% deaths due to perinatal asphyxia and 7.4% of deaths due to surgical conditions (5, 16).

Advances in neonatal intensive care have led to improved survival of neonates, but neonatal infections continue to be an important challenge to newborn survival (17). The bacterial etiology of neonatal sepsis and antimicrobial susceptibility patterns vary considerably across geographical regions and over time. Consequently, empirical treatment recommendations should be guided by local microbiological surveillance rather than relying solely on national or international guidelines. However, blood culture and antimicrobial susceptibility testing have not been routinely available in many Ethiopian healthcare facilities, particularly in conflict-affected settings (1, 12, 18, 19, 20–21).

At Ayder Comprehensive Specialized Hospital, no comprehensive study has evaluated the bacterial profile and antimicrobial resistance patterns among neonates with suspected sepsis for several years, creating a critical evidence gap for evidence-based clinical decision-making.

Understanding the local epidemiology of neonatal sepsis is essential for optimizing empirical antibiotic therapy, strengthening antimicrobial stewardship, improving infection prevention and control practices, and reducing neonatal mortality. Therefore, this study aimed to determine the bacterial profile and antimicrobial susceptibility patterns among neonates with suspected sepsis admitted to the Neonatal Intensive Care Unit of Ayder Comprehensive Specialized Hospital in Tigray, Northern Ethiopia. The findings are expected to provide evidence for revising institutional treatment protocols, enhancing infection prevention strategies, and informing regional neonatal healthcare policies in the post-conflict setting.

Materials and methods

Study setting, Period, and Design:

Study Setting

An institution-based prospective observational study was conducted at Ayder Comprehensive Specialized Hospital (ACSH), located in Mekelle, the capital city of the Tigray Region, Northern Ethiopia. ACSH is the largest tertiary referral hospital in the region, serving a catchment population of more than nine million people and receiving referrals from primary, general, and specialized hospitals across Northern Ethiopia.

The Neonatal Intensive Care Unit (NICU) has a capacity of 45 beds and manages approximately 3,500–4,000 neonatal admissions annually. The unit provides comprehensive care for preterm, term, and critically ill neonates, including mechanical ventilation, continuous positive airway pressure (CPAP), and phototherapy.

Study Period

Data were collected from January 1 to December 31, 2025. During the study period, the hospital continued to experience the consequences of the recent regional conflict, including intermittent shortages of laboratory reagents, essential antimicrobial agents, and other medical consumables. These contextual challenges are important when interpreting the microbiological findings and antimicrobial susceptibility patterns observed in this study.

Study Design

A prospective observational study design was employed to determine the bacterial profile and antimicrobial susceptibility patterns among neonates with suspected sepsis. As a non-interventional study, all clinical management, laboratory investigations, and therapeutic decisions were undertaken as part of routine patient care. The research team collected and documented clinical, laboratory, and microbiological data without influencing patient management. This design was selected to generate real-world evidence on pathogen distribution and antimicrobial resistance in a resource-limited, post-conflict setting. The study was conducted and reported in accordance with the Strengthening the Reporting of Observational Studies in Epidemiology (STROBE) guidelines.

Study Population

The source population comprised all neonates (aged 0–28 days) admitted to the Neonatal Intensive Care Unit (NICU) of Ayder Comprehensive Specialized Hospital (ACSH) during the study period. The study population included all neonates with clinically suspected sepsis, diagnosed according to the World Health Organization (WHO) neonatal sepsis screening tool and the Ethiopian National Neonatal Intensive Care Unit guideline, for which blood culture was obtained as part of routine clinical care before the initiation of empirical antimicrobial therapy. Eligible participants were neonates with complete clinical and laboratory records. Neonates were excluded if they had received systemic antibiotics within 24 hours before blood culture collection, if blood culture results were reported as contaminated, indicating skin flora rather than true pathogens, if written informed consent was not provided by a parent or legal guardian, or if essential study variables were missing and could not be retrieved from the medical records.

Sample Size and Sampling Technique

The required sample size was calculated using the single population proportion formula based on a previous Ethiopian study (18) that reported a blood culture positivity rate of 29.4% among neonates with suspected sepsis. A 95% confidence level (Z = 1.96), a margin of error of 5% (d = 0.05), and an expected prevalence (P = 0.294) were assumed. The minimum calculated sample size was 319.

To improve statistical power and compensate for possible exclusions due to contaminated cultures or incomplete records, all eligible neonates admitted during the study period were enrolled. Consequently, 526 neonates were included in the final analysis.

A consecutive sampling technique was employed, whereby every neonate meeting the eligibility criteria during the study period was recruited until the end of the data collection period. This approach minimized selection bias and ensured that the study population accurately reflected routine clinical practice in the NICU.

Variables

The primary outcome variable was the blood culture result, categorized as positive or negative. Among neonates with culture-confirmed sepsis, the isolated bacterial species and their antimicrobial susceptibility profiles, classified as susceptible, intermediate, or resistant, were evaluated as secondary outcome variables.

Independent variables included neonatal, maternal, and laboratory-related characteristics. Neonatal variables comprised; gestational age, birth weight, sex, age at symptom onset, and classification of sepsis as early-onset or late-onset.

Clinical variables included poor feeding, temperature instability, respiratory distress, lethargy, apnea, need for resuscitation at birth, use of invasive devices (including umbilical catheters, central venous lines, and mechanical ventilation), and duration of hospitalization before blood culture collection.

Maternal variables included; maternal fever, prolonged rupture of membranes, mode and place of delivery, maternal HIV status, history of genital tract infection, and maternal nutritional status.

Laboratory-related variables included; blood sample volume, timing of specimen collection, interval between specimen collection and laboratory incubation, previous culture findings, and laboratory processing conditions.

These variables were selected based on established clinical guidelines and previously published evidence demonstrating their association with neonatal sepsis.

Data Collection Procedures

Data were collected using a structured data extraction tool developed based on the study objectives and relevant literature. The data collection tool was pretested before the actual data collection process to ensure clarity, completeness, and consistency. Trained data collectors reviewed patients' medical records and collected relevant socio-demographic, clinical, laboratory, treatment-related, and outcome-related information.

The collected variables included demographic characteristics, presenting clinical features, underlying conditions, laboratory findings, microbiological results, antimicrobial treatment, clinical management, and patient outcomes. The completeness and accuracy of the collected data were checked regularly by the investigators throughout the data collection period. Any discrepancies identified during data review were verified by cross-checking the original medical records.

Microbiological Procedures and Quality Assurance

Microbiological procedures

Clinical specimens were collected from patients according to standard aseptic techniques and transported to the microbiology laboratory using appropriate procedures to maintain specimen quality. The collected samples were

processed using standard microbiological methods for the identification of bacterial pathogens and determination of antimicrobial susceptibility patterns.

Bacterial identification was performed using conventional microbiological techniques, including appropriate culture media, colony morphology assessment, Gram staining, and biochemical identification tests. Antimicrobial susceptibility testing was conducted using the recommended standard methods according to current laboratory guidelines. The interpretation of susceptibility results was performed based on established breakpoint criteria.

Quality control procedures were implemented throughout the laboratory process to ensure the reliability and accuracy of microbiological findings. Standard reference strains were used for quality assurance of culture and antimicrobial susceptibility testing procedures. All laboratory results were recorded systematically and linked with the corresponding clinical data for further analysis.

Data Processing and Analysis

Electronic data capture was performed using Kobo Toolbox. To ensure data integrity, the digital entry forms were designed with built-in logic checks and mandatory fields to minimize transcription errors and missing values. Upon completion of data collection, the dataset was cleaned, validated, and exported to IBM SPSS Statistics version 27.0 for comprehensive analysis.

Descriptive statistics were employed to summarize the study population's socio-demographic characteristics, clinical presentations, and hospital outcomes. Categorical variables, including culture positivity rates, bacterial isolate distributions, and antimicrobial susceptibility patterns, were presented as frequencies and percentages. Continuous variables were expressed as means with standard deviations or medians with interquartile ranges, depending on the normality of the distribution. Bivariate analyses, using Chi-square was conducted to compare clinical and microbiological parameters between early-onset neonatal sepsis (EOS) and late-onset neonatal sepsis (LOS) cases. While multivariable logistic regression was initially planned to identify independent predictors of culture-proven sepsis and mortality, the final analysis was restricted to descriptive and bivariate models. This decision was made due to the limited distribution and small cell counts across several key independent variables (quasi-separation of data), which precluded the development of a stable and statistically robust multivariate model. All statistical tests were two-tailed, and a p-value < 0.05 was considered statistically significant.

Results

Socio-demographic and baseline characteristics

A total of 526 neonates with suspected sepsis were enrolled in the study. Of these, 319 (60.6%) were male and 207 (39.4%) were female. Maternal age ranged from 15 to 45 years, with a mean age of 27.18 ± 5.12 years. Regarding gestational maturity, 278 (52.9%) neonates were born at term, while 159 (30.2%) were preterm. Among preterm neonates, 104 (19.8%) were moderate-to-late preterm, 48 (9.1%) were very preterm, and 7 (1.3%) were extremely preterm. Gestational age was not documented for 89 (16.9%) neonates.

Regarding birth weight, 341 (64.8%) neonates had a normal birth weight (≥ 2500 g), while 185 (35.2%) had a birth weight below 2500 g. Among the latter, 141 (26.8%) were classified as low birth weight (LBW), 43 (8.2%) as very low birth weight (VLBW), and 1 (0.2%) as extremely low birth weight (ELBW).

Geographically, 267 (50.8%) neonates originated from the Mekelle Special Zone, while 55 (10.5%) and 48 (9.1%) were from the Central and Southern Zones of Tigray, respectively.

Table 1
Socio-demographic and Baseline Characteristics (n = 526)

Characteristic	Category	N (%)
Sex	Male	319 (60.6)
	Female	207 (39.4)
Gestational age at birth	Extremely preterm (< 28 weeks)	7 (1.3)
	Very preterm (28 to < 32 weeks)	48 (9.1)
	Moderate-to-late preterm (32 to < 37 weeks)	104 (19.8)
	Term (≥ 37 weeks)	278 (52.9)
	Unknown/missing	89 (16.9)
Birth weight	Extremely low birth weight (< 1,000 g)	1 (0.2)
	Very low birth weight (1,000 to < 1,500 g)	43 (8.2)
	Low birth weight (1,500 to < 2,500 g)	141 (26.8)
	Normal birth weight (≥ 2,500 g)	341 (64.8)
Caregiver residence/zone of origin	Mekelle Special Zone	267 (50.8)
	Other zones out of Mekelle	228 (43.3)
	From Internally displaced IDP camps	31 (5.9)
Place of residence	Urban	339 (64.4)
	Rural	156 (29.7)
	Missing/unrecorded residence	31 (5.9)

Obstetric and birth characteristics

Most neonates were delivered by spontaneous vaginal delivery (SVD) (368, 70.0%), followed by cesarean section (149, 28.3%) and assisted vaginal delivery (9, 1.7%). Among the 149 cesarean deliveries, 89 (59.7%) were performed as emergency procedures and 60 (40.3%) were elective.

Prolonged rupture of membranes (ROM) lasting ≥ 18 hours was documented in 13 (2.5%) deliveries. Maternal fever during labor was recorded in 9 (1.7%) cases, while 16 (3.0%) mothers received antibiotics before delivery.

Regarding place of delivery, 455 (86.5%) neonates were delivered in public health facilities, including primary hospitals or health centers (201, 38.2%), tertiary hospitals (181, 34.4%), and general hospitals (73, 13.9%). Deliveries in private facilities accounted for 41 (7.8%), while 27 (5.1%) occurred at home or during transit.

Apgar scores at the first and fifth minutes were unavailable for 231 (43.9%) neonates. Among neonates with available fifth-minute Apgar scores, a score of 8 was most frequent (108, 20.5%), followed by a score of 9 (81, 15.4%).

Table 2
Obstetric and Delivery Characteristics (n = 526)

Variable	Category	N (%)
Mode of Delivery	SVD	368 (70.0)
	Assisted vaginal	9 (1.7)
	Caesarean section	149 (28.3)
Type of C-section	Emergency	89 (16.9)
	Elective	60 (11.4)
	Not applicable	377 (71.7)
Place of Delivery	Tertiary hospital	181 (34.4)
	Primary/health center	201 (38.2)
	General hospital	73 (13.9)
	Private facility	41 (7.8)
	Home/transit	27 (5.1)
ROM Duration	< 18 hours	513 (97.5)
	≥ 18 hours	13 (2.5)
Maternal Fever	Yes	9 (1.7)
	No	517 (98.3)
Maternal Antibiotics	Yes	16 (3.0)
	No	510 (97.0)

Clinical presentation and sepsis classification

Neonates presented with a broad range of clinical features at admission, with temperature abnormalities, poor feeding, and respiratory distress among the most frequently observed manifestations.

Temperature abnormalities were common at admission. Moderate hypothermia (32.0–35.9°C) was observed in 178 (33.8%) neonates, while 76 (14.4%) had mild hypothermia and 75 (14.3%) had severe hypothermia. A total of 197 (37.5%) neonates were normothermic.

More than half of the neonates, 264 (50.2%), were referred from other health facilities. Respiratory support was required in 438 (83.3%) neonates. The most frequently used modality was intranasal oxygen (236, 44.9%), followed by continuous positive airway pressure (CPAP) (119, 22.6%), head-box oxygen (61, 11.6%), and mechanical ventilation (22, 4.2%).

Based on the timing and setting of symptom onset, early-onset sepsis (EOS) was the most frequent presentation, occurring in 319 (60.6%) neonates. Hospital-acquired late-onset sepsis accounted for 111 (21.1%) cases, while community-acquired late-onset sepsis accounted for 48 (9.1%).

Clinically, EOS was diagnosed in 333 (63.3%) neonates, whereas late-onset sepsis (LOS) was diagnosed in 193 (36.7%). Meningitis was documented in 48 (9.1%) neonates across both EOS and LOS categories.

Table 3
Sepsis Classification and Clinical Features

Characteristic	Category	N (%)
Temperature at admission	Fever/hyperthermia (> 37.5°C)	75 (14.3)
	Normothermia (36.5–37.5°C)	197 (37.5)
	Mild hypothermia (36.0–36.4°C)	76 (14.4)
	Moderate hypothermia (32.0–35.9°C)	178 (33.8)
Admission source	Referred from another health facility	264 (50.2)
	Direct admission from same facility	262 (49.8)
Respiratory support	None	88 (16.7)
	Intranasal oxygen	236 (44.9)
	Continuous positive airway pressure (CPAP)	119 (22.6)
	Head-box oxygen	61 (11.6)
	Mechanical ventilation	22 (4.2)
Sepsis classification	Early-onset sepsis (EOS)	319 (60.6)
	Late-onset sepsis (LOS)	207 (39.4)
Meningitis	Present	48 (9.1)
	Absent	478 (90.9)

Laboratory and microbiological findings

Blood culture was performed for all 526 neonates enrolled in the study. Of these, 132 (25.1%) had positive blood culture results, while 394 (74.9%) had negative cultures.

Among the 137 culture-positive specimens, Gram-negative organisms predominated. Gram-negative rods accounted for 61 (46.2%) isolates, while Gram-negative coccobacilli accounted for 34 (25.8%). Collectively, these Gram-negative organisms represented 95 (69.3.0%) of the culture-positive isolates. Gram-positive organisms accounted for the remaining isolates, including Gram-positive cocci in clusters 35 (26.5%), Gram-positive cocci in chains 4 (3%), and Gram-positive rods 2 and 1 fungal isolate (1.5%).

The turnaround time for blood culture results was prolonged, with results for 396 (75.3%) neonates becoming available after approximately seven days.

Complete blood count was performed in 326 (62.0%) neonates, while C-reactive protein testing was performed in 148 (28.1%). Among the reported hematological abnormalities, thrombocytopenia was documented in 50 (9.5%) neonates, while a left shift was observed in 49 (9.3%).

Note

The patient denominator is 526 neonates, whereas the organism-level denominator is 137 bacterial isolates. The number of isolates exceeds the number of culture-positive neonates because more than one bacterial isolate could be recovered from an individual patient. The microbiology team report additionally documented one fungal isolate, which is reported separately from the bacterial isolates.

Antimicrobial susceptibility and resistance patterns

Antimicrobial susceptibility testing demonstrated substantial variation in susceptibility across the tested agents. Amikacin showed the highest proportion of susceptible isolates (66/69, 95.7%), followed by gentamicin (4/5, 80.0%) and Meropenem (48/64, 75.0%) among isolates with complete susceptibility results.

In contrast, high levels of resistance were observed to ampicillin (51/53, 96.2%), cefuroxime (7/7, 100%), cefazolin (24/27, 88.9%), erythromycin (16/18, 88.9%), penicillin (32/37, 86.5%), tetracycline (59/68, 86.8%), and cefotaxime (28/33, 84.8%). Resistance to Ceftazidime, ceftriaxone, ciprofloxacin, and Cotrimoxazole was also substantial, at 74.7%, 82.1%, 63.2%, and 69.7%, respectively.

Table 4

(A, B, C): Antimicrobial Susceptibility and Resistance Profiles of Bacterial Isolates Enterobacterales

Organism	Isolates	ESBL	AMK %S	AMP %S	FEP %S	CTX %S	CAZ %S	CIP %S	MEM %S	SXT %S	TZP %S	LVX %S
Citrobacter koseri	4	2	100	IR	0	0	33.3	0	100	0	0	100
Klebsiella aero genes	5	2	100	IR	50	0	100	50	100	50	50	50
Enterobacter cloacae	26	7	100	IR	42.9	42.0	50	41.7	87.5	50	57.1	78.6
Escherichia coli	6	2	88.9	25	45.5	33.3	37.5	57.1	100	0	55.6	62.5
Klebsiella oxytoca	15	1	73.9	IR	0	0	4.5	4.5	91.3	4.3	39.1	78.3
Klebsiella pneumoniae	21	5	83.9	IR	12.9	12.9	12.9	14.3	45.2	22.6	30.0	55.6
Serratia spp.	2	—	100	IR	0	0	0	0	0	0	0	0

B. Non-fermenting Gram-negative bacilli

Organism	Isolates	AMK %S	AMP %S	FEP %S	CTX %S	CAZ %S	CIP %S	MEM %S	SXT %S	SAM %S	TZP %S	LVX %S
Acinetobacter spp.	12	87.5	IR	0	0	22.2	42.9	55.6	44.4	20	22.2	55.6
Pseudomonas aeruginosa	2	100	IR	100	—	100	100	100	IR	IR	100	100
Stenotrophomonas maltophilia	2	IR	IR	NR	IR	NR	NR	IR	100	IR	IR	100

C. Gram-positive bacteria

Organism	Isolates	MRS	AMP %S	OXA %S	CIP %S	CLI %S	ERY %S	PEN %S	TCY %S	SXT %S	VAN %S	LVX %S
Enterococcus spp.	9	—	10	NR	NR	IR	NT	36.4	50	IR	60	NR
Staphylococcus aureus	12	9	NR	25	40	63.6	9.1	0	22.2	58.3	NT	42.9
CoNS	22	18	NR	19	25	63.2	0	4.8	14.3	14.3	NT	35
Streptococcus pneumoniae	1	—	—	—	100	100	0	—	0	—	—	100

Sepsis-related complications and treatment outcomes

During hospitalization, complications were documented in 466 (88.6%) neonates. These included isolated metabolic disturbances in 20 (3.8%) neonates, severe respiratory distress in 115 (21.9%), and multisystem complications, including shock and meningitis, in 331 (62.9%).

At the end of hospitalization, 272 (51.7%) neonates were discharged with clinical improvement, while 138 (26.2%) were transferred to other facilities for continued care. Forty-five (8.6%) neonates left against medical advice. Overall, 71 (13.5%) neonates died during hospitalization.

Table 5
Sepsis-Related Complications and Hospital Outcomes (n = 526)

Category	Subcategory	n	%
Sepsis Complications	Any complication	466	88.6
	No complication	60	11.4
	Mild/metabolic only (hypothermia/hypoglycaemia)	20	3.8
	Respiratory distress syndrome	115	21.9
	Severe multisystem complications*	331	62.9
Clinical Outcomes	Discharged improved	272	51.7
	Transferred for continued care	138	26.2
	Left against medical advice (LAMA)	45	8.6
	Death	71	13.5

Discussions

Summary of Primary Findings

Our study provides a comprehensive assessment of the microbiological landscape and antimicrobial resistance (AMR) patterns in a tertiary NICU in Mekelle, Tigray. Our results demonstrate a culture-proven sepsis prevalence of 25.1%, with a striking predominance of Gram-negative organisms (69.3%). The identified pathogens showed extensive resistance to the standard first-line empirical regimen (Ampicillin and Ceftazidime), while Amikacin and Meropenem retained the highest level of efficacy. The high mortality rate (13.5%) and significant proportion of patients leaving against medical advice (LAMA) (8.6%) underscore the clinical and socioeconomic challenges facing neonatal care in this post-conflict environment (19, 29).

Culture Positivity and Bacterial Profile

The culture positivity rate of 25.1% in our study aligns with findings from other sub-Saharan and low-and middle-income countries (LMICs), where rates typically range between 15% and 30%(4, 18). This rate is higher than reports from Nepal (15.1%) but lower than findings from Kanpur, India (51%), (7, 24). The variations in positivity may be attributed to differences in blood volume adequacy, prior antibiotic exposure before referral, and the specific diagnostic criteria employed across settings (19).

Consistent with recent epidemiological shifts reported in Ethiopia and globally, our findings confirm a transition toward Gram-negative dominance in neonatal sepsis (8, 22). Enterobacter cloacae (19%) and Klebsiella pneumonia (15.3%) were the primary drivers of sepsis in our cohort. This observation is consistent with studies from St. Paul's Hospital Millennium Medical College and Gondar, which also identified Klebsiella species and Coagulase-negative Staphylococci (CoNS) as leading etiologies (30, 33). The predominance of Gram-negative bacteria is particularly concerning due to their rapid clinical progression and high propensity for multidrug resistance (4, 9, and 11).

The Challenge of Antimicrobial Resistance

The most alarming finding in this study is the near-universal resistance to routinely used empirical antibiotics. Resistance to Ampicillin (96.2%) and Ceftazidime (74.7%) was significantly high, reflecting a trend observed in other Ethiopian centers where resistance rates for these drugs often exceed 70% (18). Such high resistance levels suggest that the current National Guideline-based empirical treatments may no longer be effective in our setting (1, 20).

In contrast, our isolates demonstrated high susceptibility to Amikacin (95.7%) and Meropenem. This aligns with global and regional data suggesting that aminoglycosides (specifically Amikacin) and carbapenems remain the most viable options for treating neonatal sepsis in regions with high AMR profiles (4, 16, 38). However, the detection of ESBL-producing organisms (24.2%) and MDR strains (43.3%) indicates a narrowing therapeutic window. Our MDR rate, while significant, was lower than the 72% and 69.1% reported in similar Ethiopian studies by Sorsa et al. and Gebreyesus et al. (4, 18), which may reflect differences in local antibiotic pressure or infection control practices.

Clinical Context and Vulnerabilities

The predominance of early-onset neonatal sepsis (EOS) (60.6%) in our study suggests significant gaps in maternal infection screening and intrapartum hygiene, consistent with the vertical transmission patterns recognized globally (8, 10). Furthermore, the observed male predominance (60.6%) in sepsis cases is consistent with literature from Eastern Ethiopia and other regions, which suggests a biological or genetic susceptibility of male neonates to systemic infections (3, 22).

The impact of the recent war in Tigray is evident in the study's demographic makeup. Internally displaced persons (IDPs) accounted for 5.9% of the cohort. Displacement serves as a major risk factor due to compromised access to antenatal care (ANC), nutritional deficiencies, and the breakdown of routine health services (29). The high referral rate (50.2%) from rural districts—where more than 80% of health facilities were rendered non-functional, likely explains the high severity of illness at admission and the prolonged culture turnaround times (75.3% exceeding 7 days) (1, 20) .

Morbidity, Mortality, and Socioeconomic Outcomes

The mortality rate of 13.5% reported in our study is comparable to the 13.9% observed in Dares Salaam, Tanzania (4), but lower than rates reported in some Nigerian tertiary centers (6, 9). However, the high rate of LAMA (8.6%) and transfers (26.2%) is a distinct finding that reflects the regional sociopolitical context. Financial constraints and family displacement (IDPs) often prevent the completion of care, which likely contributes to poorer long-term outcomes and the potential for community spread of resistant pathogens (29).

Strengths and Limitations

Strengths of Study

This study has several important strengths. First, it provides valuable evidence on the clinical and microbiological characteristics of neonatal sepsis in our setting, where locally generated data are essential for guiding clinical management and infection prevention practices. The study also contributes information on the distribution of bacterial pathogens and their antimicrobial susceptibility patterns, which may support the development and periodic revision of empirical antibiotic treatment protocols.

Another strength is the inclusion of clinically relevant neonatal characteristics and outcomes, allowing assessment of factors associated with poor outcomes among affected neonates. The findings may therefore provide a useful basis for strengthening neonatal sepsis management and antimicrobial stewardship within the study setting and similar healthcare facilities in the region.

Limitations of the Study

Despite its strengths, this study has some limitations that should be considered when interpreting the findings. One is being single center study; the other is the study findings may be influenced by the availability and quality of microbiological testing. Some neonates with clinical sepsis may have negative blood cultures, particularly when antibiotics were administered before specimen collection or when the bacterial load was low. Therefore, culture-confirmed infections may not represent the full burden of neonatal sepsis in the study population.

Conclusion

Neonatal sepsis remains an important cause of morbidity and mortality among hospitalized neonates. Our study demonstrates a substantial burden of culture-confirmed neonatal sepsis, with clinically important bacterial pathogens and concerning levels of antimicrobial resistance. The findings highlight the challenges of selecting effective empirical antimicrobial therapy in the context of changing bacterial profiles and increasing resistance.

Early recognition of neonatal sepsis, timely initiation of appropriate antimicrobial therapy, and effective supportive care are essential for improving outcomes. Strengthening microbiological diagnostic capacity, infection prevention and control practices, antimicrobial stewardship, and continuous surveillance of bacterial pathogens and their susceptibility patterns is critical.

Overall, locally generated evidence should be used to guide empirical antibiotic policies and clinical practice. Continued research, preferably through multicenter prospective studies, is needed to better understand the epidemiology of neonatal sepsis and develop effective strategies to reduce neonatal morbidity and mortality.

Recommendations

Improve microbiological diagnosis

Blood culture and antimicrobial susceptibility testing should be strengthened and performed whenever clinically feasible, preferably before initiation of antibiotics, without unnecessarily delaying treatment in critically ill neonates.

Develop and regularly update local antibiotic guidelines

Empirical antimicrobial therapy should be guided by current local pathogen distribution and antimicrobial susceptibility patterns and should be reviewed periodically as resistance patterns changes.

Strengthen antimicrobial stewardship

Neonatal units should implement antibiotic stewardship interventions, including appropriate antibiotic selection, dosing, duration, regular review of therapy, and de-escalation or discontinuation when microbiological and clinical findings do not support continued treatment.

Enhance infection prevention and control

Strict adherence to hand hygiene, appropriate use and care of invasive devices, environmental cleaning, isolation precautions, and other infection prevention measures should be reinforced to reduce healthcare-associated neonatal infections.

Establish continuous surveillance

Regular monitoring of neonatal sepsis pathogens, antimicrobial susceptibility patterns, treatment practices, and clinical outcomes should be incorporated into routine neonatal care and hospital surveillance systems.

Strengthen laboratory capacity

Healthcare facilities should improve access to reliable microbiological services, trained laboratory personnel, appropriate equipment, and timely reporting of culture and susceptibility results.

Promote further research

Multicenter studies with larger populations are recommended to identify modifiable risk factors, evaluate treatment outcomes, monitor antimicrobial resistance trends, and develop context-specific strategies for reducing neonatal sepsis-related morbidity and mortality.

Abbreviations

Table 6: Showing Commonly used Abbreviations in this manuscript

Abbreviation	Full term
ACSH	Ayder Comprehensive Specialized Hospital
AMR	Antimicrobial resistance
ANC	Antenatal care
CLSI	Clinical and Laboratory Standards Institute
CONS	Coagulase-negative Staphylococci
CPAP	Continuous positive airway pressure
EOS	Early-onset sepsis
ESBL	Extended-spectrum β-lactamase
HIV	Human immunodeficiency virus
IDP / IDPs	Internally displaced person / Internally displaced persons
LAMA	Left against medical advice
LBW	Low birth weight
LMICs	Low- and middle-income countries
LOS	Late-onset sepsis
MDR	Multidrug-resistant
MU-IRB	Mekelle University Institutional Review Board
NICU	Neonatal intensive care unit
ROM	Rupture of membranes
SVD	Spontaneous vaginal delivery
STROBE	Strengthening the Reporting of Observational Studies in Epidemiology
VLBW	Very low birth weight
ELBW	Extremely low birth weight
WHO	World Health Organization

Declarations

Ethical approval and consent to participate

The Ethical approval for this study was obtained from the appropriate institutional review committee [MU-IRB2301/2024] before the commencement of data collection. The study was conducted in accordance with the ethical principles outlined in the Declaration of Helsinki.

As the study involved the review of patient information, confidentiality and privacy of participants were strictly maintained throughout the research process. All collected data were analyzed and used solely for the purpose of this study. Access to the study data was restricted to the research team to ensure the protection of participants' information.

Where applicable, informed consent was obtained from participants or their legal guardians according to the requirements of the ethical review committee. No personal identifiers were included in the analysis or reporting of the

study findings.

Conflict of Interest

The authors declare that they have no competing interests.

Availability of data and materials

The datasets generated and/or analyzed during the current study are not publicly available because they contain information that could compromise participant confidentiality. However, de-identified data are available from the corresponding author upon reasonable request and with permission from the Institutional Review Board of Mekelle University, in accordance with institutional data-sharing policies.

Funding

This study was supported through collaborative support for medical education and health-system strengthening in Ethiopia, with contributions from the European Union, Spanish Cooperation, Italian Development Cooperation, and the Ministry of Health of Ethiopia. The funders had no role in the study design, data collection, analysis, interpretation of the findings, or writing of the manuscript.

Trial registration no. : This study was a prospective observational study and did not involve the allocation of interventions to participants. Therefore, **clinical trial registration no. was not applicable.**

Acknowledgments

The authors would like to express their sincere gratitude to the leadership of Mekelle University, College of Health Sciences for their institutional support throughout this study. We also gratefully acknowledge the staffs of the Department of Pediatrics and Child Health, particularly the Neonatal Intensive Care Unit, and the Department of Microbiology, for their valuable support during data collection, clinical care, laboratory processing, and antimicrobial susceptibility testing.

We sincerely thank the data collectors and all staff members who contributed to the successful completion of this study. We also acknowledge the support provided through collaborative medical education and health-system strengthening initiatives involving the European Union, Spanish Cooperation, Italian Development Cooperation, and the Ministry of Health of Ethiopia.

Finally, we extend our sincere appreciation to all neonates and their parents or legal guardians who participated in the study and made this research possible.

References

1. Richard J, Martin AA, Fanaroff MC, Walsh. Fanaroff and Martin's Neonatal-Perinatal Medicine: Diseases of the Fetus and Infant. 11th ed. Elsevier; 2020.
2. Wilar R, Lestari H. Risk Factors and Clinical Outcomes of Neonatal Sepsis in Manado Tertiary Referral Hospital: A Single-center Study. Open Access Maced J Med Sci. 2022;10:93–8. 10.3889/oamjms.2022.7993.
3. Tewabe T, Mohammed S, Tilahun Y, Melaku B, Fenta M, Dagnaw T, et al. Clinical outcome and risk factors of neonatal sepsis among neonates in Felege Hiwot referral Hospital, Bahir Dar, Amhara Regional State, North West Ethiopia 2016: A retrospective chart review. BMC Research Notes. BioMed Central Ltd.; 2017. 10.1186/s13104-017-2573-1 . PubMed PMID: 28693597.

4. Mhada TV, Fredrick F, Matee MI, Massawe A. Neonatal sepsis at Muhimbili National Hospital, Dar es Salaam, Tanzania; Aetiology, antimicrobial sensitivity pattern and clinical outcome. BMC Public Health. 2012;12(1). 10.1186/1471-2458-12-904 . PubMed PMID: 23095365.
5. Gomella TL, Eyal F. Fayez Bany-Mohammed. Gomella's Neonatology: Management, Procedures, On-Call Problems, Diseases, and Drugs. 8th ed. McGraw-Hill Education; 2020.
6. Olorukooba A, Ifusemu W, Ibrahim M, Jibril M, Amadu L, Lawal B. Prevalence and factors associated with neonatal sepsis in a tertiary hospital, North West Nigeria. Nigerian Medical Journal. 2020;61(2):60. 10.4103/nmj.nmj_31_19 PubMed PMID: 32675896.
7. Sankari SA, Vasuki V. Changes in Bacteriological Profile and Antibiotic Susceptibility of Neonatal Sepsis in a Newly Established Tertiary Care Hospital, South India. International Journal of Current Medical And Applied Sciences [Internet]. 2016. Available from:
8. Vergnano S, Sharland M, Kazembe P, Mwansambo C, Heath PT. Neonatal sepsis: An international perspective. Archives Disease Childhood: Fetal Neonatal Ed. 2005. 10.1136/adc.2002.022863 . PubMed PMID: 15846011.
9. Arowosegbe AO, Ojo DA, Dedeke IO, Shittu OB, Akingbade OA. Neonatal sepsis in a Nigerian Tertiary Hospital: Clinical features, clinical outcome, aetiology and antibiotic susceptibility pattern. S Afr J Infect Dis. 2017;32(4):127–31. 10.1080/23120053.2017.1335962.
10. Akalu TY, Aynalem YA, Shiferaw WS, Desta M, Amha H, Getaneh D et al. Prevalence and determinants of early onset neonatal sepsis at two selected public referral hospitals in the Northwest Ethiopia: a cross-sectional study. BMC Pediatr. 2023.
11. Kruse AY, Thieu Chuong DH, Phuong CN, Duc T, Graff Stensballe L, Prag J, et al. Neonatal bloodstream infections in a pediatric hospital in Vietnam: A cohort study. J Trop Pediatr. 2013;59(6):483–8. 10.1093/tropej/fmt056 . PubMed PMID: 23868576.
12. Abdelgawad samia, Saad Fathal Amgitif E. Determining the main risk factors associated with neonatal death from sepsis in Neonatal Intensive Care Unit (NICU) in Benghazi Medical Centre (BMC) during 2020. Libyan J Med Res. 2023;17–9. 10.26719/LJM17-2.16.
13. (13)Factors associated with post-neonatal mortality in Ethiopia_ Using the 2019 Ethiopia mini demographic and health survey _ PLOS One.
14. Baheru FS, Shiferaw BZ, Toru T, GebreEyesus FA. Magnitude of neonatal sepsis and its associated factors among neonates admitted to the neonatal intensive care unit in Hawassa Comprehensive Specialized Hospital, Sidama Regional State, Ethiopia, 2020. BMC Pregnancy Childbirth. 2024;24(1). 10.1186/s12884-024-06583-z . PubMed PMID: 38778246.
15. Roble AK, Ayehubizu LM, Olad HM. Neonatal Sepsis and Associated Factors Among Neonates Admitted to Neonatal Intensive Care Unit in General Hospitals, Eastern Ethiopia 2020. Clin Med Insights Pediatr. 2022;16. 10.1177/11795565221098346.
16. Nino Macharashvili E, Kourbatova M, Butsashvili T, Tsertsvadze L-A, McNutt MK. Leonard. Etiology of neonatal blood stream infections in Tbilisi, Republic of Georgia. Int J Infect Dis. 2009.
17. Nehra Y, Sachdev CP, Gulati D, Singh A, Choudhary D. Perinatal risk factors in neonatal sepsis in a tertiary care hospital. IOSR Journal of Dental and Medical Sciences (IOSR-JDMS) e-ISSN. 2020;19:50–3. 10.9790/0853-1906135053
18. (18)Blood culture result profile and antimicrobial resistance pattern_ a report from neonatal intensive care unit (NICU), Asella teaching and referral hospital, Asella, south East Ethiopia - PubMed.
19. Polin RA, Papile LA, Baley JE, Benitz W, Carlo WA, Cummings J, et al. Management of Neonates with Suspected or Proven Early-Onset Bacterial Sepsis. Pediatrics. 2012;129(5):1006–15. 10.1542/peds.2012-0541 . PubMed PMID: 22547779.

20. (20)Antimicrobial use in paediatric patients in a teaching hospital in Ethiopia _ PLOS One.
21. (21)Towards understanding global patterns of antimicrobial use and resistance in neonatal sepsis_ insights from the NeoAMR network - PubMed.
22. Jyothi P, Basavaraj MC, Basavaraj PV. Bacteriological profile of neonatal septicemia and antibiotic susceptibility pattern of the isolates. J Nat Sci Biol Med. 2013;4(2):306–9. 10.4103/0976-9668.116981.
23. Ibrahim BA, Damiri B, Allabadi H, Qadi M. Bacteriological profile and antibiotic susceptibility pattern of septicemia in neonatal intensive care units in Palestine: A retrospective study. IJID Reg. 2024;10:87–93. 10.1016/j.ijregi.2023.11.019.
24. Bhattarai S, Chapagain RH, Mishra D, Shrestha AK, Shrestha SM. Bacteriological profile and antibiotic sensitivity pattern of neonatal sepsis in central paediatric referral hospital in Nepal. J Nepal Pediatr Soc. 2019;39(1):1–5. 10.3126/jnps.v39i1.21070.
25. Sharma CM, Agrawal RP, Sharan H, Kumar B, Sharma D, Bhatia SS. Neonatal sepsis: Bacteria & their susceptibility pattern towards antibiotics in neonatal intensive care unit. J Clin Diagn Res. 2013;7(11):2511–3. 10.7860/JCDR/2013/6796.3594.
26. Basavaraj M, Mrrashmiranjan R, Gagan P. Neonatal Septicemia: Bacterial Isolates & Their Antibiotics Susceptibility Patterns: A Hospital Based Study. Journal Medical Science and Clinical Research; 2017.
27. (27)Bloodstream infections and antimicrobial resistance patterns in a South African neonatal intensive care unit - PubMed.
28. (28)Pattern of bacterial profile and antibiotic susceptibility among neonatal sepsis cases at Cairo University Children Hospital - PubMed.
29. (35)Assessing the barriers to maternal, newborn and child health service utilization in internally displaced people (IDPs)_ a community-based cross-sectional study in Banadir, Somalia - PubMed.
30. Geyesus T, Moges F, Eshetie S, Yeshitela B, Abate E. Bacterial etiologic agents causing neonatal sepsis and associated risk factors in Gondar, Northwest Ethiopia. BMC Pediatr. 2017;17(1). 10.1186/s12887-017-. 0892-y PubMed PMID: 28587631.
31. (37)Risk factors in early neonatal sepsis - PubMed.
32. Muhammad Hayun E, Alasiay D, Daud DB, Febaiani, Djauhaaiah Madjid. The Risk Factors of Early Onset Neonatal Sepsis. nAmerican J Clin Experimental Med. 2015.
33. Sherif M, Abera D, Desta K. Prevalence and antibiotic resistance pattern of bacteria from sepsis-suspected neonates at St. Paul's Hospital Millennium Medical College, Addis Ababa, Ethiopia. BMC Pediatr. 2023;23(1). 10.1186/s12887-023-04399-y . PubMed PMID: 37980512.
34. Graf RJ, Edwards A, Crowley MA, Mukherjee D, Ryan RM. Revisiting time to blood culture positivity: can we decrease antibiotic exposure in the NICU? J Perinatol. 2026. 10.1038/s41372-026-02629-6.
35. Viswanathan R, Singh AK, Ghosh C, Dasgupta S, Mukherjee S, Basu S. Profile of neonatal septicaemia at a district-level sick newborn care unit. J Health Popul Nutr. 2012;30(1):41–8. 10.3329/jhpn.v30i1.11274 . PubMed PMID: 22524118.
36. Desai KJ, Malek SS. Neonatal Septicemia: Bacterial Isolates & Their Antibiotic Susceptibility Patterns. Neonatal Septicemia 12 NJIRM. 2010;1(3).
37. Bahmani N. Bacterial Aetiology and Antibiotic Resistance Patterns in Neonatal Sepsis in the West of Iran. Original Article. 2021;9(11):14737–46. 10.22038/ijp.2020.54099.4281.
38. (43)Neonatal sepsis in a tertiary care hospital in South India_ bacteriological profile and antibiotic sensitivity pattern - PubMed.

39. Peirovifar A, Rezaee MA, Gharehbaghi MM. Prevalence of multidrug resistant extended-spectrum beta-lactamase producing gram-negative bacteria in neonatal sepsis. Int J Women's Health Reprod Sci. 2014;2(3):138–45. 10.15296/ijwhr.2014.21.
40. Flokas ME, Karanika S, Alevizakos M, Mylonakis E. Prevalence of ESBL-producing Enterobacteriaceae in pediatric bloodstream infections: A systematic review and meta-analysis. PLoS ONE. 2017;12(1). 10.1371/journal.pone.0171216 . PubMed PMID: 28141845.

Figures

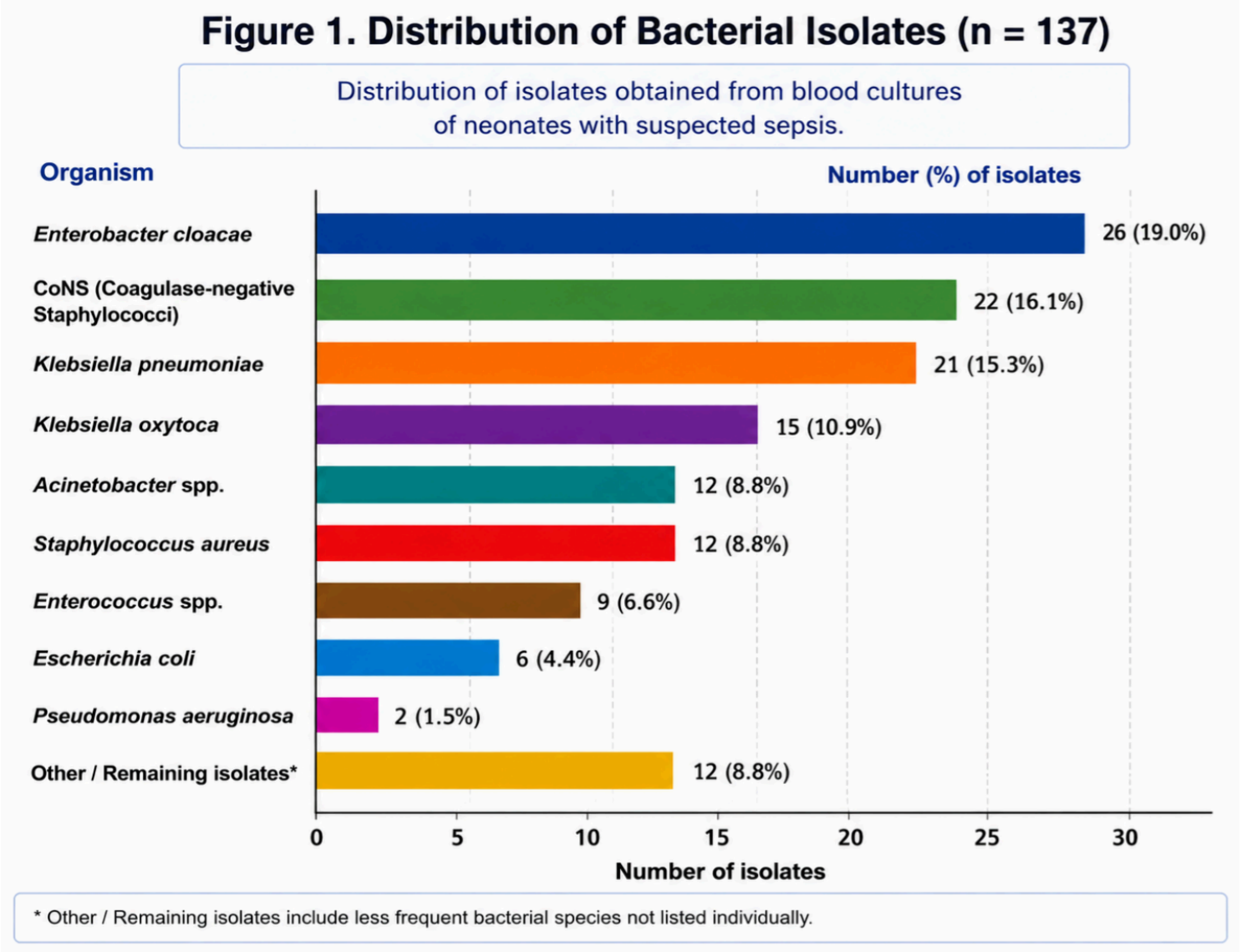


Figure 1

See image above for figure legend