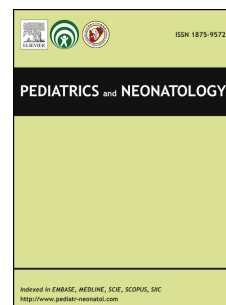


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Early-Onset Neonatal Sepsis in the Eastern Democratic Republic of the Congo:
Microbiology, Antimicrobial Resistance, and Clinical Outcomes.

Joseph Ntagerwa Ntaganzibwa, Francisca Isia Nanci, Serge Mushamuka Zigabe, Eliezer Rhuderhekuguma Kulimushi, Samuel Ndinaye, David Bahati Bashomeka, Grâce Mushagalusa Bavurhe, Christian Cito Shabani, Joy-Arsène Bwema, Lucie Nshobole Cebweru, Elyse Akonkwa Birere, Bel'Ange Assongo Wabiwa, David Lupande Mwenebitu, Oreste Battisti, Dimitri Van der Linden, Richard Mbusa Kambale



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Full Title: Early-onset neonatal sepsis in Eastern Democratic Republic of the Congo: microbiology, antimicrobial resistance, and clinical outcomes.

Running Title: Early-onset sepsis and resistance in Eastern DRC

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Word count: 3179

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HIGHLIGHTS

- Gram-negative Enterobacterales predominated among culture-confirmed EOS cases.
- High resistance to gentamicin and third-generation cephalosporins was observed.
- ESBL-producing pathogens were highly prevalent among Gram-negative isolates.
- Low birth weight independently predicted in-hospital mortality.
- Important diagnostic stewardship gaps were identified in neonatal sepsis management.

Authors' contributions

RMK, DLM, DVDL and OB designed the study and reviewed the manuscript for important intellectual content. ERK, SN, DBB, GMB, CCS, J-AB, LNC, and EAB, BAW collected the data. RMK analyzed the data. RMK wrote the manuscript. DLM, DVDL, OB, JNN, FIN, and SMZ critically revised the manuscript; All authors read and approved the final manuscript.

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Competing interest

The authors declare that they have no competing interests.

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

Ethics approval and consent to participate

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Abbreviations

EOS: Early-onset sepsis

NICU: Neonatal intensive care unit

DRC: Democratic Republic of the Congo

ESBL: Extended-spectrum beta-lactamase

LMIC: Low- and middle-income country

WHO: World Health Organization

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Abstract

Background: Early-onset neonatal sepsis (EOS) remains a major cause of neonatal morbidity and mortality in low- and middle-income countries. Data from Central Africa on microbiological patterns, antimicrobial resistance, and diagnostic stewardship are limited.

Methods: We conducted a retrospective cohort study including neonates ≤ 72 hours of life managed for suspected EOS between January 2021 and September 2025 at a tertiary neonatal intensive care unit in the Eastern Democratic Republic of the Congo. Diagnostic stewardship indicators included blood culture sampling proportion, positivity rate, and contamination rate. Multivariable logistic regression identified independent predictors of in-hospital mortality.

Results: Among 1,293 neonates with suspected EOS, blood cultures were performed in 604 (46.7%). The positivity rate for true pathogens was 17.4% (105/604), and 10.1% of cultures were classified as contaminants. Among inborn neonates, the incidence of culture-confirmed EOS was 8.4 per 1,000 live births. Gram-negative bacilli accounted for 78.1% of confirmed cases, predominantly Enterobacterales. High resistance to gentamicin and third-generation cephalosporins was documented, with frequent extended-spectrum beta-lactamase production. In the revised multivariable model, low birth weight was independently associated with increased in-hospital mortality (aOR 2.97; 95% CI 1.74–5.09), whereas culture-confirmed EOS was not independently associated with death.

Conclusion: EOS in this setting was characterized by Gram-negative predominance, substantial antimicrobial resistance, and important diagnostic stewardship constraints. The high prevalence of multidrug-resistant Enterobacterales raises concerns regarding the adequacy of current WHO-recommended empirical regimens in similar LMIC settings. Low birth weight emerged as the strongest independent predictor of in-hospital mortality. Strengthening microbiological capacity, infection prevention practices, and locally adapted antimicrobial stewardship strategies is essential to improve neonatal outcomes in this setting.

Key Words: early-onset sepsis; neonatal intensive care; antimicrobial resistance; Enterobacterales; diagnostic stewardship; Democratic Republic of the Congo

Introduction

Neonatal sepsis remains a leading cause of neonatal morbidity and mortality worldwide.¹⁻⁴ The burden is disproportionately high in low- and middle-income countries (LMICs), particularly in sub-Saharan Africa (SSA), where incidence and case-fatality rates remain elevated compared with high-income settings.^{5,6} In the Democratic Republic of the Congo (DRC), neonatal mortality remains high, and infections contribute substantially to neonatal deaths.^{7,8}

Early-onset sepsis (EOS), defined as occurring within the first 72 hours of life, is typically associated with vertical transmission from the mother.⁹⁻¹² In high-income countries (HICs), Group B *Streptococcus* (GBS) and *Escherichia coli* remain the predominant pathogens.¹³⁻¹⁵ International guidelines, including those of the World Health Organization (WHO), therefore recommend ampicillin (or benzylpenicillin) combined with gentamicin as first-line empirical therapy, with third-generation cephalosporins reserved for second-line treatment.¹⁶⁻²⁰

However, a systematic review of neonatal sepsis in LMICs identified *Klebsiella pneumoniae*, *Staphylococcus aureus*, coagulase-negative staphylococci, and *E. coli* among the most frequently isolated pathogens.²¹ More recent studies from SSA have reported a growing prevalence of extended-spectrum beta-lactamase (ESBL)-producing Enterobacterales and rising resistance to WHO-recommended empirical regimens.²²⁻²⁴ These trends raise concerns regarding the adequacy of current first-line therapies in many LMICs neonatal units.

Data from Central Africa remain limited. Earlier findings from Bukavu (Eastern DRC) reported predominance of *Enterobacter cloacae* and *K. pneumoniae* among EOS isolates,²⁵ while recent reports from neighboring countries similarly demonstrated Gram-negative predominance and increasing antimicrobial resistance.²⁶ Contemporary surveillance data from Eastern DRC are scarce.

Updated local data on pathogen distribution and antimicrobial resistance are needed to inform empirical treatment and antimicrobial stewardship. This study aimed to characterize the epidemiology, microbiological profile, antimicrobial resistance, and diagnostic stewardship of EOS in a tertiary neonatal intensive care unit (NICU) in Eastern DRC.

Methods

Study design and setting

This retrospective cohort study was conducted in the neonatal intensive care unit (NICU) of Hôpital Provincial Général de Référence de Bukavu (HPGRB), a tertiary referral hospital serving approximately 1.19 million inhabitants in the Eastern Democratic Republic of the Congo. The NICU admits both inborn and referred neonates and provides basic neonatal intensive care, although invasive mechanical ventilation, central venous access, and parenteral nutrition are not routinely available. Microbiological investigations are supported by the hospital laboratory, with variable availability of blood culture consumables.

Data were extracted from NICU admission registers, laboratory records, and medical charts using a standardized collection form. Data extraction was performed by trained physicians and cross-checked for completeness and consistency. We included all neonates aged ≤ 72 hours managed for suspected EOS between January 2021 and September 2025.

Definitions and eligibility criteria

Suspected EOS was defined as the presence of at least one maternal risk factor and/or at least one clinical sign consistent with neonatal infection, in accordance with international and WHO recommendations.^{16,20,24} The decision to perform blood culture was based on clinical judgment guided by

unit practice during the study period. A summary of the clinical indications and practical criteria used for blood culture sampling in suspected EOS cases is provided in Supplementary Appendix 1.

Maternal risk factors included prolonged rupture of membranes (>18 hours), clinical chorioamnionitis, maternal urinary tract infection during the third trimester, and preterm birth (< 37 weeks' gestation).

Clinical signs included feeding difficulties, lethargy, respiratory distress, temperature instability, gastrointestinal symptoms, and neurological manifestations.

Culture-confirmed EOS was defined as isolation of a pathogenic organism from blood culture. Coagulase-negative *staphylococci* (CoNS) and other skin commensals were considered contaminants if isolated once, unless recovered repeatedly in a symptomatic neonate.

Microbiological procedures

Blood samples (2 mL) were collected under aseptic conditions before antibiotic administration whenever feasible. During the first study period (January 2021–March 2024), samples were inoculated into BacT/Alert® PF Pediatric FAN bottles (bioMérieux, Belgium) and incubated at 37°C for up to 7 days. Positive bottles underwent Gram staining and subculture on 5% sheep blood agar. Identification was performed using standard biochemical methods.

From April 2024 onwards, semi-automated incubation and automated identification were implemented using the Render MA-120 system (Render Biotech Co., Shenzhen, China), which provides minimum inhibitory concentrations (MICs). Antimicrobial susceptibility testing results were interpreted according to the 2023 EUCAST and CLSI standards, as applicable.^{27,28} No major changes in blood sampling protocol or aseptic collection procedures occurred during the study period.

Empirical antibiotic management

Initial empirical therapy consisted of benzylpenicillin plus amikacin, following WHO and international guidelines.^{16–20} In cases of clinical deterioration or lack of improvement, second-line therapy with levofloxacin was initiated pending culture results. This choice was based on previous local

microbiological data demonstrating high resistance to third-generation cephalosporins among Gram-negative isolates.^{22,25}

Diagnostic stewardship indicators

Diagnostic stewardship was evaluated using predefined indicators:

1. Blood culture sampling rate = (number of suspected EOS undergoing blood culture / total suspected EOS) $\times$ 100
2. Positivity rate = (number of true pathogens / total blood cultures performed) $\times$ 100
3. Contamination rate = (number of contaminants / total blood cultures performed) $\times$ 100

These indicators were selected based on established diagnostic stewardship frameworks²⁹ and were defined a priori before data analysis.

Outcomes

The primary outcomes were: (i) distribution of bacterial pathogens; (ii) antimicrobial resistance patterns; and (iii) in-hospital mortality. Secondary outcomes included diagnostic stewardship indicators.

Statistical analysis

Continuous variables were expressed as median (interquartile range, IQR), whereas categorical variables were expressed as frequency and percentage. Comparisons between neonates with culture-confirmed EOS and those with sterile blood cultures were performed using the Mann–Whitney U test for continuous variables and the χ^2 test or Fisher's exact test for categorical variables, as appropriate. Missing data were not imputed, and all analyses were conducted using available-case data. No formal sample size calculation was performed because of the retrospective study design.

Comparative and mortality analyses were restricted to neonates with definitive microbiological classification after exclusion of blood culture contaminants and undocumented microbiological results.

Univariable logistic regression was first performed to estimate crude odds ratios (ORs), 95% confidence intervals (CIs), and p-values for candidate predictors of in-hospital mortality.

Given the limited number of mortality events, the primary multivariable logistic regression model was restricted to three clinically relevant covariates to reduce the risk of overfitting and maintain an adequate events-per-variable ratio. Based on clinical relevance, biological plausibility, reviewer-suggested priorities, and univariable analyses, the final parsimonious model included male sex, low birth weight (<2500 g), and culture-confirmed EOS status.

Low birth weight was preferred over gestational age because birth weight was more consistently documented and may be less prone to misclassification in this setting, where gestational age estimation may sometimes be imprecise.

Because procalcitonin values were available only for a subset of neonates, procalcitonin was not included in the primary multivariable model. A sensitivity multivariable analysis including log-transformed procalcitonin values was therefore performed among neonates with available biomarker data.

Model fit was assessed using the Hosmer–Lemeshow goodness-of-fit test and evaluation of model residuals to assess calibration and potential outliers. A two-sided p-value < 0.05 was considered statistically significant. All analyses were performed using Stata version 16.0 (StataCorp LLC, College Station, TX, USA).

Ethical considerations

The study was conducted in accordance with the Declaration of Helsinki. The study was approved by the Ethics Committee of Université Catholique de Bukavu. The requirement for informed consent was waived due to the retrospective design and anonymized data collection.

Results

Study population and diagnostic stewardship indicators

During the study period, 8,738 live births were recorded at HPGRB, of which 2,346 neonates were admitted to the NICU. Among them, 1,293 neonates aged ≤ 72 hours were managed for suspected EOS.

Blood cultures were performed in 604/1,293 suspected cases (46.7%): 105 yielded true pathogens (17.4%), 326 were sterile (54.0%), 61 were contaminants (10.1%), and 112 (18.5%) lacked documented microbiological results. After excluding contaminants and undocumented results, 431 neonates (105 culture-confirmed EOS and 326 sterile cultures) were included in comparative analyses. Among inborn neonates, 73 culture-confirmed EOS cases were identified, corresponding to an incidence of 8.4 per 1,000 live births (73/8,738).

Sociodemographic and obstetric characteristics

Comparative analyses were restricted to neonates with documented culture results (culture-confirmed EOS, $n = 105$; sterile cultures, $n = 326$). Sociodemographic and obstetric characteristics are summarized in Table 1. Several maternal and perinatal characteristics were comparable between groups; however, significant differences were observed for number of antenatal care visits, inborn delivery, birth weight, and low birth weight.

Clinical characteristics and outcomes

Clinical characteristics and laboratory findings are presented in Table 2. Procalcitonin levels were significantly higher in culture-confirmed EOS than in neonates with sterile cultures (median 1.6 vs. 0.6 ng/mL, $p = 0.008$). ROC analysis showed modest discriminatory performance (AUC 0.639, 95% CI 0.535–0.735), with an optimal threshold of 1.14 ng/mL (sensitivity 59.5%; specificity 65.1%) (Supplementary Figure 1). Culture-confirmed cases had a longer median hospital stay (8.5 vs. 5 days, $p = 0.003$). Other clinical and laboratory characteristics did not differ significantly between groups. In-hospital mortality was higher in culture-confirmed cases (26/105; 24.8%) than in those with sterile cultures (58/326; 17.8%), although the difference was not statistically significant ($p = 0.117$).

Microbiological profile

The distribution of pathogens is shown in Table 3. Gram-negative bacilli accounted for 82 of 105 isolates (78.1%). Enterobacterales predominated, with *K. pneumoniae* (25.7%), *Enterobacter* spp. (24.8%), and *E. coli* (15.2%) being the most frequently identified pathogens.

Gram-positive cocci represented 19 isolates (18.1%), mainly *S. aureus* (9.5%), followed by GBS (4.8%) and *Enterococcus* spp. (3.8%). The remaining isolates comprised Gram-positive bacilli and fungal pathogens (*Candida* spp.).

Antimicrobial susceptibility patterns

Antimicrobial resistance profiles of major Gram-negative pathogens are detailed in Table 4. High resistance rates were observed to commonly used first-line antibiotics. Resistance to gentamicin ranged from 40.0% in *E. coli* to 57.1% in *Klebsiella* spp. Substantial resistance to third-generation cephalosporins was noted, particularly for ceftazidime (71.4% in *Klebsiella* spp. and 63.0% in *Enterobacter* spp.).

The extended-spectrum beta-lactamase (ESBL) phenotype was frequently detected, occurring in 75.0% of *Klebsiella* spp., 70.4% of *Enterobacter* spp., and 46.7% of *E. coli* isolates.

Resistance to carbapenems was lower than to other β -lactams, although still present (14.3% in *Klebsiella* spp. and 25.9% in *Enterobacter* spp.). Amikacin showed comparatively lower resistance rates across species. Among the 10 *Staphylococcus aureus* isolates, resistance to penicillin, aminoglycosides, fluoroquinolones, macrolides, and clindamycin was variably observed. At least two isolates demonstrated resistance profiles compatible with methicillin-resistant *S. aureus* (MRSA), including one isolate explicitly identified as MRSA in laboratory records.

Multivariable analysis of in-hospital mortality

Crude associations between candidate predictors and in-hospital mortality are shown in Supplementary Table 1. In univariable analyses, low birth weight, very low birth weight, inborn delivery, cesarean section,

neonatal resuscitation, and number of antenatal care visits were associated with mortality. Culture-confirmed EOS was not significantly associated with mortality in crude analysis.

Results of the revised multivariable logistic regression model are presented in Table 5. After adjustment for sex, low birth weight, and culture-confirmed EOS status, low birth weight remained independently associated with increased odds of in-hospital mortality. Male sex was also associated with higher mortality, whereas culture-confirmed EOS was not independently associated with death.

In the sensitivity multivariable analysis including procalcitonin, the main findings remained broadly unchanged. Low birth weight remained independently associated with mortality, whereas procalcitonin was not independently associated with in-hospital death (Supplementary Table 2).

Discussion

This study provides contemporary data on the epidemiology, microbiological profile, antimicrobial resistance, clinical outcomes, and diagnostic stewardship of EOS in a tertiary NICU in Eastern DRC. Several findings are noteworthy.

First, only 46.7% of neonates with suspected EOS underwent blood culture, reflecting diagnostic constraints common in resource-limited settings.³⁰ The contamination rate (10.1%) exceeded the commonly recommended <5% benchmark,³¹ highlighting the need for improved aseptic sampling and staff training. Although the culture positivity rate of 17.4% was within the range reported in LMIC neonatal units,^{21,30,33} incomplete sampling may have underestimated culture-confirmed EOS and biased pathogen distribution if testing preferentially targeted clinically unstable neonates.

Second, Gram-negative bacilli predominated (78.1%), mainly *K. pneumoniae*, *Enterobacter* spp., and *E. coli*. This contrasts with HICs, where GBS and *E. coli* predominate,^{13–15} but is consistent with reports from SSA and other LMICs.^{21,32,33} The persistent predominance of Enterobacterales, also reported locally,^{22,25} may suggest healthcare-associated transmission or environmental reservoirs, reinforcing the need for infection prevention and control. Prolonged rupture of membranes was not significantly associated with culture-confirmed EOS. Although EOS is classically associated with vertical

transmission, the predominance of multidrug-resistant Enterobacterales may also suggest ultra-early acquisition from the healthcare environment or perinatal care setting, as hypothesized in other SSA neonatal units.

Third, antimicrobial resistance among Gram-negative isolates was substantial, particularly to gentamicin and third-generation cephalosporins, with frequent ESBL production among *Klebsiella* and *Enterobacter* spp., consistent with reports from LMIC neonatal units.^{24,33} These findings raise concerns about the effectiveness of WHO-recommended empirical regimens^{16–20} and support periodic adaptation of antibiotic policies to local surveillance data.^{24,34} Although carbapenem resistance was comparatively lower, its presence is concerning in settings with limited access to reserve antibiotics. The retrospective design precluded reliable attribution of mortality to antimicrobial resistance or inadequate empirical therapy. Moreover, prior antibiotic exposure before blood culture and detailed comparisons of multidrug-resistant pathogen distribution between inborn and outborn neonates were not consistently documented, limiting causal interpretation.

Fourth, culture-confirmed EOS was associated with higher procalcitonin levels and longer hospitalization, whereas the higher crude mortality among culture-positive neonates did not reach statistical significance. Procalcitonin showed only modest discriminatory performance for culture-confirmed EOS, supporting its use as an adjunct rather than a stand-alone diagnostic marker. Platelet counts did not differ significantly between groups. In the multivariable model, low birth weight emerged as the strongest independent predictor of in-hospital mortality, consistent with the greater vulnerability of these neonates to severe infection due to immunological immaturity and reduced physiological reserve.^{9,30,34} The association between low birth weight and culture positivity further supports the interaction between host vulnerability, infection risk, and adverse neonatal outcomes.

Finally, integrating blood culture sampling, positivity, and contamination rates provides a diagnostic stewardship perspective rarely reported simultaneously in Central African neonatal studies. These indicators are increasingly relevant to antimicrobial stewardship, particularly where laboratory capacity

is limited.^{30,31} Strengthening access to blood cultures, aseptic sampling, and structured audit-feedback may improve microbiological surveillance, targeted therapy, and antibiotic use.

This study has several strengths, including a large multiyear cohort, integration of microbiological, clinical, and stewardship indicators, multivariable assessment of mortality determinants, standardized EUCAST/CLSI susceptibility criteria, and explicit reporting of analytical denominators and exclusions.

Several limitations should be acknowledged. First, the retrospective single-center design may limit generalizability and carries risks of incomplete documentation and missing data. Second, blood cultures were performed in less than half of suspected EOS cases, potentially introducing selection bias. Third, contamination was relatively frequent, and a substantial proportion of blood cultures lacked documented microbiological results. Fourth, because the study relied on routine clinical data from neonates managed for suspected EOS, some deaths may have been influenced by concomitant conditions such as hypoxic-ischemic encephalopathy, congenital anomalies, or metabolic disorders that were not systematically excluded. Finally, antimicrobial susceptibility testing evolved during the study period, which may have introduced heterogeneity in resistance interpretation.

Conclusion

EOS in this tertiary NICU was predominantly associated with multidrug-resistant Gram-negative bacilli, particularly Enterobacterales, with frequent ESBL production and substantial resistance to first-line antibiotics. Low birth weight was the strongest independent predictor of mortality. Low blood culture sampling and high contamination highlight important diagnostic stewardship gaps. Strengthening microbiological capacity, infection prevention, and locally informed empirical antibiotic policies is essential in similar LMIC settings.

Table 1 Sociodemographic and obstetric characteristics.

Variable	Culture-confirmed EOS (n = 105)	Sterile (n = 326)	p-value
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Maternal age, years	28 (24–34)	29 (25–33)	0.564
No ANC follow-up	2 (2.1%)	15 (5.0%)	0.383
Number of ANC visits	3 (1–4)	3 (2–4)	0.011
Inborn (HPGRB)	61 (58.1%)	231 (70.9%)	0.015
Cesarean section	42 (42.0%)	156 (49.7%)	0.180
Male sex	53 (50.5%)	181 (55.5%)	0.416
Gestational age (weeks)	35 (32–39)	36 (33–39)	0.131
Preterm (<37 weeks)	53 (50.5%)	151 (46.3%)	0.458
Birth weight (g)	2,225 (1,565–3,050)	2,515 (1,710–3,180)	0.049
Low birth weight (<2500 g)	63 (60.0%)	156 (47.9%)	0.030
PROM	22 (28.6%)	75 (27.7%)	0.877

ANC: Antenatal Care; EOS : Early-Onset Neonatal Sepsis; HPGRB: Hôpital Provincial Général de Référence de Bukavu ;
 PROM : Premature Rupture of Membranes

Table 2 Clinical characteristics and outcomes.

Variable	Culture-confirmed EOS	Sterile	p-value
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	(n = 105)	(n = 326)	
Apgar <7 (5 min)	13 (19.1%)	25 (10.5%)	0.058
Neonatal resuscitation	25 (30.5%)	90 (33.5%)	0.616
Anemia	17 (16.2%)	41 (12.6%)	0.351
Jaundice	18 (17.1%)	59 (18.2%)	0.814
Procalcitonin	1.6 (0.4–7.2)	0.6 (0.2–2.7)	0.008
White blood cell count, cells/ μ L	11,250 (5,428–19,900)	10,745 (7,000–17,230)	0.631
Platelet count, $\times 10^3/\mu$ L	60,000 (31,600–281,000)	57,000 (24,900–262,000)	0.235
Length of stay (days)	8.5 (5–17)	5 (4–10)	0.003
In-hospital mortality	26 (24.8%)	58 (17.8%)	0.117

EOS : Early-Onset Neonatal Sepsis

Table 3 Distribution of pathogens by Gram classification (n = 105).

Pathogen	n	% of total
Gram-negative bacilli (total)	82	78.1%
<i>Klebsiella pneumoniae</i>	27	25.7%
<i>Enterobacter spp.</i>	26	24.8%
<i>Escherichia coli</i>	16	15.2%
<i>Salmonella spp.</i>	4	3.8%
<i>Acinetobacter spp.</i>	2	1.9%
<i>Citrobacter spp.</i>	2	1.9%
<i>Proteus mirabilis</i>	2	1.9%
Gram-positive cocci (total)	19	18.1%
<i>Staphylococcus aureus</i>	10	9.5%
Group B <i>Streptococcus</i>	5	4.8%
<i>Enterococcus spp.</i>	4	3.8%
Gram-positive bacilli (total)	2	1.9%
Fungi (Candida spp.)	2	1.9%

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Table 4 Antimicrobial resistance among major Gram-negative pathogens in culture-confirmed EOS.

Antimicrobial agent	<i>Klebsiella</i> spp. (% resistance)	<i>Enterobacter</i> spp. (%resistance)	<i>Escherichia coli</i> (%resistance)
Amikacin	21.4	29.6	6.7
Gentamicin	57.1	40.7	40.0
Ciprofloxacin	50.0	22.2	6.7
Levofloxacin	25.0	11.1	13.3
Piperacillin–tazobactam	35.7	51.9	53.3
Amoxicillin–clavulanate	42.9	37.0	46.7
Cefotaxime	32.1	29.6	20.0
Ceftazidime	71.4	63.0	40.0
Cefepime	57.1	33.3	26.7
Meropenem	14.3	25.9	0.0
TMP–SMX	25.0	14.8	13.3
ESBL phenotype (%)	75.0	70.4	46.7

EOS : Early-Onset Neonatal Sepsis; HPGRB: ESBL: Extended-spectrum beta-lactamase; TMP–SMX:Trimethoprim–sulfamethoxazole; Values represent percentage of resistant isolates among those tested for each antimicrobial agent.

Table 5 Multivariable logistic regression for in-hospital mortality.

Variable	aOR	(95% CI)	p-value
Male sex	1.77	(1.06–2.96)	0.029
Low birth weight <2500 g	2.97	(1.74–5.09)	<0.001
Culture-confirmed EOS	1.39	(0.81–2.40)	0.233

Legend: Adjusted odds ratios were estimated using multivariable logistic regression among neonates with definitive microbiological classification. The model was restricted to three covariates to reduce overfitting, given the limited number of in-hospital deaths. Variables were selected based on clinical relevance and reviewer-suggested priorities: sex, birth-weight category, and microbiological status. Contaminants and undocumented blood culture results were excluded. aOR: adjusted odds ratio; CI: confidence interval; EOS: early-onset sepsis.

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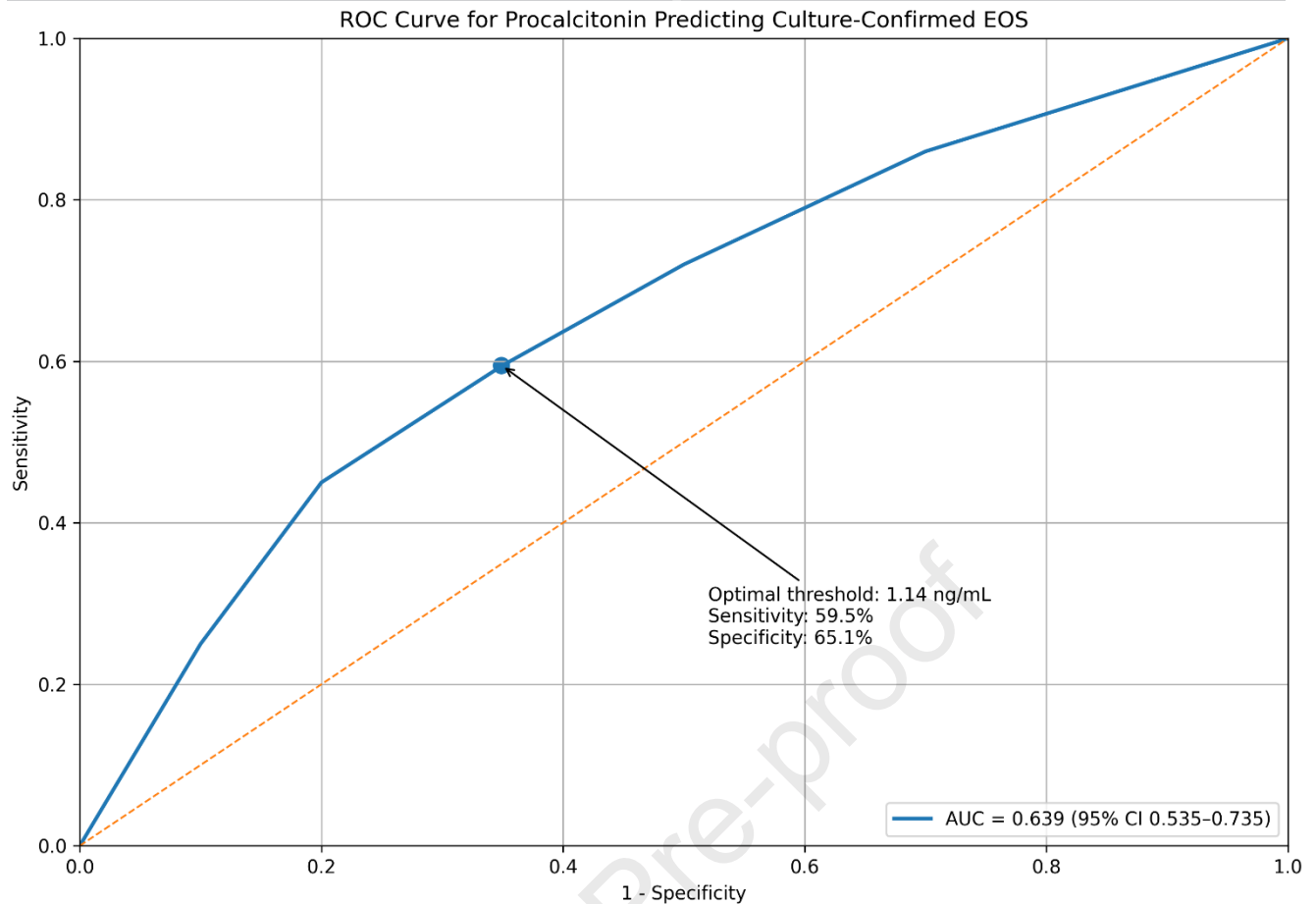
Supplementary Table 1 Univariable analysis of factors associated with in-hospital mortality, n=431.

Variable	N	Deaths	Crude OR (95% CI)	p-value
Male sex	430	84	1.30 (0.80–2.10)	0.296
Inborn delivery	430	84	0.36 (0.22–0.59)	<0.001
Cesarean section	414	80	0.59 (0.36–0.98)	0.041
Preterm <37 weeks	229	40	2.68 (1.29–5.58)	0.009
Low birth weight <2500 g	424	84	2.66 (1.59–4.44)	<0.001
Very low birth weight <1500 g	424	84	4.92 (2.88–8.39)	<0.001
Culture-confirmed EOS	431	84	1.52 (0.90–2.57)	0.118
PCT, log ₂ (PCT+0.1)	209	32	1.04 (0.88–1.22)	0.667
PCT ≥2 ng/mL	209	32	0.68 (0.29–1.62)	0.386
Apgar <7 at 5 min	306	44	1.41 (0.58–3.44)	0.450
Neonatal resuscitation	351	62	1.76 (1.01–3.09)	0.048
Anemia	431	84	0.96 (0.48–1.95)	0.914
Jaundice	431	84	1.33 (0.74–2.41)	0.343
PROM	348	61	1.21 (0.67–2.21)	0.530
Maternal age, per year	405	78	0.96 (0.92–1.00)	0.057
ANC visits, per visit	364	67	0.76 (0.64–0.92)	0.004

Legend: Crude odds ratios were estimated using univariable logistic regression among neonates with definitive microbiological classification, after exclusion of contaminants and undocumented blood culture results. The comparison group for culture-confirmed EOS was neonates with sterile blood cultures. Procalcitonin was analyzed both as a dichotomous variable using a threshold of ≥2 ng/mL and as a log-transformed continuous variable because of its skewed distribution. OR: odds ratio; CI: confidence interval; EOS: early-onset sepsis; PCT: procalcitonin; PROM: prolonged rupture of membranes; ANC: antenatal care.

Supplementary Table 2 Sensitivity analysis of the multivariable mortality model including procalcitonin.

Variable	aOR (95% CI)	p-value
Male sex	2.03 (0.89–4.63)	0.095
Low birth weight <2500 g	4.45 (1.87–10.56)	0.001
Culture-confirmed EOS	0.80 (0.27–2.36)	0.690
PCT, log2(PCT+0.1)	1.07 (0.91–1.27)	0.413



Supplementary Figure 1 Receiver operating characteristic (ROC) curve evaluating the diagnostic performance of procalcitonin for culture-confirmed early-onset neonatal sepsis among neonates with available biomarker data. The area under the curve (AUC) was 0.639 (95% CI 0.535–0.735), indicating modest discriminatory performance. The optimal threshold identified using the Youden index was 1.14 ng/mL, corresponding to a sensitivity of 59.5% and a specificity of 65.1%.