



Risk Factors for Extended-Spectrum Beta-Lactamase-Producing Bacteria among Neonates in a Referral Hospital in East Lombok, Indonesia

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Abstract

Extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria were an emerging cause of neonatal infections. However, local data from resource-limited hospitals in eastern Indonesia remained scarce. This study determined the proportion of ESBL production among Gram-negative isolates and analyzed the association of demographic and clinical characteristics with ESBL positivity in neonates. A retrospective cross-sectional study was conducted at a referral hospital in East Lombok, Indonesia, between January 2023 and December 2024. Secondary data were obtained from 117 neonates with Gram-negative isolates. Antimicrobial susceptibility testing was performed using the VITEK 2 Compact system. Data were analyzed using chi-square and multivariable logistic regression. Of 117 neonates, 56 (47.9%) harbored ESBL-producing isolates, most commonly *Klebsiella pneumoniae* and *Serratia marcescens*. Early neonatal age was the only factor independently associated with ESBL positivity (adjusted OR 49.40; 95% CI 14.86-164.17), whereas sex, gestational age, and birth weight were not. Early neonatal age was strongly associated with ESBL positivity, suggesting a possible role of maternal or perinatal colonization. These findings supported heightened vigilance and judicious empirical antibiotic use, particularly in the early neonatal period in resource-limited settings.

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Introduction

Neonatal sepsis is a major cause of mortality and long-term morbidity in newborns. This burden is especially high in low- and middle-income countries, where access to high-quality perinatal and intensive care is limited (De Rose et al., 2024; GBD 2021 Antimicrobial Resistance Collaborators,

2024). In Indonesia, sepsis, prematurity, and asphyxia are consistently reported as the top three causes of neonatal deaths (Assa et al., 2020). The growing burden of antimicrobial resistance (AMR) complicates the management of neonatal sepsis. AMR reduces the effectiveness of standard empirical antibiotic regimens and is also associated with prolonged hospitalization, higher costs, and increased mortality (GBD 2021 Antimicrobial Resistance Collaborators, 2024; Interagency Coordination Group on Antimicrobial Resistance, 2019; Siahaan et al., 2022).

Extended-spectrum beta-lactamase (ESBL)-producing Gram-negative bacteria have emerged as important causes of neonatal bloodstream infections in many regions (Castanheira et al., 2021; Herindrainy et al., 2018; Khaertynov et al., 2018). *Klebsiella pneumoniae* and *Escherichia coli* are particularly implicated. ESBL enzymes hydrolyze most beta-lactam antibiotics, including third-generation cephalosporins (Castanheira et al., 2021; Doi et al., 2017), leading to limited therapeutic options and an increased reliance on carbapenems as last-resort agents (Castanheira et al., 2021). Several studies from neonatal intensive care units (NICUs) in Asia and Africa have reported a high proportion of ESBL-producing isolates among neonates with sepsis, with prevalence rates exceeding 40-70% (Geleta et al., 2024; Lai et al., 2021; Mohakud et al., 2022). In Indonesia, a community-based study reported a substantial rate of gut colonization by ESBL-producing *Escherichia coli* in children in Surabaya (Happy et al., 2020). Several studies have identified risk factors for ESBL-producing organisms in neonatal sepsis, although they have been conducted only in Java (Miranda et al., 2024; Ratridewi et al., 2023).

Neonates are particularly vulnerable to ESBL bacteria. This vulnerability stems from their immature immune systems, frequent exposure to invasive procedures, and prolonged hospital stays (De Rose et al., 2024; Kariniotaki et al., 2025). Previous studies have identified several potential risk factors for ESBL-producing infections in neonates, including low birth weight, prematurity, prolonged NICU stay, prior antibiotic exposure, and the presence of central venous catheters (Li et al., 2017; Yan et al., 2025). However, most available data, including those from Indonesia (Miranda et al., 2024), are from large tertiary centers in urban areas. These data may not reflect the epidemiology and risk factor patterns in resource-limited hospitals such as those in eastern Indonesia (Limato et al., 2022; Siahaan et al., 2022). Understanding local risk factors is crucial for tailoring infection prevention and control strategies as well as for optimizing empirical antibiotic policies in neonatal units (Interagency Coordination Group on Antimicrobial Resistance, 2019; Kementerian Kesehatan Republik Indonesia, 2021a, 2021b; World Health Organization, 2025).

East Lombok is a district in West Nusa Tenggara Province with limited neonatal care resources (Dinas Kesehatan Kabupaten Lombok Timur, 2023). Neonates with suspected sepsis from across the district are concentrated in a single referral hospital. To our knowledge, no previous study has specifically examined the burden and risk factors for ESBL-producing bacteria in neonates in this setting. Therefore, this study aimed to determine the proportion of ESBL production among eligible Gram-negative neonatal isolates treated at a referral hospital in East Lombok, Indonesia, and to analyze the association of demographic and clinical characteristics with ESBL positivity in this population. We hypothesized that neonatal age is strongly associated with ESBL positivity.

Materials and Methods

Study Design and Setting

This was a retrospective cross-sectional study using secondary data (Sastroasmoro & Ismael, 2016). The study was conducted in the neonatal care unit, including the neonatal ward, high care unit, and NICU, of a referral hospital in East Lombok, West Nusa Tenggara, Indonesia. Data were collected from January 2023 to December 2024.

Study Population and Sampling

The study population consisted of all neonates admitted to the neonatal care unit during the study period who underwent microbiological culture examinations. Data were obtained from medical records and microbiology laboratory databases using a total sampling method (Sastroasmoro & Ismael, 2016). No formal sample size calculation was performed. The inclusion criteria were neonates aged 0-28 days with at least one positive culture result. During the study period, 437 neonatal records that met the criteria were screened for inclusion. A total of 320 records were excluded: incomplete medical record data or incomplete microbiology results (n=23), mixed growth of two bacterial species (n=19), Gram-positive isolates (n=250), and isolates of species not recognized as potential ESBL producers (n=28). This left 117 neonates for the final analysis (Figure 1).

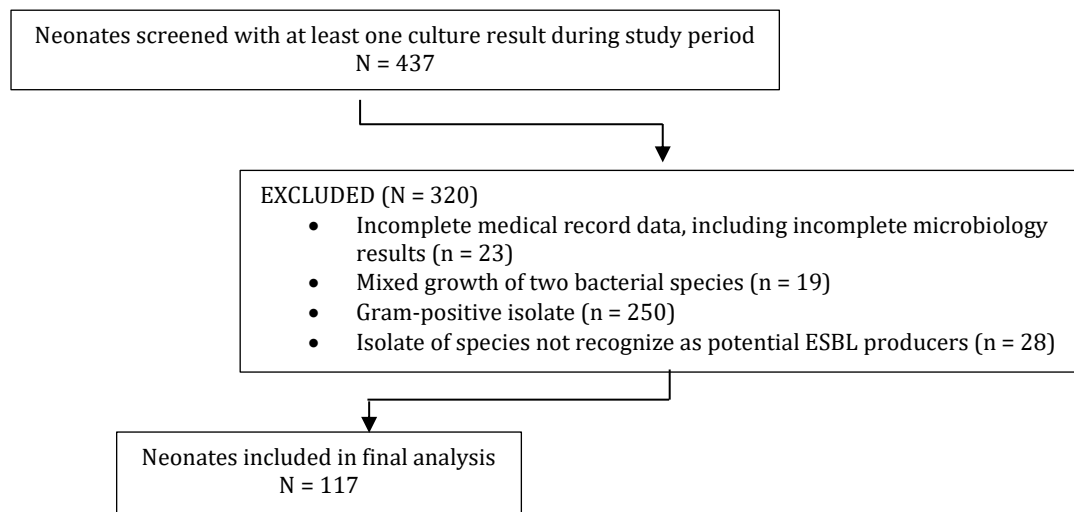


Figure 1. Flow diagram of neonatal selection for ESBL analysis

Variables and Definitions

Demographic variables included sex and age. Age at the time of culture was categorized as early neonates (0-7 days) and late neonates (8-28 days) (Pathirana et al., 2016; Shifera et al., 2023). Clinical variables included gestational age at birth and birth weight. Gestational age was categorized as preterm (<37 weeks) or term (≥37 weeks). Birth weight was categorized as low birth weight (LBW, <2,500 g) and normal birth weight (NBW, ≥2,500 g). These cut-offs are commonly used in neonatal studies (Assa et al., 2020; Kariniotaki et al., 2025; Miranda et al., 2024). Microbiological variables included bacterial species and ESBL status. Bacterial isolates were categorized into Enterobacteriaceae (*Klebsiella pneumoniae*, *Serratia marcescens*, *Escherichia coli*, *Enterobacter cloacae* complex) and non-Enterobacteriaceae, following previous studies on neonatal ESBL infections (Barbosa et al., 2025; Sau et al., 2025; Yan et al., 2025). For regression analysis, age was coded as 1 for early neonates and 0 for late neonates; sex as 1 for males and 0 for females; gestational age at birth as 1 for preterm and 0 for term; and birth weight as 1 for low birth weight and 0 for normal birth weight.

Microbiological Procedures

Microbiological cultures and antimicrobial susceptibility testing were performed in the hospital microbiology laboratory using the VITEK 2 Compact automated system, following standard laboratory procedures. Clinical specimens included blood, pus, swabs, and urine samples. Blood samples were incubated until bacterial growth was detected, then subcultured on solid media such as blood agar and MacConkey agar. Pus and swab samples were directly inoculated onto solid media.

All plates were incubated at 37 °C for 18-24 h and observed for bacterial growth. The isolates were subsequently processed for species identification and antimicrobial susceptibility testing using the same system. ESBL positivity was defined as a culture-confirmed bacterial isolate identified as ESBL-producing using this system, following the previously described criteria for ESBL detection (Castanheira et al., 2021).

Data Collection and Statistical Analysis

Data were extracted from the hospital's information system and entered into a structured electronic data collection form. Descriptive statistics summarized patient characteristics and microbiological findings as frequency and percentage. Bivariate associations between categorical variables (sex, age group, gestational age at birth, and birth weight) and ESBL status were assessed using the chi-square test (Sastroasmoro & Ismael, 2016). Neonatal age was the primary exposure of interest. Sex, gestational age at birth, and birth weight were included a priori as potential confounders based on their clinical relevance, not as competing predictors. All four candidate variables were then entered simultaneously into a multivariable binomial logistic regression model. Results are presented as odds ratios (ORs) with 95% confidence intervals (CIs). Statistical significance was set at $p < 0.05$. All analyses were performed using jamovi software version 2.6 (The jamovi project, 2024). Potential selection bias was minimized by sampling all eligible neonates, and information bias was minimized by cross-referencing medical records with microbiology laboratory databases. The study protocol was approved by the Health Research Ethics Committee of the study hospital in September 2025 (approval number 002/EC/KEPKRSU/IX/2025). Because this was a retrospective study using secondary data, informed consent was waived by the ethics committee.

Results and Discussions

A total of 117 neonates were included in the final analysis, with no missing data among the analyzed variables (Figure 1). Baseline characteristics are summarized in Table 1. The study population was relatively balanced in terms of sex and age distribution, with a modest predominance of males and late neonates. ESBL-producing isolates were identified in 56 (47.9%) of the 117 neonates. Most isolates belonged to the Enterobacteriaceae group (73.5%), predominantly *Klebsiella pneumoniae* (38.5%) and *Serratia marcescens* (23.1%) (Table 2). However, ESBL positivity was highest among *Serratia marcescens* isolates (77.8%) compared to *Klebsiella pneumoniae* isolates (44.4%). Among non-Enterobacteriaceae, ESBL positivity ranged from 25.0% to 33.3% across all tested species (Table 2).

Table 1. Baseline characteristics of neonatal subjects (n=117)

Variable	n	%
Sex		
Male	64	54.7
Female	53	45.3
Age group		
Early neonates (0–7 days)	57	48.7
Late neonates (8–28 days)	60	51.3
Gestational age at birth		
Preterm (<37 weeks)	61	52.1
Term (≥37 weeks)	56	47.9
Birth weight		
Low birth weight (<2,500 g)	52	44.4
Normal birth weight (≥2,500 g)	65	55.6

Variable	n	%
ESBL status		
ESBL	56	47.9
Non-ESBL	61	52.1

ESBL: extended-spectrum beta-lactamase.

Table 2. Distribution of ESBL positivity by bacterial species (n=117)

Species	Total isolates, n	ESBL-positive, n (%)
Enterobacteriaceae		
<i>Klebsiella pneumoniae</i>	45	20 (44.4)
<i>Serratia marcescens</i>	27	21 (77.8)
<i>Escherichia coli</i>	10	4 (40.0)
<i>Enterobacter cloacae</i> complex	4	2 (50.0)
Non-Enterobacteriaceae		
<i>Acinetobacter baumannii</i>	12	3 (25.0)
<i>Pseudomonas aeruginosa</i>	12	4 (33.3)
<i>Acinetobacter lwoffii</i>	3	1 (33.3)
<i>Pseudomonas stutzeri</i>	3	1 (33.3)
<i>Acinetobacter junii</i>	1	0 (0.0)

ESBL: extended-spectrum beta-lactamase.

Bivariate analysis showed that age was the only variable significantly associated with ESBL positivity ($\chi^2=58.8$; $p<0.001$) (Table 3). Early neonates had markedly higher odds of ESBL positivity than late neonates (crude OR 34.7; 95% CI 12.4-97.1). No significant associations were found for sex ($p=0.814$), gestational age at birth ($p=0.942$), or birth weight ($p=0.967$). In the multivariable analysis, early neonates had significantly higher adjusted odds of ESBL positivity than late neonates (adjusted OR 49.40; 95% CI 14.86-164.17; $p<0.001$) (Table 4). This model controlled for sex, gestational age at birth, and birth weight. The overall model was statistically significant ($\chi^2=68.8$; $df=4$; $p<0.001$), with a Nagelkerke R^2 of 0.593 (Table 5).

Table 3. Bivariate analysis of the association between risk factors and ESBL positivity in neonates

Variable	χ^2	df	p-value	OR	95% CI
Sex	0.055	1	0.814	1.09	0.53-2.26
Age (early vs late)	58.8	1	<0.001	34.7	12.4-97.1
Gestational age at birth	0.005	1	0.942	0.97	0.47-2.04
Birth weight	0.002	1	0.967	0.99	0.48-2.04

ORs are presented for male vs female, early vs late neonates, preterm vs term, and low vs normal birth weight. CI: confidence interval.

Table 4. Multivariable logistic regression model coefficients of risk factors for ESBL positivity in neonates

Predictor	β	p-value	OR	95% CI
Neonatal age (early vs late)	3.900	<0.001	49.40	14.86-164.17
Sex (male vs female)	1.044	0.087	2.84	0.86-9.37
Gestational age (preterm vs term)	0.109	0.875	1.12	0.28-4.37

Predictor	β	p-value	OR	95% CI
Birth weight (low vs normal)	0.228	0.744	1.26	0.32–4.95
Intercept	2.258	<0.001	–	–

Age coded as 1=early, 0=late; sex 1=male, 0=female; gestational age 1=preterm, 0=term; birth weight 1=low, 0=normal. The intercept represents the baseline log-odds when all predictors equal zero and cannot be interpreted as an odds ratio.

Table 5. Goodness-of-fit test of the multivariable logistic regression model for ESBL positivity in neonates

Null deviance	Residual deviance	χ^2	df	p-value	Nagelkerke R ²	N
162.0	93.2	68.8	4	<0.001	0.593	117

Model χ^2 tests the fitted model against the null (intercept-only) model.

Discussion

ESBL Prevalence and Predominant Pathogens

In this study, nearly half of the neonatal bacterial isolates were ESBL-producing, and early neonatal age was the strongest independent predictor of ESBL positivity. *Klebsiella pneumoniae* and *Serratia marcescens* were the predominant pathogens (Barbosa et al., 2025; Sau et al., 2025). *Klebsiella pneumoniae* has also been reported as a major multidrug-resistant pathogen in NICU settings of other resource-limited countries, frequently harboring both resistance and virulence determinants (Shrestha et al., 2024). The high proportion of ESBL positivity in our study confirms the critical role of ESBL-producing Gram-negative organisms in neonatal infections in our setting, consistent with reports from other NICUs in low- and middle-income countries where ESBL prevalence among neonatal isolates has been reported to be 40% or higher (Mohakud et al., 2022). Neonatal sepsis, including infections caused by multidrug-resistant organisms, remains a significant clinical challenge in Indonesian hospitals (Assa et al., 2020; Miranda et al., 2024). These findings underscore the need for continuous local surveillance of antibiotic resistance patterns. Furthermore, caution must be exercised when interpreting ESBL positivity rates among species with extremely limited sample sizes. For instance, *Acinetobacter junii* was represented by only a single isolate (0.0%), while *Acinetobacter lwoffii* and *Pseudomonas stutzeri* had only three isolates each in this study (Table 2)1. These small subsets are presented strictly for descriptive completeness and are insufficient to draw robust epidemiological conclusions or establish generalized resistance trends.

Early Neonatal Age as a Risk Factor

Early neonates (0–7 days) demonstrated a remarkably strong independent association with ESBL positivity compared to late neonates (8–28 days), (adjusted OR 49.40; 95% CI 14.86–164.17). However given the retrospective cross-sectional design of this study, this high odds ratio must not be interpreted as absolute risk or as definitive evidence of a causal relationship, rather, it highlights early age as a critical clinical marker strongly associated with ESBL carriage. This finding may reflect maternal or perinatal colonization by ESBL-producing organisms during the early neonatal period, consistent with previous studies showing that maternal ESBL colonization is an important predictor of neonatal ESBL acquisition and infection (Herindrainy et al., 2018; Marando et al., 2018). In Indonesia, ESBL-producing organisms have also been detected in the intestinal colonization of neonates and young children, supporting the plausibility of early perinatal acquisition in this setting (Happy et al., 2020). Possible contributing factors include antenatal antibiotic exposure or healthcare contact during pregnancy and delivery, whereas prolonged postnatal exposure to the hospital environment appears to be an unlikely explanation. In contrast, an Indonesian study of late-onset

ESBL sepsis in preterm infants pointed to nosocomial factors rather than early neonatal age, with invasive procedures, central access insertion, and parenteral nutrition as the main predictors (Ratridewi et al., 2023). The contrast with these late-onset nosocomial predictors further supports perinatal acquisition in our early neonates (Salsabila et al., 2022). This distinction is likely shaped by the unique clinical setting and referral system of our study site. In East Lombok, a district characterized by limited neonatal care resources, neonates with suspected sepsis from across the entire region are concentrated in a single referral hospital (Dinas Kesehatan Kabupaten Lombok Timur, 2023). Unlike large tertiary centers in urban areas of Java that possess advanced neonatal intensive care units where prolonged, device-associated nosocomial infections predominate (Miranda et al., 2024). Our resource-constrained referral setting experiences a high concentration of early-admission cases. Consequently, maternal or perinatal transmission during delivery emerges as a more immediate exposure in our population than long-term, hospital-acquired colonization. Data on antimicrobial resistance patterns in neonates with early-onset sepsis remain scarce in Indonesia, where limited laboratory resources have constrained the investigation of multidrug-resistant organisms in this population neonates (Salsabila et al., 2022).

Other Demographic and Clinical Variables

In the multivariable model, sex showed a non-significant trend toward higher adjusted odds of ESBL positivity in males. This shifted from a null crude association. The change suggests possible confounding or suppression once age was included. Gestational age at birth and birth weight remained non-significant in both crude and adjusted analyses. Several studies have reported prematurity and low birth weight as important risk factors for neonatal sepsis, including infections caused by multidrug-resistant organisms (Assa et al., 2020; Kariniotaki et al., 2025; Yan et al., 2025). However, the lack of independent associations in our study may reflect the strong effect of age. This effect may partly capture the influence of maternal or perinatal exposures more directly than gestational age or birth weight. The relatively small sample size may also have limited our ability to detect subtle effects (Sastroasmoro & Ismael, 2016).

Clinical and Infection Control Implications

These findings have important clinical implications for neonatal care, particularly for infection prevention and control. The high proportion of ESBL-producing pathogens and the markedly increased risk among early neonates highlight the potential value of maternal and perinatal infection prevention strategies. These may include antenatal screening for ESBL colonization where feasible. *Klebsiella pneumoniae* was numerically more common, but ESBL positivity was disproportionately higher among *Serratia marcescens* isolates (77.8% vs 44.4%). This species is intrinsically resistant to several beta-lactam antibiotics and is known to persist in hospital environment reservoirs. Documented outbreaks of ESBL-producing organisms, including this species, highlight similar risk (Montagnani et al., 2015; Stapleton et al., 2016). Infection control efforts in our setting may therefore benefit from species-specific environmental surveillance that targets potential reservoirs. Strengthening hand hygiene practices in the NICU remains essential. A modelling study of household transmission found hand hygiene improvement more effective than antibiotic restriction in reducing ESBL-*E. coli* spread (Kardaś-Słoma et al., 2020). The same principle applies to neonatal units.

Antimicrobial Stewardship

Antimicrobial stewardship is equally important. Empirical antibiotic policies for suspected early-onset sepsis should consider the local ESBL burden, balancing adequate coverage with the risk of further resistance (GBD 2021 Antimicrobial Resistance Collaborators, 2024). Newer agents such as ceftazidime-avibactam have shown promise in managing complicated infections caused by ESBL-producing organisms in pediatric populations. However, their availability remains limited in

resource-constrained settings such as ours (Bassetti et al., 2020). Regular surveillance of pathogen distribution and resistance patterns is essential to update empirical treatment protocols. This remains challenging in resource-limited Indonesian hospitals, where barriers to antimicrobial stewardship are well documented (Limato et al., 2022). Continued research on novel therapeutic strategies is critical to address the growing challenge of multidrug-resistant organisms (Elshobary et al., 2025).

Strength and Limitation

The strength of this study is that it provides the first baseline data on the burden and risk factors of ESBL-producing bacteria among neonates in a resource-limited referral hospital in eastern Indonesia. This setting is under-represented in the existing literature. The use of total sampling of all eligible neonates minimized selection bias, and cross-referencing medical records with microbiology laboratory databases minimized information bias. This study also had several limitations. First, its retrospective design depended on the completeness of medical records. Second, ESBL status was determined solely using the automated VITEK 2 system. Although this system has demonstrated high sensitivity and specificity for ESBL detection (Castanheira et al., 2021), the absence of an additional confirmatory phenotypic test cannot fully exclude misclassification. Third, the single-center design limits generalizability. Because only Gram-negative isolates were included, our findings apply to the Gram-negative fraction and should not be extrapolated to all-cause neonatal sepsis. Finally, although the association between early neonatal age and ESBL positivity was robust to adjustment, its confidence interval was wide. Specifically, the extremely wide 95% Confidence Interval (14.86–164.17) is a mathematical consequence of a very large effect size (adjusted OR 49.40) evaluated within our modest clinical sample of 117 neonates. While this wide range points to a strong direction of risk, the lower statistical precision means the exact magnitude of the effect remains uncertain and must be interpreted with caution. These wide estimates warrant confirmation in larger studies (De Rose et al., 2024; Li et al., 2017; Shrestha et al., 2024).

Conclusion

ESBL-producing Gram-negative bacteria were common among neonates in a referral hospital in East Lombok, Indonesia, accounting for almost half of all isolates. Early neonatal age was the only independent factor significantly associated with ESBL positivity, suggesting that maternal or perinatal colonization may play an important role in acquiring these pathogens. Given the observational nature of this study, these findings indicate a strong clinical linkage rather than a direct causal pathway. These findings highlight the need to strengthen infection prevention and control strategies and reinforce antimicrobial stewardship in this resource-limited setting. In particular, hospital infection prevention teams and clinicians in comparable neonatal units should heighten vigilance for ESBL-producing organisms during the early neonatal period and adopt judicious empirical antibiotic use. Future multicenter studies incorporating maternal colonization data are recommended to confirm these findings and inform targeted prevention strategies.

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