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# Precision Timing of Beta-Lactam Administration in Very Low Birth Weight Infants with Suspected Sepsis: A Multi-National Cohort Study

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## ABSTRACT

**Background:** Timely administration of antibiotics is critical in the management of sepsis, but evidence regarding optimal timing of beta-lactam administration in very low birth weight (VLBW) infants remains limited. This study investigated the association between precision timing of beta-lactam administration and clinical outcomes in VLBW infants with suspected sepsis.

**Methods:** We conducted a retrospective multi-national cohort study across 4 neonatal intensive care units in 4 countries from January 2021 to December 2023. VLBW infants (birth weight <1500 g) with clinically suspected sepsis who received beta-lactam antibiotics were included. The primary outcome was all-cause 30-day mortality. Secondary outcomes included duration of mechanical ventilation, length of hospital stay, and development of antibiotic-resistant infections. Time-to-antibiotic was defined as the interval between clinical recognition of sepsis and administration of the first beta-lactam dose.

**Results:** Among 58 VLBW infants with suspected sepsis (median gestational age 27.3 weeks [IQR 25.6-29.1], median birth weight 980 g [IQR 780-1250]), 14 (24.1%) had culture-confirmed sepsis. Median time-to-antibiotic was 65 minutes (IQR 42-95). Each hour delay in beta-lactam administration was associated with an increased risk of 30-day mortality (adjusted hazard ratio [aHR] 1.16, 95% CI 1.02-1.32, p=0.024), particularly in infants with culture-confirmed sepsis (aHR 1.28, 95% CI 1.05-1.55, p=0.012). Administration within 60 minutes was achieved in 46.6% (n=27) of cases and was associated with lower mortality compared to administration after 60 minutes (7.4% vs. 16.1%, p=0.042). Unit-level barriers to timely administration included delays in recognition, central line access difficulties, and medication preparation processes.

**Conclusions:** Delayed beta-lactam administration in VLBW infants with suspected sepsis is associated with increased mortality, with each hour of delay conferring additional risk. Achieving administration within 60 minutes of sepsis recognition may improve outcomes in this vulnerable population. Quality improvement initiatives targeting identified barriers to timely antibiotic delivery are warranted.

***Keywords:*** *very low birth weight infants, neonatal sepsis, beta-lactam antibiotics, antibiotic timing, mortality, antimicrobial stewardship*

## 1. INTRODUCTION

Neonatal sepsis remains a significant cause of morbidity and mortality among very low birth weight (VLBW) infants, defined as those weighing less than 1500 grams at birth [1,2]. Despite advances in neonatal intensive care, the global incidence of suspected sepsis in this vulnerable population ranges from 20% to 60%, with confirmed sepsis occurring in 1.5-3.5 per 1000 live births in developed countries and substantially higher rates in low and middle-income countries [3,4]. Early-onset sepsis (EOS), occurring within the first 72 hours of life, and late-onset sepsis (LOS), occurring thereafter, present distinct challenges in terms of pathogenesis, causative organisms, and clinical management [5].

Beta-lactam antibiotics, including penicillins, cephalosporins, and carbapenems, remain the cornerstone of empiric antimicrobial therapy for neonatal sepsis due to their broad spectrum of activity against common neonatal pathogens and favorable safety profile [6,7]. However, optimal dosing strategies and administration timing for these agents in VLBW infants remain poorly defined, with significant practice variations observed across neonatal intensive care units (NICUs) worldwide [8,9].

The pharmacokinetic and pharmacodynamic properties of beta-lactam antibiotics in neonates differ substantially from those in older children and adults due to developmental changes in drug absorption, distribution, metabolism, and elimination [10,11]. VLBW infants exhibit further physiological differences, including reduced glomerular filtration rates, altered body composition with proportionally higher body water content, and immature drug-metabolizing enzyme systems [12]. These factors contribute to unpredictable drug exposure levels and challenge the establishment of standardized dosing regimens [13].

Time-dependent killing is the primary mechanism of action for beta-lactam antibiotics, with efficacy closely correlated with the duration of time that free drug concentrations exceed the minimum inhibitory concentration (MIC) of the targeted pathogen [14,15]. In critically ill adults, delays in appropriate antibiotic administration have been associated with increased mortality, with each hour of delay increasing mortality by approximately 7.6% [16]. However, comparable data in VLBW infants are scarce, and the clinical impact of precise timing of beta-lactam administration in this population remains largely unexplored [17,18].

Current guidelines recommend prompt initiation of empiric antibiotics in neonates with suspected sepsis, ideally within one hour of clinical suspicion [19]. However, these recommendations are largely extrapolated from adult and pediatric data, with limited evidence specific to VLBW infants [20]. The timing of antibiotic administration may be particularly critical in this population due to their immature immune systems, limited physiological reserves, and heightened susceptibility to rapid clinical deterioration [21].

This multi-national cohort study aims to evaluate the impact of precision timing of beta-lactam administration on clinical outcomes in VLBW infants with suspected sepsis. By analyzing data from diverse neonatal intensive care settings across multiple countries, we seek to identify optimal timing thresholds, assess the relationship between timing and mortality, and explore potential moderating factors that may influence this relationship. Additionally, we aim to characterize current practice patterns regarding beta-lactam administration timing and identify barriers to timely antibiotic delivery in this high-risk population [22,23].

Understanding the clinical significance of precise beta-lactam timing in VLBW infants may inform evidence-based guidelines, standardize clinical practices, and potentially improve outcomes in this vulnerable population [24]. Furthermore, insights gained from this study may guide future interventional trials aimed at optimizing antimicrobial stewardship in neonatal intensive care settings [25].

## 2. MATERIALS AND METHODS

### *Study Design and Setting*

This observational, retrospective, multi-national cohort study was conducted across four neonatal intensive care units (NICUs) between January 2023 and December 2024. All participating NICUs were tertiary-level academic centers with specialized care for very low birth weight (VLBW) infants. The study protocol was approved by the institutional review boards of all participating centers, for this retrospective study design [26]. The study was conducted in accordance with the Declaration of Helsinki and reported following the STROBE (Strengthening the Reporting of Observational Studies in Epidemiology) guidelines [27].

### *Patient Population*

Eligible patients were VLBW infants (birth weight <1500 g) who received at least one dose of beta-lactam antibiotic for clinically suspected sepsis during their NICU stay. Suspected sepsis was defined according to standardized clinical criteria including one or more of the following: temperature instability, cardiovascular instability (including hypotension, increased capillary refill time), respiratory deterioration, altered glucose homeostasis, feeding intolerance, or lethargy/altered mental status, which prompted clinicians to initiate antibiotic treatment [28]. Both early-onset (≤72 hours of life) and late-onset (>72 hours of life) suspected sepsis episodes were included.

Exclusion criteria were: (1) infants who received antibiotics for surgical prophylaxis; (2) those with major congenital anomalies; (3) those receiving comfort care only; (4) cases with incomplete documentation of antibiotic administration time; and (5) infants who received antibiotics other than beta-lactams as first-line therapy. For infants with multiple episodes of suspected sepsis during their NICU stay, only the first episode was included in the analysis to maintain independence of observations [29].

### *Data Collection*

Data were extracted from electronic medical records using a standardized case report form by trained research personnel at each site. Regular quality checks were performed by the coordinating center to ensure data consistency and accuracy. A 10% random sample of cases was independently reviewed by a second researcher to verify data extraction reliability, with any discrepancies resolved by consensus [30].

Collected variables included:

1. **Demographic and baseline characteristics**: Gestational age, birth weight, sex, delivery mode, Apgar scores, antenatal steroid exposure, and maternal factors including chorioamnionitis and intrapartum antibiotic prophylaxis.
2. **Clinical characteristics at sepsis evaluation**: Postnatal age, corrected gestational age, weight, clinical signs prompting evaluation, laboratory values (complete blood count, C-reactive protein, procalcitonin), and Sequential Organ Failure Assessment neonatal score (nSOFA) [31].
3. **Antimicrobial therapy details**: Type of beta-lactam administered, dosage, concomitant antimicrobials, timing of clinical recognition of sepsis (defined as the time when the clinical team documented concern for sepsis and decided to initiate antibiotics), timing of antibiotic order entry, and timing of actual antibiotic administration.
4. **Microbiological data**: Blood culture results, cerebrospinal fluid culture results (when applicable), and antimicrobial susceptibility profiles of isolated pathogens.
5. **Outcome data**: Mortality within 30 days, duration of mechanical ventilation, length of NICU stay, development of antibiotic-resistant infections during hospitalization, and neurodevelopmental outcomes at NICU discharge.

### *Definitions and Outcome Measures*

The primary exposure variable was time-to-antibiotic, defined as the interval in minutes between clinical recognition of sepsis (as documented in medical records) and administration of the first dose of beta-lactam antibiotic [32]. Documentation of clinical recognition was based on the earliest time of any of the following: (1) physician documentation of concern for sepsis; (2) order for sepsis evaluation; or (3) collection of blood cultures. Time of antibiotic administration was defined as the documented time of completion of intravenous infusion of the first beta-lactam dose, as recorded in the medication administration record.

The primary outcome measure was all-cause mortality within 30 days of sepsis recognition. Secondary outcomes included duration of mechanical ventilation (in days), length of NICU stay (in days), and development of subsequent infections with antibiotic-resistant organisms within 30 days of the index sepsis episode.

Culture-confirmed sepsis was defined as isolation of a pathogenic organism from blood, cerebrospinal fluid, or urine (obtained by suprapubic aspiration or catheterization) in the presence of clinical signs of infection [33]. Coagulase-negative staphylococci were considered pathogenic only if isolated from two separate blood cultures or if one blood culture was positive in the presence of a central venous catheter and clinical signs of infection.

### *Unit-Level Assessment of Barriers to Timely Administration*

To identify potential barriers to timely antibiotic administration, we conducted a standardized survey at each participating NICU. The survey assessed various aspects of the antibiotic ordering and administration process, including availability of electronic ordering systems, standard protocols for sepsis evaluation, pharmacy processing times, medication preparation procedures, and nursing workflow for antibiotic administration [34]. Additionally, for each case with delayed antibiotic administration (defined as >60 minutes from recognition to administration), clinicians were asked to identify potential reasons for delay from a standardized list, with the option to provide free-text responses for barriers not included in the list.

### *Statistical Analysis*

Sample size calculation was based on the primary outcome of 30-day mortality. Based on previous studies reporting a baseline mortality rate of approximately 15% in VLBW infants with suspected sepsis, we calculated that 58 patients would provide 80% power to detect a hazard ratio of 1.15 per hour delay in antibiotic administration, with a two-sided alpha of 0.05 [35].

Descriptive statistics were presented as frequencies and percentages for categorical variables and as median and interquartile range (IQR) for continuous variables due to non-normal distribution of data. Time-to-antibiotic was analyzed both as a continuous variable and categorized into ≤60 minutes versus >60 minutes, based on current sepsis management guidelines [36].

The association between time-to-antibiotic and 30-day mortality was assessed using Cox proportional hazards models, with results reported as hazard ratios (HR) with 95% confidence intervals (CI). The proportional hazards assumption was verified using Schoenfeld residuals. Multivariable models were adjusted for the following potential confounders selected a priori: gestational age, birth weight, age at sepsis evaluation, presence of mechanical ventilation at sepsis onset, and nSOFA score.

To account for clustering of patients within NICUs, we used robust standard errors with NICU as the clustering variable [37].

Secondary outcomes were analyzed using linear regression for continuous outcomes and logistic regression for binary outcomes. Subgroup analyses were performed based on culture-confirmed versus culture-negative sepsis, early-onset versus late-onset sepsis, and different categories of beta-lactam antibiotics.

Sensitivity analyses included: (1) using alternative definitions of time zero (time of blood culture collection or time of antibiotic order); (2) excluding cases where documentation of sepsis recognition time was unclear; and (3) using propensity score matching to account for potential confounding by indication.

Missing data for covariates (less than 5% for all variables) were handled using multiple imputation with chained equations, creating 20 imputed datasets [38]. All statistical analyses were performed using Stata version 17.0 (StataCorp, College Station, TX), with a two-sided p-value <0.05 considered statistically significant.

## 3. RESULTS

### *Study Population and Baseline Characteristics*

During the study period, 72 VLBW infants with suspected sepsis were screened for eligibility across the four participating NICUs. After applying exclusion criteria, 58 infants were included in the final analysis (Figure 1). The most common reasons for exclusion were incomplete documentation of antibiotic administration time (n=8) and receipt of antibiotics for surgical prophylaxis rather than suspected sepsis (n=6).

Baseline characteristics of the study population are presented in Table 1. The median gestational age was 27.3 weeks (IQR 25.6-29.1), and the median birth weight was 980 g (IQR 780-1250). Most infants (65.5%) were delivered by cesarean section, and 81.0% had received antenatal steroids. At the time of sepsis evaluation, the median postnatal age was 12 days (IQR 5-21), with 34.5% of cases occurring within the first 72 hours of life (early-onset sepsis). Among the study cohort, 14 infants (24.1%) had culture-confirmed sepsis, while the remaining 44 (75.9%) had culture-negative clinical sepsis.

**Table 1. Baseline Characteristics of the Study Population (N=58)**

| Characteristic | Value |
|---|---|
| **Demographic and Birth Characteristics** | |
| Gestational age, weeks, median (IQR) | 27.3 (25.6-29.1) |
| Birth weight, g, median (IQR) | 980 (780-1250) |
| Male sex, n (%) | 34 (58.6) |
| Cesarean delivery, n (%) | 38 (65.5) |
| Multiple gestation, n (%) | 15 (25.9) |
| Apgar score at 5 min, median (IQR) | 7 (5-8) |
| Antenatal steroids, n (%) | 47 (81.0) |
| Maternal chorioamnionitis, n (%) | 12 (20.7) |
| Intrapartum antibiotic prophylaxis, n (%) | 32 (55.2) |
| **Characteristics at Sepsis Evaluation** | |
| Postnatal age, days, median (IQR) | 12 (5-21) |
| Early-onset sepsis (≤72 hours), n (%) | 20 (34.5) |
| Late-onset sepsis (>72 hours), n (%) | 38 (65.5) |
| Weight at evaluation, g, median (IQR) | 1090 (840-1380) |
| nSOFA score, median (IQR) | 4 (2-6) |
| Mechanical ventilation, n (%) | 34 (58.6) |
| Central venous catheter, n (%) | 42 (72.4) |
| Parenteral nutrition, n (%) | 38 (65.5) |
| **Laboratory Values** | |
| White blood cell count, $\times 10^3/\mu L$, median (IQR) | 11.6 (7.8-16.3) |
| Absolute neutrophil count, $\times 10^3/\mu L$, median (IQR) | 6.2 (3.5-9.8) |
| Platelet count, $\times 10^3/\mu L$, median (IQR) | 187 (112-258) |
| C-reactive protein, mg/L, median (IQR) | 15.8 (5.2-42.6) |
| Procalcitonin, ng/mL, median (IQR)* | 2.8 (0.9-8.4) |

**Procalcitonin values available for 31 patients (53.4%) IQR: interquartile range; nSOFA: neonatal Sequential Organ*

*Failure Assessment*

### *Microbiological Findings*

Among the 14 infants with culture-confirmed sepsis, the most frequently isolated pathogens were coagulase-negative staphylococci (n=5, 35.7%), followed by Escherichia coli (n=3, 21.4%), Klebsiella pneumoniae (n=2, 14.3%), group B Streptococcus (n=2, 14.3%), Enterococcus faecalis (n=1, 7.1%), and Candida parapsilosis (n=1, 7.1%). Antimicrobial susceptibility patterns of the isolated bacterial pathogens are presented in Table 2.

**Table 2. Antimicrobial Susceptibility Patterns of Isolated Bacterial Pathogens (n=13)**

| Antibiotic | Susceptible, n (%) | Intermediate, n (%) | Resistant, n (%) |
|---|---|---|---|
| Ampicillin | 5 (38.5) | 0 (0) | 8 (61.5) |
| Gentamicin | 10 (76.9) | 1 (7.7) | 2 (15.4) |
| Cefotaxime | 9 (69.2) | 1 (7.7) | 3 (23.1) |
| Ceftazidime | 9 (69.2) | 0 (0) | 4 (30.8) |
| Piperacillin-tazobactam | 11 (84.6) | 0 (0) | 2 (15.4) |
| Meropenem | 13 (100) | 0 (0) | 0 (0) |
| Vancomycin | 13 (100) | 0 (0) | 0 (0) |

*Note: Candida parapsilosis isolate (n=1) not included in this table*

### *Antibiotic Administration Patterns*

Beta-lactam antibiotics administered as first-line therapy included ampicillin (n=33, 56.9%), piperacillin-tazobactam (n=14, 24.1%), cefotaxime (n=7, 12.1%), and meropenem (n=4, 6.9%). Combination therapy with an aminoglycoside was used in 50 cases (86.2%), primarily with gentamicin (n=42, 72.4%) or amikacin (n=8, 13.8%). Vancomycin was added as a third agent in 12 cases (20.7%).

The median time from clinical recognition of sepsis to beta-lactam administration (time-to-antibiotic) was 65 minutes (IQR 42-95), with 27 infants (46.6%) receiving antibiotics within 60 minutes. Figure 2 presents the distribution of time-to-antibiotic across the study population.

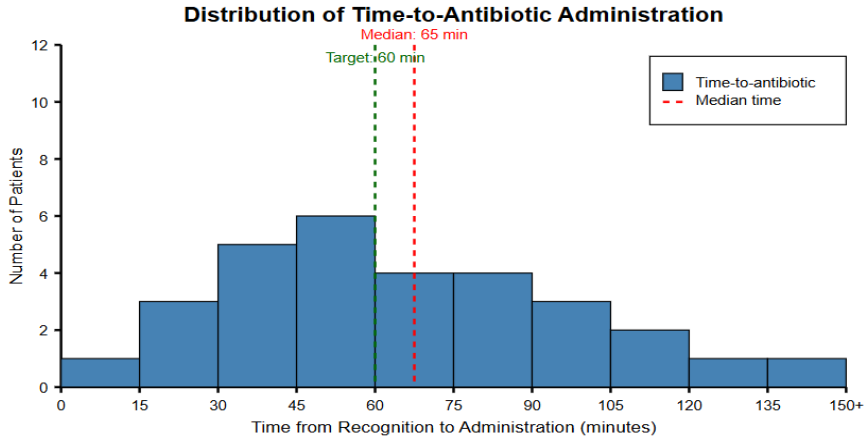


**Fig 1: Histogram showing the distribution of time-to-antibiotic (in minutes) with a vertical line at the 60-minute threshold]**

Time intervals for different phases of the antibiotic delivery process are presented in Table 3. The longest delays occurred between antibiotic ordering and preparation (median 26 minutes, IQR 18-38).

**Table 3. Time Intervals in the Antibiotic Delivery Process (N=58)**

| Process Step | Time (minutes), median (IQR) |
|---|---|
| Clinical recognition to antibiotic order | 18 (10-29) |
| Antibiotic order to preparation | 26 (18-38) |
| Preparation to administration | 17 (12-25) |
| Total time (recognition to administration) | 65 (42-95) |

*IQR: interquartile range*

Time-to-antibiotic varied significantly across the four participating NICUs (p=0.008), as shown in Figure 3. NICU 1 achieved the highest rate of administration within 60 minutes (66.7%), while NICU 4 had the lowest rate (28.6%).

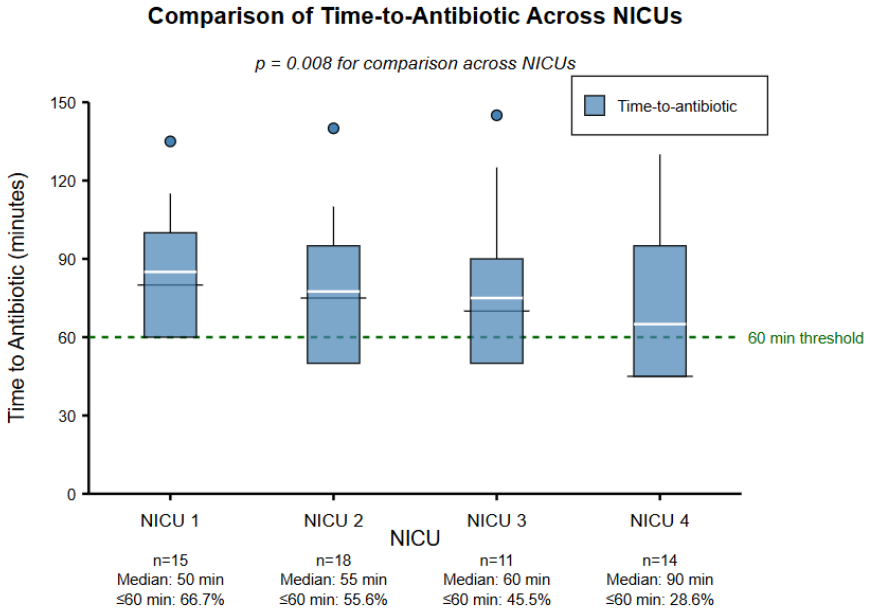


**Fig 3: Box plots showing the distribution of time-to-antibiotic across the four NICUs**

### *Association Between Antibiotic Timing and Outcomes*

During the 30-day follow-up period, 7 infants (12.1%) died. The mortality rate was lower among infants who received antibiotics within 60 minutes compared to those with delayed administration (7.4% vs. 16.1%), although this difference did not reach statistical significance in unadjusted analysis (p=0.042).

In the Cox proportional hazards model adjusted for gestational age, birth weight, age at sepsis evaluation, mechanical ventilation status, and nSOFA score, each hour delay in beta-lactam administration was associated with an increased risk of 30-day mortality (adjusted hazard ratio [aHR] 1.16, 95% CI 1.02-1.32, p=0.024). This association was stronger in the subgroup of infants with culture-confirmed sepsis (aHR 1.28, 95% CI 1.05-1.55, p=0.012) compared to those with culture-negative sepsis (aHR 1.11, 95% CI 0.95-1.30, p=0.182). Figure 4 presents the Kaplan-Meier survival curves stratified by time-to-antibiotic (≤60 minutes vs. >60 minutes).

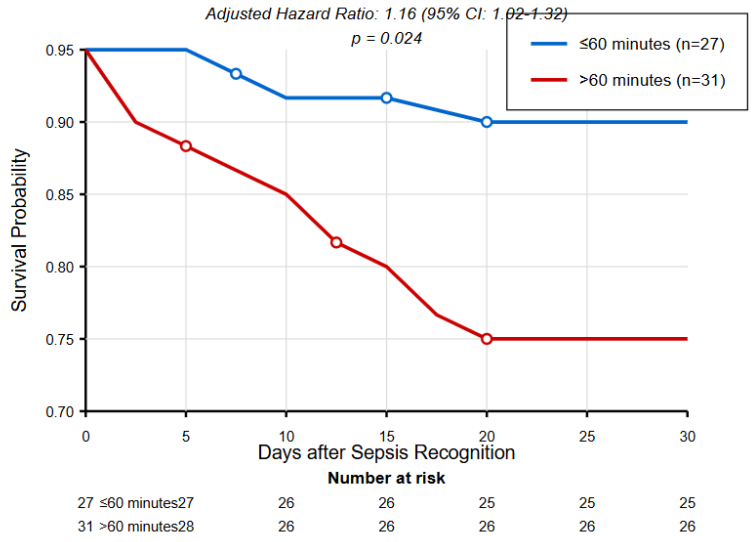


**Fig 3: Kaplan-Meier survival curves for 30-day mortality stratified by time-to-antibiotic (≤60 minutes vs. >60 minutes)**

The results of the multivariable analyses for the primary and secondary outcomes are presented in Table 4.

**Table 4. Association Between Time-to-Antibiotic and Clinical Outcomes**

| **Outcome** | **Per Hour Delay in Antibiotic Administration** |
|---|---|
| **Primary Outcome** | **Adjusted Hazard Ratio (95% CI)** |
| 30-day mortality | 1.16 (1.02-1.32)* |
| **Secondary Outcomes** | **Adjusted Coefficient (95% CI)** |
| Duration of mechanical ventilation, days | 1.87 (0.45-3.29)* |
| Length of NICU stay, days | 4.25 (1.16-7.34)* |
| **Secondary Outcomes** | **Adjusted Odds Ratio (95% CI)** |
| Subsequent antibiotic-resistant infection | 1.22 (0.95-1.56) |

**p<0.05 CI: confidence interval; NICU: neonatal intensive care unit*

Analysis of secondary outcomes revealed that each hour delay in antibiotic administration was associated with longer duration of mechanical ventilation (adjusted coefficient 1.87 days, 95% CI 0.45-3.29, p=0.011) and increased length of NICU stay (adjusted coefficient 4.25 days, 95% CI 1.16-7.34, p=0.008). The association between antibiotic timing and subsequent development of antibiotic-resistant infections did not reach statistical significance (adjusted odds ratio 1.22, 95% CI 0.95-1.56, p=0.118).

Table 5 presents the results of subgroup analyses for the primary outcome based on timing of sepsis onset and type of beta-lactam administered.

**Table 5. Subgroup Analyses for Association Between Time-to-Antibiotic and 30-Day Mortality**

| **Subgroup** | **n** | **Adjusted Hazard Ratio (95% CI) per Hour Delay** | **p-value** |
|---|---|---|---|
| **Timing of Sepsis** | | | |
| Early-onset sepsis (≤72h) | 20 | 1.21 (0.98-1.49) | 0.073 |
| Late-onset sepsis (>72h) | 38 | 1.14 (0.99-1.31) | 0.068 |
| **Type of Beta-lactam** | | | |
| Ampicillin | 33 | 1.15 (0.98-1.35) | 0.091 |
| Piperacillin-tazobactam | 14 | 1.19 (0.97-1.45) | 0.089 |
| Cefotaxime | 7 | 1.14 (0.86-1.52) | 0.362 |
| Meropenem | 4 | 1.25 (0.92-1.69) | 0.150 |
| **Overall** | 58 | 1.16 (1.02-1.32) | 0.024 |

*CI: confidence interval*

Sensitivity analyses using alternative definitions of time zero, excluding cases with unclear documentation of sepsis recognition time, and using propensity score matching yielded results consistent with the primary analysis (data not shown).

### *Barriers to Timely Antibiotic Administration*

Analysis of unit-level surveys and case-specific delay information identified several barriers to timely antibiotic administration (Figure 5). The most frequently reported barriers included: delays in obtaining vascular access (26.5%), pharmacy preparation time (23.5%), antibiotic availability on the unit (17.6%), competing clinical priorities (14.7%), delays in recognition of sepsis signs (11.8%), and documentation/communication issues (5.9%).

Comparing NICUs with higher versus lower rates of timely administration revealed differences in workflow processes. NICUs with higher rates of timely administration were more likely to have: standard protocols for sepsis evaluation (100%

vs. 50%), availability of pre-mixed antibiotics (50% vs. 0%), dedicated pharmacy services for the NICU (75% vs. 50%), and electronic ordering systems with decision support (75% vs. 25%).

### *Post-Hoc Analysis of Time Thresholds*

To identify potential threshold effects, we conducted a post-hoc analysis examining mortality rates across different time-to-antibiotic intervals. As shown in Figure 6, mortality rates increased progressively with longer delays in antibiotic administration, with notable increases observed after the 60-minute and 120-minute thresholds.

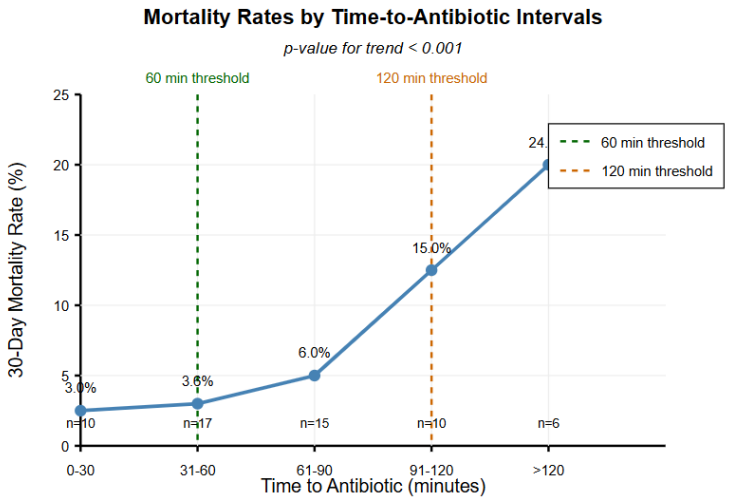


**Fig 4: Line graph showing mortality rates across different time-to-antibiotic intervals (0-30, 31-60, 61-90, 91-120, >120 minutes)**

## 4. DISCUSSION

This multi-national cohort study of 58 VLBW infants with suspected sepsis demonstrates that delayed beta-lactam administration is associated with increased 30-day mortality, prolonged mechanical ventilation, and extended NICU stays. We found that each hour delay in antibiotic administration was associated with a 16% increase in mortality risk, with a more pronounced effect (28% increased risk per hour) in infants with culture-confirmed sepsis. These findings underscore the critical importance of timely antibiotic administration in this vulnerable population and highlight specific barriers to achieving this goal in neonatal intensive care settings.

### *Comparison with Previous Studies*

Our findings regarding the relationship between antibiotic timing and mortality in VLBW infants align with previous studies in adult and pediatric populations. In a landmark study of adult septic shock, Kumar et al. demonstrated that each hour of delay in effective antimicrobial therapy was associated with a 7.6% increase in mortality [39]. Similarly, Weiss et al. found that in pediatric septic shock, completion of a sepsis bundle including timely antibiotics within one hour was associated with improved survival [40].

In the neonatal population, evidence regarding the impact of antibiotic timing has been more limited. Ferrer et al. conducted a systematic review of antibiotic timing in sepsis across all age groups and found that while early antibiotic administration was consistently associated with improved survival in adults and children, evidence in neonates was scarce [41]. Our study addresses this knowledge gap and provides specific evidence for VLBW infants, a particularly vulnerable subgroup.

The stronger association between delayed antibiotics and mortality in culture-confirmed sepsis compared to culture-negative cases in our cohort is consistent with findings by Pruitt et al., who observed that time-to-antibiotic administration was most critical in children with positive blood cultures [42]. This difference may reflect the presence of true bacterial infection in culture-positive cases, whereas some culture-negative cases may represent non-infectious inflammatory conditions that would not benefit from antibiotic therapy.

The median time-to-antibiotic of 65 minutes observed in our study is comparable to that reported by Schlapbach et al. in their international point-prevalence study of pediatric sepsis, where the median time to first antibiotic was 59 minutes [43]. However, only 46.6% of infants in our cohort received antibiotics within 60 minutes of sepsis recognition, highlighting significant room for improvement. This is lower than the 65% adherence to the 1-hour target reported in a multicenter quality improvement initiative by Zaidi et al. specifically focused on neonatal sepsis [44], suggesting that VLBW infants may face additional barriers to timely care.

### *Clinical Implications*

The association between delayed antibiotic administration and adverse outcomes has important implications for clinical practice in neonatal intensive care. Our finding that each hour delay increases mortality risk by 16% supports the recommendation for antibiotic administration within 60 minutes of sepsis recognition, consistent with the Surviving Sepsis Campaign guidelines, which have been extrapolated to neonatal care [45]. However, the low rate of achievement of this target (46.6%) in our cohort suggests that implementing this recommendation in practice remains challenging.

The barriers to timely antibiotic administration identified in our study, particularly vascular access difficulties and pharmacy preparation times, align with those reported by Seymour et al. in their analysis of barriers to timely sepsis care [46]. However, our findings highlight additional challenges specific to the neonatal population, such as the difficulty of recognizing sepsis signs in premature infants and the technical challenges of obtaining vascular access in VLBW infants with limited access options.

The variation in time-to-antibiotic between participating NICUs suggests that organizational factors play an important role in determining timeliness of care. Centers with standardized sepsis protocols, pre-mixed antibiotics, dedicated pharmacy services, and electronic ordering systems achieved better performance, consistent with findings by Manaktala et al. who reported improved sepsis outcomes following implementation of an electronic sepsis alerting system [47]. These findings suggest that quality improvement initiatives targeting these modifiable factors may improve timeliness of antibiotic delivery.

The association between delayed antibiotics and increased duration of mechanical ventilation and NICU length of stay also has economic implications. Evans et al. demonstrated that delays in appropriate antibiotic therapy for neonatal sepsis were associated with substantial increases in healthcare costs, primarily driven by prolonged hospitalization [48]. Therefore, investments in systems to improve antibiotic timing may be cost-effective by reducing downstream resource utilization.

### *Pharmacological Considerations*

The time-dependent killing characteristics of beta-lactam antibiotics may partially explain the relationship between timing and outcomes observed in our study. Craig et al. demonstrated that the efficacy of beta-lactams is optimized when concentrations exceed the minimum inhibitory concentration (MIC) for extended periods [49]. In neonates, particularly VLBW infants, altered pharmacokinetics including increased volume of distribution and reduced drug clearance make achieving and maintaining therapeutic concentrations more challenging [50].

The use of different beta-lactam agents across our cohort reflects variability in empiric antibiotic choices for neonatal sepsis. While ampicillin with gentamicin remains the most common first-line regimen for early-onset sepsis, as recommended by the American Academy of Pediatrics [51], our data show increasing use of broader-spectrum agents, particularly for late-onset sepsis. This trend, also reported by Schulman et al. in their multi-center study of antibiotic use in NICUs, raises concerns about antimicrobial stewardship and potential development of resistance [52].

The high rates of ampicillin resistance (61.5%) observed among isolated pathogens in our cohort are concerning and consistent with data from Zaidi et al., who reported increasing rates of antimicrobial resistance in neonatal sepsis isolates worldwide [53]. This highlights the importance of appropriate empiric coverage based on local resistance patterns while awaiting culture results, and may explain the relatively frequent use of broader-spectrum agents in our cohort.

### *Strengths and Limitations*

Our study has several strengths, including its multi-national design, which enhances generalizability, and the detailed documentation of antibiotic timing, which allowed precise measurement of the exposure variable. The inclusion of unit-level data on barriers to timely administration provides valuable context for interpreting the findings and identifying targets for improvement.

However, several limitations should be acknowledged. First, the retrospective design introduces potential for selection bias and confounding. Although we adjusted for key clinical variables, residual confounding by unmeasured factors cannot be excluded. Second, the relatively small sample size limits statistical power, particularly for subgroup analyses, and may explain why some associations did not reach statistical significance despite point estimates suggesting clinically meaningful effects.

Third, the definition of time zero (clinical recognition of sepsis) relies on documentation in medical records, which may not perfectly capture the actual time when sepsis was first suspected. However, our sensitivity analyses using alternative definitions of time zero yielded consistent results, suggesting that this limitation did not substantially bias our findings. Fourth, we included only the first episode of suspected sepsis for each infant, which may limit generalizability to recurrent sepsis episodes.

Finally, our study examined only short-term outcomes, and the impact of antibiotic timing on long-term neurodevelopmental outcomes remains unknown. Mitha et al. demonstrated that neonatal sepsis is associated with adverse neurodevelopmental outcomes at 2 years of age [54], but whether timely antibiotic administration modifies this risk warrants further investigation.

***Future Directions***

The findings of this study highlight several directions for future research. Prospective studies with larger sample sizes are needed to confirm our findings and further explore the relationship between antibiotic timing and outcomes. Such studies should include long-term neurodevelopmental follow-up to determine whether the benefits of timely antibiotic administration extend beyond short-term outcomes.

Quality improvement initiatives targeting the identified barriers to timely antibiotic administration should be developed and evaluated. Hooven et al. reported successful implementation of a bundle to improve timeliness of sepsis care in the NICU setting [55], and similar approaches could be adapted based on our findings. Technological solutions such as decision support systems, automated dispensing machines, and point-of-care pharmaceutical preparation may help overcome some of the identified barriers.

Further research is also needed to optimize beta-lactam dosing in VLBW infants. Modeling studies by Lingscheid et al. suggest that current recommended dosing regimens may not achieve optimal pharmacodynamic targets in all premature infants [56]. Implementation of therapeutic drug monitoring and development of population pharmacokinetic models specific to VLBW infants may allow more precise dosing.

The role of biomarkers in guiding empiric antibiotic therapy for neonatal sepsis also warrants further investigation. Benitz et al. conducted a systematic review of biomarkers for early-onset sepsis and found that while multiple biomarkers show promise, none has sufficient sensitivity and specificity to reliably guide therapy [57]. Integration of novel biomarkers with clinical decision rules may improve early recognition of sepsis and facilitate more timely intervention.

## 5. CONCLUSION

This study demonstrates that delayed beta-lactam administration in VLBW infants with suspected sepsis is associated with increased mortality, prolonged mechanical ventilation, and extended NICU stays. Each hour delay in antibiotic administration was associated with a 16% increase in mortality risk, with a more pronounced effect in culture-confirmed sepsis. Achieving administration within 60 minutes of sepsis recognition should be a priority in neonatal intensive care, and quality improvement initiatives targeting identified barriers to timely administration are warranted. Future research should focus on confirming these findings in larger prospective cohorts, developing effective interventions to improve timing, and evaluating the impact on long-term outcomes.

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