ORIGINAL INVESTIGATION

Access this article online
Quick Response Code:

Website:
www.jcritintensivecare.org
DOI:
10.14744/dcybd.2025.97149

Association Between Meropenem Exposure and Necrotizing Enterocolitis in Infants: A Retrospective Cohort Study

Ana Beatriz Dantas Gomes,[1] Flavia Evelyn Medeiros Fernandes,[2] Fabiana Ariston Filgueira,[2] Daniel Paiva Marques,[2] Iris Ucella de Medeiros,[2] Francisca Sueli Monte Moreira,[2] Rand Randall Martins[1]

Abstract

Aim: This study aimed to identify specific antimicrobial agents associated with the development of necrotizing enterocolitis in critically ill neonates and to characterize independent clinical and demographic factors associated with its occurrence in this vulnerable population.

Study Design: This retrospective cohort study included neonates admitted for more than 24 hours to a Brazilian neonatal intensive care unit between January 2020 and December 2021. Only cases of necrotizing enterocolitis classified as Bell stage IIA or higher were analyzed. Antimicrobial exposure was assessed by days of therapy, considering only agents administered before the diagnosis of necrotizing enterocolitis. Univariate and multivariate logistic regression analyses were performed.

Results: Among 594 neonates included, 15 developed necrotizing enterocolitis (incidence: 1.7%). Meropenem exposure was significantly associated with necrotizing enterocolitis (adjusted odds ratio 3.74; 95% confidence interval: 1.14–12.2; p=0.03). Additional associated factors included lower gestational age, lower birth weight, presence of congenital heart disease, *Methicillin-resistant Staphylococcus aureus* detection in blood culture, and prolonged hospitalization. No evidence of multicollinearity was found among variables.

Conclusions: Meropenem exposure prior to necrotizing enterocolitis onset was associated with a higher likelihood of developing the disease. These findings reinforce the importance of judicious use of broad-spectrum antibiotics and careful monitoring of antimicrobial stewardship in neonatal units.

Keywords: Bacterial resistance; Broad-spectrum antibiotics; Meropenem; Necrotizing enterocolitis; Neonatal intensive care units.

[1]*Pharmaceutical Services and Policies, Health Science Center, Federal University of Rio Grande do Norte, Natal, Brazil*
[2]*School Maternity Januário Cicco, Health Science Center, Federal University of Rio Grande do Norte, Natal, Brazil*

Address for correspondence:
Rand Randall Martins, MD. Pharmaceutical Services and Policies, Health Science Center, Federal University of Rio Grande do Norte, Natal, Brazil.
E-mail: rand.martins@ufrn.br

Received: 29.07.2025
Accepted: 21.10.2025
Published: 22-12-2025

Introduction

Necrotizing enterocolitis (NEC) remains a predominant cause of gastrointestinal morbidity and mortality in preterm neonates.[1] Globally, its incidence is estimated at approximately 7.0% among very low birth weight infants and between 2% to 13% in preterm populations.[1,2] The condition carries significant

How to cite this article: *Gomes ABD, Fernandes FEM, Filgueira FA, Marques DP, de Medeiros IC, Moreira FSM, Martins RR. Association Between Meropenem Exposure and Necrotizing Enterocolitis in Infants: A Retrospective Cohort Study. J Crit Intensive Care 2025;16(3):109—117.*

mortality risk, increasing the likelihood of death 16-fold in neonates requiring intensive care.[3] Multiple factors have been associated with NEC development, including prematurity, low birth weight, sepsis, blood transfusions, respiratory distress syndrome, and pneumonia.[4] Of particular pathophysiological importance is prior antibiotic exposure, which has emerged as a significant modifiable factor.[5,6]

In clinical practice, empirical antimicrobial therapy for suspected early-onset sepsis (EOS) in preterm neonates frequently involves broad-spectrum antibiotics.[7] These agents profoundly impact the developing gut microbiome and have been mechanistically linked to NEC pathogenesis.[5,6] Their effects include suppression of beneficial commensal microorganisms, overgrowth of potential pathogens, and induction of dysbiosis, all established mechanisms in NEC development.[3,8] Nevertheless, the relationship between early antibiotic exposure and NEC demonstrates considerable complexity. Current evidence remains conflicting regarding risk stratification by specific antimicrobial agents,[3,8,9] highlighting persistent gaps in our understanding of this critical neonatal complication.

Given the paucity of robust evidence regarding antimicrobial-specific risks, this study primarily aims to identify specific antimicrobial agents associated with NEC development in critically ill neonates. Secondarily, we seek to characterize independent clinical and demographic variables associated with NEC in this vulnerable population.

Materials and Methods

Study Design and Participants

This is a retrospective cohort study that assessed the relationship between the occurrence of NEC and exposure to specific antimicrobials in neonates receiving intensive therapy. The research was conducted in the Neonatal Intensive Care Unit (NICU) of a Maternity School, a reference facility for high-risk pregnancies in Natal, Brazil. The institution has 20 level II neonatal Intensive Care Unit (ICU) beds, approximately 400 admissions per year, and exclusively serves patients from the Unified Health System (SUS). All neonates admitted to the NICU between January 2020 and December 2021 with a hospitalization period longer than 24 hours were eligible. Inclusion required documented exposure to at least one antimicrobial agent during hospitalization. No restrictions were applied regarding gestational age, birth weight, congenital anomalies, or palliative care status. Neonates were excluded if they received only supportive therapies (such as parenteral nutrition, electrolytes, and/or blood products) without antimicrobial exposure, or if they had a diagnosed immunodeficiency. The study was approved by the Ethics Committee of Onofre Lopes University Hospital of The Federal University of Rio Grande Do Norte (Approval Number: 5.173.658, Date: 17.12.2021), in accordance with the Declaration of Helsinki and Resolution No. 466/12 of the Brazilian National Health Council. In line with national regulations for retrospective studies, attempts were made to obtain informed consent from legal representatives by telephone contact. When formal consent could not be obtained after repeated attempts, data access was permitted by the ethics committee.

Data Collection

The data were collected from electronic medical records and microbiological test results. Information recorded included gestational age, birth weight, gender, occurrence of ruptured membranes, Apgar score (Appearance, Pulse, Grimace, Activity, and Respiration) score, clinical diagnoses (cardiac, neurological, renal conditions, congenital malformations, early and late-onset sepsis), and length of hospital stay.

The prescribed antimicrobials and their duration of use were measured in days of therapy (DOT), defined as the administration of any antimicrobial agent, counted once per agent for each 24-hour period.[10] Days of therapy is a preferred measure of antimicrobial consumption in children, as it accounts for differences in dosage related to age and weight.[11] At the study institution, antimicrobial prescribing in the NICU follows strict internal protocols based on national and international guidelines. These include predefined indications for empirical and targeted antimicrobial therapy, daily clinical reassessment, and regular review by the hospital's antimicrobial stewardship team.

Necrotizing enterocolitis was defined according to the modified Bell's criteria, and only cases classified as stage IIA or higher were included in the analysis. These stages correspond to definite NEC, characterized by the presence of at least one clinical sign (e.g., abdominal distension, gastric residuals, bloody stools) combined with radiographic findings such as pneumatosis intestinalis, portal venous gas, or pneumoperitoneum. Cases classified as stage I ("possible NEC"), which involve only non-

specific systemic or gastrointestinal signs without radiographic confirmation, were excluded from this study.[12] For all cases, only antimicrobials administered before the clinical and radiographic diagnosis of NEC were considered as exposure variables in the analysis.

The blood, cerebrospinal fluid, and urine samples used for microbiological characterization were obtained from hospitalized patients at the institution and analyzed in the institution's Microbiology Laboratory. For isolation and characterization, the samples were incubated using an automated system (Phoenix, BD) designed for the rapid detection of bacteria and fungi through a phenotypic method. Resistance markers for extended-spectrum β-lactamases (ESBL) and methicillin-resistant *Staphylococcus aureus* (MRSA) were identified using the same automated methodology. For the phenotypic identification of carbapenemase-producing *Enterobacteriaceae* (KPC), a confirmatory method using the modified Hodge test was employed, although it is no longer recommended due to its low specificity and, in some cases, less than ideal sensitivity.[13] Lastly, the vancomycin resistance marker for *Enterococcus faecium* or *Enterococcus faecalis* (VRE) was identified via strip sensitivity testing using vancomycin.

Statistical Analysis

With a presumed prevalence of 7% for NEC,[2] the sample size was defined as 626 individuals, ensuring a maximum error of ±2 percentage points with 95% confidence. Data analysis was conducted using Stata version 15 (Stata Corporation, College Station, TX, USA). The data were presented as median and interquartile range, absolute and relative frequencies, and mean and standard deviation, depending on the type of variable analyzed. The incidence of NEC was expressed as an incidence rate (number per 1,000 patient-days) with a 95% confidence interval (CI). To compare population characteristics between neonates with and without NEC, normality of continuous variables was first assessed using the Shapiro–Wilk test. Depending on the distribution, either Student's t-test or the Mann–Whitney U test was applied. For categorical variables, Pearson's chi-square test or Fisher's exact test was used. A p-value <0.05 was considered statistically significant.

Univariate logistic regression was used to determine factors associated with the occurrence of NEC, estimating respective odds ratios (OR) and 95% confidence intervals. All variables with an association test showing a p-value <0.10 were included in a multivariate logistic regression model, and a significance level of $p<0.05$ was adopted. The factors identified in the previous stage were used as adjustment variables in investigating the relationship between the DOT of the most prescribed antimicrobials and the occurrence of NEC via a multivariate logistic regression model ($p<0.05$). Collinearity between variables included in the multivariable logistic regression model was assessed using the variance inflation factor (VIF). VIF values remained below the conventional threshold of 5, indicating no relevant multicollinearity among the variables analyzed. Late-onset sepsis cases included in the analysis all occurred prior to the diagnosis of NEC. Cases of sepsis diagnosed concurrently with or after NEC onset were not included as potential risk factors. To assess causality between the initiation of antimicrobial use and the period of NEC diagnosis, Student's t-test was used for mean comparison ($p<0.05$).

AI Use Statement

No generative artificial intelligence (AI) tools were used in the conception, design, data analysis, or writing of this manuscript. All content was produced exclusively by the authors.

Results

During the study period, 594 neonates were included, with a mean gestational age of 33.2±4.4 weeks and a birth weight of 1978.3±1010.2 g (Table 1). Regarding the diagnosis of sepsis, 173 neonates were identified with early-onset sepsis (29.1%), and 206 had late-onset sepsis (34.7%). The length of hospital stay was 24.5±32.3 days, and 15 neonates presented with NEC, corresponding to a cumulative incidence of 1.7%. The incidence rate was 40.85 cases per 1,000 patient-days (95% CI: 22.89–68.47).

In Table 2, the prescription profile of antimicrobials and DOT are described. The combination of ampicillin and gentamicin was the most common prescription (359 neonates, 55.4%), followed by the regimen of oxacillin and ampicillin (200, 33.7%), meropenem (150, 25.3%), and vancomycin (148, 24.9%). Among the most frequently used antimicrobials, meropenem and vancomycin showed the longest durations of use prior to NEC diagnosis, with median durations of 15 and 13 days, respectively. These two agents also stood out for their prevalence among neonates who developed NEC: 13 of the 15 affected neonates had received meropenem, and 14 had received vancomycin prior to NEC onset.

Table 1. Population characteristics (n=594)

Characteristics	Total Feature	Without NEC (n=579)	With NEC (n=15)	p
Gestational age in weeks (Mean±SD)	33.2±4.4	33.3±4.4	30.7±3.7	0.03
Gestational age in weeks (n, %)				
≤28 weeks	108 (18.1%)	103 (17.8%)	5 (33.3%)	0.12
29–35 weeks	290 (48.5%)	282 (48.7%)	8 (53.3%)	0.72
≥36 weeks	196 (33.0%)	194 (33.5%)	2 (13.3%)	0.10
Birth weight in grams (Mean±SD)	1978.3±1010.2	1998.7±1009.4	1191.0±688.9	<0.01
Birth weight in grams (n, %)				
≤1000 grams	118 (19.9%)	111 (19.2%)	7 (46.7%)	<0.01
1001–2500 grams	304 (51.2%)	297 (51.3%)	7 (46.7%)	0.72
≥2501 grams	172 (30.0%)	171 (29.5%)	1 (6.7%)	0.06
Female gender (n, %)	277 (46.9%)	270 (46.9%)	7 (46.7%)	0.98
Ruptured membranes (n, %)	85 (15.7%)	82 (15.5%)	3 (21.4%)	0.55
Apgar score at 1 minute (Mean±SD)	6.4±2.3	6.4±2.3	5.7±2.3	0.51
Apgar score at 5 minutes (Mean±SD)	7.9±1.5	7.9±1.5	7.7±1.6	0.50
Clinical diagnosis (n, %)				
Cardiac diseases	74 (12.5%)	71 (12.6%)	3 (20.0%)	0.37
Malformations	76 (12.8%)	73 (12.1%)	3 (20.0%)	0.40
Neurological diseases	30 (5.1%)	30 (5.1%)	0 (0.0%)	0.37
Renal diseases	46 (7.7%)	43 (7.4%)	3 (20.0%)	0.11
Early-onset sepsis (n, %)				
Clinical diagnosis	163 (27.4%)	159 (27.5%)	4 (26.7%)	0.83
Laboratory diagnosis	10 (1.7%)	10 (1.7%)	0 (0.0%)	0.77
Late-onset sepsis (n, %)				
Clinical diagnosis	101 (17.0%)	99 (17.1%)	2 (13.3%)	0.77
Laboratory diagnosis	105 (17.7%)	95 (16.4%)	10 (66.7%)	<0.01
Bacterial resistance marker (n, %)				
ESBL*	80 (13.5%)	72 (12.4%)	8 (53.3%)	<0.01
MRSA#	44 (7.4%)	39 (6.7%)	5 (33.3%)	<0.01
KPC=	17 (2.9%)	14 (2.4%)	3 (20.0%)	<0.01
Length of hospitalization in days (Mean±SD)	24.5±32.3	24.4±33.5	63.7±35.5	<0.01
Necrotizing enterocolitis (n, IR per 1,000 patient-days, 95% CI)	15; 40.85 (22.89–68.47)	-	-	-
Death (n, %)	83 (14.0%)	76 (13.1%)	7 (46.7%)	<0.01

*ESBL: Extended-Spectrum Beta-Lactamases; #MRSA: Methicillin-resistant *Staphylococcus aureus*; =KPC: Carbapenemase-producing *Enterobacteriaceae*; IR: Incidence Rate. Missing data: sex (n=3), ruptured membranes (n=51), Apgar score at 1 min (n=2), Apgar score at 5 min (n=2), and length of hospitalization (n=8).

Regarding factors associated with the occurrence of NEC (Table 3), univariate analysis identified lower gestational age (OR: 0.956, 95% CI: 0.914-0.999), birth weight (OR: 0.999, 95% CI: 0.998-0.999), congenital heart disease (OR: 1.811, 95% CI: 1.043-3.145), congenital malformations (OR: 1.923, 95% CI: 1.160-3.218), and late-onset sepsis (OR: 3.367, 95% CI: 1.917-5.913). Additionally, the presence of ESBL (OR: 3.586, 95% CI: 2.339-5.496), KPC (OR: 3.357, 95% CI: 1.903-5.923), MRSA (OR: 3.464, 95% CI: 2.197-5.460), and longer hospitalization (OR: 1.007, 95% CI: 1.004-1.010) were associated with NEC. However, the multivariate model confirmed only gestational age (OR: 1.250, 95% CI: 1.134-1.377), birth weight (OR: 0.998, 95% CI: 0.997-0.999), congenital heart disease (OR: 4.777, 95% CI: 2.217-10.293), MRSA (OR: 2.748, 95% CI: 2.067-7.705), and longer hospitalization (OR: 1.004, 95% CI: 1.000-1.008) as related to the occurrence of NEC. The collinearity analysis revealed no evidence of multicollinearity among the variables included in the multivariable model. All VIF values were below 2, supporting the independence of the effects observed (Table 1).

Table 2. Prevalence of antimicrobials, their respective prescription days, and occurrence of necrotizing enterocolitis

Antimicrobial	Neonates		Therapy Days			NEC Occurrence	
	n	%	Median	p25	p75	n	%
Ampicillin + Gentamicin	329	55.4	6	4	8	8	1.3
Oxacillin + Amikacin	200	33.7	7	4	9.5	10	1.7
Meropenem	150	25.3	15	8	25	13	2.2
Vancomycin	148	24.9	13	8.5	19.5	14	2.4
Fluconazole	110	18.5	22	10	33	7	1.2
Penicillin	106	17.8	7	4	9	0	0.0
Cefepime	82	13.8	8	4	12	9	1.5
Amphotericin B	62	10.4	19.5	12	30	6	1.0
Polymyxin	25	4.2	14	4	18	2	0.3
Ciprofloxacin	22	3.7	9	5	15	2	0.3
Piperacillin-Tazobactam	21	3.5	6	3	8	3	0.5
Liposomal Amphotericin B	20	3.4	17	11	22	5	0.8
Others	21	3.5	6	4	11	0	0.0

Table 3. Multivariate logistic regression model to investigate factors associated with the occurrence of necrotizing enterocolitis

Characteristics	Univariate				Multivariate			
	OR	95% CI		p	OR	95% CI		p
Gestational age in weeks	0.956	0.914	0.999	0.049	1.250	1.134	1.377	<0.001
Birth weight in grams	0.999	0.998	0.999	<0.001	0.998	0.997	0.999	<0.001
Female gender	0.885	0.579	1.350	0.570	-	-	-	-
Ruptured membranes	1.161	0.686	1.967	0.576	-	-	-	-
Apgar score at 1 minute	0.964	0.884	1.051	0.413	-	-	-	-
Apgar score at 5 minutes	0.979	0.866	1.107	0.740	-	-	-	-
Patent ductus arteriosus (PDA)	1.733	0.478	6.287	0.403	-	-	-	-
Mechanical ventilation	1.428	0.400	5.097	0.549	-	-	-	-
Clinical diagnosis					-	-	-	-
Cardiac diseases	1.811	1.043	3.145	0.035	4.777	2.217	10.293	<0.001
Malformations	1.923	1.160	3.218	0.011	-	-	-	-
Neurological diseases	1.000	-	-	-	-	-	-	-
Renal diseases	1.525	0.892	2.607	0.123	-	-	-	-
Early-onset sepsis	0.925	0.588	1.456	0.738	-	-	-	-
Late-onset sepsis	3.367	1.917	5.913	<0.001	-	-	-	-
Bacterial resistance marker					-	-	-	-
ESBL*	3.586	2.339	5.496	<0.001	-	-	-	-
KPC=	3.357	1.903	5.923	<0.001	-	-	-	-
MRSA#	3.464	2.197	5.460	<0.001	2.748	2.067	7.705	<0.001
Length of hospitalization in days	1.007	1.004	1.010	<0.001	1.004	1.000	1.008	0.050

*ESBL: Extended-Spectrum Beta-Lactamases; #MRSA Methicillin-Resistant Staphylococcus aureus; =KPC: Carbapenemase-Producing Enterobacteriaceae.

The characteristics identified in the previous model were used as adjustment variables to identify antimicrobials associated with the occurrence of NEC (Fig. 1). Only meropenem (OR: 1.075, 95% CI: 1.006-1.148) and vancomycin (OR: 1.067, 95% CI: 1.003-1.136) were found to be related to NEC. In Figure 2, it is observed that meropenem is commonly prescribed around the 17th day of hospitalization, preceding the diagnosis period of

NEC around the 34th day (17.5, 95% CI: 10.6-24.4 vs. 34.5, 95% CI: 20.3-42.7 days; p=0.03). Despite vancomycin showing a relationship with NEC, the analysis in Figure 2 demonstrates that only meropenem was statistically significant.

Discussion

In this retrospective cohort of 594 neonates, we identified that prior exposure to meropenem was associated with the development of NEC. In addition to previous meropenem use, other independent variables associated with NEC were identified: lower gestational age, very low birth weight, presence of congenital heart disease, MRSA positivity in blood culture, and longer hospital stay. Our findings reinforce this knowledge by further highlighting the potential role of a broad-spectrum antibiotic in this association.

The necrotizing enterocolitis incidence observed in our study (2%) was slightly lower than that reported in previous studies, which range between 2% and 13% in preterm populations. For instance, Alsaied et al.[2] in 2020 estimated a pooled global prevalence of 7% among very low birth weight infants, while Zozaya et al.[1] in 2020 reported an incidence of 8.8%, with 5.5% requiring surgical treatment.We emphasize the use of strict diagnostic criteria for NEC, including only cases classified as Bell stage IIA or higher (modified Bell criteria), with clinical and radiological confirmation.[3,12] By applying this definition, we ensured comparability with most published studies and avoided the inclusion of mild or equivocal cases that could artificially increase incidence estimates. We also documented a high rate of early-onset neonatal sepsis (29.1%) in our cohort—higher than rates typically reported in Brazilian neonatal units.[14] This discrepancy may reflect the use of broader clinical criteria for early-onset sepsis diagnosis and the higher severity profile of

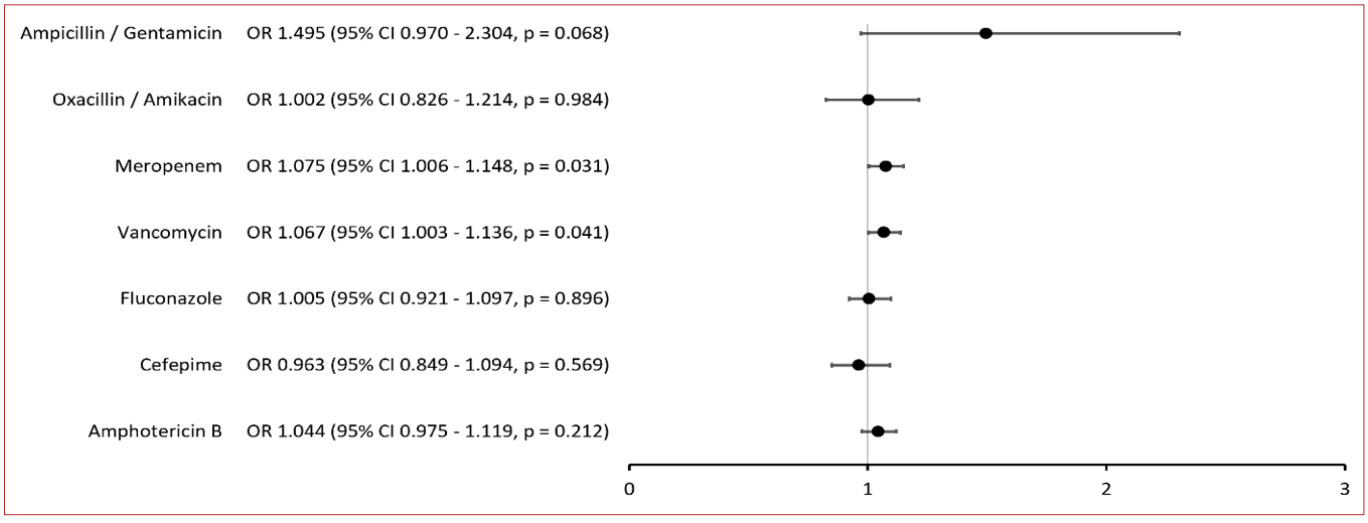

Figure 1. Multivariate logistic regression model investigating antimicrobials associated with the occurrence of necrotizing enterocolitis, adjusted for gestational age, birth weight, diagnosis of congenital heart disease, infection related to the presence of methicillin-resistant Staphylococcus aureus, and treatment days.

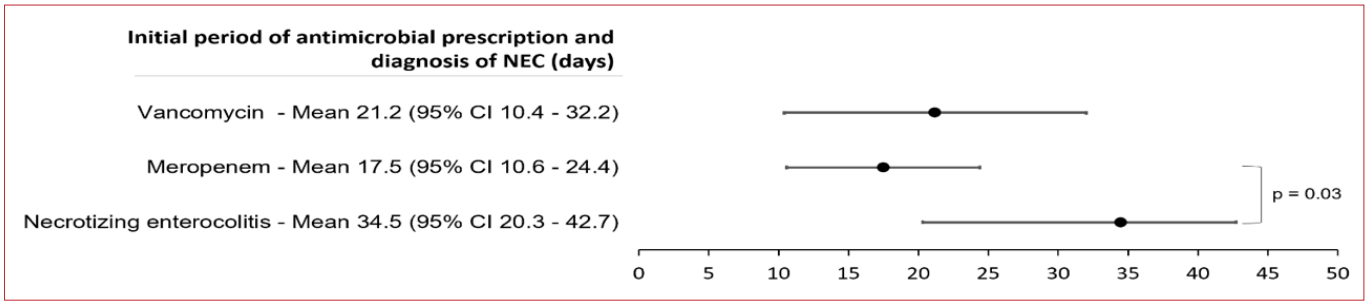

Figure 2. Comparison between the means for the start of antimicrobial administration (meropenem and vancomycin) and the diagnosis period of necrotizing enterocolitis (Student's t-test; p<0.05)*.
***No adjusted analyses were performed; results reflect univariate comparisons.**

patients treated at our referral center. The elevated sepsis rate indicates that a large proportion of patients received empirical antibiotic therapy in the first days of life, a crucial context for interpreting the impact of antimicrobials on subsequent NEC development.

Extensive antibiotic exposure has been consistently associated with NEC, likely due to its disruption of the neonatal gut microbiota. Broad-spectrum antimicrobials may suppress beneficial commensal bacteria and promote dysbiosis, favoring colonization by pathogenic microorganisms.[5,15] In preterm infants, this dysbiosis can trigger mucosal injury and intestinal inflammatory responses, contributing to NEC pathophysiology.[16] Among extremely low birth weight infants, prolonged antibiotic use (≥5 days) for suspected early-onset sepsis is associated with increased NEC incidence.[6,9,17] Our findings expand this evidence by identifying a specific antibiotic—meropenem—as independently associated with NEC. In our cohort, meropenem was initiated, on average, around the 17th day of life, while NEC diagnosis occurred at a median of 34 days. This temporal sequence strengthens the plausibility of a causal association, though it does not confirm it.

Although antibiotic use in general has been previously associated with NEC, few studies have evaluated the association with specific agents. To our knowledge, this is one of the first studies to identify meropenem as independently associated with NEC using a multivariate model. Previously, Raba et al.[9] in 2019 reported a similar association in a case-control study. Our results corroborate these findings in a larger cohort with a study design that better establishes the temporal relationship between antimicrobial exposure and disease development.

It is important to note that meropenem is typically reserved for patients with severe or refractory infections due to its broad-spectrum activity.[18] Thus, its association with NEC may partially reflect the underlying severity of patients who receive it. However, by adjusting for variables such as gestational age, birth weight, and comorbidities, we sought to control for these potential confounders. The persistence of the association even after adjustment suggests that meropenem (or the clinical circumstances warranting its use) may directly contribute to NEC development. Given its broad spectrum of activity against gram-negative, gram-positive, and anaerobic bacteria,[20] its impact on the neonatal intestinal ecosystem is likely substantial.

Our study also identified other factors associated with NEC. Prematurity and low birth weight showed strong associations with the disease, consistent with extensive literature.[1-6,8,9] These factors are linked to immaturity of the intestinal barrier, mesenteric perfusion, and immune system.[5,16] Moreover, extremely preterm infants often require invasive interventions and early antibiotic use, which may compound the risk of gut dysbiosis and NEC.[6,20,21] In this context, MRSA colonization may reflect a history of prolonged broad-spectrum antimicrobial use, commonly associated with resistant organism colonization in NICUs.[22,23] Such colonization indicates an imbalanced microbiota, a central feature in NEC pathophysiology.[5,15] Thus, MRSA likely acts not as a direct causative agent but rather as an indirect marker of intense antimicrobial pressure and gut dysbiosis. Congenital heart disease emerged as another associated factor, supporting previous studies reporting higher NEC incidence even among term infants with cardiac conditions.[24] Finally, prolonged hospitalization was associated with NEC, as expected, since extended hospital stays reflect greater clinical severity, increased exposure to nosocomial infections, and repeated cycles of antibiotic therapy.

The results underscore the importance of vigilant use of broad-spectrum antibiotics in NEC prevention. In clinical practice, this requires careful evaluation of meropenem initiation criteria in neonates and prompt discontinuation when feasible. Identifying empirical regimens with lower impact on the gut microbiota and their association with NEC, as suggested by other authors,[8,20] would be ideal. Concurrently, protective strategies like exclusive breastfeeding, a key factor for intestinal health promotion and consistently associated with lower NEC incidence,[25] should be encouraged.

Several limitations should be considered when interpreting our findings. First, this was a single-center retrospective study, which may restrict generalizability beyond our institutional context. Additionally, the final sample size did not reach the originally planned number of neonates, mainly due to logistical barriers in data collection, and this shortfall may have reduced statistical power and the precision of estimates. Although strict diagnostic criteria were applied, NEC diagnosis relied on a single neonatologist without independent review, and the possibility of misclassification with spontaneous intestinal perforation cannot be completely excluded. The inclusion of neonates with congenital anomalies and those receiving palliative

care may also have influenced clinical management and outcomes. Moreover, important clinical variables such as feeding type, blood product transfusions, and validated severity-of-illness scores were not available for analysis, which limited adjustment for established NEC risk factors. Another limitation is the absence of stratified analyses by gestational age or birth weight, and survivor bias may explain the unexpectedly higher NEC incidence at greater gestational ages, as extremely preterm neonates often died before the typical onset window. Regarding antimicrobial exposure, cumulative or combined use of broad-spectrum agents was not formally analyzed, and confounding by indication remains a concern, since meropenem was frequently prescribed to more severely ill neonates. Finally, local antimicrobial stewardship policies may have influenced antibiotic prescribing patterns, thereby limiting the external validity of our results. Despite these limitations, our study contributes valuable preliminary evidence by evaluating recent data from a tertiary NICU with standardized antimicrobial protocols and advanced neonatal care, which enhance the internal validity of the findings.

Conclusion

In summary, this study identified prior meropenem exposure as an independent associated factor for NEC, alongside established factors including lower gestational age, very low birth weight, congenital heart disease, MRSA infection, and prolonged hospitalization. These findings support the hypothesis that broad-spectrum antibiotics—particularly meropenem—may promote gut dysbiosis and NEC pathogenesis, underscoring the need for judicious use of these agents in neonates. Future research should investigate the underlying pathophysiological mechanisms and validate these associations across diverse populations.

Ethics Committee Approval: Ethics committee approval was obtained from Ethics Committee of Onofre Lopes University Hospital of The Federal University of Rio Grande Do Norte (Approval Number: 5.173.658, Date: 17.12.2021).

Informed Consent: Informed consent from legal representatives by telephone contact.

Conflict of Interest: The authors have no conflicts of interest to declare.

Funding: This study was financially supported by Conselho Nacional de Desenvolvimento Científico e Tecnológico (CNPq; Grant 001) and Coordenação de Aperfeiçoamento de Pessoal de Nível Superior (CAPES).

Use of AI for Writing Assistance: No use of AI-assisted technologies were declared by the authors.

Author Contributions: Concept – A.B.D.G., R.R.M.; Design – A.B.D.G., R.R.M.; Supervision – F.S.M.M., R.R.M.; Data Collection and/or Processing - A.B.D.G., F.A.F., D.P.M., I.C.M.; Analysis and/or Interpretation - A.B.D.G., F.A.F., I.C.M., R.R.M.; Literature Review – A.B.D.G., F.E.M.F.; Writing – A.B.D.G., D.P.M., R.R.M.; Critical Review – F.E.M.F., F.S.M.M.

Acknowledgements: The authors would like to express their sincere gratitude to the healthcare professionals from Maternidade Escola Januário Cicco for their valuable collaboration throughout the study. We also thank the Gerência de Ensino e Pesquisa (Education and Research Management) for their institutional support. Finally, we are especially grateful to the patients who generously agreed to participate in this research.

Peer-review: Externally peer-reviewed.

References

1. Zozaya C, García González I, Avila-Alvarez A, Oikonomopoulou N, Sánchez Tamayo T, Salguero E, et al. Incidence, Treatment, and Outcome Trends of Necrotizing Enterocolitis in Preterm Infants: A Multicenter Cohort Study. Front Pediatr 2020;8:188. [CrossRef]
2. Alsaied A, Islam N, Thalib L. Global incidence of Necrotizing Enterocolitis: a systematic review and Meta-analysis. BMC Pediatr 2020;20(1):344. [CrossRef]
3. Pei X, Gao Y, Kou Y, Ding Y, Li D, Lei P, et al. Association of necrotizing enterocolitis with antimicrobial exposure in preterm infants <32 weeks gestational age:A multicenter prospective case-control study. Front Pharmacol 2022;13:976487. [CrossRef]
4. Su Y, Xu RH, Guo LY, Chen XQ, Han WX, Ma JJ, et al. Risk factors for necrotizing enterocolitis in neonates: A meta-analysis. Front Pediatr 2023;10:1079894. [CrossRef]
5. Cuna A, Morowitz MJ, Sampath V. Early antibiotics and risk for necrotizing enterocolitis in premature infants: A narrative review. Front Pediatr 2023;11:1112812. [CrossRef]
6. Zhu K, Gao H, Yuan L, Wang L, Deng F. Prolonged antibiotic therapy increased necrotizing enterocolitis in very low birth weight infants without culture-proven sepsis. Front Pediatr 2022;10:949830. [CrossRef]
7. Darlow CA, da Costa RMA, Ellis S, Franceschi F, Sharland M, Piddock L, et al. Potential Antibiotics for the Treatment of Neonatal Sepsis Caused by Multidrug-Resistant Bacteria. Paediatr Drugs 2021;23(5):465-84. [CrossRef]
8. Raba AA, O'Sullivan A, Miletin J. Pathogenesis of necrotising enterocolitis: The impact of the altered gut microbiota and antibiotic exposure in preterm infants. Acta Paediatr 2021;110(2):433-40. [CrossRef]
9. Raba AA, O'Sullivan A, Semberova J, Martin A, Miletin J. Are antibiotics a risk factor for the development of necrotizing enterocolitis-case-control retrospective study. Eur J Pediatr 2019;178(6):923-8. [CrossRef]
10. Morris AM, Brener S, Dresser L, Daneman N, Dellit TH, Avdic E, et al. Use of a structured panel process to define quality metrics for antimicrobial stewardship programs. Infect Control Hosp Epidemiol 2012;33(5):500-6. [CrossRef]
11. Pakyz AL, Gurgle HE, Ibrahim OM, Oinonen MJ, Polk RE. Trends

in antibacterial use in hospitalized pediatric patients in United States academic health centers. Infect Control Hosp Epidemiol 2009;30(6):600-3. [CrossRef]

12. Walsh MC, Kliegman RM. Necrotizing enterocolitis: treatment based on staging criteria. Pediatr Clin North Am 1986;33(1):179-201. [CrossRef]
13. Brazilian Committee on Antimicrobial Susceptibility Testing (BRCAST). EUCAST guidelines for detecting resistance mechanisms and specific resistances of clinical and/or epidemiological importance. Portuguese. Available at: https://brcast.org.br/wp-content/uploads/2022/07/Orientacoes-do-EUCAST-para-a-deteccao-de-mecanismos-de-resistencia-e-resistencias-especificas-de-importancia-clinica-e-ou-epidemiologica.pdf. Accessed November 5, 2025.
14. Camargo JF, Caldas JPS, Marba STM. Early neonatal sepsis: prevalence, complications and outcomes in newborns with 35 weeks of gestational age or more. Rev Paul Pediatr 2021;40:e2020388. [CrossRef]
15. Hou S, Wang X, Wang F, Li Z, Wang H, Li J, et al. Excessively Prolonged Early Antibiotic Duration in Very-Low-Birth-Weight Infants: A Multicenter Prospective Cohort Study in a Developing Country. Infect Drug Resist 2022;15:1921-31. [CrossRef]
16. Chaaban H, Patel MM, Burge K, Eckert JV, Lupu C, Keshari RS, et al. Early Antibiotic Exposure Alters Intestinal Development and Increases Susceptibility to Necrotizing Enterocolitis: A Mechanistic Study. Microorganisms 2022;10(3):519. [CrossRef]
17. Stout G, Lambert DK, Baer VL, Gordon PV, Henry E, Wiedmeier SE, et al. Necrotizing enterocolitis during the first week of life: a multicentered case-control and cohort comparison study. J Perinatol 2008;28(8):556-60. [CrossRef]
18. Raza MA, Yao BF, Shi HY, Xu HY, Hao GX, Van Den Anker J, et al. Optimal dose of meropenem for the treatment of neonatal sepsis: Dosing guideline variations and clinical practice deviations. Br J Clin Pharmacol 2022;88(7):3483-9. [CrossRef]
19. Steffens NA, Zimmermann ES, Nichelle SM, Brucker N. Meropenem use and therapeutic drug monitoring in clinical practice: a literature review. J Clin Pharm Ther 2021;46(3):610-21. [CrossRef]
20. Dierikx TH, Deianova N, Groen J, Vijlbrief DC, Hulzebos C, de Boode WP, et al. Association between duration of early empiric antibiotics and necrotizing enterocolitis and late-onset sepsis in preterm infants: a multicenter cohort study. Eur J Pediatr 2022;181(10):3715-24. [CrossRef]
21. Mulinge MM, Mwanza SS, Kabahweza HM, Wamalwa DC, Nduati RW. The impact of NICU antibiotics on gut bacterial microbiota of preterm infants: a systematic review. Front Microbiome 2023;2:1180565. [CrossRef]
22. Balamohan A, Beachy J, Kohn N, Rubin LG. Risk factors for nosocomial methicillin resistant Staphylococcus aureus (MRSA) colonization in a neonatal intensive care unit: A Case-control study. Am J Infect Control 2021;49(11):1408-13. [CrossRef]
23. Romanelli RMC, de Souza GG, Fontis JP, Rodrigues JHP, Viana JPR, Rocha KO, et al. Risk factors for colonization/infection by resistant microorganisms in outbreaks in neonatal unit-a systematic review and meta-analysis. J Pediatr (Rio J) 2025;101(3):297-311. [CrossRef]
24. Kelleher ST, McMahon CJ, James A. Necrotizing Enterocolitis in Children with Congenital Heart Disease: A Literature Review. Pediatr Cardiol 2021;42(8):1688-99. [CrossRef]
25. Chae H, Kim SY, Kang HM, Im SA, Youn YA. Dysbiosis of the initial stool microbiota increases the risk of developing necrotizing enterocolitis or feeding intolerance in newborns. Sci Rep 2024;14(1):24416. [CrossRef]