RESEARCH ARTICLE

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Multidrug-Resistant Gram-Negative Bacteria Rate and Risk Factors in the Neonatal Intensive Care Unit: A Single-Center Ten-Year Experience

ABSTRACT

Objective: Multidrug resistance (MDR) in gram-negative neonatal infections is difficult to manage, and the risk factors differ among different studies. Therefore, we aim to investigate the demographics, mortality, MDR status of gram-negative isolates, and risk factors for MDR gram-negative infections.

Methods: We conducted a retrospective single-center study about MDR gram-negative infections in neonates between January 2012-January 2022 at Duzce University Hospital in Turkey. This study evaluates neonates with MDR gram-negative infections' risk factors and clinical features. All analyses were performed using IBM SPSS V23. In addition, univariate analyses and multivariate logistic regression models were studied to determine MDR's risk factors.

Results: Of 107 gram-negative bacteria, 41 (38.3%) accounted for *Enterobacter*, 30 (28%) for *Klebsiella pneumonia,* and 22 (20.6%) for *Escherichia coli.* Additionally, 61 (56.5%) were MDR microorganisms. Among the susceptibility tests performed for selected isolates, 41 (77.4%) had resistance to piperacillin, 57 (75%) showed resistance to amoxiclav, and 16 (72.7%) had cefoxitin resistance. In addition, carbapenemase resistance was found in 24 (43.6%) and meropenem resistance in 13 (36.1%). Colistin, aztreonam, and tigecycline resistances were the least frequent. In addition, the following dependent risk factors increased the multidrug resistance risk in gram-negative infections; late-onset sepsis 3.547 fold (p=0.005), use of mechanical ventilation 3.143 fold (p=0.007), blood transfusion 3.587 fold (p=0.013), bronchopulmonary dysplasia 6.702 fold, (p= 0.015) and total parenteral nutrition 5.591 fold (p=0.001), lower gestational age 1.122 (1/0.891) fold (p=0.026), and birth weight 1.001 (1/0.999) fold, (p=0.013). Similarly, antibiotherapy duration was significantly higher in the MDR group than in the non-MDR group.

Conclusions: The reported risk factors for MDR in gram-negative neonatal infections are all dependent risk factors. Hence clinicians must be alert to all potential risk factors.

Keywords: Gram-Negative Bacterial Infections, Neonates, Multidrug Resistance.

Yenidoğan Yoğun Bakım Ünitesinde Çok İlaca Dirençli Gram-Negatif Bakteri Oranı ve Risk Faktörleri: Tek Merkezli On Yıllık Deneyim

ÖZET

Amaç: Gram-negatif yenidoğan enfeksiyonlarında çoklu ilaç direncinin (ÇİD) yönetimi zordur ve risk faktörleri farklı çalışmalar arasında farklılık göstermektedir. Bu çalışmanın amacı, gram-negatif izolatların demografik özelliklerini, mortalitesini, ÇİD durumunu ve ÇİD gram-negatif enfeksiyonlar için risk faktörlerini araştırmaktır.

Gereç ve Yöntem: Düzce Üniversitesi Hastanesi'nde Ocak 2012-Ocak 2022 tarihleri arasında yenidoğanlarda ÇİD gram-negatif enfeksiyonlarla ilgili retrospektif tek merkezli bir çalışma yapıldı. Bu çalışmada ÇİD gram-negatif enfeksiyonu olan yenidoğanların risk faktörleri ve klinik özellikleri değerlendirildi. Tüm analizler IBM SPSS V23 kullanılarak gerçekleştirildi. Ayrıca, ÇİD risk faktörlerini belirlemek için tek değişkenli analizler ve çok değişkenli lojistik regresyon modelleri incelenmiştir.

Bulgular: Yüzyedi gram-negatif bakterinin 41'ini (%38,3) *Enterobacter*, 30'unu (%28) *Klebsiella pneumonia* ve 22'sini (%20,6) *Escherichia coli* oluşturmuştur. Ayrıca, 61'i (%56,5) ÇİD mikroorganizmalardır. Seçilen izolatlar için yapılan duyarlılık testleri sonucunda 41'inde (%77,4) piperasilin, 57'sinde (%75) amoksiklav ve 16'sında (%72,7) sefoksitin direnci tespit edilmiştir. Ayrıca, 24'ünde (%43,6) karbapenemaz direnci ve 13'ünde (%36,1) meropenem direnci tespit edilmiştir. Kolistin, aztreonam ve tigesiklin dirençleri en az görülen dirençlerdi. Aşağıdaki bağımlı risk faktörleri gram-negatif enfeksiyonlarda çoklu ilaç direnci riskini arttırmıştır; geç başlangıçlı sepsis 3. 547 kat (p=0. 005), mekanik ventilasyon kullanımı 3. 143 kat (p=0. 007), kan transfüzyonu 3. 587 kat (p=0. 013), bronkopulmoner displazi 6. 702 kat, (p=0. 015) ve total parenteral beslenme 5. 591 kat (p=0. 001), düşük gebelik yaşı 1. 122 (1/0. 891) kat (p=0. 026) ve doğum ağırlığı 1. 001 (1/0. 999) kat (p=0. 013). Benzer şekilde, antibiyoterapi süresi ÇİD grubunda ÇİD olmayan gruba göre anlamlı uzun bulundu.

Sonuç: Gram-negatif yenidoğan enfeksiyonlarında ÇİD için bildirilen risk faktörlerinin tümü bağımlı risk faktörleridir. Bu nedenle klinisyenler tüm potansiyel risk faktörleri çoklu ilaç direncini öngörmekte önem taşımaktadır.

Anahtar Kelimeler: Gram Negatif Bakteriyel Enfeksiyonlar, Yenidoğanlar, Çoklu İlaç Direnci.

INTRODUCTION

Two-thirds of the isolates in neonatal sepsis are gram-negative. The most common organisms of sepsis in neonates are *Acinetobacter baumannii (A. baumannii), Klebsiella pneumonia (K. pneumonia), Staphylococcus aureus (S. Aureus), and Escherichia coli (E.coli).* Multidrug resistance (MDR), defined as resistance to any three of five antibiotic classes, is detected in 20% of gram-negative infections in neonates (1). In the multicenter research from China and Brazil, more than half of the late-onset conditions (after the three days of life) are caused by gram-negative microorganisms, *Enterobacterales*. In sub-Saharan Africa and India, gram negatives are the isolates in 40% of neonatal infections(2).

Antibiotic resistance, a rising problem, has different mechanisms in *Enterobacterales. E.coli, K. Pneumonia and Proteus mirabilis* have *Extended-spectrum ß lactamases (ESBL)* genes that inactive cephalosporins, aztreonam, and penicillin. *Carbapenem-resistant Enterobacterales (CRE)* has two main resistance mechanisms; carbapenemase or having poor membrane permeability with the production of an ESBL or AmpC *ß lactamases*. *K. Pneumonia* is the most common carbapenemase-producing bacteria. Independent risk factors for gram-negative microorganisms are gestational age ≤37 weeks, very low birth weight (<1500 grams), and prolonged hospitalization (more than 15 days). The inconsistent risk factors for gram-negative infections are mechanical ventilation, central venous catheters, parenteral nutrition, renal diseases, and cytopenias. Prolonged antibiotic administrations or prior cephalosporin exposures are also associated with MDR gram-negative infections (2). There is a debate on risk factors for MDR in neonatal sepsis. In one study, Gestational age, neurologic sequelae, and aminoglycoside were found to be essential risk factors (3). In another study, except for the variables of gestational age (>2500 g), cesarian labor, cytopenias, maternal infection, early onset sepsis, and antibiotic exposure were risk factors for MDR (4).

MDR gram-negative infections in neonates may be mortal, and the risk factors are contradictory. Therefore, this study aimed to represent the demographics of the neonates with gram-negative infections and determine the independent risk factors for MDR gram-negative infections and the mortality of MDR gram-negative infections.

MATERIAL AND METHODS

We conducted a retrospective single-center study about MDR gram-negative infections in neonates at Duzce University Hospital in Turkey. Demographic and bacteriological data were obtained from electronic records among neonates hospitalized in the neonatal intensive care unit between January 2012-January 2022; newborns infected with gram-negative microorganisms were included in the study. In addition, positive gram-negative cultures (blood, urinary, conjunctival, deep tracheal aspiration, catheter, tissue biopsy) were recorded. Exclusion criteria were the positive blood cultures inconsistent with the clinical manifestations or suspected to be contaminated bacteria.

Samples were inoculated on 5% sheep blood agar, chocolate agar, and eosin methylene blue agar (EMB) (Oxoid, UK) and incubated at 35°C for 18-24 hours in an aerobic environment. The microorganisms grown were identified by conventional microbiological methods (carbohydrate fermentation, citrate utilization, presence of urease, indole positivity, movement test) or automated system (VITEK 2, bioMérieux, France, BD Phoenix). In addition, antibiotic susceptibilities were performed by the Kirby-Bauer disk diffusion method and determined according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria.

This study evaluates neonates with MDR gram-negative infections' risk factors and clinical features. Patients infected with gram-negative bacteria were divided into; the MDR and the non-MDR groups. An MDR gram-negative organism was defined as an isolate that was non-susceptible to at least one agent in at least three antimicrobial classes. All antibiotic classes included penicillins, penicillins + ß lactamase inhibitors, non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins, extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins, fluoroquinolones, carbapenemase, cephamycins, glycylcyclines, folate pathway inhibitors, monobactam, polymyxin (1).

All analyses were performed using IBM SPSS V23. Univariate analyses were performed for each of the variables. The multivariate logistic regression model studied all the variables with a P value of ≤0.05 in the univariate analyses. The strength of the associations was shown by the odds ratio (OR) and 95% confidence interval (CI)—Fisher's Exact test calculated mortality rates in MDR gram-negative infections. Statistical significance was defined with a P value of <0. 05. Duzce University Ethics Committee approved the study on 20.06.2022 with approval number 2022/120.

RESULTS

The number of gram-negative infections was 105 between January 1, 2012, to January 1, 2022. For neonates, 61 (58.1%) of the patients were males, and 82 (75.9%) were born with a cesarian section. Fifteen newborns (14.4%) developed early-

onset sepsis and 53 (51%) developed late-onset sepsis. Thirty-six had gram-negative infections without sepsis. Of 105 infants, the median gestational age was 35 weeks, and the birth weight was 2503 grams. The cumulative mortality rate was 4.7% (n=5) (Table 1).

Table 1. Demographics and prognosis of neonates with gram-negative infections

	n	%
Sex		
Male	61	58.1
Female	44	41.9
Type of delivery		
Vaginal	26	24.1
Caesarian Section	82	75.9
Onset of sepsis		
None	36	34.6
Early-onset sepsis	15	14.4
Late-onset sepsis	53	51
Death		
Survivor	101	95.3
Exitus	5	4.7
	Mean ± standard deviation	Median (minimum-maximum)
Birth weight (gram)	2503.35 ± 940.15	2645 (730 - 5580)

Most patients (n=101) (98.1%) with gram-negative infection were afebrile, and the majority (n=69) (65.7%) of them received combination antibiotic therapy. Transfusion rates were 25%, and total parenteral nutrition was administered to 33 (30.8%) neonates. Table 2 includes laboratory abnormality rates and prognosis. Thrombocytopenia occurred in 16 (15.2%) and neutropenia in 9 (8.5%) (Table 2).

Table 2. Clinical features and laboratory abnormalities

	n	%
Period of pyrexia (days)		
0	101	98.1
1	2	1.9
Antibiotic Treatment		
One drug	36	34.3
Combination	69	65.7
Transfusion		
Absent	81	75
Present	27	25
Total parenteral nutrition		
Absent	74	69.2
Present	33	30.8
Surgery		
Absent	103	95.4
Present	5	4.6
Mechanical ventilation		
Absent	61	58.1
Present	44	42
Neutropenia		
Absent	97	91.5
Present	9	8.5
Thrombocytopenia		
Absent	89	84.8
Present	16	15.2
Acidosis in blood gas		
Absent	65	78.3
Present	18	21.7
Bronchopulmoner dysplasia		
Absent	92	85.2
Present	16	14.8
Prognosis	Mean ± standard deviation	Median (minimum-maximum)
The Day of positive culture	11.99 ± 12.18	8 (0 - 60)
Duration of antibiotic use (Day)	12.95 ± 15.1	7 (1 - 90)
Duration of antibiotic use before positive culture (Day)	9.11 ± 13.61	4 (0 - 78)
Length of hospital stay before positive culture	12.33 ± 16.39	5 (0 - 70)
Length of hospital stay (Day)	22.63 ± 25.65	10 (0 - 100)
Mean-time for culture negativization (Day)	9.94 ± 13.59	7 (0 - 60)

Before culture positivity, the median duration of antibiotic use was 4 (0 - 78) days, and the median length of hospital stay was 5 (0 - 70) days (Table 2). Additionally, 64 (59.3%) had positive urinary cultures, and 30 (27.8%) had positive blood cultures.

Of 107 gram-negative bacteria, 41 (38.3%) accounted for *Enterobacter,* 30 (28%) for *Klebsiella pneumonia,* and 22 (20.6%) for *Escherichia coli.* Additionally, 61 (56.5%) were MDR microorganisms. Therefore, the gram-negative isolates were mainly (n=71) (66.4%) studied between 2017-2022 (Table 3). Mortality rates were similar between the MDR (n=2) (4.3%) and non-MDR groups (n=3)(5%) (p=1).

Table 3. Microbiological findings

	n	%
Isolated site		
Missing	2	1.9
Blood	30	27.8
Urine	64	59.3
Conjunctival	1	0.9
Deep tracheal aspiration	4	3.7
Catheter	4	3.7
Tissue	2	1.9
MDR (multidrug resistance)		
Absent	47	43.5
Present	61	56.5
Period of cultural positivities		
2012-2016	36	33.6
2017-2021	71	66.4
Prevalence of gram-negative bacterial species		
Enterobacteria	41	38.3
Klebsiella pneumonia	30	28
Escherichia coli	22	20.6
Acinetobacter baumannii	6	5.6
Pseudomonas auroginosa	5	4.7
Stenotrophomonas maltophilia	1	0.9
Shigomonas paucimobilis	1	0.9
Serratia marsencens	1	0.9

Among the susceptibility tests performed for selected isolates, 41 (77.4%) had resistance to piperacillin, 57 (75%) showed resistance to amoxiclav, and 16 (72.7%) had cefoxitin resistance. In addition, carbapenemase resistance was found in 24 (43.6%) and meropenem resistance in 13 (36.1%). Colistin, aztreonam, and tigecycline resistances were the least frequent (Table 4).

Tablo 4. Antibiotic resistance among the gram-negative isolates

	Susceptible		Resistant	
	n	%	n	%
Antibiotic resistance	6	5.6	101	94.4
Penicillins				
Piperasilin	12	22.6	41	77.4
Penicillins + ß lactamase inhibitors				
Amoxilav	19	25	57	75
Antipseudomonal penicillins + ß-lactamase inhibitors				
Piperasilin/tazobaktam	23	39	36	61
Aminoglycosides				
Gentamicin	47	58	34	42
Amikacin	35	55.6	28	44.4
Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins				
Cefuroksime, Cefazolin	24	35.8	43	64.2
Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins				
Ceftazidime	15	34.1	29	65.9
Cefotaxim	17	32.1	36	67.9
Extended-spectrum cephalosporins; 3rd cephalosporin + ß-lactamase inhibitors				
Cefaperazone/sulbactam	9	69.2	4	30.8
Fluoroquinolones				
Ciprofloksasin	22	68.8	10	31.3
Carbapenemase	31	56.4	24	43.6
Meropenem	23	63.9	13	36.1
Cephamycins				
Cefoxitin	6	27.3	16	72.7
Glycylcyclines				
Tigecycline	9	90	1	10
Folate pathway inhibitors				
Cotrimoxazole	34	65.4	18	34.6
Monobactam				
Aztreonam	5	62.5	3	37.5
Polymyxin				
Colistin	15	88.2	2	11.8

Overall, resistance to antibiotics among gram-negative isolates is shown in Table 4. *Klebsiella and Enterobacter,* the primary isolates, primarily resisted penicillins (53.3% and 34.1%), penicillins + ß lactamase inhibitors (30% and 68.2%), piperacillin/tazobactam (26.7%, and 39%), amikacin (16.7%, and 39%), and gentamicin 20%, and 42.5%), respectively. Additionally, Enterobacter showed marked resistance to cefuroxime/cefazolin (51.2%) and ceftazidime (41.5%) while having resistance to cotrimoxazole (26.8%) and carbapenems (22.3%). Acinetobacter demonstrated marked resistance to piperacillin-tazobactam (83.3%) and ceftazidime (83.3%). In addition, Escherichia coli showed significant resistance to commonly used antibiotics. Gentamycin (60%), amikacin (40%), and ceftazidime (60%) were the leading antibiotics to which Pseudomonas was resistant. *Stenotrophomonas maltophilia (n=1)* and *Serratia marsencens (n=1)* were susceptible to commonly used antibiotics. However, Shigomonas paucimobilis resisted ampicillin, amoxicillin, gentamicin, amikacin, cefotaxime, and trimethoprim/sulfometaxole (Table 5).

Tablo 5. Antibiotic resistance among the gram-negative isolates

Isolated gram-negative microorganisms	*Enterobacteria (n=41) (38.3%)*	*Klebsiella pneumonia (n=30) (28%)*	*Escherichia coli (n=22) (20.6)*	*Acinetobacter baumannii (n=6)(5.6%)*	*Pseudomonas auroginosa (n=5) (4.7%)*	Others (n=3) (2.8%)
Antibiotic classes						
Penicillins						
Piperacillin	14 (34.1%)	16 (53.3%)	11 (50%)	0 (0%)	0 (0%)	1 (0%)
Penicillins + ß lactamase inhibitors						
Amoxilav	28 (68.2%)	15 (30%)	11 (50%)	1 (16%)	1 (20%)	1 (33.3%)
Antipseudomonal penicillins + ß-lactamase inhibitors						
Piperasilin/tazobactam	16 (39%)	8 (26.7%)	6 (27.2%)	5 (83.3%)	0 (0%)	1 (33.3%)
Aminoglycosides						
Gentamicin	17 (42.5%)	6 (20%)	6 (27.2%)	1 (16%)	3 (60%)	1 (33.3%)
Amikacin	16 (39%)	5 (16.7%)	3 (13.6%)	1 (16%)	2 (40%)	1 (33.3%)
Non-extended spectrum cephalosporins; 1st and 2nd generation cephalosporins						
Cefuroksime Cefazolin	22 (51.2%)	14 (43.3%)	7 (31.8%)	0 (0%)	0 (0%)	0 (0%)
Extended-spectrum cephalosporins; 3rd and 4th generation cephalosporins						
Ceftazidime	17 (%41.5)	3 (10%)	1 (4.5%)	5 (83.3%)	3 (60%)	0 (0%)
Extended-spectrum cephalosporins; 3rd cephalosporin + ß-lactamase inhibitors						
Cefaperazone/sulbactam	1 (2.4%)	2 (6.7%)	0 (0)	1 (16%)	0 (0%)	0 (0%)
Fluoroquinolones						
Ciprofloxacin	3 (7.3%)	5 (16.7%)	1 (4.5%)	1 (16%)	0 (0%)	0 (0%)
Carbapenemase	12 (22.3%)	7 (23.3%)	3 (13.6%)	1 (16%)	1 (20%)	0 (0%)
Meropenem	8 (19.5%)	4 (13.3%)	0 (0)	0 (0%)	1 (20%)	0 (0%)
Cephamycins						
Cefoxitin	6 (14.6%)	5 (16.6%)	3 (13.6%)	0 (0%)	0 (0%)	1 (33.3%)
Glycylcyclines						
Tigecycline	0 (0)	0 (0)	1 (4.5%)	0 (0%)	0 (0%)	0 (0%)
Folate pathway inhibitors						
Cotrimoxazole	11 (26.8%)	3 (10%)	2 (9%)	0 (0%)	1 (20%)	1 (33.3%)
Monobactam						
Aztreonam	2 (4.9%)	1 (3.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)
Polymyxin						
Colistin	1 (2.4%)	1 (3.3%)	0 (0%)	0 (0%)	0 (0%)	0 (0%)

Univariate and multivariate analyses analyzed risk factors associated with MDR gram-negative bacteria. Univariate analyses showed that late-onset sepsis increased the multidrug resistance risk 3.547 fold (p=0.005) than the non-sepsis group; mechanical ventilation increased the risk. 3.143 fold (p=0.007). The risk of multidrug resistance was 3.587-fold higher among neonates with blood transfusion. Bronchopulmonary dysplasia and total parenteral nutrition increased the risk of multidrug resistance rates 6.702 fold and 5.591 fold with a p-value of 0.015 ve 0.001, respectively. In addition, lower gestational age [1.122 (1/0.891) fold, p=0,026] and birth weight [1.001 (1/0.999) fold, p=0.013) increased the multidrug resistance risk. Similarly, antibiotherapy duration was significantly longer in the MDR group than in the non-MDR group (p=0.027). However, multivariate analyses concluded that none of the variables were independent risk factors (Table 6).

Table 6. Risk factors for multidrug-resistant gram-negative infections

	MDR (multidrug resistance)		Univariate		Multivariate	
	Absent	Present	OR (% 95 CI)	p	OR (% 95 CI)	p
	Mean± SD	Mean± SD				
Gestational age	36.4 ± 3.3	34.5 ± 4.6	0.891 (0.805 – 0.986)	**0.026**	1.285 (0.927 – 1.78)	0.132
Birth weight	2764.4 ± 887.3	2295.4 ± 936.2	0.999 (0.999 - 1)	**0.013**	0.999 (0.998 - 1)	0.147
Antibiotic delivery time (Day)	8.2 ± 3.9	16.9 ± 19.3	1.086 (1.009 – 1.168)	**0.027**	1.043 (0,954 - 1,139)	0.355
	n (%)	n (%)				
Sex						
Female	18 (40.9)	26 (59.1)	Ref.		Ref.	
Male	27 (44.3)	34 (55.7)	0.872 (0.398 – 1.912)	0.732	1.227 (0.335 – 4.494)	0.757
Onset of sepsis						
None	21 (58.3)	15 (41.7)	Ref.		Ref.	
Early onset of sepsis	7 (46.7)	8 (53.3)	1.6 (0.476 – 5.374)	0.447	0,918 (0,136 - 6,199)	0.930
Late onset of sepsis	15 (28.3)	38 (71.7)	3.547 (1.453 – 8.657)	**0.005**	2.653 (0.531 – 13.246)	0.234
Mechanical ventilation						
Absent	33 (54.1)	28 (45.9)	Ref.		Ref.	
Present	12 (27.3)	32 (72.7)	3.143 (1.366 – 7.229)	**0.007**	1.409 (0.301 – 6.591)	0.663
Neutropenia						
Absent	42 (43.3)	55 (56.7)	Ref.		Ref.	
Present	3 (33.3)	6 (66.7)	1.527 (0.361 – 6.465)	0.565	1.019 (0.062 – 16.787)	0.990
Thrombocytopenia						
Absent	39 (43.8)	50 (56.2)	Ref.		Ref.	
Present	5 (31.2)	11 (68,8)	1.716 (0.55 – 5.35)	0.352	2.311 (0.268 – 19.904)	0.446
Acidosis						
Absent	30 (46.2)	35 (53,8)	Ref.		Ref.	
Present	4 (22.2)	14 (77,8)	0.988 (0.59 – 1.656)	0.964	3.001 (0.418 – 21.535)	0.274
Type of labor						
Vaginal	15 (57.7)	11 (42,3)	Ref.		Ref.	
Cesarian	32 (39.0)	60 (61.0)	2.131 (0.87 – 5.218)	0.098	0.793 (0.193 – 3.256)	0.747
Blood Transfusion						
Absent	41 (50.6)	40 (49.4)	Ref.		Ref.	
Present	6 (22.2)	21 (77.8)	3.587 (1.311 – 9.815)	**0.013**	0.808 (0.092 – 7.124)	0.848
Bronchopulmonary dysplasia						
Absent	45 (48.9)	47 (51.1)	Ref.		Ref.	
Present	2 (12.5)	14 (87.5)	6.702 (1.441 – 31.168)	**0.015**	---	
Surgery						
Absent	46 (44.7)	57 (55.3)	Ref.		Ref.	
Present	1 (20.0)	4 (80.0)	3.228 (0.349 – 29.885)	0.302	---	
Total parenteral nutrition						
Absent	41 (55.4)	33 (44.6)	Ref.		Ref.	
Present	6 (18.2)	27 (81.8)	5.591 (2.064 – 15.142)	**0.001**	3.53 (0.304 – 40.979)	0.313

DISCUSSION

In the present study, the most common three gram-negative organisms for infections were *Enterobacterium, Klebsiella pneumonia,* and *Escherichia coli,* respectively, similar to the findings of previous studies (5,6,7,8). In contrast, Solomon showed that the most prevalent gram-negative bacterial species were *Klebsiella spp, Escherichia coli, and Acinetobacter baumannii* (9). *Klebsiella pneumonia and Acinetobacter baumanii* predominance were reported in previous studies (3,9). In contrast to our research, studies by Pokhrel et al. demonstrated *Klebsiella* and *Enterobacter* as the most common organisms (11).

Fifteen newborns in our study (14.4%) developed early-onset sepsis, and 53 (51%) developed late-onset sepsis. The overall mortality rates of gram-negative infections and MDR isolates were 4.7% vs. 4.3%, respectively, consistent with the report of Liu et al. (4.4% for hospital-acquired late-onset sepsis, 7.4% for early sepsis) (8).

In our study, gram-negative organisms showed high susceptibility to colistin (88.2%) and tigecycline (90%), followed by carbapenem (63.9%), which is consistent with the findings of Pokhrel et al. (11).

Our research showed that most gram-negative microorganisms resisted the frequently used antibiotics ampicillin, amoxiclav, cephalosporins, and colymixins. Our study is consistent with studies from China and Ethiopia and showed high resistance rates to commonly used antibiotics (9). Solomon et al. showed higher resistance rates to 3 and 4-generation cephalosporins (88% vs. 65.9%), amoxiclav (92% vs. 75%), gentamicin (85% vs. 42%) compared with our study. However, the rates of carbapenemases (43.6%) were markedly higher in our neonatal population than those reported in Solomon's study (1%) (12).

We report that *Enterobacteria,* the primary gram-negative isolate, showed significant resistance (more than >50%) to amoxiclav, 1st and 2nd generation cephalosporins, and carbapenemase rates of 22.3%. A recently published review about neonates recommended colistin, high-dose meropenem., and ceftazidime-avibactam in carbapenem-resistant enterobacteria (13). However, higher Carbapenemase rates were reported in several countries; Brazil, Egypt, Ghana, Greece, Japan, Poland, Taiwan (100%), and Turkey (72.6%) among toddlers, infants, and neonates, much more than our center (14).

Our study's second most typical organism of gram-negative infections, *Klebsiella pneumonia,* showed high resistance toward piperacillin. However, in another study, *Klebsiella pneumonia* demonstrated resistance to third-generation cephalosporins, aztreonam, beta-lactam, gentamycin, and tobramycin (15).

Our findings also showed that *E. coli* had high resistance to piperacillin and amoxiclav, showing susceptibility towards colistin and tigecycline, consistent with the meta-analysis results (14).

The emergence of infections with MDR bacteria neonates remains a significant challenge (16). The MDR rate in our study was 56.5%, similar to the survey from New Delhi (17).

MDRGN has been mainly reported in preterm newborn infants. Underlying disease, extended length of stay, surgery, prior use of antibiotics, invasive procedures, line central venous catheter and urinary catheter, BPD, and cytopenias, prolonged antibiotic use were the most commonly associated conditions.

Our work showed that lower gestational age, low birth weight, late onset of sepsis, use of mechanical ventilation, blood transfusions, bronchopulmonary dysplasia, and total parenteral nutrition were significantly associated with multidrug resistance. However, none were independent risk factors for MDR gram-negative infections, unlike Solomon et al., who found that low birth weight and late-onset sepsis were significantly associated with MDR resistance in sepsis (12). Zou et al. demonstrated that late-onset sepsis and antibiotic exposure were risk factors for MDR infection (9). Prior antibiotic exposure, underlying disease, invasive procedures, medical devices, and demographics were dominant risk factors for CRE infection (18,19,20).

The limitations are the retrospective and single-centered setting, small populations, and limited yield of some pathogens.

CONCLUSIONS

Important GNB pathogens, such as Enterobacteria, Klebsiella, and E.coli, are worth mentioning. Acinetobacter, and *Pseudomonas auroginosa*, were less reported. Risk factors should be avoided, despite their dependence on other variables. Hence clinicians must be alert to all potential risk factors.

REFERENCES

1. Wattal C, Kler N, Oberoi JK, Fursule A, Kumar A, Thakur A. Neonatal Sepsis: Mortality and Morbidity in Neonatal Sepsis due to Multidrug-Resistant (MDR) Organisms: Part 1. Indian J Pediatr. 2020.
2. Flannery DD, Chiotos K, Gerber JS, Puopolo KM. Neonatal multidrug-resistant gram-negative infection: epidemiology, mechanisms of resistance, and management. Pediatr Res. 2022;91(2):380-91.
3. Thatrimontrichai A, Premprat N, Janjindamai W, Dissaneevate S, Maneenil G. Multidrug-resistant Gram-negative bacilli sepsis from a neonatal intensive care unit: a case-case-control study. J Infect Dev Ctries. 2019;13(7):603-11.
4. Zou H, Jia X, He X, Su Y, Zhou L, Shen Y, et al. Emerging Threat of Multidrug-Resistant Pathogens From Neonatal Sepsis. Front Cell Infect Microbiol. 2021;11:694093.
5. Li JY, Chen SQ, Yan YY, Hu YY, Wei J, Wu QP,et al. Identification and antimicrobial resistance of pathogens in neonatal septicemia in China-A meta-analysis. Int J Infect Dis. 2018;71:89-93.
6. Oliveira PMN, Buonora SN, Souza CLP, Simões Júnior R, Silva TCD, Bom GJT,et al . Surveillance of multidrug-resistant bacteria in pediatric and neonatal intensive care units in Rio de Janeiro State, Brazil. Rev Soc Bras Med Trop. 2019;52:e20190205.
7. Aizawa Y, Shoji T, Ito K, Kasai M, Sakurai H, Toyofuku E,et al. Multidrug-resistant Gram-negative Bacterial Bloodstream Infections in Children's Hospitals in Japan, 2010-2017. Pediatr Infect Dis J. 2019;38(7):653-9.
8. Liu J, Fang Z, Yu Y, Ding Y, Liu Z, Zhang C, et al. Pathogens distribution and antimicrobial resistance in

bloodstream infections in twenty-five neonatal intensive care units in China, 2017-2019. Antimicrob Resist Infect Control. 2021;10(1):121.
9. Zou H, Jia X, He X, Su Y, Zhou L, Shen Y, et al. Emerging Threat of Multidrug-Resistant Pathogens From Neonatal Sepsis. Front Cell Infect Microbiol. 2021;11:694093.
10. Berberian G, Brizuela M, Rosanova MT, Travaglianti M, Mastroiani A,et al. Multidrug-resistant gram-negative infections in neonatology. Arch Argent Pediatr. 2019;117(1):6-11. English, Spanish.
11. Pokhrel B, Koirala T, Shah G, Joshi S, Baral P. Bacteriological profile and antibiotic susceptibility of neonatal sepsis in neonatal intensive care unit of a tertiary hospital in Nepal. BMC Pediatr. 2018;18(1):208.
12. Solomon S, Akeju O, Odumade OA, Ambachew R, Gebreyohannes Z, Van Wickle K, et al. Prevalence and risk factors for antimicrobial resistance among newborns with gram-negative sepsis. PLoS One. 2021;16(8):e0255410.
13. Chiusaroli L, Liberati C, Caseti M, Rulli L, Barbieri E, Giaquinto C, et al. Therapeutic Options and Outcomes for the Treatment of Neonates and Preterms with Gram-Negative Multidrug-Resistant Bacteria: A Systematic Review. Antibiotics (Basel). 2022;11(8):1088.
14. Osei Sekyere J, Reta MA, Bernard Fourie P. Risk factors for, and molecular epidemiology and clinical outcomes of, carbapenem- and polymyxin-resistant Gram-negative bacterial infections in pregnant women, infants, and toddlers: a systematic review and meta-analyses. Ann N Y Acad Sci. 2021;1502(1):54-71.
15. Asensio A, Oliver A, González-Diego P, Baquero F, Pérez-Díaz JC, Ros P, et al. Outbreak of a multiresistant Klebsiella pneumoniae strain in an intensive care unit: antibiotic use as risk factor for colonization and infection. Clin Infect Dis. 2000;30(1):55-60.
16. Magiorakos AP, Srinivasan A, Carey RB, Carmeli Y, Falagas ME, Giske CG, et al. Multidrug-resistant, extensively drug-resistant and pan drug-resistant bacteria: an international expert proposal for interim standard definitions for acquired resistance. Clin Microbiol Infect. 2012;18(3):268-81.
17. van Loon K, Voor In 't Holt AF, Vos MC. A Systematic Review and Meta-analyses of the Clinical Epidemiology of Carbapenem-Resistant Enterobacteriaceae. Antimicrob Agents Chemother. 2017;62(1):e01730-17.
18. Predic M, Delano JP, Tremblay E, Iovine N, Brown S, Prins C. Evaluation of patient risk factors for infection with carbapenem-resistant Enterobacteriaceae. Am J Infect Control. 2020;48(9):1028-31.
19. Barber KE, Wagner JL, Larry RC, Stover KR. Frequency of and risk factors for carbapenem-resistant *Enterobacteriaceae*. J Med Microbiol. 2021;70(2):001286.
20. Zhang AR, Wang Q, Zhou CE, Zhang JG, Wang XJ, Zhao JK, et al. [Risk factors and clinical prognosis analysis of carbapenem-resistant *Enterobacterales* bacteria nosocomial infection]. Zhonghua Yi Xue Za Zhi. 2021;101(21):1572-82.