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Neonatal Intestinal Colonization with Extended-Spectrum B-Lactamase-Producing Enterobacteriaceae: Molecular Analysis and Risk Factors in NICU neonates

Parvin Askari [1], Atiyeh Khosravi [2], Sedighe Rastaghi [3], Gholamreza Faal [4], * Mohammad Hasan Namaei [5]

[1] Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran.

[2] Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.

[3] Department of biostatistics and epidemiology, Mashhad University of medical science, Mashhad, Iran.

[4] Department of Pediatrics, Faculty of Medicine, Birjand University of Medical Sciences, Birjand, Iran.

[5] Infectious Diseases Research Center, Birjand University of Medical Sciences, Birjand, Iran.

Abstract

Background: The goal of this project was to assay the prevalence of fecal carriage of ESBL-producing Enterobacteriaceae (ESBL-PE), and to identify the risk factors for carriage in neonates hospitalized in a Neonatal Intensive Care Unit (NICU) of an educational therapeutic hospital (Vali-e-Asr) of Birjand city, east of Iran.

Methods: Rectal swabs were taken from 200 neonates at the beginning of hospitalization, every week in case of hospitalization and at the time of discharge. Bacterial isolates were identified using different biochemical experiments. Screening of ESBL-PE was first done by phenotypic test (DD test) and then antibiotic resistance genes were detected by PCR assay.

Results: In our research, 42 Enterobacteriaceae were obtained from 200 neonates. The total prevalence rate of neonatal rectal carriage of ESBL-PE was 42/200 (21%), mostly Escherichia coli, 18 (42.8%). blaCTX-M and blaCTX-M-15 were the most prevailing β-lactamase-encoding genes recognized by PCR tests. Intestinal carriage of ESβL among neonates displayed a statistically significant relationship with the use of the mechanical ventilation (p=0.025), APGAR score (p=0.005) and gestational age (weeks) (p=0.044).

Conclusion: Our findings highlighted the importance of consistent screening for resistant ESBL-PE among neonates (especially preterm newborns) and minimizing invasive ventilation whenever possible.

Key Words: Enterobacteriaceae, Extended-spectrum beta-lactamase, Intestinal carriage, Iran, Neonate, NICU.

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****Corresponding Author:***

Mohammad Hasan Namaei, Infectious Diseases Research Center, Birjand University of Medical Sciences (BUMS), Birjand, Iran. Email: mhnamaei@hotmail.com

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1- INTRODUCTION

Severe bacterial infections are one major reason for morbidity and mortality among newborns in neonatal intensive care units (NICU). Based on recent statistical analysis, globally, more than 1.4 million neonatal deaths occur due to invasive infections. Infections are responsible for 35% of all neonatal deaths in developing regions.

Neonatal infections are primarily caused by Enterobacteriaceae. Escherichia coli (E. coli) and Klebsiella pneumoniae were the most frequent species isolated from neonatal specimens (1-5). The most common significant adverse outcome in infections emerged from these bacteria is resistance to multiple drugs limiting the therapeutic options. Of special concern is the extended-spectrum beta-lactamase-producing Enterobacteriaceae (ESBL-PE) which is the most recurrently isolated multidrug resistant bacteria. ESBL-PE is resistant to cephalosporins and beta-lactams. ESBLs are classified in several beta-lactamase genes; usually CTX-M, SHV and TEM. Distribution of the ESBL genotypes varies in different geographical areas and time zones. CTX-M-type ESBLs are dominant in European, American and African countries, as well as some Asian countries like Japan, Saudi Arabia (1, 4, 6, 7). Within the CTX-M family, CTX-M-9 and CTX-M-15 are the most widely disseminated genotypes, respectively.

The gastrointestinal tract of the newborn can be easily colonized by ESBL-PE. A high fecal carriage of ESBL-PE strains can be a precursor to invasive infections in NICU. As a result, intestinal colonization with ESBL-PE has been extensively studied in neonates. The outbreak of ESBL-PE intestinal carriage in previous studies has been reported as 11-58.0% (1, 3, 8, 9). Prolonged hospitalization, prematurity (gestational age, 37 weeks), cesarean section, low birth weight, previous use of antibiotics, comorbidity, type of feeding, and necessary intensive care related to the use of invasive devices (particularly mechanical ventilators and intravenous catheters) are already established risk factors for intestinal ESBL-PE colonization (2, 4, 10-12). Colonized healthcare providers have also been identified as sources of ESBL-PE transmission to neonates (13). So, investigation of intestinal colonization of ESBL-PE and its associated risk factors are critical preventive strategies in the control of neonatal infections.

Neonatal infections are one of the major reasons of death in Iran and the most common cause of infections in neonates has been gram-negative bacilli (GNB) (14). There has been no research in Iran to estimate the prevalence of ESBL-PE rectal colonization and its associated risk factors in neonates in intensive care units. Therefore, the target of the current research was to study the outbreak of intestinal ESBL-PE colonization and to ascertain associated risk factors and antimicrobial resistance patterns among neonates in NICU.

2- MATERIALS AND METHODS

2-1. Setting

This cross-sectional analysis was carried out in the NICU of the educational therapeutic hospital (Vali-e-Asr) of Birjand. This hospital has 15 fully equipped beds for the most severe conditions, and the intermediate intensive care unit has 12 beds. Two specialist neonatologists and five nurses are assigned to this ward diurnal.

2-2. Design and Sampling

All neonates admitted to NICU during 04/03/2019 to 03/11/2019, and who remained hospitalized participated in the study. 610 rectal swabs were taken from 200 neonates at the beginning of hospitalization, every week in case of

hospitalization and at the time of discharge by NICU staff (as clinically indicated). Medical and demographic information, containing age, sex, APGAR score, gestational age, administration route, length of hospital stay, reason for hospitalization, and antibiotic therapy were documented for each neonate.

For environmental screenings, 96 swabs were taken from NICU equipment, including incubators, intravenous catheter, and umbilical vein catheter, feeding tube, oxyhood and radiant warmer; moreover, 256 stool specimens were obtained from healthcare workers (HCWs) per week during the study period. The swabs and stool samples were immediately transported to the microbiology laboratory.

2-2-1. Inclusion and exclusion criteria

The inclusion criteria encompassed neonates who were hospitalized, had a hospital stay of at least 24 hours, had absence of congenital major anomalies and chromosomal abnormalities which prevent swab sampling (such as anal abnormalities), and had parental permission for participation. Patients discharged before 24 h of hospitalization and those whose parents were dissatisfied to complete the study were excluded.

2-3. Microbial screening

Specimens were inoculated on two supplemented MacConkey agar with cefotaxime (4 µg/ml) and ceftazidime (4 µg/ml) and incubated at 37 C for 24 h. Suppositional Enterobacteriaceae (oxidase-negative, facultative aerobic, Gram-negative rods) were identified and confirmed by standard biochemistry methods (15). Then, Enterobacteriaceae colonies were stored in Tryptic Soy Broth (TSB) with 15% Glycerol at -25° C.

2-4. Antimicrobial Susceptibility Testing

Antimicrobial sensitivity was conducted for Enterobacteriaceae isolates using the disc diffusion method as recommended by the guidelines of the Clinical and Laboratory Standards Institute (CLSI, 2017). Antimicrobial agents (Mast, UK) including imipenem (10 µg), gentamicin (10 µg), ciprofloxacin (5 µg), amikacin (30 mg) and levofloxacin (5 µg) were tested. Escherichia coli ATCC 25922 were used as quality control strains (15).

2.-5. Phenotypic Determination of ESBLs

ESBL production was detected using the double-disk synergy test with disks of cefotaxime and ceftazidime alone and with cefotaxime + clavulanic acid and ceftazidime + clavulanic acid (Mast group, UK) on a Muller–Hinton agar plate (16). K. pneumoniae ATCC 700603 and Escherichia coli ATCC 25922 were used as positive and negative reference strains.

2-6. DNA Extraction

Total DNA was prepared from fresh colonies of Enterobacteriaceae with a positive phenotypic test by boiling method as defined formerly (17). Few fresh colonies of overnight growth of isolates resuspended in 500 mL of distilled water. The bacterial suspension was boiled at 100°C for 10 min in a dry block incubator, then centrifuged at 14,000 g for 10 min. Consequently, supernatant was used as a DNA template for PCR. The extracted DNA was stored at -20°C till analysis (15).

2-7. Detection of b-lactamase-encoding genes

PCR screening was done for detection of beta-lactamase encoding genes (blaCTX-M, blaTEM, blaSHV, and blaCTX-M-15) in the phenotypic ESBL positive strains using specific primers (**Table 1**). Amplification reactions were performed within the PeqSTAR 2X thermal cycler (PEQLAB, Germany) in a final volume of 20 µL containing 10 µL of Taq 2X Master Mix, 1µL of every primer (10 pmol), 2 µL of sample DNA, and 6 µL of nuclease-free water.

Table-1: Target genes and their primers used in the current research.

Primer	Sequence (5´-3´)	Products sizes (bp)	Annealing (°C)	Reference
bla_{TEM}	Fw-ATGAGTATTCAACATTTCCG Rv- CTGACAGTTACCAATGCT TA	868	54	(22)
bla_{SHV}	Fw- ATGCGTTATATTCGCCTGTGTAT Rv- TTAGCGTTGCCAGTGCTCGATCAG	868	56	(23)
bla_{CTX-M}	Fw- ATGTGCAGCACCAGTAAAGT Rv- ACCGCGATATCGTTGGTGG	542	55	(24)
$bla_{CTX-M-15}$	Fw- ATAAAACCGGCAGCGGTG Rv- GAATTTTGACGATCGGGG	483	55	(25)

Cycling parameters included denaturation at 94 C for 5 min, followed by 35 cycles of denaturation at 94 C for 30 seconds, annealing at 55 C for 40 seconds for CTX-M, and CTX-M-15 or at 54 C for 1 min for TEM and SHV, and extension at 72 C for 1 min, ending with a final extension period of 72 C for 10 min. The amplicons were analyzed by agarose gel electrophoresis stained with green viewer stain (parstous Co., Tehran, Iran) and visualized using an ultraviolet gel documentation device (Herolab, Germany). Positive control genes were obtained from Iran's Pasteur Institute.

2-8. Statistical Analysis: SPSS software Version 22.0 was utilized for statistical analysis. The Chi-square test and Fisher's exact test, where appropriate, were conducted to compare variables. A p-value < .05 was considered statistically significant.

3- FINDINGS

3-1. Baseline Characteristics

During the research course, 200 neonates were screened for fecal carriage of ESBL-PE, among whom, there were 98 male infants (49%) and 102 female infants (51%). The mean age of neonates was 2.64±7.0 days and their gestational age varied between 24-41 weeks (mean 34.46±4.8 weeks). In total, 77 (38.5%) neonates had respiratory distress which was the most common reason for hospitalization. Average length of hospital stay was 13.9 with SD of ±7.22 weeks. More than half of neonates were born by normal vaginal delivery. Characteristics of the neonates and risk factors for colonization with ESBL-PE during NICU care are summarized in **Table 2**.

Table-2: Features of the neonates and risk factors for ESBL-PE colonization.

Characteristics and Categories		ESBL + [n (%)]	ESBL - [n (%)]	Total N=200(%)	Chi-Square Tests/ a:Fisher's Exact Test
Sex	Male	16(16.3)	82(83.67)	98(49)	p-value=0.438
	Female	21(20.6)	81(79.4)	102(51)	
Age (days)	Newborn	26(20.2)	103(79.8)	129(64.5)	p-value=0.837[a]
	1-5	10(19.23)	42(80.76)	52(26)	
	5-10	0(0)	3(100)	3(1.5)	
	10-20	0(0)	7(100)	7(3.5)	
	20 <	1(11.2)	8(88.8)	9(4.5)	
Gestational age (weeks)	< 30	13(28.88)	32(71.11)	45(22.5)	p-value=0.044
	30-35	11(21.56)	40(78.43)	51(25.5)	

Characteristics and Categories		ESBL + [n (%)]	ESBL - [n (%)]	Total N=200(%)	Chi-Square Tests/ a:Fisher's Exact Test
	35-40	8(11.76)	60(88.23)	68(34)	
	40 <	4(11.12)	32(88.88)	36(18)	
Mode of delivery	Vaginal	18(48.6)	99(60.7)	117(58.5)	p-value=0.178
	Cesarean	19(51.4)	64(39.3)	83(41.5)	
APGAR score	10	20(13.33)	130(86.66)	150(75)	p-value=0.005
	9	13(35.2)	24(64.8)	37(18.5)	
	≤ 8	4(30.76)	9(69.23)	13(6.5)	
Type of feeding	Breast of feeding	3(16.6)	15(83.4)	18(9)	p-value=0.890[a]
	Breast milk through the syringe	7(19.44)	36(80.55)	43(21.5)	
	Formula milk	2(13.33)	15(86.66)	17(8.5)	
	Breast of feeding Breast milk through the syringe	18(23.37)	77(76.62)	95(47.5)	
	Breast milk & Breast milk through the syringe & formula feeding	6(28.57)	21(71.42)	27(13.5)	
Reason of hospitalization	Respiratory distress	21(27.3)	56(72.7)	77(38.5)	p-value=0.525
	Transient Tachypnea of Neonatal	2(11.76)	15(88.23)	17(8.5)	
	Prematurity	3(16.66)	15(83.33)	18(9)	
	Intrauterine growth restriction	2(22)	7(77.8)	9(4.5)	
	Sepsis	5(29.41)	12(70.58)	17(8.5)	
	Pneumonia	2(12.5)	14(87.5)	16(8)	
	Icterus	1(5.2)	18(94.73)	19(9.5)	
	Neonatal dehydration	0(0)	6(100)	6(3)	
	Meconium aspiration	0(0)	6(100)	6(3)	
	Mother's addiction	0(0)	5(100)	5(2.5)	
	asphyxia	1(25)	3(75)	4(2)	
	Convulsion	0(0)	2(100)	2(1)	
	Inguinal Hernia	0(0)	2(100)	2(1)	
	Arrhythmia	0(0)	2(100)	2(1)	
Length of NICU stay	≤5	3(12)	22(88)	25(12.5)	p-value=0.932[a]
	6-10	11(20)	44(80)	55(27.5)	
	11-15	14(21.9)	50(78.1)	64(32)	
	16-20	2(18.18)	9(81.81)	11(5.5)	
	21-25	4(16.66)	20(83.33)	24(12)	
	26 ≤	3(14.28)	18(85.71)	21(10.5)	
Exposure to invasive device					
Mechanical ventilation	Yes	18(27.3)	48(72.7)	66(33)	p-value=0.025
	No	19(14.2)	115(85.8)	134(67)	
Central venous catheter	Yes	2(33.33)	4(66.66)	6(3)	p-value=0.307a
	No	35(18.1)	159(81.9)	194(97)	
History of	Yes	3(21.5)	11(78.5)	14(7)	p-value=0.726

Characteristics and Categories		ESBL + [n (%)]	ESBL - [n (%)]	Total N=200(%)	Chi-Square Tests/ a:Fisher's Exact Test
hospitalization	No	34(18.3)	152(81.7)	186(93)	
Antibiotic therapy	Ampicillin & Gentamicin	33(18.9)	142(81.1)	175(87.5)	p-value=0.283
	Cefotaxime & Vancomycin	0(0)	5(100)	5(2.5)	
	Cefotaxime & Ampicillin	0(0)	5(100)	5(2.5)	
	Meropenem & Vancomycin	1(33.33)	2(66.67)	3(1.5)	
	Amikacin & Ceftazidime	0(0)	2(100)	2(1)	
	Cefotaxime & Meropenem	0(0)	1(100)	1(0.5)	
	Ceftriaxone & Gentamicin	0(0)	1(100)	1(0.5)	
	Amikacin	1(100)	0(0)	1(0.5)	
	Fluconazole	1(100)	0(0)	1(0.5)	
	Cefotaxime	1(100)	0(0)	1(0.5)	
	Meropenem	0(0)	1(100)	1(0.5)	
	Ceftazidime & Gentamicin & Ampicillin	0(0)	1(100)	1(0.5)	
	Cefotaxime & Vancomycin & Metronidazole	0(0)	1(100)	1(0.5)	
	Cefotaxime & Cefazolin & Amikacin & Metronidazole	0(0)	1(100)	1(0.5)	
	Cefotaxime & Cefazolin & Amikacin & Metronidazole & Vancomycin	0(0)	1(100)	1(0.5)	

a: Fisher exact test

3-2. Prevalence and Antimicrobial Susceptibility Patterns of ESβL-Producing Enterobacteriaceae

A total of 42 GNB were isolated from 200 hospitalized neonates. Mono-bacteria were isolated from 32 (16%) neonates and double bacteria were isolated from 5 (2.5%) neonates. The results of DDT displayed that 42 (100%) isolates were phenotypically confirmed as ESβL producers. All strains (42 strains with positive DDT) were confirmed as ESBL producers by PCR amplification. The overall prevalence of ESBL-PE among hospitalized neonates was 42/200 (21%). Out of 200 neonates hospitalized at the NICU in this study, 18.5% were ESBL-PE carrier.

The dominant ESBL-producer organism was Escherichia coli, 18(42.8%), followed by Serratia marcescens, 12(28.5%), Salmonella enterica, 5(11.9%), Citrobacter freundii, 3(7.14%), Enterobacter cloacae, 3(7.14%), and Klebsiella pneumoniae, 1(2.4%).

The most commonly used antibiotics were ampicillin + gentamicin in 175 neonates (87.5%), and gentamicin resistance occurred in 59.5% of ESBL-PE. The antibiotic resistance rates of quinolones occurred in 38.8% of Escherichia coli. All Serratia marcescens and Klebsiella

pneumoniae strains were susceptible to quinolones and imipenem (**Table 3**).

21.4% of ESBL-PE isolates were multidrug resistant, with resistance to amikacin, and quinolones being common; and only 2.4% were resistant to imipenem (**Table 3**).

Table-3: Frequency of resistance in ESBL-PE isolated

Enterobacteriaceae	No. (%) of resistant isolates				
	AMK	GEN	CIP	LVX	IPM
Escherichia coli (n=18)	1(5.5)	6(33.3)	4(22.2)	3(16.6)	1(5.5)
Serratia marcescens (n=12)	1(8.3)	10(83.3)	0(0)	0(0)	0(0)
Salmonella enterica (n=5)	1(20)	4(80)	2(40)	2(40)	0(0)
Citrobacter freundii (n=3)	0(0)	3(100)	1(33.3)	0(0)	0(0)
Enterobacter cloacae (n=3)	1(33.3)	1(33.3)	1(33.3)	1(33.3)	0(0)
Klebsiella pneumoniae (n=1)	1(100)	1(100)	0(0)	0(0)	0(0)
Total (n=42)	5(11.9)	25(59.5)	8(19)	6(14.3)	1(2.8)

Abbreviations: AMK, amikacin; GEN, gentamicin; CIP, Ciprofloxacin; LVX, Levofloxacin; IPM, Imipenem

Molecular analysis of the Enterobacteriaceae strains disclosed that 100% (42/42) had two of the four b-lactamase genes studied. Between all species, the bla_{CTX-M} was the most common gene detected (41/42; 97.6%), followed by $bla_{CTX-M-15}$ (40/42; 95.2 %), bla_{TEM} (36/42; 85.7%) and bla_{SHV} (12/42; 28.6%). The combinations of ESBL genes detected were bla_{CTX-M}/$bla_{CTX-M-15}$/bla_{TEM}/bla_{SHV} (23.8%, n=10), bla_{CTX-M}/ $bla_{CTX-M-15}$/ bla_{TEM} (54.7%, n=23), bla_{CTX-M}/$bla_{CTX-M-15}$ (14.3%, n=6), bla_{TEM}/bla_{SHV} (4.7%, n=2), and bla_{CTX-M}/bla_{TEM} (2.4%, n=1) as shown in **Table 4**.

Table-4: Multidrug Resistance Patterns of ESBL-PE among Hospitalized Neonates

Bacteria	Multiple antibiotics Resistance				Total
	R0	R1	R2	R3 and above	
Escherichia coli	2	12	3	1	18
Serratia marcescens	2	9	1	0	12
Salmonella enterica	0	3	1	1	5
Citrobacter freundii	0	3	0	0	3
Enterobacter cloacae	1	1	0	1	3
Klebsiella pneumoniae	0	0	1	0	1
Total	5	28	6	3	42

Out of 42 (21%) colonized neonates, 31 (15.5%) were found colonized with ESBL-PE on admission, which on the first screen (one week after admission) were negative for beta-lactamase enzymes. In the second screening (second week after admission), 5 (16.1%) infants were colonized with ESBL-PE. In 3 infants, the identified bacteria with the isolates at the beginning of admission were of the same species and also in terms of antibiotic susceptibility patterns and the antibiotic resistance genes were similar. But the bacterial isolates in 2 neonates were of different species from

those detected at the time of admission. Rectal swabs of neonates who were colonized on admission, were negative for the ESBL-PE in the third and subsequent screens.

Among the noncarriers on admission, 3% (5/169) acquired ESBL-PE in the second week of hospitalization, but none of these neonates at the time of discharge were identified as carriers of ESBL-PE (**Fig. 1**).

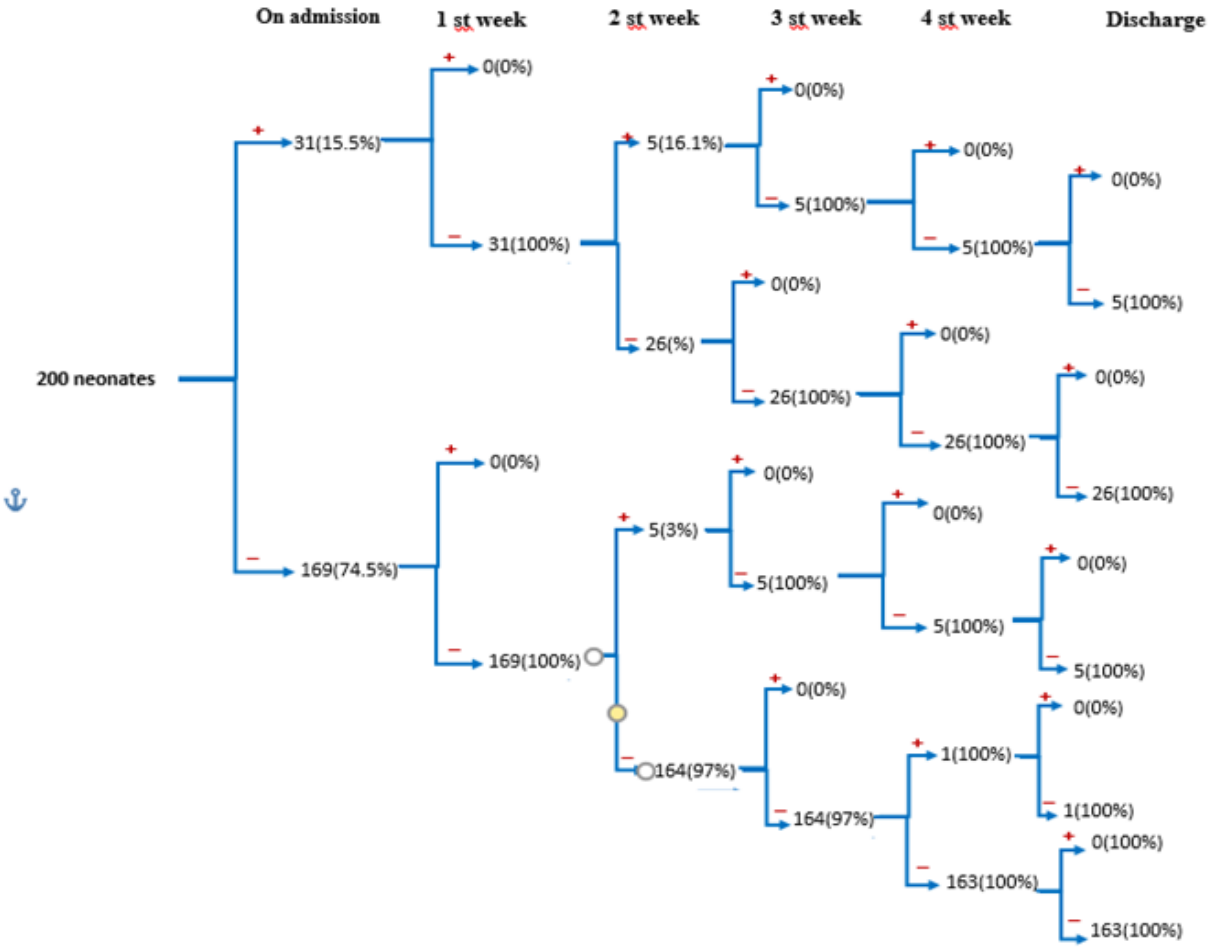

Fig. 1: Neonates' distribution in accordance to ESBL-PE carriage status during follow-up time

A co-colonization with ESBL-PE was later seen in two of 42 neonates during follow-up time.

All NICU staff fecal cultures (N=256) and 96 environmental cultures were negative for ESBL-PE.

3-3. Factors associated with ESβL-Producing Enterobacteriaceae Carriage

Bivariate analysis showed that the following variables were related to carriage rate of ESβL-PE during hospitalization in NICU: APGAR score (p-value= 0.005), use of the mechanical ventilation (p-value= 0.025), gestational age (p-value= 0.044). Other variables did not affect the rate of rectal carriage by ESBL-PE (**Table 2**). The relationship between all three variables and the type of antibiotic use was also investigated. Two variables, gestational age (p-value=0. 764) and APGAR score (p-value= 0.856), were

not associated with antibiotic use, but there was a significant relationship between the use of the mechanical ventilation and type of antibiotic received (p-value= 0.004).

Table-5: Distribution of genotypes according to species of ESBL-PE

GNB	% of isolates by genotype			
	CTX-M	CTX-M-15	SHV	TEM
Escherichia coli	100	100	66.7	88.9
Serratia marcescens	100	100	16.7	83.3
Salmonella enterica	100	80	0	60
Citrobacter freundii	66.7	66.7	66.7	100
Enterobacter cloacae	100	100	33.3	100
Klebsiella pneumoniae	100	100	100	100
Total	97.6	95.2	28.6	85.7

4- DISCUSSION

Neonatal course is the most critical time for child survival. Bacterial infections caused by multidrug-resistant GNB that produce ESBL enzymes are one of the major causes of death in Iran (14). Few information has been published regarding risk factors for neonatal colonization or infection with ESBL-PE. Based on the findings of this research, the prevalence of ESBL-PE fecal carriage was (18.5%); a similar range (10.4-58%) was reported by multiple studies from rectal swabs of neonates in various Asian and African countries (1-3, 18). In the present study, ESBL E. coli was the most prevalent isolate, which is consistent with previous findings in the Saudi Arabia, Ecuador, Czech Republic and Madagascar (2-4, 7). However, in studies conducted in Mexico City, Ethiopia, and Morocco, K. pneumoniae isolates were found to be the major ESβL producers among hospitalized neonates (1, 18, 19). Differences in geographical region, sample size, hospital setup and methodological changes can be the major reasons for these changes.

In present study ESBL-PE bacilli displayed a higher resistance against gentamicin (59.5%), Ciprofloxacin (19%). These outcomes are in line with previous studies done in Ecuador, Ethiopia and Morocco (1, 7, 18). Though, these results are completely different from the outcomes of the study carried out by Talat Elkersh (2). Among ESBL-PE isolates in our study, 24 isolates were MultiDrug Resistant (MDR) (57.1%). Our result showed a lower prevalence of MDR ESBL-PE compared to studies conducted in Ethiopia (87.5%) (18) and Morocco (>91%) (1). The lower prevalence rate in our region can be attributed to the setting of standard diagnosis and the unavailability of some antibiotics.

Out of the four ESBL genes (bla_{CTX-M}, $bla_{CTX-M-15}$, bla_{TEM}, and bla_{SHV}) analyzed, bla_{CTX-M} was the predominant gene (97.6%), followed by $bla_{CTX-M-15}$ and bla_{TEM} gene with a prevalence of 95.2% and 85.7%, respectively. These outcomes are in agreement with other studies on the pediatric population in Qatar (20) and NICU neonates at Bugando Medical Center, Tanzania (13), and the department of Neonatology, Olomouc (4). In contrast, Arhoune et al. found that bla_{SHV} and bla_{TEM} demonstrated rates of 67.3% and 42.8%, respectively, among all ESBL-PE strains originating from neonates in NICU in Fez, Morocco (1).

In the current study, we observed that 85.7% of ESBL-PE strains isolated from rectal swabs, harbor multiple ESBL genes

(bla_{CTX-M}, bla_{TEM}, and bla_{SHV}), similar to a study from Tanzania and Ecuador (7, 13). We found no other similar studies among hospitalized neonates in NICU in Iranian hospitals.

Also, for the first time in Iran, we evaluated the risk factors (sex, age in days, gestational age, mode of delivery, APGAR score, type of neonatal feeding, reason of hospitalization, length of NICU stay, exposure to invasive device, history of hospitalization, antibiotic therapy) on the intestinal colonization of ESBL-PE. There was no statistically noteworthy difference in ESBL-PE carriage rate between females and males. This data is congruent with the finding of former research from Kenya, Ecuador, and Ethiopia (1, 7, 18). Results from our study revealed that the frequency of intestinal colonization of ESBL-producing GNB in the first day of life is upper than fecal carriage in the following days of life; and, there is no relationship between the neonatal age and intestinal carriage percentages of ESBL-PE. This observation is close to former studies demonstrating that the neonatal gastrointestinal tract colonization with ESBL-PE is transient in the first days of life (2, 18). However, Kagia's study revealed that increasing the age of neonates has a positive effect on the fecal carriage of ESBL-PE.

We did not observe a correlation between rectal carriage of ESBL-PE and mode of delivery. Our results are in line with those previously published by Nordberg et al (7). But in conflict with some other studies (2, 3, 11). Attention to infection control programs in the investigated hospital can be the reason for this difference in results between studies.

There are quite different results from previous studies regarding the type of feeding and its effect on ESBL-PE intestinal colonization. Elkersh et al. (2015) found that colonization rate among breastfed neonates were higher as compared with formula feeding alone or mixed with breastfeeding (2). In Ecuador, Nordberg et al. reported that nourishing with a mixture of formula and breastfeeding was a risk factor for ESBL-colonization (7). However, the present study revealed that different feeding methods had no effect on the ESBL-PE fecal carriage, which is similar to previous findings in Ethiopia (18).

Since overuse and misuse of antibiotics are the primary stimulus in the expansion of drug-resistant bacteria, we looked for the effects of antibiotics usage on ESBL enteric carriage rate in neonates. In other reports, the antimicrobial combination therapy (aminoglycosides plus third-generation cephalosporin or aminoglycoside plus ampicillin) was utterly related to the resistant gram-negative bacilli acquisition (9, 18). But in our study, there was no correlation between the consumption of different antibiotic compounds and the rectal carriage of ESβL by neonates. The discrepancy in the results can be due to the hospital-based antimicrobial stewardship programs in the NICU department of our study.

Other studies done in Ethiopia, Brazil, the Czech Republic, and México have demonstrated that long time stay in the NICU was associated with Gastro-Intestinal Tract (GIT) ESBL-PE colonization in the NICU (4, 9, 18, 19). But our results showed that the long stay in NICU had no effect on the rate of intestinal acquisition ESBL-PE. Since all the stool cultures of NICU staff fecal cultures and environmental cultures were negative for ESBL-PE during the study period, the above finding is confirmed.

We found that using mechanical ventilation is a risk factor for ESBL-PE acquisition which is in line with previous studies on ESBL-PE carriage (4, 7, 9, 18). APGAR score, which is a quick measure for doctors to evaluate the health of

neonates, is related to carriage percentages of ESBL-PE in the stool. To our knowledge, there is no research investigating the relationship between the Apgar score and neonatal ESBL-PE carriage. We also found that gestational ages (weeks) less than 30 are associated with an intestinal carriage rate of ESβL-PE during hospitalization in NICU, similar to a recent report (18).

Several studies have demonstrated that maternal–neonatal transmission is one of the significant risk factors for transferring ESBL-PE to fetus (11, 21). One limitation of our study is that pregnant women were not examined for recto-vaginal carriage of ESBL-PE.

5- CONCLUSION

In this research, the prevalence of neonatal intestinal colonization with ESBL-PE was (18.5%). CTX-M type enzymes are the most detected enzymes among ESβL-PE. Risk factors related to intestinal carriage of ESBL-PE in neonates were use of the mechanical ventilation, APGAR score, and gestational ages (weeks) less than 30.

6- ETHICAL CONSIDERATIONS

The study was approved by the ethics committee of Birjand University of Medical Sciences (IR.BUMS.REC.1397.233). Written informed consent to participate in the study was obtained from the parents or guardians of all participants. Also, no additional cost was imposed on the participants.

7- CONFLICTS OF INTERESTS

None.

8- FUNDING

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9- SUPPLEMENTARY MATERIALS

Table-6: Associations between the type of antibiotic received and gestational age

Type of Antibiotic received	Age_gestational_Level				Total	Fisher's Exact Test p-value
	30>	30-35	35-40	40 <		
Vancomycin + Meropenem	2	0	1	0	3	0.764
Gentamicin + ampicillin	40	42	59	34	175	
Fluconazole	1	0	0	0	1	
Cefotaxime	0	1	0	0	1	
Ceftrixon + Chentamycin	0	0	1	0	1	
Meropenem	1	0	0	0	1	
Amikacin	0	1	0	0	1	
Cefotaxime + Vancomycin	2	2	0	1	5	
Cefotaxime + ampicillin	0	1	3	1	5	
Cefotaxime + Meropenem	0	1	0	0	1	
Cefotaxime + cefazolin + amikacin + metronidazole	0	0	1	0	1	
Multidrug therapy	0	1	0	0	1	
Cefotaxime + vancomycin + metronidazole	0	0	1	0	1	
Ceftazidime + amikacin	0	1	1	0	2	
Ampicillin + gentamicin + ceftazidime	0	0	1	0	1	
Total	46	50	68	36	200	-

Table-7: Associations between the type of antibiotic received and APGAR score

Type of Antibiotic received	APGAR score			Total	Fisher's Exact Test p-value
	Score 10	Score 9	Score 8 or less		
Vancomycin + Meropenem	3	0	0	3	0.856
Gentamicin + ampicillin	127	36	12	175	
Fluconazole	1	0	0	1	
Cefotaxime	1	0	0	1	
Ceftrixon + Chentamycin	1	0	0	1	
Meropenem	1	0	0	1	
Amikacin	1	0	0	1	
Cefotaxime + Vancomycin	5	0	0	5	
Cefotaxime + ampicillin	5	0	0	5	
Cefotaxime + Meropenem	0	0	1	1	
Cefotaxime + cefazolin + amikacin + metronidazole	1	0	0	1	
Multidrug therapy	1	0	0	1	
Cefotaxime + vancomycin + metronidazole	1	0	0	1	
Ceftazidime + amikacin	1	1	0	2	
Ampicillin + gentamicin + ceftazidime	1	0	0	1	
Total	150	37	13	200	

Table-8: Association between the type of antibiotic received and use of ventilation

Type of Antibiotic received	Negative	Use ventilation		Fisher's Exact Test p-value
		Positive	Total	
Vancomycin + Meropenem	0	3	3	0.004
Gentamicin + ampicillin	121	54	175	
Fluconazole	0	1	1	
Cefotaxime	1	0	1	
Ceftrixon + Chentamycin	0	1	1	
Meropenem	1	0	1	
Amikacin	1	0	1	
Cefotaxime + Vancomycin	2	3	5	
Cefotaxime + ampicillin	5	0	5	
Cefotaxime + Meropenem	0	1	1	
Cefotaxime + cefazolin + amikacin + metronidazole	1	0	1	
Multidrug therapy	0	1	1	
Cefotaxime + vancomycin + metronidazole	0	1	1	
Ceftazidime + amikacin	2	0	2	
Ampicillin + gentamicin + ceftazidime	0	1	1	
Total	134	66	200	

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