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The Association Between Genetic Variants in Extended Spectrum Beta-Lactamases and AmpC-producing Gram-Negative Bacilli and Antibiotic Resistance

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ABSTRACT

Background and Aim: A large group of genes associated with extended-spectrum beta-lactamases (ESBL) and AmpC expression is involved in inducing antibiotic resistance in various bacteria. Identifying these genes and assessing their harboring will be crucial in determining the pattern of antibiotic resistance. This study aimed to characterize genetic variants in extended-spectrum beta-lactamases, AmpC-producing gram-negative bacilli, and associated antibiotic resistance.

Materials and Methods: This cross-sectional research was conducted on clinical samples of patients admitted to Rasool-e-Akram Hospital, Tehran, Iran (2019 and 2020). The genetic variants were assessed by the Polymerase chain reaction method. The pattern of durability to antibiotics was determined by the disk diffusion system.

Results: Of the 80 isolates that produced ESBL and AmpC beta-lactamase, 75 cases were ESBL producers and 5 cases co-producers of ESBL and AmpC. The most common cultivated strains included *Escherichia coli* (61.2%) and *Klebsiella pneumoniae* (31.3%). The highest antibiotic resistance in ESBL producers was related to cefotaxime, trimethoprim-sulfamethoxazole, and cefazolin, and the lowest resistance level was related to colistin, cefoxitin, and ceftriaxone. The total frequencies of common genes of ESBL-producing gram-negative bacilli were bla_{CTX-M} (76.0%), bla_{TEM} (46.7%), and bla_{SHV} (26.7%), while multigenic harboring was also observed in 49.3%. The level of drug resistance to Imipenem, Amikacin, and Ceftazidime in people who harbored the bla_{SHV} gene was significantly higher than in other cases.

Conclusion: The most common cultivated strains included *E. coli* and *K. pneumoniae*. bla_{CTX-M}, bla_{TEM}, bla_{SHV}, and multigenic expression were observed. The detection of the bla_{SHV} gene is associated with increased antibiotic resistance to Imipenem, Amikacin, and Ceftazidime.

Keywords: AmpC beta-lactamase, Antibiotic resistance, Extended-spectrum beta-lactamase, Gram-negative bacilli

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1 Introduction

Bacterial durability to antibiotics is an essential scientific issue related to community health as well as hospitals, which has led to the failure of treatments for dangerous infections (1,2). Fast detection of organisms' resistance to antimicrobial is important in clinical laboratories (3). Characterization of the resistance mechanism can facilitate innovation in antimicrobials design. Three general pathways for antibiotic resistance have been identified: A) The generation of beta-lactamases, which break the beta-lactam loop and thus inactivate the beta-lactam antibiotic, B) Lack or decrease in the exposition of outer membrane proteins called OMP in gram-negative pathogens and C) Increased expression of membrane pumps (4-7). ESBL production is an important resistance mechanism that prevents the antimicrobial treatment of gram-negative infections and shows a significant hazard to available antibiotics,

particularly β-lactam antibiotics, including penicillins, cephalosporins, and monobactams **(8-10)**. AmpC β-lactamases preferentially hydrolyze narrow, broad, and expanded-spectrum cephalosporins and cephamycins and abide blockage by clavulanate, sulbactam, and tazobactam. AmpC-producing strains are generally resistant to oxyimino-beta lactams and cephamycins and are sensitive to carbapenems, but decreased porin expression will build such a strain carbapenem-persistent as well **(11)**.

The introduction of carbapenems to the medical world has been the main breakthrough in the remedy of diseases caused by beta-lactam-resistant bacteria. Carbapenems are the Preferred medicine for treating infections caused by penicillin-resistant or cephalosporin-resistant gram-negative bacilli due to their wide range of activity and resistance to hydrolysis by most beta-lactamases **(12,13)**. Resistance to carbapenem has been attributed to various factors such as decreased expression of outer membrane proteins, increased efflux activity, and production of carbapenemase beta-lactamases **(14)**.

According to the Ambler molecular classification, beta-lactamases are divided into four groups: class A, C, and D are serine beta-lactamase, and class B is Metallo-β-lactamase (MBL) **(15)**. Extended-spectrum Beta-lactamases are interdicted by clavulanic acid, sulbactam, and tazobactam and are encoded by different genes (including bla_{SHV}, bla_{TEM}, bla_{OXA}, bla_{PER}, bla_{VEB-1}, *and* bla_{BES-1}) that can vary between bacteria **(16-19)**. Among the various types of ESBL, the most common subgroups *are* bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, which are crucial factors in antibiotic resistance in important strains such as *Escherichia coli* and *Klebsiella pneumoniae* **(20,21)**.

AmpC Beta-lactamases are encoded by different genes such as bla_{CMY}, bla_{FOX}, bla_{DHA}, bla_{MOX}, *and* bla_{CIT}. However, there is little information about the frequency of these plasmid genes and their genetic pattern in Iran.

The outbreak of antibiotic resistance is increasing, and one of the essential resistance mechanisms is the generation of extended-spectrum ESBL and AmpC by gram-negative bacilli. Therefore, determining its prevalence in each region, like hospitals, is crucial. Also, it seems that gene changes of resistant microorganisms can be different in each region, and monitoring the changes can help determine better and more appropriate treatments, especially in more sensitive units such as intensive care units and infectious diseases units. Hence, this research attempted to characterize the outbreak of fast-growing gram-negative bacilli producing ESBL and AmpC and evaluate their common genetic variants.

2.Materials and Methods

Bacterial Isolates

This cross-sectional research was carried out on 9424 clinical samples (including blood, urine, BAL, trachea, or Shaldon) of patients admitted to different wards of Rasool-e-Akram Hospital (Tehran, Iran) from 2019 to 2020. The samples were sent to the hospital's laboratory at the request of physicians to detect and isolate various fast-growing gram-negative bacilli. The isolates were recognized using phenotypic techniques.

Antibiotic Susceptibility Testing

The pattern of resistance to 8 antibiotics was identified considering Clinical and Laboratory Standards Institute (CLSI) guidelines by disk diffusion method by Rosco Neosensitabs (Rosco Diagnostica, Taastrup, Denmark) (Table 1). Imipenem (10mcg), Meropenem (10mcg), Ampicillin (10mcg), Penicillin (10IU), Aztreonam (30mcg), Cefotaxime (30mcg), Cefoxitin(30mcg), and Ceftazidime (30mcg) were used in this study.

Table 1. CLSI guidelines (2020) of current antibiotics against gram-negative bacilli

Antibiotic	S(mm or more)	I(mm)	R(mm or more)
Ceftazidime	21	18–20	17
Cefotaxime	26	23–25	22
Ampicillin	17	14–16	13
Penicillin	17	14–16	13
Imipenem	23	20-22	19
Meropenem	23	20-22	19
Aztreonam	21	18-20	17
Cefoxitin	18	15-17	14

If the activity of the above antibiotics, except for Imipenem (meropenem) and Cefoxitin, were inhibited in the culture medium, the presence of ESBL was suspected. In this case, their existence was confirmed by confirmatory tests, which was the use of clavulanic acid (beta-lactamase inhibitor).

Screening for ESBL-producing Strains

Suspicious specimens were first cultured on Müller-Hinton agar (Condalab, Spain). Therefore, after suspecting the presence of ESBL, we used a disc containing clavulanic acid and compared the diameter of the inhibitory zone with and without inhibitors so that if the difference was more than 5 mm, ESBL was confirmed (Figure 1).

Figure 1. Evaluation of antibiotic resistance for ESBL-Producing gram-negative bacilli

Screening for AmpC-producing Strains

In addition, we were suspected of having AmpC if, in the first stage, all antibiotics, except imipenem (or meropenem), were inhibited in the culture medium, or the second stage, ESBL confirmation tests were negative. Therefore, we used cefoxitin-cloxacillin (CFO-CX) disc. A distinction between the cefoxitin-cloxacillin inhibition zone and the cefoxitin alone zone of ≥4 mm indicated AmpC production (Figure 2).

Figure 2. Evaluation of antibiotic resistance for AmpC-Producing gram-negative bacilli

Molecular Specification of ESBL and AmpC-betalactamases Genes

Finally, special primers were planned for the extracted bacteria to study the genes of bla_{TEM}, bla_{SHV}, *and* bla_{CTX-M} in ESBL, and bla_{FOX}, bla_{MOX}, bla_{CIT}, bla_{DHA}, and $_{bla}EBC$ for AmpC (Table 2) **(22, 23)**.

Table 2. Oligonucleotide sequences used in this study

Target Gene	Primer Sequencing	Annealing Temperature	Product Size(bp)
CTX-M	F:GGT TAA AAA ATC ACT GCG TC R:TTG GTG ACG ATT TTA GCC GC	58	863
TEM	F:CGC CGC ATA CAC TAT TCT CAG AAT GA R:ACG CTC ACC GGC TCC AGA TTT AT	65	445
SHV	F:CTT TAT CGG CCC TCA CTC AA R:AGG TGC TCA TCA TGG GAA AG	58	237
CIT	F:TGG CCA GAA CTG ACA GGC AAA R:TTT CTC CTG AAC GTG GCT GGC	61	462
FOX	F:AAC ATG GGG TAT CAG GGA GAT G R:CAA AGC GCG TAA CCG GAT TGG	63	190
MOX	F:GCT CAA GGA GCA CAG GAT R:CAC ATT GAC ATA GGT GTG C	55	517
EBC	F:TCG GTA AAG CCG ATG TTG CGG R:CTT CCA CTG CGG CTG CCA GTT	63	302
DHA	F:AAC TTT CAC AGG TGT GCT GGG T R:CCG TAC GCA TAC TGG CTT TGC	63	405

Multiplex PCR (Bio-Rad Thermal Cyclers, Singapore) was utilized to identify the major ESBL and AmpC beta-lactamase genes. Single colonies of each isolate were cultivated, and bacterial cells were bulleted by centrifugation (14000 g for 4 min) after removing supernatants. DNA extraction was carried out exploitation the microorganism GeneAll KIT (Seoul, Korea). 3 µL of Total DNA (10 ng) was added to every multiplex PCR reaction. The reaction mixture contained 12 µL mastermix (Tempase hot begin, Amplicon, Denmark), 0.7 µL specific primer (10 Pmol), and 5 µL nuclease-free water. In this study, thermal cycling PCR (BIO-RAD) was used according to the following protocol:

The primary denaturation was executed at 95°C for 15 min, followed by 29 cycles of denaturation at 94°C for 30 s, tempering at 54°C for 80 s, extension at 72°C for 1 min and final extension at 72°C for 10 min.

Finally, the Amplified genes were loaded on 2% agarose gel and electrophoresed (Gelldoc, Iran). The PCR product was stained by SYBR stain and visualized by a gel documentation system according to standard procedures.

It is necessary to explain that to carry out this project, no additional sampling was taken from the hospitalized patients. All the steps were taken on the same samples sent to the hospital laboratory at the request of the physicians.

Results of the actuarial assay were reported as mean ± standard deviation (SD) for quantitative variables and were epitomized by frequency (percentage) for classified variables. Contiguous variables were evaluated using t-test or Mann-Whitney test whenever the data did not appear to have normal dispensation or when the presumption of similar variances was breached across the study groups. On the other hand, categorical variables were evaluated using the Chi-square test. A P-value of ≤ 0.05 was considered statistically significant. The statistical software SPSS version 23.0 for windows (IBM, Armonk, New York, USA) was used for the actuarial assay.

3.Results

Bacterial Isolation

In the present research, Out of 9424 samples sent to the laboratory, 288 suspected cases were identified and after confirmatory tests, finally 80 ESBL and AmpC beta-lactamase productive isolates were detected. From these isolates, 75 cases are ESBL producers, and 5 cases are co-producers of ESBL and AmpC.

Because of few cases of co-producers, a total of 75 specimens were extracted and studied. In total, 31 cases (41.3%) were male with mean age 56.8±35.23 years, and 44 cases (58.7%) were female with mean age 55.21±46 years. Specimens were accumulated from various hospital wards (details in Table 3). Also, the extracted samples included urine, BAL, blood, trachea, and Shaldon secretions that the details are described in Table 3.

Table 3. Characteristics of the studied samples

Character	Category	Number (percent)
Gender	Man	31 (41.3)
	Female	44 (58.7)
Inpatient department	Emergency	15 (20.0)
	Respiratory disease	15 (20.0)
	Internal medicine	9 (12.0)
	Infectious disease	6 (8.0)
	Respiratory ICU	7 (9.3)
	MICU	5 (6.7)
	Gynecology	4 (5.3)
	Surgery	4 (5.3)
	Pediatrics	3 (4.0)
	post CCU	2 (2.7)
	Surgical ICU	1 (1.3)
	Open heart ICU	1 (1.3)
	Ophthalmology	1 (1.3)

Character	Category	Number (percent)
Sample type	Skin disease	1 (1.3)
	Urine	57 (76.0)
	Trachea	1 (1.3)
	Blood	4 (5.3)
	BAL	12 (16.0)
	Shaldon	1 (1.3)
Mean age, year	55.43±20.36	

The types of bacilli identified were *E. coli* in 44 cases (58.7%), *K. pneumoniae* in 25 samples (33.3%), *Pseudomonas aeruginosa* in 3 samples (4.0%), *Enterobacter cloacae* in 2 samples (2.2%) and *Acinetobacter baumannii* in 1 sample (1.3%). The above study and the information obtained were the results of 75 ESBL producers. The frequency of gram-negative bacilli in terms of primary characteristics is listed in detail in Table 4.

Table 4. Distribution of ESBL gram-negative bacilli according to study characteristics

Bacillus	*E. coli*	*Klebsiella*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Acinetobacter baumannii*	P-value
Gender						0.270
Man	54.8%	32.3%	9.7%	3.2%	0.0%	
Female	61.4%	34.1%	0.0%	2.3%	2.3%	
Mean age, year	54±20	57±18	56±30	72±11	60±10	0.779
Inpatient department						0.001
Emergency	80.0%	13.3%	6.7%	0.0%	0.0%	
Respiratory disease	53.3%	33.3%	6.7%	6.7%	0.0%	
Internal medicine	55.6%	33.3%	11.1%	0.0%	0.0%	
Infectious disease	66.7%	33.3%	0.0%	0.0%	0.0%	
Respiratory ICU	14.3%	71.4%	0.0%	0.0%	14.3%	
MICU	20.0%	80.0%	0.0%	0.0%	0.0%	
Gynecology	75.0%	25.0%	0.0%	0.0%	0.0%	
Surgery	100%	0.0%	0.0%	0.0%	0.0%	
Pediatrics	100%	0.0%	0.0%	0.0%	0.0%	
post CCU	50.0%	50.0%	0.0%	0.0%	0.0%	
Surgical ICU	100%	0.0%	0.0%	0.0%	0.0%	
Open heart ICU		100%	0.0%	0.0%	0.0%	
Ophthalmology	100%	0.0%	0.0%	0.0%	0.0%	
Skin disease	0.0%	100%	0.0%	0.0%	0.0%	
Sample type						0.001
Urine	73.7%	22.8%	1.8	0.0%	1.8%	
Trachea	100%	0.0%	0.0%	0.0%	0.0%	
Blood	0.0%	50.0%	0.0%	50.0%	0.0%	
BAL	8.3%	75.0%	16.7%	0.0%	0.0%	
Shaldon	0.0%	100%	0.0%	0.0%	0.0%	

Antibiotic Susceptibility Results

First, there was no difference in the frequency of bacterial strains between men and women based on different age groups. However, isolated Bacillus strains have a great variety in other hospital wards, which are described in detail in Table 4, with the names of each ward. Of course, as detailed in Table 4, the isolated strains also varied according to the type of samples. For example, in urine, the most common isolated strain was *E. coli* (73.7%), and there were no *Enterobacter cloacae*, while in blood, *E. cloacae* (50%) and *Klebsiella pneumoniae* (50%) were the most common. Regarding antibiotic resistance against ESBL-producing gram-negative bacilli, the frequency of antibiotic resistance in detail is expressed in Table 5. For example, the highest antibiotic resistance against gentamycin is seen in *Enterobacter cloacae* (100%) and *Acinetobacter baumannii* (100%), while *Pseudomonas aeruginosa* had no resistance to gentamycin.

Table 5. Distribution of antibiotic resistance by type of gram-negative bacilli

Antibiotic	*E. coli*	*Klebsiella*	*Pseudomonas aeruginosa*	*Enterobacter cloacae*	*Acinetobacter baumannii*	P-value
Gentamycin	34.7%	76.0%	0.0%	100%	100%	0.002
Cefepime	77.6%	44.0%	33.3%	0.0%	100%	0.009
Imipenem	8.2%	56.0%	66.7%	0.0%	100%	0.001
Ampicillin-Sulbactam	34.7%	84.0%	66.7%	50.0%		0.002
Nitrofurantoin	8.2%	16.0%	0.0%	0.0%	100%	0.049
Amikacin	6.1%	60.0%	66.7%	0.0%	100%	0.001
Cefoxitin	0.0%	0.0%	33.3%	0.0%		0.001
Ciprofloxacin	75.0%	88.0%	66.7%	0.0%	100%	0.050
Cefazolin	95.9%	52.0%	33.3%	0.0%	100%	0.001
Cefotaxime	100%	100%	66.7%	100%	100%	0.001
Trimethoprim-sulfamethoxazole	83.7%	88.0%	100%	100%	100%	0.862
Piperacillin-tazobactam	4.1%	48.0%	66.7%	66.7%	66.7%	0.001
Ceftriaxone				50.0%	0.0%	0.001
ceftazidime	4.1%	48.0%	33.3%	0.0%	0.0%	0.001
Meropenem		44.0%	66.7%	0.0%	0.0%	0.001
Cefuroxime	2.0%	0.0%	0.0%	0.0%	0.0%	0.958

The total frequencies of common genes of ESBL-producing gram-negative bacilli were bla_{CTX-M} in 76.0%, bla_{TEM} in 46.7%, and bla_{SHV} in 26.7%. While in 14.7%, the type of gene detected was unknown. Also, cases of simultaneous detection of multiple genes were observed in 49.3% of gram-negative ESBL-producing bacilli; of this number, triple gene detection was reviewed in 16.0% (Figures 3, 4, 5, and 6).

In this article, we used positive control to identify bla_{SHV}, bla_{CTX-M}, and bla_{TEM} genes. Still, for bla_{DHA}, bla_{MOX}, bla_{CIT}, bla_{EBC}, and bla_{FOX} genes, positive control species could not be found because no study was done previously. The frequency of genes by sex, age, type of bacilli, and sample evaluated are described in Table 6.

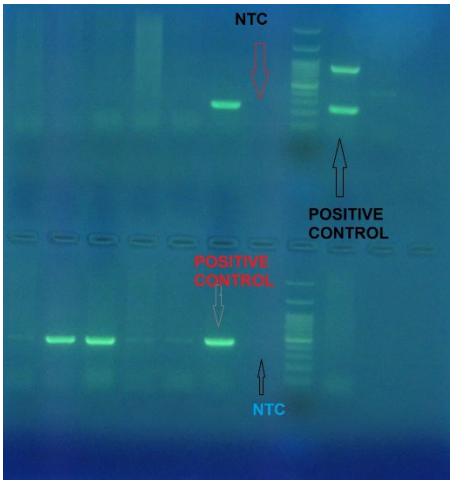

Figure 3. PCR Multiplex for detection of *TEM* (lower part) & *SHV*(upper part) gene

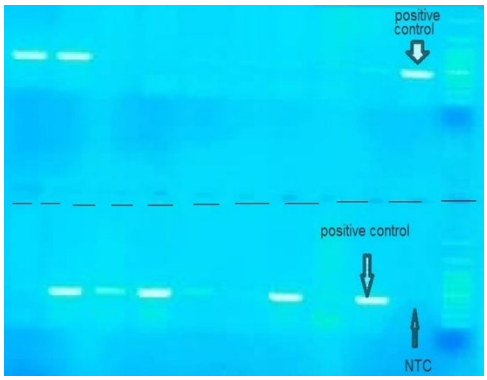

Figure 4. PCR Multiplex for detection of *CTX-M* gene

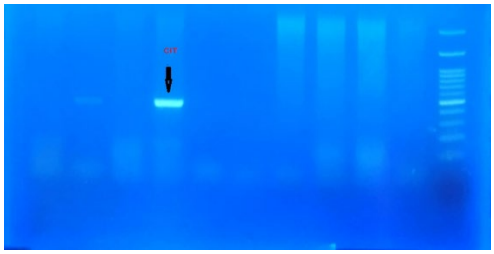

Figure 5. PCR Multiplex for detection of *CIT&EBC&-DHA&FOX* gene

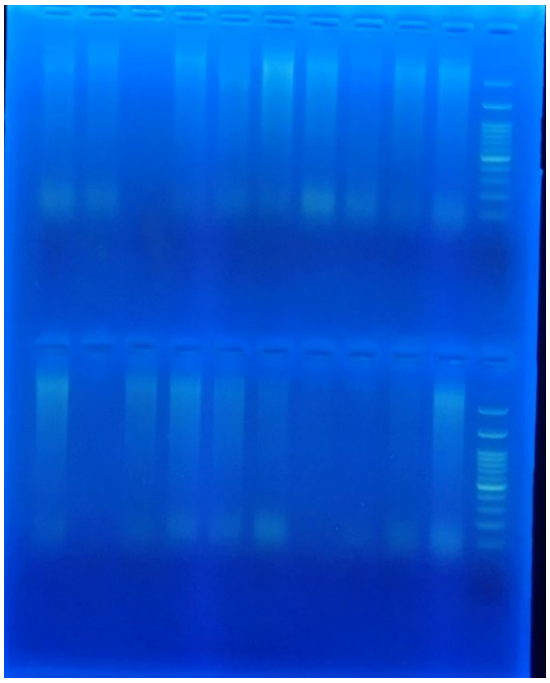

Figure 6. PCR Multiplex for detection of *MOX* gene

Table 6. Frequency of common genes of ESBL gram-negative bacilli according to study characteristics

GENE	*CTX-M*	*TEM*	*SHV*	unknown	Multi
Total	76.0%	46.7%	26.7%	14.7%	49.3%
Gender					
Man	64.5%	48.4%	22.6%	25.8%	47.4%
Female	84.1%	45.5%	29.5%	6.8%	50.0%
Mean age, year	56.56	54.97	58.86	55.40	55.39
Sample type					
Urine	77.2%	50.9%	17.5%	14.0%	47.4%
Trachea	0.0%	100%	0.0%	0.0%	0.0%
Blood	100%	0.0%	0.0%	0.0%	0.0%
BAL	66.7%	33.3%	58.3%	25.0%	58.3%
Shaldon	100%	100%	100%	0.0%	100%
Type of bacilli					
E. coli	77.3%	45.5%	9.1%	13.6%	43.2%
Klebsiella	72.0%	52.0%	60.0%	20.0%	64.0%
Pseudomonas aeruginosa	66.7%	33.3%	33.3%	0.0%	33.3%
Enterobacter cloacae	100%	0.0%	0.0%	0.0%	0.0%
Acinetobacter baumannii	100%	100%	0.0%	0.0%	100%

The relationship between gram-negative bacilli's common genes and antibiotic resistance is illustrated in Table 7.

Table 7. Frequency of antibiotic resistance according to common genes of gram-negative bacilli

Antibiotic	*CTX-M*	*TEM*	*SHV*	Unknown	Multi
Gentamycin	45.9%	45.0%	61.9%	20.0%	45.2%
Cefepime	68.9%	60.0%	61.9%	40.0%	66.7%
Imipenem	23.0%	27.5%	42.9%	0.0%	23.8%
Ampicillin-Sulbactam	47.5%	42.5%	61.9%	0.0%	42.9%
Nitrofurantoin	9.8%	12.5%	9.5%	0.0%	9.5%
Amikacin	23.0%	35.0%	42.9%	20.0%	28.6%
Cefoxitin	1.6%	2.5%	0.0%	0.0%	2.4%
Ciprofloxacin	75.4%	71.8%	81.0%	100%	75.6%
Cefazolin	82.0%	80.0%	66.7%	100%	83.3%
Cefotaxime	98.4%	67.5%	100%	100%	97.6%
Trimethoprim-sulfamethoxazole	88.5%	92.5%	85.7%	100%	92.9%
Piperacillin-tazobactam	16.4%	20.0%	33.3%	0.0%	16.7%
Ceftriaxone	0.0%	0.0%	0.0%	0.0%	0.0%
Ceftazidime	16.4%	17.5%	33.3%	0.0%	0.0%
Meropenem	13.1%	15.0%	28.6%	0.0%	14.3%
Cefuroxime	1.6%	0.0%	0.0%	0.0%	0.0%

First, there was no observed association between the detection of any of the *blaCTX-M, bla*$_{TEM}$, or *bla*$_{SHV}$ genes, as well as the detection of a multigenic pattern with resistance to gentamicin, nitrofurantoin, ciprofloxacin, cefazolin, cefotaxime, trimethoprim-sulfamethoxazole, piperacillin-tazobactam, or cefuroxime. Regarding imipenem, the drug resistance was remarkably greater in those who harbored the *bla*$_{SHV}$ gene. Drug persistence was also remarkably greater in those who harbored the *bla*$_{SHV}$ gene regarding amikacin. Also, the drug resistance was significantly greater for ceftazidime in those who detected the *bla*$_{SHV}$ gene. It is necessary to explain that in co-producers of ESBL and AmpC, *bla*$_{CIT}$ gene was the main gene, in which in 3 cases is accompanied by *CTX-M* and *TEM*, in 1 case with *bla*$_{CTX-M}$ and another case is associated with *bla*$_{TEM}$. In these cases, resistance to cefotaxime, trimethoprim-sulfamethoxazole, ciprofloxacin, and ceftazidime was common, but these relations were not statistically significant.

4.Discussion

A large group of genes associated with ESBL and AmpC beta-lactamases expression is involved in inducing antibiotic resistance in various bacteria. Identifying these genes and assessing their detection will be crucial in determining the pattern of antibiotic resistance. In other words, a significant proof for the change in the drug resistance of bacteria (intensification or reduction of resistance) is the change and occurrence of mutations in these genes and the change in their expression. Therefore, detection of these genes can be important at the clinical level and facilitate the traceability of changes in antibiotic resistance in different parts of the hospital. The current research identified the frequency of fast-growing gram-negative bacilli producing ESBL and studied their common genetic changes in Rasool-e-Akram Hospital. In this evaluation, ESBL-producing gram-negative Bacilli strains were first identified in various blood, urine, trachea, Shaldon, and BAL samples by cultivating in the mentioned methods.

Remarkable results in the present study were:

A) The most common cultivated strains were *E. coli* (58.7%), *K. pneumoniae* (33.3%), *P. aeruginosa* (4.0%), *Enterobacter cloacae* (2.7%), and *Acinetobacter baumannii* (1.3%);

B) Regarding antibiotic resistance evaluated in different sections, the highest antibiotic resistance was related to cefotaxime with a resistance of 98.8%, trimethoprim-sulfamethoxazole with 86.2%, cefazolin with 77.5%, ciprofloxacin with 76.0%, and cefepime with the resistance of 63.8%. In this regard, the lowest level of resistance was related to colistin with no

identified resistance, cefoxitin and ceftriaxone with 1.2% resistance;

C) The communication between detecting some bacterial genes and antibiotic resistance was notable. First, the level of drug resistance to imipenem in people who harbored the bla_{SHV} gene was significantly higher than in the cases that did not harbor it. Also, similarly, drug resistance to amikacin and ceftazidime was significantly higher in those who detected the bla_{SHV} gene. Therefore, studying the role of the bla_{SHV} bacterial gene in increasing antibiotic resistance to drugs such as imipenem, amikacin, and ceftazidime, which are widely used drugs in various sectors, suggests that the bla_{SHV} bacterial gene evaluation can be routinely used to evaluate the antibiotic resistance pattern of ESBL-producing gram-negative bacilli.

Based on the different characteristics of different communities and the genomic pattern of bacteria in other communities, a different expression of bacterial genes was obtained simultaneously with antibiotic resistance, which could be specific to that community. In the study by Gundran *et al*. **(24)**, the highest prevalence was related to the $bla_{CTX\text{-}M\text{-}1}$ gene, with a prevalence of 72.4%, followed by the $bla_{CTX\text{-}M\text{-}2}$ gene. The bla_{TEM} and bla_{SHV} genes frequency were 57.9% and 27.5%, respectively. Simultaneous expression of $bla_{CTX\text{-}M}$ was observed in 73.5% of patients, utterly different from the values obtained in the existing research. In the study by Pishtiwan *et al*. **(25)**, of the ESBL-producing *E. coli* cases, 81% displayed the bla_{TEM} gene, 16.2% the bla_{SHV} gene, and 32.4% the $bla_{CTX\text{-}M}$ gene. Similarly, 64.7% showed bla_{TEM} gene, 35.2% bla_{SHV} gene, and 41.1% $bla_{CTX\text{-}M}$ gene in *K. pneumoniae* samples. In the study of Farzi *et al*. **(26)**, from 100 urine samples, 21 ESBL producer cases (21%) were detected, and the presence of $bla_{CTX\text{-}M}$ and bla_{TEM} genes in the isolates were 21% and 20%, respectively. In the study of Roshdi Maleki *et al.* **(27)**, of 120 strains of *K. pneumoniae*, 71 (59.2%) were established for ESBL. The bla_{TEM} and bla_{SHV} ESBLs were identified in 35 (49.3%) and 31 (43.7%) strains respectively. finally, the co-existence of bla_{TEM} and bla_{SHV} was detected in 5 (7%) isolates. In a study by Bajpai *et al.* **(28)** in India, genes related to beta-lactamase production were examined, including $bla_{CTX\text{-}M\text{-}M}$ (48.7%), bla_{TEM} (7.6%), and bla_{SHV} (1.5%). In a study by Ghaima *et al.* **(29)** that investigated ESBL genes in *Acinetobacter baumannii* isolates, $bla_{CTX\text{-}M\text{-}M}$, bla_{TEM}, and bla_{SHV} genes were discovered in 45%, 75%, and 27.5%, respectively. In a study by Kumarss Amini *et al.* **(30)** in Iran, from 200 diarrhea specimens, 60 (30%) *Shigella sonnei* strains were found. The molecular test results showed that 40 (66.6%) and 33 (55%) of the strains harbored the $bla_{CTX\text{-}M\text{-}8}$ and *CMY* genes, respectively.

The $bla_{CTX\text{-}M\text{-}2}$ gene was not identified in any of the specimens. Also, in the study of Mubarak Saif Alfaresi *et al.* **(31)** in the UAE, out of 240 cases of ESBL, 228 cases carried the ESBL *bla* gene. Also, 87% of the strains carried the $bla_{CTX\text{-}M\text{-}15}$ gene, and the $bla_{SHV\text{-}28}$ gene was identified in 13% of these strains. Kooshesh *et al.* **(32)** found that in ESBL and AmpC positive isolates in Kerman in 2015, 76.1% were positive for bla_{TEM}, 14.2% positive for $bla_{OXA,}$ and 2.3% positive for bla_{SHV}, and none of the isolates were found positive for bla_{PER}. The outbreak of ESBL, AmpC, $bla_{TEM,}$ and bla_{OXA} in inpatients isolates was 7.2%, 0.2%, 37.2%, and 8.5, respectively, which differed from our study terms of genotype and destination.

As it is evident, in different societies and even in a society with different geographical conditions, both the rate of antibiotic resistance and the expression of bacterial genes associated with these resistances have been reported quite differently, which can be related to both the genomic conditions associated with that community and differences in the techniques used to assess genomic expression.

5. Conclusion

In conclusion, in our study population, first of all, we faced high antibiotic resistance to cefotaxime, trimethoprim-sulfamethoxazole, cefazolin, ciprofloxacin, and cefepime. Also, the prevalence of multi-antibiotic resistance in our society is high. In terms of identifying bacterial genes associated with ESBL-producing gram-negative bacilli, the outbreak of common ESBL-producing gram-negative bacilli genes was $bla_{CTX\text{-}M}$ in 76.0%, bla_{TEM} in 46.7%, and bla_{SHV} in 26.7% of bacilli. Also, cases of simultaneous harboring of multiple genes were observed in 49.3% of gram-negative ESBL-producing bacilli. Finally, bla_{SHV} gene detection will be associated with increased antibiotic resistance to imipenem, amikacin, and ceftazidime.

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Conflict of Interest

The authors declared no conflict of interest.

Authors Contribution

Study Conception, Design, Data Analysis and Interpretation of Results: Dr. Pegah Babaheidarian, Majid Mehdinejad. Design and Interpretation of Results: Dr. Ali Zare-Mirzaie. Data Collection: Roya Mokarinejad, Ensieh Jafari.

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Reference

1. Dancer SJ, Coyne M, Robertson C, Thomson A, Guleri A, Alcock S. Antibiotic use is associated with resistance of environmental organisms in a teaching hospital. J Hosp Infect; 2006; 62(2):200-6. [DOI:10.1016/j.jhin.2005.06.033] [PMID]
2. Klugman KP, Madhi SA. Emergence of drug resistance: impact on bacterial meningitis. Infect Dis Clin North Am. 1999;13(3): 637-646. [DOI:10.1016/S0891-5520(05)70098-2]
3. Jalalpoor SH, Abousaidi H. Survey Role and Important of Surfaces Structure and β-lactamase of Bacillus cereus in Drug Resistant. J Microb World. 2009; 2(3): 169-76.
4. Massova I, Mobashery S. Kinship and diversification of bacterial penicillin-binding proteins and β-lactamases. Antimicrob Agents Chemother. 1998; 42(1):1-17. [DOI:10.1128/AAC.42.1.1] [PMID] [PMCID]
5. Fedarovich A, Nicholas RA, Davies C.The role of the β5-α11 loop in the active-site dynamics of acylated penicillin-binding protein A from Mycobacterium tuberculosis. J Mol Biol. 2012; 418(5): 316-30. [DOI:10.1016/j.jmb.2012.02.021] [PMID] [PMCID]
6. Zapun A,Contreras-Martel C,Vernet T. Penicillin-binding proteins and β-lactam resistance. FEMS Microbiol Rev. 2008; 32(2):361-85. [DOI:10.1111/j.1574-6976.2007.00095.x] [PMID]
7. Delcour AH. Outer membrane permeability and antibiotic resistance. Biochim Biophys Acta Proteins Proteom. 2009; 1794(5):808-16. [DOI:10.1016/j.bbapap.2008.11.005] [PMID] [PMCID]
8. Jalalpoor S, Kermanshahi RK, Noohi AS, Isfahani HZ. The Prevalence of Nano-structure Surface Layer in Bacillus Cereus Strains Isolated from Staff Hands and Hospital Surfaces. J Isfahan Med School. 2009;27(100):632-45.
9. Samaha-Kfoury JN, Araj GF. Recent developments in β lactamases and extended spectrum β lactamases, BMJ. 2003; 327(7425):1209-13. [PMCID] [DOI:10.1136/bmj.327.7425.1209] [PMID]
10. Parija S. Textbook of Microbiology & Immunology. 2th ed. Elsevier Health Sciences; 2014.
11. Thomson KS. Extended-spectrum-β-lactamase, AmpC, and carbapenemase issues. J Clin Microbiol. 2010; 48(4):1019-25. [DOI:10.1128/JCM.00219-10] [PMID] [PMCID]
12. Li X-Z, Nikaido H. Efflux-mediated drug resistance in bacteria. Drugs. 2009;69(12):1555-623. [DOI:10.2165/11317030-000000000-00000] [PMID] [PMCID]
13. Bush K, Jacoby GA. Updated functional classification of β-lactamases. Antimicrob Agents Chemother. 2010; 54(3):969-76. [DOI:10.1128/AAC.01009-09] [PMID] [PMCID]
14. Pitout JD. Infections with extended-spectrum β-lactamase-producing Enterobacteriaceae. Drugs. 2010; 70(3): 313-33. [DOI:10.2165/11533040-000000000-00000] [PMID]
15. Ambler RP, et al.A standard numbering scheme for the class A β-lactamases. Biochem J. 1991; 276(Pt 1): 269-70. [DOI:10.1042/bj2760269] [PMID] [PMCID]
16. Jacoby GA. AmpC β-lactamases. Clin Microbiol Rev. 2009; 22(1): 161-82. [DOI:10.1128/CMR.00036-08] [PMID] [PMCID]
17. Chew KL , Lin RT, Teo JW. Klebsiella pneumoniae in Singapore: hypervirulent infections and the carbapenemase threat. Front Cell Infect Microbiol. 2017;7: 515. [DOI:10.3389/fcimb.2017.00515] [PMID] [PMCID]
18. Cho JC, Fiorenza MA, Estrada SJ. Ceftolozane/Tazobactam: A Novel Cephalosporin /β-Lactamase Inhibitor Combination. Pharmacotherapy. 2015; 35(7):701-15. [DOI:10.1002/phar.1609] [PMID]
19. Geisinger E, Isberg RR. Interplay between antibiotic resistance and virulence during disease promoted by multidrug-resistant bacteria. J Infect Dis. 2017; 215(suppl_1):S9-17. [DOI:10.1093/infdis/jiw402] [PMID] [PMCID]
20. D'Andrea MM, Arena F, Pallecch L, Rossolini GM. CTX-M-type β-lactamases: a successful story of antibiotic resistance. Int J Med Microbiol. 2013; 303(6-7): 305-17. [DOI:10.1016/j.ijmm.2013.02.008] [PMID]
21. Levy SB. Factors impacting on the problem of antibiotic resistance. J Antimicrob Chemother. 2002; 49(1):25-30. [DOI:10.1093/jac/49.1.25] [PMID]
22. Rajivgandhi G, Maruthupandy M, Ramachandran G, Priyanga M, Manoharan N. Detection of ESBL genes from ciprofloxacin resistant Gram negative bacteria isolated from urinary tract infections. J Front Lab Med. 2018;2(1):5-13. [DOI:10.1016/j.flm.2018.01.001]
23. Gunjan G, Vibhor T, Purva M. Detection of AmpC betalactamases in gram negative bacteria. J Lab Physicians. 2014;6(01):001-6 [DOI:10.4103/0974-2727.129082] [PMID] [PMCID]

24. Gundran RS et al. Prevalence and distribution of bla CTX-M, bla SHV, bla TEM genes in extended-spectrum β-Lactamase-producing E. coli isolates from broiler farms in the Philippines. BMC Vet Res. 2019; 15(1):1-8. [DOI:10.1186/s12917-019-1975-9] [PMID] [PMCID]

25. Pishtiwan AH, Khadija K.M. Prevalence of blaTEM, blaSHV, and blaCTX-M genes among ESBL-producing Klebsiella pneumoniae and Escherichia coli isolated from thalassemia patients in Erbil, Iraq Mediterr J Hematol Infect Dis. 2019;11(1). [DOI:10.4084/mjhid.2019.041] [PMID] [PMCID]

26. Farzi S,Ranjbar R,Niakan M,Ahmadi MH. Molecular Characterization of Antibiotic Resistance Associated with TEM and CTX-M ESBL in Uropathogenic E. coli Strains Isolated from Outpatients. Iran J Pathol. 2021; 16(4):386-91 [DOI:10.30699/ijp.2021.521669.2556] [PMID] [PMCID]

27. Roshdi Maleki M, Taghinejad J. Prevalence of Extended-spectrum Beta-lactamases (ESBL) Types blaTEM and blaSHV in Klebsiella pneumoniae Strains Isolated from Clinical Samples by PCR in Miandoab, West Azerbaijan. Iran J Med Microbiol. 2021; 15(4): 458-64. [DOI:10.30699/ijmm.15.4.458]

28. Bajpai T, Pandey M, Varma M, Bhatambare G. Prevalence of TEM, SHV, and CTX-M Beta-Lactamase genes in the urinary isolates of a tertiary care hospital. Avicenna J Med. 2017; 7(1):12. [DOI:10.4103/2231-0770.197508] [PMID] [PMCID]

29. Ghaima K. Distribution of extended spectrum beta-lactamase (ESBL) genes among Acinetobacter baumannii isolated from burn infections. MOJ Cell Sci Rep. 2018; 5(3):42-6. [DOI:10.15406/mojcsr.2018.05.00112]

30. Amini K, Konkori M. Identification of Broad-Spectrum Beta-lactamase CTX-M-2, CTX-M-8, and Ampc-dependent CMY Genes in Shigella sonnei Isolated from Pediatric Diarrhea Specimens by Multiplex-PCR and Antibiotic Resistance Pattern Determination. Iran J Med Microbiol. 2020; 14(5): 501-11. [DOI:10.30699/ijmm.14.5.501]

31. Alfaresi MS, Elkoush AA, Alshehhi HM, Abdulsalam AI. Molecular characterization and epidemiology of extended-spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae isolates in the United Arab Emirates. Med Princ Pract. 2011; 20(2):177-80. [DOI:10.1159/000319912] [PMID]

32. Koshesh M, Mansouri S,Hashemizadeh Z, Kalantar-Neyestanaki. Identification of extended-spectrum β-lactamase genes and ampc-β-lactamase in clinical isolates of escherichia coli recovered from patients with urinary tract infections in Kerman, Iran. Arch Pediatr Infect Dis. 2017; 5(2): e37968. [DOI:10.5812/pedinfect.37968]