Original Article | Iran J Med Microbiol. 2023; 17(1): 39-49

10.30699/ijmm.17.1.39

Iranian Journal of Medical Microbiology | ISSN:2345-4342

Frequency of Extended-Spectrum Beta-Lactamase-producing Genes associated in gram-negative bacteria isolated from infectious patients in Kermanshah (2019-2020)

Sepide Kadivarian[1], Somayeh Hosseinabadi[1], Ramin Abiri[2], Sara Kooti[3], Amirhooshang Alvandi[4*]

1. *Department of Microbiology, School of Medicine, Kermanshah University of Medical Sciences, Kermanshah, Iran*
2. *Department of Microbiology, School of Medicine, Fertility and Infertility Research Center, Research Institute for Health Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran*
3. *Behbahan Faculty of Medical Sciences, Behbahan, Iran*
4. *Department of Microbiology, School of Medicine, Medical Technology Research Center, Research Institute for Health Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran*

ABSTRACT

Background and Aim: Nosocomial infections caused by antibiotic-resistant bacteria and their rapid spread threaten public health. This study aimed to determine the frequency of genes encoding Extended-Spectrum Beta-Lactamase (ESBL) in gram-negative bacteria in Kermanshah city, west of Iran.

Materials and Methods: Identification and antibiotic susceptibility pattern of 165 isolates were performed by biochemical and disk diffusion methods, respectively. Screening and confirming the presence of ESBL genes were performed according to the double disk combination test (DDCT) method. The presence of genes encoding ESBL in each isolate was identified by Polymerase Chain Reaction (PCR) method.

Results: Out of 165 isolates, 83 strains were resistant to all antibiotics. The lowest frequency of resistance was observed for Gentamicin, while the highest frequency was observed for Cefotaxime and Cefazolin. Among all strains, 50 (30.30 %) and 80 (48.48%) isolates were phenotypically and genotypically ESBL-positive, respectively. The most prevalent genes encoding ESBL were *SHVOS* and *SHV-1,* with a frequency of 20.61 % and 21.82 %, respectively.

Conclusion: The frequency of producing ESBL bacteria and the prevalence of *blaSHV* and *blaCTX-M* genes in our studied *Klebsiella pneumoniae* and *Escherichia coli* isolates were high. However, unlike some previous reports from Kermanshah, the prevalence of ESBL-encoding genes in *Pseudomonas aeruginosa* was low, and the *blaVEB* gene was not found.

Keywords: Antibiotic resistance, ESBL-encoding genes, ESBL-producing Enterobacteriaceae, Frequency

Received: 2022/02/26; **Accepted**: 2022/10/08; **Published Online:** 2023/01/20

Corresponding Information: **Amirhooshang Alvandi**, *Department of Microbiology, School of Medicine, Medical Technology Research Center, Research Institute for Health Technology, Kermanshah University of Medical Sciences, Kermanshah, Iran*
Email: ah_alvandi@kums.ac.ir

Copyright © 2023, This is an original open-access article distributed under the terms of the Creative Commons Attribution-noncommercial 4.0 International License which permits copy and redistribution of the material just in noncommercial usage with proper citation.

Use a device to scan and read the article online

Kadivarian S, Hosseinabadi S, Abiri R, Kooti S, Alvandi A. Frequency of Extended-Spectrum Beta-Lactamase-producing Genes associated in gram-negative bacteria isolated from infectious patients in Kermanshah (2019-2020). Iran J Med Microbiol. 2023; 17(1);39-49.

Download citation: BibTeX | RIS | EndNote | Medlars | ProCite | Reference Manager | RefWorks
Send citation to: Mendeley Zotero RefWorks

1. Introduction

Nowadays, antibiotic resistance is considered as one of the major problems of nosocomial infections and a threat to public health **(1)**. The incidence of bacterial resistance and their rapid spreading among different parts of the hospitals are performed by various mechanisms, such as the production of beta-lactamase enzymes **(2)**. In the early 1980s, a new family of beta-lactamases was recognized as the Extended-Spectrum Beta-Lactamase (ESBL) in *Klebsiella* and their spreading was discussed as an epidemiological phenomenon since the second half of the 1980s **(3)**. The plethora of taking cephalosporins iAn treating infections caused by gram-negative bacilli has significantly increased drug resistance rates. Thus, it has caused global health and clinical challenges **(4)**. According to the studies and epidemiological stewardship, *Escherichia coli, Klebsiella pneumoniae*, and *Pseudomonas aeruginosa*, are the main ESBL-producing organisms **(5)**. These organisms have been registered in the critical group of the World Health Organization (WHO) Global Priorities List as a particular threat to hospitals and are the major causes of nosocomial infections **(6, 7)**. Heretofore, various types of ESBL have been identified. *SHV*, *TEM*, and *CTXM* are the major beta-lactamase genes **(8)**. Although the most common ESBL genes are found in most parts of the world, such as Europe and Asia, some of them, such as *VEB*, *PER*, and *GES* genes, have limited geographical distribution and are rare types of plasmid-mediated ESBLs **(9)**.

The spread of ESBL-producing bacteria in different regions has led to the emergence of antibiotic resistance leading to difficulties in treating infections caused by ESBL-producing organisms. Therefore, more accurate monitoring of ESBL-producing bacteria and ESBL-encoding genes is essential for managing infection treatment **(9, 10)**. Some studies are on the prevalence of ESBL-producing gram-negative bacteria and ESBL-encoding genes in Kermanshah city, west of Iran **(11-18)**. Based on these studies, the frequency of ESBL-producing gram-negative bacteria in Kermanshah varies, ranging from 22% to 45%. Also, there is a different frequency pattern of ESBL-encoding genes reported for bacteria isolated in Kermanshah. Since the pattern of antibiotic susceptibility of ESBL-producing gram-negative bacteria may change over time due to several reasons, such as the widespread use of antibiotics and the bacterial genome changing, the annular (or occasionally) monitoring of their antibiotic susceptibility is required to better management of the infections caused by them. Therefore, the present study aimed to evaluate the recent frequency of ESBL-encoding genes among clinical isolates of gram-negative bacteria in Kermanshah, using phenotypic and genotypic methods.

2. Materials and Methods

Patient and Samples

This cross-sectional study was conducted in Imam Reza and Imam Khomeini Hospitals and Reference Laboratory in Kermanshah from August 2019 to August 2020 from different clinical samples like urine, blood, sputum, trachea, tissue, wound biopsies, and other secretions from inpatients and outpatients. Demographic data, including age, gender, and type of infection, were obtained from the medical records. The samples were inoculated in Müller-Hinton broth medium in a shaker incubator at 37°C for 18-24 hours. Then, samples were incubated aerobically on Müller Hinton Agar, MacConkey Agar, and EMB (Merck, Germany) at 37°C for 18-24 hours. All isolates were identified by standard microbiological methods, including gram staining. *P. aeruginosa* ATCC 27853, *E. coli* ATCC 25922, and *K. pneumoniae* ATCC 700603 were used as a positive control, and for evaluation of results were purchased from Pasteur Institute, Tehran, Iran.

For quality control and the evaluation of results, E. coliATCC25922 was used as a positive control.

Antimicrobial Susceptibility Test

The pattern of antibiotic resistance was determined based on Clinical Laboratory Standard Instructions 2018 (CLSI 2018) by the Kirby-Bauer disk diffusion method. Antibiotic discs were examined as follow: Cefotaxime (30 µg), Cefazolin (30 µg), Tobramycin (10 µg), Ceftriaxone (30 µg), Ceftazidime (30 µg), Cefopodoxime (30 µg), Co-trimoxazole (25 µg), and Gentamiaycin (10 µg).

Detection of ESBL-producing Bacteria

Double Disc Combination Test (DDCT) with Cefopodoxime (30 µg)/ Cefopodoxime- clavulanic acid or Ceftazidime (30 µg)/ Ceftazidime-clavulanic acid discs was the method of choice for assessment of ESBL-producing bacteria. A five-millimeter difference between the inhibition zones around the two discs shows the production of ESBL enzyme by the bacterium (CLSI2018).

PCR

The genomic DNA of bacterial isolates was extracted using the AccuPrep Genomic DNA extraction kit (Bioneer, Korea) based on the kit instructions. The primers used to identify ESBL genes,

including *CTXM-1*, *SHVOS*, *TEM*, *SHV-1*, *PER-1*, and *VEB*-1 are depicted in Table 1. The characteristics and specificity of each pair of primers were checked and confirmed by online software.

Table 1. Sequence of primers.

No.	Name	Sequence(5′ to 3′)	Product size	Reference
1	CTXM-1 F	GCAGCACCAGTAAAGTGATGG	591 bp	(19)
	CTXM-1 R	GCTGGGTGAAGTAAGTGAACC		
2	TEM A	TAAAATTCTTGAAGACG	1074 bp	(39)
	TEM B	TTACCAATGCTTAATCA		
3	SHVOS 5	GATTTGCTGAATTCGCTC	797 bp	(39)
	SHVOS 6	TTATCTCCCTGTTAGCCA		
4	SHV-1 F	ATGCGTTATATTCGCCTGTGTA	855 bp	This study
	SHV-1 R	TTGCCAGTGCTCGTACAGC		
5	VEB F	CGACTTCCATTTCCCGATGC	927 bp	(40)
	VEB R	GGACTCTGCAACAAATACGC		
6	PER-1 F	ATGAATGTCATTATAAAAGCT	643 bp	(40)
	PER-1 R	TTAATTTGGGCTTAGGG		

PCR was performed by Thermal cycler (Bio-Rad, Singapore) in 30 cycles in a final volume of 25 microliters, including 8.5 µL Master Mix 1X (Amplicon, Denmark) of each pair of 0.5mm primer (Bioneer, Korea) and 2 µL Template DNA of bacterial isolates. In addition, the PCR plan of the genes was performed by Initial denaturation at 94°C for 3 minutes, denaturation temperature at 94°C for 45 seconds, annealing temperature at 54.5°C for *CTXM-1* gene, 42°C for *TEM* gene, 49.5°C for *SHVOS* gene, 55.5°C for *SHV-1* gene, 45.5°C for *PER-1* gene, and 55°C for *VEB-1* gene for 45 seconds, extension temperature of 72°C for 1 minute and final extension of 72°C for 10 minutes. The PCR products were detected by Electrophoresis gel (Bio-Rad) with voltage 85 for 45 minutes on 1.5% agarose gel containing a safe stain.

Sequencing

The PCR product was sequenced by Dye-terminator sequencing (Bioneer, Korea) to confirm the presence of genes. After editing each sequence, the similarity was determined with the BLAST database in the next step.

Statistical Analysis

The results were analyzed and compared by Chi-square test using SPSS software version 16 (SPSS Inc., Chicago, Ill., USA). The threshold of P-value <0.05 were considered statistical significance.

3. Results

Totally, 165 bacterial strains, including *P. aeruginosa* (n=55), *K. pneumoniae* (n=55), and *E. coli* (n = 55), were isolated from various clinical samples. Most of the bacteria were isolated from females (n=78). The most common age group was 55-65 (n = 29).

The bacteria were isolated from urine (n=106, 64.24%), sputum (n=5, 3.03%), trachea (n=23, 13.94%), blood (n=18, 10.91%), tissue/wound (n=10, 6.06%), and other secretions (n=3, 1.82%) (Table 2). The prevalence of isolates in immatures and adults were 6 (10.91%) and 49 (89.09%) for *K. pneumoniae*, 8 (14.55%) and 47 (85.45%) for *E. coli*, and 8 (14.55%) and 47 (85.45%) for *P. aeruginosa*, respectively.

Most samples were isolated from Imam Reza hospital (91 cases, 55.15%). Most *K. pneumoniae* isolates were collected from Reference Laboratory (24 cases), while most *E. coli* isolates (40 cases) and *P. aeruginosa* isolates (36 cases) were collected from Imam Reza hospital. It is worth noting that most samples from the ICU, surgical and infectious wards belonged to men, while most outpatient samples and the samples from the burn ward belonged to women. The bacteria were isolated from different hospital wards as follows: ICU (n=36, 21.82%), surgery (n=27, 16.36%), pediatrics (n=20, 1212%), infectious diseases (n=10, 6.06%), and burns (n=9, 5.45%) wards.

The number of phenotypic/genotypic positive samples and the frequency of ESBL genes in studied isolates based on the hospital wards shown in Table 3.

Table 2. Distribution of clinical samples, bacterial isolates, and ESBL production

Specimen type	Total No. of Isolates	Organism	No. of Isolate (%)	No. of ESBL positive phenotype	No. of ESBL positive genotype
Urine	106	*P. aeruginosa*	16 (15.1)	2	1
		K. pneumoniae	46 (43.4)	14	43
		E. coli	44 (41.5)	17	15
Sputum	5	*P. aeruginosa*	5 (100)	3	0
		K. pneumoniae	0 (0)	0	0
		E. coli	0 (0)	0	0
Tracheal	23	*P. aeruginosa*	13 (56.52)	2	0
		K. pneumoniae	3 (13.05)	2	2
		E. coli	7 (30.43)	3	3
Blood	18	*P. aeruginosa*	11 (61.11)	1	1
		K. pneumoniae	4 (22.22)	0	3
		E. coli	3 (16.67)	1	1
Burnt	10	*P. aeruginosa*	10 (100)	3	1
		K. pneumoniae	0 (0)	0	0
		E. coli	0 (0)	0	0
Other body fluid	3	*P. aeruginosa*	0 (0)	0	0
		K. pneumoniae	3 (100)	1	3
		E. coli	0 (0)	0	0

Table 3. Phenotypic and genotypic relationship and frequency of ESBL genes in studied isolates based on types of hospital wards

Unit	*K. pneumoniae*			*E. coli*			*P.aeruginosa*				Total Phenotype positive ESBL (%)	Total Genotype positive ESBL (%)
	Phenotype positive ESBL (%)	Genotype positive ESBL (%)	Total isolates (%)	Phenotype positive ESBL (%)	Genotype positive ESBL (%)	Total isolates (%)	Phenotype positive ESBL (%)	Genotype positive ESBL (%)	Phenotype + Genotype positive ESBL (%)	Total isolates (%)		
ICU	3 (15)	5 (11.1)	6 (10.1)	6 (26.08)	7 (26.9)	13 (23.6)	1 (8.3)	0 (0)	8 (20.5)	16 (29.1)	10 (20)	9 (14.7)
Childrens	2 (10)	5 (11.1)	5 (9.1)	4 (17.4)	4 (15.3)	7 (12.7)	2 (16.6)	1 (25)	6 (15.3)	8 (14.5)	8 (16)	9 (14.7)
Other wards	6 (30)	2 (4.4)	7 (12.7)	3 (13.04)	2 (7.7)	8 (14.5)	1 (8.3)	1 (25)	5 (12.8)	3 (5.4)	6 (12)	3 (4.9)
Outpatiet	7 (35)	27 (60)	29 (52.7)	2 (8.7)	3 (11.5)	8 (14.5)	0 (0)	0 (0)	8 (20.5)	6 (10.1)	9 (18)	5 (8.2)
Surgery	1 (5)	3 (6.7)	3 (5.4)	7 (30.4)	9 (34.6)	16 (29.1)	1 (8.3)	0 (0)	8 (20.5)	8 (14.5)	9 (18)	10 (16.4)
Burn	0 (0)	0 (0)	2 (3.6)	0 (0)	0 (0)	0 (0)	4 (33.3)	2 (50)	2 (5.1)	7 (12.7)	3 (6)	9 (14.7)
Infectious	1 (5)	3 (6.7)	3 (5.4)	1 (4.3)	1 (3.8)	3 (5.4)	3 (12)	0 (0)	2 (5.1)	7 (12.7)	5 (10)	16 (26.2)
Total	20 (100)	45 (100)	55 (100)	23 (100)	26 (100)	55 (100)	12 (100)	4 (100)	39 (100)	55 (100)	50 (100)	61 (100)

The results of antibacterial susceptibility testing are shown in Table 4. The results indicated that 47.75%, 2.01%, and 50.24% of isolates were sensitive, intermediate, and resistant to all antibiotics, respectively. The percentage of resistance in *K. pneumoniae*, *E.* coli, and *P. aeruginosa* isolates was 41.14%, 58.86%, and 73.64 %, respectively. The most effective antibiotic against ESBL-producing isolates was gentamycin (n=115, 69.69%). The highest resistance in ESBL-producing isolates was observed for Ceftazidime (n=88, 53.33%).

Table 4. The characterization of antibiotic resistance of bacterial isolates

Antibiotic	*K. pneumoniae*			*E. coli*			*P. aeruginosa*		
	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)	S (%)	I (%)	R (%)
GM	43 (78.18%)	0 (0%)	12 (21.82%)	27 (49.09%)	2 (3.64%)	26 (47.27%)	45 (81.82%)	0 (0%)	10 (18.18%)
TN	38 (69.09%)	0 (0%)	17 (30.91%)	7 (12.73%)	4 (7.27%)	44 (80%)	45 (81.82%)	0 (0%)	10 (18.18%)
CZ	30 (54.55%)	2 (3.64%)	23 (41.81%)	10 (18.18%)	1 (1.82%)	44 (80%)	-	-	-
CAZ	27 (49.09%)	0 (0%)	28 (50.91%)	23 (41.82%)	0 (0%)	32 (58.18%)	34 (61.82%)	0 (0%)	21 (38.18%)
CPD	27 (49.10%)	3 (5.45%)	25 (45.45%)	12 (21.82%)	4 (7.27%)	39 (70.91%)	-	-	-
CTX	28 (50.91%)	1 (1.82%)	26 (47.27%)	12 (21.82%)	0 (0%)	43 (78.18%)	-	-	-
CRO	32 (58.18%)	1 (1.82%)	22 (40%)	12 (21.82%)	2 (3.64%)	41 (74.54%)	-	-	-
SXT	34 (61.82%)	1 (1.82%)	20 (36.36%)	13 (23.64%)	0 (0%)	42 (76.36%)	-	-	-
Total	259 (58.86%)	8 (1.82%)	173 (39.32%)	116 (26.36%)	13 (2.95%)	311 (70.69%)	124 (75.15%)	0 (0%)	41 (24.85%)

Abbreviations: S: sensitive, I: Intermediate, R: Resistance, GM: Gentamiaycin, TN: Tobramycin, CZ: Cefazolin, CAZ: Ceftazidime, CPD: Cefopodoxime, CTX: Cefotaxime, CRO: Ceftriaxone, SXT: Co-trimoxazole.

A total of 165 bacterial isolates were studied according to CLSI instructions for phenotypic detection of ESBL production based on the combined disk method. The results showed that 50 (30.30%) isolates were ESBL-positive, and 115 (69.7%) isolates were ESBL-negative. Of these, 17 (30.91%), 22 (40%), and 11 (20%) strain of *K. pneumoniae*, *E. coli*, and *P. aeruginosa* were ESBL positive. The most phenotypically ESBL-positive isolates related to women (n=23, 46%), *E. coli* strains (n=22, 44%), ICU ward (n=10, 20%), and Imam Reza hospital (n=34, 68%).

Detection of beta-lactamase genes using PCR showed that out of 165 bacterial isolates, 80 (48.48%) were ESBL-producing isolates. Of ESBL-producing isolates, 51 (63.75%), 26 (5.5%), and 3 (3.75%) isolates of *K. pneumoniae*, *E. coli,* and *P. aeruginosa* produced ESBL. In general, 39 (23.64%) isolates were both genotypically and phenotypically ESBL positive.

Most of the isolates harbored *CTXM-1* (n=26, 52%). Interestingly, none of the ESBL-positive *P. aeruginosa* and *E. coli* isolates harbored *CTXM-1*. The frequency of the studied genes and distribution of the simultaneous presence of several genes in bacterial isolates are shown in Figure 1. The highest frequency of ESBL genes in Imam Khomeini hospital, Imam Reza hospital, and the reference laboratory was for *CTXM-1* and *SHV-1* genes.

According to the results of the present study, in general, a significant relationship was observed between ESBL-producing strains and resistance to cephalosporins. There was a significant relationship between resistance to ceftriaxone and trimethoprim-sulfamethoxazole, cefotaxime and cefpodoxime, and Ceftazidime in ESBL-producing stains of *E. coli*, *K .pneumoniae,* and *P. aeruginosa* (Figure 2). The results showed a significant difference in the prevalence of *K. pneumoniae* and *P. aeruginosa*. In terms of gender, a significant relationship was observed between women and ceftazidime resistance. The results of agarose gel electrophoresis of PCR products are shown in Figure 3.

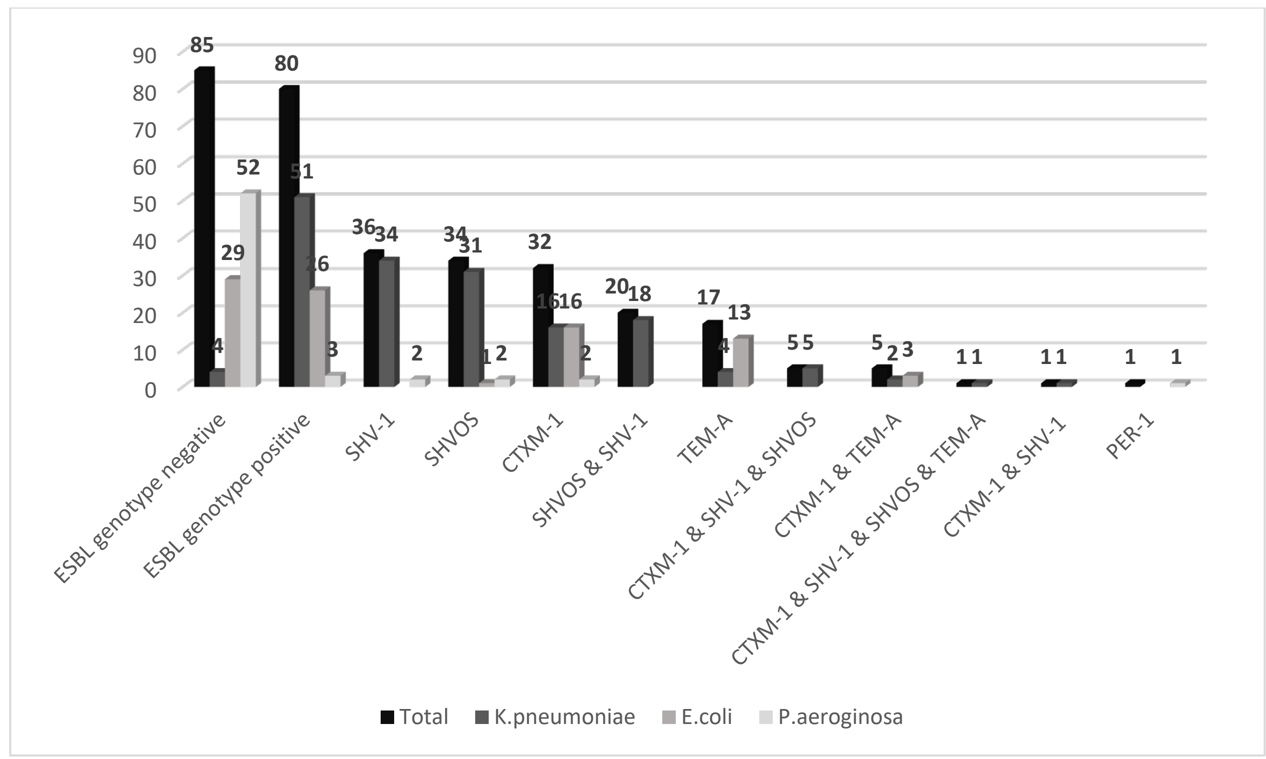

Figure 1. The frequency of the studied genes and distribution of the simultaneous presence of several genes in bacterial isolates.

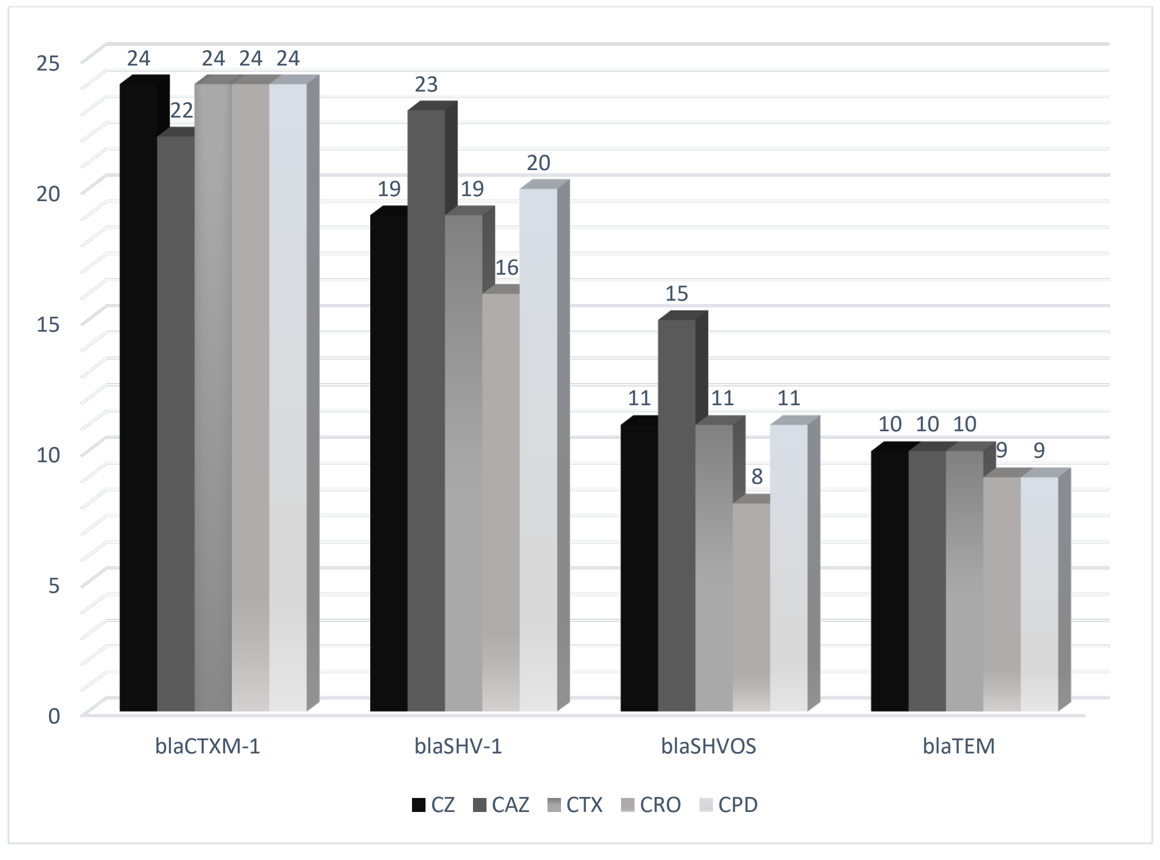

Figure 2. The relationship between resistance to antibiotics and presence of ESBL genes in the studied strains.

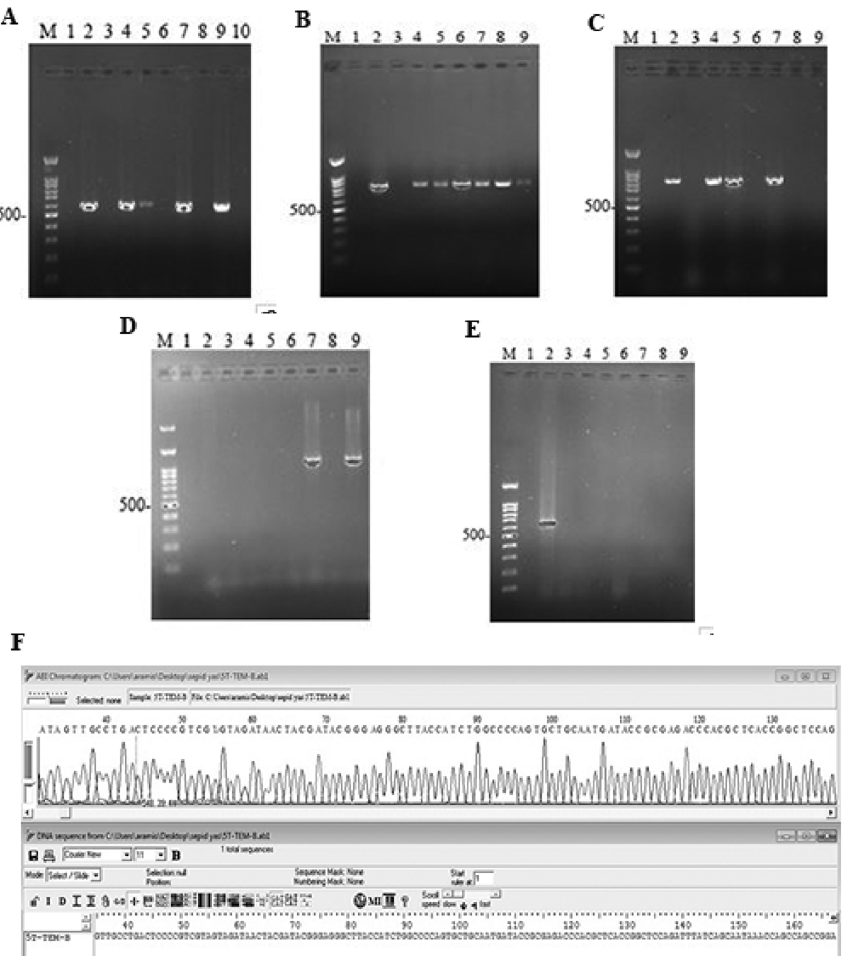

Figure 3. Agarose gel electrophoresis of PCR product of *CTXM-1* genes with 591 bp, *SHVOS* with 797 bp, *SHV-1* with 855 bp, *TEM-A* with 1074 bp and *PER-1* with 643 bp in the gram-negative bacilli studied. A: Well M **:** marker 100bp, Well 1: negative control, Wells 2, 4, 5, 7, and 9: positive samples, Wells 3, 6, 8, and 10: negative samples. B: Well M **:** marker 100bp, Well 1: negative control, Wells 2, 4, 5, 6, 7, 8, 8: positive samples, Wells 3**:** negative sample. C: Well M **:** marker 100bp, Well 1: negative control, Wells 2, 4, 5, and 7: positive samples, Wells 3, 6, 8, 9: negative samples. D. Well M **:** marker 100bp, Well: negative control, Wells 7, 9: positive samples, Wells 2, 3, 4, 5, 6, 8: negative samples, E. Well M **:** marker 100 bp, Well 1: negative control, Well 2: positive sample. Wells 3, 4, 5, 6,7,8,9 negative samples.

4. Discussion

Klebsiela pneumoniae, *E. coli*, and *P. aeruginosa* are the most important gram-negative bacteria that are often associated with urinary tract infections (UTIs), respiratory tract infections (pneumonia), bloodstream infections (bacteremia), and others. Identification of infectious agents and detection of genes encoding beta-lactamase play an important role in the management of infectious diseases and the selection of appropriate antibiotics. Increasing antibiotic resistance among gram-negative bacteria, associated with acquiring resistant genes such as ESBLs, is a global challenge **(19)**.

In the present study, the frequency of antibiotic resistance to common antibiotics was 50.24%. The highest and lowest resistance was observed in *E. coli* (73.6%) and *P. aeruginosa* (24.8%), respectively. High resistance to Ceftazidime was observed in *K. pneumoniae* (50.9%) and *P. aeruginosa* (38.18%), while in *E. coli* isolates, the resistance to tobramycin and cefazolin was very high (80%). Similarly, in the studies conducted from 2013 to 2019 in Kermanshah **(11-18)** the resistance of gram-negative bacteria to tobramycin and third-generation cephalosporins was high. The highest rate of antibiotic resistance to third-generation cephalosporins in Kermanshah was reported in 2017 **(20)**. In addition, 70% resistance of *P. aeruginosa* to Ceftazidime and 76% resistance of *E. coli* to cotrimoxazole was observed in the study of Heidari **(21)** and Yousefi Fatemeh Seri **(13)** from Kermanshah. Resistance of *K. pneumoniae*, *E. coli*, and *P. aeruginosa* isolates varies in different provinces of Iran. Studies from Tehran **(22)**, Kashan **(23)**, and Islamshahr **(24)** have reported the highest resistance of *K. pneumoniae* isolates to ampicillin, ciprofloxacin, Ceftazidime, and cefotaxime. Also, the

resistance of *E. coli* isolates was different in studies from Tehran **(25)** (high resistance to ampicillin) and Karaj **(26)** (high resistance to cotrimoxazole and ciprofloxacin). Factors involved in the diversity of antibiotic resistance to different antibiotics include the type of samples, the number of samples, the origin of the samples, the time of the study, and regional diversity **(27)**.

In the present study, the prevalence of ESBL gene-carrying *K. pneumoniae* was higher than in other isolates, but more than 63% were not phenotypically positive. It shows that although ESBL protein is not expressed in these strains, the ability of the ESBL gene to be transmitted to other bacteria may be important.

The present study showed that 30.3% of the isolates were ESBL-positive. In a 6-year study in Kermanshah, 31.4% of gram-negative bacteria were ESBL-positive. Contrary to the present study, the highest and lowest isolates producing ESBL were *E. coli* and *P. aeruginosa*. In studies from Kermanshah, *P. aeruginosa* (51.7%) was the most frequent, and *E. coli* (23.4%) was the lowest frequent strain producing ESBL. The results of studies from West Azerbaijan **(28)** and Karaj **(29)** showed that 59.2% and 42.8% of *K. pneumoniae* isolates were ESBL-positive. *P. aeruginosa* isolates in Zahedan **(30)** were 57% ESBL-positive.

The results of PCR in the present study showed that 48.48% of the isolates carried ESBL genes. The results showed that 36 isolates carried *blaSHV-1* gene, and one isolate carried *blaPER-1* gene. In previous studies in Kermanshah, it was found that *blaSHV-1* gene (30.5%) had the highest frequency and *blaPER* gene (8.3%) had the lowest frequency. According to the results of studies from Tehran **(31)**, Kerman **(32)**, and Mazandaran **(33)** *blaCTXM*, *blaTEM*, and *blaSHV* were the most common ESBL genes among *E. coli* isolates, respectively. The frequency of *blaCTXM-1* gene in *E. coli* isolates in Arak (94.4%) was higher than in the present study (29.1%). In the present study, the *blaVEB* gene was not found and the *blaPER-1* gene was found only in one *P. aeruginosa* isolate. In studies from Kermanshah in 2016 **(34)** and 2017 **(14)**, the *blaPER* gene was not isolated from any of the isolates of *K. pneumoniae* and *P. aeruginosa*. The emergence of the *blaPER-1* gene in the present study is worrying and alarming.

In addition, the prevalence of ESBL genes in *P. aeruginosa* isolates in a study in Bandar-Abbas **(11)** was higher than the results of the present study. The difference in the frequency of ESBL-producing genes in the results of the present study and other studies in Iran may be due to the temporal effect of increased infections and clonal diffusion **(35)**. The prevalence of ESBL-producing genes plays a key role in creating a different pattern of antibiotic resistance in bacteria. Screening for ESBL-causing bacteria and antibiotic resistance genes in healthcare systems around the world could help implement a program to control and treat threatening infections in the future. It also allows for extensive analysis of antibiotic resistance gene maps. Therefore, the transfer and diffusion of antibiotic-resistance genes can be prevented as much as possible **(3)**.

In a systematic review and meta-analysis study in Iran, it was shown that the high presence of *blaSHV* in the west of the country is a worrying issue. In contrast, *blaTEM* and *blaCTXM* in the central and east of Iran, and *blaTEM*, *blaSHV*, and *blaCTX-M* in southern regions of Iran were more frequent. In general, classes A and D are the most common classes of beta-lactamases in the northern, southern, and central parts of the country. While in the west and east of the country, class A is predominant **(3)**. In the present study, similar to other studies **(36, 37)**, the simultaneous presence of several ESBL genes was observed in *K. pneumoniae* and *E. coli* isolates. As observed here, *blaSHVOS* and *blaSHV-1* alone and together were predominant in *K. pneumoniae*. Also, in our study, the *blaCTXM-1* and *blaTEM* genes alone and together were predominant in *E. coli* isolates. This significantly increases the number of clones carrying ESBL genes, increases bacterial infections, and increases treatment costs. It seems that the high frequency of ESBL genes in gram-negative bacteria isolated from patients in different wards of hospitals in Kermanshah and antibiotic resistance is a serious problem. It has been shown that the trend in the level of antibiotic resistance is consistence with the trend of antibiotic consumption; therefore, it needs to strengthen policy planning to control and optimize use of antibiotics **(38)**.

5. Conclusion

The frequency of producing ESBL and the prevalence rate of *blaSHV* and *blaCTX-M* genes in *K. pneumoniae* and *E. coli* in the mentioned bacterial isolates are high in Kermanshah. However, unlike some of the previous reports from Kermanshah, the prevalence of ESBL-encoding genes in *P. aeruginosa* was low, and *blaVEB* gene was not found.

Acknowledgment

This research was supported by Vice-Chancellor for Research and Technology of Kermanshah University of Medical Sciences (IR.KUMS.REC.1397.861).

Funding

This study was supported by Vice-Chancellor for Research and Technology of Kermanshah University.

Conflict of Interest

The authors declare no conflict of interest.

Reference

1. Prestinaci F, Pezzotti P, Pantosti A. Antimicrobial resistance: a global multifaceted phenomenon. Pathog Glob Health. 2015;109(7):309-18. [DOI:10.1179/204-7773215Y.0000000030] [PMID] [PMCID]
2. Carrasco-Anabalón S, Vera-Leiva A, Quezada-Aguiluz M, Morales-Rivera MF, Lima CA, Fernández J, et al. Genetic Platforms of blaCTX-M in Carbapenemase-Producing Strains of K. pneumoniae Isolated in Chile. Front Microbiol. 2018;9. [DOI:10.3389/fmicb.2018.00324] [PMID] [PMCID]
3. Leylabadlo HE, Pourlak T, Aghazadeh M, Asgharzadeh M, Kafil HS. Extended-spectrum beta-lactamase producing gram negative bacteria In Iran: A review. Afr J Infect Dis. 2017; 11(2):39-53. [DOI:10.21010/ajid.v11i2.6] [PMID] [PMCID]
4. Pavez M, Troncoso C, Osses I, Salazar R, Illesca V, Reydet P, et al. High prevalence of CTX-M-1 group in ESBL-producing enterobacteriaceae infection in intensive care units in southern Chile. Braz J Infect Dis. 2019;23(2):102-10.[PMID] [DOI:10.1016/j.bjid.2019.03.002] [PMCID]
5. Nachimuthu R, Kannan VR, Bozdogan B, Krishnakumar V, S KP, Manohar P. CTX-M-type ESBL-mediated resistance to third-generation cephalosporins and conjugative transfer of resistance in Gram-negative bacteria isolated from hospitals in Tamil Nadu, India. Access Microbiol. 2021;3(3). [DOI:10.1099/acmi.0.000142] [PMID] [PMCID]
6. Nordmann P, Poirel L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria. Clin Infect Dis. 2019;69(7): S521-S8. [DOI:10.1093/cid/ciz824] [PMID] [PMCID]
7. Taraghian A, Nasr Esfahani B, Moghim S, Fazeli H. Characterization of Hypervirulent Extended-Spectrum β-Lactamase-Producing Klebsiella pneumoniae Among Urinary Tract Infections: The First Report from Iran. Infect Drug Resist. 2020; 13:3103-11. [DOI:10.2147/IDR.S264440] [PMID] [PMCID]
8. Abrar S, Ain NU, Liaqat H, Hussain S, Rasheed F, Riaz S. Distribution of blaCTX − M, blaTEM, blaSHV and blaOXA genes in Extended-spectrum-β-lactamase-producing Clinical isolates: A three-year multi-center study from Lahore, Pakistan. Antimicrob Resist Infect Control. 2019;8(1):80. [DOI:10.1186/s13756-019-0536-0] [PMID] [PMCID]
9. Mirkalantari S, Masjedian F, Irajian G, Siddig EE, Fattahi A. Determination of the frequency of β-lactamase gen-es (bla SHV, bla TEM, bla CTX-M) and phylogenetic gro-ups among ESBL-producing uropathogenic Escherichia coli isolated from outpatients. J Lab Med. 2020;44-(1):27-33. [DOI:10.1515/labmed-2018-0136]
10. Molton JS, Tambyah PA, Ang BSP, Ling ML, Fisher DA. The Global Spread of Healthcare-Associated Multidrug-Resistant Bacteria: A Perspective From Asia. Clin Infect Dis. 2013;56(9):1310-8. [DOI:10.1093/cid/cit020] [PMID]
11. Sarshar S, Mirnejad R, Babapour E. Frequency of bla (CTX-M) and bla (TEM) Virulence Genes and Antibiotic Resistance Profiles among Klebsiella pneumoniae Isolates in Urinary Tract Infection (UTI) Samples from Hashtgerd, Iran. Rep Biochem Mol Biol. 2021;10(3):412-9. [DOI:10.52547/rbmb.10.3.412] [PMID] [PMCID]
12. Mohajeri P, Kavosi S, Esmailzadeh T, Farahani A, Dastranj M. Molecular characteristics of extended-spectrum-beta-lactamase-producing Klebsiella pneum-oniae isolates in the West of Iran. Advances Hum Biol. 2018;8(3):175-9. [DOI:10.4103/AIHB.AIHB_20_18]
13. Yousefi Fatmesari G, Hemmati M, Mortazavi SH, Mansouri F, Azizi M, Etemadimajed M, et al. Frequency of blaCTX-M, blaTEM, and blaSHV Genes in Escherichia Coli Isolated from Urine Samples of Children in Kermanshah City, Iran. J Isfahan Med Sch. 2017;35(430):551-7.
14. Vaziri S, Mansouri F, Abiri R, Alvandi A, Mortazavi SH, Ahmadi K, et al. Prevalence Study of Extended Spectrum Beta-Lactamase in Klebsiella Pneumonia Isolated from Patients with Ventilator-Associated Pneumonia in Kermanshah City, Iran. J Isfahan Med Sch. 2017;35(444):1113-9.
15. Akya A, Ahmadi M, Khodamoradi S, Rezaei M, Karani N, Elahi A, et al. Prevalence of bla CTX-M, bla CTX-M-2, bla CTX-M-8, bla CTX-M-25 and bla CTX-M-3 Genes in Escherichia coli Isolated from Urinary Tract Infection in Kermanshah City, Iran. J clin diagn Res. 2019(13):8.

16. Akya A, Jafari S, Ahmadi K, Elahi A. Frequency of blaCTX-M, blaTEM and blaSHV genes in Citrobacters isolated from Imam Reza Hospital in Kermanshah. J Maz Univ Med Sci. 2015;25(127): 65-73.

17. Khodadoost M, Akya A, Ale Taha SM, Adabagher S. The frequency of antibiotic resistance and ctx-m gene in Escherichia coli isolated. Studies Med Sci. 2013;24(5):318-28.

18. Mohajeri P, Rostami Z, Farahani A, Norozi B. Distribution of ESBL producing Uropathogenic Escherichia coli and carriage of selected [beta]-lactamase genes in Hospital and community isolates in west of Iran. Ann Trop Med Public Health. 2014;7(5):219. [DOI:10.4103/1755-6783.154823]

19. Khurana S, Mathur P, Kapil A, Valsan C, Behera B. Molecular epidemiology of beta-lactamase producing nosocomial Gram-negative pathogens from North and South Indian hospitals. J Med Microbiol. 2017;66(7):999-1004. [DOI:10.1099/jmm.0.000513] [PMID]

20. Sarvazad H, Darbouy M. Correlation of Antibiotic Resistance with SHV, CTX-M and TEM Extended-Spectrum Beta Lactamases Genes among Klebsiella pneumoniae Isolates from Patients in Kermanshah Hospitals. J Ardabil Univ Med Sci. 2017;17(3):353-62.

21. Haidari E, Akya A. The frequency of broad-spectrum beta-lactamase CTX-M genotypes in Pseudomonas aeruginosa isolated from Kermanshah hospitals (2013-14). J Kermanshah Univ Med Sci. 2015;19(4):e69866.

22. Shams E, Firoozeh F, Moniri R, Zibaei M. Prevalence of Plasmid-Mediated Quinolone Resistance Genes among Extended-Spectrum β -Lactamase-Producing Klebsiella pneumoniae Human Isolates in Iran. J Pathog. 2015;2015: 434391. [DOI:10.1155/2015/434391] [PMID] [PMCID]

23. Sedaghatpishe S, Ghane M, Babaeekhou L. Identification and sequencing of bla CTX-M genes in clinical isolates of Klebsiella pneumoniae from Milad hospital. J Birjand Univ Med Sci. 2019;26(4):315-26. [DOI:10.32592/JBirjandUnivMedSci.2019.26.4.103]

24. Shivaee A, Shahbazi S, Soltani A, Ahadi E. Evaluation of the prevalence of broad-spectrum beta-lactamases (ESBLs) and carbapenemase genes in Klebsiella pneumoniae strains isolated from burn wounds in patients referred to Shahid Motahari Hospital in Tehran. Med Sci J Islamic Azad Univ. 2019;29(3). [DOI:10.29252/iau.29.3.232]

25. Ghane M, Adham F. Frequency of TEM and PER Beta-Lactamase Genes in Urinary Isolates of Escherichia Coli Producing Extended-Spectrum Beta-Lactamases. J Arak Univ Med Sci. 2020; 22(6):218-29. [DOI:10.32598/JAMS.22.6.2891.1]

26. Wintermans BB, Reuland EA, Wintermans RGF, Bergmans AMC, Kluytmans JAJW. The cost-effectiveness of ESBL detection: towards molecular detection methods? Clin Microbiol Infect. 2013;19(7):662-5. [DOI:10.1111/j.1469-0691.2012.03998.x] [PMID]

27. Kashefieh M, Hosainzadegan H, Baghbanijavid S, Ghotaslou R. The Molecular Epidemiology of Resistance to Antibiotics among Klebsiella pneumoniae Isolates in Azerbaijan, Iran. J Trop med. 2021;2021:9195184. [DOI:10.1155/2021/9195184] [PMID] [PMCID]

28. 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]

29. Haddadi A. Frequency determination of blaTEM & blaSHV in the Extended-Spectrum Beta-Lactamase producing Escherichia coli isolates from Karaj. Iran J Med Microbiol. 2017;11(2):75-80.

30. Shoeib N, Fereshteh S, Mehdi FM, Sadat NV, Leila N. Molecular characterization of CTX-M β-lactamases among Klebsiella pneumoniae isolated from patients at Tehran hospitals. Indian J Med Microbiol. 2011;29(3):254-7. [DOI:10.4103/0255-0857.83908] [PMID]

31. Koshesh M, Mansouri S, Hashemizadeh Z, Kalantar Neyestanaki D. 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]

32. Bagheri Nesami M, Rafiei A, Eslami G, Ahangarkani F, Rezai MS, Nikkhah A, et al. Assessment of extended-spectrum β-lactamases and integrons among Enterobacteriaceae in device-associated infections: multicenter study in north of Iran. Antimicrob Resist Infect Control. 2016;5(1):52. [DOI:10.1186/s13756-016-0143-2] [PMID] [PMCID]

33. Davodian E, Sadeghifard N, Ghasemian A, Noorbakhsh S. Presence of blaPER-1 and blaVEB-1 beta-lactamase genes among isolates of Pseudomonas aeruginosa from South West of Iran. J Epidemiol Glob Health. 2016;6(3):211-3. [DOI:10.1016/j.jegh.2016.02.002] [PMID] [PMCID]

34. Bahrami M, Mmohammadi-Sichani M, Karbasizadeh V. Prevalence of SHV, TEM, CTX-M and OXA-48 β-Lactamase genes in clinical isolates of Pseudomonas aeruginosa in Bandar-Abbas, Iran. vicenna J Clin Microbiol Infect. 2018;5(4):86-90. [DOI:10.34172/ajcmi.2018.18]

35. Mansury D, Motamedifar M, Sarvari J, Shirazi B, Khaledi A. Antibiotic susceptibility pattern and identification of extended spectrum β-lactamases (ESBLs) in clinical isolates of Klebsiella pneumoniae from Shiraz, Iran. Iran J Microbiol. 2016;8(1):55-61.

36. Musa MA, Tahir OM. Detection of CTX-M, TEM and SHV genes in gram negative bacteria isolated from nosocomial patients at port sudan teaching hospital. European J Clin Biomed Sci. 2017;3(6): 101-8. [DOI:10.11648/j.ejcbs.20170306.11]

37. Ugbo EN, Anyamene CO, Moses IB, Iroha IR, Babalola OO, Ukpai EG, et al. prevalence of blaTEM, blaSHV, and blaCTX-M genes among extended spectrum beta-lactamase-producing Escherichia coli and Klebsiella pneumoniae of clinical origin. Gene Rep. 2020;21-:100909. [DOI:10.1016/j.genrep.2020.100909]

38. Antimicrobial resistance: Policy insights 2016 Available from: [https://www.oecd.org/health/health-systems/AMR-Policy-Insights-November2016.pdf]

39. Abayneh M, Worku T. Prevalence of multidrug-resistant and extended-spectrum beta-lactamase (ESBL)-producing gram-negative bacilli: A meta-analysis report in Ethiopia. Drug Target Insights. 2020;14:16-25. [DOI:10.33393/dti.2020.2170] [PMID] [PMCID]

40. Amer R, El-Baghdady K, Kamel I, El-Shishtawy H. Prevalence of Extended Spectrum Beta-Lactamase Genes among Escherichia coli and Klebsiella pneumoniae Clinical Isolates. Egypt J Microbiol. 2019;54(1):91-101. [DOI:10.21608/ejm.2019.16460.1113]