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Association between the CRISPR-associated *cas* genes and beta-lactamase genes (*bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$) in uropathogenic *Escherichia coli* (UPEC)

Neda Merikhi[1], Jamileh Nowroozi[*1], Ali Nazemi[2], Mehrdad Hashemi[3], Robab Rafiei Tabatabaei[1]

1. Department of Microbiology, North Tehran Branch, Islamic Azad University, Tehran, Iran
2. Department of Biology, Tonekabon Branch, Islamic Azad University, Tonekabon, Iran
3. Department of genetics, Faculty of advanced sciences and technology, Islamic Azad University, Tehran medical sciences branch, Tehran, Iran

** Corresponding author's E-mail: Nowroozij@yahoo.com*

ABSTRACT

The purpose of this work was to assess the prevalence of *cas* genes (*cas* 1, 2, 3, 5 and 7) in UPEC isolates, as well as the connection between CRISPR-associated *cas* genes and beta-lactamase genes (*bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$). The Kirby–Bauer disk diffusion technique was used to assess the susceptibility of isolates to targeted antibiotics. For isolates that were resistant to at least one of the third-generation of cephalosporins in the antibiotic susceptibility test, the combined disk test was conducted. The *bla*$_{TEM}$, *bla*$_{SHV}$, *bla*$_{CTX-M}$, and *cas* genes (*cas* 1, 2, 3, 5 and 7) were identified molecularly using the polymerase chain reaction technique. A total of 106 uropathogenic *Escherichia coli* isolates were identified from 437 positive urine culture samples. Overall, 64.1 % of isolates harbored *bla*TEM genes. Nevertheless, 46.2 % and 35.9 % of isolates, respectively, carried *bla*$_{SHV}$ and *bla*$_{CTX-M}$. The *cas* 1, 2, 3, 5 and 7 genes were detected in 96.2 %, 48.1 %, 60.4 %, 65.1%, and 50% of isolates, respectively. We discovered a statistically significant association between the presence of *cas*2 and the presence of *bla*$_{SHV}$ (p-value = 0.01) and *bla*$_{TEM}$ (p-value = 0.02) viruses. We observed a statistically significant association between the presence of at least three *cas* genes and the presence of *bla*$_{SHV}$ (p-value = 0.001) gene. At the end, we detected a statistically significance between simultaneous presence of at least five *cas* genes and simultaneous presence of beta-lactamase genes (*bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$; Pv: 0.01). The results of this study indicated that the presence of *cas* genes linked with the CRISPR system may help lower the presence of beta-lactamase genes (*bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$) in UPEC isolates.

Keywords: CRISPR-associated (*cas*) genes, Uropathogenic *Escherichia coli*, UPEC, ESBL, Beta-lactamase genes.
Article type: Research Article.

INTRODUCTION

Clustered regularly interspaced short palindromic repeats (CRISPR) is a bacterial adaptive immune response system that mediates bacterial immunological responses (Hsu *et al*. 2014). CRISPR was discovered in *Escherichia coli* in 1987 during the iap gene study, which was conducted as part of a research project (Ishino *et al*. 1987). CRISPR is made up of two major components: the repeats and the spacer DNA (also known as the spacer RNA; Dyda & Hickman 2015). Essentially, the spacers serve as a repository for the history of previous infections, whilst the repetitions serve as small portions of DNA (20 to 40 letters in length). According to studies conducted in the early 2000s, the spacer DNA, which is critical DNA, was shown to be a perfect match with viral DNA, particularly bacteriophage DNA. They also discovered a number of genes that are related to CRISPR, which are known as CRISPR-associated or *cas* genes (Makarova *et al*. 2019). These genes create Cas proteins, and Cas proteins in general are helicases, which are proteins that destroy foreign DNA, such as that of bacteriophages, plasmids, and

other conjugative elements, when they come into contact with them. Then there are nucleases, which cleave the DNA. This is one of the ways in which bacteria may defend themselves against invading DNA (Dyda & Hickman 2015; Samson *et al*. 2015). Based on a study of 13,116 full genomes, researchers discovered that CRISPR–Cas loci are present in 85.2 % of archaea and between 10 and 40% of bacteria (Burstein *et al*. 2016). As with other biological defense mechanisms, there are significant distinctions between archaeal and bacterial CRISPR–Cas systems, including changes in Cas protein sequences, gene components, and locus genetic architecture (Makarova *et al*. 2019). The CRISPR-Cas system is divided into six distinct subtypes: CRISPR-Cas system types I, II, III, IV, V, and VI (Makarova *et al*. 2019). Type I is the most basic of the CRISPR-Cas systems, while type II is the most advanced (Makarova *et al*. 2011). Cas1 and Cas2 proteins are found in all forms of CRISPR-Cas systems, and they play an important role in the capture and integration of novel spacers during the adaptation phase of the genome editing process (Almendros *et al*. 2019). Class 1 of the CRISPR-Cas system includes 12 subtypes, which are distinguished by comparing effector protein clusters, locus structures, and repeat sequences. CRISPR-Cas systems are divided into three types: types I, III, and IV, with 12 subtypes included in class 1. In addition, class 2 of the CRISPR-Cas system is composed of types II, V, and VI, as well as 17 subtypes. In *E. coli*, four CRISPR arrays, including CRISPR1, CRISPR2, CRISPR3, and CRISPR4, as well as the *cas* genes, *cas*3, *cse*1, *cse*2, *cas*7, *cas*5, *cas*6e, *cas*1 and *cas*2, have been identified. These genes are members of the Type I-E strain and are related to the CRISPR1 loci, which are found in the CRISPR1 array (Haft *et al*. 2005; Díez-Villaseñor *et al*. 2010; Touchon *et al*. 2011). Several studies have shown that the CRISPR-Cas system is involved in the development of antibiotic resistance.

The CRISPR-Cas system has the potential to restrict genetic elements ingested while also defending against intrusive foreign DNA (Garneau *et al*. 2010; Barrangou 2015; Samson *et al*. 2015). Several studies have shown that the CRISPR-Cas system can limit the presence of foreign genetic material in bacteria such as *Enterococcus faecalis*, *Enterococcus faecium*, *Pseudomonas aeruginosa*, and *Streptococcus pneumoniae* (Palmer & Gilmore 2010; Cady *et al*. 2011; Burley & Sedgley 2012; Bikard *et al*. 2012). Human urinary tract infections (UTIs) are caused by strains of the bacteria uropathogenic *E. coli* (UPEC), which are the most prevalent agents causing UTIs (Tabasi *et al*. 2016). Recently, the emergence of extended-spectrum beta-lactamase (ESBL)-producing *E. coli* and the development of multi-drug resistance (MDR) among UPEC patients have posed a significant treatment challenge, particularly in women who suffer from recurrent urinary tract infections. The high frequency of MDR-UPEC in many countries, especially in developing nations, leads to the improper use of extended-spectrum antimicrobial drugs, which increases hospitalization and treatment expenses as well as the number of people admitted to hospitals (Bartoletti *et al*. 2016). The increasing incidence of resistance to β-lactam antibiotics in UPEC indicates that the study of novel strategies for reducing antibiotic resistance may be required in the future. There are also some other reports about *E. coli* occurrence in other organisms in Iran and other parts of the world (Bayas-Morejón *et al.* 2020; Khezri *et al.* 2021; Tonekabony *et al.* 2021; Yousif *et al.* 2022). As a result, the purpose of this study was to assess the frequency of *cas* genes in UPEC isolates, as well as the link between CRISPR-associated (*cas*) genes (*cas* 1, 2, 3, 5 and 7) and beta-lactamase genes (bla_{TEM}, bla_{SHV}, and $bla_{\text{CTX-M}}$), in addition to the frequency of *cas* genes.

MATERIALS AND METHODS

Samples collection

During the period of one year from December 2018 to November 2019, this cross-sectional research was conducted in Iranian hospitals located in western Tehran and also Chalus, Mazandaran Province, Iran. Positive urine cultures were found from individuals who had urethra infection in a total of 437 instances. The sample collection techniques used were in accordance with the ethical norms of the Islamic Azad University's Ethics Committee in Tehran, Iran (IR.IAU.TNB.REC.1398.027) and the Helsinki Moral Principles. Antibiotic resistance surveillance data for all samples were obtained from the Study Center's Medical Records, then analyzed.

Identification of bacteria

All samples were cultivated on eosin methylene blue (EMB) and blood agar (Merck, Darmstadt, Germany) plates to determine the presence of eosin methylene blue. The cultures having a colony count of more than 100000 CFU mL^{-1} were next tested for the presence of uropathogenic *E. coli* (UPEC). The detection of UPEC strains was accomplished by the employment of routine biochemical assays and an Enterobacteriaceae commercial diagnostic kit (Padtan-Teb, Tehran, Iran).

Antibiotics Susceptibility test

Each clinical isolate of UPEC was subjected to an antimicrobial susceptibility test with cefepime (PEP, 30 µg), ceftazidime (CAZ, 30 µg), cefotaxime (CTX, 30 µg), and cefixime (CFM, 5 µg) according to the Clinical and Laboratory Standards Institute (CLSI) instruction using the Kirby–Bauer disk diffusion method. The *E. coli* ATCC 25922 strain was utilized as the quality control strain for the tests. Results of susceptibility testing were evaluated in accordance with CLSI standards (XIONG *et al*. 2019).

Phenotypic detection for ESBL production

According to Livermore & Brown (2001) the combined disk test (CDT) was used to identify isolates resistant to at least one of the third generation cephalosporins in the antibiotic susceptibility test. Mueller-Hinton agar (MHA) was used to grow the isolates, and cefotaxime (CTX, 30 µg) and ceftazidime (CAZ, 30 µg) discs were placed individually on the surface of the culture medium. Then, at a distance of 20 mm, these disks were combined with 10 µg clavulanic acid (center to center). If the diameter difference between the constraint zone and the surrounding zone was higher than 5 mm, it was termed positive ESBL. As negative and positive controls, *E. coli* ATCC 25922 and *Klebsiella pneumoniae* ATCC 700603 ESBL positive were utilized, respectively.

Molecular detection of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes

All of the isolates had their DNA extracted using a commercial kit (PZP, Daneshbonyan, Iran), following the manufacturer's instructions. Based on Ojdana *et al*. (2014) and produced by Macrogen/Korea, each primer pair was designed to target beta-lactamase genes. For all isolates, the *bla*$_{TEM}$, *bla*$_{SHV}$, and *bla*$_{CTX-M}$ genes were identified using the PCR (polymerase chain reaction) technique. The following was the PCR master mix: 12.5 µL Ampliqon master mix Red, 2.5 µL DNA templet, 4 µL distilled water, and 1 µL each of the forward and reverse primers (Macrogen, South Korea) in a total volume of 20 µL (Table 1). An amount of 1.5% agarose gel electrophoresis was used to analyze the PCR results. The gels were stained with a mixture of 5 µL of PCR products and 2 µL of RedSafe dye (Khamesipour *et al*. 2015; Doosti *et al*. 2016b; Chehelgerdi & Doosti 2020). LED trans-illuminator was used to evaluate these gels.

Molecular detection of *cas genes*

The primers, developed and produced by the Macrogen Company in Korea, were used in the PCR test for detection of *cas* genes (*cas* 1, 2, 3, 5 and 7; Table 1; Merikhi *et al*. 2022). Following was the PCR master mix: 12.5 µL of Ampliqon master mix Red, 2.5 µL of DNA templet, 4 µL of distilled water, and 1 µL each of the forward and reverse primers in a total volume of 20 µL. As previously noted, the gels were stained and assessed using an LED trans-illuminator. After that, 25 µL of PCR products were sent for sequencing, along with 5 µL of forward primer (0.1 concentration) for each gene (Macrogen, South Korea). Chromas software version 1.45 was used to analyze the sequences. Afterward, the sequences were BLASTed against the NCBI database.

Nucleotide sequence accession numbers

The nucleotide sequences have the following GenBank accession numbers: MT885230 for *cas*1, MW044948 for *cas*2, MT980728 for *cas*3, MT980729 for *cas*7, and MW030688 for *cas*5.

Statistical analysis

Stata Version 14.0 (IBM, Armonk, NY, USA) was used to conduct the statistical analysis. The categorical variables were evaluated using the Chi-square test and Fisher's exact test (Version 2021). The strength of the link was measured using the odds ratio (OR) with a 95% confidence level. Significant was defined as a p-value of less than 0.05.

RESULTS

Distribution of the uropathogenic *Escherichia coli* strains

Between December 2018 and November 2019, 437 positive urine cultures yielded 106 (24.3 %) uropathogenic *Escherichia coli* (UPEC) strains. The patients' average age was 19.5 - 39.5 years (range: 4 to 78 years). Females made up around 76.4 % of the patients. The majority of female patients (16.9%) were between the ages of 35 and 44.

Table 1. List of primers used for detection of β-lactamase and *cas* genes in this study.

Primer	Oligonucleotide	Sequence (5´–3´)	length (bp)	PCR reaction conditions	References
TEM-F	bla_{TEM}	CCA TCT GGC CCC AGT GCT GC	686	95℃ 300s (94℃ 60s-58℃ 60s-72℃ 60s) ×35, 72℃ 600s	(Ojdana *et al.*, 2014)
TEM-R		GCT CAC CCA GAA ACG CTG GT			
SHV-F	bla_{SHV}	CAT GCTCGCCGGSGTATCCC	733	95℃ 300s (94℃ 60s-58.5℃ 60s-72℃ 60s) ×35, 72℃ 600s	
SHV-R		CCCGCAGCCGCTTGAGCAAA			
CTX-M-F	bla_{CTX}	SCVATGTGCAGYACCAGTAA	581	94℃ 180s (94℃ 30s-55℃ 30s-72℃ 45s) ×35, 72℃ 600s	
CTX-M-R		ACCAGAAYVAGCGGBGC			
Cas1-F	*cas*1	GG GTT GCC TCG CTG CTA TT	198	95℃ 300s (95℃ 30s-55℃ 30s-72℃ 30s) ×30, 72℃ 300s	Macrogen company, Korea
Cas1-R		GTT GTG AAT CGC TGC ATC AGT G			
Cas3-F	*cas3*	GCG CTT ACG TGG ACG GCT C	387		
Cas3-R		ACT CGG TAT TGG TCG CCC AGG			
Cas2-F	*cas2*	GCG CTT ACG TGG ACG GCT C	157		
Cas2-R		ACT CGG TAT TGG TCG CCC AGG			
Cas7-F	*cas7*	CGT CCT TCA TGC TTC CCT GTT TG	243		
Cas7-R		CAA CCG GCA AAC AGA ACA GCT			
Cas5-F	*cas5*	ACC GCC ACC ATC CAC CAG	297		
Cas5-R		GCG TCA TAC CCA TGA ACT GCC TTC			

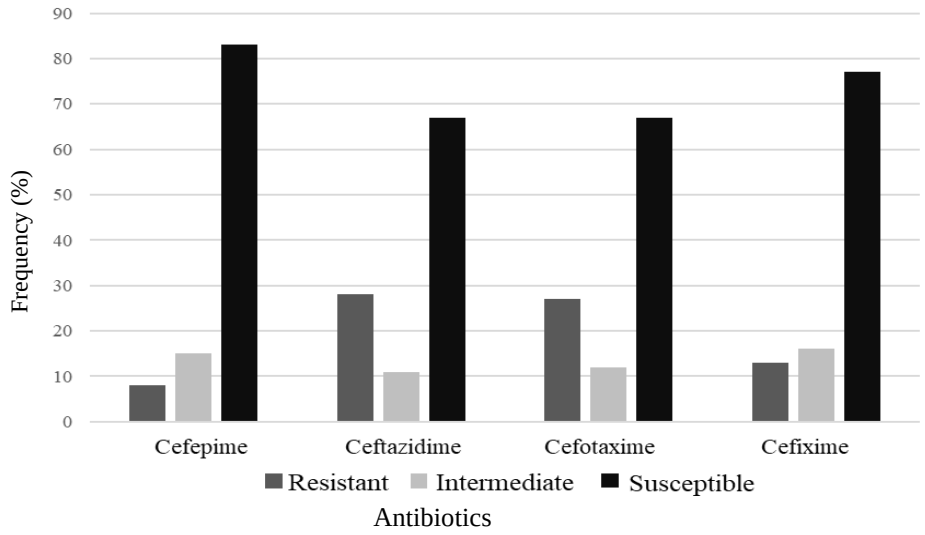

Fig. 1. Age groups and sex distribution of the patients

Antimicrobial susceptibility tests

Fig. 2 depicts the distributions of antibiotic resistance for cefepime, ceftazidime, cefotaxime, and cefixime. Thirty-six isolates (33.96%) were found to be antibiotic-resistant, compared to cefixime (12.2%) and cefepime (7.6%). Resistance to ceftazidime (26.4%) and cefotaxime (25.5%) was more common (7.6%). Ceftazidime and cefotaxime resistances were found in 12 isolates (11.3%). All antibiotic resistances were found in two isolates (1.9 %).

Association between antibiotic resistance and ESBL phenotype

CDT phenotypic identification of ESBL production revealed that 30 isolates (28.3%) were ESBL producers, whereas 76 isolates (71.7%) were non-ESBL producers. The majority of ESBL-producing isolates (40%) were resistant to ceftazidime and cefotaxime, but not to other antibiotics (Table 2). In three groups of patterns (CAZ, CAZ+CTX, and CAZ+CTX+CFM), the distribution of ESBL positive isolates was statistically significant.

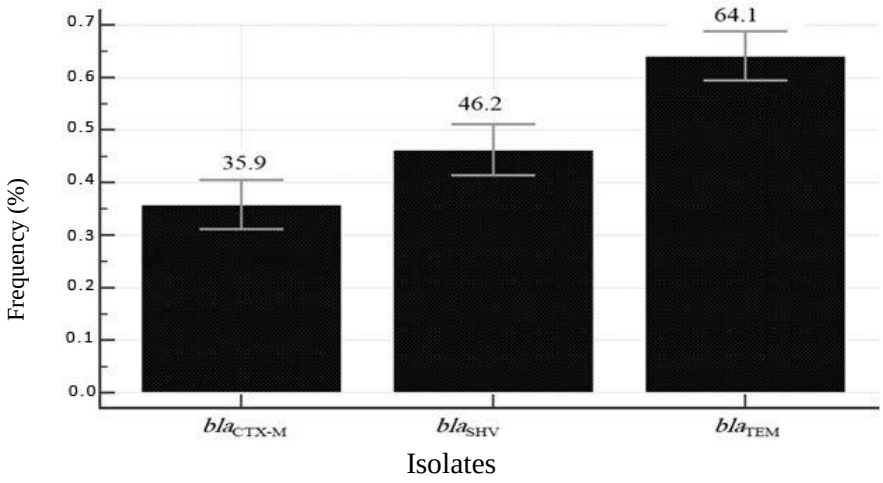

Fig. 2. The rate of isolates resistant to each antimicrobial agent tested.

Table 2. Drug resistance patterns of uropathogenic *Escherichia coli* isolates from patients and percentage of ESBL positive in these patterns.

Patterns of antibiotic resistant	Non-ESBL producing	ESBL producing	p-value
	Number (%)		
PEP*	0 (0.0)	0 (0.0)	-
CAZ	4 (3.8)	3 (10.0)	0.035
CTX	3 (2.8)	2 (6.67)	0.13
CFM	2 (1.9)	0 (0.0)	0.37
PEP+CAZ	1 (0.9)	0 (0.0)	0.53
PEP+CTX	2 (1.9)	1 (3.3)	0.49
PEP+CFM	1 (0.9)	0 (0.0)	0.53
CAZ+CTX	12 (11.3)	12(40)	<0.001
CAZ+CFM	0 (0.0)	0 (0.0)	-
CTX+CFM	0 (0.0)	0 (0.0)	-
PEP+CAZ+CTX	1 (0.9)	1 (3.3)	0.11
PEP+CAZ+CEM	1 (0.9)	1 (3.3)	0.11
PEP+ CEM +CTX	0 (0.0)	0 (0.0)	-
CAZ+CTX+CFM	7 (6.6)	6 (20.0)	<0.001
PEP+CAZ+CTX+CFM	2 (1.9)	2 (6.7)	0.023

* Resistant to PEP (cefepime), but susceptible or intermediate to other antibiotics.

Prevalence of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes in uropathogenic *E. coli* and relation to ESBL production isolates

The prevalence of ESBL genes among UPEC isolates is shown in Fig. 3. Overall, 64.1% of the isolates had *bla*$_{TEM}$ genes; however, 46.2 % and 35.9% of the isolates carried *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes, respectively. All of the ESBL-positive UPEC isolates carried one or more ESBL genes, according to a PCR analysis of ESBL genotypes. In general, 17% of isolates (no. 5) examined were positive for one ESBL gene, 40% (no. 12) for two ESBL genes, and 43% (no. 13) for three ESBL genes (Table 3).

Table 3. Distribution of β-lactamase gene patterns in different ESBL phenotypes of UPEC isolates.

β-lactamase genes	ESBL phenotype	Number (%)	p-value
SHV	ESBL positive	0 (0)	0.006
	ESBL negative	16 (21)	
TEM	ESBL positive	2 (7)	0.014
	ESBL negative	22 (29)	
CTX-M	ESBL positive	3 (10)	0.38
	ESBL negative	4 (5)	
CTX-M, SHV	ESBL positive	0 (0)	0.37
	ESBL negative	2 (3)	
CTX-M, TEM	ESBL positive	6 (20)	0.13
	ESBL negative	7 (9)	
SHV, TEM	ESBL positive	6 (20)	0.28
	ESBL negative	9 (12)	
CTX-M, TEM, SHV	ESBL positive	13 (43)	<0.001
	ESBL negative	3 (4)	

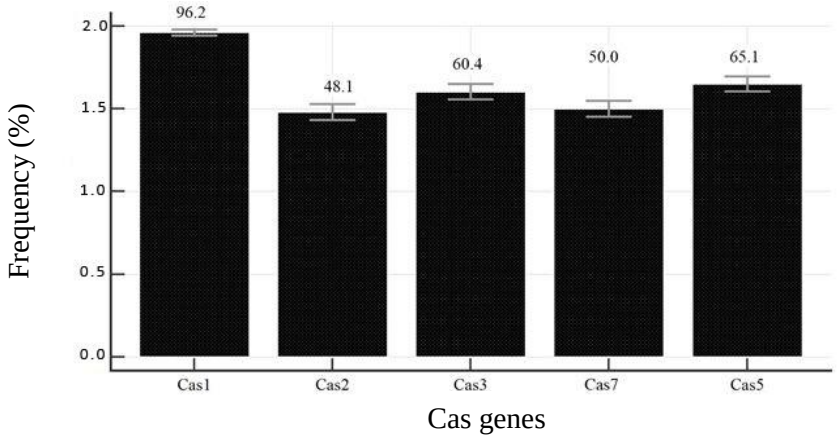

Fig. 3. Distribution of *bla*$_{TEM}$, *bla*$_{SHV}$ and *bla*$_{CTX-M}$ genes with their percentages among UPEC isolates.

Prevalence of *cas genes* (*cas* 1, 2, 3, 5 and 7) in uropathogenic *E. coli* and association between the ESBL and *cas* genes

Using a PCR technique to identify *cas* genes, we discovered that 96.2 % of isolates examined were positive for the *cas*1 gene; however, 48.1 %, 60.4 %, 65.1 %, and 50 % of isolates for the *cas*2, *cas*3, *cas*5, and *cas*7 genes, respectively (Figs. 4-5).

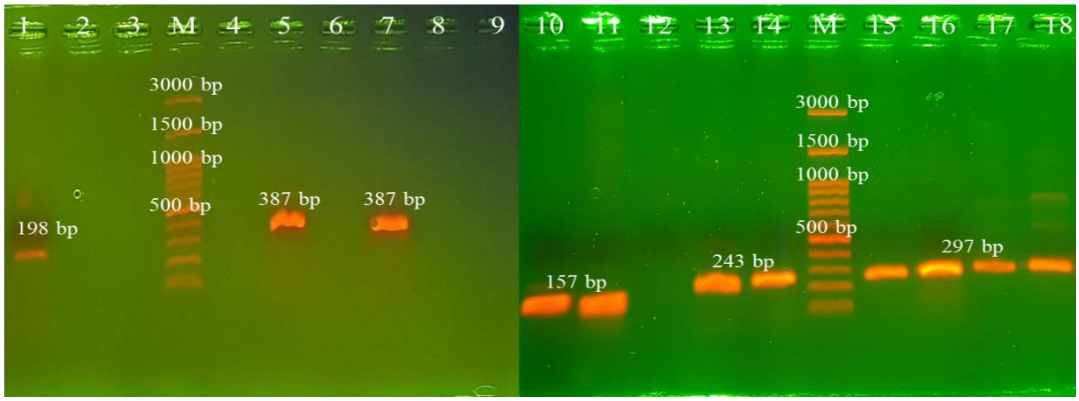

Fig. 4. Polymerase chain reaction amplification of *cas* genes in UPEC isolates M; 100 bp + 3k DNA ladder; lanes 2, 3, 4, 6, 8, 9, and 12 were negative controls; lane 1 *cas1* (198 bp), lanes 5 and 7 *cas3* (387 bp), lanes 10 and 11 *cas2* (157 bp), lanes 13 and 14 *cas7* (243 bp), and lanes 15, 16, 17, and 18 *cas5* (297 bp), were amplification products.

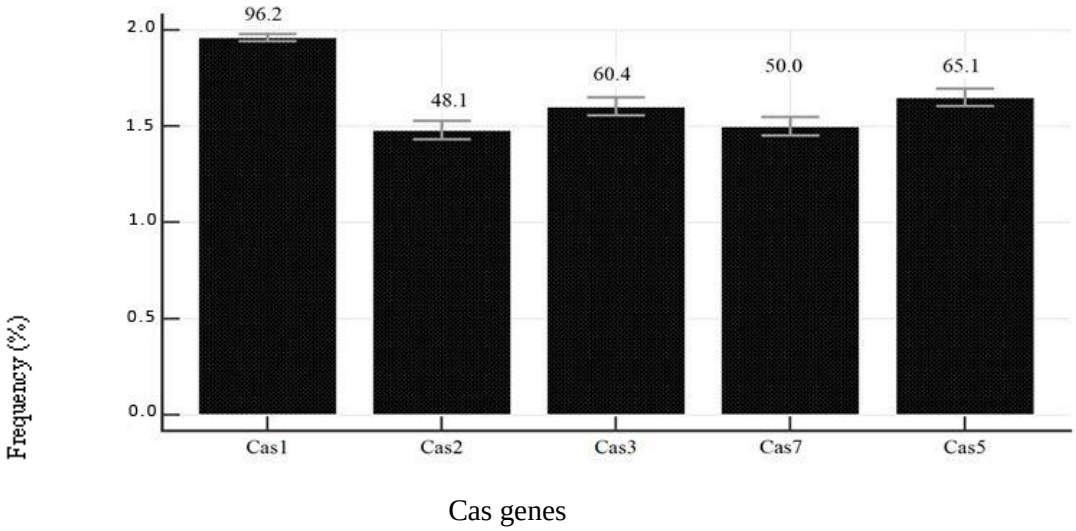

Fig. 5. Distribution of *cas genes* with their percentages among UPEC isolates.

In each sample, at least one of the *cas* genes was found to be present. As depicted in Table 4, only 33.3 % of bla_{SHV} positive isolates and 52.9 % of bla_{TEM} positive isolates examined were also positive for the *cas*2 gene. On the other hand, 66.7 % of bla_{SHV} negative isolates and 47.1 % of bla_{TEM} negative isolates examined were positive for the *cas*2 gene. In this study, there was a statistically significant relationship between the presence of *cas*2 gene and that of the bla_{SHV} (p-value = 0.01, odds ratio: 0.35, confidence interval (CI): 0.16-0.79) and bla_{TEM} (p-value = 0.02, odds ratio: 0.38, CI: 0.17-0.87) genes. It was found that bla_{SHV} positive gene isolates were 35 % less likely to be present in isolates with *cas*2 gene than bla_{SHV} negative gene isolates, and that bla_{TEM} positive gene isolates were 38 % less likely to be present in isolates with *cas*2 gene when compared to bla_{TEM} negative gene isolates. Also, the presence of at least three *cas* genes was found in conjunction with the bla_{SHV} gene (p-value: 0.001, OR: 0.10, 95% CI: 0.03-0.35; Table 5). There is a statistically significant association between the simultaneous presence of at least three *cas* genes and the simultaneous presence of at least three bla_{SHV} and bla_{TEM} genes (Table 6; p-value: 0.016; OR: 3.81; CI: 1.21-11.95). A statistically significant relationship (p-value: 0.01, odds ratio: 0.19, CI: 0.05-0.69) was discovered between the simultaneous presence of at least five *cas* genes and the simultaneous presence of beta-lactamase genes (bla_{TEM}, bla_{SHV}, and bla_{CTX-M}).

DISCUSSION

We believe that this is the first cross-sectional investigation to examine the association between beta-lactamase genes and *cas* genes (*cas* 1, 2, 3, 5 and 7) among uropathogenic *Escherichia coli* (UPEC) isolates, to the best of our knowledge. In our investigation, we discovered that UPEC isolates were more prevalent in females than in males (76.4 %). This result has also been discovered in a number of other studies (Issazadeh *et al*. 2015; Pourzare *et al*. 2017). The frequency of UPEC in females was greater in the age range of 35-44 years than in any other group (16.9 %). This conclusion was validated by additional studies conducted by Dehbanipour *et al.* (2016), and Tabasi *et al.* (Tabasi *et al*. 2015). Anatomical features, normal vaginal flora, and the closeness of the vagina to the anus are all possible explanations for the increased occurrence of UPEC in females (Foxman *et al*. 2000; Hooton 2001). During this study, we discovered that the majority of isolates were sensitive to the cephems (66.04 %), although earlier studies revealed less susceptibility to this antibiotic (Zhao *et al*. 2015; Hassuna *et al*. 2020). We discovered that 40 % of ESBL-producing isolates were also resistant to ceftazidime and cefotaxime, and that 20% of ESBL-producing isolates were also resistant to ceftazidime, cefotaxime, and cefepime, respectively. We found that 28.3 % of UPEC isolates were ESBL producers, while in a previous study in Iran Kashef Nejad *et al*. (2017) reported that 40.6 % of UPEC isolates were ESBL-positive. Based on the results of the PCR technique used to determine the frequency of three ESBL genes (bla_{TEM}, bla_{CTX-M}, and bla_{SHV}), it was determined that bla_{TEM} (64.1 %) was the most prevalent ESBL gene, followed by bla_{SHV} (46.2 %) among all UPEC isolates. The bla_{CTX-M} gene is more prevalent in various geographic locations than other ESBL genes, such as the bla_{TEM} and bla_{SHV} genes, which are also prevalent (D'Andrea *et al*. 2013; Liu *et al*. 2015; Bush 2018). Despite the fact that our findings were not verified by these studies, the bla_{TEM} gene was shown to be dominant in a number of studies (Jena *et al*. 2017; Yazdi *et al*. 2012). This mechanism was originally discovered in an *E. coli* investigation (Brouns *et al*. 2008), and has since been reported in a number of other studies involving bacteria of this kind (Haft *et al*. 2005; Díez-Villaseñor *et al*. 2010). The *cas*1 gene was found in virtually all of the UPEC isolates examined in our study (96.2 %). Our results revealed that the presence of the *cas*2 gene was associated with a decrease in bla_{SHV} (p-value = 0.01, OR: 0.35, CI: 0.16-0.79) and bla_{TEM} (p-value = 0.02, OR: 0.38, CI: 0.17-0.87) in UPEC isolates (p-value = 0.02, OR: 0.38, CI: 0.17-0.87; Chen *et al*. 2010). We also found an association between the simultaneous presence of bla_{SHV} and bla_{TEM} genes and the simultaneous presence of at least three *cas* genes in UPEC isolates (p-value= 0.016, OR: 3.81, CI: 1.21-11.95). Touchon *et al*. (2012), reported that there was no correlation between the presence or lack of *cas* genes and antibiotic resistance. A link between the CRISPR-Cas system and antibiotic resistance has been identified by Palmer & Whiteley (2011). We found that the CRISPR-Cas system in *E.coli* might be latent; as a result, the expression of this system may not be detected, at least under laboratory settings, at this time (Westra *et al*. 2010; Pougach *et al*. 2010). As a result, it is possible that the lack of statistically significant variations in the presence/absence of additional *cas* genes in UPEC isolates is attributable to a decrease in the expression of this system in these isolates. Based on the presence of beta-lactamase genes in *E. coli*, we compared the co-presentation of *cas* genes in the bacteria.

Table 4. Percentage of *cas genes (cas* 1, 2, 3, 5 and 7*)* and β-lactamase genes in UPEC isolates.

Genes		***bla*SHV N (%)**		p-value	OR	CI95%	***bla*TEM N (%)**		p-value	OR	CI95%	***bla*CTX-M N (%)**		p-value	OR	CI95%
		-	+				-	+				-	+			
*cas*1	+	55 (53.9)	47 (46.1)	1.00	0.85	0.11-6.30	36 (35.3)	66 (64.7)	0.61	1.80	0.24-13.5	65 (63.7)	37 (36.3)	1.00	1.70	0.17-17.0
	_	2 (50)	2 (50)				2 (50.0)	2 (50.0)				3 (75.0)	1 (25.0)			
*cas*2	+	34 (66.7)	17 (33.3)	0.01	0.35	0.16-0.79	24 (47.1)	27 (52.9)	0.02	0.38	0.17-0.87	32 (65.5)	19 (37.2)	0.77	1.12	0.50-2.40
	_	23 (41.8)	32 (58.2)				14 (25.5)	41 (74.5)				36 (65.5)	19 (34.5)			
*cas*3	+	34 (53.1)	30 (46.9)	0.86	1.06	0.48-2.33	26 (40.6)	38 (59.4)	0.20	0.58	0.25-1.34	39 (60.9)	25 (39.1)	0.39	1.42	0.62-3.26
	_	23 (54.8)	19 (45.2)				12 (28.6)	30 (71.4)				13 (30.9)	29 (69.1)			
*cas*7	+	33 (62.3)	20 (37.7)	5.07	0.5	0.23-1.08	16 (30.2)	37 (69.8)	0.22	1.64	0.73-3.65	32 (60.4)	21 (39.6)	0.41	1.38	0.62-3.08
	_	24 (45.3)	29 (54.7)				22 (41.5)	31 (58.5)				36 (67.9	17 (32.1)			
cas5	+	39 (56.5)	30 (43.5)	0.43	0.72	0.32-1.6	25 (36.2)	44 (63.8)	0.91	0.95	0.41-2.19	44 (63.8)	25 (36.2)	0.91	1.04	0.45-2.41
	_	18 (48.6)	19 (51.4)				13 (35.1)	24 (64.9)				24 (64.9)	13 (35.1)			

Table 5. Percentage of simultaneous presence *cas genes (cas* 1, 2, 3, 5 and 7*)* and positive β-lactamase genes in UPEC isolates.

Genes		***bla*SHV N (%)**		p-value	OR	CI95%	***bla*TEM N (%)**		p-value	OR	CI95%	***bla*CTX-M N (%)**		p-value	OR	CI95%
		-	+				-	+				-	+			
At least two *cas* genes	+	55 (54.5)	46 (45.5)	0.66	0.55	0.08-3.48	38 (37.6)	63 (2.93)	0.15	1.00	-	65 (64.4)	36 (35.6)	1.00	0.83	0.13-5.20
	_	2 (40.0)	3 (60.0)				0 (0.0)	5 (100.0)				3 (60.0)	2 (40.0)			
At least three *cas* genes	+	53 (64.6)	29 (35.4)	<0.001	0.10	0.03-0.35	31 (37.8)	51 (62.2)	0.44	0.67	0.25-1.81	52 (63.4)	30 (36.6)	0.77	1.15	0.44-3.01
	_	4 (16.7)	20 (83.3)				7 (29.2)	17 (70.8)				16 (66.7)	8 (33.3)			
At least four *cas* genes	+	23 (60.5)	15 (39.5)	0.29	0.65	0.29-1.45	18 (47.4)	20 (52.6)	0.06	0.46	0.2-1.05	23 (60.5)	15 (39.5)	0.56	1.27	0.56-2.90
	_	34 (50.0)	34 (50.0)				20 (29.4)	48 (70.6)				45 (66.2)	23 (33.8)			
At least five *cas* genes	+	7 (53.3)	5 (41.7)	0.74	0.81	0.24-2.74	2 (16.7)	10 (83.3)	0.20	3.10	0.64-14.9	4 (33.3)	8 (66.7)	0.026	4.26	1.19-15.28
	_	50 (25.3)	44 (46.8)				58 (61.7)	36 (38.3)				64 (68.1)	30 (31.9)			

Table 6. Percentage of simultaneous presence *cas genes (cas* 1, 2, 3, 5 and 7*)* and simultaneous presence of positive β-lactamase genes in UPEC isolates.

gene		***bla*SHV and *bla*TEM N (%)**		**p-value**	**OR**	**CI95%**	***bla*SHV and *bla*CTX-M N (%)**		**p-value**	**OR**	**CI95%**	***bla*TEM and *bla*CTX-M N (%)**		**p-value**	**OR**	**CI95%**	***bla*TEM and *bla*CTX-M and *bla*SHV N (%)**		**p-value**	**OR**	**CI95%**
		-	+				-	+				-	+				-	+			
At least two *cas* genes	+	87 (86.1)	14 (13.9)	0.54	1.55	0.16-14.93	99 (98.0)	2 (2.0)	1.00	1.00	-	88 (87.1)	13 (12.9)	1.00	1.00	-	87 (86.1)	14 (13.9)	0.16	4.14	0.63-27.04
	–	4 (80.0)	1 (20.0)				5 (100.0)	0 (0.0)				5 (100.0)	0 (0.0)				3 (60.0)	2 (40.0)			
At least three *cas* genes	+	74 (90.2)	8 (9.8)	0.016	3.81	1.21-11.95	81 (98.8)	1 (1.2)	0.40	3.52	0.21-58.51	70 (85.4)	12 (14.6)	0.29	0.25	0.31-2.06	72 (87.8)	10 (12.2)	0.12	2.4	0.77-7.47
	–	17 (70.8)	7 (29.2)				23 (95.8)	1 (4.2)				23 (95.8)	1 (4.20)				18 (75.0)	6 (25.0)			
At least four *cas* genes	+	36 (94.7)	2 (5.3)	0.079	4.25	0.91-19.98	38 (100.0)	0 (0.0)	0.54	1.00	-	32 (84.2)	6 (15.8)	0.41	0.61	0.19-1.97	32 (84.2)	6 (15.8)	0.88	0.92	0.31-2.76
	–	55 (80.9)	13 (19.1)				66 (97.1)	2 (2.1)				61 (89.7)	7 (10.3)				58 (85.3)	10 (14.7)			
At least five *cas* genes	+	12 (100.0)	0 (0.0)	0.21	1.00	-	92 (97.9)	2 (2.1)	1.00	1.00	-	9 (75.0)	3 (25.0)	0.16	0.36	0.08-1.54	7 (58.3)	5 (41.7)	0.01	0.19	0.05-0.69
	–	79 (84.0)	15 (16.0)				12 (100.0)	0 (0.0)				84 (89.4)	10 (10.6)				83 (88.3)	11 (11.7)			

We found that the simultaneous presence of *cas* genes (at least three *cas* genes) might reduce the presence of the -lactamase (bla_{SHV}) gene by up to 50%. (OR: 0.10, CI: 0.03-0.35). Other studies has revealed that the lack of the CRISPR/Cas system may result in the development of drug resistance in *K. pneumoniae* isolates and *E.coli* strains (Aydin *et al*. 2017; Wang *et al*. 2020).

CONCLUSION

The existence of *cas*2 genes was shown to be associated with the presence of bla_{SHV} and bla_{TEM} genes, according to our findings. On the other hand, we discovered a link between the simultaneous existence of the bla_{SHV} and bla_{TEM} genes and the presence of at least three *cas* genes in the same individual. A further finding was that there was a correlation between the simultaneous presence of at least five *cas* genes and the simultaneous presence of beta-lactamase genes, i.e., bla_{TEM}, bla_{SHV}, and bla_{CTX-M} in UPEC isolates. The findings of our study exhibited that the existence of *cas* genes might have a variety of consequences on the presence or absence of beta-lactamase genes. However, we were unable to detect any statistically significant link between the existence of independent *cas* genes and the presence of bla_{CTX-M}. Furthermore, once compared to previous studies, we found a significant level of sensitivity to the antibiotics examined. In general, the inclusion of *cas* genes in the CRISPR-Cas system does not guarantee that beta-lactamase genes will not be present in a given strain. In order to establish a link between the existence of *cas* genes and lower beta-lactamase resistance, further studies are required.

ABBREVIATION

CRISPR: Clustered regularly interspaced short palindromic repeats, **Cas**: CRISPR-associated, **UPEC**: Uropathogenic *Escherichia coli*, **ESBL**: Extended-spectrum beta-lactamases, **EMB**: Eosin methylene blue, **CDT**: Combined disk test, **PCR**: Polymerase chain reaction, **MDR**: Multidrug resistance, **PEP**: Cefepime, **CAZ**: Ceftazidime, **CTX**: Cefotaxime, **CFM**: Cefixime, **CLSI**: Clinical and Laboratory Standards Institute, **MHA**: Mueller-Hinton agar, NCBI: The National Center for Biotechnology Information, **OR**: Odds ratio, **CI**: Confidence interval.

DECLARIATIONS

ETHICAL APPROVED

IR.IAU.TNB.REC.1398.027.

AVAILABILITY OF DATA

The datasets generated and/or analysed during the current study are available in the Neda Merikhi repository; the GenBank accession numbers for the nucleotide sequences are as follows: MT885230 for *cas*1, MW044948 for *cas*2, MT980728 for *cas*3, MT980729 for *cas*7, and MW030688 for *cas*5 gene.

CONFLICT OF INTEREST

The authors declare that they have no competing interests**.**

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AUTHOR'S CONTRIBUTION

J.N. and A.N. conceived of the presented idea. N.M. developed the theory, performed the computations, and was a major contributor in writing the manuscript. M.H. and A.N. performed and verified the analytical methods. R.R.T. collected all samples. All authors discussed the results and contributed to the final manuscript.

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