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Molecular detection of bla_{TEM}, bla_{SHV},and bla_{CTX-M} genes among Uropathogenic Escherichia coli isolated from cases with urinary tract infection in Erbil city-Iraq

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Abstract

Background: Urinary tract infections (UTIs) are the most popular type of diagnosed bacterial illness, and the most frequent cause of bacteria responsible for UTIs is *Escherichia coli* (*E. coli*). β-lactamases are the most frequent resistance for gram-negative bacteria to beta-lactam antibiotics, especially in *E. coli*. The number of patients infected by extended-spectrum β-lactamases (ESBLs) producing *E. coli* was rising and regarded as a significant global health problem.

Objective: evaluate how frequently blaTEM, blaSHV, and blaCTX-M genes were detected in *E. coli* isolated from UTIs.

Patients and Methods: We collected 54 midstream urine samples from patients with symptomatic UTIs, in all age groups, from the outpatient department in Erbil hospitals from October 1, 2021 to April 1, 2022 for the isolation of *E. coli.* All samples were analyzed for the detection of blaTEM, blaSHV, and blaCTX genes using the polymerase chain reaction (PCR) method.

Results: Most of the samples were taken from females (61.11%); according to their ages, they were divided into two groups, and most of the samples (74.07%) were taken from patients below 40 years old. PCR testing for all ESBL-producing *E. coli* isolate samples revealed that 16S rRNA 797 was the most frequently detected gene in all analyzed samples (100%), while it was less frequently detected in blaCTX 585 (48.15%).

Conclusion: Colonization with *S. aureus* and MRSA inversely correlated with younger This found that elevated ESBL genes in *E. coli* isolated from symptomatic UTIs in our community increase the risk of possible resistance.

Keywords: Uropathogenic *Escherichia coli*, Urinary tract infections, *blaTEM*, *blaSHV*, *blaCTX-M*, PCR assay

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Introduction

One of the most prevalent infections caused by pathogenic bacteria is urinary tract infection (UTI), which can lead to major health issues, high costs, increased morbidity,

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and even significant mortality. Except for infancy, UTI affects females more than males. In young females, nearly 90 percent of all UTIs are caused by *E. coli*, a normal intestinal microorganism, and a rod-shaped, gram-negative bacterium. The most important factor in severe UTI cases is virulence factors [1,2]. Numerous microorganisms are causative of UTIs; bacteria are the major related causes. The most popular bacteria are E. coli, responsible for up to 80% of infections [3]. Still, UTIs are a general severe health concern, especially in underdeveloped nations, as reported; in most UTI cases, the causative organism could be *E. coli*, primarily caused by a group of bacteria known as Uropathogenic Escherichia coli (UPEC), which accounts for about 90% of community-acquired UTIs [4]. UPEC is one of the strains of E. coli that frequently and strongly invade the urinary tract system, a specific factor that empowers bacteria to remain alive. UPEC pathogenesis has been documented and is regulated by Virulence Factors (VFs) diversity products [5].

Antibacterial resistance development is one of the primary issues with antibiotic usage [6]. Many reasons lead to increased interest in novel, non-antibiotic-based ways of limiting and managing UTIs, for example, high antibiotic drug costs, increasing antibiotic resistance, and deficient therapeutic alternative options [7].

Although a wide range of antibiotics are utilized globally, β-lactams are the most commonly accepted antibiotics because of their minimal toxicity and complications. And they are regarded as a treatment for about 50% of the total antibiotics in use worldwide. One of the essential resistance processes for β-lactam agents is the production of β-lactamases by many gram-positive and gram-negative bacteria [8].

Globally, *E. coli* was mentioned as the leading source of general health problems among all ESBL-producing uropathogenic bacteria. Although ESBL genes mainly originate from blaSHV or blaTEM cases, in 1998, the frequency of bl;aCTX-M forms was raised critically in most areas worldwide [9].

Most ESBL-producing bacteria are *E. coli*, mainly found in urine samples. In the case of infection produced by ESBL-producing *E. coli*, the patients are in danger of treatment failure or death because of postponement and the wrong drug [10]. The main hazard factors for the rise of antibiotic resistance include overtreatment and incorrect empiric therapy, in addition to the high prevalence of UTIs and under-treatment of the infection, which also causes serious complications [11].

In this study, the purposes are: to demonstrate the frequency of ESBL-producing *E. coli* detected from urine specimens of symptomatic UTIs cases to expose their vulnerability to the antibacterial widely utilized for UTIs therapy; to identify the popularity of *blaTEM* (temoneira β-lactamase), *blaSHV* (sulfhydryl variable β-lactamase); and *blaCTX_M* (cefotaximase β-lactamase) genes. Those help detect markers of drug resistance that microbiology laboratories can diagnose to decrease complications caused by inappropriate treatments used by patients.

Patients and Methods

Collection of samples

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We prospectively analyzed and recorded 54 samples of urine taken from cases ages 1-65 years old with symptomatic UTIs; samples were collected from the outpatient department in Erbil hospitals (Erbil Teaching Hospital, Rzgari Teaching Hospital, Raparin Hospital, and Bio center in Erbil city) during the period from October 1, 2021 to April 1, 2022 for isolation of *E. coli.* After we get the official permission, data will be collected, and further details will be included.

Extraction of bacterial DNA

Clinic Cell SV small kit (Songpa-gu, Seoul, Korea) has been used for Genomic DNA extraction from pure cultures through the GeneAll® ExgeneTM kit. It is sufficient to grow bacterial cells by incubating the culture sample at 37°C for 12-24 hrs. and with dynamic shaking until the cells reach the log phase. Then, bacterial cells will likely be prepared to be utilized directly or stored at -20°C or -80°C for subsequent utilization.

Estimation of Extracted DNA

Before the PCR run, agarose gel electrophoresis was used to evaluate the extracted genomic DNA in E. coli. 1.5% agarose gel was used and ran on 85V for 45 min [12].

Preparation of primers for PCR

Required from Macrogen (Korea), the primers mentioned in Table (1) and utilized in this study were produced by adding the suggested volume of free nuclease water indicated in the datasheet to lyophilized primers to make 100 µM (stock solution). As an appropriate solution for the PCR process, a ten µM concentration was then created. Every primer aliquot was stored at -20°C.

Amplification of DNA

It's an enhanced ready to utilize 2× PCR mixture of Taq DNA polymerase, gel-loading dyes, deoxynucleotide triphosphates, PCR buffer, and a novel green dye that generates a fluorescent stain that could be seen right away after the DNA electrophoresis utilizing a blue-light transilluminator or ultraviolet light. With the exclusion of the primers and DNA templates, the Master Mix includes all components necessary for PCR.

Table (1): The four primers were utilized in this study

Target genes	The sequence of the primers (5' to 3')	Size of the product by base pairs
16SrRNA	F- AGT TTG ATC MTG GCT CAG R- GGA CTA CHA GGG TAT CTA AT	797bp
blaTEM	F- ATGAGTATTCAACATTTCCGTGT R- TTACCAATGCTTAATCAGTGAGG	861bp
bla$_{CTX-M}$	F- AACGCACAGACGCTCTACC R- GGGTAGCCCAGCCTGAAT	517bp
blaSHV	F- TCGTTATGCGTTATATTCGCC R- GGTTAGCGTTGCCAGTGCT	868bp

PCR amplification of genes

Table (2) shows the amplicon size and PCR conditions for every gene under analysis. The 16S rRNA gene was amplified by utilizing DNA in the thermal cycler at 94°C for 5 min. to completely denaturize the DNA templates before being used to identify *E. coli.* The following program was then used to continue the PCR: 30 sec. at 94°C, annealing at 55°C for 1 min. and 72°C for 1 min., and 35 cycles of these segments were repeated, an end extension of 10 min. at 72°C. Eventually, PCR tubes were kept at -20°C until the additional analysis [13].

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Detection of ESBL genes of *E. coli*

Three ESBL genes (blaTEM, blaSHV, and blaCTX-M) were among those that PCR detected during the screening of all isolates used in this study, and the PCR programs for all three of these genes are described in Table (2). The PCR products of all genes were visualized using 1.2% agarose stained with Red Safe dye under transilluminator UV light [14].

Table (2): Amplification processes of ESBL genes in E. coli used in the PCR program

Genes	Stages						
	No. of cycles	Denaturation		Annealing		Extension	
		Temperature	Time	Temperature	Time	Temperature	Time
bla_{TEM}	35	94°C	30 sec.	50°C	30 sec.	72°C	40 sec
bla_{SHV}	40	95°C	30 sec.	50°C	30 sec.	72°C	45 sec
bla_{CTX-M}	40	95°C	30 sec.	58°C	30 sec.	72°C	45 sec

Statistical Analysis

The chi-square exact test was utilized to analyze the consequences of our work, with a p-value of <0.05 measured as substantial differences.

Results

The total number of analyzed samples was 54, taken from patients who attended the laboratory with symptomatic UTIs. The mean age of cases was (29.86), ranging from 1 to 68 years old. Most of the samples were taken from females (61.11%), and 21 (35.2%) specimens were collected from male cases. Depending on the age of the patients, 40 (74.07%) samples are below 40 years old, and the rest 14 (29.6%) are above 40 years old, as shown in Table (3).

Table (3): The age and gender distribution of the positive E. coli patients were approved by PCR assay in the present study

	Number (n)	Percentage %
Gender		
Female	33	61.11
Male	21	38.89
Age (years)		
<40	40	74.07
≥40	14	25.93

Regarding distribution according to age and gender, most of the female cases, 28 (70%), are in the group aged <40 years, and inpatients aged ≥ 40, most of them are males 9 (64.29%), which is more detailed in Figure (1).

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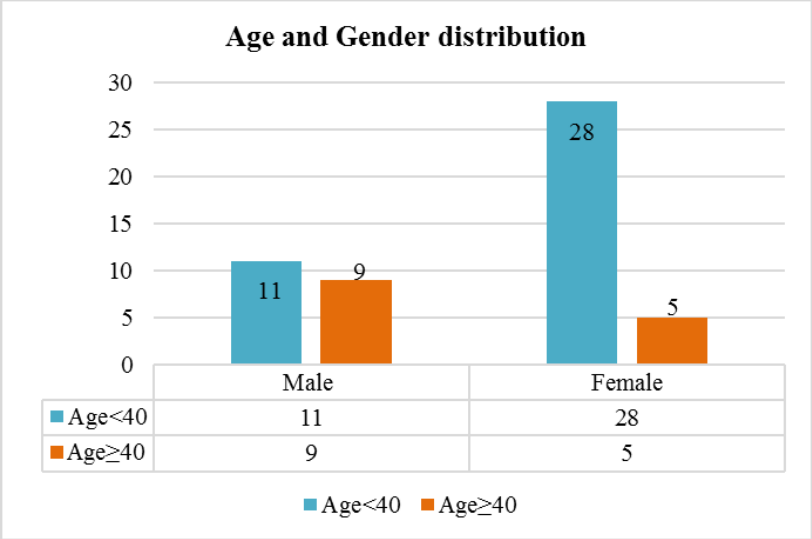

Figure (1): Distribution of data according to age and gender

Regarding the result of the PCR test using 16S rRNA 797 for detecting the *E. coli* genome, 54 samples showed 100% positivity, as shown in Table (4), which also revealed 48.15 % positivity and 51.8 % negativity for blaCTX 585. As well, 77.7% of the sample is positive, and 22.3 % is negative for the blaTEM 861 gene. And showed 75.9% of the sample as positive and 24.6 % as negative for the blaSHV 686 gene, as shown in Table (4).

Table (4): The estimation of amplification genes for diagnostic *E. coli*

Name of gene	16S rRNA 797	*bla*CTX 585	*bla*TEM 861	*bla*SHV 686
Positive	54 (100%)	26 (48.15%)	42 (77.7%)	41 (75.9%)
Negative	0 (0%)	28 (51.8%)	12 (22.3%)	13 (24.6%)
p-value	< 0.0001 (N.S)			

Regarding the results of the PCR test using 16S rRNA for detecting the presence of the *E. coli* genome among 54 urine samples, 54 (100%) as positive, as revealed in Figure (2).

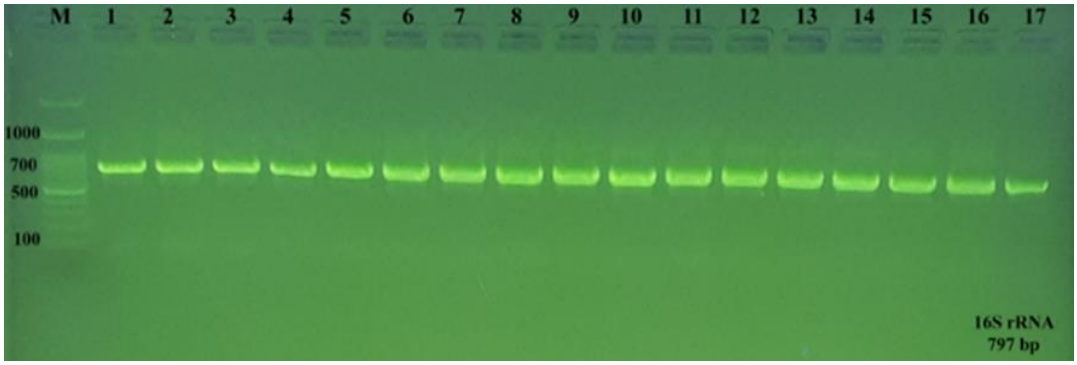

Figure (2): Electrophoresis picture of 16S rRNA gene amplification (797 bps) for molecular detection of E. coli isolates from clinical samples. M: DNA ladder (100 bp), 1-17: positive 16S rRNA gene samples

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Results of a PCR test using a specific primer to detect the blaSHV gene among 54 positives *E. coli* showed 41 (75.4%) positive, as shown in Figure (3). PCR test for the blaTEM gene showed 42 (77.7%) positive results, as shown in Figure (4). And finally, the PCR test for the blaCTX gene showed 26 (48.15%) positive results, as shown in Figure (5).

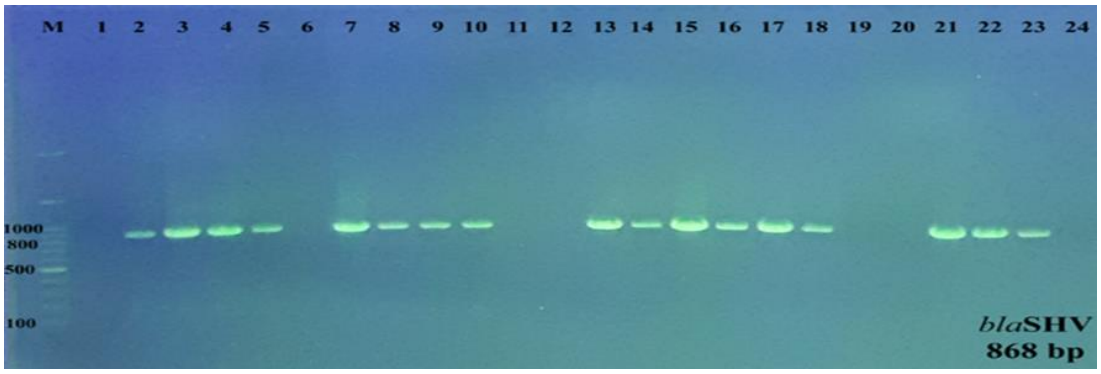

Figure (3): *E. coli* isolates blaSHV gene amplified using PCR test, amplified product (868 bps) of E. coli isolates represented by lanes (2, 3, 4, 5, 7, 8, 9, 10, 13, 14, 15, 16, 17, 21, 22, 23), while negative for blaSHV gene were represented in following lines (1, 6, 11, 12, 19, 20, 24), M: ladder 100 bp

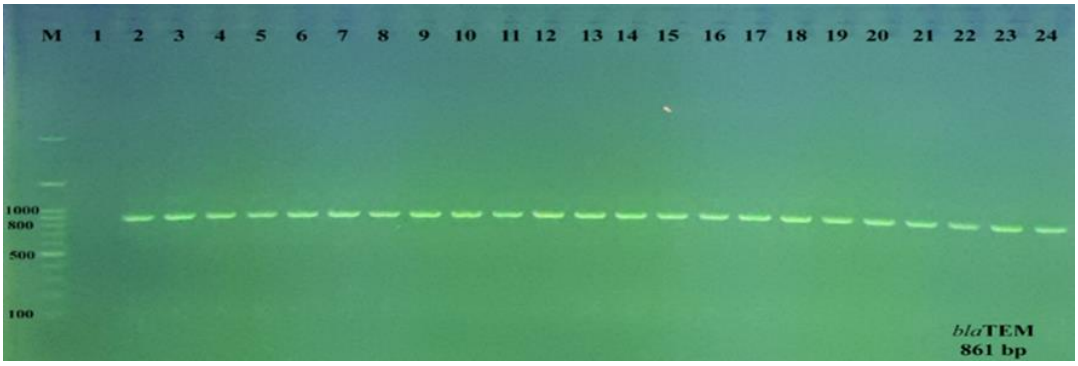

Figure (4): PCR amplification of bla_{TEM} gene of *E. coli* isolates, amplified product (861 bps) of *E. coli* isolates represented by lanes from 2 to 24, while lane 1 was negative for bla_{TEM} gene. *M*: ladder 100 bp

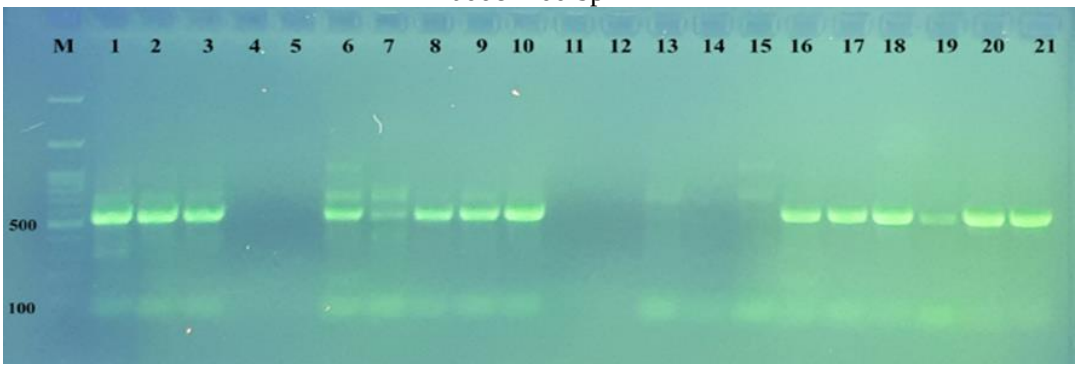

Figure (5): PCR amplification of blaCTX gene of E. coli isolates, amplified product (517 bps) of E. coli isolates represented by lanes (1, 2, 3, 6, 7, 8, 9, 10, 16, 17, 18, 19, 20 21), while other lines (4, 5, 11, 12, 13, 14, 15) represent negative for blaCTX gene, M: ladder 100 bp

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Discussion

In the present study, 54 patients were included, and all 54 samples showed positive results for *E. coli* in UTIs. According to prior studies, *E. coli* was the most significant reason for UTIs [15]. Regarding the gender of the patients as shown in Figure (6), 21 (38.89%) of the cases were males, and 33 (61.11%) were females, which is a statistically not significant difference (p=0.56) that is comparable to what has been reported in previous studies [16,17,18]. It is most likely due to the proximity of the female urethra to the anus, and the shorter female urethra [16, 17,18]. In our analysis, the most common age was found in patients younger than 40 years, similar to previous studies [18, 19].

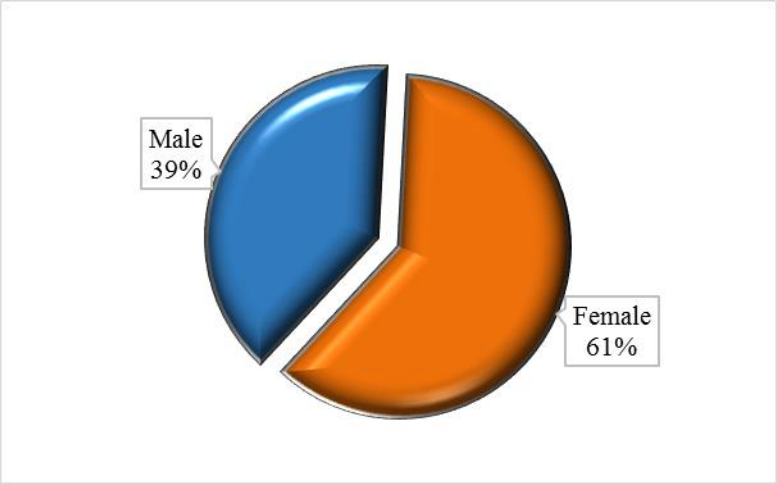

Figure (6): Female to male distribution in the current study

Regarding gene estimation, we reported in our study that blaSHV was isolated in 41 (75.9%) of the UPEC isolates utilizing the boiling process. This is comparable to Pakzad *et al.* (2011), who showed that 95.2 percent of E. coli isolates included the blaSHV gene. Still, Pongpech *et al.* (2008) said that the blaSHV gene was identified in just 8 percent of the provided ESBL-producing *E. coli* isolates [20].

In the current investigation, we mentioned that result of the *blaTEM* gene was 42 (77.7%) of the UPEC isolates, which is more than the outcome of Almohana (2013), which revealed the *blaTEM* gene was (57.1%) of *E. coli* isolates. The prevalence rate of *blaSHV* and *blaTEM* variation in this research likened to prior work might be increased for many explanations, including the variation in the category and number of antimicrobial agents taken and the variation in the period throughout which the isolates were obtained [21]. Chaudhuri *et al.* (2011) mentioned that the blaCTX-M gene was identified in 63.5% of ESBL isolates, which was lower than the result of the *blaCTX-M* gene 26 (48.15%) in the current study. *blaCTX-M* is the most frequent type of *blaCTX-M* from Asia, Europe, and North and South America among multidrug-resistant *E. coli* [22].

Molecular characteristics of the β-lactamase genes could be necessary for the dependable epidemiological examination of antibiotic resistance [23]. It was reported from various investigations that the prominent ESBL gene was different. Prior

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studies revealed that the most standard types of ESBL genes are *blaSHV*, *blaTEM*, and *blaCTX*. While the *blaCTX* and *blaSHV* categories were the most frequent kinds of β-lactamase genes over the previous decade, the *blaCTX* kind has been more globally spread compared to the *blaSHV* and *blaTEM* genotypes [24]. In general, blaCTX was frequent in a variety of regions; multiple outcomes were obtained from Iran (74%) [25] Morocco, North Africa (70%) [26], and India (93.7%). Iran had the highest prevalence rate, followed by Morocco, North Africa, and India [26]. In addition, the present study demonstrated that the *blaTEM* type β-lactamase gene was the most prevalent ESBL gene in UPEC, which is consistent with the findings of several previously published studies (27-30). In Italy (45.4%)[31], Portugal (40.9%) [32], and Turkey (72.7%), the blaTEM type β-lactamase gene was the most prevalent [33].

Conclusions

The results suggest that most of the samples obtained from the cases of UTI were under 40 years of age, with the predominant sex being female. Regarding the outcome of the PCR test among the four tested genes (16S rRNA, *blaCTX*, *blaTEM*, and *blaSHV* genes), the 16S rRNA gene has been detected 100% in accurate tests, while the least detected gene is blaCTX (48.15%). It means that ESBL-producing uropathogenic *E. coli* is an increasing health issue with an increased risk of global dissemination.

Recommendations

To understand the evolution of multiple antibiotic resistances in Uropathogenic *E. coli*, more study on the multiplicity of antibiotic resistance is necessary. Also, future research should advocate phenotypic or genotypic testing to identify ESBL-producing isolates in laboratories in order to choose the best antibiotics to treat UTIs due to the high frequency of ESBL-producing Uropathogenic *E. coli* (UPEC) in the examined location.

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Ethical clearance: Ethical approval was obtained from the College of Medicine / University of Diyala ethical committee for this study.

Conflict of interest: Nil

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الكشف الجزيئي عن جينات bla_{TEM} وbla_{SHV} و bla_{CTX-M} بين التهاب البولية لبكتريا القولون Escherichia coli المعزولة من حالات عدوى المسالك البولية في أربيل - العراق

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الملخص

خلفية الدراسة: عدوى المسالك البولية هي أكثر أنواع الأمراض البكتيرية التي يتم تشخيصها شيوعًا ، والبكتيريا الأكثر شيوعًا المسؤولة عن عدوى المسالك البولية هي الإشريكية القولونية (E. coli). β-Lactamases هي أكثر أنواع البكتيريا سالبة الغرام مقاومة للمضادات الحيوية بيتا لاكتام ، خاصة في الإشريكية القولونية. ارتفع عدد المرضى المصابين بـ β-lactamases (ESBLs) المنتجة للإشريكية القولونية واعتبرت مشكلة صحية عالمية كبيرة.

اهداف الدراسة: لتقييم مدى تكرار اكتشاف جينات bla_{TEM} و bla_{SHV} و bla_{CTX-M} في الإشريكية القولونية المعزولة من عدوى المسالك البولية.

المرضى والطرائق: جمعنا 54 عينة في منتصف البول من مرضى يعانون من أعراض التهاب المسالك البولية ، من جميع الفئات العمرية ، في أقسام العيادات الخارجية في مستشفيات أربيل من 1 أكتوبر 2021 إلى 1 أبريل 2022 لعزل الإشريكية القولونية. تم تحليل جميع العينات للكشف عن جينات bla_{TEM} و bla_{SHV} و bla_{CTX} باستخدام طريقة تفاعل البلمرة المتسلسل (PCR).

النتائج: تم تجنيد معظمهم من الإناث (61.11٪)؛ تم تقسيمهم إلى مجموعتين حسب أعمارهم ومعظم العينات (74.07٪) كانت من مرضى تقل أعمارهم عن 40 سنة. كشف اختبار PCR لجميع عينات E. coli المنتجة لـ ESBL أن الجين S rRNA 16 797 هو الجين الأكثر اكتشافًا في جميع العينات التي تم تحليلها (100٪) ، بينما كان أقل تواترًا في blaCTX 585 (48.15٪).

الاستنتاجات: أظهر هذا أن زيادة تنظيم جينات ESBL في E. coli المعزولة من التهاب المسالك البولية المصحوب بأعراض في بيئتنا تزيد من خطر المقاومة المحتملة.

الكلمات المفتاحية: الإشريكية القولونية , التهابات المسالك البولية, bla_{CTX-M}, bla_{SHV}, bla_{TEM}, فحص PCR

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