Original Article

Molecular Detection of *blaTEM*, *blaSHV*, and *blaCTX-M* among *Escherichia coli* Isolated from Urinary Tract Infection Patients in Babylon Province

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Abstract

Background: The antimicrobial resistant bacteria, *Escherichia coli*, is a significant health care problem worldwide. **Objectives:** Current study's objective is to assess phenotypically and molecularly the characteristics of carbapenem-resistant genes among *E. coli* isolates in Al-Hilla city. **Materials and Methods:** During six-month study, 320 (49.5%) isolates were identified with *E. coli* that were obtained from 646 midstream urine specimens. Carbapenems resistance gene detected phenotypically that confirmed by modified Hodge test. Kirby-Bauer disk diffusion method was employed to evaluate 11 antimicrobial compounds for antibiotic susceptibility. The prevalence of *E. coli* expressing the Extended-spectrum β-lactamases was assessed by polymerase chain reaction for the following genes: bla_{TEM}, bla_{SHV}, and bla_{CTX-M}. **Results:** Only 320 (49.5%) of 646 clinical isolates were identified as *E. coli*. All isolates were resistant to amoxicillin (279) 87.1% and ampicillin (243) 75.9%, and the majority of these isolates were sensitive to meropenem (255) 79.6% and imipenem (209) 65.3%. Only 20 isolates were resistant to carbapenems antibiotics based on phonotypic detections methods, whereas genotypic results revealed positive for special primers including the bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes. **Conclusions:** Through our research, it was determined that women suffer from higher rates of infections of the urinary tract than men undertake. Our research also revealed that whereas the majority of the isolates were sensitive to the antibiotics meropenem and imipenem, the majority of the isolates were also resistant to the antibiotics amoxicillin and ampicillin. Only twenty *E. coli* isolates have been found that are resistant to carbapenem antibiotics, according to antibiotic sensitivity tests. However, genetic detections revealed that these isolates have high prevalence of the genes bla_{CTX-M}, bla_{TEM}, and bla_{SHV}, respectively.

Keywords: Antibiotics susceptibility testing, conventional PCR, *Escherichia coli*, modified Hodge test

Introduction

The public health risks that the world faces include the presence of intestinal hosts of various types of Carbapenem-resistant Enterobacteriaceae (CRE) which poses serious risks to the general population.[1] Antimicrobial of carbapenem features beta-lactam ring with a connected hydroxyl-subunits, which is essential in the treatment of Gram -ve bacteria (GNB) infections.[2] Antimicrobial of carbapenems possess wide spectrum activity and shows best effectiveness toward all gram-negative and gram-positive microorganisms.[2] These medications are only used as a last option for people who are very unwell or are suspected of having ESBL and AmpC-resistant bacteria.[3] Antimicrobial carbapenem-resistant Enterobacteriaceae is documented in an increasing number of nations in recent years.[4,5]

Basic carbapenem-resistant mechanisms are as follows: (1) reduced permeable; (2) exporter channels; (3) modify penicillin-binding protein; and (4) β-lactamase synthesis.[6] Additionally, in 2017 the World Health Organization (WHO) classified carbapenem-resistant

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Enterobacteriaceae as an emergency disease for greater study and the advancement of innovative therapies.[7]

Bacteria from the Enterobacteriaceae family are the most prevalent cause of an acquired through community involvement infection of the urinary tract.[8] A infection of the urinary tract is regarded as one of the significantly prevalent sicknesses that are infectious, furthermore to having an upper respiratory infection.[9]

Terms of promoting impedance gene horizontal transmission.[10] Bacterial carbapenemase-related impedance genes increased as a result of nonstandard antibiotic prescriptions and abuse, particularly in countries with limited resources.[11] It is essential to analyze carbapenem resistance trends using traditional laboratory techniques and to develop a comprehensive antibiotic management and control program because carbapenem-resistant organisms play a critical part in the spread of healthcare- and public-related diseases. Carbapenem-resistant Enterobacteriaceae (CRE) are intestinal bacteria that are resistant to at least one carbapenem antibiotic, such as ertapenem, doripenem, meropenem, or imipenem, and/or have a carbapenemase gene, according to the Centers for Disease Control and Prevention.[12]

During the past few years, hospital acquired infections have become a major issue in critical care units. Human medicine has advanced as a result of unneeded and long-term antibiotic therapy and extended hospitalization. This might have a substantial impact on death rates and treatment results in the case of immunocompromised individuals having surgery.[13] Meanwhile, because of increased antibiotic resistance, carbapenems have emerged as the most essential therapeutic option for Gram-negative bacilli (GNB) infections.[14]

The global appearance and collective prevalence of antimicrobial resistant to β-lactam antimicrobial within members of the *Pseudomonas aeruginosa*, *Acinetobacter baumannii*, and Enterobacteriaceae families, are regarded a severe medical and public health concern.[15] For infection prevention and control, monitoring, and epidemiological research, it is essential that genes of carbapenemase in gram-negative bacteria are discovered as soon as possible. It would also have a significant impact on choosing the optimal course of treatment for individuals who are severely ill.[16] Various diagnostic approaches based on carbapenem-hydrolyzing activity tests have been discovered.[17] The aim of the study is to investigate the phenotypic and genetic characteristics of *Escherichia coli* bacteria that isolated from urinary tract infections patients by using the specifics primers including bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes.

Materials and Methods

During a period of 6 months, from February to the end of July 2022, a cross-sectional survey was conducted at three hospitals in the Babylon province: Marjan Teaching Hospital, Alamam Al-Sadeq Hospital, and Al-Hilla General Teaching Hospital.

Midstream urine samples taken from those with urinary tract infections (UTIs) comprise both men and women of various ages.

Bacterial identification and antibiotic susceptibility testing

The earliest methods used to identify *E. coli* isolates were morphological features on MacConkey and Eosin Methylene Blue agar, as well as biochemical assays based on Clinical & Laboratory Standards Institute (CLSI) 2022 recommendations.[18] The Vitek-2 method (BioMerieux, Marcy d'Etoile, France) was then used to validate the *E. coli* identification. The antibiotic susceptibility test (AST) GN76 kit, Kirby-Bauer disk diffusion techniques, and Vitek-2 system (all from BioMerieux) were used for the antibiotic susceptibility testing, and the results were interpreted in accordance with CLSI standards.[18]

Antibiotic susceptibility testing was performed on Mueller Hinton agar (bioMerieux), using overnight cultures at a 0.5 McFarland standard followed by incubation at 35°C for 16–18 h. The antimicrobial tests include: gentamicin, trimethoprim, nitrofurantoin, ciprofloxacin, ceftriaxone, cefepime, imipenem, meropenem, amoxicillin, ampicillin, and piperacillin [Table 2].

Phenotypic testing modified Hodge test

All 20 carbapenem-resistant *E. coli* isolates were tested by using modified Hodge test (MHT) for phenotypic confirmation test. The conventional suspending (*E. coli* ATCC 25,922 at a 0.5 McFarland density) has been diluted 1:10 in a sterile solution of saline. Following then, the bacteria were inoculated on Mueller-Hinton agar plates that had been treated with zinc sulfate. For each plate, colonies were streaked out from the edge of a meropenem disk (10 mg) toward the plate boundary. Indentation of the inhibitory zone(s) after overnight incubation revealed that the test strain attacked carbapenems.

Extraction of DNA

Only one colony from each isolates was suspended in 400 µL sterile DW and heated for 10-15 min at 98-100°C in a heating block (Eppendorf, Germany). The lysates were then centrifuged (13,000×*g*, 2 min), and the supernatants were transferred to a new Eppendorf tube and stored at -20°C as DNA templates for the subsequent steps.[19]

DNA extraction and polymerase chain reaction protocol

PCR detection of ESBLs genes among carbapenems resistance E. coli isolates

Particularly bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes are used to detect of Extended-spectrum β-lactamases (ESBLs) genes among carbapenems resistance *E. coli* isolates via polymerase chain reaction (PCR) testing.

Table 1: Primers that involve of this research

No.	Primer ID	Sequences from 5′ to 3′	Production size (bp)	References
1-	CTX-M	Forward: 5′-CAGAGATTTTGCCGTCTAAG-3′R	(946) bp	[20]
		Reverse: 3′-GGCCCATGGTTAAAAAATCACTGC-5′		
2-	TEM	Forward: 5′-TCGGGGAAATGTGCG -3′R	(971) bp	[20]
		Reverse: 3′-TGCTTAATCAGTGAGGCACC-5′		
3-	SHV	Forward: 5′- GCCTTTATCGGCCTTCACTCAAG-3′R	(898) bp	[20]
		Reverse: 3′-TTAGCGTTGCCAGTGCTCGATCA-5′		

The PCR was then carried out using the oligonucleotide primers listed in Table 1. Amplify the interesting genes by using a conventional PCR reaction of 20 µL of pre-mixed PCR reagents, 1 µL of each primer, and 2 µL of DNA template was employed.

This was done in a total volume of 20 L. DNA from *E. coli* was amplified using forward and reverse primers for the bla_{CTX-M}, bla_{TEM}, and bla_{SHV} genes, respectively.

In the PCR procedure, the following parameters were used: The substance was first incubated at 95ºC for 15 min.

PCR protocol

The PCR conditions were set up in 25 µL monoplex maximum combination of reactions volume and 50 µL multiplex maximum mixture of reactions amount, with 25 µL containing 12.5 µL of Taq DNA Polymerase Master Mix green (Promega, UK), primers, 2 µL (10 pmol/L) from each with 6 µL (50 ng) sterile deionized water to produce a final volume of 25 µL. A thermocycler (Bio-Rad C1000 Touch) was used to do the amplifications.

The results of the PCR were separated on 1.5% agarose gel electrophoresis and observed with UV transillumination A 1500. Table 1 summarizes the primers used, the size of the product, and the search for PCR conditions used to identify genes.

Ethical considerations

Before beginning sample collection for this study, the project received preliminary permission. Furthermore, the Scientific Research Methodology Ethics Committee of Babylon University's Scientific College, Department of Biology, and Microbiology Branch approved the study's design. In February 21, 2022, reference No.7/17/1336.

Results

The three main medical centers in Babylon province, Marjan Teaching Hospital, Al-Hilla General Teaching Hospital, and Alamam Al-Sadeq Hospital, contributed a total of 646 clinical samples over the course of the 6-month period.

The numbers of samples from each hospital were 230 (35.6%), 170 (26.3%), and 246 (38.1%), respectively. 646 urine samples from patients suspected of having UTIs were among the clinical sample types gathered throughout the research period.

Table 2: Antibiotic susceptibility test (AST) among *E. coli* isolates

No.	Antibiotics	Sample	Concentration	Resistance No. (%)
1	Amoxillin	AMC	20/10 mg	279 (87.1%)
2	Ampicillin	AMP	10 mg	243 (75.9%)
3	Gentamycin	GM	10 mg	214 (66.8%)
4	Trimethiprim	STX	1.25/23.75 mg	159 (49.6%)
5	Nitrofurantion	NIT	300 mg	138 (43.1%)
6	Ciprofloxacine	Cip	5 mg	110 (34.3%)
7	Ceftriaxone	CRO	30 mg	103 (32.1%)
8	Piperacillin	PI	100 mg	98 (30.6%)
9	Cefepime	CPT	30 mg	85 (26.5%)
10	Meropenem-	ME	20/10 mg	20 (6.2%)
11	Imipenem	IM	10/25 mg	20 (6.2%)

The rate of UTI among female patients was more than that of male patients female patients were having shorter urethras than men so that the majority of these isolates caused female UTIs. The investigation revealed that only 320 (49.5%) out of 646 isolates were determent *E. coli* isolates, and the results of this study showed 47 (14.7%) males and 273 (85.3%) female patients.

Antibiotic susceptibility test and the modified Hodge test

The disk diffusion AST findings for all isolates (n = 320) demonstrated resistance to the following antibiotics. Amoxicillin 279/320 = 87.1%, Ampicillin 243/320 = 75.9%, Gentamycin 214/320 = 66.8%, Trimethoprim 159/320 = 49.6%, Nitrofurantoin 138/320 = 43.1%, Ciprofloxacin 110/320 = 34.3%, Ceftriaxone 103/320 = 32.1%, Piperacillin 98/320 = 30.6%, Cefepime 85/320 = 26.5%. As shown in Table 2, only 20 isolates were resistance carbapenem antibiotics including (meropenem and imipenem).

E. coli isolates were screened for the production of β-lactamase by Imipenems (MHT). The results revealed that only 20 (6.2%) *E. coli* isolates were positive for the MHT.

Molecular identification ESLBs genes among carbapenems resistance *E. coli* isolates

PCR approach was applied on a total of twenty resistant of carbapenem *E. coli* isolates to explore molecular pattern of resistant carbapenem genes.

Table 3: Detection of ESBLs genes in 20 carbapenems resistant *E. coli* isolates

No.	Primer ID	Positive isolates	No. positive isolates	Applicant size (bp)	Denaturation	Annealing
	CTX-M	All 20 isolates except isolate No. 16	19/20	946 bp	94ºC	56ºC
2	TEM	1, 2, 3, 4, 5, 6, 7, 9, 11, 12, 14, 15, 16, 17, 18 and 19	16/20	971 bp	95ºC	56ºC
3	SHV	3, 9, 11, 14, and 18	5\20	898 bp	94ºC	55ºC

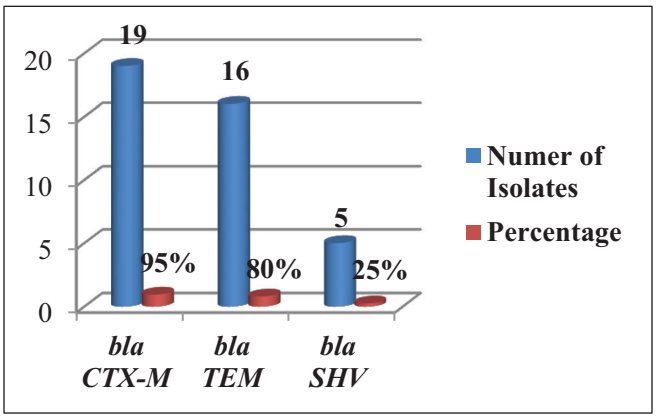

Figure 1: Distribution ESBLs genes among carbapenems resistance *E. coli* isolates

Primers utilized during our study including: bla_{CTX-M}, $bla_{TEM,}$ and bla_{SHV} [Table 1]. Molecular detections revealed significant distributions of bla_{CTX-M} was 19 (95%) genes, followed by bla_{TEM} was 16 (80%) genes, and finally less distributions of bla_{SHV} was 5 (25%) genes among 20 carbapenem resistance *E. coli* isolates. Figure 1 depicts the gene distributions among 20 *E. coli* isolates.

PCR results

The detection of *blaCTX-M*, *blaTME*, and *blaSHV* genes were effectively amplified using the primers stated in Table 3.

PCR results observed a band of *blaCTX-M* (946 bp) had extremely significant distributions across 19 (95%) isolates, as illustrated in Figure 2.

PCR results showed effectively amplify *blaTME*, with a band (946 bp) and extremely significant distributions across 16 (80%) isolates when compared to the standard ladder, as shown in Figure 3.

Finally PCR results showed less effectively amplify of *blaSHV* (946 bp) with fewer significant distributions among 5 (25%) isolates as shown in Figure 4.

ESBLs genes distribution among 20 carbapenems resistant *E. coli* isolates according to Ambler classification

Molecular results revealed following distribution Ampc β-Lactmase genes among 20 carbapenems resistance *E. coli* isolates: Eco(1, 2, 4, 5, 6, 7, 12, 15, 17, and 19) were harbored two different genes encoding for the following enzymes: $bla_{CTX-M-1}$ and bla_{TME}, both of which belong to class A.

Eco (3, 9, 11, 14, and 18) harbored three different genes encoding for the following enzymes: $bla_{CTX-M-1}$, bla_{TME}, and bla_{SHV}, and all of which belong to class A.

Eco (8, 10, 13, and 20) only have one gene encoding the class A enzyme $bla_{CTX-M-1}$, whereas bla_{TME} and bla_{SHV} were not discovered by PCR, Eco (16) was found to have just one gene encoding for the enzyme bla_{TME}, which belongs to the class A [Table 4].

Discussion

According to research conducted by international health organizations, most microorganisms, particularly resistant of antimicrobial, represent a serious risk to worldwide public health.[1,21] According to bacterial classification, *E. coli* is considered a part of Enterobacteriaceae family and may be transferred to people in a variety of ways.[22] *E. coli* is responsible for approximately 80% of all UTI worldwide.[23] Recent research aimed to determent morphological and genetic features of resistance carbapenem among *E. coli* obtained UTI patients in Babel Governorate, Iraq. Taking into account the biological and chemical tests, only 320 of 646 isolates were identified as *E. coli*, including 273 (85.3%) female isolates and 47 (14.7%) male isolates that had significant multi drugs resistance.

Only 20 carbapenem-resistant isolates were examined using disc-diffusion and broth microdilution methods. However, all 20 *E. coli* isolates were positive for MHT in our study. Several research studies performed to establish the global frequency resistance to carbapenem phenotypically. Ripabelli *et al.*[24] published their result in 2018. During 2019, Murugan *et al.*[25] found only 29.03% carbapenem-resistant *E. coli* isolates in India. Sahu *et al.*[26] (India) observed *E. coli* isolates obtained from urine showed imipenem resistance results. Sudanese researchers Mahmoud *et al.*[27] observed about 33% of *E. coli* isolates showed resistance results towards imipenem antibiotics. Murugan *et al.*[28] observed 29% *E. coli* strains mostly showing resistance to one carbapenem antibiotic at least. Resistance to the antibiotics investigated was found to be 23.3%, 2.1%, and 1.4% for meropenem, imipenem, and ertapenem, respectively. Gurung *et al.*[29] reported in 2020 *E. coli* strains comprised 28.6% resistance toward carbapenem antibiotics.

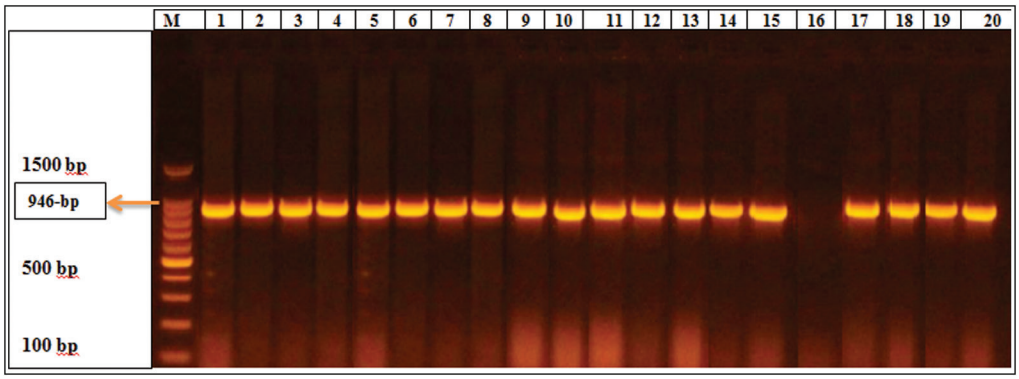

Figure 2: Agarose gel electrophoresis of $bla_{CTX-M-1}$ specific PCR product for 946 bp 1.5% agarose gel at 90 V for 60 min in 1× TBE buffer, observed under transilluminator UV after staining with red safe. Lane L: 100 bp DNA ladder. All of the lines (1, 2, 3, 4, 5, 6, 7, 8, 9, 10, 11, 12, 13, 14, 15, 17, 18, 19, and 20) provide a good result (946-bp fragment). Line (16) represents a negative outcome, whereas line (M) represents a marker (1500-100) base pair

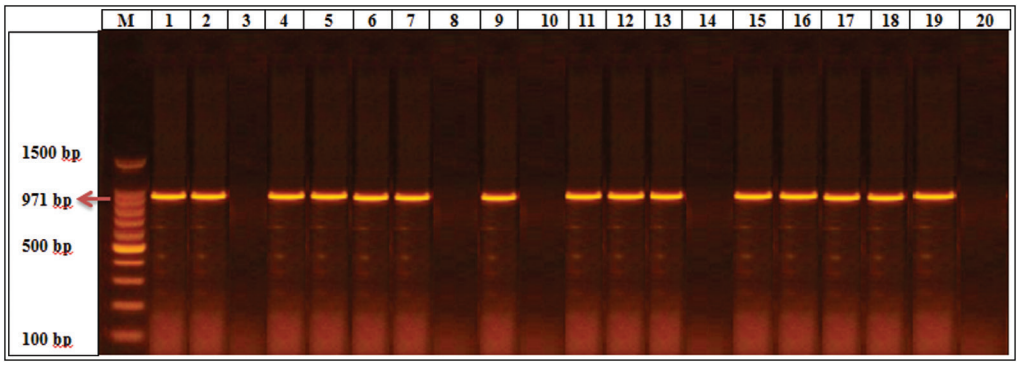

Figure 3: Agarose gel electrophoresis of bla_{TEM} specific PCR product for 971 bp. 1.5% agarose gel at 90 V for 60 min in 1× TBE buffer, observed under transilluminator UV after staining with red safe. Lane L: 100 bp DNA ladder. All of the lines (1, 2, 4, 5, 6, 7, 9, 11, 12, 13, 15, 16, 17, 18, and 19) provide a good result (971-bp fragment). Lines (3, 8, 10, 14, and 20) yield negative findings, whereas line (M) represents a marker (1500-100) base pair

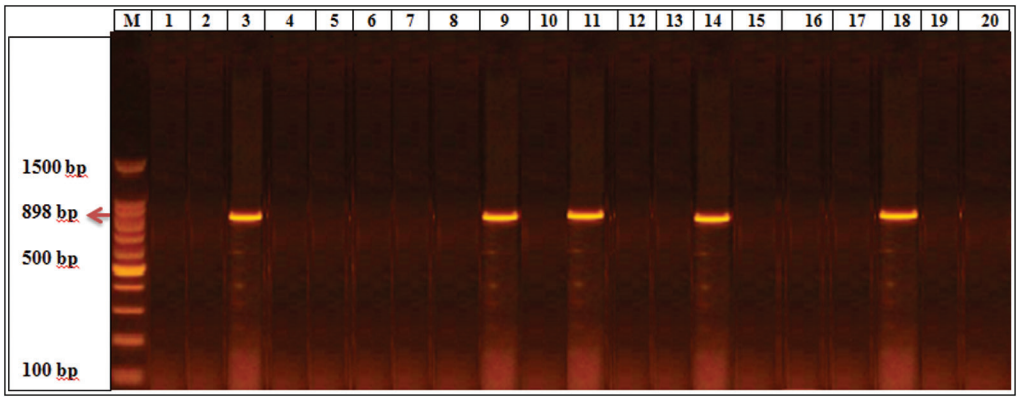

Figure 4: Agarose gel electrophoresis of bla_{SHV} specific PCR product for 898 bp using 1.5% agarose gel at 90 V for 60 min in 1× TBE buffer, and visualized under transilluminator UV after staining with red safe. Lane L is a 100 bp DNA ladder. All of the lines (3, 9, 11, 14, and 18) 5 isolates indicate a positive result (898-bp fragment), whereas the lines (1, 2, 4, 5, 6, 7, 8, 10, 12, 13, 15, 16, 17, 19, and 20) 15 isolates show a negative result. Line (M) represented a marker (1500-100) bp

The results of our research were close to some studies that were carried out by Khulaif and Al-Charrakh in 2023. They found out antibiotic resistance of *E. coli* isolates to β-lactam drugs which revealed that isolates were resistant to ampicillin (100%), amoxicillin (100%), piperacillin (99.1%), amoxicillin clavulanate (100%), cephazolin (89.3%), ceftriaxone (78.5), and aztreonam (67.9%).[29] Regarding the antibiotic resistance of the isolates to non-β-lactam drugs, the results revealed that isolates were resistant to nalidixic acid (90.5%), norfloxacin (70.5%),

Table 4: ESBLs genes distribution among 20 carbapenems resistant *E. coli* isolates

No.	Isolates symbol	Carbapenemase resistance genes according to the Ambler classification			Total genes
		Class A			
		bla_{TME}	bla_{SHV}	$bla_{CTX-M-1}$	
1	E.co-1	+	-	+	2
2	E.co-2	+	-	+	2
3	E.co-3	+	+	+	3
4	E.co-4	+	-	+	2
5	E.co-5	+	-	+	2
6	E.co-6	+	-	+	2
7	E.co-7	+	-	+	2
8	E.co-8	-	-	+	1
9	E.co-9	+	+	+	3
10	E.co-10	-	-	+	1
11	E.co-11	+	+	+	3
12	E.co-12	+	-	+	2
13	E.co-13	-	-	+	1
14	E.co-14	+	+	+	3
15	E.co-15	+	-	+	2
16	E.co-16	+	-	-	1
17	E.co-17	+	-	+	2
18	E.co-18	+	+	+	3
19	E.co-19	+	-	+	2
20	E.co-20	-	-	+	1
Total positive genes		(80%) 16	(25%) 5	(95%) 19	40

trimethoprim–sulfamethoxazole (68%), doxycycline (67.8%), and ciprofloxacin (65%).[30]

According to Azimi *et al.*[31] in Iran, Carba-NP examination confirmed 11% resistance toward carbapenem antibiotics through GNBs. Resistance to antimicrobial agents and carbapenem-resistant isolates might vary due to a variety of circumstances, including sample size, sample type, diagnostic method variation, variation in equipment's utilized, and evolution kind infections among patients.[32,33]

MDR genes and genes β-lactamase observed significant results indicated bla_{TEM} and bla_{CTX-M} genes existence among *E. coli* isolates, whereas bla_{SHV} had a low frequency.[34,35] Several countries and areas, particularly northern and eastern Europe, have seen a high frequency of bla_{TEM} and bla_{CTX-M} positive isolates.[36] Several countries reported that the bla_{CTX-M} group of genes predominate and rapidly spread among the many Enterobacteriaceae which trigger UTIs.[37] According to certain research, *E. coli* causes UTI in more than 85% of hospitalized patients in the United States.[38,39] A recently published study's findings on the genes bla_{TEM} and bla_{CTX-M} exceeded prior South African and Nigerian studies.[40,41] The bla_{SHV} prevalence was similar to that of Sri Lanka.[42] Horizontal transfer of gene through genetic components that are mobile such as plasmids with conjugation or a transposons is frequently used for acquiring these genes from different bacteria.[43]

Beta lactams' genes have been observed in medical facilities all over the world and typically are accountable for Beta lactam antibiotic resistance phenotypes.[44] Furthermore, an important connection between abundance bla_{CTX-M} gene and the ability to antibiotics resistant phenotypically to ceftazidime, cefotaxime, and ceftriaxone has been established [Table 2].

Some previous studies have demonstrated the ability of blaCTX-M enzymes to hydrolyze and degrade cephalothin, cephaluridine, penicillin, ceftriaxone, and ceftriaxone.[45] *E. coli*, an essential component of commensal populations of gut microbiota, has a stable genetic structure with relatively limited recombination occurring in its genome. This genetic feature promotes clonal diversity in bacterial populations, which may be used to find strong phylogenetic groupings.[46]

Conclusion

E. coli that was isolated from clinical samples of UTIs displayed variable drug sensitivity patterns and exhibited strong resistance to widely used antimicrobial agents. Imipenem and meropenem were the medicines that worked the best against isolates of *E. coli.*

The clinical isolates of *E. coli*, however, have substantial levels of amoxicillin and ampicillin resistance.

Twenty isolates out of the ESBL-producing *E. coli* isolates tested positive in the MHT. Almost of UTI cases involved women.

In this investigation, the bla_{CTX-M} and bla_{TME} genes were more often found than the bla_{SHV} gene. The following *E. coli* isolates were discovered to have significant gene distributions: 3, 9, 11, 14, and 18.

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Conflicts of interest

There are no conflicts of interest.

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