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Detection of Antimicrobial Resistance Genes by Using Molecular Methods in Urinary Tract Infections (UTI) patients in the Babil Site

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ABSTRACT

Around the world, there is growing worry over beta-lactamase resistance in Enterobacteriaceae, specifically in Escherich coli (E. coli). The present study aimed to determine the frequency of the beta-lactamase-resistant bla-TEM and bla-AMPC genes in the Enterobacteriaceae family using PCR. Thirty isolates from the Babil Teaching Hospital were obtained for identification since E. coli beta-lactamase genes were discovered in certain laboratories among the 71.6 positive isolates. The current study's findings demonstrated that patients (2 to 18 years old) constituted the majority of the isolates of beta-lactamase-resistant E. coli. Across all bacterial isolates, bla-AMPC and bla-TEM were significantly more common than bla-CTX-M and bla-SHV-5 ($p < 0.05$). bla-AMPC and bla-TEM had prevalence rates of 93.33% and 71.66%, respectively, but bla-SHV-5 and bla-AMPC had lower rates of 20% and 46.66%, respectively. In certain pathogenic bacteria, the potential relationships between virulence genes and antibiotic resistance were investigated. Considering Given that the existence of established infectious factors in clinical isolates could potentially facilitate infection and pathogen persistence, virulence factors were analyzed in relation to these genes and a few additional genes that were studied in the same lab. The current study demonstrated a statistically significant association between antibiotic resistance profile and virulence, with a person correlation of 0.957 between virulence and antibiotic determinants, according to the statistical analysis. The biofilm gene, which was found in 95% of the bacterial isolates, was the most prevalent gene according to the gene virulence data, followed by the iss gene (86.6%) and the fimH gene 85%. Therefore, the discovery of a relationship between virulence profile and resistance may contribute to the development of various bacterial infection rates and their recurrence in the clinical setting.

1. INTRODUCTION

Gram-negative, rod-shaped Escherichia coli (E. coli) bacteria are commonly encountered in warm-blooded species' lower intestines [1–3]. While the majority of E. Coli strains are safe, certain serotypes, particularly in developing nations, can cause dangerous food poisoning in their hosts. their pathogenicity is due to their ability to cause infections in many sites of human body, such as urinary tract, blood stream and surgical sites. E. coli has the ability of construction many different enzymes and toxins which assist bacteria to seepage immune response and antibiotics conduct [4,5] It is the most prevalent infectious agent by far. causing some 90% of urinary tract infections (UTI) in outpatients and approximately 50% in hospitalized patients. Multiple drug-resistant pathogens have resulted in the emergence of resistance strains, and there is a dearth of molecular studies, particularly to comprehend the process by which opportunistic bacteria, which can be lethal pathogens, prevent infection in Iraq [1,3,6–8] The expression of intrinsic low-affinity penicillin-binding proteins is the usual source of β-lactam resistance gene expression in gram-positive bacteria. In gram-negative bacteria, however, the expression of acquired β-lactamases poses a unique challenge because some natural spectra practically consist of β-lactam classes. [4,7,9,10]. A serious public health concern is antibiotic resistance, particularly in developing nations. where there is a significant incidence of bogus and spurious medications of dubious quality in addition to high levels of poverty, ignorance, and poor hygiene habits. [11–15]. Antibiotics known as beta-lactams are used to treat and manage

bacterial infections [14, 15]. Over time, β_lactam drugs' significance in treating bacterial infections has grown. Once often responsive to β-lactam chemotherapy, three Gram-positive human pathogens (Streptococcus pneumoniae, Enterococcus faecium, and Staphylococcus aureus) are no longer [9,10,15]. The conceptual underpinning for developing novel β-lactam antibiotics is derived from the growing molecular-level comprehension of the defense mechanisms that these microorganisms use. The establishment of plasmid-mediated penicillin resistance in the Streptococcus genus has been demonstrated by recent study. [10,16,17] the existence of an enzyme that the antimicrobial agent does not inhibit; a change in the target of the antimicrobial agent that lessens the antimicrobial agent's binding. Natural selection drives the evolution of antibiotic resistance by haphazard mutation [15,18]. Furthermore, resistance could be brought on via a mechanism that hasn't been identified yet. However, a gene that does not express itself in vitro might do so in vivo [19–21]. UTIs are among the most prevalent ailments in the globe., impacting about 150 million people annually [20, 21]. Severe infections have the potential to extended to the kidneys, enter, the, circulation, can result in uremia and bacteremia. [22,23] To control UTIs, early and appropriate antibiotic therapy is essential.

However, the development of multidrug-resistant (MDR) bacteria and the rates at which their resistance builds up, especially in the case of Gram-negative bacteria that are resistant to extended-spectrum β-lactamases (ESBL) or carbapenems, can stimulate therapeutic management [24,25]. As a result, antimicrobial in recent years, resistance has become a significant public health concern. [26, 27]. This has led to a rise in the demand for techniques and tools that enable quick assessment of an antibiotic's susceptibility [26,27]. Enhancing the diagnosis of critically Patients and shorter hospital stays necessitate quick pathogen recognition and efficient antibiotic administration. Many bacterial strains cannot be recognized by commercial multiplex PCR-based pathogen boards because they are unable to detect all known infection-causing pathogens [28,29]. Pathogen recognition in public health laboratories may change as a result of the burgeoning field of metagenomics. while both laboratory techniques and bioinformatics advancements have progressed [30,31]. Previous studies have validated the objective pathogen identification using metagenomic next-generation sequencing, which yields results faster that are important for public health and medical diagnostics [32,33].

2. MATERIALS

Kits

Table 1: *The study's kits together with the manufacturers and nations of origin*

No.	Kit	(Company)	(Country)
1	Genomic DNA Extraction Kit (Bacteria)	Gene aid	,USA,
	GT buffered		
	GB buffered		
	W1 buffered		
	Wash buffered		
	Elution buffered		
	GD column		
	Two-milliliter collection tube		
3	Accu Power ™ PCR Premix	Pioneer	Korea,
	DNA polymerase Taq		
	dNTPs ($dTTP$, $dATP$, $dCTP$, and $dGTP$)		
	pH 9.0 $Tris-HCl$, KCl, and $MgCl2$		
	Tracking dye and stabilizer		

Primers

The primers used in this work were created using Primer 3 and NCBI Gene-Bank, which were made available online by Pioneer Company, Korea, and are shown in the following table.

1. Escherichia coli strain AS713010 beta-lactamase ($blaTEM$) gene, complete cds GenBank: JN037848.1
2. Escherichia coli strain 154297 extended-spectrum beta lactamase ($blaampC$) gene, complete cds GenBank: AY533245.1.

Table 2: *Primers Used for Amplification of $blaTEM$ and $blaAmpC$ Genes and Their Corresponding GenBank References*

blaTEM	F	GGTGCACGAGTGGGTTACAT	531bp
	R	TGCAACTTTATCCGCCTCCA	
$blaAMPC$	F	AAACGACGCTCTGCACCTTA	670bp
	R	TGTACTGCCTTACCTTCGCG	

3. METHODS

Sample gathering

The bacteria samples were collected from patients in Al-Hella Educational Hospital which were cultured in microbiology laboratory. We collected 30 samples from the patient their ages are ranged between (2-18) years old. The bacteria which were collected is E. Coli from an individual with a serious urinary tract infection.

Bacterial isolation

The bacterial isolation was taken from patient urine with sever urinary tract infection after cultured the bacteria on CLED agar and incubated for the entire night at 37°C. Colonies of bacteria were examined on the palate in the hospital. These sample diagnosed by biochemical test. After we ensure the bacterial isolation is E. Coli, the bacteria then were taken by swab media (cottoning semi – sold media) to save the bacterial requirements (food) and saved from harsh weather. Then new culture media (MacConkey agar) was prepared and culturing on the media

in micro lab in the university and incubated at 370C overnight. Next day plate was checked for the growth then we prepared brain heart broth media to transfer these bacteria to this media and incubated in the same condition after one day we extract genomic DNA that explain in next step.

Extraction of genomic DNA

Using the Genomic DNA Mini Bacteria Kit, genomic DNA was extracted from Escherichia coli bacterial isolates using the procedures listed below:

1. A 1.5 ml microcentrifuge tube was filled with 1 ml of cultivated bacterial cells that had been incubated for 18 hours. The tube was then centrifuged at high speed for 1 minute at 15000 rpm, and the supernatant was disposed of.
2. The tube was filled with 200µl of lysozyme buffer, and the cell pellet was re-suspended by violently shaking it with a vortex. The tube was then incubated for 10 minutes at room temperature, with the tubes being inverted every three minutes during the incubation time.
3. Each tube was filled with 200 µl of GB buffer, which was violently agitated for five seconds. Subsequently, the tubes underwent a 10-minute incubation period at 60ºC, with one inversion every three minutes.
4. After adding 200 µl of absolute ethanol and aggressively shaking the mixture, the clear lysate was pipetted with precipitates to break it up.
5. The entire mixture—including any precipitate—was put into a 2 ml collection tube and placed into a GD column. centrifuged at 15,000 rpm for a duration of two minutes. After that, the GD column was placed in a brand-new 2 ml collection tube and the 2 ml collection tube containing the flow-through was disposed of.
6. Once 400µl of W1 buffer has been added to the GD column, centrifuge at 15,000 rpm for 30 seconds. Reintroducing the GD column into the 2 ml collection tube came after disposing of the flow-through.
7. A 600µl wash buffer was added to the GD column. centrifuged for 30 seconds at 15,000 rpm after that. After discarding the flow-through, the GD column was reinserted into the 2 ml collecting tube. and to dry the column matrix, the tubes were centrifuged one more for three minutes at 15,000 rpm.
8. After the GD column was dried, it was placed in a sterile 1.5 ml microcentrifuge tube and 100 µl of hot elution buffer was added to the middle of the matrix of the column.
9. The tubes were allowed to stand for at least three minutes in order to ensure that the matrix had absorbed the elution buffer. then, to extract the pure DNA, centrifuged for 30 seconds at 15,000 rpm

Estimation of DNA extracts

The following steps were taken to verify the extracted DNA: utilizing Nanodrop (THERMO. USA) to determine the concentration of DNA (ng/µl) and verifying the purity of the DNA by reading the absorbance at 260 /280 nm:

1. Once the Nanodrop software has opened, select the relevant application (DNA, nucleic acid).
2. The measurement pedestals were cleaned multiple times with a dry chem-wipe. Next, carefully pipette 2µl of free nuclease water over the lower measurement pedestals' surface to completely blank the apparatus.
3. After lowering the sampling arm and selecting OK to begin the Nanodrop, the pedestals were cleaned, and one microliter of DNA was inserted for measurement.

Making the PCR master mix

The following table shows how the PCR master mix was created using the AccuPower® PCR Premix Kit and the company's instructions.

Table 3: *Composition of PCR Master Mix Used for Gene Amplification*

Master mix for PCR	Volume
template for DNA	5 µL
Forward primer with 10 pmol	1.5µL
Reveres primer (10pmol)	1.5µL
Water for PCR	12µL
Total volume	20 µL

Following that, the components of the PCR master mix listed in the above table were added to a standard AccuPower PCR PreMix Kit, which also included loading dye, dNTPs, Tris-HCl pH: 9.0, KCl, MgCl2, and Taq DNA polymerase, among other components required for a PCR reaction. After that, each PCR tube was moved into an existing vortex centrifuge and spun at 3000 rpm for three minutes. The PCR thermocycler (MyGene, Bioneer, Korea) was then used.

Thermocycler Conditions for PCR

For each gene, the conventional PCR thermocycler system was used to create the PCR thermocycler settings listed in the following table:

Table 4: *summarizes PCR thermocycling conditions for gene amplification, including temperature, duration, and cycle number for each step.*

PCR step	Temp.	Time	Repeat
nitial Denaturation	95°c	Five minutes	1
Denaturation	95°c	30 seconds.	30 cycle
Annealing	58°c	30 seconds.	
Extension	72°c	One minutes	
Final extension	72°c	Five minutes	1
Hold	4°c	Forever	-

Chain Reaction Polymerase

The beta lactase antibiotic resistance genes in E. coli were found using a PCR experiment. This experiment was conducted in accordance with the protocol, which was detailed in full in the section above (2.2.4), using a conventional PCR thermocycler system for each gene.

Analysis of PCR products

The following procedures were used to analyze the PCR results using agarose gel electrophoresis:

1. After dissolving a 1% Agarose gel with 1X TBE in a water bath at 100°C for fifteen minutes, the temperature was lowered to 50°C.
2. Subsequently, 3µL of Ethidium Bromide dye was combined with the agarose gel solution...
3. Following proper comb alignment and agarose gel solution pouring into the tray, the comb was gently taken out and left to set at room temperature for 20 minutes.
4. One TBE buffer was added after the gel tray of the electrophoresis chamber was fixed. Each comb well received five microliters of the 100 bp ladder and ten microliters of the PCR product. Following that, an hour was spent running electricity at 80 AM and 100 volts.
5. The PCR products were seen using a UV transilluminator.

4. RESULTS

Samples diagnosed in the Al-Hella hospital. They were 30 as shown in figure (1).

Figure 1: *E. coli samples were cultured on MacConkey agar. The picture shows the growth of bacteria on this media.*

after that we examine these samples for known it is resistance for antibiotics (penicillin, cefotaxime, ceftriaxone) as illustrated in figure (2).

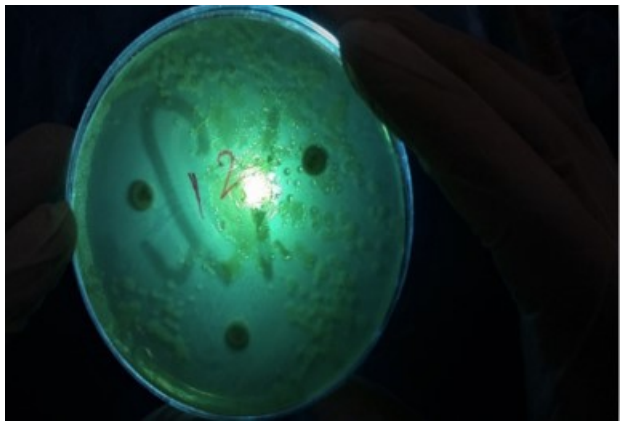

Figure 2: *The E. Coli bacteria in Sample No. 12 are susceptible to ceftriaxone and cefotaxime, two beta-lactam antibiotics, as can be seen on the left. The E. Coli bacteria on the right, however, are resistant to penicillin and other beta-lactam medicines.*

Table 5: *Shows samples were classified using: percentage Law (part/All × 100%).*

Isolate No.	CRO	P	CTX	Res No.	SEN No.	INTER No.
1	res	res	res	3	0	0
2	res	res	res	3	0	0
3	inter	res	inter	1	0	2
4	res	res	sen	2	1	0
5	res	res	inter	2	0	1
6	res	sen	res	2	1	0
7	res	res	res	3	0	0
8	res	res	res	3	0	0
9	res	res	res	3	0	0
10	res	res	res	3	0	0
11	res	res	inter	2	0	1
12	res	inter	sen	1	1	1
13	res	res	res	3	0	0
14	res	res	res	3	0	0
15	res	res	sen	2	1	0
16	inter	res	res	2	0	1
17	res	res	res	3	0	0
18	sen	sen	res	1	2	0
19	res	res	res	3	0	0
20	sen	res	res	2	1	0
21	sen	res	res	2	1	0
22	res	res	res	3	0	0
23	res	res	res	3	0	0
24	res	res	inter	2	0	1
25	res	sen	inter	1	1	1
26	res	res	res	3	0	0
27	res	res	res	3	0	0
28	res	res	res	3	0	0

29	res	sen	res	2	1	0
30	res	sen	res	2	1	0

***Table 6**: Phenotype percentage of all samples were calculated in the following table 1B.*

Antibiotic	Resistance	Intermediate	Sensitive
Penicillin	80.00%	3.33%	16.66%
Cefotaxime	76.67%	16.66%	10.00%
Ceftriaxone	83.33%	6.66%	10.00%

Table 6: Shows phenotype percentage.
Diagram Shows phenotype percentage in more details

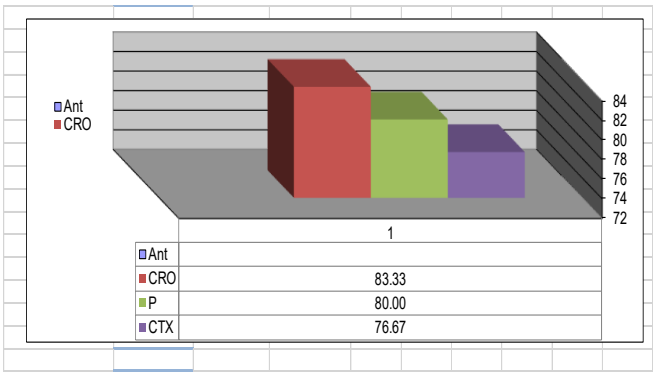

***Figure 3:** This digram shows phenotype percentage in columns for moor illustration.*

DNA was extracted then its purification and concentration were measured using the Nanodrop as shown in the following table

***Table 7:** Shows the measurement of the DNA purification and concentration of DNA for 30 samples using the Nano drop*

NO.	The concent-ration ng/µl	The purity	NO.	The concent-ration ng/µl	The purity
1	16.6	1.9	16	46	1.8
2	24.0	1.4	17	119	1.8
3	21.0	1.8	18	46.4	1.5
4	33.4	1.6	19	77	1.9
5	42.3	1.5	20	69	1.9
6	44.6	1.6	21	30	1.8
7	44.1	1.6	22	44	1.9
8	19.8	1.7	23	96	1.2
9	35.9	1.6	24	39	1.9
10	60.2	1.4	25	89	1.9
11	35.9	1.4	26	50	1.8
12	90	1.5	27	44	1.8
13	36	1.3	28	57	1.9
14	37	1.7	29	38	1.7
15	24	1.7	30	45	1.6

The accompanying images and table show the results of Agarose gel electrophoresis used to detect PCR products.

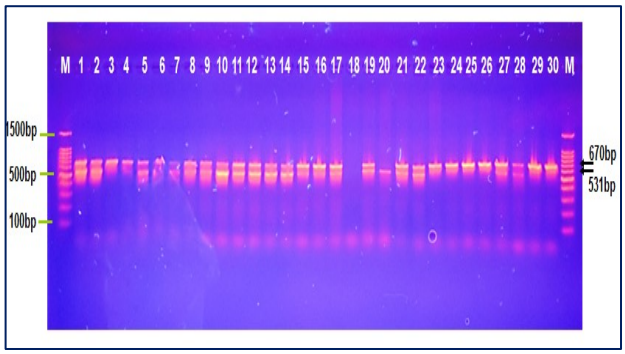

***Figure 4:** A picture from an agarose gel electrophoresis that displays the results of a multiplex PCR product analysis of the antibiotic resistance genes bla − TEM and bla-AMPC in beta lactamases found in isolates of Escherichia coli. where the marker M is situated (1500-100bp). The positive amplification for bla − TEM gene at (531bp) PCR product size and positive amplification for bla − AMPC gene at (670bp) PCR product size.*

This table shows the PCR products that agarose gel electrophoresis was able to identify.

***Table 8:** Illustrated the PCR products that an agarose gel electrophoresis revealed.*

Isolate No.	*bla − TEM*	*bla − AmpC*	Gene bla No.	BLA %
1	Positive	Positive	4	100%
2	Positive	Positive	4	100%
3	Negative	Positive	3	75%
4	Negative	Positive	1	25%
5	Positive	Positive	3	75%
6	Negative	Positive	2	50%
7	Positive	Positive	3	75%
8	Positive	Positive	4	100%
9	Positive	Positive	4	100%
10	Positive	Positive	4	100%
11	Positive	Positive	4	100%
12	Positive	Positive	2	50%
13	Positive	Positive	4	100%
14	Positive	Positive	3	75%
15	Positive	Positive	2	50%
16	Positive	Positive	3	75%
17	Positive	Positive	3	75%
18	Negative	Negative	1	25%
19	Positive	Positive	3	75%
20	Positive	Negative	2	50%
21	Positive	Positive	3	75%
22	Positive	Positive	3	75%
23	Negative	Positive	3	75%
24	Negative	Positive	3	75%
25	Negative	Positive	2	50%
26	Negative	Positive	3	75%

27	Positive	Positive	3	75%
28	Positive	Positive	3	75%
29	Negative	Positive	2	50%
30	Negative	Positive	2	50%

Parameter	$bla-TEM$	$bla-AmpC$
Total Positive	20	28
Percent (%)	66.67%	93.33%

Gene	Percent (%)
$bla-TEM$	66.67%
$bla-AmpC$	93.33%

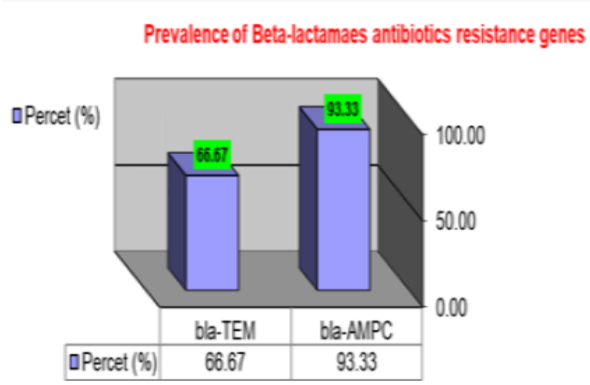

Figure 5: *The Percent (%) of detection of gene* $bla-TEM$ *(66.67),* $bla-AMPC$ (93.33) *in E. coli for 30 sample.*

Beta-Lactam's gene were compared with other genes such as is and Fim in E. Coli to see if there any existence of any virulence factors.

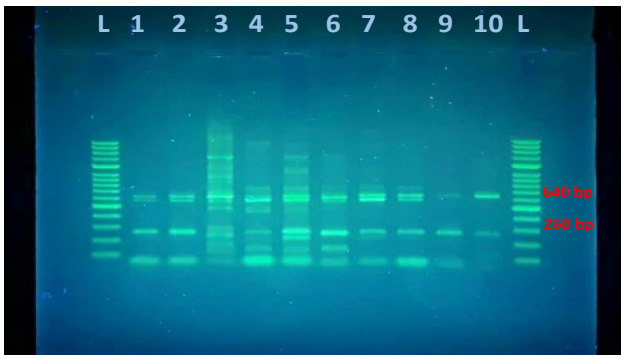

Figure 6: *The Multiplex PCR product analysis of virulence iss and fim in Escherichia coli isolates is depicted in this Agarose gel electrophoresis picture. M is the marker (1500-100bp) here. The advantage*

amplification for is gene at (260bp) PCR product size and positive amplification for fim gene at (640bp) PCR product size.

5. Discussion

After multiplex PCR, agarose gel electrophoresis was employed in order to find ESBL. Two isolates of E. coli genes that create ESBL when infected with UTIs. That's right—$bla-TEM$ and $bla-AMP$. Furthermore, a recent investigation involving over 30 samples revealed that 15 phenotypically non-ESBL isolates, of which 70.5% were genotypically positive, had the genes $bla-TEM$ and bla-SHV. Among these, (21.5%) isolates had bla-TEM in combination with tem gene, while 14 (42%) isolates had bla-TEM gene alone. In the north and center of Portugal, 39% of E. Coli isolates resistant to expanded-spectrum cephalosporins have been found to have ESBL [1]. About 38–39% of the E. coli isolates that produced ESBLs were in Emirate [2]. Significantly higher frequency of ESBL has been recorded in 2005–6, with reports citing 30–60% in America, 58% in Turkey, and 56% in India [4–6]. Low rate of ESBL production, however (5-8%). In the Netherlands, less than 1% the isolates of E. coli were found to be ESBL positive. [7,34,35] In Korea, E. coli has been identified., Japan, Malaysia, and Singapore [36,37] The disparity between the two approaches (phenotypic and genotypic) for identifying ESBL-positive isolates may be due to the phenotypic method's lower sensitivity as well as the impact of external elements on the development of resistance. The phenotypic methods employed to detect ESBL in bacterial isolates may also have an impact on it. Some ESBLs might not build up to a certain amount. where disk diffusion tests can identify them, which would lead to therapy failure for the afflicted individuals. The current work demonstrated a lack of association between disk diffusion susceptibility results and ESBL production, which highlights the necessity of integrating an enhanced ESBL detection technique into standard susceptibility protocols [38] In comparison, the genotypic approach that employs targeted Resistance gene PCR amplification seems to have 100% sensitivity and specificity. A recent investigation revealed that all 30 genotypically positive isolates have $bla-TEM$. It was found that neither the SHV nor the CTX-M genes were present in any isolate. With their rapid proliferation among Enterobacteriaceae in recent years, CTX-M beta lactamases have emerged as the most common Beta-lactamases with an Extended Spectrum ESBLs globally. Antibiotic-resistant genes are introduced and spread, which reduces treatment options, raises patient mortality and morbidity, lengthens hospital stays, and raises expenses. Alice Elena Ghenea [35] has confirmed the beta-lactamases that are circulating and encoded by the genes bla-CTX-15, bla-shv-1, and bla-TEM-1 in strains of K. pneumoniae and E. coli. Moreover, the isolates carried only SHV and CTX-M or the combination of TEM, SHV, and CTX-M genes. Our study's findings demonstrated that all genotypically positive isolates (66.67%) carried the $bla-TEM$ gene. Under our circumstances, the $bla-TEM$ gene may be a good choice for molecular screening of ESBL-positive samples because it is present in every genotypically positive isolate. [39] The enzymes CTX-M have been linked to outbreaks all around the world, including Iraq, and are becoming recognized as a major global public health risk. Beta-lactamases that show a greater inclination towards cefotaxime and ceftriaxone in comparison to ceftazidime, as well as a greater vulnerability to tazobactam in contrast to clavulanate. Clinicians should be concerned about them as they persist in the Iraqi bacterial community, particularly in our environments. These findings were in agreement with (7) According to Zeynudin A, et al.'s work, the majority of isolates carrying $bla-CTX-M$ carrying Enterobacteriaceae are susceptible to imipenem and

meropenem, while 13.1% for tigecycline, 10.9% for Fosfomycin, and 1.6% for amikacin have moderate resistance rates. But all of the Enterobacteriaceae that tested positive for $blaCTX-M$ had an MDR phenotype, exhibiting notable non-susceptibility rates, or co-resistances, to trimethoprim/sulfamethoxazole (92.2%), aminoglycosides (92.2%), and fluoroquinolones (78.1%). Finding and isolating ESBL-producing bacteria is crucial to choose the best antibiotic for the patient. roughly 15% of the isolates that produced ESBL in this investigation were found to be susceptible to pencil line, and roughly 9% to ceftriaxone and cefotaxime. Our results demonstrated the prevalence of the CTX-M type of β-lactamases in Iraq. Among E. Coli isolates that tested positive for ESBL, the frequency of CTX-M was 68% in France [40], and 66% in Portugal [41] However, compared to certain industrialized nations, Mashhad, in northern Iran, and Iraq exhibited higher CTX-M prevalence, according to another study. The frequency of CTX-type, TEM-type, SHV-5, and $bla-ampc$ type enzymes was $90\%, 63\%, 36\%,$ and $93\%,$ respectively, among E. isolates in Al-Hilla Hospital; The most prevalent beta-lactamase among Gram-negative bacteria is called TEM-1. Up to 88% of E. coli ampicillin resistance is caused by the growth of TEM-1. [25]. also, in charge of the penicillin resistance shown in K. pneumonia and E. Coli. The CTX-M Given that cefotaxime was a substrate of these enzymes with more activity than other oxyimino-beta-lactams (such ceftazidime, ceftriaxone, or cefepime), they were given this name. instead, then developing by mutation. Since these two frequently isolated beta-lactamases only share 40% of their identity, there is no significant relationship between these enzymes and SHV or TEM beta-lactamases. CTX-M-15 is the most prevalent type of E. coli detected in the UK in 2006. which is also highly prevalent in the population (38). According to a recent study published in Roman, the majority of the CTXM-15 enzyme is present in Enterobacteriaceae that produce ESBLs and are circulating in Romania's Oltenia region. These bacteria have significant levels of antibiotic resistance, especially E. coli. The SHV enzyme was uncommon in E. coli but present in all strains of K. pneumoniae, whereas the TEM enzyme was more prevalent in E. coli than in K. pneumoniae. [30] The SHV-1 and TEM-1 have a similar general structure and share 68% of their amino acid composition. The SHV-1 beta-lactamase is responsible for up to 20% of the plasmid-mediated penicillin resistance in K. pneumoniae., which is mostly present in this species. Conversely, Enterobacteriaceae produced a lot of ESBL, with the $bla\,TEM$ and the higher frequency of $bla\,CTX-M$ genotypes. It needs both public health and high-caliber laboratory standards to stop the spread of resistant infections [18]. The amino acids surrounding the active site of ESBLs in this family are also altered; these modifications typically occur at 238 or 238 and 240 places. About sixty different SHV variations are known to exist. The two most common ones are SHV-12 and SHV-5. [39]. Bacteria resistant to cephalosporins frequently develop extended-spectrum $AmpC$ type β-lactamases. Class C, or group 1, AmpC β-lactamases are generally found on the chromosomes of various Gram-negative bacteria, such as Enterobacter, Citrobacter, and Serratia species, where they are usually stimulated to express themselves. They can also be found on Escherichia coli, but their expression is typically not stimulated and may even be hyper expressed. Amp C type β-lactamases can also be present in plasmids [38] In contrast to extended-spectrum β-lactamases (ESBLs) like $AmpC$ β-lactamases, have the ability to hydrolyze a wide range of cephalosporins, such as oxyimino-β-lactams and cephamycin's, without being hindered by β-lactamase inhibitors such clavulanic acid. The majority of ESBL synthesis is carried by plasmids, and these genes could readily spread among hospitalized patients, according to research that is currently accessible [4,5,7]. This is a key element contributing to the ESBL producers' growing dispersion. Consequently, it's critical to identify ESBL-producing bacteria in communities and to properly manage the prescription of antibiotics in order to prevent them. They have come to the conclusion that resistant strains of Escherichia coli ESBL were quite common in UTIs acquired in hospitals and the community, especially in female patients. The following medications worked well against E. coli ESBL: levofloxacin, colistin, tigecycline, amikacin, meropenem, imipenem, and nitrofurantoin. These results offer more proof that, in order to properly prescribe effective antibiotics, earlier and more accurate approaches for identifying ESBL-producing bacteria must be used [40,41]

6. CONCLUSIONS

This is a key element contributing to the ESBL producers' growing dispersion. Consequently, it's critical to identify ESBL-producing bacteria in communities and to properly manage the prescription of antibiotics in order to prevent them. They have come to the conclusion that resistant strains of Escherichia coli ESBL were quite common in UTIs acquired in hospitals and the community, especially in female patients. The following medications worked well against E. coli ESBL: levofloxacin, colistin, tigecycline, amikacin, meropenem, imipenem, and nitrofurantoin. These results offer more proof that, in order to properly prescribe effective antibiotics, earlier and more accurate approaches for identifying ESBL-producing bacteria must be used (40). a highly significant relationship between the profile of antibiotic resistance and pathogenicity. m the statistical study showed that the virulence and antibiotic components had a 0.957-person association. As a result, the rise in beta-lactamase resistance emphasizes the necessity of developing regional guidelines for antibiotic therapy by strengthening the national resistance surveillance system and streamlining the policy for the use of antibiotics.

AUTHORS' DECLARATION

We confirm that all the Figures and Tables in the manuscript belong to the current study.

CONFLICT OF INTEREST

The authors declare that there are no conflicts of interest regarding the publication of this study.

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