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ESBL, AmpC β-Lactamases, and NDM Genes' Co-Existence and its Association with Multidrug Resistance among E. coli Isolated from UTI

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Abstract

The occurrence of multidrug-resistant *Escherichia coli* in urinary tract infections is considered the emergent health concern among world countries, particularly in the regions with limited clear antimicrobial resistance surveillance. The aime is assessed the β-lactamase-production profile of the *E. coli* and evaluate the complicated resistance pattern as gene co-occurrence outcomes among UTI patients. This study includes 212 urine samples collected from males and females, where 168 samples exhibited microbial growth, where the *E. coli* demonstrated a predominance (68%) among the isolated pathogens. However, the antimicrobial susceptibility reveals an elevated resistance rate toward most tested antibiotics, including amoxicillin/clavulanic acid (80%) and third-generation cephalosporins (≥61%). While nitrofurantoin (13%), carbapenem (≤12%), and aminoglycoside resistance (≤8%) remained the most susceptible ones. Additionally, the phenotypic confirmation demonstrated 67% of isolates were ESBL producers and AmpC producers. The genotypic investigation exposed a high frequency of ESBL genes: *CTX-M-1* (86%), *TEM-1* (85%), and *OXA-1* (75%), with frequent co-occurrence between them. *SHV-1* was the least distinguished gene (30%). The *pAmpC* is predominantly revealed (97%) among the isolates, in contrast to the *CMY* gene and the *NDM* gene, which were noticed in 19% and 9% of the isolates, respectively. However, a strong correlation has been found between the existence of ESBL genes and the resistance pattern of tested beta-lactam antibiotics *($p< 0.001$)*. In conclusion, the high occurrence of ESBL, AmpC, and NDM genes contributes to increasing the intensity and complexity of resistance patterns, therefore accelerating the occurrence of MDR among *E. coli* isolates.

الوجود المشترك لجينات ESBL وAmpC β-Lactamases وNDM وعلاقته بالمقاومة المتعددة للأدوية في عزلات الاشريكية القولونية في حالات عدوى المسالك البولية

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الخلاصة

يُعَدّ ظهور الإشريكية القولونية (***Escherichia coli***) المتعددة المقاومة للأدوية في حالات عدوى المسالك البولية مشكلة صحية ناشئة على مستوى العالم، وخاصةً في المناطق التي تفتقر إلى أنظمة واضحة لمراقبة مقاومة المضادات الحيوية. يهدف هذا البحث إلى تقييم النمط الإنتاجي للـ **β-lactamase** في عزلات ***E. coli***، ودراسة النمط المعقد للمقاومة كنتيجة للتشارك الجيني بين المرضى المصابين بعدوى المسالك البولية. شملت الدراسة 212 عينة بول جُمِعت من الذكور والإناث، حيث أظهرت 168 عينة نمواً ميكروبيّاً، وكانت ***E. coli*** هي السائدة بنسبة 68% بين العزلات البكتيرية. وكشفت اختبارات الحساسية للمضادات الحيوية عن ارتفاع معدلات المقاومة تجاه معظم المضادات المختبرة، بما في ذلك الأموكسيسيلين/كلافولانيك أسيد (80%) والسيفالوسبورينات من الجيل الثالث (≤61%). في المقابل، ظلّت أقل معدلات المقاومة تجاه النيتروفورانتوين (13%)، والكاربينيمات (≤12%)، والأمينوغليكوزيدات (≤8%). كما أظهرت الفحوصات الظاهرية أن 67% من العزلات منتجة لإنزيمات **ESBL** ومنتجة لـ **AmpC.** أما الفحوصات الجينية فقد بيّنت ارتفاع معدلات وجود جينات **ESBL** وهي **CTX-M-1** بنسبة (86%)، و **TEM-1** بنسبة (85%)، و **OXA-1** بنسبة (75%)، مع تسجيل تكرار واضح للتشارك الجيني بينها، في حين كان جين **SHV-1** الأقل انتشاراً (30%). كما ظهر الجين **pAmpC** في معظم العزلات (97%)، في حين تم الكشف عن جيني **CMY** و **NDM** في 19% و9% من العزلات على التوالي. وقد وُجد ارتباط قوي بين وجود جينات **ESBL** ونمط المقاومة تجاه المضادات الحيوية من فئة **β-lactam (p<0.001).** وباستنتاج، فإن الارتفاع الكبير في معدلات جينات **ESBL** و **AmpC** و **NDM** يسهم في زيادة شدة وتعقيد أنماط المقاومة، مما يسرّع من انتشار المقاومة المتعددة للأدوية بين عزلات ***E. coli***.

1.Introduction

Urinary tract infections (UTIs) are considered the most common bacterial infections challenging public health worldwide and posing a significant problem on healthcare systems, predominantly in the developing countries (Foxman, 2002).

As *Escherichia coli* (*E. coli*) was highlighted as the major uropathogen inducing both community-acquired and nosocomial UTIs, the extensive evolution in the emergence of multidrug resistance (MDR) has impacted the therapeutic process and hence the increase in the severity and complexity of UTIs (Whelan et al., 2023). Moreover, in *E. coli*, the acquisition of β-lactamase enzymes is the primary motivation of MDR, as it inactivates a extensive range of broad-spectrum antibiotics, and as its co-occurrence with other antibiotic resistance mechanisms on the same plasmids (Ibrahim et al., 2023). Extended-spectrum β-lactamases (ESBLs), plasmid-mediated AmpC β-lactamases (pAmpCs), New Delhi metallo-β-lactamases (NDMs), and the OXA-type (oxacillin-hydrolyzing) β-lactamases are distinguished as the major classes of β-lactamase enzymes that confer resistance to penicillins, cephalosporins, carbapenems, and beta-lactams with inhibitors. Generally, ESBLs are primarily encoded by SHV, CTX-X, and TEM genes; however, the CTX-M gene displays a predominance among ESBL-producing *E. coli* worldwide. AmpC β-lactamases exhibit resistance to cephamycins and β-lactamases/β-lactamase inhibitor blends and contribute to the sophisticated β-lactam resistance, as it may be associated with the acquisition of ESBL genes, which are frequently undetectable by phenotypic assays (Alanazi et al., 2024). However, the most alarming subject facing the globe is the carbapenemase, such as NDMs, which has emerged as a precarious threat, putting up a therapeutic challenge as it confers ineffectiveness to the last resort carbapenem antibiotics (Gondal et al., 2020). The Centers for Disease Control and Prevention (CDC) has published a report in 2019, it states that carbapenem-resistant *Enterobacteriaceae* is an urgent threat responsible for 11,000 annual deaths in 2017 and considers the ESBL-producing *Enterobacteriaceae* as a serious threat causing 9,100 deaths in 2017; however, these numbers are at an accelerating rate yearly (*Antibiotic resistance threats in the United States, 2019*, 2019).

The coexistence of ESBL, AmpC and NDM genes within *E. coli* is driving public health toward exhausted, challenging levels, narrowing the therapeutic choices, and accelerating the death rate among patients (Alara and Alara, 2024). Although over-antibiotic prescriptions, excessive empirical treatment, and wrong patient consumption habits aid in the evolution of more complicated resistance strategies in the bacteria that are mainly associated with horizontal transfer of multidrug resistance plasmids or integrons (Shrestha et al., 2020; Tavares et al., 2025). In Iraq, a recent study published by Rajal Dave, *et al*. (Dave and Joshi, 2025) revealed a shocking result where ESBL and carbapenemase producers were 73.9% and 47.8%, respectively, and this reflects the disturbing decline of Iraqi health and limited strategies to control this catastrophe, which is not only caused by the society's health culture but also the limited and underdeveloped antimicrobial stewardship programs and multidrug resistance surveillance. However, this study aims to monitor the existence of molecular co-occurrence of ESBL, AmpC, and NDM genes among E. coli isolates from UTI cases in Iraq and evaluate the consequence of these genes on the antimicrobial resistance by assessing the β-lactamase-production profile of the *E. coli* and considering the complicated resistance pattern as gene co-occurrence outcomes among UTI patients.

2. Methodology

2.2. Bacterial Identification

This study was conducted in the Microbiology Department of the Faculty of Medicine/Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences. Consequently, 212 urine samples from four hospitals were collected, including Alhakem General Hospital, Al-Najaf Al-Ashraf Teaching Hospital, Al-Forat Al-Awsat Teaching Hospital, and Imam Al Hassan Al Mujtaba Teaching Hospital, located in Al-Najaf and Karbala city from September 2024 to December 2024 from symptomatic inpatients and outpatients confirmed to have a UTI. Although the study incorporated both females and males. The isolation of *E. coli* was conducted through ordinary microbiological culture methods and biochemical assays; the confirmation of the diagnosis was achieved through the VITEK 2 identification system (Biomerieux France).

2.3. Antimicrobial Susceptibility Testing

Antibiotic susceptibility testing was conducted using a Kirby-Bauer disc diffusion method as recommended by the Clinical and Laboratory Standards Institute guidelines of 2024 (Clinical and Laboratory Standards Institute, 2024). The antibiotics used were gentamicin (10 µg), amikacin (30 µg), ciprofloxacin (30 µg), levofloxacin (10 µg), ceftriaxone (30 µg), ceftazidime (30 µg), nitrofurantoin (300 µg), piperacillin/tazobactam (100/10 µg), cefepime (30 µg), imipenem (10 µg), meropenem (10 µg), ertapenem (10 µg), and trimethoprim/sulfamethoxazole (1.25/23.75 µg), and plates were incubated under aerobic conditions at 37 °C for 24 h. The organisms were identified as sensitive, resistant, and intermediate based on zone diameter measurement in millimeters as per CLSI 2024 guidelines. *E. coli* strain ATCC 25922 was used as a control strain.

2.4. Phenotypic Screening for AmpC, ESBL and Carbapenem Resistance.

Screening and confirmation of AmpC, ESBL, and carbapenemases were carried out using the disk diffusion assay as recommended by the CLSI 2024 guidelines; as for AmpC beta-lactamase, detection was performed by examination of reduced susceptibility of bacteria to cefoxitin (30 µg) inhibition zone < 18 mm. As for ESBL screening was done by observing a reduced susceptibility to 3rd-generation cephalosporins, including ceftriaxone (30 µg), ceftazidime (30 µg), cefpodoxime (10 µg), and cefotaxime (30 µg). The isolates showed a nonsusceptibility to any of the tested antibiotics and were confirmed for ESBL production by using the modified double-disk synergy (MDDS) method by placing amoxicillin/clavulanic acid (20 µg amoxicillin plus 10 µg clavulanic acid) in the center of a Mueller-Hinton agar plate that has been inoculated with a potentially ESBL-producing *E. coli* isolate and being surrounded by 4 antibiotic discs, including cefotaxime, ceftazidime, ceftriaxone, and aztreonam (30 µg each). The distance between them was kept at 15mm. A keyhole phenotype between the amoxicillin/clavulanic acid disc and the cephalosporin disc was considered an ESBL-producer isolate. However, the decreased susceptibility to imipenem (10 µg), meropenem (10 µg), or ertapenem (10 µg) was considered carbapenemase-producer.

2.5. Molecular Recognition of pAmpC, ESBL and Carbapenem Resistance Genes

The extracted genomic DNA from the isolates that showed positive results for the phenotypic detection using the HiPure Bacterial DNA Kit (Magen, China) and then subjected to detection of responsible genes using the polymerase chain reaction technique PCR, which included the following: for AmpC (pAmpC and CMY-1) (Shahid et al., 2009a; Zhao et al., 2001), for ESBL (TEM-1, SHV-1, CTX-M, and OXA-1) (Ogutu et al., 2015a; Saladin et al., 2002a), and for Metallo-

beta lactamase (NDM) (Gondal et al., 2020). Furthermore, all genes' primers are listed in Table1, and the amplification protocol is listed in Table2. Both multiplex PCR and monoplex PCR were used in this study. All amplified genes' products were documented by electrophoresis in 1.5% agarose gel containing 3 mg/ml of ethidium bromide at 70 V for 70 min.

2.6. Statistical Analysis

The statistical analysis was achieved using various bioinformatics programs and software packages, which included Statistical Package for Windows (SPSS) v.23.0 and Microsoft Excel. The Chi-square x^2 test was used to analyze the correlation in this study. The results of $p\ value < 0.05$ were considered statistically significant.

Table 1: Ampc, ESBL And New Delhi Metallo-Beta Lactamase Genes Primers

Gene	pimer	Primer sequence	Ref.
bla TEM	*bla TEM-F*	CATTTCCGTGTCGCCCTTATTC	(Ogutu et al., 2015b)
	bla TEM-R	CGTTCATCCATAGTTGCCTGAC	(Saladin et al., 2002b)
bla SHV	*bla SHV-F*	AGCCGCTTGAGCAAATTAAAC	(Saladin et al., 2002b)
	bla SHV-R	ATCCCGCAGATAAATCACCAC	
bla CTX-M	*bla-CTX-M-F*	SCSATGTGCAGYACCAGTAA	(Ogutu et al., 2015b)
	bla CTX-M-R	CCGCRATATGRTTGGTGGTG	
bla OXA-1	*bla OXA-1-F*	GGCACCAGATTCAACTTTCAAG	(Saladin et al., 2002b)
	bla OXA-1-R	GACCCCAAGTTTCCTGTAAGTG	
blaNDM	*blaNDM-F*	GGTTTGGCGATCTGGTTTTC	(Ogutu et al., 2015b)
	blaNDM-R	CGGAATGGCTCATCACGATC	
blaAmpC	*blaAmpC-F*	CCCCGCTTATAGAGCAACAA	(Shahid et al., 2009b)
	blaAmpC-R	TCAATGGTCGACTTCACACC	
bla CMY	*bla CMY-F*	GACAGCCTCTTTCTCCACA	(Shahid et al., 2009b)
	bla CMY-R	TGGAACGAAGGCTACGTA	

Table2: Amplification Condition of Beta-Lactamase Genes PCR Cycle

Monoplex gene	Temperature (ºC)/Time						
	Initial denaturation	Cycle No.	Denaturation	Annealing	Extension	Final extension	Amplicon size
bla TEM	94 °C/10min	30	94 °C/30sec	61°C/35 s	72 °C/1 min	72 °C/9 min	800
bla SHV	94 °C/10min	30	94 °C/40sec	60°C/40 s	72 °C/1 min	72°C/7 min	713
bla CTX-M	94 °C/10min	30	94 °C/30sec	61°C/35 s	72 °C/1 min	72 °C/9 min	554
bla OXA-1	94/10min	30	94/40 sec	59/40 sec	72/1 min	72/5 min	564
blaNDM	95°C/5min	30	95°C/30sec	62/30 sec	72°C/1min	72°C/5min	621
blaAmpC	95/15 min	35	94/1 min	58/1 min	72/1 min	72/7 min	634
bla CMY	95/15 min	35	94/1 min	58/1 min	72/1 min	72/7 min	1000

3. Results and Discussion

3.1. Bacterial Isolation and Sample Collection

During the study duration, 212 urine samples have been collected, which included both female and male, with prevalence of 170 (80%) and 42 (20%), respectively. Moreover, 168 urine samples reveal a positive microbial growth, while 44 of them show no growth. Although *E. coli* was the most prevalent pathogen, 106 (63%), followed by *Klebsiella pneumoniae* and Staphylococcus aureus, 14 (8%) and 12 (7%), respectively, all microbial growth prevalence is demonstrated in Fig.1. this quietly aligns with a Chinese study published in 2024 (Mei et al., 2024). Likewise, in Egypt by Masoud S. *et al.*

(Masoud et al., 2021) and Saudi Arabia by Alanazi *et al*. (Alanazi et al., 2018).Among E. coli isolates, the mean age group in the study was 40±19 years, ranging from 16 to 104, the age groups represented in Table3. The outpatients were the most predominant among E. coli isolates, 100 (94%), compared with hospitalized patients, 6 (6%), the highest prevalence was among females (92%) compared to males (8%). This reveals a significant association of both age and sex in apathogenic E. coli infections.

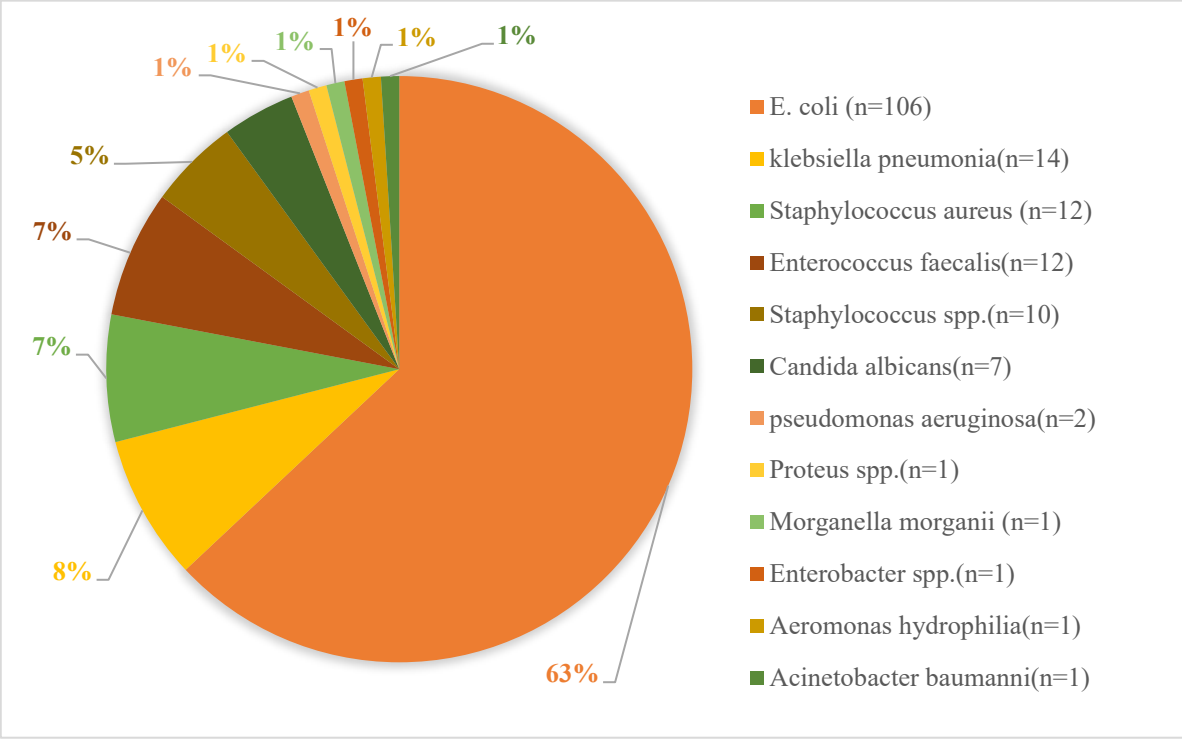

Figure1: Microbial Growth Distribution Among Urine Samples

Table3: Age Group, Sex, And Hospitalization Prevalence of *E. Coli* Isolates

	Group	no.(%)
Age group	(16-25)	22(21%)
	(26-35)	31(30%)
	(36-45)	13(12%)
	(46-55)	13(12%)
	(56-65)	13(12%)
	over 66	14(13%)
Sex	female	97(92%)
	male	9(8%)
Hospitalization	outpatients	100(94%)
	inpatients	6(6%)

3.2. Antimicrobial Susceptibility Testing

As the antimicrobial susceptibility testing of E. coli in this study demonstrates, an elevated level of resistance was detected in amoxicillin/clavulanic acid (80%), cefoxitin (66%), ceftazidime (80%), cefotaxime (62%), ceftriaxone (61%), and cefpodoxime (66%). Compared to ertapenem (11%), imipenem (12%), meropenem (8%), amikacin (8%), gentamicin (18%), and nitrofurantoin (13%), which seem to be more effective against E. coli, although this emphasizes a misfortune and frustrating health burden since this narrows the therapeutic choices that have been in daily use for UTIs. Moreover,

the findings of this study were in accordance with three Iraqi studies published by Alkhudhairy M et al. (Alkhudhairy et al., 2019), Hammadi A et al. (Hammadi et al., 2015), and Pishtiwan A et al. (Pishtiwan and Khadija, 2019a), all of which exhibit similar findings to the current study. Table4 shows resistance patterns of E. coli isolated from UTI patients. The isolates show reduced susceptibility to any of the 3rd generation cephalosporins were submitted to the MDDS test to confirm ESBL results. Also, all the isolates that resist cefoxitin are identified as AmpC producers and were subjected to plasmid-mediated AmpC gene detection. Extended-spectrum β-lactamase production was phenotypically confirmed in 71 (67%) E. coli isolates. The results demonstrate four isolates with an increased inhibition zone toward beta-lactamase inhibitor (typical ESBL results); however, the rest, 67/71 (94%), expose misleading, false negative results by resisting all or most of the tested antibiotics, including AMC. These phenomena basically rely on other beta-lactamase enzymes' interference leading to masking the actions of ESBL in E. coli isolates, and what confirms these results is the genotypical identification of underlying genes. Besides ESBL and AmpC beta-lactamase, in this study about 16% of the E. coli isolates were resistant to carbapenem antibiotics; moreover, all ESBL-producing E. coli isolates were also plasmid-mediated AmpC producers. Furthermore, a strong significant correlation has been found between ESBL production and quinolone resistance, 40/71 (56%), compared to ESBL production and quinolone susceptibility, 31/71 (44%) ($p < 0.001$). This correlation is mainly mediated by co-localization of the causative resistance genes on the same mobile elements, such as plasmids, which also has been proven by numerous studies published locally and in other countries (Azargun et al., 2018; Onishi et al., 2022; Salah et al., 2019). The prevalence of ESBL in this study (67%) represents a risky increase that is fundamentally reflected in the deterioration of the community's health; furthermore, this prevalence demonstrates an elevation compared to 2019, as mentioned by Alkhudhairy M et al. (Alkhudhairy et al., 2019). Which has noticed that ESBL occurrence was 23.7% in pregnant women with UTI. However, these occurrences in this study seem to be lower than those mentioned in an Egyptian study, where it was 100% in the urine samples (Masoud et al., 2021). Instead, this study shows accordance with other studies published in Iraq (Anwar, 2025), Cuba (Carmona-Cartaya et al., 2022), Sri Lanka (Perera et al., 2022), and Pakistan (Naeem et al., 2021). In contrast to America, Europe, Canada, and the Pacific Western region, which were 8%, 23%, 5%, and 25%, respectively, Fig.2.

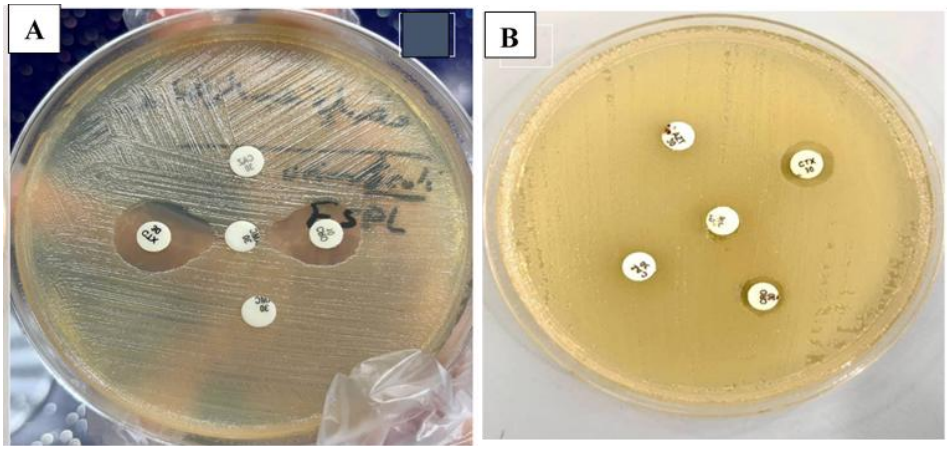

Figure2: MDDS Test Result of ESBL Production Phenotypic Confirmation. A/ Demonstrate the typical synergy effect between AMC and CRO/CTX (keyhole phenotype). B/ Exhibit a complete resistance to all tested antibiotics (atypical ESBL phenotype)

Table4: Antimicrobial Sensitivity Testing of The Study *E. Coli* Isolates.

Antimicrobial Susceptibility of *E. Coli* (No.=106)			
Antibiotic	**Sensitive**	**Intermediate**	**Resistant**
Amoxicillin/clavulanic acid	21(20%)	-	85(80%)
Ceftazidime	18(17%)	3(3%)	85(80%)
Cefoxitin	29(27%)	7(7%)	70(66%)
Cefpodoxime	33(31%)	3(3%)	70(66%)
Cefotaxime	33(31%)	7(7%)	66(62%)
Ceftriaxone	37(35%)	4(4%)	65(61%)
Aztreonam	37(35%)	15(14%)	54(51%)
Cefepime	58(55%)	7(7%)	41(39%)
Trimethoprim/Sulfamethoxazole	64(60%)	1(1%)	41(39%)
piperacillin/tazobactam	59(56%)	8(8%)	39(37%)
Ciprofloxacin	66(62%)	2(2%)	32(30%)
Levofloxacin	68(64%)	5(5%)	27(25%)
Gentamicin	87(82%)	-	19(18%)
Nitrofurantoin	83(78%)	9(8%)	14(13%)
Imipenem	91(86%)	2(2%)	13(12%)
Ertapenem	92(87%)	2(2%)	12(11%)
Meropenem	95(90%)	2(2%)	9(8%)
Amikacin	85(80%)	12(11%)	9(8%)

3.3. Molecular Detection of ESBL, Pampc, And Metallo-Beta Lactamase Genes

As the molecular determination is crucial to confirm the phenotypical identification of ESBL, AmpC, and the existence of the NDM gene among carbapenem-resistant isolates. Although the ESBL encoding genes primarily are TEM-1, SHV-1, and CTX-M-1, the study also included the identification of Class D β-lactamases of the OXA-1 type, which confer a resistance against amoxicillin/clavulanic acid. In this study all phenotypically identified ESBL producers harbored at least one ESBL gene determinant, moreover, the most predominant gene was found to be CTX-M-1 61(86%), followed by TEM-1 60 (85%) and OXA-1 53(75%). Though the SHV-1 gene was the least found 21(30%). Yet, the CTX-M and TEM are more likely to occur together in the study isolates, with a frequency of 36 (51%), followed by the presence of the three genes TEM-1/SHV-1/CTX-M-1 with a frequency of 17 (24%). The findings of this study were in contradiction to an Egyptian study published in 2020 where TEM-1 and SHV-1 were the most prevalent among E. coli, with 59.7% and 38.4%, respectively. Yet, the combinations of TEM/CTX-M were the highest and followed by TEM-1/SHV-1. Conversely, the finding of this study is consistent with the results of Livermore D, et al. (Livermore et al., 2019), Owusu F, et al. (Owusu et al., 2023), and Ibrahim D, et al. (Ibrahim et al., 2023), who reported a predominance of the CTX-M gene, followed by the TEM gene, with an elevated level of the OXA-1 gene and the lowest level of the SHV gene that barely existed among E. coli isolates. Erbil city, the studies display a predominance of the TEM gene rather than SHV and CTX-M genes, which contrasts with the predominance of the genes in this study but with the same frequency as it (K Ahmed and A Hawezy, 2023; Pishtiwan and Khadija, 2019b). The disparity of these results is primarily motivated by the existence and horizontal transfer of certain types of plasmids including these genes; also, it's related to the difference in E. coli clones, and consequently, clonal expansion of multidrug-resistant E. coli lineages is an essential handler of the epidemiological success of ESBL genes among society. Still, the incidence of these genes' patterns reflects on the resistance pattern of the antibiotics as demonstrated in Table5, which revealed an abundant dissemination of CTX-

M/TEM and CTX-M/SHV/TEM in the first and second groups compared to the other groups, reflecting the essential role of these patterns in motivations of high resistance levels, proven by the significant correlation between the existence of CTX-M/SHV/TEM genes and the resistance pattern of tested beta-lactam antibiotics ($p< 0.001$). A study published in Qatar in 2018 (Eltai et al., 2018) reveals an abundant distribution of the CTX-M/TEM gene combination followed by CTX-M/TEM/SHV gene combinations in E. coli, which aligns with the finding of this study. When it comes to Iraq, a study by Alkhudhairy M, et al. (Alkhudhairy et al., 2019) stated a predominant dissemination of the CTX-M gene. While different findings were reported in two studies accomplished in Erbil city, the studies display a predominance of the TEM gene rather than SHV and CTX-M genes, which contrasts with the predominance of the genes in this study but with the same frequency as it (K Ahmed and A Hawezy, 2023; Pishtiwan and Khadija, 2019b). The disparity of these results is primarily motivated by the existence and horizontal transfer of certain types of plasmids including these genes; also, it's related to the difference in E. coli clones, and consequently, clonal expansion of multidrug-resistant E. coli lineages is an essential handler of the epidemiological success of ESBL genes among society. Still, the incidence of these genes' patterns reflects on the resistance pattern of the antibiotics as demonstrated in Table5 and Fig.3, which revealed an abundant dissemination of CTX-M/TEM and CTX-M/SHV/TEM in the first and second groups compared to the other groups, reflecting the essential role of these patterns in motivations of high resistance levels, proven by the significant correlation between the existence of CTX-M/SHV/TEM genes and the resistance pattern of tested beta-lactam antibiotics ($p< 0.001$).

Table5: Distribution of ESBL, Ampc, And OXA-1 Genes Among Antibiotic Resistance Pattern of ESBL Positive *E. Coli* Isolates

ESBL (+) *E. coli* isolates (no.=71)	n	SHV	TEM	CTX-M	SHV/ TEM	TEM/ CTX-M	SHV/TEM /CTX-M	OXA -1	AmpC	CM Y
AMC/CAZ/CTX/CRO/CPD/ FEP*	38	1	0	2	0	17	14	29	34	8
AMC/CAZ/CTX/CRO/CPD	23	0	4	2	0	15	2	18	23	4
AMC/CAZ/CTX/CPD	4	1	1	2	0	0	0	1	2	1
AMC/CAZ/CRO/FEP/CPD	1	0	1	0	0	0	0	0	1	0
AMC/CAZ/FEP/CPD	1	0	0	0	0	1	0	1	1	0
AMC/CAZ/CPD	4	0	1	0	2	1	0	2	4	2

*AMC: amoxicillin/clavulanic acid, CAZ: ceftazidime, CTX: cefotaxime, CRO: ceftriaxone, CPD: cefpodoxime, FEP:Cefepime

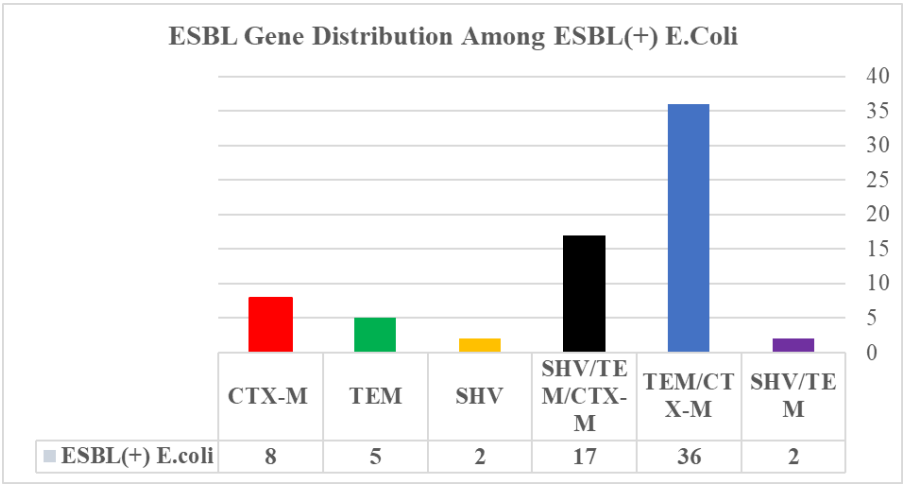

Figure3: Dissemination of ESBL Genes Among Phenotypically ESBL-Positive *E. Coli* Isolates.

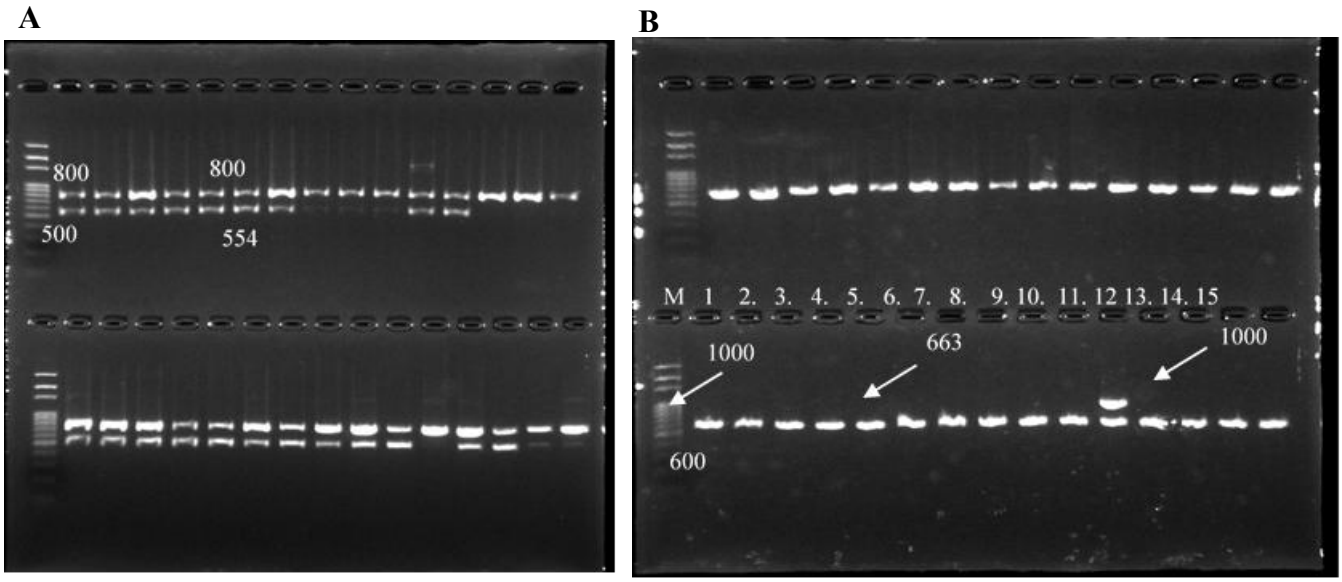

Figure4: Gel Electrophoresis Analysis of Multiplex PCR Products. (A) Multiplex PCR amplification of TEM-1 and CTX-M-1 genes. Lane M represents the 100 bp DNA molecular size marker; CTX-M-1 amplicon was observed at 554 bp, and TEM-1 at 800 bp.(B) Multiplex PCR amplification of pAmpC and CMY genes. Lane M represents the 100 bp DNA molecular size marker; pAmpC amplicon was detected at 634 bp, and CMY at 1000 bp.

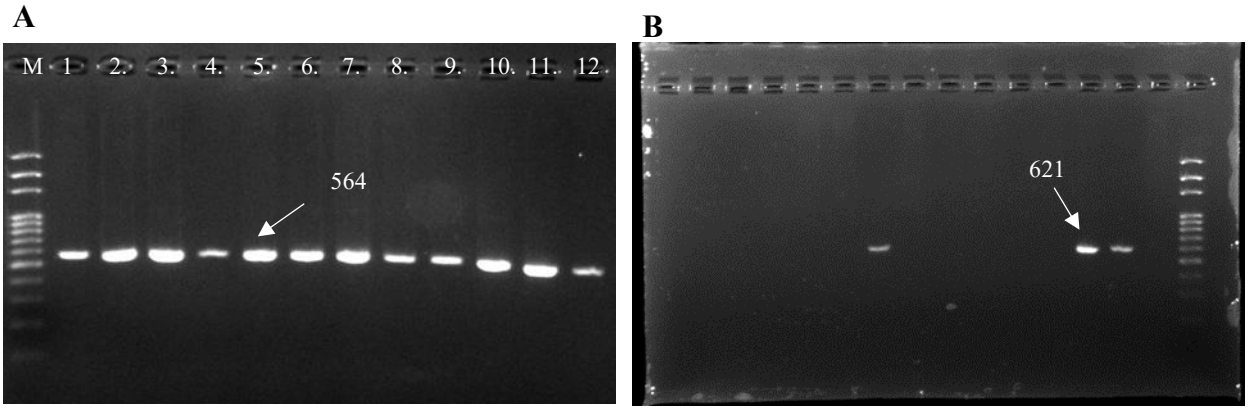

Figure5: Gel Electrophoresis Analysis of Monoplex PCR Products. (A) Monoplex PCR amplification of the OXA-1 gene. Lane M represents the 100 bp DNA molecular size marker; the OXA-1 amplicon was observed at 564 bp. (B) Monoplex PCR amplification of the NDM gene. Lane M represents the 100 bp DNA molecular size marker; the NDM amplicon was detected at the expected size.

Although what also has a significant role in such a strong resistance pattern is the occurrence of both the AmpC genes and the OXA-1 gene, which also incorporate increasing the complexity of the expected outcome, in other words, evolving a more sophisticated resistant action toward beta-lactam antibiotics. In this study, the frequency of the pAmpC gene was 68 (97%) among ESBL-producing isolates, although the CMY gene exhibited a low incidence, 13 (19%), among the E. coli isolates, which aligns with Perera P, et al. (Perera et al., 2022), which states that the predominance of the CMY gene was 20% among E. coli isolates. On the other hand, a strong significant association has been found between the existence of OXA-1 and amoxicillin/clavulanic acid resistance 53/82 (65%) compared to those isolates that showed resistance to

amoxicillin/clavulanic acid without harboring OXA-1 29/82 (35%) ($p< 0.001$), and all OXA genes were associated with the CTX-M gene in the isolates, which aligns with the result reviewed by Owusu F, et al.(Owusu et al., 2023). As the New Delhi Metallo-beta lactamase (NDM) poses a crucial and devastating public health threat in the context of antimicrobial resistance due to the extended hydrolyzing ability of them on beta-lactam antibiotics, cephalosporins, and the most significant carbapenem antibiotics, as it's considered the last therapeutic choice. In this study the prevalence of NDM was 6 (9%) out of ESBL-producing isolates and 5/17 (29%) out of carbapenem-resistant isolates. These findings were supported by those of Heshu J, et al. (Heshu J. Ahmed, 2023). Although other mechanisms accompany carbapenem resistance, this study was representing the demonstration of NDM among E. coli isolates, resistance among urinary tract infections in Iraq. The study emphasizes a considerable increase in the existence of CTX-M/TEM/OXA-1 and pAmpC genes among E. coli isolates and what's relay on the sophisticated resistance pattern of antibiotics as an outcome. Likewise, the existence of β-lactamase-encoding genes was accompanied by other antibiotic resistance mechanisms, as demonstrated by the limited number of antibiotics that display a high susceptibility level affordable as an alternative therapeutic choice. Which further applies challenging antibiotic stewardship strategies to restrict the outbreak of multidrug-resistant E. coli in Iraq. Fig.4A-B and Fig.5A-

4. Conclusion

In conclusion, this study highpoints the complexity of the co-occurrence of ESBL, pAmpC, and metallo-beta-lactamase genes in E. coli isolates and its contribution to the emerging problem of antimicrobial resistance among urinary tract infections in Iraq. The study emphasizes a considerable increase in the existence of CTX-M/TEM/OXA-1 and pAmpC genes among E. coli isolates and what's relay on the sophisticated resistance pattern of antibiotics as an outcome. Likewise, the existence of β-lactamase-encoding genes was accompanied by other antibiotic resistance mechanisms, as demonstrated by the limited number of antibiotics that display a high susceptibility level affordable as an alternative therapeutic choice. Which further applies challenging antibiotic stewardship strategies to restrict the outbreak of multidrug-resistant E. coli in Iraq.

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6. Ethics

This study was conducted after achieving the approval for the medical ethics committee from the Faculty of Medicine/Jabir Ibn Hayyan University for Medical and Pharmaceutical Sciences (2024). The patients' verbal and written consent approval was also provided for each participant.

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