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Evaluation of the Relationship Between Integrons and Class A and B Carbapenem Resistance Genes in Escherichia coli Isolated from Patients with Urinary Tract Infections

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Abstract

Recently Carbapenem resistant reports increased among E.coli isolates from patients with urinary tract infection. Otherwise integrons are genetic elements with ability of integration of resistance gene cassettes in their structure facilitating the dissemination of antibiotic resistance genes. The study highlighting the detection of carbapenem resistance genes in Escherichia coli and how they are affected by the bacterial integron from 200 patients with Urinary tract infection. This cross-sectional study was conducted in Al-Sadder Medical City in Al-Najaf from the first of September to end of December 2024. *E .coli* isolates were identified using conventional biochemical tests and VITEK-2®System, while biofilm formation tested by Congo red agar. Antimicrobial susceptibility and phenotypic detection of carbapenemase were done using VITEK-2 AST card with disk diffusion and mCIM methods respectively. The presence of carbapenemase gene (*bla-KPC*, *bla-GES*, *bla-NDM*, *bla-VIM* and *bla-IMP*), Int-1and Int-2 were examined by polymerase chain reaction. Approximately 49 (24.5%) isolates of *Eschericichia coli* were identified from which 38(78%) were biofilm producers. Antibiotic resistant recorded in low rate to imipenem (20.4%), meropenem (22%) and ertapenem (24%). All *E.coli* isolates were MDR 46(94%) except 3(6%) were XDR. The positive results of Modified Carbapenem inactivation method (mCIM) recorded in 13 isolates. The gene *bla-KPC* found in 6(46%) isolates, *bla-NDM* in 11(85%) isolates and *bla-VIM* in 3(23%) isolates. Neither *bla-GES* nor *bla-IMP* genes were found. All isolate have Int-1 while only 1 isolate have Int-2. The presence of integrons mainly class-1 encourage Carbapenem and multi-drug resistant, indicating their role in enhancing and spreading resistant genes.

تقييم العلاقة بين الإنتغرون وجينات مقاومة الكاربابينيم من الفئة A، B في الإشريكية القولونية المعزولة من المرضى المصابين بعدوى المسالك البولية

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الخلاصة

في الآونة الأخيرة، ازدادت التقارير حول مقاومة الكاربابينيم بين عزلات الإشريكية القولونية (E. coli) المعزولة من مرضى التهاب المسالك البولية.من جهة اخرى تُعَدّ الإنتجرونات عناصر وراثية قادرة على دمج أشرطة (كاسيتات) الجينات المقاومة للمضادات الحيوية ضمن بنيتها، مما يسهم في انتشار هذه الجينات. تهدف هذه الدراسة إلى تسليط الضوء على الكشف عن جينات مقاومة الكاربابينيم في E. coli ودراسة مدى تأثرها بوجود الإنتجرون البكتيري، وذلك في 200 مريض مصاب بالتهاب المسالك البولية. أُجريت هذه الدراسة المقطعية في مدينة الصدر الطبية في النجف، من الأول من أيلول وحتى نهاية كانون الأول 2024. تم تشخيص عزلات E. coli باستخدام الاختبارات الكيميائية الحيوية التقليدية ونظامVITEK-2® ، بينما تم الكشف عن إنتاج البايوفيلم باستخدام وسط Congo red agar. تم إجراء اختبارات الحساسية للمضادات الحيوية والكشف الظاهري عن إنتاج الكاربابينيميز باستخدام بطاقة VITEK-2 AST مع طريقة انتشار الأقراص (Disk diffusion) وطريقة mCIM على التوالي. تم فحص وجود جينات الكاربابينيميز(*bla-KPC* ، *bla-GES*، *bla-NDM*، *bla-VIM*، *bla-IMP*) وجيني الإنتجرون Int-1 و Int-2 بواسطة تفاعل البلمرة المتسلسل (PCR) .من بين العينات، تم التعرف على 49 عزلة من E. coli (24.5%)، كان منها 38 عزلة (78%) منتجة للبايوفيلم. سُجّلت معدلات منخفضة نسبيًا لمقاومة الكاربابينيم: الإيميبينيم 20.4%، الميروبينيم 22%، والإرتابينيم 24%. جميع العزلات كانت متعددة المقاومة للمضادات الحيوية MDR بنسبة 46 عزلة (94%)، باستثناء 3 عزلات (6%) كانت شديدة المقاومة XDR. أعطت طريقة mCIM نتائج إيجابية في 13 عزلة. وُجد جين-bla KPCفي 6 عزلات (46%)، وجين bla-NDM في 11 عزلة (85%)، وجين bla-VIM في 3 عزلات (23%)، بينما لم يُكتشف أي من جيني bla-GES أو bla- IMP . وُجد جين Int-1 في جميع العزلات، في حين وُجد جين Int-2 في عزلة واحدة فقط. تشير النتائج إلى أن وجود الإنتجرونات، وخاصةً من الصنف الأول، يساهم في تعزيز مقاومة الكاربابينيم والمقاومة المتعددة للمضادات الحيوية، مما يبرز دورها في انتشار جينات المقاومة

1. Introduction

Urinary tract infections considered one of the most significant bacterial infection that requiring antimicrobial treatment. Almost UTI infections were more in female rather than male (Ahmed and Hawezy, 2023). As many as 80-90% of UTIs from the community and 30-50% of UTIs from the hospital originates from *Escherichia coli* (Ahmed and Hawezy, 2023; Katongole et al., 2020). The exceptional abilities of *E .coli* to gain transposon, integron and plasmids combined with clustering of resistant genes could result in multidrug resistant phenomena, a significant condition due to the restricted number of medical treatment options (Pourbaghi et al., 2022). A variety of organism utilize the mechanism of biofilm formation as a way to survive. Uropathogenic bacteria that produce a biofilm may be the reason for recurrent infection with UTI contributing to their resistant to antibiotics and complicating the treatment of infections (Neupane et al., 2016). Carbapenem exhibit elevated level of activity against gram negative bacteria with multidrug resistance. Carbapenem hydrolysing enzymes represent one of the most mechanism for Carbapenem resistant in *E .coli* (Al-Sa'ady et al., 2020). The WHO defines Carbapenem resistant *E .coli* (CREC) as a member of the nine bacteria of global concern (Kazemian et al., 2019; Khalaf and Al-Mayahi, 2025). Carbapenemase genes were divided into three classes according to ambler classification A, B and D. Class A carbapenemase involved several genes located either on chromosome like SME, IMI-1, SFC-1and BIC-1 or plasmid like GES and KPC. This class require serine to become active and confer resistant to a variety of beta-lactam drug but still inhibited by clavulanic acid (Garsevanyan and Barlow, 2024). while class B called metallo-β-lactamase need zinc factor to become active conferring resistant to several beta lactam antibiotic but inhibited by ethylene di-amine tetra acetic acid (EDTA). The most prevalent genes in class B were VIM, IMP and NDM carbapenemase enzymes (Gurung et al., 2020; Mohanna and Al-Yasseen, 2024). ClassD carbapenemase are particularly dangerous in the clinic by conferring reisistance to Carbapenem, the antibiotic that are the last choice. Enzymes fron the 0XA-23, OXA-48, OXA-50 and OXA-58 are among the clinically significant family owing to their broad spread within bacterial pathogenes (Hirvonen et al., 2021) Integrons are non-motile genetic situated on transposon, chromosome and plasmids supporting their transmission among bacterial isolates promoting the spread of resistance (Khulaif and Al-Charrakh, 2023). When the incidence of Carbapenem resistant *E .coli* continues to increase worldwide, epidemiological tracking along with researches to understanding the methods behind these strains is necessary for the global control of Carbapenem Resistant Enterobacteriacae (CRE). This study aims to investigate the prevalence of class A and class B carbapenemse in relation with integron in *E .coli* isolated from Urinary tract infection in Al- Najaf, Iraq. The process employed phenotypic based investigation and antimicrobial resistant pattern to assess the prevalence of carbapenemase genes using polymerase chain reaction.

2.Patients, Materials and Methods

2.1. Sample Collection (200 samples)

Midstream urine samples were preferred to collect in a wide sterile container. The study participants involve inpatient and outpatient with clinical manifeatation of urinary tract infection

2.2. Questionnaire

The question are includes questions directed to the patient such as name, age, weight, other injuries, antibiotics taken,

2.3. Inclusion Criteria

Involve all patients with symptoms for urinary tract infection

2.4. Exclusion Criteria

The patient with negative growth from microbiology unit or those with mixed growth

2.5. Bacterial Isolation

The identification of bacterial strain started by culturing urine samples on Blood and MacConkey agar with the using of conventional biochemical tests (Indole, simmon citrate, oxidase and motility test) to differentiate gram negative from gram positive bacteria. Furthermmore confirmation of *E .coli* isolates were done using Eosin methylene blue agar and HiCrome UTI agar (Himedia, India). Final identification was done using VITEK-2 system ID card (BioMerieux – France).

2.6. Biofilm Formation Assay Using Congo Red Agar

An easy qualitative assay based on changes in colony colour in Congo red agar were used for detection of bacteria that have the ability to produce a biofilm. By applying Congo red dye (Himedia, India) (0.8 g/l) and glucose to brain heart infusion agar 37 g/L, the formation of biofilm has been examined. While non biofilm producers form pink colonies, biofilm producers reflects black, dry crystalline colonies.

2.7. Antibiotic Susceptibility Test

Susceptibility to various antimicrobials was measured using Vitek-2 system AST417 card and Kirby buaer disk method. The tested antibiotic were: Imipenem (IMP, 10µg), Meropenem (MEM, 10µg), Ertapenem (ERT, 10µg), Amoxicillin/Clavulanic Acid (AMC, 20\10µg), Cefepime (FEP), Ceftazidime (CAZ, 30µg), cefotaxime (CTX, 30µg), azteronam (ATM, 30µg), Ceftrixone (CRO, 30µg), Cefazolin (CZ, 30µg), Norfloxacin (NOR, 30µg), Fosfomycin (FOS, 200µg), Amikacin (AK, 30µg), Gentamicin(GN, 10µg), Ciprofloxacin (CIP), Cefuroxime (CXM, 30µg), Tetracycline (TET, 30µg), Piperacillin-tazobactam (TZP, 100/10µg), Trimethoprim-Sulfamethoxazole (TMP, 30µg) and Nitro-furantoin (NIT, 300µg). The interpretation of results were done according to CLSI guidelines (Humphries et al., 2021; Wayne and PA, 2007).

2.8. Colistin Broth Disk Elution Test

Firstly four tubes each containing 10 ml of Muller Hinton broth were prepared. Then colistin disk 10 µg were added to produce final concentration of 0,1,2,3 and 4µg/ml respectively. The tubes were left for 30 minutes to aid in the elution of colistin. Next, 50µl volume of bacterial suspension adjusted to 0.5 McFarland standard were added to each tube and incubated at 35°C for 24 hours. The MIC or minimum inhibitory concentrations described as the lowest concentration at which no visible growth occurs and interpretation employed CLSI of 2µg/ml as intermediate and 4µg/ml as resistant. (Humphries et al., 2021; Wayne and PA, 2007).

2.9. Modified Carbapenem In Activation Method (Mcim)

The mCIM is a new phenotypic test which was developed and conducted according to CLSI demands for detecting the presence of carbapenemase genes. A total of two milliliters of tryptone soy broth were added to tubes with few colonies of *E .coli* from overnight blood agar. Then 10 µg meropenem disk were applied to bacterial suspension and incubated for four hours at 35°C. After that the disks have been removed and placed on previously streaked Muller Hinton agar plates with sensitive control strain *E .coli* ATCC®25922. The plates were re-incubated overnight for 24 hrs at 35°C. The results were positive if a clear zone were formed within 15-16 mm or with the formation of pin point colonies within 16-18 mm.

2.10. PCR Amplification for Carbapenemase and Integron Genes

Carbapenemase and integron genes were detected by specific primers for bla KPC, bla GES, bla NDM, blaVIM, bla IMP, Int-1 and Int-2 from (Macrogen, Korea) as listed in Table1. The DNA was extracted using Silica membrane column (Hi pure) of the DNA extraction kit (Magen, China) and performed according to the protocol provided by the manufacturer. Then the extracted DNA assessed for purity by nanodrop spectrophotometer (Biodrop, USA). The monoplex PCR technique was done by making mixture of 4 µL FIREPol® Master Mix Ready to Load (Solis.biodyne, Tartu, Estonia), 3 µL of DNA template, 0.5 µL of each specific primer group, and 12 µL of nuclease-free water. The reaction conditions showed in Table2.

Table1: Primers Used in The Current Study From (Macrogen, Korea)

Classes	Gene	Primer	Oligo sequence (5'-3')	Product Size
Class A	bla_{KPC}	KPC-F	ATG TCA CTG TAT CGC CGT CT	893
		KPC-R	TTTTCA GAG CCT TAC TGC CC	
	bla_{GES}	GES-F	GCTTCATTCACGCACTATT	323
		GES-R	CGATGCTAGAAACCGCTC	
Class B	bla_{IMP}	IMP-F	TTGACACTCCATTTACDG	139
		IMP-R	GATYGAGAATTAAGCCACYCT	
	bla_{NDM}	NDM-F	GGTTTGGCGATCTGGTTTTC	621
		NDM-R	CGGAATGGCTCATCACGATC	
	bla_{VIM}	VIM-F	GATGGTGTTTGGTCGCATA	390
		VIM-R	CGAATGCGCAGCACCAG	
Integrons Genes	Int-1	Int-1-F	CAGTGGACATAAGCCTGTTC	160
		Int-1-R	CCCGAGGCATAGACTGTA	
	Int-2	Int-2-F	CACGGATATGCGACAAAAAGGT	789
		Int-2-R	GTAGCAAACGAGTGACGAAATG	

Table2: The Protocol Followed in PCR

Monoplex gene	Temperature					
	Initial denaturation	Cycle No.	denaturation	Annealing	Extension	Final extension
***bla*KPC**	94/5 min	35	94/1 min	60/1 min	72/1 min	72/10 min
bla GES	94/5 min	25	94/30 sec	50/30 sec	72/1 min	72/7 min
***bla*NDM**	94/10 min	32	94/30 sec	52/40 sec	72/50 sec	72/5 min
***bla*VIM**	94/10 min	36	94/30 sec	52/40 sec	72/50 sec	72/5 min
***bla*IMP**	95/10 min	30	95/40 sec	55/40 sec	72/1 min	72/10 min
***Int*-1**	94/5 min	35	94/30 sec	55/30 sec	72/1 min	72/7 min
***Int*-2**	94/5 min	30	94/30 sec	62/30 sec	72/1 min	72/10 min

2.11. Statistical Analysis

Analyzing of data and the design of graphs were done by utilizing Microsoft Excel software. Chi square test was used to assess the association between the presences of *E .coli* with gender, however, Fisher's exact test was used to investigate the relationship between *E .coli* and age groups of patients and also between the biofilm formation and type of multiple resistance using SPSS V26.

3. Results and Discussion

3.1. Distribution of E. coli According to Sex and Ages

A total of 200 urine samples were examined for the growth of *E. coli* in patients with urinary tract infection. After incubation overnight at 37°C, 49 isolates of *E. coli* were identified appearing as small and pink colonies on MacConkey agar based on lactose fermentation, green metallic sheen on Eosin methylene blue agar based on acid formation while give pink-purple color on HiCrome UTI agar based on chromogenic substrate cleaved by enzyme β-D-galactosidase produced by *E .coli* in the media. The isolates were further confirmed using VITEK 2 compact system GN ID card. A study on different clinical samples detect 49 isolates of *E coli* from urine sample, which is the same to our results (Ghaffoori and Suleiman, 2022). The frequent presence of *E. coli* was in the age group (63-71) years in 15 isolates and in female 32% more than male 14%. The results were statistically significant using Chi square test observing that *p* value was (0.003). This is because female was more prone to infection with UTI, often due to the shorter of women's urethra, which made the bacteria rapidly infect the bladder and causing disease in addition to their closeness to the rectum (Ghaffoori and Suleiman, 2022; Puca et al., 2019). Also, the association between *E. coli* and age groups observed to be significant (P-value: 0.05) by using Fisher's exact test as in Table3.

Table3: Observed the Distribution of E. coli According to Sex and Ages

Variable	Category	Total of specimen(n=200)	E coli(n=49)	P value
Gender	Male	80(40%)	11(14%)	0.003
	Female	120(60%)	38(32%)	
Age	18-26	12(6%)	5(42%)	<0.05
	27-35	13(6.5%)	3(23%)	
	36-44	34(17%)	6(18%)	
	45-53	35(17.5%)	8(23%)	
	54-62	61(30.5%)	9(15%)	
	63-71	37(18.5%)	15(41%)	
	72-80	8(4%)	3(38%)	

3.2. Biofilm Formation

Congo red agar was qualitative method employed to determine the formation of biofilm based on converting the red color of the medium at the site of bacterial colonies to black color after 24 hr. of incubation. The present study observed that 38 (78%) isolates of *E. coli* were biofilm producers. The dark color formed due to the presence of sucrose in the medium as shown in Fig.1. A study conducted by Katongole in. (2020) observe biofilm producing *E. coli* isolates in (62.5%), which is similar to our results (Katongole et al., 2020).

3.3. Antibiotic Susceptibility Test

The results of antibiotic susceptibility test were half gained from the disk method and the other from vitek AST card for all 49 isolates of *E. coli* recovered from urine samples. The results were summarized in Fig.2. The rate of resistant was 98% for cefazolin and cefuroxime, 96% for cefotaxime, 92% for ceftriaxone and cefotaxime, while resistance recorded to be 90% for amoxicillin-clavulanic acid followed by cefepeme 80%. However, the rate of resistance was 73% for tetracycline, 71% for aztreonam and norfloxacin, 67% for trimethoprime-sulfamethoxazole and piperacillin-tazobactam, and 59% for ciprofloxacin. The resistant to Carbapenem antibiotics were 20.4% for imipenem, 22% for meropenem and 24% for ertapenem. The lowest resistant recorded for nitrofurantoin 10%, colistin 2% and fosfomycin 0%. Approximately 13 isolates of *E. coli* showed resistant to imipenem and meropenem in 10 and 11 isolates respectively, which was similar to previous study by Haji in (2025) who reported both imipenem and meropenem in 10 isolates (Haji et al., 2025a).

Figure1: Growth of *Escherichia coli* on Congo Red Agar. Black bacterial colonies indicate a positive test, while red colonies indicate a negative test

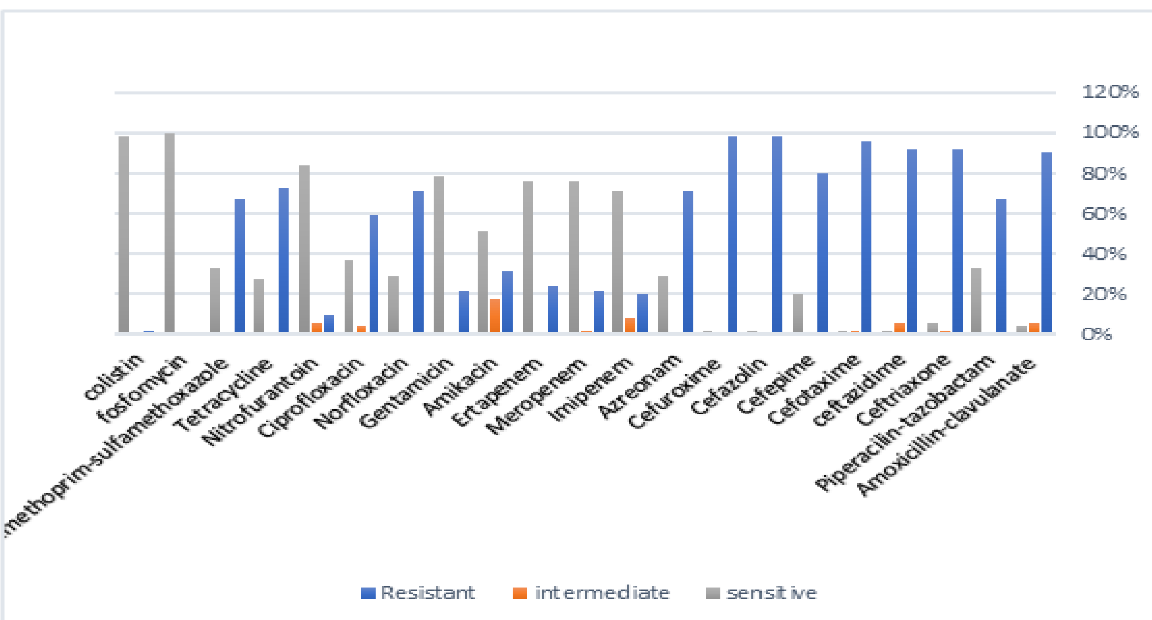

Figure2: Antibiotic susceptibility profile of *E. coli*

A cording to Centre for Disease Control and Prevention (CDC) and European Centre for Disease Control (ECDC). MDR meaning that isolates were resistant to three or more classes of antibiotics while XDR meaning that isolates showed resistant to at least one agent in each group but only two groups or less. However, PDR indicate the resistance to all antibiotics in all Magiorakos, A.P. *et al.* (2012) (Magiorakos et al., 2012; Poulikakos et al., 2014). The results of *E. coli*, showed that 46 (94%) and 3 (6%) of isolates were MDR and XDR respectively. The number of MDR and PDR in biofilm and non- biofilm producing *E. coli* isolates were showed in Table4. The study isolates showed that 38(78%) were biofilm producers from which 36 isolates were MDR and 2 isolates were XDR and 11 isolates were non-biofilm producers from which 10 isolates were MDR and 1 isolate was XDR. Statistical analysis using Fisher's exact test did not reveal statistically significant association (*p*-value: 0.707) between biofilm formation and resistance even though the majority of isolate were biofilm producers. One possible explanation might because many of the isolates had resistant genes that made it difficult to determine a statistically significant connection between antibiotic resistant and biofilm formation. Also previous investigations have suggest that the correlation between biofilm and resistance cannot always be direct. This could be affected by different things like how much the resistance genes has been expressed, the environment in which they grow and the characteristic features of bacterial strains (Azeredo et al., 2017; Poulikakos et al., 2014)

Table4: MDR and XDR distribution according to biofilm formation

Status (n)	MDR n (46)	XDR n (3)	P value
Biofilm producers (38)	36	2	0.707
Non- biofilm producers (11)	10	1	

3.4. Modified Carbapenem Inactivation Method (Mcim)

Phenotypic detection is critical for quickly identifying and stopping the dissemination of Carbapenem resistant organism. Therefore, 49 isolates of *E. coli* were tested by mCIM method. The protocol of mCIM was conducted in accordance with CLSI 2024 and the results revealed that 13 isolates confirmed to have carbapenemase genes. The results were considered positive if a clear zone were formed within 15-16 mm or with the formation of pin point colonies within 16-18 mm, while the isolates considered negative for the production of carbapenemase if their plates develop inhibition zone more than 19mm Fig.3.

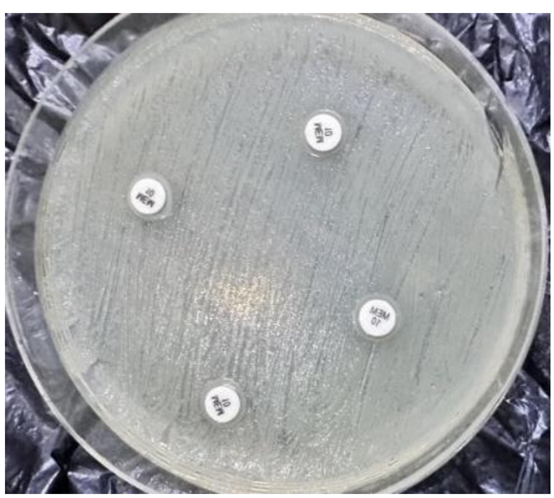

Figure3: Positive results of mCIM method, each disk represents one sample test

3.5. Detection of Carbapenemase and Integron Genes Using Polymerase Chain Reaction Technique

Bacterial DNA was extracted from 13 isolates of *E .coli* that confirmed to have carbapenemase genes by (mCIM). The results from molecular detection in *E .coli* as follows: bla NDM in 11 (85%) followed by bla KPC in 6 (46%) and bla VIM in 3(23%). Moreover, there is 7(54%) isolate of *E .coli* co-harbouring multiple carbapenemase genes with one *E .coli* isolate harboured 3 resistant genes as in Table5. Also the results showed that the tested genes were not found in one isolate of *E .coli*. Furthermore, integron-1 gene was found in all isolate with resistant phenotype to Carbapenem, while only 1 isolate have integron-2 in E .coli. Gel electrophoresis of agarose gel (in 1X TBE buffer) stained with ethidium bromide for 1 hours at 70 volt for bla-KPC, bla-NDM, bla-VIM and Int-1 were showed in Fig.4, 5, 6 and 7.

Table5: The Distribution of Resistant Gens in E. Coli Isolates

Number of *E .coli* isolate	Number of Genes per isolate	bla-genes	Int-1	Int-2
1	3	KPC, NDM and VIM	1	
4	2	KPC and NDM	4	
1	2	KPC and VIM	1	
1	2	VIM and NDM	1	
5	1	NDM	5	1
1	0	None	1	

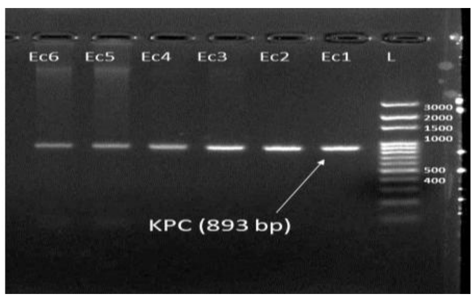

Figure4: Agarose gel electrophoresis of bla-KPC (893bp), lane L, 100 bp ladder, other lanes positive for bla-KPC

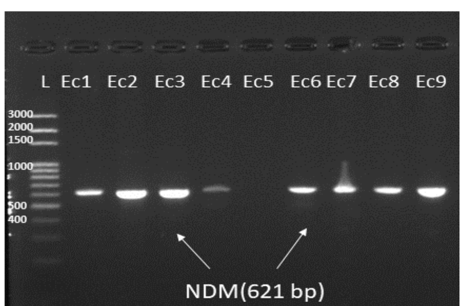

Figure5: Agarose gel electrophoresis of bla-NDM (621bp), lane L, 100 bp ladder, other lanes positive for bla-NDM.

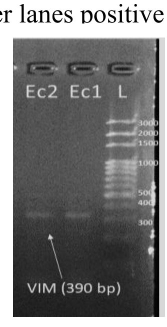

Figure6: Agarose gel electrophoresis of bla-VIM (390bp), lane L, 100 bp ladder, other lanes positive for bla-VIM

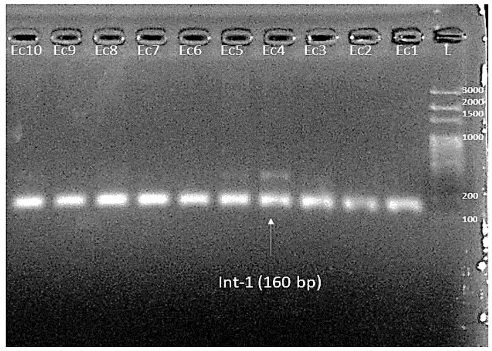

Figure7: Agarose gel electrophoresis of Int-1 (160bp), lane L, 100 bp ladder, other lanes positive for Int-1 gene

The emergence of gram-negative bacteria producing carbapenemase posed a significant threat to the health. Recently one of the main pathogen causing Carbapenem resistant was *E .coli*.The most critical problems attributed to *E .coli* include the increasing number of *E .coli* that are multi-drug resistance, the capability to transfer it's resistant genes to other bacterial species and the capability to colonize both human and animal gut conferring it's the characteristic ability to be used as genetic material donor to other bacteria (Ghaffoori Kanaan et al., 2020; Li et al., 2021; Sugawara et al., 2017). Monoplex PCR was done to 13 isolate of *E .coli*. The study recorded carbapenmase production by 12 isolates, this is slightly different from mCIM. For isolates in which no resistant gene was found, their phenotypic resistant to Carbapenem might be caused by other carbapenemase encoding genes that were not screened in the current study. PCR results showed that 7 (54%) isolate of *E .coli* found with multiple carbapenemase genes. These isolates recognized to show resistant to all types of Carbapenem. Among class A carbapenemase genes *bla-KPC* recorded in 6(46%) isolate of *E .coli*. This is the same with (Haji et al., 2025b) who recorded KPC gene in 5 isolates of *E .coli*. The gene *bla-NDM* have been spreading among bacterial species worldwide by horizontal gene transfer. In this study *bla-NDM* was reported in 11(85%) isolate of *E .coli*. A similar results reported by (Abas and Al-Hamdani, 2017) that detected *bla-NDM* gene in 14 isolates of *E .coli* isolated from different clinical sources, however, our results appear more than those in Erbil city (Ho et al., 2016; "Molecular Characterization of Carbapenem resistant Escherichia coli and Klebsiella pneumoniae in Erbil, Iraq," 2023) who reported NDM in 12.2% in *E .coli* isolates. The increasing number of strains producing NDM has been evolved towards a global issue for public health because the plasmid containing NDM contains accumulation of other genes resistant to all beta lactam, aminoglycoside and fluoroquinolone (Krawczyk et al., 2022). In addition Cetazidime avibactam sodium as a highly effective new agent towards KPC, OXA-48, ESBL and AmpC. Despite this, it is ineffective at combating MBL genes (Krawczyk et al., 2022; Zhou et al., 2022). This may be attributed in that NDM gene depend on zinc ions as a substrate. Other characteristic feature that sets NDM apart from other carbapenemsaes in that it has joined to gram negative's outer membrane. From that blaNDM genes and NDM enzymes from that it could be secreted using outer membrane vesicles conferring beta lactam resistant to the closer bacterial cells (Alvisi et al., 2025). The lowest prevalent of resistant to Carbapenem was for *bla-VIM* which appeared in three isolate of *E .coli* (23%). Likewise, a study conducted by Al-Mayahie in (2022) in Al-Kut City reported *bla-VIM* in 4 (10.5%). A similar observation recorded by Khalifa et al., (2021) [34] in Egypt who noted VIM gene in 35%. VIM gene was found on plasmid and gene cassette mainly class-1 integron. Upon screening of integron genes by PCR, all isolates of *E .coli* were found to harbor integron genes.

Several study reported that class 1 integron was predominant among *E .coli* isolates in 99.1%. A study such that conducted in Iran by Mohebi in (2023) revealed that class-1 integron is more prevalent than class-2 because integrase gene in class-2 become non-functional when it comes with stope codon at position 179 resulting in in activated amino acid polypeptide. The ongoing acquirement of antibiotic resistant genes mostly develop in plasmid has been triggered by integron facilitating 63.15% of antibiotic resistant transfer among plasmid. A lot of resistant gene sets can be assembled and transmitted into the genomic cassette through integrons allowing the resistant to several genes simultaneously. The existence of class-1 integron may be the key factor that explaining the prevalence of NDM along with multidrug resistant in *E .coli* isolates from UTI patients, facilitating the transmission of resistant genes (Al-Mayahie et al., 2022b; Caliskan-Aydogan and Alocilja, 2023; Mohebi et al., 2023; Wang et al., 2024; Zhang et al., 2024).

4. Conclusion

Our study highlight the prevalence of carbapenemase genes and integrons class1 and 2 among E .coli isolates in patients with urinary tract infection so as to understand the diversity of CREC. The isolate were resistant to most antibiotics including Carbapenem except 99osfomycin, colistin and nitrofurantoin being the most effective antibiotics. The prevalence of integron class 1in E .coli considered a serious risk factor for dissemination of resistance like Carbapenem resistant. The most recorded carbapenemase gene was bla-NDM then bla-KPC and less bla-VIM attributed to the presence of integron class 1 and also to the overdose and abuse of antibiotics in the absence of recommendations and suitable instructions regarding antibiotic administration.

References

Abas, I.J., Al-Hamdani, M.A., 2017. Carbapenem Resistance-Determining Genes Among Multi-Drug Resistant Bacterial Isolated From Clinical Samples in Basrah Governorate. European Journal of Biomedical and Pharmaceutical Sciences 4, 60–72.

Ahmed, K.K., Hawezy, A.A., 2023. Molecular detection of blaTEM, blaSHV, and blaCTX-M genes among Uropathogenic Escherichia coli isolated from cases with urinary tract infection in Erbil city-Iraq. Diyala Journal of Medicine 25. https://doi.org/10.26505/djm.v25i1.1021

Al-Mayahie, S.M.G., Al-Guranie, D.R.T., Hussein, A.A., Bacha, Z.A., 2022a. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic Escherichia coli phylogroup B2 isolates from outpatients in Wasit Province/ Iraq. PLOS ONE 17, e0262984. https://doi.org/10.1371/journal.pone.0262984

Al-Mayahie, S.M.G., Al-Guranie, D.R.T., Hussein, A.A., Bachai, Z.A., 2022b. Prevalence of common carbapenemase genes and multidrug resistance among uropathogenic Escherichia coli phylogroup B2 isolates from outpatients in Wasit Province/ Iraq. PLoS One 17. https://doi.org/10.1371/journal.pone.0262984

Al-Sa'ady, A.T., Mohammad, G.J., Hussen, B.M., 2020. Genetic relation and virulence factors of carbapenemase-producing Uropathogenic Escherichia coli from urinary tract infections in Iraq. Gene Rep 21. https://doi.org/10.1016/j.genrep.2020.100911

Alvisi, G., Curtoni, A., Fonnesu, R., Piazza, A., Signoretto, C., Piccinini, G., Sassera, D., Gaibani, P., 2025. Epidemiology and Genetic Traits of Carbapenemase-Producing Enterobacterales: A Global Threat to Human Health. Antibiotics. https://doi.org/10.3390/antibiotics14020141

Azeredo, J., Azevedo, N.F., Briandet, R., Cerca, N., Costa, A.R., Desvaux, M., Bonaventura, G. Di, Jaglic, Z., Kačániová, M., Knøchel, S., Mergulhão, F., Meyer, R.L., Nychas, G., Tresse, O., Sternberg, C., Azeredo, J., Azevedo, N.F., Briandet, R., Cerca, N., Costa, A.R., Desvaux, M., Bonaventura, G. Di, Hébraud, M., Kačániová, M., Knøchel, S., Lourenço, A., Mergulhão, F., Meyer, L., Nychas, G., Simões, M., Tresse, O., Sternberg, C., Rita, A., Di, G., Michel, H., Meyer, R.L., Nychas, G., Sim, M., Tresse, O., 2017. Critical Reviews in Microbiology Critical review on biofilm methods a. Crit Rev Microbiol 7828.

Caliskan-Aydogan, O., Alocilja, E.C., 2023. A Review of Carbapenem Resistance in Enterobacterales and Its Detection Techniques. Microorganisms. https://doi.org/10.3390/microorganisms11061491

Garsevanyan, S., Barlow, M., 2024. The Klebsiella pneumoniae carbapenemase (KPC) β-Lactamase Has Evolved in Response to Ceftazidime Avibactam. Antibiotics 13. https://doi.org/10.3390/antibiotics13010040

Ghaffoori, H.A., Suleiman, A.A., 2022. Isolation , Identification and antibiotic resistance profile distribution of clinical E . coli in Iraqi patients. Eurasian Medical Research Periodica 8.

Ghaffoori Kanaan, M.H., Al-Shadeedi, S.M.J., Al-Massody, A.J., Ghasemian, A., 2020. Drug resistance and virulence traits of Acinetobacter baumannii from Turkey and chicken raw meat. Comp Immunol Microbiol Infect Dis 70. https://doi.org/10.1016/j.cimid.2020.101451

Gurung, S., Kafle, S., Dhungel, B., Adhikari, N., Shrestha, U.T., Adhikari, B., Banjara, M.R., Rijal, K.R., Ghimire, P., 2020. Detection of oxa-48 gene in carbapenem-resistant escherichia coli and klebsiella pneumoniae from urine samples. Infect Drug Resist 13. https://doi.org/10.2147/IDR.S259967

Haji, S.H., Ganjo, A.R., Abdulaziz, S.M., Abdullah, Z.A., Smail, S.B., 2025a. Molecular Assessment Using the MASTDISCS® Combi D72C Set for the Phenotypic Detection of Extended-Spectrum Beta-Lactamases, AmpC Beta-Lactamases, and Carbapenemase Enzymes in Escherichia coli and Klebsiella pneumoniae. Cureus. https://doi.org/10.7759/cureus.77269

Haji, S.H., Ganjo, A.R., Abdulaziz, S.M., Abdullah, Z.A., Smail, S.B., 2025b. Molecular Assessment Using the MASTDISCS ® Combi D72C Set for the Phenotypic Detection of Beta-Lactamases , and Carbapenemase Enzymes in Escherichia coli and Klebsiella pneumoniae. Cureus 17, 1–11. https://doi.org/10.7759/cureus.77269

Hirvonen, V.H.A., Spencer, J., van der Kamp, M.W., 2021. Antimicrobial Resistance Conferred by OXA-48 β-Lactamases: Towards a Detailed Mechanistic Understanding. Antimicrob Agents Chemother 65, 1–18. https://doi.org/10.1128/AAC.00184-21

Ho, P.L., Cheung, Y.Y., Wang, Y., Lo, W.U., Lai, E.L.Y., Chow, K.H., Cheng, V.C.C., 2016. Characterization of carbapenem-resistant Escherichia coli and Klebsiella pneumoniae from a healthcare region in Hong Kong. European Journal of Clinical Microbiology and Infectious Diseases 35. https://doi.org/10.1007/s10096-015-2550-3

Humphries, R., Bobenchik, A.M., Hindler, J.A., Schuetz, A.N., 2021. Overview of Changes to the Clinical and Laboratory Standards Institute Performance Standards for Antimicrobial Susceptibility Testing, M100, 31st Edition. J Clin Microbiol. https://doi.org/10.1128/JCM.00213-21

Katongole, P., Nalubega, F., Florence, N.C., Asiimwe, B., Andia, I., 2020. Biofilm formation, antimicrobial susceptibility and virulence genes of Uropathogenic Escherichia coli isolated from clinical isolates in Uganda. BMC Infect Dis 20. https://doi.org/10.1186/s12879-020-05186-1

Kazemian, H., Heidari, H., Ghanavati, R., Ghafourian, S., Yazdani, F., Sadeghifard, N., Valadbeigi, H., Maleki, A., Pakzad, I., 2019. Phenotypic and Genotypic Characterization of ESBL-, AmpC-, and Carbapenemase-Producing Klebsiella pneumoniae and Escherichia coli Isolates. Medical Principles and Practice 28. https://doi.org/10.1159/000500311

Khalaf, M.M., Al-Mayahi, F.S.A., 2025. Phenotypic and genotypic characterization of carbapenemase-producing Escherichia coli clinical isolates in Thi-Qar, Iraq. Iran J Microbiol 17. https://doi.org/10.18502/ijm.v17i2.18387

Khulaif, M., Al-Charrakh, A., 2023. Detection of class 1 integron and antibiotic resistance of β-lactamase-producing Escherichia coli isolated from four hospitals in Babylon, Iraq. Medical Journal of Babylon 20. https://doi.org/10.4103/MJBL.MJBL_155_23

Krawczyk, B., Wysocka, M., Michalik, M., Gołębiewska, J., 2022. Urinary Tract Infections Caused by K. pneumoniae in Kidney Transplant Recipients – Epidemiology, Virulence and Antibiotic Resistance. Front Cell Infect Microbiol. https://doi.org/10.3389/fcimb.2022.861374

Li, F., Ye, K., Li, X., Ye, L., Guo, L., Wang, L., Yang, J., 2021. Genetic characterization of Carbapenem-Resistant Escherichia coli from China, 2015–2017. BMC Microbiol 21. https://doi.org/10.1186/s12866-021-02307-x

Magiorakos, A.P., Srinivasan, A., Carey, R.B., Carmeli, Y., Falagas, M.E., Giske, C.G., Harbarth, S., Hindler, J.F., Kahlmeter, G., Olsson-Liljequist, B., Paterson, D.L., Rice, L.B., Stelling, J., Struelens, M.J., Vatopoulos, A., Weber, J.T., Monnet, D.L., 2012. Multidrug-resistant, extensively drug-resistant and pandrug-resistant bacteria: An international expert proposal for interim standard definitions for acquired resistance. Clinical Microbiology and Infection 18. https://doi.org/10.1111/j.1469-0691.2011.03570.x

Mohanna, Z.A., Al-Yasseen, A.K., 2024. Distribution of Carbapenemase Genes among Carbapenem-Resistant Klebsiella pneumoniae Isolates from the Patients in Najaf, Iraq. Biomedical and Biotechnology Research Journal 8. https://doi.org/10.4103/bbrj.bbrj_212_24

Mohebi, S., Golestani-Hotkani, Z., Foulad-Pour, M., Nazeri, P., Mohseni, F., Hashemizadeh, Z., Moghani-Bashi, Z., Niksefat, N., Rastegar, S., Khajedadian, M., Lotfian, Z., Hosseini-Nave, H., 2023. Characterization of integrons, extended spectrum beta lactamases and genetic diversity among uropathogenic Escherichia coli isolates from Kerman, south east of Iran. Iran J Microbiol 15. https://doi.org/10.18502/ijm.v15i5.13867

Molecular Characterization of Carbapenem resistant Escherichia coli and Klebsiella pneumoniae in Erbil, Iraq, 2023. . Journal of Population Therapeutics and Clinical Pharmacology 30. https://doi.org/10.47750/jptcp.2023.30.04.044

Neupane, S., Pant, N.D., Khatiwada, S., Chaudhary, R., Banjara, M.R., 2016. Correlation between biofilm formation and resistance toward different commonly used antibiotics along with extended spectrum beta lactamase production in uropathogenic Escherichia coli isolated from the patients suspected of urinary tract infections visiting Shree Birendra Hospital, Chhauni, Kathmandu, Nepal. Antimicrob Resist Infect Control 5. https://doi.org/10.1186/s13756-016-0104-9

Poulikakos, P., Tansarli, G.S., Falagas, M.E., 2014. Combination antibiotic treatment versus monotherapy for multidrug-resistant, extensively drug-resistant, and pandrug-resistant Acinetobacter infections: a systematic review. European Journal of Clinical Microbiology and Infectious Diseases. https://doi.org/10.1007/s10096-014-2124-9

Pourbaghi, E., Doust, R.H., Rahbar, M., Farzami, M.R., 2022. Investigation of OXA-23, OXA-24, OXA-40, OXA-51, and OXA-58 Genes in Carbapenem-Resistant Escherichia coli and Klebsiella pneumoniae Isolates from Patients with Urinary Tract Infections. Jundishapur J Microbiol 15. https://doi.org/10.5812/jjm-119480

Puca, Entela, Bitri, S., Puca, Edmond, 2019. An overview of urinary tract infection in diabetic patients. Endocrine Abstracts. https://doi.org/10.1530/endoabs.63.p985

Sugawara, Y., Akeda, Y., Sakamoto, N., Takeuchi, D., Motooka, D., Nakamura, S., Hagiya, H., Yamamoto, N., Nishi, I., Yoshida, H., Okada, K., Zin, K.N., Aye, M.M., Tonomo, K., Hamada, S., 2017. Genetic characterization of blaNDM-harboring plasmids in carbapenem-resistant Escherichia coli from Myanmar. PLoS One 12. https://doi.org/10.1371/journal.pone.0184720

Wang, X., Zhang, H., Yu, S., Li, D., Gillings, M.R., Ren, H., Mao, D., Guo, J., Luo, Y., 2024. Inter-plasmid transfer of antibiotic resistance genes accelerates antibiotic resistance in bacterial pathogens. ISME Journal 18. https://doi.org/10.1093/ismejo/wrad032

Wayne, PA, 2007. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. 17th informational supplement. Wayne, PA: CLSI.

Zhang, Y., Li, W., Wu, Y., Tian, X., Li, G., Zhou, Y., Sun, J., Liao, X., Liu, Y., Wang, Y., Yu, Y., 2024. Chitosan oligosaccharide accelerates the dissemination of antibiotic resistance genes through promoting conjugative plasmid transfer. J Hazard Mater 469. https://doi.org/10.1016/j.jhazmat.2024.133922

Zhou, S., Ren, G., Liu, Y., Liu, X., Zhang, L., Xu, S., Wang, T., 2022. Challenge of evolving Klebsiella pneumoniae infection in patients on hemodialysis: from the classic strain to the carbapenem-resistant hypervirulent one. Int J Med Sci. https://doi.org/10.7150/ijms.69577