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ORIGINAL ARTICLE

Phenotypic and genotypic characterization of carbapenemase-producing *Escherichia coli* clinical isolates in Thi-Qar, Iraq

Moslim Mohsin Khalaf[1,2*], Firas Srhan Abd Al-Mayahi[2]

[1]*Department of Pathological Analysis, College of Applied Medical Sciences, Al-Shatrah University, Thi-Qar, Iraq*
[2]*Department of Biology, College of Science, University of Al-Qadisiya, Al-Qadisiya, Iraq*

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ABSTRACT

Background and Objectives: The emergence of carbapenem resistance in *Escherichia coli (E. coli)* poses an urgent threat. The study aims to assess carbapenem resistance and the presence of carbapenemase genes in *E. coli* clinical isolates from Thi-Qar Hospital, Iraq.

Materials and Methods: A total of 2203 specimens were collected from patients at two hospitals between January and October 2024. *E. coli* was identified via biochemical tests and confirmed with the Vitek2® system. Antibiotic sensitivity was evaluated using disc diffusion, and carbapenemase production was investigated through combined disc tests (CDT) and modified Hodge tests (MHT). PCR was used to detect carbapenemase genes.

Results: Out of 2203 specimens, 1212 (55.02%) exhibited bacterial growth, with *E. coli* accounting for 15.35% (186/1212) of isolates. Among these, 40 (21.51%) were resistant to at least one carbapenem. CDT identified 10, and MHT identified 1 as a carbapenemase producer. The most detected gene was bla_{NDM} (60.00%), followed by bla_{OXA} (40.00%) and bla_{OXA-48} (15.00%). bla_{OXA-51} and bla_{VIM} were found in 5.00% of isolates each. No bla_{KPC}, bla_{NMC}, bla_{IMI}, bla_{GES}, bla_{SPM}, bla_{GIM}, or bla_{SIM} was detected.

Conclusion: The high prevalence of carbapenem resistance and the corresponding encoding genes in *E. coli* in Thi-Qar province pose a concerning challenge for managing serious infections caused by this pathogen.

Keywords: Antibiotic resistance; Beta-lactam resistance; Carbapenems; Doripenem; *Escherichia coli*; Imipenem; Meropenem

INTRODUCTION

Antimicrobial resistance is an increasingly significant health issue due to the improper use of antibiotics in recent decades (1). Until recently, carbapenems were considered one of the last resort antibiotics for managing multi-drug resistant Gram-negative infections (2). These antibiotics belong to the β-lactam class and have broad-spectrum activity due to their distinct chemical structure, composed of a carbapenem linked to a β-lactam ring which renders them resistance and slow hydrolysis by many β-lactamase enzymes (3). The emergence of carbapenem-resistant bacteria is of significant concern due to the limited choice of antimicrobials available for treating infections caused by them (4).

*Corresponding author: Moslim Mohsin Khalaf, M.Sc, Department of Pathological Analysis, College of Applied Medical Sciences, Al-Shatrah University, Thi-Qar, Iraq; Department of Biology, College of Science, University of Al-Qadisiya, Al-Qadisiya, Iraq. Tel: +9647809818106 Email: moslimmohsin@shu.edu.iq

Although *Escherichia coli (E. coli)* is a part of normal intestinal flora, some strains are pathogenic and can cause intestinal and/or extra-intestinal diseases in hosts (5). Unfortunately, *E. coli* has developed extensive antibiotic resistance, particularly to the β-lactam class (6). In a recent global surveillance report by the World Health Organization (WHO), carbapenem resistance *E. coli* is one of the nine bacteria of international concern (7). Resistance to carbapenem can be mediated by various mechanisms, including the production of carbapenemase enzymes or other β-lactamases, along with porin deficiency (8). Production of carbapenemases is the main resistance mechanism (9). Carbapenemases are defined as a diverse group of β-lactamase enzymes that can hydrolyze and inactivate carbapenems and they belong to different Ambler classes (A, B, and D) (10). Therefore, rapid recognition of carbapenem-resistant strains is vital for clinical and public health. Carbapenem resistance may be identified through phenotypic (such as modified Hodge test and EDTA-based inhibition tests) or molecular techniques (11).

Due to limited data, this study aims to investigate the prevalence of carbapenem resistance and identify carbapenemase-encoding genes in *E. coli* clinical isolates from Thi-Qar province, Iraq.

MATERIALS AND METHODS

Specimen collection and bacterial isolates. The study involved 2203 clinical specimens obtained from two main hospitals in Thi-Qar, Iraq (Al-Hussein Teaching Hospital and Al-Nasiriyah Teaching Hospital) from January to October 2024. The clinical specimens for this study were collected from hospital microbiology laboratories, with patient consent for research use. All patient data remained anonymous. Each specimen was transferred to the microbiology laboratory/college of dentistry/Thi-Qar University for processing as described by (12). The specimens were distributed as follows: 814 (36.95%) urine, 516 (23.42%) blood, 495 (22.47%) sputum, 223 (10.12%) body fluids, 72 (3.27%) swabs from various regions, 58 (2.63%) wound and burn exudates, and 25 (1.13%) cerebrospinal fluid. All specimens were collected in sterile containers. A loopful of each specimen was immediately streaked onto a blood agar plate (Himedia, India), a MacConkey agar plate (Oxoid, UK), and a Methylene Blue Eosin agar plate (Himedia, India). The plates were then incubated aerobically for 24 hours at 37°C. *E. coli* isolate identification was based on growth characteristics, Gram reactions, and routine standard biochemical tests according to the schemes provided by (13). In addition, *E. coli* isolates also underwent confirmatory identification using the Vitek2® system (BioMerieux, France).

***E. coli* antibiotic sensitivity testing.** The antibiotic sensitivity test of each *E. coli* isolate was assessed using the Kirby-Bauer disc diffusion method (14) on Mueller-Hinton agar (Oxoid, UK), with the results being interpreted according to CLSI (CLSI, 2023) guidelines (15). The antibiotics used includes: ampicillin (25 µg), amoxicillin-clavulanic acid (20/10 µg), ampicillin/sulbactam (10/10 µg), ceftazidime (30 µg), cefotaxime (30 µg), ceftriaxone (30 µg), cefepime (10 µg), cefoxitin (30 µg), aztreonam (30 µg), imipenem (10 µg), meropenem (10 µg), doripenem (10 µg), amikacin (10 µg), gentamicin (10 µg), tobramycin (10 µg), ciprofloxacin (5 µg), levofloxacin (5 µg), nalidixic acid (30 µg), doxycycline (10 µg), tetracycline (30 µg), trimethoprim (10 µg), chloramphenicol (10 µg), nitrofurantoin (100 µg). These were obtained from Bioanalyse/Turkey.

Phenotypic screening of carbapenemases. Carbapenemase production was assessed in *E. coli* isolates that revealed non-susceptibility (resistance or intermediate susceptibility) to carbapenem antibiotics by using a combined disk test (CDT) as described by (16) and Modified Hodge Test (MHT) (17).

DNA extraction and genotypic screening of carbapenemase genes. DNA was extracted from carbapenem-resistant *E. coli* isolates using the Presto™ Mini gDNA Bacteria Kit (Geneaid, USA). PCR was performed for carbapenemase genes with primers indicated in Table 1.

PCR was performed using Go Taq Green Master mix (Promega, USA). Following the manufacturer's guidelines, 4 µl of template DNA (~100 ng/µl), 2 µl of each primer (10 pmoles/µl), and 4.5 µl of nuclease-free water were mixed with 12.5 µl of Master Mix to achieve a final volume of 25 µl. The optimal condition is identified depending on their reference procedure, the program specific to each single primer set is indicated in Table 2.

The presence of the carbapenemase genes was verified by PCR apparatus (Bioneer, Korea). All com-

Table 1. Forward and reverse primers used for detecting carbapenemase genes (Macrogen, Korea).

Class	Gene name	Primer name	Sequence (5`→3`)	Product size (bp)	Reference
A	*bla*$_{KPC}$	KPC-F	ATGTCACTGTATCGCCGTCT	540	(18)
		KPC-R	TTTTCAGAGCCTTACTGCCC		
	bla$_{NMC}$	NMC-F	GCATTGATATACCTTTAGCAGAGA	371	(19)
		NMC-R	CGGTGATAAAATCACACTGAGCATA		
	bla$_{IMI}$	IMI-F	CCATTCACCCATCACAAC	440	(20)
		IMI-R	CTACCGCATAATCATTTGC		
	bla$_{GES}$	GES-F	ATGCGCTTCATTCACGCAC	846	(21)
		GES-R	CTATTTGTCCGTGCTCAGG		
B	*bla*$_{NDM}$	NDM-F	GGTTTGGCGATCTGGTTTTC	621	(22)
		NDM-R	CGGAATGGCTCATCACGATC		
	bla$_{IMP}$	IMP-F	CATGGTTTGGTGGTTCTTGT	448	(22)
		IMP-R	ATAATTTGGCGGACTTTGGC		
	bla$_{VIM}$	VIM-F	GATGGTGTTTGGTCGCATA	390	(22)
		VIM-R	CGAATGCGCAGCACCAG		
	bla$_{GIM}$	GIM-F	TCGACACACCTTGGTCTGAA	477	(23)
		GIM-R	AACTTCCAACTTTGCCATGC		
	bla$_{SPM}$	SPM-F	GGGTGGCTAAGACTATGAAGCC	447	(24)
		SPMR	GCCGCCGAGCTGAATCGG		
	bla$_{SIM}$	SIM-F	TACAAGGGATTCGGCATCG	250	(23)
		SIM-R	TAATGGCCTGTTCCCATGTG		
D	*bla*$_{OXA}$	OXA-F	GGCACCAGATTCAACTTTCAAG	564	(25)
		OXA-R	GACCCCAAGTTTCCTGTAAGTG		
	bla$_{OXA-48}$	OXA-48-F	TTGGTGGCATCGATTATCGG	744	(24)
		OXA-48-R	GAGCACTTCTTTTGTGATGGC		
	bla$_{OXA-51}$	OXA-51-F	TAATGCTTTATCGGCCTTG	353	(26)
		OXA-51-R	TGGATTGCACTTCATCTTGG		

Table 2. The optimum condition for detection of carbapenemase genes.

Gene	Initial denaturation	Cycling conditions			Final extension	Cycles
		Denaturation	Annealing	Extension		
bla$_{KPC}$	95/15 min	94/30 sec	57/1 min	72/90 sec	72/10 min	30
bla$_{NMC}$	94/10 min	95/1 min	52/30 sec	72/1 min	72/6 min	35
bla$_{IMI}$	94/5 min	95/1 min	52/30 sec	72/1 min	72/5 min	30
bla$_{GES}$	93/3 min	93/1 min	55/1 min	72/1 min	72/7 min	40
bla$_{NDM}$	94/10 min	94/60 sec	60/30 sec	72/30 sec	72/10 min	35
bla$_{IMP}$	94/10 min	94/60 sec	60/30 sec	72/30 sec	72/10 min	35
bla$_{VIM}$	94/10 min	94/60 sec	60/30 sec	72/30 sec	72/10 min	35
bla$_{GIM}$	95/2 min	95/30 sec	59/30sec	72/80 sec	72/5 min	30
bla$_{SPM}$	95/15 min	94/30 sec	60/60 sec	72/90 sec	72/5 min	30
bla$_{SIM}$	95/2 min	95/30 sec	58/30 sec	72/1 min	72/5 min	30
bla$_{OXA}$	94/10 min	94/40 sec	60/40 sec	72/1 min	72/5 min	30
bla$_{OXA-48}$	94/5 min	94/1 min	50/1 min	72/1 min	72/10 min	35
bla$_{OXA-51}$	94/3 min	94/30 sec	57/30 sec	72/36 sec	72/5 min	35

ponents for the PCR were collected in the PCR tube and mixed under sterile conditions in an ice bag. The amplified PCR product was separated using a 1% agarose gel on the electrophoresis unit (FSF-SPBT, UK) and observed under an ultraviolet transilluminator (E-Graph, Japan).

Statistical analysis. Data analysis was conducted using SPSS-24 (IBM, Chicago, USA), and Chi-square analysis was used to evaluate the data.

RESULTS

Bacterial isolates. Of 2203 clinical samples, 1212 (55.02%) were positive for bacterial culture, while 991 (44.98%) clinical specimens showed no bacterial growth. Among all positive bacterial cultures, *E. coli* was the second most commonly cultured bacterium, representing 15.36% (186/1212) of all isolates. All of these *E. coli* isolates were identified morphologically and biochemically by the Vitec2® system.

Demographic characteristics of the study population. The distribution of *E. coli* within collected samples is indicated in Table 3. Urine samples were a major source of *E. coli* isolates (63.98%); the remaining isolates were recovered as a culture of sputum (19.35%), body fluid (7.53%), swabs from different sources (4.30%), blood (2.15%), wound and burns exudates (2.15%), and cerebrospinal fluid (0.54%). The study population comprised females (53.23%) and males (46.77%). Patients above the age of 60 years had the highest rate of *E. coli* infections (34.41%), followed by those between the ages of 21-40 years (26.88%). The majority of the *E. coli* infections were community-acquired infections (54.30%), while health-care-associated *E. coli* infections represented (45.70%).

Table 3. Prevalence and demographic characteristics of patients with *E. coli* infections (n=186).

Variable	Category	No. of *E. coli* (%)
Type of specimens	Blood Body fluids	4 (2.15%)
	Cerebrospinal Fluid	14 (7.53%)
	Sputum	1 (0.54%)
	Swabs	36 (19.35%)
	Urine	8 (4.30%)
	Wounds and burns	119 (63.98%)
	<1	4 (2.15%)
Age (Years)	1-20	5 (2.69%)
	21-40	27 (14.52%)
	41-60	50 (26.88)
	>60	40 (21.51)
	Female	64 (34.41)
Gender	Male	99 (53.23)
	Inpatient	87 (46.77)
Hospitalization	Outpatient	85 (45.70)
		101 (54.30)

Carbapenem-resistant *E. coli* isolates. The majority of carbapenem-resistant *E. coli* isolates were collected from urine (27, 67.50%) followed by sputum (6, 15.00%), body fluids (5, 12.5%), swabs (1, 2.50%) and cerebrospinal fluid (1, 2.50%). While no carbapenem resistance isolates were identified in wound and burn exudates and blood. There was no statistical association ($P<0.05$) between the type of specimens and the rate of carbapenem-resistant *E. coli* (Table 4).

Resistance susceptibility test of carbapenem-resistant *E. coli* isolates. Antibiotic susceptibility data for the 186 *E. coli* isolates revealed that 40 (21.62%) were resistant to carbapenems used (imipenem, meropenem, and doripenem). Among these isolates, 21 (52.50%) of 40 carbapenem-resistant isolates exhibited resistance to imipenem, 22 (55.00%) to meropenem, and 32 (80.00%) to doripenem (Fig. 1).

The sensitivity results for other antibiotic used showed high resistance of *E. coli* for ampicillin, cefotaxime (100% each of them), ceftazidime, ceftriaxone (97.50% each of them), cefepime (95.00%), trimethoprim (87.50%), nalidixic Acid (85.00%), amoxicillin-clavulanic acid (82.50%), amikacin, gentamicin, ciprofloxacin, levofloxacin, tetracycline (80.00% each of them), tobramycin (72.50%), doxycycline (70.00%), aztreonam (67.50%), nitrofurantoin (65.00%); and moderate resistance for cefoxitin (57.50%), ampicillin-sulbactam (55.00%); while low resistance for chloramphenicol (32.50%).

Phenotypic detection of carbapenemase production. All the carbapenem-resistant *E. coli* (40, 21.62%) isolates detected were further subjected to carbapenemase production detection phenotypically by CDT and MHT. The result revealed that 10 (25.00%) of *E. coli* isolates were possible carbapenemase producers by CDT, while only one of the *E. coli* isolates (2.50%) was carbapenemase producer by MHT.

Table 4. Distribution of carbapenem-resistant *E. coli* isolates from different clinical specimens (n=40).

Type of specimen	No. specimens	No. of *E. coli* isolates (%)	No. of Carbapenem resistant *E. coli* (%)
Blood	516	4 (0.18)	0 (0.00)
Body fluids	223	14 (0.64)	5 (35.71)
Cerebrospinal fluid	25	1 (0.05)	1 (100.00)
Sputum	495	36 (1.63)	6 (16.67)
Swab	72	8 (0.36)	1 (12.50)
Urine	814	119 (5.40)	27 (22.69)
Wounds and burns	58	4 (0.18)	0 (0.00)
Total	2203	186 (8.44%)	40 (21.51%)
Chi-square	X^2= 8.50, D.f.=6, P≤0.05		Significant P = 0.20

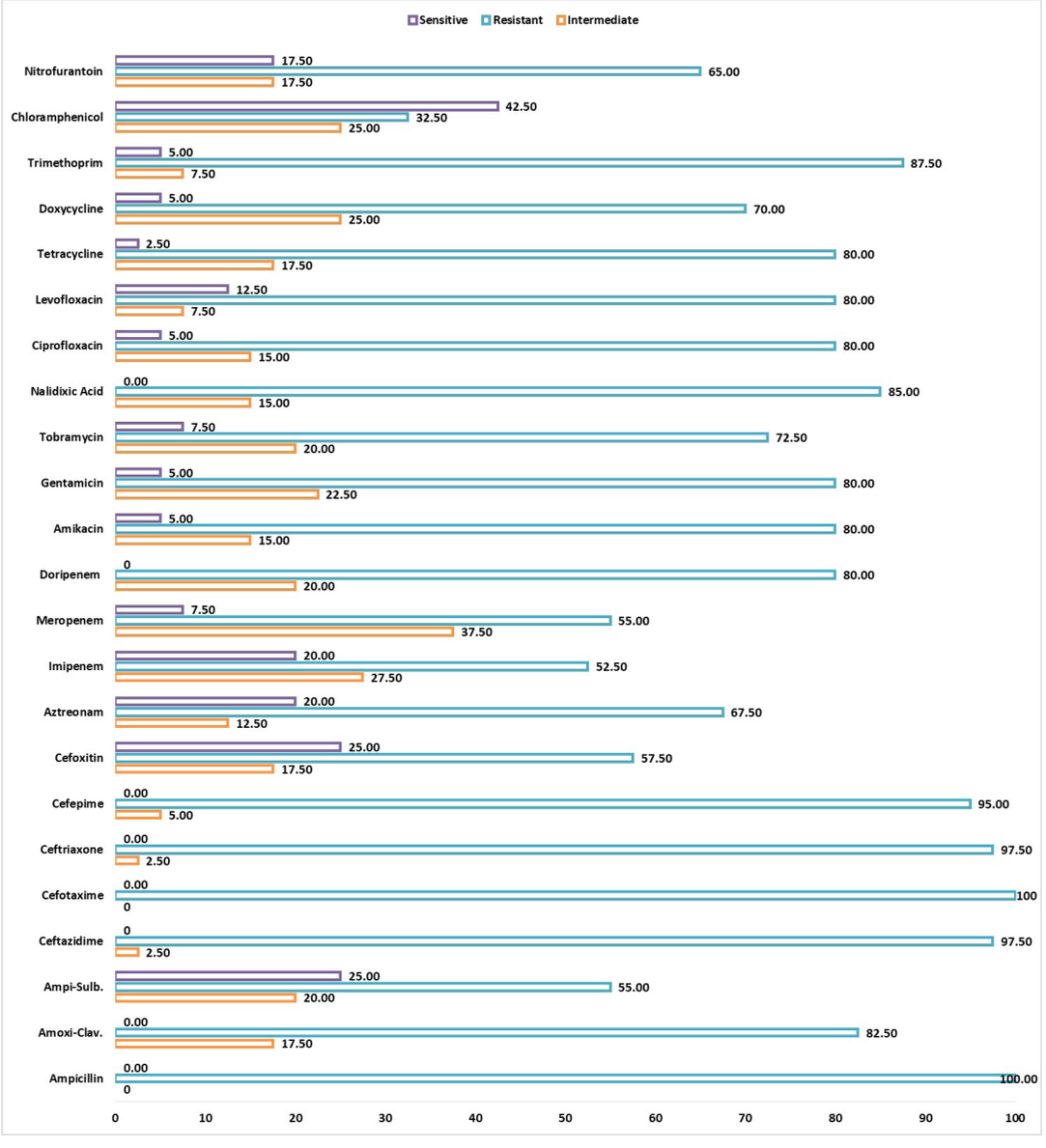

Fig. 1. Antibiotic sensitivity test of the *E. coli* isolates (n=40).

Genotypic detection of carbapenem-resistant genes. Based on PCR results obtained, 34 (85.00%) of the carbapenem-resistant *E. coli* isolates carried carbapenemase genes that were heterogeneously distributed, while 6 (15.00%) of the isolates did not carry any of carbapenemase genes tested. Predominantly, bla_{NDM} was the most frequent 24 (60.00%), followed by bla_{OXA} 16 (40.00%) and bla_{OXA-48} 6 (15.00%), 2 (5.00%) isolates harbor both bla_{OXA-51} and bla_{VIM}. No PCR-amplification products were noticed with bla_{KPC}, bla_{NMC}, bla_{IMI}, bla_{GES}, bla_{SPM}, bla_{GIM}, and bla_{SIM} (Table 5). Figs. 2-6 show PCR amplification for gene products.

Coproduction of these enzymes occurred among 18 (52.94%) isolates. The combination of bla_{NDM} and bla_{OXA} was the most frequent (11, 61.11). Other combinations included bla_{VIM}-bla_{OXA} and bla_{OXA}-bla_{OXA-48} each present in 2 isolates (11.11%). A single combination was observed for bla_{VIM}-bla_{NDM}.

Table 5. Distribution of carbapenemase gene among *E. coli* isolate (n=40).

Carbapenemase gene	No (%) of isolates
bla_{KPC}	0
bla_{NMC}	0
bla_{IMI}	0
bla_{GES}	0
bla_{NDM}	24 (60.00%)
bla_{IMP}	0
bla_{VIM}	2 (5.00%)
bla_{GIM}	0
bla_{SPM}	0
bla_{SIM}	0
bla_{OXA}	16 (40.00%)
bla_{OXA-48}	6 (15.00%)
bla_{OXA-51}	2 (5.00%)

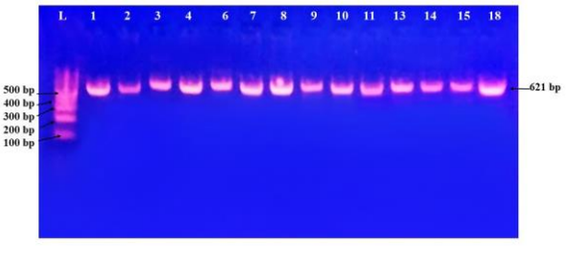

Fig. 2. PCR product of carbapenemase gene (bla_{NDM}, 621 bp).
Line (L): DNA ladder 100 base-pair.
Lines (1-37): Positive result of bla_{NDM} gene.

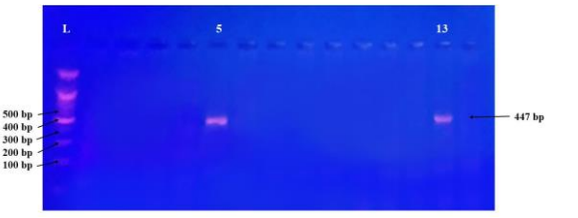

Fig. 3. PCR product of carbapenemase gene (bla_{VIM}, 447 bp).
Line (L): DNA ladder 100 base-pair.
Lines (5-13): Positive result of bla_{VIM} gene.

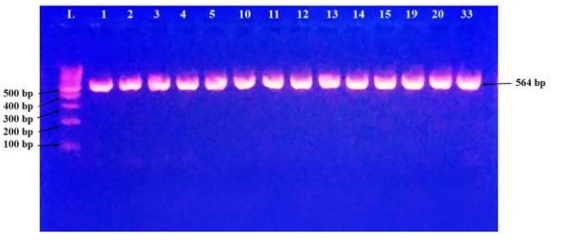

Fig. 4. PCR product of carbapenemase gene (bla_{OXA}, 564 bp).
Line (L): DNA ladder 100 base-pair.
Lines (1-39): Positive result of bla_{OXA} gene.

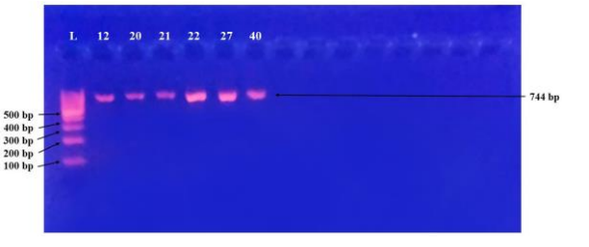

Fig. 5. PCR product of carbapenemase gene (bla_{OXA-48}, 744 bp).
Line (L): DNA ladder 100 base-pair.
Lines (12-40): Positive result of bla_{OXA-48} gene.

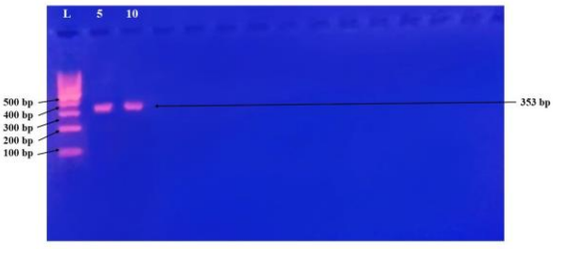

Fig. 6. PCR product of carbapenemase gene (bla_{OXA-51}, 353 bp).
Line (L): DNA ladder 100 base-pair.
Lines (5-10): Positive result of bla_{OXA-51} gene.

bla$_{NDM}$-*bla*$_{OXA-51}$, and *bla*$_{OXA}$-*bla*$_{OXA-51}$, each accounting for 5.56% of the isolates.

DISCUSSION

Currently, *E. coli* is considered one of the most encountered bacteria in community-acquired and hospital-acquired infections (27). In this context, the findings of this study revealed that *E. coli* was the second most commonly cultured bacterium (186/1212, 15.35%). This result was similar to a study by Kibret and Abera (2011), which identified that 14.2% of bacterial isolates from various clinical samples were *E. coli* (28). In contrast, a previous study by Atif et al. (2023) found that 49% of clinical specimens contained *E. coli* isolates (29), contradicting the current study's findings. Differences in the size and types of clinical specimens, along with the timing and geographic location of specimen collection, can lead to variations in incidence.

The spectrum of *E. coli* isolated from urine specimens in this study was the most frequent (119/186, 63.98%), followed by sputum (19.35%). This result concurs with a study conducted by Xu et al. (2023), which found that the majority of *E. coli* were isolated from urine specimens (67.9%); other specimen types included sputum (11.0%), pus (7.2%), blood (5.7%), and wound secretion (3.0%) (30). Another study revealed that out of 232 *E. coli* isolates, the majority (65.1%) were isolated from urine (31).

Since many infections caused by *E. coli* are resistant to traditional antibiotics, treating them is challenging. Therefore, there is a growing concern regarding the emergence and spread of carbapenemase-producing bacteria, which pose serious threats to public health, leading to higher costs and mortality rates worldwide (32). The present study focused on antimicrobial resistance to carbapenems, as they are the drug of choice for treating infections caused by multi-drug-resistant bacteria. In agreement with the global increase, carbapenemase-producing organisms are believed to be linked to the extensive use and misuse of antibiotics, often without proper diagnosis or due to self-medication by patients (33, 34). Our study detected that 40/186 (21.62%) *E. coli* isolates were resistant to the carbapenems tested. Similarly, 61 out of 294 (20.72%) were identified as carbapenem-resistant gram-negative bacteria in Basrah, southern Iraq (35). Additionally, a study conducted in the Kurdistan region of northern Iraq found a frequency of carbapenem resistance of 34 out of 110 (30.9%) (36).

The antibiotic susceptibility testing showed that the carbapenem-resistant *E. coli* isolates in this study displayed high resistance rates to all tested antibiotics. A previous study explains that most carbapenem-resistant Enterobacteriaceae are often resistant to all antibiotics except colistin and a few others (37). These antibiotic resistances may result from the co-occurrence of carbapenemase production and other resistance mechanisms (38).

In the present study, the resistance rates to the carbapenem antibiotics were as follows: imipenem at 52.50%, meropenem at 55.00%, and doripenem at 80.00%. These rates were slightly lower than those reported by Alizadeh et al. (2021), which found resistance rates of 58.2% for imipenem and 64.3% for meropenem (39). In contrast, Murugan et al. (2019) reported that out of 81 isolates, 29.03% were resistant to at least one of the three carbapenem antibiotics, specifically 23.30% for meropenem, 2.15% for imipenem, and 1.43% for ertapenem (40).

All carbapenem-resistant *E. coli* isolates were analyzed using PCR to identify the presence of carbapenemase genes. The results indicated a prevalence of Class B carbapenemases, specifically *bla*$_{NDM}$, and *bla*$_{VIM}$, while *bla*$_{GIM}$, *bla*$_{SPM}$, and *bla*$_{SIM}$ were not detected. Additionally, Class D genes, including *bla*$_{OXA}$, *bla*$_{OXA-48}$, and *bla*$_{OXA-51}$, were also identified.

Regarding Class A carbapenemase in this study, none of the *E. coli* isolates possessing Class A carbapenemase (*bla*$_{KPC,}$ *bla*$_{NMC}$, *bla*$_{IMI}$, and *bla*$_{GES}$) was observed. Similarly, previous study did not detect the *bla*$_{KPC}$ gene (41). In contrast, a study conducted in Turkey found that among 24 carbapenem-resistant *E. coli* isolates, only 2 isolates were positive for KPC, and both were obtained from the same patient (42).

This study demonstrated that 40.00% of *E. coli* isolates carried the *bla*$_{NDM}$ gene. Similarly, a previous study by Adam and Elhag (2018) reported a high prevalence of NDM, with 26 isolates (36.1%) testing positive (43). In contrast, a study from Iran identified *bla*$_{NDM-1}$ in 94% of 114 carbapenem-resistant Enterobacteriaceae isolates (44). This high prevalence may be due to highly mobile conjugative plasmids that encode NDM enzymes, promoting horizontal transfer between bacteria instead of clonal spread (45).

The prevalence of the *bla*$_{VIM}$ is low, and it was found in 2 out of 40 isolates (5.00%), as illustrated in Fig. 4. This finding was similar to the results of Alsaadi et al. (2020), who reported that 4 of carbapenem-re-

sistant isolates in Diyala hospitals carried the bla_{VIM} gene (46). Notably, this study did not detect the bla_{IMP} gene among the tested isolates, which is consistent with the results from a similar study (47).

The detection of OXA β-lactamase in Enterobacteriaceae is a significant public health concern due to the rapid mutation capability of these bacteria, which leads to an expanded spectrum of activity (48). Among the isolates studied, 16 (40.00%) expressed bla_{OXA} Class D β-lactamases. These genes are commonly found in Enterobacteriaceae and *Pseudomonas aeruginosa* in Iraq, as described in a previous study (49).

The prevalence of OXA-48 in Enterobacteriaceae is increasing. Studies have shown that among the major carbapenemases in *E. coli* cases of urinary tract infections (UTIs), the bla_{OXA-48} gene is the most common, accounting for 53% of *E. coli* strains in South East Iran (50). In our study, however, the prevalence was found to be 15.00%. Additional studies conducted in Turkey revealed a high prevalence of bla_{OXA-48}, with 96.8% of *E. coli* isolates harboring this gene (51).

OXA-51 is considered as intrinsic in Acinetobacter species. Recently, the gene encoding this enzyme has been identified in plasmids, which allows it to spread among various bacterial species (52). In this study, only two (5.00%) isolates harbored this carbapenemase gene. A similar study revealed that some strains of *E. coli* appear to have the bla_{OXA-51} gene (3/37) (53).

Furthermore, these isolates exhibited the coexistence of the bla_{NDM} gene alongside bla_{VIM} and several oxacillinase genes, including bla_{OXA}, bla_{OXA-48} and bla_{OXA-51}. It has been previously reported that plasmids carrying the bla_{NDM} gene are diverse and can contain multiple resistance determinants, such as bla_{OXA}, bla_{OXA-48}, and bla_{VIM} (54, 55).

The evolution of carbapenemases may be influenced by insertion elements or transposons that aid their transmission into chromosomes or plasmids. Moreover, carbapenem resistance is not solely caused by the presence of carbapenemase genes; it can also result from porin loss and efflux pump overexpression (7). All these alternative mechanisms aim to block the penetration of the antibiotic within the bacterial cell. This explains why some isolates (6, 15.00%) resist carbapenems despite lacking carbapenemase genes.

CONCLUSION

With the increasing use of carbapenem, the emergence of carbapenemase-producing *E. coli* has become a concern for treating infections and accounts for 21.51% of the total 186 *E. coli* clinical isolates in Thi-Qar Hospitals, Iraq. Among carbapenemase genes, bla_{NDM}, bla_{VIM}, bla_{OXA}, bla_{OXA-48}, and bla_{OXA-51} were present, which were associated with a high resistance rate to antimicrobials. By identifying the types of genes responsible for resistance, more effective approaches to treating infectious diseases may be developed. Therefore, proper implementation of monitoring programs is crucial for limiting their dissemination.

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