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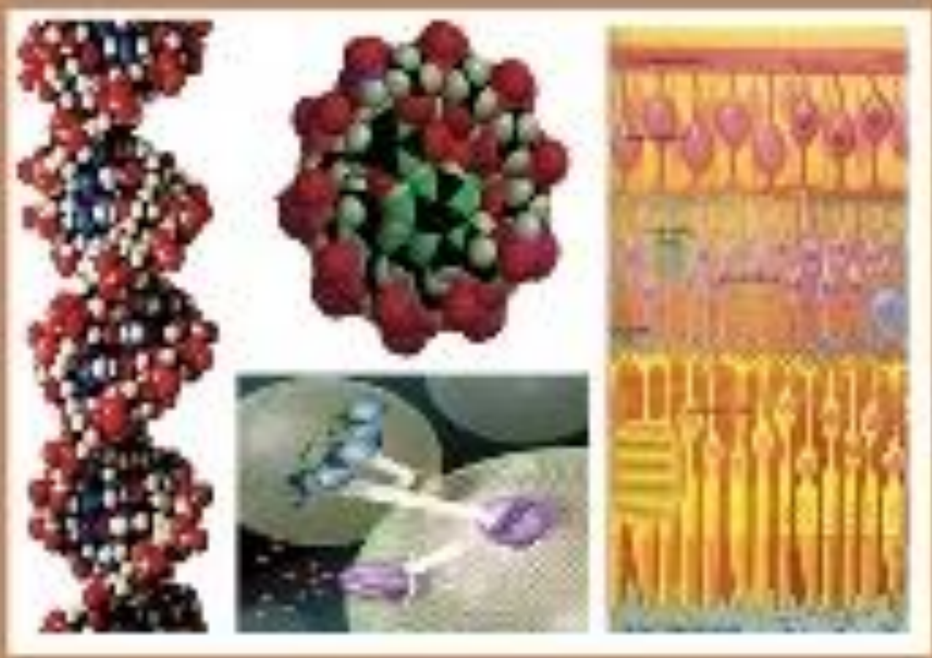

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Genotypic Detection of New-Delhi Metallo-β-Lactamase Producing Carbapenem Resistant *Escherichia coli* in Holy Karbala Province-Iraq

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ABSTRACT

Background: Carbapenem is a beta-lactam antibiotic that works similarly to Penicillins and Cephalosporins by binding to Penicillin-binding sites and subsequently inhibiting cell wall synthesis. Due to their high potency and extraordinarily broad antibacterial action, Carbapenem antibiotics, which belong to the most recent generation of β-lactam antibiotics, are frequently used in clinics to treat bacterial infections. The current study aimed to shed light on *Escherichia coli*'s resistance to β-lactam antibiotics, especially Carbapenem, and the potential link between Carbapenem resistance and the production of β-lactamase variants bla (NDM-1, NDM-2, NDM-3, NDM-4, NDM-5, GIM-1, VIM-1, SPM-1, and IMP-1) in Karbala province, Iraq. **Materials and methods:** Four hundred-eight isolates have been identified according to traditional methods (colonial morphology on MacConkey and Eosin methylene blue (EMB) agar, and biochemical reactions), and confirmed by the Vitek2® system. Carbapenem susceptibility was assayed by using the Vitek2® system. Isolates were subjected to multiplex PCR targeting β-lactamase variants genes. **Results:** Out of a total of 408 samples randomly collected from different clinical sources, 24 (19.51%) *Escherichia coli* isolates were diagnosed. Four (16.67%) of the *Escherichia coli* isolates tested positive for imipenem and meropenem resistance. PCR experiments indicated that only two (50%) isolates contained the blaNDM-1 gene and four (100%) carried the blaNDM-3 gene; no additional beta-lactamases were found.

INTRODUCTION

Resistance to carbapenems in *Enterobacteriaceae* is a serious problem, as these medications are often the last line of battle against infections caused by these bacteria. There are three types of β-lactamases that inactivate carbapenems (also known as carbapenemases): KPCs, metallo-β-lactamases (MBLs), and Oxacillinases (Peirano *et al.*, 2011). The VIM and IMP forms of MBL are the most prevalent types discovered in *Enterobacteriaceae* (Cornaglia *et al.*, 2007). The phenomenon in which the microorganisms grow in the presence of therapeutic agents that earlier negatively affected them, this known as antimicrobial resistance (Dadgostar, 2019). As a result of developing microorganisms resistant to antibiotics, it annually generates significant rates of mortality, morbidity, and economic costs. Identification and comprehension of antibiotic resistance are critical for medical treatments for managing disorders caused by antibiotic-resistant bacteria and for public health measures to stop the development of resistance (Boolchandani *et al.*, 2019).

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Antibiotic-resistant bacteria have emerged globally as a result of the improper and excessive usage of antibiotics in the medical, and agricultural industries. This trend affects a wide variety of microorganisms with a high prevalence, endangering human health (Pulingam *et al.*, 2022).Due to their high potency and extraordinarily broad antibacterial action, carbapenem antibiotics, which belong to the most recent generation of β-lactam antibiotics, are frequently used in clinics to treat bacterial infection (Coulthurst *et al.*, 2005; Cornaglia *et al.*, 2011). Carbapenem is a beta-lactam antibiotic that works similarly to penicillins and cephalosporins by binding to penicillin-binding sites and subsequently inhibiting cell wall synthesis (Zhanel *et al.*, 2007; Meletis, 2016). However, in contrast to penicillins and cephalosporins, carbapenem is believed to have a broader spectrum. Carbapenem is one of the newest beta-lactam antibiotics to be constructed. It has an unusual structure that provides high stability against β-lactamases, particularly extended-spectrum β-lactamases such as ceftazidime, ceftriax-one, and cefepime (INGGRAINI *et al.,* 2021). Carbapenemase is split into three types based on functional structure, including β-lactamase class A, B, and D (Whitley *et al.*, 2020). Serine β-lactamases are the name given to Class A and D β-lactamases, which have serine residues at the active site. At the active site of Class B β-lactamase, there are metal ions (zinc) (Queenan and Bush, 2007). The most important carbapenem-resistant components that hydrolyze practically all β-lactam antibiotics are known as New Delhi metallo-beta-lactamases (NDMs), and they are extensively spread throughout the world (Van Duin and Doi, 2017; Logan and Weinstein, 2017).

MATERIALS AND METHODS

A total of 408 samples were randomly collected from the two main hospitals (Imam Al-Hussein, peace be upon him, Medical City and Imam Al-Hassan Al-Mujtaba, peace be upon him, Hospital) in the holy Karbala province from September to December 2022. Those samples were obtained from several clinical sources (i.e. urine, stool, blood, sputum, CSF, body fluid, seminal fluid, abscess, and swabs from different regions of the body).

Identification of the *Escherichia coli* :

Escherichia coli were presumptively identified by traditional method (colonial morphology on MacConkey and Eosin methylene blue (EMB) agar, biochemical reactions) (as mentioned by Tille, 2015). The Vitek2® technology was used for confirmatory identification.

Antimicrobial Susceptibility Testing:

The antibiotic susceptibility of all *Escherichia coli*-positive isolates has been evaluated via the Vitek2® technology, which has been approved by the Clinical and Laboratory Standards Institute (CLSI) (Clinical and Laboratory Standards Institute, 2017).

The antimicrobial agents used in antimicrobial susceptibility testing include amikacin, ampicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, meropenem, minocycline, piperacillin, piperacillin/ tazobactam, ticarcillin/clavulanic acid, ticarcillin, tobramycin, and trimethoprim /sulfamethoxazole.

Phenotypic Detection of Carbapenem Resistance Strains:

All *Escherichia coli* isolates were tested for carbapenem antibiotics (imipenem and meropenem) by using the Vitek2® system in accordance with global Clinical and Laboratory Standards Institute (CLSI) guidelines(Clinical and Laboratory Standards Institute, 2017).

Genotypic Detection of Carbapenem Resistance Genes:

MBLs were detected in *E. coli* by bla PCR amplification (NDM-1, NDM-2, NDM-3, NDM-4, NDM-5, GIM-1, VIM-1, IMP-1, and SPM-1) as previously described (Hornsey *et al.*, 2011; Kaase *et al.*, 2011; Qin *et al.*, 2016; Vural *et al.*, 2020; Thapa *et al.*, 2022).

RESULTS

Growth Rate of Microorganisms Among Collected Samples:

From a total of the 408 collected samples, there were 260 (63.72%) female and 148 (36.28%) male. The bacterial growth rate was 30.15% in all cultivated samples (123/408). Of these 13.24 % (54/123) were recorded to be Gram-positive bacterial isolates and 16.91% (69/123) were Gram-negative isolates. Table 1, illustrates ten age groups that ranged from 1-98 years and the growth rate with sex distribution for each group.

Table 1: Sample distribution by sex, age, positive and negative bacterial growth.

Age in years	Recorded-Sample's		Growth rate		Total No. (%)
	Male No. (% to Male total)	Female No. (% to female total)	Positive bacterial growth rate for both sex samples No. (%)	Negative bacterial growth rate for both sex samples No. (%)	
1-10	11 (7.43)	14 (5.38)	8 (6.5)	17 (68)	25 (6.13)
10-20	13 (8.78)	32 (12.31)	12 (9.76)	33 (73.34)	45 (11.03)
21-30	25 (16.89)	75 (28.85)	19 (15.45)	81 (81)	100 (24.51)
31-40	20 (13.51)	57 (21.92)	28 (22.76)	49 (63.64)	77 (18.87)
41-50	27 (18.24)	40 (15.38)	20 (16.26)	47 (70.1)	67 (16.42)
51-60	28 (18.92)	21 (8.08)	18 (14.63)	31 (63.26)	49 (12.01)
61-70	14 (9.46)	13 (5)	12 (9.76)	15 (55.56)	27 (6.62)
71-80	10 (6.76)	6 (2.31)	6 (4.88)	10 (62.5)	16 (3.92)
81-90	0 (0)	1 (0.38)	0 (0)	1 (100)	1 (0.25)
91-100	0 (0)	1 (0.38)	0 (0)	1 (100)	1 (0.25)

Escherichia coli, *Staphylococcus* species, *Candida* species, *Enterobacter* species, *Pseudomonas* species, *Klebsiella* species, *Streptococcus* species, *Proteus* species, and unidentified bacteria have been identified in 24 (19.51%), 22 (17.88%), 18 (14.63%), 16 (13.01%), 15 (12.2%), 11 (8.95%), 10 (8.13%), 2 (1.62%), and 5 (4.07%), respectively. According to the statistics above, the most common isolate was *E. coli*.

Distribution of *Escherichia coli* Isolates Among Positive Samples:

Escherichia coli isolates were distributed among sex as 18(75%) female and 6(25%) male, with female to male ratio 3:1, and among sample type as 13(54.17%) urine, 4(16.67%) body fluid, 4(16.67%) swab, 2(8.33%) sputum, and 1(4.16%) stool. Table 2, shows the distribution of *Escherichia coli* among sex and sample type.

The age of patients who carry *Escherichia coli* ranged from 4-80 years and are separated into 4 age groups, Table 3, will demonstrate the number (%) and the sex ratio of each age group.

Table 2: Distribution of *Escherichia coli* isolates among patient's sex and sample type**.**

Sample type	*E. coli* distribution among sex		Total No. (%) of *E. coli* isolates
	Male No. (% to Male total)	Female No. (% to female total)	
Urine	3 (50%)	10 (55.56%)	13 (54.17%)
Body fluid	0 (0%)	4 (22.22%)	4 (16.67%)
Swabs	2 (33.33%)	1 (5.55%)	3 (12.5%)
Sputum	1 (16.67%)	1 (5.55%)	2 (8.33%)
Wound swab	0 (0%)	1 (5.55%)	1 (4.16%)
Stool	0 (0%)	1 (5.55%)	1 (4.16%)

Table 3: Patient's age and sex distribution of *Escherichia coli*-positive isolates.

Age group (years)	Sample's No. (%)	Male (%)	Female (%)
1-20	6 (25%)	2 (33.33%)	4 (22.22%)
21-40	4 (16.67%)	0 (0%)	4(22.22%)
41-60	11 (45.83%)	3 (50%)	8 (44.44%)
61-80	3 (12.5%)	1 (16.67%)	2 (11.11%)
Total	24	6	18

Antimicrobial Resistance of *Escherichia coli* Positive Isolates:

Antimicrobials have been employed for treating bacterial infections for over seventy years, although these low-molecular-weight bioactive chemicals also have a variety of other medical uses (Uddin *et al.*, 2021). One of the most serious global health challenges, antibiotic resistance affects food security, human, and animal health, and results in significant economic losses. It is primarily brought on by the inappropriate use of antibiotics in agriculture, the environment, and animal and human medicine (Caniça *et al.*, 2019). The following antimicrobial agents were tested *Escherichia coli* isolates: amikacin, ampicillin, aztreonam, cefepime, ceftazidime, ceftriaxone, ciprofloxacin, gentamicin, imipenem, meropenem, minocycline, piperacillin, piperacillin/tazobactam, ticarcillin/clavulanic acid, ticarcillin, tobramycin, and trimethoprim/sulfamethoxazole. Table 4, presents the outcomes of antimicrobial susceptibility among sex.

Table 4: Antimicrobial susceptibility profile of *Escherichia coli* isolates among sex.

Antimicrobial classes		Antimicrobial agents	Sensitive No. (%)		Intermediate No. (%)		Resistant No. (%)	
			Male	Female	Male	Female	Male	Female
β-lactam	Cephalosporins	Cefepime	4(16.67%)	12(54.17%)	0 (0%)	0 (0%)	2 (8.33%)	6 (20.83%)
	Penicillin	Ceftazidime	2 (8.33%)	7 (29.17%)	0 (0%)	0 (0%)	4(16.67%)	11(45.83%)
		Ceftriaxone	2 (8.33%)	5 (20.83%)	0 (0%)	0 (0%)	4(16.67%)	13(54.17%)
		Ampicillin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (25%)	18 (75%)
	Carbapenems	Piperacillin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (25%)	18 (75%)
		Ticarcillin	0 (0%)	0 (0%)	0 (0%)	0 (0%)	6 (25%)	18 (75%)
		Imipenem	5(20.83%)	15 (62.5%)	0 (0%)	0 (0%)	1 (4.17%)	3 (12.5%)
	β-lactam/β-lactamase inhibitor	Meropenem	5(20.83%)	15 (62.5%)	0 (0%)	0 (0%)	1 (4.17%)	3 (12.5%)
		Piperacillin/ tazobactam	3 (12.5%)	11(45.83%)	0 (0%)	0 (0%)	3 (12.5%)	7 (29.17%)
		Ticarcillin /clavulanic acid	1 (4.17%)	7 (29.17%)	2(8.33%)	1 (4.17%)	3 (12.5%)	10(41.66%)
	Aminoglycosides	Aztreonam	1 (4.17%)	4 (16.67%)	0 (0%)	0 (0%)	5(20.83%)	14(58.33%)
Non- β-lactam	Fluoroquinolones	Amikacin	4(16.67%)	11(45.83%)	0 (0%)	3 (12.5%)	2 (8.33%)	4 (16.67%)
		Gentamicin	4(16.67%)	5 (20.83%)	0 (0%)	0 (0%)	2 (8.33%)	13(54.17%)
		Tobramycin	4(16.67%)	5 (20.83%)	0 (0%)	0 (0%)	2 (8.33%)	13(54.17%)
	Tetracycline	Ciprofloxacin	2 (8.33%)	4 (16.67%)	0 (0%)	0 (0%)	4(16.67%)	14(58.33%)
	Sulfonamide	Minocycline	1 (4.17%)	8 (33.33%)	0 (0%)	0 (0%)	5(20.83%)	10(41.67%)
	Cephalosporins	Trimethoprim/ sulfamethoxazole	3 (12.5%)	5 (20.83%)	0 (0%)	0 (%)	3 (12.5%)	13(54.17%)

Distribution of Beta-Lactamase Variants Among Carbapenem-Resistant *Escherichia coli*:

Due to their wide variety of therapeutic indications, β-lactam antibiotics are one of the most commonly recommended drug groups. Since its debut in the 1930s, the fight against bacterial infectious diseases has undergone a significant change (Pandey and Cascella, 2022). Several β-lactam-inactivating-lactamases have developed as a result of the widespread usage of β-lactam antibiotics as medications during the past 90 years (Bush and Bradford, 2020). The widespread usage of β-lactams has facilitated the development and spread of resistance, much like with other antimicrobial groups (Tooke *et al.*, 2019). The beta lactamases used in the present study are *bla*(NDM-1, NDM-2, NDM-3, NDM-4, NDM-5, GIM-1, VIM-1, IMP-1, and SPM-1), discovered by PCR technique and gel electrophoresis system. Table 5, contains information regarding β-lactamase variants found in individuals infected with *Escherichia coli* in the current study**.**

Table 5: Beta-lactamase variants profile for all *Escherichia coli* isolates.

Isolate code	Hospital types in Karbala province	Sex	Age in years	Sample type	Beta lactamase variants								
					NDM-1	NDM-2	NDM-3	NDM-4	NDM-5	GIM-1	VIM-1	IMP-1	SPM-1
67	Imam Al-Hasan	F	6	Urine	-	-	-	-	-	-	-	-	-
99	Imam Al-Hasan	F	17	Urine	-	-	-	-	-	-	-	-	-
119	Imam Al-Hussein	F	19	Fluid	-	-	+	-	-	-	-	-	-
216	Imam Al-Hussein	F	20	Urine	-	-	-	-	-	-	-	-	-
202	Imam Al-Hussein	F	26	Fluid	-	-	-	-	-	-	-	-	-
70	Imam Al-Hussein	F	31	Urine	-	-	-	-	-	-	-	-	-
37	Imam Al-Hussein	F	35	Fluid	-	-	-	-	-	-	-	-	-
95	Imam Al-Hussein	F	38	Urine	-	-	-	-	-	-	-	-	-
14	Imam Al-Hasan	F	45	Urine	-	-	-	-	-	-	-	-	-
146	Imam Al-Hussein	F	45	Fluid	-	-	-	-	-	-	-	-	-
223	Imam Al-Hussein	F	47	Swab	-	-	-	-	-	-	-	-	-
200	Imam Al-Hussein	F	49	Urine	-	-	-	-	-	-	-	-	-
147	Imam Al-Hussein	F	51	Swab	-	-	-	-	-	-	-	-	-
66	Imam Al-Hussein	F	52	Urine	-	-	-	-	-	-	-	-	-
162	Imam Al-Hussein	F	52	Urine	-	-	+	-	-	-	-	-	-
40	Imam Al-Hasan	F	53	Stool	-	-	-	-	-	-	-	-	-
154	Imam Al-Hussein	F	63	Sputum	-	-	-	-	-	-	-	-	-
29	Imam Al-Hussein	F	80	Urine	+	-	+	-	-	-	-	-	-
78	Imam Al-Hussein	M	4	Swab	+	-	+	-	-	-	-	-	-
40	Imam Al-Hussein	M	15	Urine	-	-	-	-	-	-	-	-	-
163	Imam Al-Hussein	M	50	Urine	-	-	-	-	-	-	-	-	-
189	Imam Al-Hussein	M	60	Urine	-	-	-	-	-	-	-	-	-
196	Imam Al-Hussein	M	60	Sputum	-	-	-	-	-	-	-	-	-
170	Imam Al-Hussein	M	70	Swab	-	-	-	-	-	-	-	-	-

F: females; M: males; NDM: New Delhi metallo beta lactamase; GIM: German-imipenemase; VIM: Verona Integron-Borne Metallo-Lactamase; IMP: Imipenemase; SPM: São Paulo metallo-beta-lactamase.

As demonstrated in Table 5, four patients (16.67%) carried carbapenem-resistant *Escherichia coli*, which was caused by *β*-lactamases (specifically New Delhi metallo beta-lactamase). Those four NDM-positive *Escherichia coli* isolates were collected from three females and one male. There was no relationship between sex and infection with NDM-positive *Escherichia coli*, with males (16.67%) and females (16.67%) having the same percentage (one of six isolates harbored NDM in both males and females).

Escherichia coli-positive-NDM was found in individuals of varying ages, with four isolates collected from patients aged four, nineteen, fifty, and eighty years. *β*-lactamases found in Karbala by the current investigation are exclusively NDM, particularly NDM-1, and NDM-3, with NDM-3 having the highest incidence. NDM-1 was detected in one urine sample and one swab, whereas NDM-3 was detected in two urine samples, one swab, and one fluid.

Two patients were infected with *Escherichia coli* that expressed both *bla*NDM-1 and *bla*NDM-3, as shown in Figure 1A , 1C and Table 5, while the other two patients were infected with *Escherichia coli* that expressed only *bla*NDM-3, as shown in figure 1C and table 5; no additional beta-lactamases were detected (Figs. 1B, 1D, 1E, 1F, 1G, 1H, and 1I).

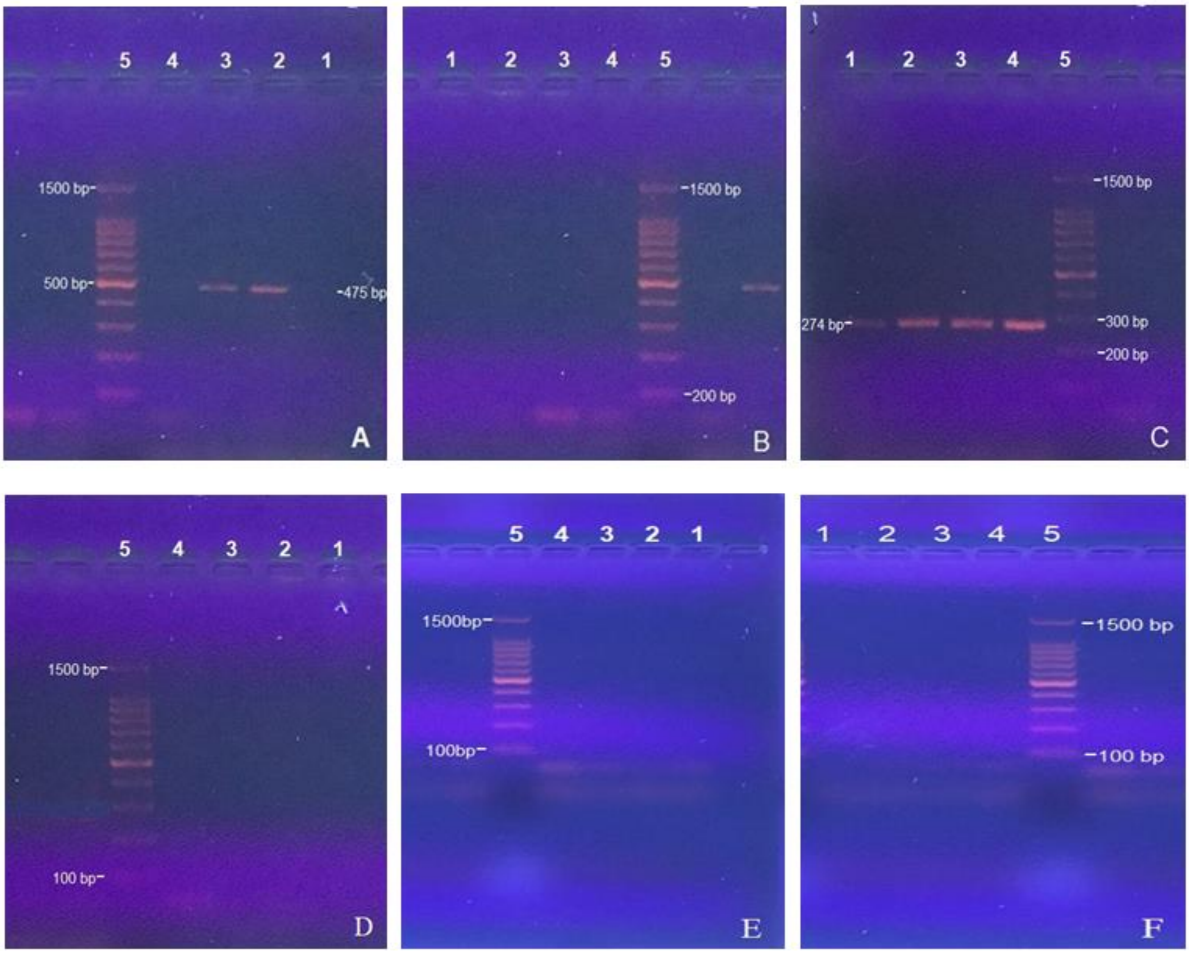

Fig. 1: Gel electrophoresis of PCR amplicon of (A) *bla*NDM-1, (B) *bla*NDM-2, (C) *bla*NDM-3, (D)*bla*NDM-4, (E)*bla*NDM-5, (F)*bla*GIM-1, (G)*bla*VIM-1, (H)*bla*IMP-1, and (I) *bla*SPM-1. Lane 1: Sample no. 119; Lane 2: sample no. 29; lane 3: sample no. 78; lane 4: sample no. 162; lane 5: marker.

DISCUSSION

Globally, AMR issues are becoming worse, even with carbapenems, the final line of defense. It has been demonstrated that widespread inappropriate use and misuse of antibiotics is the major source of drug resistance among microorganisms in countries with low or middle incomes (LMICs), where appropriate infection control and antibiotic stewardship are severely lacking (Pokharel and Adhikari, 2020). The development of carbapenem-resistant *Enterobacteriaceae* is the result of selective pressure from improper carbapenem usage. The majority of pathogens that are found in almost all common diseases brought on by Gram-negative bacteria (GNB) belong to the family *Enterobacteriaceae*. More precisely, the primary agents identified from infections caused by GNB are *Enterobacterales*, such as *E. coli* and *K. pneumoniae* (Thapa *et al.*, 2022). This investigation was carried out to discover carbapenem-resistant *E. coli* as well as the potential acquisition of MBL carbapenemase genes (NDM variants, GIM-1, VIM-1, SPM-1, and IMP-1) among such strains in Karbala province, Iraq, in order for this study to become a useful reference in the study region to determine the overall prevalence of drug resistance. In this present study, about 30.15 percent of the cultured samples tested positive for considerable bacterial growth. Similar findings were seen in earlier studies carried out in several Iraqi districts (Sabry *et al.*, 2021), while some other studies reported higher growth rates (Alzaidi and Mohammed, 2022; Al-Asady *et al.*, 2022). This ratio of no growth results from those patients who probably uptake antibiotics, suffering from a viral infection, immunological disorder, or other conditions.

The sex ratio of the current study was higher in females and this corresponds with the results published in Italy that showed females were more infected by *Escherichia coli* than males (Magliano *et al.*, 2012), and in Kirkuk City, Iraq that illustrates *Escherichia coli* strains were isolated from 76.4% females and 23.5% males (Aljebory and Mohammad, 2019). The body physiology and lifestyle of females make them most susceptible to disease and infection, this reason may be the most acceptable cause pushing females to visit hospitals more than males.

The infection rate was higher than 30% in children under the age of ten and in patients over the age of 51, because the immunity in children is low and in the growth stage in addition the children's hygiene is low especially when they meet their peers and have fun playing, whereas in the elderly, immunity is gradually weakened and become slower in responding to the pathogen in addition to many changes that happen for the body physiology such as menstrual cycle stopping in female. Age group 31-40 also have an infection rate of more than 30%. Comparable with the results of previous studies include the infection rate among age groups was 1.3% for 10-20 years, 8.1% for 20-30 years, 8.8% for 30-40 years, 5% for 40-50 years, and 1.3% for 50-60 years (Ranjit *et al.,* 2018). Another research in Karbala province, Iraq reported that the greatest occurrence (33.33%) of bacterial infection among patients occurred in the age group 31-45 years (Abdul-Husin, 2021), while in Shahrekord, Iran recorded that the age group 30-39 years occupy the highest rate 54.78% of infection (Tajbakhsh *et al.*, 2015). This age group of patients is considered a productive age group, as they have passed the stage of adolescence and have become more mature and interested in life. They realize that age is progressing and time is passing, so it must be used properly, and its perseverance is often the use of time in order to develop in all aspects of life, for example, increasing wealth and building social relationships, and therefore all these interests and goals can make a person forget his health or ignore it. On the other hand, achieving these goals often requires communication with many people, some of whom may be infected with a specific pathogen, and thus the rate of infection transmission increases for them. The infection rate for the age groups 81-90 years and 91-100 years cannot be evaluated since the sample size was insufficient to be depended on.

Escherichia coli was the most prevalent isolate among positive samples with a 19.51% ratio, according to the data of the current study. Many studies support these results and have similar results that *E. coli* were the predominant isolates in various provinces in Iraq (Karbala, Baghdad, and Basra), Iran, Syria, and Nepal (Thapa *et al.*, 2022; and Mahdi *et al.*, 2020; Salman *et al.*, 2022; Jasim *et al.*, 2022; Hassanzadeh *et al.*, 2009; Abbood *et al.,* 2022). This high percentage of *Escherichia coli* infection is because *Escherichia coli* are the normal bowel flora, therefore, it can easily spread from person to person in case of low personal hygiene in both males and females and in females this ratio becomes higher which in addition to the previous reason the body physiology of reproductive system of female (shorter urinary tract and a closer distance between the anus and the urethral opening) simplify the self-infection.

In the present study, *E. coli* were mostly infecting females and this corresponds with the results of several previous studies (Magliano *et al.,* 2012; Aljebory and Mohammad, 2019). According to the findings of the present investigation, urinary tract infections 13(54.17%) were the most prevalent source of *Escherichia coli*, similar to many previous studies (Abdul-Husin, 2021; Ronald, 2002; Naqid *et al.,* 2020; Karam *et al.*, 2019). Body fluid infections are mostly caused by *Escherichia coli* bacteria findings and these findings are also reported in many published research (Rouf and Nazir, 2019). Swabs obtained from different clinical sources bear the same percentage of body fluid and occupy the second stage of infections in humans after the urine samples. Results of the study in Kurdistan, Iraq showed that *Escherichia coli* was most detected after urine at 92.2% in the wound 3.9% and cervical 1.5% swabs (Naqid *et al.,* 2020). Another study in Najaf province, Iraq, illustrated that the occurrence of *E. coli* infections in the urinary tract was 91%, wounds 7%, and burns 2% (Najm and Hussein, 2023), while research published in Egypt showed that 28.7% of all *Escherichia coli* isolates obtained during their study were swabbed from wounds, throat, and vagina (El-Baz *et al.,* 2022). During the present study from all *Escherichia coli* isolates 8.33% were obtained from the respiratory tract as sputum. Results of a study done in Egypt demonstrate that the respiratory tract has 3.3% of *Escherichia coli* isolates (El-Baz *et al.,* 2022). In accordance with the prior research, acceptable respiratory tract infections include *Streptococcus pneumoniae* the most usually identified, followed by *Moraxella catarrhalis* and *Haemophilus influenza*, whereas the non-acceptable were *Escherichia coli*, *Staphylococcus aureus*, and *Pseudomonas aeruginosa* (Popova *et al.,* 2019). *Escherichia coli* is normal bowel flora, but it is opportunistic and can cause infection in many conditions in addition to several strains that can cause intestinal and extra-intestinal infections such as EPEC, ETEC, etc.

The age group 41-60 years had the highest rate of *Escherichia coli* infection, accounting for 45.83% of all *Escherichia coli* isolates, followed by the 1-20 years with 25%, 21-40 years with 16.67%, and 61-80 years with 12.5%. The males (33.33%) infection rate of the 1-20 years age group was higher than females (22.22%) and these results were parallel to the previous study (Hossain *et al.,* 2020), and differ from others (Yadav and Prakash, 2017). *Escherichia coli* primarily affects females (22.22%) in patients aged 21 to 40. The research performed in Bangladesh (Hossain *et al.,* 2020) and in Nepal (Yadav and Prakash, 2017), showed that infection rates were in females 32.4%, males 17.7%, and females 48.45%, males 43.80%, respectively. The prevalence rate of the age group 41-60 years reveals that males (50%) are mostly infected by *Escherichia coli*, which agrees with the findings prior study (Yadav and Prakash, 2017). The rate of infection in the age group 61-80 years demonstrates that males (16.67%) are the majority infected by *Escherichia coli*, which is supported by the results published in many recent research (Hossain *et al.,* 2020).

In this current study, a majority of the *E. coli* isolates displayed resistance against frequently suggested medicines. All *Escherichia coli* isolates were resistant to ticarcillin, piperacillin, and ampicillin, and they were resistant to at least three distinct antibiotic classes, in this situation, the bacteria termed multidrug resistance. These findings concord with many previous reports (Mohammed *et al.,* 2021; Abdelmongy *et al.,* 2022). Multidrug resistance is described as resistance to at least three medications from different antibacterial classes, primarily cephalosporins, penicillins, carbapenems, β-lactam/β-lactamase inhibitors, monobactam, aminoglycosides, fluoroquinolones, tetracycline, and sulfonamides (Saderi and Owlia, 2015). *Escherichia coli* isolates intriguingly displayed worrisome penicillin resistance, with a 24 (100%) resistance rate for all penicillin antibiotics. The outcome is identical to the prior study (Puvača and de, 2021). Aztreonam 19 (79.16%) and ciprofloxacin 18 (75%) were both less effective against *Escherichia coli* which was highly resistant to those antibiotics, followed by ceftriaxone 17 (70.84%), trimethoprim/ sulfamethoxazole 16 (66.67%), 15 (62.5%) for each ceftazidime, gentamicin, tobramycin, and minocycline, while ticarcillin/clavulanic acid resistant rate was 13 (54.16%), and piperacillin/tazobactam 10(41.67 %). Previous studies showed slightly different rates of resistance to aztreonam (85%), and minocycline (60%) (Biagi *et al.,* 2022; Popova *et al.,* 2019). Ciprofloxacin (93.81%), trimethoprim/sulfamethoxazole (87%), ceftazidime (95.88%), and Piperacillin/Tazobactam (90%), showed higher resistance rates in previous reports

(Mohammed *et al.,* 2021; Ahmad *et al.,* 2022; and Ruaa, 2023). The resistance rate to ceftriaxone (58%), gentamycin (28%), tobramycin (20%), and ticarcillin/clavulanic acid (27.27%), increased when compared to previously published research (Mohammed *et al.,* 2021; Ali and Al-Dahmoshi, 2021; and Abdul-Jabar *et al.,* 2020). The lowest rate of resistance was to cefepime, amikacin, imipenem, and meropenem with 8(29.16%), 6(25%), 4(16.67%), and 4(16.67%) resistant rate, respectively. Previous studies found a higher rate of resistance to cefepime (65%), while a lower rate of amikacin (11%), imipenem (5%), and meropenem (0%) were reported (Mohammed *et al.,* 2021; Ahmad *et al.,* 2022). However, there was the lowest degree of resistance to carbapenems, which are regarded as one of the most strong and effective β-lactams.

Resistance to carbapenems can emerge through three different processes: efflux pump-over activity, porin loss (mutation), and carbapenemase enzyme production. Nonetheless, the production of this enzyme (or variations of this enzyme) is considered to be the fundamental mechanism of resistance in carbapenem-resistant *E. coli* (Hammoudi and Ayoub, 2020). In the present study among 24 *E. coli* positive isolates, four (16.67%) were carried carbapenem-resistant *Escherichia coli*, which was caused by β-lactamases (specifically New Delhi metallo beta-lactamase). Those four NDM-positive *Escherichia coli* isolates were collected from three females and one male. There was no relationship between sex and infection with NDM-positive *Escherichia coli*, with males (16.67%) and females (16.67%) having the same percentage (one of six isolates harbored NDM in both males and females). *Escherichia coli*-positive-NDM was found in individuals of varying ages, with four isolates collected from patients aged four, nineteen, fifty, and eighty years. The findings aligned with prior research done by Ismail and Mahmoud, 2018, in which three blaNDM-1 positive isolates and one blaNDM-2 positive isolate among six carbapenem-resistant isolates in Baghdad, Iraq. Another study in Najaf, Iraq discovered among 12 phenotypically MBL-producing *P. aeruginosa* isolates, 4 (33.3%) and 3 (25%), respectively, carried blaVIM and blaIMP genes. Furthermore, two isolates had the blaSPM and blaSIM genes, while one had the blaNDM gene (Alkhudhairy and Al-Shammari, 2020). In Al Jouf, Saudi Arabia, the researcher reported that 71 (74%) had blaNDM-1, and 24 (25%) carried blaNDM gene variants (Junaid, 2021. NDM-producing *Escherichia coli* isolates were reported to make up 18% (22/122) and 20% (14/71), respectively, of the carbapenemase-producing *Enterobacteriaceae* identified in Switzerland and the Hesse area of Germany. Additionally, in Switzerland and the Hesse region, respectively, the overall numbers of NDM-like producing *Escherichia coli* isolates were 77% (17/22) and 93% (13/14) for NDM-5 producers (Chakraborty *et al.,* 2021).

Metallo-βlactamases (MBLs) were encountered from clinical isolates globally with increasing frequency during the past several years (Thapa *et al.,* 2022).

CONCLUSIONS

According to the present study's findings, the age group 61-70 years had the greatest percentage of infection in general, at 44.4 %, when compared to other age groups. *Escherichia coli* is the most common bacterium in the province of Karbala, per the findings. Although *Escherichia coli* is the most prevalent cause of urinary tract infection, it can also cause respiratory tract infection, wound infection, and burn infection. *Escherichia coli* isolates are resistant to the majority of antimicrobial agents. Throughout this study, *Escherichia coli* showed 100% resistance to ampicillin and ticarcillin. Antibiotics including ciprofloxacin, gentamicin, minocycline, and trimethoprim/ sulfamethoxazole all have significant rates of antibiotic resistance. The percentages are lowest in amikacin, cefepime, imipenem, meropenem, and piperacillin/tazobactam. All carbapenem-resistant *Escherichia coli* in Karbala province produce NDM-3, and 50% only produce

NDM-1, with no other NDM variants observed.

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The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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