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Phenotypic Detection of Effective Antibiotics against *Escherichia coli* and Determination of the Minimum Inhibitory Concentration of these Antibiotics in Holy Karbala Province

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Abstract

Antimicrobial resistance among microorganisms causes large rates of morbidity, mortality, and financial costs every year. To treat illnesses brought on by antibiotic-resistant bacteria and public health activities to prevent the development of resistance need the identification and understanding of antibiotic resistance. The current study aimed to shed light on the effectiveness of antibiotics against *Escherichia coli* and the minimum inhibitory concentration of these antibiotics. In this study, 408 samples were collected randomly from different clinical sources; females (63.72%) were most susceptible to various disease and infection than males (36.28%). From total samples 24 isolates were positive for Escherichia coli, from those 18 (75%) samples were obtained from females and 6 (25%) samples from males. These 24 isolates were tested for antibiotic susceptibility via Vitek2®. All Escherichia coli isolates were multidrug-resistant. All isolated strains of Escherichia coli were 100% resistant to Ampicillin, Piperacillin, and Ticarcillin. Nineteen (79.16%) isolates were reported to be resistant to Aztreonam. Ciprofloxacin resistance was recorded in 18 (75%) isolates. The lowest rate of resistance was recorded for Cefepime, Amikacin, Imipenem, and Meropenem with 8(29.16%), 6(25%), 4(16.67%), and 4(16.67%) resistant rates, respectively. In conclusion to the previous results, penem antibiotics are still the most effective agent among the tested antibiotics.

Keywords: Antibiotic Resistance, Minimum Inhibitory Concentration (MIC), *Escherichia Coli*

1. Introduction

The genus *Escherichia*, named after Theodor Escherich, a German pediatrician who discovered *Escherichia coli* (E. coli), is made up of facultative anaerobic Gram-negative bacilli belonging to the *Enterobacteriaceae* family [1]. The majority of the facultative anaerobic bacteria in the human colon is *E. coli*, normally within hours of birth, it often colonizes the infant's gastrointestinal tract (GIT) and helps both sides [2]. However, it is also one of the most prevalent human and animal infections, causing a wide range of illnesses [3].

The knowledge of microorganisms and infectious diseases was lacking during the period before antibiotics. Millions of people died as a result of the ineffectiveness of the methods used to treat and stop the spread of these lethal diseases, which frequently neared epidemic levels [4]. Antibiotics are natural products produced mainly by living organisms including plant microflora. Several antibiotics can be utilized to eliminate bacteria *in vivo* due to their powerful and specialized biologic activity against microorganisms in addition to their minimal toxicity [5]. The inappropriate and excessive consumption of antibiotics in the healthcare and agriculture sectors has led to the development of antibiotic-resistant bacteria on a worldwide scale. This development affects a wide variety of microorganisms with a high prevalence, endangering human health [6]. The ability of microorganisms, such as bacteria, viruses, fungi, and parasites, to survive and adapt in the presence of therapeutic drugs that had previously negatively affected them is referred to as antimicrobial resistance (AMR) [7]. AMR among microorganisms causes large rates of morbidity, mortality, and financial costs every year. To treat illnesses brought on by antibiotic-resistant bacteria and public health activities to prevent the development of resistance need the identification and understanding of antibiotic resistance [8].

Carbapenem antibiotics, which are part of the newest generation of beta-lactam (β-lactam) antibiotics, are often used in clinics to treat bacterial infections due to their powerful and unusually broad antibacterial activity [9, 10]. Similar to penicillins and cephalosporins, carbapenem is a β-lactam antibiotic that inhibits the synthesis of cell walls by engaging in penicillin-binding proteins (PBPs) [11, 12]. However, carbapenem appears to have a wider spectrum of activity when compared to penicillins and

cephalosporins [13]. In contrast to penicillin, the most recent β-lactam antibiotic, carbapenem, has a β-lactam ring and a five-group ring, this particular structure offers high stability against β-lactamases, particularly extended-spectrum β-lactamases (ESBL) such as ceftazidime, ceftriaxone, and cefepime [12].

2. Materials and Methods

From September to December 2022, 408 samples were randomly collected from the two main governmental hospitals in the holy Karbala province (Imam Al-Hussein, peace be upon him, Medical City and Imam Al-Hassan Al-Mujtaba, peace be upon him, Hospital). Those samples were from a variety of clinical sources (urine, stool, blood, sputum, cerebral spinal fluid, bodily fluids, seminal fluid, abscess, and swabs from various parts of the body).

Excluded patients are those who have recently received antibiotic therapy. All those patients were excluded because antibiotic consumption interfered with the result of the treatment.

2.1 Identification of the *Escherichia coli*

Traditional methods (colonial morphology on MacConkey and Eosin methylene blue (EMB) agar, biochemical reactions) were used to presumptively identify Escherichia coli [as indicated in 14]. Confirmatory identification was accomplished using Vitek2® technology.

2.2 Antimicrobial Susceptibility Testing

The antimicrobial susceptibility to 17 antimicrobial drugs was examined using the Vitek2® technology to assess the resistance profiles of E. coli isolates. The chosen agents included: ampicillin, piperacillin, amikacin, ceftazidime, ciprofloxacin, ceftriaxone, cefepime, gentamicin, minocycline, aztreonam, imipenem, meropenem, ticarcillin, tobramycin, piperacillin/tazobactam, ticarcillin/clavulanic acid, and trimethoprim/sulfamethoxazole. The susceptibility data were evaluated following the Clinical and Laboratory Standards Institute (CLSI) guidance [15].

2.3 Phenotypic Detection of Carbapenem Resistance Strains

The Vitek2® system was used to test all Escherichia coli isolates for carbapenem antibiotics (imipenem and meropenem) susceptibility according to global Clinical and Laboratory Standards Institute (CLSI) guidance [15].

3. Results

The following are the sample types distributions for the current study: 252/408 (61.76%) urine, 41/408 (10.04%) sputum, 36/408 (8.83%) swabs from different regions of the body, 21/408 (5.14%) vaginal swabs, 20/408 (4.9%) body fluid, 18/408 (4.41%) blood, 7/408 (1.71%) diarrheal stool, 3/408 (0.73%) for each wound and burn swabs, 2/408 (0.5%) for each semen and abscess samples, 1/408 (0.25%) sample from each cerebrospinal fluid, synovial fluid and cervical swab. The sex ratio was 16:9, with 260/408 (63.72%) females and 148/408 (36.28%) males. The patients' ages varied from 1 to 98 years, and they were divided into 10 groups. Table 1 shows each age group's infection rate and sex distribution.

Table 1: Sample distribution according to patient age, sex, and bacterial growth rate

Age groups (years)	Recorded-Sample's		Growth rate		
	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. (%)	Total 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)

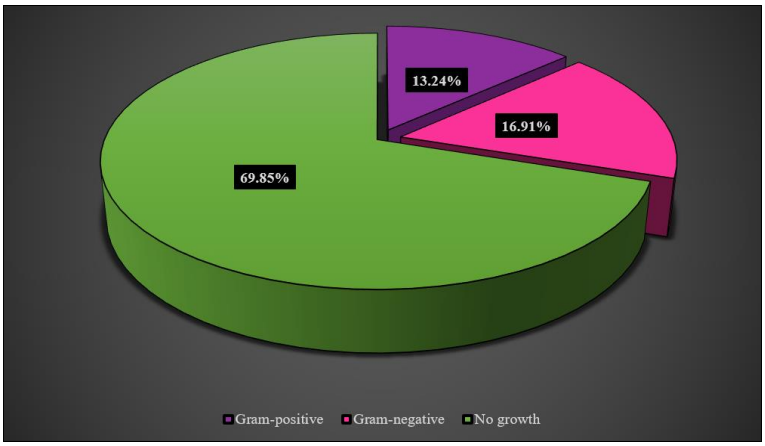

Fig 1: The growth rate of microorganisms among total study samples

Out of the total samples, 123/408 (30.15%) were positive for bacterial growth, whereas 285/408 (69.85%) were negative. Positive isolates were distributed over total samples as 54/408 (13.24%) gram-positive bacteria isolates, whereas 69/408 (16.91%) isolates were recognized as gram-negative bacteria, as shown in Fig 1. All gram-positive and lactose-non fermenting bacteria were ignored, as the *E. coli* is gram-negative, lactose-fermenting bacteria.

The following microorganisms had been identified in the positive samples (123): 24/123 isolates (19.51%) *Escherichia coli*, 22/123 isolates (17.88%) *Staphylococcus* species, 18/123 isolates (14.63%) *Candida* species, 16/123 isolates (13.01%) *Enterobacter* species, 15/123 isolates (12.2%) *Pseudomonas* species, 11/123 isolates (8.95%) *Klebsiella* species, 10/123 isolates (8.13%) *Streptococcus* species, 5/123 isolates (4.07%) other bacteria, and 2/123 isolates (1.62%) *Proteus* species (Fig 2).

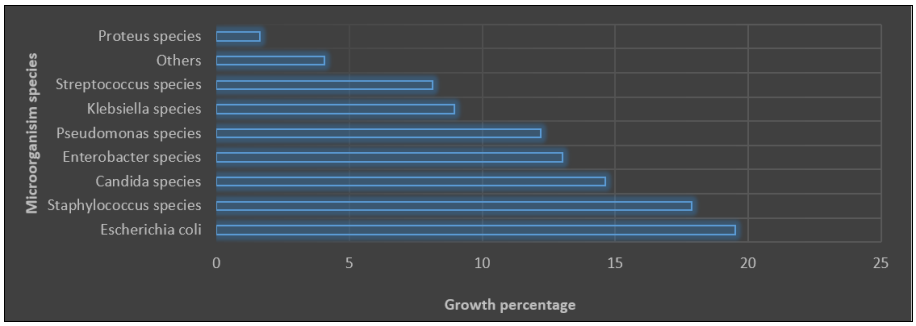

Fig 2: Bacterial isolate distribution among positive samples

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 No. (%)	Female No. (%)
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

Escherichia coli isolates were distributed among sex as 18/24 (75%) female and 6/24 (25%) male, with female to male ratio 3:1, and among sample type as 13/24 (54.17%) urine, 4/24 (16.67%) body fluid, 4/24 (16.67%) swab, 2/24 (8.33%) sputum, and 1/24 (4.16%) stool. Table 2 shows the distribution of *Escherichia coli* among sex and sample type.

The age of patients carrying *E. coli* varied from 4 to 80 years and is divided into four age groups; Table 3 shows the number (%) and sex ratio of each age group.

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. Tables 4 and 5 show the findings of antimicrobial susceptibility testing.

The minimum inhibitory concentration (MIC) of an antibacterial agent is measured in milligrams per liter or µg/mL, and it is the lowest concentration at which the test strain of a microorganism is unable to grow in any way [16]. MIC of all used antibiotics are shown in the Table 6.

Table 4: Antimicrobial susceptibility 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	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%)

	inhibitor	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%)

Table 5: Antimicrobial susceptibility profile for all *Escherichia coli* isolate

Isolate code	**Hospital types in Karbala province**	**sex**	**Age in years**	**Sample type**	**Antimicrobial agents susceptibility**															
					AMK	**AMP**	**ATM**	**FEP**	**CAZ**	**CRO**	**CIP**	**GEN**	**IPM**	**MEM**	**MIN**	**PIP**	**TZP**	**TIC/CLA**	**TIC**	**TOB**	**SXT**
67	Imam Al-Hasan	F	6	Urine	iIi	iR	Ri	iR	iR	Ri	Ri	Ri	iS	iS	Ri	Ri	iS	iS	Ri	Ri	Ri
99	Imam Al-Hasan	F	17	Urine	iIi	iR	Ri	iS	iS	Ri	Ri	Ri	iS	iS	iS	Ri	R	R	Ri	Ri	Ri
119	Imam Al-Hussein	F	19	Fluid	iR	iR	Ri	iR	iR	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri
216	Imam Al-Hussein	F	20	Urine	iS	iR	Si	iS	iiS	Si	S	Ri	iS	iS	Ri	Ri	iS	iS	Ri	Ri	iS
202	Imam Al-Hussein	F	26	Fluid	iS	iR	Si	iS	iS	Ri	Ri	Ri	iS	iS	iS	Ri	iS	iS	Ri	Ri	Ri
70	Imam Al-Hussein	F	31	Urine	iS	iR	Si	iS	iS	Si	Ri	Ri	iS	iS	iS	Ri	iS	R	Ri	iS	Ri
37	Imam Al-Hussein	F	35	Fluid	iR	iR	Ri	iS	iR	Ri	Ri	Ri	iS	iS	Ri	Ri	Ri	Ri	Ri	Ri	S
95	Imam Al-Hussein	F	38	Urine	iS	iR	Ri	iS	iR	iS	iS	iS	iS	iS	S	Ri	S	S	Ri	S	R
14	Imam Al-Hasan	F	45	Urine	iS	iR	Ri	iR	iR	Ri	Ri	iS	iS	iS	Ri	Ri	S	R	Ri	R	S
146	Imam Al-Hussein	F	45	Fluid	iI	iR	Ri	iS	iR	Ri	Ri	Ri	iS	iS	S	Ri	R	R	Ri	R	Ri
223	Imam Al-Hussein	F	47	Swab	iS	iR	Ri	iiS	iR	Ri	S	S	iS	iS	Ri	Ri	S	R	Ri	S	S
200	Imam Al-Hussein	F	49	Urine	iS	iR	Ri	iS	iR	Si	S	S	iS	iS	S	Ri	S	I	Ri	S	Ri
147	Imam Al-Hussein	F	51	Swab	iS	iR	Si	iS	iS	Ri	Ri	Ri	iS	iS	Ri	Ri	S	S	Ri	Ri	Ri
66	Imam Al-Hussein	F	52	Urine	iS	iR	Ri	iR	iR	Ri	Ri	Ri	iS	iS	Ri	Ri	Ri	Ri	Ri	Ri	Ri
162	Imam Al-Hussein	F	52	Urine	iR	iR	Ri	iR	iR	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri	Ri
40	Imam Al-Hasan	F	53	Stool	iS	iR	Rii	iS	iS	Si	Ri	S	iS	iS	S	Ri	S	S	Ri	S	S
154	Imam Al-Hussein	F	63	Sputum	iS	iR	Ri	iS	iS	Rii	Ri	Ri	iS	iS	R	Ri	S	S	Ri	R	R
29	Imam Al-Hussein	F	80	Urine	iR	iR	Ri	iR	iR	Ri	Ri	Ri	Ri	Ri	S	Ri	R	R	Ri	R	R
78	Imam Al-Hussein	M	4	Swab	iR	iR	Ri	iR	iR	Ri	Ri	Ri	Ri	Ri	Ri	Ri	R	R	Ri	R	S
40	Imam Al-Hussein	M	15	Urine	iS	iR	Si	iS	iS	iS	iS	iS	iS	iS	Ri	Ri	S	I	Ri	S	R
163	Imam Al-Hussein	M	50	Urine	iR	iR	Ri	iS	iR	Ri	Ri	Ri	iS	iS	Ri	Ri	R	R	Ri	R	R
189	Imam Al-Hussein	M	60	Urine	iS	iR	Ri	iR	iR	Ri	iS	iS	iS	iS	Ri	Ri	S	I	Ri	S	S
196	Imam Al-Hussein	M	60	Sputum	iS	iR	Ri	iS	iS	iS	Ri	iS	iS	iS	S	Ri	S	S	Ri	S	S
170	Imam Al-Hussein	M	70	Swab	iS	iR	Ri	iS	iR	Ri	Ri	iS	iS	iS	Ri	Ri	Ri	Ri	Ri	S	Ri

F: female; M: male; AMK: amikacin; AMP: ampicillin; ATM: aztreonam; FEP: cefepime; CAZ: ceftazidime; CRO: ceftriaxone; CIP: ciprofloxacin; GEN: gentamicin; IPM: imipenem; MEM: meropenem; MIN: minocycline; PIP: piperacillin; TZP: piperacillin/tazobactam; TIC/CLA: ticarcillin/clavulanic acid; TIC: ticarcillin; TOB: tobramycin; SXT: trimethoprim/sulfamethoxazole; S: sensitive; R: resistant; I: intermediate

Table 6: Minimum inhibitory concentration (µg/mL) of *Escherichia coli* isolates

Isolate code	Antimicrobial agents susceptibility																
	AMK	AMP	ATM	FEP	CAZ	CRO	CIP	GEN	IPM	MEM	MIN	PIP	TZP	TIC/CLA	TIC	TOB	SXT
67	16	>=32	16	32	16	>=64	>=4	>=16	1	<=0.5	>=16	>=128	<=4	16	>=128	>=16	>=320
99	8	>=32	16	2	4	>=64	>=4	>=16	<=0.25	<=0.25	<=1	>=128	8	>=128	>=128	>=16	>=320
119	>=64	>=32	16	>=64	>=64	>=64	>=4	>=16	>=16	>=16	>=16	>=128	>=28	>=128	>=128	>=16	>=320
216	<=2	>=32	<=1	<=1	<=1	<=1	0.5	>=16	<=0.25	<=0.25	>=16	>=128	<=4	16	>=128	8	<=20
202	<=2	>=32	4	2	4	>=64	>=4	>=16	<=0.25	<=0.25	<=1	>=128	<=4	16	>=128	8	>=320
70	<=2	>=32	4	2	4	<=1	>=4	>=16	<=0.25	<=0.25	<=1	>=128	<=4	>=128	>=128	<=1	>=320
37	>=64	>=32	16	4	16	>=64	>=4	>=16	<=0.25	<=0.25	>=16	>=128	32	>=128	>=128	>=16	<=20
95	<=2	>=32	>=64	2	16	<=1	<=0.25	<=1	<=0.25	<=0.25	<=1	>=128	<=4	16	>=128	<=1	>=320
14	<=2	>=32		>=64	>=64	>=64	>=4	<=1	<=0.5	<=0.25	>=16	>=128	16	>=128	>=128	>=16	<=20
146	16	>=32	16	2	16	>=64	>=4	>=16	<=0.25	<=0.25	4	>=128	8	>=128	>=128	>=16	>=320
223	<=2	>=32	16	8	32	>=64	<=0.25	<=1	<=0.25	<=0.25	>=16	>=128	<=4	>=128	>=128	<=1	<=20
200	<=2	>=32	16	2	16	<=1	<=0.25	<=1	<=0.25	<=0.25	<=1	>=128	<=4	32	>=128	<=1	>=320
147	<=2	>=32	4	<=1	4	>=64	>=4	>=16	<=0.5	<=0.25	>=16	>=128	<=4	16	>=128	>=16	>=320
66	<=2	>=32		>=64	>=64	>=64	>=4	>=16	<=0.25	<=0.5	>=16	>=128	>=128	>=128	>=128	>=16	>=320
162	>=64	>=32	>=64	>=64	>=64	>=64	>=4	>=16	>=16	>=16	>=16	>=128	>=128	>=128	>=128	>=16	>=320
40	<=2	>=32	16	<=1	4	<=1	>=4	<=1	<=0.25	<=0.25	<=1	>=128	<=4	16	>=128	<=1	<=20
154	<=2	>=32	16	<=1	4	>=64	>=4	>=16	<=0.25	<1=0.5	>=16	>=128	<=4	16	>=128	<=1	<=20
29	>=64	>=32	16	>=64	>=64	>=64	>=4	>=16	>=16	8	4	>=128	>=128	>=128	>=128	>=16	>=320
78	>=64	>=32	16	>=64	>=64	>=64	>=4	>=16	>=16	8	>=16	>=128	>=128	>=128	>=128	>=16	<=20
40	<=2	>=32	<=1	<=1	<=1	<=1	<=0.25	<=1	<=0.25	<=0.25	>=16	>=128	<=4	32	>=128	<=1	>=320
163	>=64	>=32	16	2	>=64	>=64	>=4	>=16	<=0.25	<=0.25	>=16	>=128	>=128	>=128	>=128	>=16	>=320
189	<=2	>=32	>=64	>=64	>=64	>=64	<=0.25	<=1	<=0.25	<=0.25	>=16	>=128	<=4	64	>=128	<=1	<=20
196	<=2	>=32	16	2	4	<=1	>=4	<=1	<=0.25	<=0.25	<=1	>=128	<=4	16	>=128	<=1	<=20
170	<=2	>=32	>=64	2	>=64	>=64	>=4	<=1	<=0.25	<=0.25	>=16	>=128	>=128	>=128	>=128	<=1	>=320

F: female; M: male; AMK: amikacin; AMP: ampicillin; ATM: aztreonam; FEP: cefepime; CAZ: ceftazidime; CRO: ceftriaxone; CIP: ciprofloxacin; GEN: gentamicin; IPM: imipenem; MEM: meropenem; MIN: minocycline; PIP: piperacillin; TZP: piperacillin/tazobactam; TIC/CLA: ticarcillin/clavulanic acid; TIC: ticarcillin; TOB: tobramycin; SXT: trimethoprim/sulfamethoxazole; S: sensitive; R: resistant; I: intermediate

4. Discussions

Globally, AMR is worsening, even with carbapenems, the last line of defense. It has been proven that widespread improper use and abuse of antibiotics is the leading cause of drug resistance among microorganisms in low and middle-income countries (LMICs), where infection control and antibiotic stewardship are severely weak [17]. The emergence of carbapenem-resistant Enterobacteriaceae is a consequence of selective pressure caused by inappropriate carbapenem use. The majority of pathogens detected in practically all prevalent infections caused by Gram-negative bacteria (GNB) are members of the Enterobacteriaceae family. More specifically, the major agents implicated in GNB infections are Enterobacterales such as E. coli and Klebsiella pneumoniae [18]. The purpose of this study was to find carbapenem-resistant E. coli among such strains in holy Karbala province, Iraq so that this study might become a valuable reference in the study region to evaluate the general prevalence of drug resistance.

In this investigation, around 30.15 percent of the cultivated samples revealed positive for significant bacterial growth. Earlier research in numerous Iraqi districts found similar results [19], while some studies found greater rates of growth [20, 21]. This ratio of no growth is caused by patients who probably intake antibiotics in the recent period before sampling, suffering from a viral infection, immunological disorder, or other conditions.

The current study's sex ratio was higher in females, which corresponds with findings published in Italy that showed females were more commonly infected than males [22], and in Kirkuk City, Iraq, where bacterial strains were isolated from 76.4% females and 23.5% males [23]. Females' bodily physiology and lifestyle make them more susceptible to sickness and infection; this may be one of the most acceptable reasons for females visiting hospitals more than males.

The incidence of infection was greater than 30% among kids under the age of ten years and in patients who exceeded the age of 51 years, because immunity in children is low and in the growth stage, and children's hygiene is low, especially when they meet their peers and have fun playing, while among the elderly, immunity gradually declines and becomes slower in responding to the pathogen, in addition to many changes that occur for the body physiology such as menstrual cycle stopping in females. Infection rates in the age range 31-40 years are similarly higher than 30%. In comparison to earlier research, the infection rate 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 [24]. Another study in Karbala province, Iraq found that the age range 31-45 years had the highest percentage of bacterial infection (33.33%) [25], whereas in Shahrekord, Iran, the age group 30-39 years had the highest rate of infection (54.78%) [26]. Patients in this age range (31-40 years) are deemed productive since they have passed the adolescent stage and have grown more mature and engaged in life. They

recognize that time is passing and that age is progressing, so it must be used properly, and perseverance to use the time to develop in all aspects of life, such as increasing wealth and building social relationships, and all of these interests and goals can cause a person to forget or ignore them health. On the other hand, attaining these goals frequently necessitates communication with a large number of people, some of whom may be infected with a specific disease, increasing the rate of infection transmission for them. The infection rate for the age categories 81-90 years and 91-100 years cannot be assessed because the sample size was inadequate.

According to the current study's findings, E. coli was the most common isolate among positive samples, with a 19.51% ratio. Many investigations confirm these findings and show that E. coli was the most prevalent isolate in various areas in Iraq (Karbala, Baghdad, and Basra), Iran, Syria, and Nepal [18, 27-31]. Because E. coli is a normal bowel flora, it can easily spread from person to person in cases of poor personal hygiene in both males and females, and in females this ratio becomes higher because, in addition to the previous reason, the body physiology of the female reproductive system (shorter urinary tract and a closer distance between the anus and the urethral opening) simplifies self-infection.

In the current investigation, E. coli primarily infected females, which matches the findings of numerous earlier studies [22, 32]. According to the current study's outcomes, urinary tract infections 13 (54.17%) were the most prevalent source of E. coli, which is consistent with many prior studies [25, 33-35]. According to data from current investigation, E. coli is the most common pathogens found in body fluid, which are also described in numerous published studies [36]. Swabs from various clinical sources contain the same portion of bodily fluid and are the second stage of infection in people after urine samples. According to the findings of a study conducted in Kurdistan, Iraq, E. coli was most commonly discovered after urine at 92.2% in wound 3.9% and cervical 1.5% swabs [34]. Another study in Iraq's Najaf province found that 91% of E. coli infections were detected in the urinary tract, 7% in wounds, and 2% in burns [37], while research published in Egypt found that 28.7% of all E. coli isolates obtained during their study were swabbed from wounds, throat, and vagina [38]. During the current investigation, 8.33% of all E. coli isolates were collected as sputum from the respiratory tract. According to the findings of an Egyptian investigation, 3.3% of E. coli isolates are discovered in the respiratory system [38]. According to previous research, acceptable respiratory tract infections include Streptococcus pneumoniae, Moraxella catarrhalis, and Haemophilus influenza, while unacceptable were Escherichia coli, Staphylococcus aureus, and Pseudomonas aeruginosa [39]. E. coli is typical bowel flora, but it is opportunistic and may cause infection under a variety of situations, in addition to numerous strains that can cause intestinal and extra-intestinal infections such as enteropathogenic E. coli, enterotoxigenic E. coli, and others.

The 41–60-year age group had the greatest incidence of E. coli infection, accounting for 45.83% of all E. coli isolates, followed by the 1–20-year age group with 25%, 21–40-year age group with 16.67%-, and 61-80-year age group with 12.5%. Males (33.33%) had a greater infection rate than females (22.22%) in the 1–20-year age group, which was similar to the previous study [40] but different from others [41]. In patients aged 21 to 40, E. coli mostly affects females (22.22%). According to studies conducted in Bangladesh [40] and Nepal [41], infection rates in females were 32.4%, males 17.7%, and females 48.45%, males 43.80%, respectively. According to the prevalence rate of the age range 41-60 years, males 3/6 (50%) are primarily infected with E. coli than females 8/18 (44.44%), which accords with the findings of a previous research [41]. The incidence of infection in the age range 61-80 years shows that men (16.67%) are the majority infected by E. coli, which is supported by the findings of many recent studies [40].

A significant percentage of the E. coli isolates in this investigation were resistant to commonly prescribed antibiotics. All E. coli isolates were resistant to ticarcillin, piperacillin, and ampicillin, and they were resistant to at least three different antibiotic classes, a condition known as multidrug resistance (MDR) in bacteria. These findings are consistent with several earlier studies [42, 43]. Resistance to at least three antimicrobial classes, particularly cephalosporins, penicillins, carbapenems, β-lactam/β-lactamase inhibitors, monobactam, aminoglycosides, fluoroquinolones, tetracycline, and sulfonamides, is defined as multidrug resistance [44]. With a 24 (100%) resistance rate for all penicillin drugs, E. coli isolates intriguingly displayed concerning penicillin resistance. The results are the same as in the previous study [45]. Aztreonam 19 (79.16%) and ciprofloxacin 18 (75%) were both less potent against E. coli which was strongly resistant to those antimicrobial agents, 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 research found somewhat different rates of resistance to aztreonam (85%) and minocycline (60%) [40, 46]. Ciprofloxacin (93.81%), trimethoprim/sulfamethoxazole (87%), ceftazidime (95.88%), and Piperacillin/ Tazobactam (90%), showed higher resistance rates in previous reports [43, 46, 49]. The resistance rate to ceftriaxone (58%), gentamycin (28%), tobramycin (20%), and ticarcillin/clavulanic acid (27.27%), increased when compared to previously published researches [50, 47, 48]. The lowest rate of resistance were to cefepime, amikacin, imipenem, and meropenem with 8(29.16%), 6(25%), 4(16.67%), and 4(16.67%) resistant rate, respectively. Previous studies recorded a higher rate of resistance to cefepime (65%), while a lower rate of amikacin (11%), imipenem (5%), and meropenem (0%) were reported [43, 46]. 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 [50].

In the present investigation, imipenem and meropenem were shown to be the most effective antibiotics, with sensitivity for 83.33% of the isolates at MICs of <=0.25, 0.5, and 1 µg/mL, and resistance for 16.67% of the isolates at MICs >=16 and 8 µg/mL. Cefepime followed imipenem and meropenem in the power of action against E. coli with MICs <=1, 2, and 4, µg/mL of sensitivity (70.84%) while showing resistance at MICs 32 and >=64 µg/mL. Following imipenem, meropenem, and cefepime, amikacin

demonstrates strong sensitivity for 62.5% of all E. coli isolates at MIC <=2 µg/mL, resistance for 25% of all isolates at MIC >=64 µg/mL, and intermediate activity for the remaining 12.5% of isolates at MICs 16 and 8 µg/mL. Piperacillin/tazobactam and ticarcillin clavulanic acid were moderately influencing E. coli, which piperacillin/tazobactam at MICs <=4 and 16 µg/mL demonstrate sensitivity for 58.33% of all E. coli isolates and at MICs 8, >=28, 32< and >=128 µg/mL resistant, on the other hand, E. coli were sensitive (33.34%) to ticarcillin/clavulanic acid at MIC 16 µg/mL, resistant (54.16%) at MIC >=128 µg/mL, and intermediate at MICs 32 and 64 µg/mL. Ceftazidime, gentamicin, tobramycin, and minocycline all show the same sensitivity rate (37.5%) among E. coli with variant minimum inhibitory concentration as illustrated in Table 6. E. coli in this present study possessed different MICs for ceftazidime (sensitivity <=1 and 4 µg/mL; resistance 16, 32, >=64 µg/mL), tobramycin (sensitivity <=1 µg/mL; resistance 8 and >=16 µg/mL), and minocycline (sensitivity <=1 and 4 µg/mL; resistance >=16 µg/mL), however for gentamicin MIC was <=1 µg/mL for sensitivity and >=16 µg/mL for resistance. The elimination of E. coli employed in this investigation needed trimethoprim/sulfamethoxazole and ceftriaxone to have minimum inhibitory concentrations of <=20 µg/mL and <=1 µg/mL, respectively, whereas resistant E. coli was tolerant of these medications even at MICs of >=320 and >=64 µg/mL, respectively. The least inhibitory doses of ciprofloxacin and aztreonam needed to kill the E. coli employed in this investigation were <=0.25, 0.5 µg/mL and <=1, 4 µg/mL, respectively. However, resistant E. coli tolerated these medicines even at MICs of >=4 and 16, >=64 µg/mL, respectively. All E. coli isolates in the current investigation were resistant to the antibiotics ampicillin, piperacillin, and ticarcillin, with MIC values of >=32 µg/mL for ampicillin and >=128 µg/mL for piperacillin and ticarcillin, respectively, as a result, there was no impact of prescribed antibiotics on E. coli. Many of the current study results about parallel to results of Egyptian researchers, and other differs [51].

The current study were have little limitations, among these limitations is the difficulty of obtaining information from some patients, especially women, for example, some samples such as samples of vaginal and cervical smears are done by a doctor, and the difficulty of the researcher, especially the male researcher, to reach the patient.

5. Conclusions

Females were most susceptible to various disease and infection than males. 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. But for E. coli infections the age group 41-60 demonstrate the higher percentage of infection and age group 61-80 years the lowest rate. E. coli is the most common bacterium in the province of holy Karbala, per the findings. Although E. coli is the most prevalent cause of urinary tract infection, it can also cause respiratory tract infection, wound infection, and burn infection. E. coli isolates are resistant to the majority of antimicrobial agents, and all isolates show multidrug resistant. Throughout this study E. coli showed 100% resistance to ampicillin, piperacillin, and ticarcillin. Antibiotics including aztreonam, ciprofloxacin, gentamicin, minocycline, ticarcillin/clavulanic acid, tobramycin, and trimethoprim/sulfamethoxazole all have significant rates of antibiotic resistance. The percentages are lowest in amikacin, cefepime, imipenem, meropenem, and piperacillin/tazobactam.

6. Recommendations

The findings of the current investigation recommend that:-

- Antibiotics must not be used at random by patients without a physician's prescription, and even when they feel becoming well, they should closely follow the doctor's instructions for dosage and time.
- The identification of carbapenemase-producing bacteria should be available in all hospitals and primary care settings since they are frequently linked to treatment failure.
- Before treating a bacterial infection, it is necessary to identify which bacteria are resistant and susceptible to antibiotics to select the correct antibiotic.

7. References

1. Gomes TA, Elias WP, Scaletsky IC, Guth BE, Rodrigues JF, Piazza RM, *et al*. Diarrheagenic escherichia coli. Brazilian Journal of Microbiology. 2016; 47:3-30.
2. Nataro JP, Kaper JB. Diarrheagenic escherichia coli. Clinical microbiology reviews. 1998; 11(1):142-201.
3. Allocati N, Masulli M, Alexeyev MF, Di Ilio C. Escherichia coli in Europe: an overview. International Journal of Environmental Research and Public Health. 2013; 10(12):6235-6254.
4. Uddin TM, Chakraborty AJ, Khusro A, Zidan BRM, Mitra S, Emran TB, *et al*. Antibiotic resistance in microbes: History, mechanisms, therapeutic strategies and future pro-spects. Journal of Infection and Public Health. 2021; 14(12):1750-1766.
5. Korzybski T, Kowszyk-Gindifer Z, Kurylowicz W. Antibiotics: Origin, nature and properties. Elsevier, 2013.
6. Pulingam T, Parumasivam T, Gazzali AM, Sulaiman AM, Chee JY, Lakshmanan M, *et al*. Antimicrobial resistance: Prevalence, economic burden, mechanisms of resistance and strategies to overcome. European Journal of Pharmaceutical Sciences. 2022; 170:p106103.
7. Dadgostar P. Antimicrobial resistance: implications and costs. Infection and drug resistance, 2019, 3903-3910.
8. Boolchandani M, D'Souza AW, Dantas G. Sequencing-based methods and resources to study antimicrobial resistance. Nature Reviews Genetics. 2019; 20(6):356-370.
9. Coulthurst SJ, Barnard AM, Salmond GP. Regulation and biosynthesis of carbapenem antibiotics in bacteria. Nature Reviews Microbiology. 2005; 3(4):295-306.
10. Cornaglia G, Giamarellou H, Rossolini GM. Metallo-β-lactamases: A last frontier for β-lactams. The Lancet infectious diseases. 2011; 11(5):381-393.
11. Zhanel GG, Wiebe R, Dilay L, Thomson K, Rubinstein E, Hoban DJ, *et al*. Comparative review of the carbapenems. Drugs. 2007; 67:1027-1052.
12. Meletis G. Carbapenem resistance: Overview of the problem and future perspectives. Therapeutic advances in infectious disease. 2016; 3(1):15-21.
13. Inggraini M, Nurfajriah S, Priyanto JA, Ilsan NA.

Antimicrobial susceptibility and molecular species identification of clinical carbapenem-resistant bacteria. Biodiversitas Journal of Biological Diversity. 2021; 22(2).
14. Tille P. Bailey & Scott's diagnostic microbiology-E-Book. Elsevier Health Sciences, 2015.
15. Clinical and Laboratory Standards Institute. Performance standards for antimicrobial susceptibility testing. CLSI supplement M100, 2017, 106-112.
16. Kowalska-Krochmal B, Dudek-Wicher R. The minimum inhibitory concentration of antibiotics: Methods, interpretation, clinical relevance. Pathogens. 2021; 10(2):p165.
17. Pokharel S, Adhikari B. Antimicrobial resistance and over the counter use of drugs in Nepal. Journal of Global Health. 2020; 10(1).
18. Thapa A, Upreti MK, Bimali NK, Shrestha B, Sah AK, Nepal K, *et al*. Detection of NDM Variants (bla NDM-1, bla NDM-2, bla NDM-3) from Carbapenem-Resistant Escherichia coli and Klebsiella pneumoniae: First Report from Nepal. Infection and Drug Resistance, 2022, 4419-4434.
19. Sabry NN, Owaied YH, Mahmoud AJ. Epidemiological Study of Common Bacte-rial and Parasitic Infections in Some Areas of Salah Al-Din Governorate, Iraq. In IOP Conference Series: Earth and Environmental Science. IOP Publishing. 2021; 923(1):p012081.
20. Alzaidi JR, Mohammed AS. First Record of Dissemination of BLBLI-Resistant from Public Hospitals in Baghdad, Iraq. The Open Microbiology Journal. 2022; 16(1).
21. Al-Asady FM, Al-Saray DA, Al-Araji AE. Screening of urinary tract bacterial infections and their antibiogram among non-pregnant women admitted to Al-Sadiq hospital, Iraq. In AIP Conference Proceedings. AIP Publishing.2386; 2022 (1).
22. Magliano E, Grazioli V, Deflorio L, Leuci AI, Mattina R, Ro-mano P, Cocuzza CE. Gender and age-dependent etiology of community-acquired urinary tract infections. The Scientific World Journal, 2012.
23. Aljebory IS, Mohammad KA. Molecular Detection of Some Virulence Genes of Escherichia coli Isolated from UTI Patients in Kirkuk City, Iraq. Journal of Global Pharma Technology. 2019; 11(3):349-355.
24. Ranjit E, Raghubanshi BR, Maskey S, Parajuli P. Prevalence of bacterial vaginosis and its as-sociation with risk factors among nonpregnant women: A hospital-based study. International Journal of Mi-crobiology, 2018.
25. Abdul-Husin F. Plasmid-Mediated Mechanism of Quinolone Resistance on E. coli Isolates from Different Clinical Samples. Archives of Razi Institute. 2021; 76(3):p561.
26. Tajbakhsh E, Tajbakhsh S, Khamesipour F. Isolation and molecular detection of Gram-negative bacteria causing urinary tract infection in patients referred to Shahrekord hospitals, Iran. Iranian Red Crescent Medical Journal. 2015; 17(5).
27. Mahdi MS, Mohammed SH, Ahmed MM, Al-Deen AN, Fadhil N, Zaid NYA, *et al*. Resistance Rates of the most Isolated Bacteria from different Clinical Samples, Kerbala, Iraq, 2015.
28. Salman HA, kamil Alhameedawi A, Muhamad SMSG, Taha Z. Prevalence of multi-antibiotic resistant bacteria isolated from children with urinary tract infection from Baghdad, Iraq, 2022.
29. Jasim HA, Ibrahim RS, Abbas BA, Ali B, Najim Q, Ali A. Isolation and identification of some bacterial species from dialysis unit in Basrah. In AIP Conference Proceedings. AIP Publishing LLC.; 2022 2386(1):p020007.
30. Hassanzadeh P, Motamedifar M, Hadi N. Prevalent bacterial infections in intensive care units of Shiraz University of medical sciences teaching hospitals, Shiraz, Iran. Jpn J Infect Dis. 2009; 62(4):249-253.
31. Abbood A, Malek Z, Thallaj N. Antibiotic resistance of urinary tract pathogens in Syrian children. Research Journal of Pharmacy and Technology. 2022; 15(11):4935-4939.
32. Badulla WF, Alshakka M, Mohamed Ibrahim MI. Antimicrobial resistance profiles for different isolates in Aden, Yemen: a cross-sectional study in a resource-poor setting. BioMed Research International, 2020.
33. Aljebory IS, Mohammad KA. Molecular Detection of Some Virulence Genes of Escherichia \coli Isolated from UTI Patients in Kirkuk City, Iraq. Journal of Global Pharma Technology. 2019; 11(3):349-355.
34. Ronald A. The etiology of urinary tract infection: traditional and emerging pathogens. The American Journal of Medicine. 2002; 113(1):14-19.
35. Naqid IA, Balatay AA, Hussein NR, Saeed KA, Ahmed HA, *et al*. Antibiotic Susceptibility Pattern of Esche-richia coli Isolated from Various Clinical Samples in Duhok City, Kurdistan Region of Iraq. Int J Infect. 2020; 7(3):e103740.
36. Karam MRA, Habibi M, Bouzari S. Urinary tract infection: Pathogenicity, antibiotic resistance and development of effective vaccines against Uropathogenic Escherichia coli. Molecular immunology. 2019; 108:56-67.
37. Rouf M, Nazir A. Aerobic bacteriological profile and antimicrobial sensitivity pattern of bacteria isolated from sterile body fluids: A study from a tertiary care hospital in North India. Microbiology Research Journal International. 2019; 28(1):1-10.
38. Najm R, Hussein JM. Isolation and Identification of Pathogenic Escherichia coli from Different Sources in Najaf Hospital. Journal of Population Therapeutics and Clinical Pharmacology. 2023; 30(8):459-464.
39. El-Baz R, Said HS, Abdelmegeed ES, Barwa R. Characterization of virulence determinants and phylogenetic background of multiple and extensively drug resistant Escherichia coli isolated from different clinical sources in Egypt. Applied Microbiology and Biotechnology. 2022; 106(3):1279-1298.
40. Popova G, Boskovska K, Arnaudova-Danevska I, Smilevska-Spasova O, Jakovska T. Sputum quality assessment regarding sputum culture for diagnosing lower respiratory tract infections in children. Open Access Macedonian Journal of Medical Sciences. 2019; 7(12):1926.
41. Hossain A, Hossain SA, Fatema AN, Wahab A, Alam MM, Islam MN, *et al*. Age and gender-specific antibiotic resistance patterns among Bangladeshi patients with urinary tract infection caused by Escherichia coli. Heliyon. 2020; 6(6):e04161.
42. Saderi H, Owlia P. Detection of multidrug resistant (MDR) and extremely drug resistant (XDR) P.

aeruginosa isolated from patients in Tehran, Iran. Iranian Journal of Pathology. 2015; 10(4):p265.
43. Mohammed EJ, Allami M, Sharifmoghadam MR, Bahreini M. Relationship between antibiotic resistance patterns and O-serogroups in uropathogenic Escherichia coli strains isolated from Iraqi patients. Jundishapur Journal of Microbiology. 2021; 14(8).
44. Yadav K, Prakash S. Screening of ESBL producing multi-drug resistant E. coli from urinary tract infection suspected cases in southern Terai of Nepal. J Infect Dis Diagn. 2017; 2(2):p116.
45. Abdelmongy M, Zaki M, Hamouda R. Antibacterial activity of multidrug resistance Escherichia coli by some natural products. Research Journal of Applied Biotechnology. 2022; 8(2):5-12.
46. Biagi M, Lee M, Wu T, Shajee A, Patel S, *et al*. Aztreonam in combination with imipenem-relebactam against clinical and isogenic strains of serine and metallo-β-lactamase-producing enterobacterales. Diagnostic Microbiology and Infectious Disease. 2022; 103(2):p115674.
47. Ahmad S, Ali F, Qureshi SA, Uzma B, Shakeela Q, Sabir MS, *et al*. The evaluation of antibiotic susceptibility pattern and associated risk factors of UTI in tertiary care hospital of Peshawar. Pakistan Journal of Pharmaceutical Sciences. 2022; 35(3):897-903.
48. Kareem Raheem Z, Abdulhamid Said L. Antibiotic Susceptibility Profile of Bacteria Causing Aerobic Vaginitis in Women in Iraq. Archives of Razi Institute. 2023; 78(1):31-43.
49. Abdul-Jabar HH, Abd AH, Abdulamir AS. Efficacy of Combinations of Piperacilline/Tazobactam, Ceftazidime, Amikacin and Bacteriophage against Enterobacteriaceae Sepsis in Neonates: *In Vitro* Study. Systematic Reviews in Pharmacy. 2020; 11(10).
50. Ruaa SH. Screening of some bacterial and fungal infections in neutropenic cancer patients in Al-Najaf governorate-Iraq. Iranian Journal of War and Public Health. 2023; 15(2):1001-1007.
51. Zafer MM, El-Mahallawy HA, Abdulhak A, Amin MA, Al-Agamy MH, Radwan HH. Emergence of colistin resistance in multidrug-resistant Klebsiella pneumoniae and Escherichia coli strains isolated from cancer patients. Annals of Clinical Microbiology and Antimicrobials. 2019; 18:1-8.