International Journal of Environmental Sciences
ISSN: 2229-7359
Vol. 11 No. 10s, 2025
https://theaspd.com/index.php

Occurrence Of Colistin Resistance Genes Among Clinical Isolates Escherichia Coli In Najaf

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ABSTRACT
Background: The last resort against both the infections with carbapenem resistant Enterobacteriaceae regards the polymyxins and the colistin. In the recent past, the transmissible colistin resistance has been reported in Enterobacteriaceae through the m.c.r-genes.

OBJECTIVE
The aim of the present study is to evaluate the frequency of plasmid mediated colistin resistance genes among *E. coli* isolates obtained from clinical isolates in Najaf hospitals
Subjects and Methods: Cross-section study was conducted between November 2024 to February 2025 at Al-Najaf hospitals. The study population consist 150 clinical specimens were gathered from individuals who were either admitted to or visited hospitals, and the specimens immediately transported to the microbiology laboratory. All Gram-negative isolates in the current study had been identified by depending on morphological characteristic, examination by light microscope, and biochemical studies in accordance to the normal procedure guided by Mac.Faddin and Hart. The disk diffusion procedure was used which is based on recommendations made by international Clinical Laboratory Standards Institute regarding antibiotics susceptibility (CLSI, 2024). For this purpose, twenty-six different antibiotics disks of 10 classes were used. Colistin broth disk elution test as recommended by CLSI (2023) on polymyxin cut points isolates having a MIC of ≥ 4 μg/ml is considered colistin resistant and colistin Kirby Bauer were done to assess phenotypic colistin resistance. The *E. coli* and *K. pneumonia* isolates exhibited resistance to colistin were examined for the presences of plasmid mediated mcr-1 to mcr-8 genes. Extended spectrum beta-lactamase (ESBL), AmpC beta lactamase and carbapenemase were inialy detected by the phenotypic methods. *bla* genes (*bla* $_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{OXA}$, and *bla*$_{IMP}$) were detected by PCR technique.

RESULTS
A total of 150 isolate were collected during the study period, from main hospital in Najaf, these isolates were from clinical specimens which included urine (47, 53%), burn wound (8, 66%), seminal fluid (4,50%), sputum (5, 62.5%), and wound abscesses(8, 80%). Bacterial isolates obtained from clinical specimens, 72(48%) were recognized as *E. coli*. Antibiotic resistance among isolates diverse from 11.1% for colistin to 100% for ampicillin, amoxicillin and amoxicillin-clavulanic. 48 (66.6%) E.coli isolates were identified as MDR and 24(33.3%) isolates considered as XDR. Initial screening of ESBL indicated that all isolates were putatively assigned as ESBL producers. (70.8%) E. coli isolates were AmpC β-lactamase producers. Genotype bla gene positive ESBL for E. coli isolates 11 (15.2%) have blaOXA, 12 (16.6%) blaCTX-M isolates have and 15(20.8%) have blaSHV, the frequency of carbapenemase-encoding genes among the isolates, blaIMP was negative for all isolates.
Among screening all phenotype colistin resistance isolates 8 *E. coli*, the frequencies of plasmid mediated colistin resistance mcr genes. No isolates were positive for plasmid encoded colistin resistance gene mcr1, mcr-2, mcr-3, mcr-4, mcr-5, mcr-7, mcr-8, while all the eight-colistin resistant isolates harbored the plasmid mediated colistin resistance gene *mcr-6*.

CONCLUSION
Results revealed that the prevalence of colistin resistance E. coli isolates in Al-Najaf hospital is concerning; further limiting potential therapeutic options. This is the first report of plasmid encoded colistin resistance E.coli isolate that carries mcr-6 in Al-Najaf hospital

Background: Antibiotic resistance has emerged as a significant global public health issue, driven by the rapid adaptation of microorganisms to commonly prescribed antibiotics(Mondal et al., 2024). These genes can be rapidly disseminated among the strains because they exist on the mobile genetic elements (Gröndahl-Yli-Hannuksela et al., 2018). The case of superbugs with extended-spectrum beta-lactamases, AmpC beta lactamases, or Metallo-beta lactamases has minimized treatment options (Shad., 2018). Carbapenems are a type of beta-lactam antibiotics with a wide spectrum of action on numerous aero- and anaerobic bacteria, Gram-positive and Gram-negative bacteria, and are utilized in the management of serious infections that are life-threatening and unresponsive to the conventional antibiotic therapy (Wanger et al., 2017). Carbapenems are widely utilized in the infections that are caused by multidrug resistant strains of the Enterobacteriaceae and they were being taken as the last resort antibiotics to treat the multidrug resistant Gram-negative bacteria (Chiu et al., 2018, Armin et al., 2023).Polymyxins such as colistin are believed to be the final resort of antibiotics to cure infections caused by carbapenem resistant Enterobacteriacea (CRE). In 2012 the World Health Organization reclassified colistin as critically important to human medicine (Newton-Foot et al., 2017).The mechanism of action of colistin utilizes the interaction with the outer-side of the bacteria to remove the divalent cations on negative phosphate groups of the Lipid A of the lipopolysaccharide membrane resulting in cell lysis (Olaitan et al., 2014).Colistin resistance has previously only been regarded as a consequence of chromosomal mutation in the genes that encode either the P.m.r.A/Pmr.B and PhoP/PhoQ signalling systems or the negative regulator MgrB. These mutations lead to an alteration of the Lipid A molecule (Olaitan et al., 2014). In the recent past, resistance by the polymyxins that is transferable through the m.c.r- genes has been observed. The location of these m.c.r- genes was found on the plasmid and this increases the fear that the resistance might be conveyed into Gram-negative bacteria. Mcr-1 gene was initially detected in Escherichia coli and Klebsiella pneumoniae in China(Srijan et al., 2018), and then the existence of mcr-1 gene was also detected in other bacteria in countries around the globe e.g. United States, European Union, Turkey, South Africa, Malaysia, Greece, Italy, Algeria, Tunisia and Kuwait(Wang et al., 2018).The first study carried out in Egypt in 2016 reported the presence of mcr-1 gene in E. coli strain taken from an intensive care unit (ICU) patient(Elnahriry et al., 2016). In 2016, the mcr-2 plasmid-mediated colistin resistance was detected in E. coli first in Belgium from pigs and then from patients (Xavier et al., 2016).

MATERIALS AND METHODS:

Subjects and Methods:

Cross-section study was conducted between November 2024 to February 2025 at Al-Najaf hospitals. The study population consist 150 clinical specimens were gathered from individuals who were either admitted to or visited hospitals, and the specimens immediately transported to the microbiology laboratory. All Gram-negative isolates in the current study had been identified by depending on morphological characteristic, microscopically examination, and biochemical tests according to standard method described by MacFaddin and Hart. Antibiotics susceptibility was performed by disk diffusion methods according to the recommendations of international Clinical Laboratory Standards Institute (CLSI, 2024). For this purpose, twenty-six different antibiotics disks of 10 classes were used. Colistin broth disk elution test as recommended by CLSI (2023) on polymyxin cut points isolates having a MIC of ≥ 4 μg/ml is considered colistin resistant and colistin Kirby Bauer were done to assess phenotypic colistin resistance. The *E. coli* isolates exhibited resistance to colistin were examined for the presences of plasmid mediated mcr-1 to mcr-8 genes. Extended spectrum beta-lactamase (ESBL), AmpC beta lactamase and carbapenemase were inialy detected by the phenotypic methods. *bla* genes (*bla* $_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{OXA}$, and *bla*$_{IMP}$) were detected by PCR technique. Antimicrobial susceptibility testing: Antibiotic susceptibility testing to *E. coli* was performed by using disc diffusion method, (Kirby-Bauer method). The selection of antibiotics disks (table1). For the colistin antibiotic the Colistin Broth Disk Elusion Method, the sensitivity then tested in which tube the growth of bacteria is inhibited if ≤ 20 ul/ml intermediate, ≥40ul/ml resistant (CLSI, 2023). were performed according to Clinical and Laboratory Standards Institute (CLSI 2024). All susceptibility results were interpreted according to standard values performed by CLSI (2024). The reference strain for the quality control of the antibiotics under test was *E. coli* ATCC

25922. Identification of Multidrug resistant (MDR) and extensively drug resistant (XDR) of the isolates were according to the guideline of CDC and ECDC (18).

Phenotypic Detection of ESBL Production:
Screening of ESBL Production Initial screening test for the detection of ESBL production by *E. coli* isolates was performed using third-generation cephalosporins including ceftazidime (30μg), cefotaxime (30μg) and ceftriaxone (30μg) as per the CLSI (2024) guidelines. Isolate showing zone of inhibition of ≤17mm for ceftazidime and/or ≤19mm for ceftriaxone and/or ≤22mm for cefotaxime was considered as a potential ESBL producer.

Screening for AmpC β-Lactamase Producing Isolates:
E. coli isolates were screened for the probable production of AmpC β-lactamases using a Kirby-Bauer disk diffusion test, in which cefoxitin (30 μg) was used. Based on the CLSI 2024) criteria, all isolates showing an inhibition zone of <14 mm were suspected of being AmpC β-lactamase producers (Polsfuss *et al.*, 2011), and were subjected to further molecular evaluation.

Phenotypic Detection of Carbapenemase Production:
E. coli isolates exhibited inhibition zone less than19 mm for imipenem and meropenem, the isolates were subjected to tests for confirmation of carbapenemase.

Molecular Analysis Techniques
DNA Extraction
Prior to DNA extraction, the bacterial isolates were cultivated in 5 ml LB broth at 37°C for 24 hours, then 0.5-2 ml of the culture were transferred to Eppendorf tubes. The tubes were centrifuged at 10,000 rpm for 1 minutes and the supernatant was removed. The genomic DNA extraction was carried out according to manufacturing origin company protocol (Magen, China). The yield and purity of a DNA was determined using bio photometer plus (Nanodrop). Finally, extracted DNA was stored in a freezer at -20°C, ready to be used for PCR.

Detection of Resistance Genes by PCR
It should be that, all components of PCR were accumulated in PCR tube and mixed under sterile conditions on ice container.
PCR Amplification Primers: The list of primers used in this study is given in Table 1

Table (1): Sequences of primers used in this study:

Primer Name		Primer sequence	Product(bp)	Reference
Mcr-1	F	5'-AGTCCGTTTGTTCTTGTGGC-3'	320	(Rebelo et al., 2018)
	R	5'-AGATCCTTGGTCTCGGCTTG-3'		
Mcr-2	F	5'-CAAGTGTGTTGGTCGCAGTT-3'	715	
	R	5'-TCTAGCCCGACAAGCATACC-3'		
Mcr-3	F	5'-AAATAAAAATTGTTCCGCTTATG-3'	929	
	R	5'-AATGGAGATCCCCGTTTTT-3		
Mcr-4	F	5'-TCACTTTCATCACTGCGTTG-3'	1116	
	R	5'-TTGGTCCATGACTACCAATG-3		

Mcr-5	F	5'-ATGCGGTTGTCTGCATTTATC-3'	1644	
	R	5'-TCATTGTGGTTGTCCTTTTCTG-3		
Mcr-6	F	5'-GTCCGGTCAATCCCTATCTGT-3'	556	(AbuOun et al., 2017)
	R	5'-ATCACGGGATTGACATAGCTAC-3'		
Mcr-7	F	5'-TGCTCAAGCCCTTCTTTTCGT-3	892	(Yang et al., 2018)
	R	5'-TTCATCTGCGCCACCTCGT -3'		
Mcr-8	F	5'-AACCGCCAGAGCACAGAATT-3'	667	(Wang et al., 2018)
	R	5'-TTCCCCCAGCGATTCTCCAT-3		
bla $_{CTX-M}$	F	SCS ATG TGC AGY ACC AGT AA	554	(Saladin et al., 2002)
	R	CCG CRA TAT GRT TGG TGG TG		
bla_{SHV}	F	GGGTTATTCTTATTTGTCGC	930	(Hadi,2015)
	R	TTAGCGTTGCCAGTGGTC		
bla_{OXA}	F	GGCACCAGATTCAACTTTCAAG	554	(Dallenne et al., 2010)
	R	GACCCCAAGTTTCCTGTAAGTG		
bla_{IMP}	F	TTGACACTCCATTTACDG	139	Dallenne *et al.* (2010)
	R	GATYGAGAATTAAGCCACYCT		

Polymerase Chain Reaction (PCR): Monoplex and multiplex patterns of the PCR assay were used in this investigation. A total of 10 µl of PCR master mix, 2.5µl primer forward,2.5µl primer reverse, and 2 µl of extracted DNA. The mixture was then topped up with 8µl of sterile deionized distilled water were used to prepare the PCR 25ul reaction mixture. Following a brief centrifugation to guarantee adequate mixing of the contents, the PCR reaction tubes were added to the thermocycler PCR program in accordance with the instructions listed in Table 3-6.

Table (3-6): PCR Cycling Conditions:

Gene	Initial Denaturation Temp/Time	Denaturation Temp/Time	Annealing Temp/Time	Extension Temp/Time	Final Denaturation Temp/Time	Cycle number
bla_{CTX-M}	94/4min	94/30sec	63/1min	72/1min	72/5min	35
bla $_{SHV}$	94/1 min	94 /1 min	56 / 1min	72 /1 sec	72 /10 min	30
bla_{OXA}	94/10 min	94/40 sec	60/40 sec	72/1 min	72/5 min	30

Mcr-1	94/15min	94/30	58/90	72/1	72/10	32
Mcr-2	94/15min	94/30	58/90	72/1	72/10	32
Mcr-3	94/15min	94/30	58/90	72/1	72/10	32
Mcr-4	94/15min	94/30	58/90	72/1	72/10	32
Mcr-5	94/15min	94/30	58/90	72/1	72/10	32
Mcr-6	94/15min	94/30	58/90	72/1	72/10	32
Mcr-7	94/15min	94/30	58/90	72/1	72/10	32
Mcr-8	94/15min	94/30	58/90	72/1	72/10	32

Statistical Analysis: In the present study, statistical analysis was performed using Microsoft Office Excel 2019 for certain calculations and Chi square calculator. A significant threshold of 0.05 was used, and *p-values* below this threshold were deemed statistically significant.

Ethical Issues
Ethical issues for this study were obtained from the ethical committee of Al- Najaf Hospital. Teaching.

RESULTS
A total of 150 isolate were collected during the study period, from main hospital in Najaf, these isolates were from clinical specimens which included urine (112), burn wound (12), seminal fluid (10), sputum and wound abscesses (8). 72(48%) were recognized as E. coli obtained from clinical specimens, included urine (47, 53%), burn wound (8, 66%), seminal fluid (4,50%), sputum (5, 62.5%), and wound abscesses(8,80%).

Antibiotics susceptibility patterns
The result of antibiotic susceptibility profile in *E. coli* showed that (100%) of the isolates were completely resistant to ampicillin and amoxicillin. Resistance to beta-lactam/beta lactamase inhibitor combinations was also high at 100% for amoxicillin-clavulanic acid, 93% for ampicillin-sulbactam, 85% for piperacillin-tazobactam and 71% for ticarcillin-clavulanic acid. *E.coli* demonstrated a significant rate of resistance to cephalosporins III and IV in the current investigation, exhibiting rates of 85.3% for ceftazidime and 88% for cefotaxime, 76.6% ceftriaxone, 78% cefepime and cephamycin 70% for cefoxitin. The resistance proportions to monobactams was 43.3 % for aztreonam and carbapenem antibiotics were 17% for imipenem and meropenem.In addition to about 21% of *E. coli* were resistant to amikacin, 26.3% to gentamicin, 41.6 % to tobramycin, 43% to kanamycin and 34.7% to netilmicin when it came to aminoglycoside antibiotics. Quinolones class was with resistance rates of 54% for ciprofloxacin, 53.3% for levofloxacin, and 46% for nalidixic acid. Trimethoprim/sulfamethoxazole exhibited a resistance rate of 42% and 33.3% of isolates were resistance to chloramphenicol. *E. coli* isolates were resistance to Colistin 11.1% Table (2).

Table (2): The characteristics of the Antibiotic Susceptibility Profile for E.coli (n=72).

Antibiotic classes	Antibiotic disk	No. (%) of isolates exhibited:		
		Resistance	Intermediate	Susceptible
Penicillins	Ampicillin	72(100)	0	0
	Amoxicillin	72(100)	0	0
Penicillins + β-lactamase inhibitors	Amoxicillin-clavulanic acid	72(100%)	(0)	(0)
	Ampicillin-sulbactam	67 (93%)	(0)	5 (7%)
	Piperacillin-tazobactam	61 (84.7%)	(0)	11 (15.2%)
	Ticarcillin-clavulanic acid	51 (70.8%)	(0)	21 (29.1%)
Cephalosporins	Cefotaxime	63 (87.5%)	3(4.1%)	6(8.3%)
	Ceftazidime	61 (85.3 %)	4 (6%)	7(10%)
	Ceftriaxone	56 (78%)	7 (10%)	10 (14%)
	Cefepime	56 (78%)	8(11.11%)	8(11.11%)
Cephamycins	Cefoxitin	50(70.8%)	(0)	22 (31%)
Monobactams	Aztreonam	31 (43)	(0)	41(57%)
Carbapenems	Imipenem	12(17%)	2 (3)	58 (80.5%)
	Meropenem	12(17%)	1(1.3%)	59 (81.9 %)
Quinolones	Nalidixic acid	33(46%)	(0)	39(54%)
	Ciprofloxacin	39(54.1%)	(0)	33(46%)
	Levofloxacin	38(53%)	3(4.1%)	31(43%)
Aminoglycosides	Amikacin	15(21%)	(0)	57(79.1%)
	Tobromycin	30 (41.6%)	(0)	42(58.3%)
	Gentamicin	19 (26.3%)	(0)	53(74%)
	Kanamycin	31 (43%)	(0)	41(57%)
	Netilmicin	25 (34.7%)	(0)	47(65.2%)
Phenicols	Chloramphenicol	24 (33.3%)	(0)	48 (67%)
Folate pathway inhibitors	Sulfamethoxazole Trimethoprim	30(42%)	(0)	42 (58.3%)
Lipopeptide	Colistin	8(11.11%)	(0)	64(89%)

Phenotype and Genotype of ESBLs and AmpC β-Lactamases Producing Isolates:

The phenotypic methods of screening the resistant E. coli clinical pathogens were done as per the CLSI (2024) recommendations (Table 3). All the isolates that were tested on susceptibility to cefotaxime, ceftazidime in the presumptive test of determination of potential ESBL producers were screened, ceftriaxone, Cefepime and Aztreonam using disc diffusion susceptibility testing. The isolates were classified as potential ESBL producers if the zone diameter for ceftazidime was ≤17 mm, ≤22 mm for cefotaxime, ≤19mm for ceftriaxone, Cefepime≤ 18 and ≤17mm for Aztreonam. Initial screening indicated that all isolates were putatively assigned as ESBL producers. Subsequently, all isolates resistant to third-generation cephalosporins were subjected to confirmatory tests using the disk approximation test as described in the CLSI guidelines. All the same, based on the disk approximation test, no isolate was found to be ESBL producer. Isolates exhibited resistance to cefoxitin by disc diffusion test (≤14mm) considered as putative AmpC β-lactamase producers. In this study, all isolates demonstrated resistance to cefoxitin and were considered as potential AmpC β-lactamase producers. The result of cefoxitin resistance in *E. coli* (70.8%), Table (3).

Table (3): phenotype ESBLs and AmpC β-Lactamases Producing Isolates

Name of isolate	No. (%) of ESBL producers					No. (%) of AmpC producers
	Initial screening test					
	Ceftazidime ≤ 17 mm	Cefotaxime ≤ 22 mm	Ceftriaxone ≤ 19 mm	Cefepime≤ 18	Aztreonam ≤17mm	Cefoxitin ≤ 14 mm
E. coli	61 (85.3 %)	63 (87.5%)	56 (78%)	56 (78%)	31 (43%)	50 (70.8%)

Genotype bla gene positive ESBL for and E. coli isolates According to the CLSI the isolate is considered to be a potential ESBL producers Monoplex PCR analysis was performed on 72 isolates to verify the precision of the tests and procedures for genus identification, the findings showed that the isolates had the following genes: bla_{OXA} 11 (15.2%), bla_{CTX-M} 12 (16.6%) and bla_{SHV} 15(20.8%) of the isolates, to determine the frequency of carbapenemase-encoding genes among the isolates, bla_{IMP} was negative for all isolates.

Phenotype and Genotype Carbapenem Resistant in *E. coli*:

According to the CLSI (2024) criteria, carbapenem resistant *E. coli* were defined as those isolates that showed resistance to one or more of the tested carbapenems (imipenem and meropenem) via Kirby Bauer's disk diffusion method. Based on these inclusion criteria, out of 72 examined *E. coli*, a total of 12 (17%) isolates, which showed phenotypic resistance to at least one agent belonging to carbapenem class. The Genotype Carbapenem resistance genes in *E. coli* was negative for bla_{IMP} in all isolate.

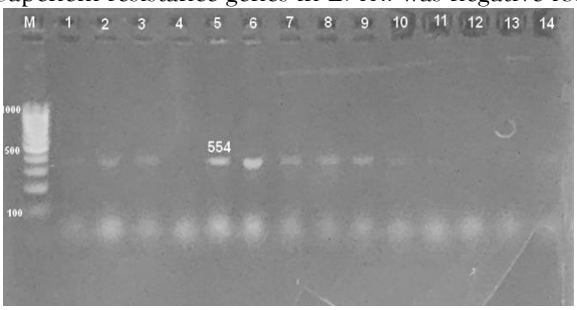

Figure (1): Ethidium bromide-stained agarose gel of monplex PCR amplified products from extracted DNA of *E. coli* isolates and amplified with bla_{CTX-M} gene primers. The electrophoresis was performed at 65 volts for 1 hr, 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (M), DNA molecular size marker (100bp ladder), Lanes (2,3, 5,6,7,8,9, and10) show positive results with (554bp).

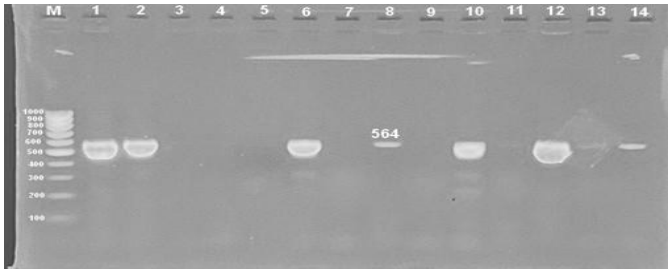

Figure (2): Ethidium bromide –stained agarose gel of monoplex PCR amplified products from extracted DNA of of *E. coli* isolates and amplified with bla_{OXA} genes primers. The electrophoresis was performed at 65 volts for 1 hr, 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (L), DNA molecular size marker (50 bp ladder), Lanes (1,2, 4,6,8,10,12,13and14) show positive results with (564bp)

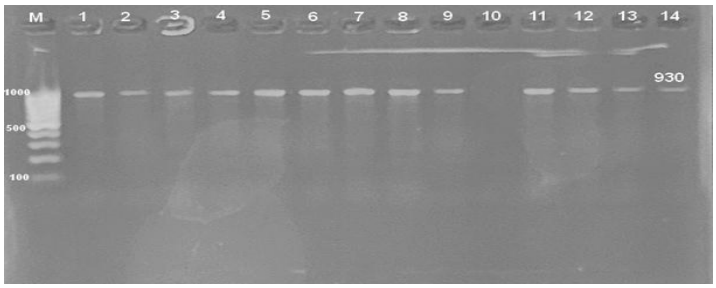

Figure (3): Ethidium bromide-stained agarose gel of monplex PCR amplified products from extracted DNA of of *E. coli* isolates and amplified with *bla* $_{SHV}$ genes primers. The electrophoresis was performed at 65 volts for 1 hr, 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (M), DNA molecular size marker (100 bp ladder), Lanes (1,2,3,4,5,6,7,8,9,11,12,13,14) show positive results with (930bp).

Phenotype and Genotype colistin Resistant in E. coli:

Colistin susceptibility assessment was carried out by colistin broth disk elution test as recommended by CLSI (2023) on polymyxin breakpoint (≥ 4µg/ml). Isolates having a MIC of ≥ 4 µg/ml is considered colistin resistant. From 72 clinical *E.coli* isoates, we identified 8(11.1%) ioslates that phenotype colistin resistant. To identify the plasmid mediated colistin resistance mcr genes, mcr-1to mcr-8 were investigated by PCR for 8 *E.coli* isolates exhibited MIC (≥ 4 µg/ml) breakpoint for colistin. mcr-1to mcr-8 except mcr-6 gene was not detected in any isolates were mcr-6 gene was detectec in 8(11.5%) *E.coli*, and maximum number two isolates had a combination of mcr-6 and bla_{OXA}, bla_{CTX-M}, bla_{SHV}, followed by bla_{OXA}, and bla_{SHV} and bla_{CTX-M}, bla_{SHV} in two and one isolates respectively.

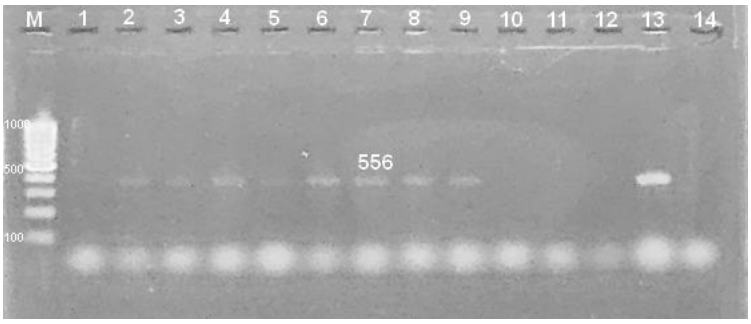

Figure (4-7): Ethidium bromide-stained agarose gel of monplex PCR amplified products from extracted DNA of of *E. coli* isolates and amplified with blaIMP genes primers. The electrophoresis was performed at 65 volts for 1 hr, 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (M), DNA molecular size marker (100 bp ladder), Lanes (2,3,4,5,6,7,8,9,and 13) show positive results with (556bp).

DISCUSSION

This cross-sectional study was initially to identify the occurrence of *E. coli* in clinical isolates in Najaf province and ultimately emphasis on colistin resistant isolates. During the study period and based on morphological and biochemical characteristics, 72 consecutive, non-repeat, discrete E. coli isolates were isolated from various clinical specimens of patients had a clinical indication of infections. *Escherichia coli* is still among the most common causes of various common bacterial infections among human beings and animals. The main cause of enteritis, urinary tract infection and septicemia among others is E. coli and severe neonatal meningitis. (Allocati et al., 2013). Also In this study, it was reported that, the frequency of the isolates were 72(48%). In the present study the frequency of isolation of E.coli from urine was 47 (35%), this result was in agreement with studies achieved by Lukey and Abbas (2021) and Kasanga et al. (2024) who reported that the prevalence of E. coli in clinical sample was (30%) and (37%) respectivly. Studies across the world show varying occurrence of E.coli in clinical specimens, in France 19.9% (Lienard et al., 2021), 15.7% in India (Patil and Y A, 2021), 14.4% in Romanian (Uivaraseanu et al., 2020), 13% in UK (Arias et al.,2019). Additional, the prevalence of E.coli was lower than that establish

from Democratic Republic of Congo who reported that the frequency of E. coli in clinical 58.9% (Irenge et al., 2014).*E. coli* resistance to Penicillin; ampicillin (100%) and amoxicillin (100%). This finding is not surprising; penicillins are the extensive used antibiotics in the community and hospitals in Iraq.The current resut was agreement with several studies Al mohana (2024) and Jubair et al (2020) in Najaf, Al Bshabshe et al. (2020) in Saudi Arabia. Also existence of AmpC β-lactamases, which are encoded by the *E. coli* chromosome, may also contribute to the bacteria's resistance to penicillins. The fact that penicillins are frequently obtained without a prescription and are frequently used improperly may be a factor in the reported resistance to these medications (Ardillon et al., 2023; Jacoby, 2009). Carbapenems, a class of antibiotics considered the last resort for treating infections caused by multidrug-resistant bacteria (Tompkins & van Duin, 2021; Elshamy & Aboshanab, 2020). In 2017, the WHO published a list of bacteria urgently needing new antibiotics. Imipenem and meropenem of the carbapenems group are the commonly antibiotics using for the management of severe form of infections caused by ESBL producing *E coli* isolates worldwide. The present study found that the E.coli isolates were highly susceptible to imipenem and meropenem (17%). Susceptiblility of E.coli isolates to carbapenems has been reported in several studies (Naqid et al., 2020; Dodoo et al., 2023). Aminoglycoside are broad-spectrum antibacterial antibiotics for Gram-negative organisms and important option for treating colistin resistance *E. coli*. That include: Amikacin, Gentamicin, Tobramycin, Kanamycin and Netilmicin. In the current study, variable resistance patterns were found for the aminoglycosides. Antibiotic sensitivity pattern in *E. coli* revealed resistant to gentamicin (26.3%) amikacin (21%), Tobramycin (41.6%), Kanamycin (43%) and Netilmicin (34.7%). The previously report emerging from in Najaf city, Iraq have shown an alarming rise of aminoglycoside antibiotics resistance (Tuwaij, 2020).Quinolones constitute a large class of synthetic antimicrobial agents that are highly effective in the treatment of many types of infectious diseases, particularly those caused by bacteria. The resistance rates to nalidixic acid, ciprofloxacin and levofloxacin, in isolates were 46%, 54.1% and 53%, respectively. The data reported in this study consistent with the previous study described by (Tuwaij, 2020). Who found *E. coli* isolated from patients with significant bacteriuria the level of resistance to fluoroquinolone: ciprofloxacin (68%) and Levofloxacin (36%).

Colistin is a polymyxin antibiotic, which has not been used clinically on a large scale until now, being associated with nephrotoxicity, but is increasing prescribed because of rising cases of multidrug resistant (MDR) infections globally with no alternative antibiotic treatment (Paterson & Harris, 2016; Lowe et al., 2018),). Mobile colistin resistance (mcr) gene first described followed the recognition of the causal factor of colistin resistance in a strain of Escherichia coli way back in an intensive pig farm in Shanghai China (Liu et al., 2016).

In this study, antibiotic susceptibility tests found that 11.1% of *E.coli* isolates were resistant to colistin. There are very few reports available on colistin-resistant E. coli isolated from Iraq. However, Zakaria et al., (2021) found 4.5% of uropathogenic *E. coli* isolates were resistance to colistin and Zafer et al (2019) in Egypt found 4.5% of *E.coli* isolates were resistance to colistin. Colistin resistance was detected in 10.8% of E.coli and *K. pneumoniae* clinical isolates in Iran according to Moosavian and Emam (2019).

The current investigation determined that approximately 48(66.6%) of *E.coli* isolates were MDR and 24(33.3%) were XDR , which is camparable with a previous study condecated in Zambia by Kasanga et al. (2024), which found that MDR prevalence was 66.5% in *E.coli*. Furthermore, various investigations condecated in the Europe and India have ervealed that MDR is most widespread in E.coli and *K.pneumoniae* (Folgori et al., 2014; Pondi et al., 2016). Besides, a higher preavlence of MDR in *E.coli* was previously reported in Zimbibwe by Mbanga et al (2023) who found frequency of MDR in E.coli was 84% . In contrast Anwar etal (2016) did a study in Pakistan that found MDR was 37.5% and XDR was 56.5%. MDR *E. coli* is a threat to public health it has been associated with increased morbidity and mortality globally. A further objective of the present study was focused to screen the proportion of colistin resistant *E. coli* isolates that possessed ESBL resistant phenotypes as well as their resistant determinants. the result of ESBLs in E. coli revealed resistance to the ceftazidime (85.3 %), cefotaxime (87.5%), ceftriaxone (78%), Cefepime (78%) and Aztreonam (43) these result agreement with result by (Al-Shamarti, 2024; Khulaif & Al-Charrakh, 2023;Najm and Hussein, 2023),. CLSI recommens multiple

confimatory test for ESBL identification due to the difficulty of detecting beta lactam resistance. The disk approximation test yielded negative findings for all *E.coli* isolates tested for verified ESBL production. The current finding were consistent with several studies have demonstrated that although initial screening tests are positive, confirmatory testing are negative (Hadi, 2015, Al-Shammari, 2021). The existence of an ESBL was not verified in all isolates examined, perhaps due to the concomitant development of other forms of β-lactamases, such as AmpC β-lactamase or inhibitor resistant cephalosporinases. However, present study revealed that identification of ESBL producing isolates based on phenotype method is a simple way, but it may be ambiguous. Still, PCR assay for acquired ESBLs genes appears to be a more direct way of assessing the β-lactamase resistance potential of isolates (Oliveira *et al.,* 2022). Out of 72 of E.coli isolates exhibited carrying bla-genes, bla_{SHV}15 (20.8%), bla_{OXA} 12(16.6%) and $bla_{CTX\text{-}M}$ 11(15.2 %). the result of *bla* -genes in present study was lower than other previous study done by Alasady et al (2022) who found the rate of bla_{OXA} was (33%) and $bla_{CTX\text{-}M}$ (35.7%), as well as with the result bla_{SHV} who found the frequency of bla_{SHV} (11%) that was low compare withe current result. Carbapenem resistance poses a potential public-health emergency, especially in developing countries, accounting for high morbidity, mortality, and healthcare costs (Das, S. 2023). The present investigation found that. Out of 72 E.coli, 12 isolates were resistant to carbapenems, the result of carbapenems resistance in *E. coli.* The high susceptibility of *E. coli* isolates to carbapenems has been reported in other studies. (Naqid et al., 2020; Dodoo el al., 2023). The gold standard to confirm the presence of known carbapenemases by the detection og genes encoding these enzymes, using most common carbapenemases specific primers. In contrast with present study bla_{IMP} was detected in 43.1% of *E. coli* and 25.7% of *K. pneumoniae* isolates found in study conducted (Ahmad et al., 2024) in Iraq. The present study reveald that the rate of colistin resistance in *E. coli* (11.1%). present result are in contrast with astudy conducted by (Fadhil and Hadi, 2024) who found that the colistin resistance in E. coli was (0%). Molecular method by using PCR was performed for the detection of plasmid encoded (mcr-1 to mcr-8) genes, result show that of mcr-1, mcr-2, mcr-3, mcr-4, mcr5, mcr-7 and mcr-8 genes were not detected in any of the isolates, although mcr-6 gene was detected in 16(10.6 %) of *E.coli* isolates. The mcr-1 gene was initially found in an E. coli strain that was obtained from Chinese food animals towards the end of 2015. for evaluated molecular mechanisms of colistin resistance among the clinical isolates of *E.coli* from hospitalized patients admmitted to tertiary care hospitals in Iran. As well as this results are consistent with observations from other studies (Haeili et al., 2017; Esposito et al., 2018). Although low frequency, numerous variants og mcr genes have been described from differint regions of the world (Stoesser et al., 2016; Di Pilato et al., 2016; Moosavian et al., 2019; Pishnian et al., 2019). Besides studies reported in Türkiye and Malaysia by (Afyoncu & Eryıldız, 2024; Lemlem et al., 2023), which in line with observations. Other investigation for detection mcr genes found that the mcr-7 was (14.5%) and mcr-8 was (18.8%) (Lemlem et al., 2023). A review article conducted by Rahimi et al (2024) was gathered from associated publications about isolated colistin-resistant E. coli isolates from humans, animals, and food-producing animals. Reported colistin-resistant *E. coli* isolates have recently been identified from the Middle East between (2010 and 2023) Turkey, Egypt, Saudi Arabia, Algeria, Iran, Iraq, Bahrain, Qatar, Oman, Kuwait and Lebanon (Elnahriry *et al.,* 2016; Alfouzan *et al.,* 2018; Yürüyen *et al.,* 2018; Mohsin *et al.,* 2018; Bachiri *et al.,* 2018; Zakaria *et al.,*2019; Moosavian and, Emam, 2019; El-Mokhtar *et al.,* 2021; Tsui *et al.,* 2020; Eltai *et al.,* 2020).

REFERENCES

1- A Review on Colistin Resistance: An Antibiotic of Last Resort
Mondal A, Khare K, [...] Yadav D Microorganisms (2024) 12(4) 772.
2-Gröndahl-Yli-Hannuksela K, Lönnqvist E, Kallonen T, Lindholm L, Jalava J, Rantakokko-Jalava K, Vuopio J. The first human report of mobile colistin resistance gene, mcr-1, in Finland. APMIS. 2018 May;126(5):413-417. doi: 10.1111/apm.12834. PMID: 29696722.
3- MCR-1 Colistin Resistance in Escherichia coli Wildlife: A Continental Mini-review
Shad A
Journal of Drug Metabolism & Toxicology (2018) 09(03).
4- Antibiotics, Antimicrobial Resistance, Antibiotic Susceptibility Testing, and Therapeutic Drug Monitoring for Selected Drugs
Wanger A, Chavez V, [...] Dasgupta A
Elsevier, (2017), 119-153.

5- Sheu C. C., Lin S. Y., Chang Y. T., Lee C. Y., Chen Y. H., Hsueh P. R. (2018). Management of infections caused by extended-spectrum beta-lactamase-producing *Enterobacteriaceae*: current evidence and future prospects. *Expert Rev. Anti Infect. Ther.* 16 205–218. 10.1080/14787210.2018.1436966.
6- Antibiotic Susceptibility Patterns for Carbapenem-Resistant Enterobacteriaceae
Shahnaz Armin 1, Fatemeh Fallah 1, Abdollah Karimi 1, Fereshteh Karbasiyan 2, Masoud Alebouyeh 1, Sedigheh Rafiei Tabatabaei 1, Maryam Rajabnejad 1, Roxana Mansour Ghanaie 1, Seyed Alireza Fahimzad 1, Nafiseh Abdollahi 1, Hannan Khodaei 1, Leila Azimi 1
7- Plasmid-mediated mcr-1 colistin resistance in Escherichia coli and Klebsiella spp. clinical isolates from the Western Cape region of South Africa Newton-Foot M, Snyman Y, [...] Whitelaw A Antimicrobial Resistance & Infection Control (2017) 6(1) 78.
8- Mechanisms of polymyxin resistance: acquired and intrinsic resistance in bacteria
Olaitan A, Morand S, Rolain Frontiers in Microbiology (2014).
9- Genomic Characterization of Nonclonal mcr-1- Positive Multidrug-Resistant Klebsiella pneumoniae from Clinical Samples in Thailand Srijan A, Margulieux K, [...] Swierczewski BMicrobial Drug Resistance (2018) 24(4) 403-410.
10- The global distribution and spread of the mobilized colistin resistance gene mcr-1
Wang R, van Dorp L, [...] Balloux F Nature Communications (2018) 9(1) 1179.
11- Emergence of Plasmid-Mediated Colistin Resistance Gene mcr-1 in a Clinical Escherichia coli Isolate from Egypt Elnahriry S, Khalifa H, [...] Shimamoto Antimicrobial Agents and Chemotherapy (2016) 60(5) 3249-3250.
12- Identification of a novel plasmid-mediated colistin-resistance gene, mcr-2, in Escherichia coli, Belgium, June 2016 Xavier B, Lammens C, [...] Malhotra-Kumar Eurosurveillance (2016) 21(27).
13- Multiplex PCR for detection of plasmid-mediated colistin resistance determinants, mcr-1, mcr-2, mcr-3, mcr-4 and mcr-5 for surveillance purposes Rebelo A, Bortolaia V, [...] Hendriksen R Eurosurveillance (2018) 23(6).
14- mcr-1 and mcr-2 (mcr-6.1) variant genes identified in Moraxella species isolated from pigs in Great Britain from 2014 to 2015 AbuOun M, Stubberfield E, [...] Anjum M Journal of Antimicrobial Chemotherapy (2017) 72(10) 2745-2749.
15- Novel plasmid-mediated colistin resistance gene mcr-7.1 in Klebsiella pneumoniae
Yang Y, Li Y, [...] Wang H Journal of Antimicrobial Chemotherapy (2018) 73(7) 1791-1795.
16. Emergence of a novel mobile colistin resistance gene, mcr-8 , in NDM-producing Klebsiella pneumoniaeWang X, Wang Y, [...] Wang Y emerging Microbes & Infections (2018) 7(1) 1-9
17-Diversity of CTX-M beta-lactamases and their promoter regions from Enterobacteriaceae isolated in three Parisian hospitals. Michèle Saladin 1, Van Thi Bao Cao, Thierry Lambert, Jean-Luc Donay, Jean-Louis Herrmann, Zahia Ould-Hocine, Charlotte Verdet, Françoise Delisle, Alain Philippon, Guillaume Arlet.
18-Surgers L, Boyd A, Girard P-M, Arlet G, Decré D. ESBL-producing strain of hypervirulent Klebsiella pneumoniae K2, France. Emerg Infect Dis. 2016;22(9):1687.
19-Xu H, Huo C, Sun Y, et al. Emergence and molecular characterization of multidrug-resistant Klebsiella pneumoniae isolates harboring blaCTX-M-15 extended-spectrum β-lactamases causing ventilator-associated pneumonia in China. Infect Drug Resist. 2019;12:33.
20-Dallenne, C., Da Costa, A., Decré, D., Favier, C. and Arlet, G. (2010). Development of a set of multiplex PCR assays for the detection of genes encoding important beta-lactamases in *Enterobacteriaceae*. J. Antimicrobe. Che mother., 65: 490-495.
21- Detection of Class 1 Integrons and Antibiotic Resistance of β-Lactamase-Producing Escherichia coli Isolated from Four Hospitals in Babylon, Iraq Khulaif, Mohammed Jasim; Al-Charrakh, Alaa H.
22- Hadi, Zainab & Turaihi, Thanaa & Alsherees, Hashim & Al-Sherees, Abdualmeer & Bashboosh, Alia & Suaad, & Rasheed, Suaad & Al-khateeb, Sumaya. (2020). Prevalence of Integrons and Antibiotic Resistance among Escherichia coli isolated from patient suspected with urinary tract infection from Al-Najaf hospitals. 10.13140/RG.2.2.31854.84805.
23-Al-Shamarti, M. (2024). Activity Assessment of Antibiotics Used Against Different Bacterial Etiological Agents of UTI in Najaf, Iraq. *Iranian Journal of Pathology*, *19*(3), 348-354. doi: 10.30699/ijp.2024.2027209.3293
24- Rusul Najm, & Jinan Mohammed Hussein. (2023). Isolation and Identification of Pathogenic Escherichia coli from Different Sources in Najaf Hospital. Journal of Population Therapeutics and Clinical Pharmacology, 30(8), 459-464. https://doi.org/10.47750/jptcp.2023.30.08.049.
25- Microbiological study and antibiotic susceptibility pattern among lethargic neonates in Najaf city, Iraq. Isam A, Aasam A, [...] Nasrawi M.
26- Molecular Study of Some Virulence Genes of Escherichia coli Isolated from Women with Urinary Tract Infection in AL-Najaf City Al-Nasrawi M, Al-Hashimy A (2020) 42-48.
27- Peter-Getzlaff S, Polsfuss S, Poledica M, Hombach M, Giger J, Böttger EC, Zbinden R, Bloemberg GV. Detection of AmpC beta-lactamase in Escherichia coli: comparison of three phenotypic confirmation assays and genetic analysis. J Clin Microbiol. 2011 Aug;49(8):2924-32. doi: 10.1128/JCM.00091-11.
28- Treatment for carbapenem-resistant Enterobacterales infections: recent advances and future directions Tompkins K, van Duin DEuropean Journal of Clinical Microbiology & Infectious Diseases (2021) 40(10) 2053-20...
29- A Review on Bacterial Resistance to Carbapenems: epidemiology, Detection and Treatment Options Elshamy A, Aboshanab K Future Science OA (2020) 6(3).
30- Phenotypic and genotypic characterization of carbapenem resistant natively isolated Acinetobacter baumannii Chessab J, Shubbar E Materials Today: Proceedings (2023) 80 3655-3659.

31- Molecular profile of aminoglycoside, fluoroquinolone, and class 1 integron genes among gentamicin-resistant Escherichia coli in Najaf City, Iraq Tuwaij N International Journal of Research in Pharmaceutical Sciences (2020) 11(2) 2558-2567.
32- International Clinical Practice Guidelines for the Treatment of Acute Uncomplicated Cystitis and Pyelonephritis in Women: A 2010 Update by the Infectious Diseases Society of America and the European Society for Microbiology and Infectious Diseases Gupta K, Hooton T, [...] Soper D Clinical Infectious Diseases (2011) 52(5) e103-e120.
33- Colistin resistance: a major breach in our last line of defense Paterson D, Harris P The Lancet Infectious Diseases (2016) 16(2) 132-133.
34- Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study Liu Y, Wang Y, [...] Shen J The Lancet Infectious Diseases (2016) 16(2) 161-168.