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Emergence of Plasmid Mediated Colistin Resistance Genes in Klebsiella Pneumoniae Clinical Isolates from Najaf Hospitals

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ABSTRACT

Background: The "last resort" medications for treating infections caused by carbapenem-resistant Enterobacteriaceae are polymyxins, such as colistin.

Objective: prevalence of plasmid-mediated colistin resistance genes that contribute to colistin resistance in *Klebsiella pneumoniae* that produce carbapenemase in clinical isolates in Najaf hospitals

Subjects and Methods: Cross-section study was conducted between November 2024 to February 2025 at Al-Najaf hospitals. Clinical specimens were gathered from individuals who were either admitted to or visited hospitals, All isolates in the current study had been identified by depending on morphological characteristic, microscopically examination, and biochemical tests. Antibiotics susceptibility was performed by disk diffusion methods. Colistin broth disk elution test for colistin antibiotic. The *K. pneumoniae* isolates exhibited resistance to colistin were examined for the presences of plasmid mediated mcr-1 to mcr-8 genes. And *bla* genes (*bla* $_{CTX-M}$, *bla*$_{SHV}$, *bla*$_{OXA}$, and *bla*$_{IMP}$) were identified using the PCR technique.

Results: A total of 150 isolates were obtained during the study period from the major hospitals in Najaf; 78(52%) were recognized as *K. pneumoniae*. Antibiotic resistance among isolates diverse from 11.5% for colistin to 100% for ampicillin, amoxicillin and amoxicillin-clavulanic. Genotype bla gene positive ESBL for *K. pneumoniae* isolates 13 (16.6%) have *bla*$_{OXA}$, 13 (16.6%) *bla*$_{CTX-M}$ isolates have and 17(21.8%) have *bla*$_{SHV}$, the frequency of carbapenemase-encoding genes among the isolates, *bla* $_{IMP}$ was negative for all isolates. All phenotype colistin resistance isolates 9 *K. pneumoniae*, 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: The proportion of colistin-resistant *K. pneumoniae* isolates in Al-Najaf hospital is alarming. This is the first report of plasmid encoded colistin resistance *K. pneumoniae* isolate that carries mcr-6 in Al-Najaf hospital.

KEYWORDS

Plasmid Mediated Colistin resistance genes, Klebsiella pneumoniae, polymyxins

Introduction

Antibiotic resistance has emerged as a significant global public health issue, driven by the rapid adaptation of microorganisms to commonly prescribed antibiotics (1). These genes can be rapidly disseminated among the strains because they exist on the mobile genetic elements (2). The advent of superbugs harbouring

extended-spectrum beta-lac AmpC beta-lactamases, and metallo-beta-lactamases has diminished therapeutic options (3). Carbapenems are beta-lactam antibiotics, with a broad spectrum of action versus numerous Gram-positive and Gram-negative aerobic and anaerobic organisms, utilised for the treatment of serious illnesses unresponsive to normal antibiotic therapy (4). Carbapenems are frequently employed in the medical management of infections that result from multidrug-resistant varieties of Enterobacteriaceae and are regarded as antibiotics of last option for multidrug-resistant Gram-negative bacteria (5,6).

The polymyxins, especially colistin, are regarded as the final option of antibiotics in the treatment of illnesses caused by carbapenem-resistant Enterobacteriaceae (CRE). In 2012, the World Health Organisation classed colistin as vital for the health of humans (7). Colistin reacts with the bacterial outermost membrane via displacing cations that are divalent from the negatively charged phosphate groups of Lipid A in the lipopolysaccharide membrane, resulting in cell lysis (8).

Historically, colistin resistance was attributed solely to chromosomal mutations in the genes responsible for the PmrA/PmrB and PhoP/PhoQ signalling pathways, as well as the negative regulator MgrB. These mutations induce alterations to the Lipids A molecule (8). Recently, transferable resistance to polymyxins mediated by mcr-genes has been identified. The mcr- genes were situated on the plasmid, raising concerns about the potential transmission of resistance to Gram-negative bacteria. The mcr-1 gene was initially identified in *Escherichia coli* and Klebsiella pneumoniae in China (9). Subsequently, numerous studies globally have documented the presence of the mcr-1 gene in various bacteria from countries including the US, the European Union, the nation of Turkey, the Republic of South Africa, the nation of Malaysia, Greece, Italy, the country of Algeria, Tunisia, and Kuwait (10). The initial investigation conducted in Egypt in 2016 identified the mcr-1 gene in an E. coli strain obtained from a patient in an intensive care unit (ICU) (11). In 2016, the mcr-2 plasmid-mediated colistin resistance was initially identified in *E. coli* in Belgium, originating from pigs and subsequently from patients (12). Limited research has recorded colistin resistance throughout individuals in Iraq, despite the antibiotic's vital role as a last option. The aim of the present study is to evaluate the frequency of plasmid mediated colistin resistance genes that contribute to colistin resistance in Klebsiella pneumoniae

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, The specimens were promptly sent to the microbiology laboratory. All Gram-negative isolates in the present investigation have been determined based on morphological characteristics, microscopic examination, and biochemical assays using the conventional methods outlined by MacFaddin and Hart (13).

Antimicrobial susceptibility testing:

Antibiotic susceptibility testing to *K.pneumoniaei* was performed by using disc diffusion method, (Kirby-Bauer method). (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 (14). were performed according to Clinical and Laboratory Standards Institute (15). All sensitivity findings were analysed in accordance with established accepted standards CLSI (14). 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 (16).

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 (14) 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:

Klebsiella pneumoniae 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 (17), and were subjected to further molecular evaluation.

Phenotypic Detection of Carbapenemase Production:

Klebsiella pneumoniae 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

Extracted of DNA following the guidelines set forth by the manufacturing origin business (Magen, China), the genomic DNA extraction was completed. Bio Photometer Plus (Nanodrop) was used to measure the yield and purity of a DNA sample. In order to prepare it for PCR, the isolated DNA was lastly kept in a freezer at -20ºC.

Detection of Resistance Genes by 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 primers sequence and PCR condition were published elsewhere (18-24). Amplicons were analzed by 1.5% agaros gel electrophorese in one X TBE buffer and stained with ethidum bromide.

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	18
	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	19
	R	5’-ATCACGGGATTGACATAGCTAC-3’		
Mcr-7	F	5’-TGCTCAAGCCCTTCTTTTCGT-3	892	20
	R	5’-TTCATCTGCGCCACCTCGT -3’		
Mcr-8	F	5’-AACCGCCAGAGCACAGAATT-3’	667	21
	R	5’-TTCCCCCAGCGATTCTCCAT-3		
bla CTX-M	F	*SCS ATG TGC AGY ACC AGT AA*	554	22
	R	*CCG CRA TAT GRT TGG TGG TG*		

bla_{SHV}	F	GGGTTATTCTTATTTGTCGC	930	23
	R	TTAGCGTTGCCAGTGGTC		
bla_{OXA}	F	GGCACCAGATTCAACTTTCAAG	554	24
	R	GACCCCAAGTTTCCTGTAAGTG		
bla_{IMP}	F	TTGACACTCCATTTACDG	139	24
	R	GATYGAGAATTAAGCCACYCT		

Statistical Analysis: The current study utilised Microsoft's Office Excel 2019, which was used for specific computations and a Chi-square calculator for the analysis of statistics. 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). 78(52%) were recognized as K. pneumoniae obtained from clinical specimens, included urine (65, 58%), burn wound (4, 33%), seminal fluid (4,50%), sputum (3, 37.5%), and wound abscesses (2,20%).

Antibiotics susceptibility patterns:

The result of antibiotic susceptibility profile in *K. pneumoniae* showed that 100% of the isolates were completely resistant to ampicillin, amoxicillin, amoxicillin-clavulanic acid, and ampicillin-sulbactam, piperacillin-tazobactam 88.4% and 76% for ticarcillin-clavulanic acid combinations were resistant to beta-lactam combinations *K. pneumoniae* demonstrated a significant rate of resistance to cephalosporinsIII &IV in the current investigation, exhibiting rates of 90% for ceftazidime and 91% for cefotaxime, 81% for ceftriaxone, 77% cefepime and cephamycin 76% for cefoxitin. The resistance rates to monobactams 48.4% for aztreonam and carbapenem antibiotics were 17% for imipenem and for meropenem.

Furthermore about 26% of K. pneumoniae was resistant to amikacin, 35% to gentamicin, 46% to tobramycin, 48.7% to Kanamycin and 39.9% to netilmicin when it came to aminoglycoside antibiotics. Quinolones class was with resistance rates of 58% for ciprofloxacin, 58% for levofloxacin, and 50% for nalidixic acid, trimethoprim/sulfamethoxazole exhibited a resistance rate of 47% and 38% to Chloramphenicol. Colistin resistance to K. pneumoniae was 11.5% Table (2). 54 (69.2%) *K. pneumonia* isolates were identified as MDR and 24(30.7%) isolates were considered as XDR

Tables (2): **The characteristics of the Antibiotic Susceptibility Profile for *Klebsiella pneumoniae*(n=78).**

Antibiotic classes	**Antibiotic disk**	**No. (%) of isolates exhibited:**		
		R	**I**	**S**
Penicillins	Ampicillin	78(100)	0	0
	Amoxicillin	78(100)	0	0
	Amoxicillin-clavulanic acid	78(100%)	(0)	(0)
	Ampicillin-sulbactam	78(100%)	(0)	(0)

Penicillins + β-lactamase inhibitors	Piperacillin-tazobactam	69 (88.4%)	(0)	9(11.5%)
	Ticarcillin-clavulanic acid	59 (76%)	(0)	13(17%)
Cephalosporins	Cefotaxime	71(91%)	3(4%)	4 (5.1%)
	Ceftazidime	70 (90%)	4 (5.1%)	4 (5.1%)
	Ceftriaxone	63 (81%)	5(6.4%)	10 (13%)
	Cefepime	60 (77%)	2(3%)	15(19.2%)
Cephamycins	Cefoxitin	59 (76%)	(0)	13(17%)
Monobactams	Aztreonam	38 (48.7)		34 (44%)
Carbapenems	Imipenem	13 (17%)	(0)	59 (76%)
	Meropenem	13 (17%)	(0)	59 (76%)
Quinolones	Nalidixic acid	39 (50%)	(0)	39(50%)
	Ciprofloxacin	45 (58%)	(0)	33(42.3%)
	Levofloxacin	45 (58%)	3(4%)	30 (38.4%)
Aminoglycosides	Amikacin	20 (26%)	(0)	58 (74.3 %)
	Tobromycin	36 (46. %)	(0)	47 (60%)
	Gentamicin	27(35%)	(0)	51 (65.3%)
	Kanamycin	38 (48.7%)	(0)	40 (51%)
	Netilmicin	31(39.7%)	(0)	47(60%)
Phenicols	Chloramphenicol	30(38%)	(0)	48 (62%)
Folate pathway inhibitors	Sulfamethoxazole Trimethoprim	37 (47%)	(0)	41(53%)
Lipopeptide	Colistin	9 (11.5 %)		69 (88.4%)

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

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, No isolate was identified as an ESBL generator according to the disc approach test. 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 K. pneumoniae (76%).

Genotype bla gene positive ESBL for and K. pneumonia isolates According to the CLSI the isolate is considered to be a potential ESBL producers Monoplex PCR analysis was performed on 78 isolates to verify the precision of the tests and procedures for genus identification, the findings showed that the isolates had the following genes*:* 13 (16.6%) have *bla*$_{OXA}$, 13(16.6%) *bla*$_{CTX-M}$ isolates have and 17(21.8%) have *bla*$_{SHV}$ of the isolates.

Phenotype and Genotype Carbapenem Resistant in *K. pneumonia*

According to the CLSI (2024) criteria, carbapenem resistant *K. pneumonia* 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 78 examined *K. pneumonia*, a total of 13 (17%) isolates, which showed phenotypic resistance to at least one agent belonging to carbapenem class. The Genotype Carbapenem resistance genes in *K. pneumonia* were negative for *bla*$_{IMP}$ in all isolate**.**

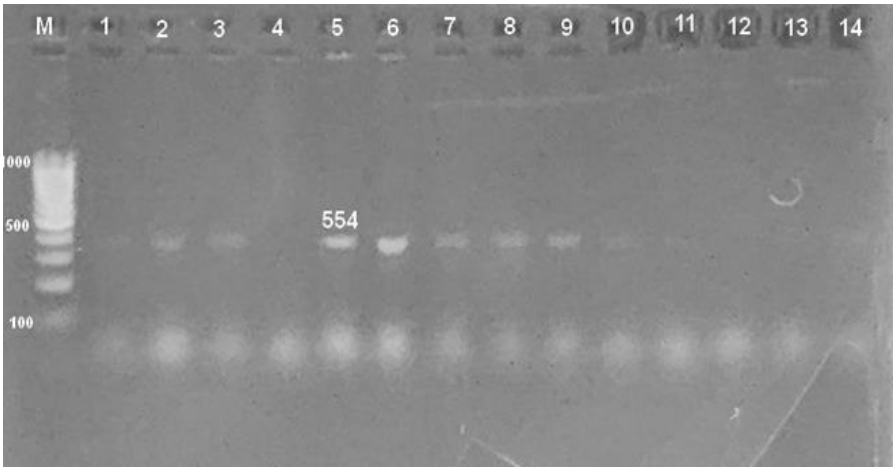

Figure (1): Ethidium bromide-stained agarose gel of Monoplex PCR amplified products from extracted DNA of *K. pneumonia* isolates and amplified with *bla*$_{CTX-M}$ gene primers. The electrophoresis was performed at 65 volts for 1 hr.; 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (M),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

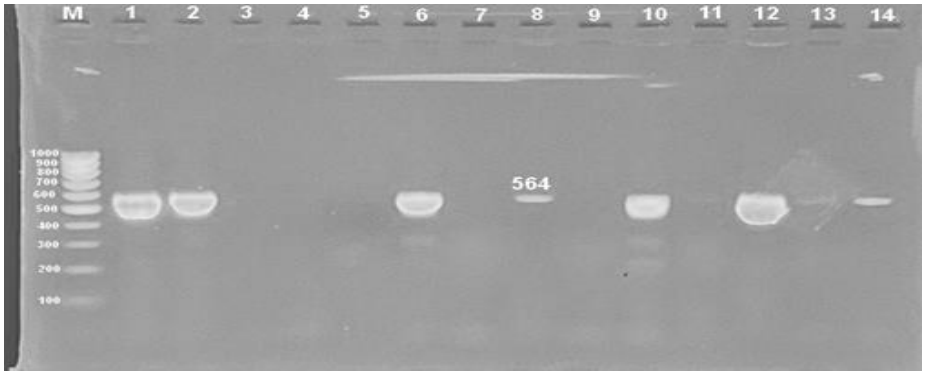

Figure (2): Ethidium bromide-stained agarose gel of Monoplex PCR amplified products from extracted DNA of *K. pneumonia* isolates and amplified with *bla* $_{OXA}$ genes primers. The electrophoresis was performed at 65 volts for 1 hr.; 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (L),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

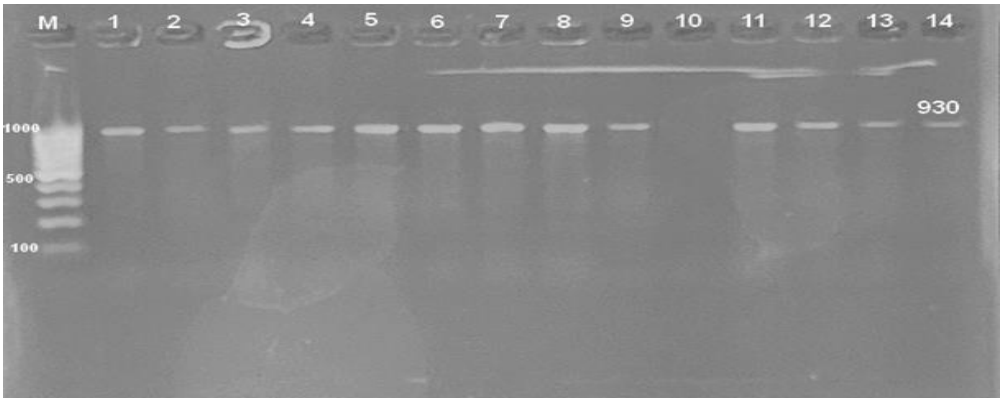

Figure (3): Ethidium bromide-stained agarose gel of Monoplex PCR amplified products from extracted DNA of *K. pneumonia* isolates and amplified with *bla* $_{SHV}$ genes primers. The electrophoresis was performed at 65 volts for 1 hr.; 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (M), 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 *K. pneumoniae*

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 78 clinical *K. pneumoniae* isolates, we identified 9(11.5%) isolates 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 detected in 8(11.1%) *K. pneumoniae*, and maximum number two isolates had a combination of mcr-6 and bla_{OXA}, $bla_{CTX\text{-}M}$, bla_{SHV}, followed by bla_{OXA}, and bla_{SHV} and blaCTX$_{\text{-M}}$, blaSHV in two and one isolates respectively.

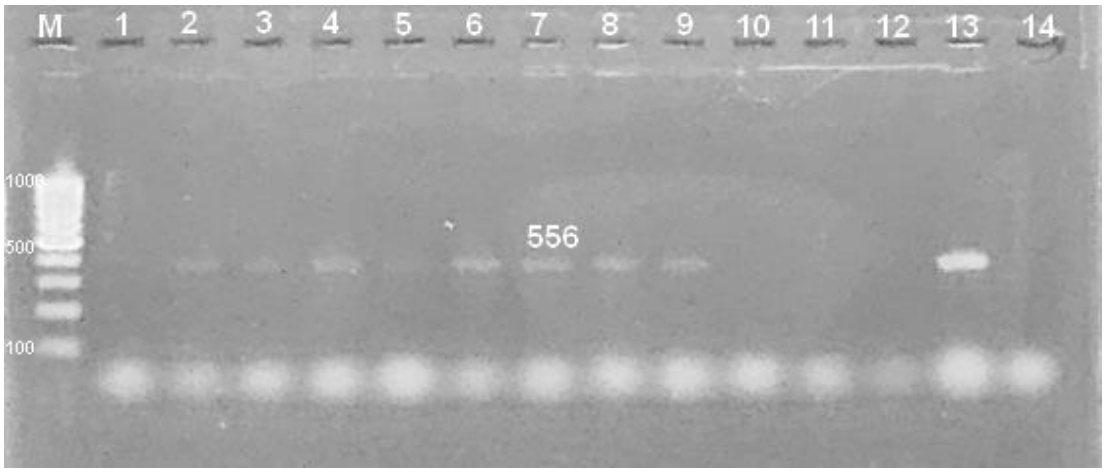

Figure (4-7): Ethidium bromide-stained agarose gel of Monoplex PCR amplified products from extracted DNA of *K. pneumonia* isolates and amplified with blaIMP genes primers. The electrophoresis was performed at 65 volts for 1 hr.; 1.5 g of agarose powder was added to 100 ml of TBE buffer. Lane (M), DNA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

Discussion

During the study period and based on morphological and biochemical characteristics, 78 consecutive, non-repeat, discrete K. pneumoniae isolates were isolated from various clinical specimens of patients had a clinical indication of infections.

Klebsiella pneumoniae is increasingly important opportunistic pathogens that cause a variety of communities and hospitl-acquierd infections, responsible for 30% of all Gram-negative bacterial infection (25, 26). In this study, it was reported that, the frequency of the *K. pneumoniae* isolates were 78 (52%) among clinical specimens. This outcome was line with a study goes along with study in Pakistan 40% (27). On the other hand, current result was higher than a study displayed in Iraq 20.8% (28), Pakistan 20% (29), Northwest Ethiopia 20% (30) Indonesia 17.8% (31),

Eastern Ethiopia 15% (32), Iran10.2% (33), Saudi Arabia 4% (34). However, present study was lower than a study conducted in Iran which was 88% (35). This disagreement might be due to variations in geographic location, sample size, study design, study period, and diagnostic methods.

Antibiotic-resistant bacteria emergence is increasing drastically worldwide, thus limiting the efficacy of antibiotics. Isolates demonstrated a high rate of resistance to antimicrobials The resistance rates (highest and lowest) were observed for Ampicillin (100%) and colistin (17%). K. pneumoniae resistance to Penicillin; ampicillin (100%), amoxicillin (100%), amoxicillin-clavulanic acid (100%), ampicillin-sulbactam (100%), piperacillin-tazobactam (88.4%) and Ticarcillin-clavulanic acid (76%). This result was found to be in agreement with other results in Najaf (36, 37) and 100% of ampicillin sulbactam in K. pneumoniae in Al-Diwaniyah (38).

Cephalosporins and monobactams have been widely used for the treatment of Gram-negative bacteria including K. pneumoniae infections, essentially worthless today. According to the current study, aztreonam and third and fourth generation cephalosporins (such as ceftriaxone, cefotaxime, ceftazidime, and cefepime) one of the high antibiotic resistances were against third and fourth generation cephalosporins and monobactams (including ceftriaxone, cefotaxime, ceftazidime, cefepime) and aztreonam, antibiotic sensitivity test revealed maximum resistant to third and fourth generation cephalosporins; cefotaxime (91%), ceftazidime (90%), ceftriaxone (81%), cefepime (77%) and aztreonam (48.7%). This result was in line with those reported in other studies were conducted in Najaf found high rate of resistance to cefotaxime (93%%), ceftazidime (94%), ceftriaxone (100%) by (39). As well as, the resistance to aztreonam (66.1%) reported by (38). That high when compare with present result. On the other hand, the study conducted in Al-Ramadi Teaching Hospital, Iraq shows the resistance to cefepime (75.6% %) by (40) that agreement with present result. These high rates of resistance may be due to the lack of antibiotic policy and the irrational use of third and fourth generation cephalosporins and monobactams in Iraqi hospitals.

Result shows the resistance rate to cefepime (77%) in K. pneumoniae isolates this result was similar with other local studies done by Al-Qaysi et al (2024) (41) who found 75.6% of K. pneumoniae isolates exhibited resistance to cefepime. Despite the limited use of cefoxitin (cephamycin) in the therapy of bacterial infection in Iraq, the findings of this study indicated a higher resistance (76%) of K. pneumoniae isolates. This result of cefoxitin in K. pneumoniae is consistent with the findings recorded in Najaf by (42). Cefoxitin resistance may indicate the possibility of AmpC-mediated resistance but it can also be a suggestion of reduced outer membrane permeability (43).

Susceptibility of K. pneumoniae different percentages were carried out by local studies, such as Mohsin (2024) who establish 24.3% of K. pneumoniae isolated from clinical specimens had carbapenem-resistant. Similarly to a study by Jubair et al. (2020) who found 15% of the K. pneumoniae isolates were carbapenem resistant.(43,44)

This investigation also indicated that K. pneumonia had highest and lowest resistance to kanamycin (48.7%) and amikacin (26%). This result was agreement with local previous study conducted by Alasady et al (2022) (40).

Colistin, a the polymyxin antibiotic infrequently utilised in clinical settings up until recently because of its nephrotoxic effects, is now increasingly frequently administered due to the rising global incidence of multidrug-resistant, or MDR, infections for which other antibiotic therapies are unavailable (45; 46). The mobile resistance to colistin (mcr) gene was first recognised as the source of colistin resistant in Escherichia coli obtained from an intensive pig farm in Shanghai, China(47).

In this study, antibiotic susceptibility tests found that 11.5% K. pneumonia isolates were resistant to colistin. There are very few reports available on colistin-resistant K. pneumoniae isolated from Iraq. Colistin resistance was detected in 10.8% of E. coli and K. pneumoniae clinical isolates in Iran according to Moosavian and Emam (2019) (48). Furthermore Jubair et al. (2020) found 13.6% colistin-resistant carbapenem-resistant K. pneumoniae isolated from Najaf hospitals. According to Al-Jubouri (2023), 4.3% of XDR K. pneumoniae in Najaf showed resistance to colistin. In comparison, the rates of colistin-resistant K. pneumoniae isolates from various countries were found to be: 35.1% in Saudi Arabia (47), 31.4% in the United Arab Emirates (48), 39% in Egypt (49), 28.8% in Iran (50), 14.6% in India (51), and 13% in the United States (52). The current investigation used established laboratory methods (colistin broth disk elution test) to determine the MIC values of colistin according to the CLSI. The current data demonstrate that colistin is the most effective antibiotic against these isolates. Furthermore, limited resistance to colistin has been shown in local research, owing to the fact that colistin is not commonly prescribed in Najaf and is frequently reserved for inpatients.

The percentage of MDR K. pneumoniae isolates in present study was 69.2% and 30.7% was XDR. The

frequency of multiple resistant K. pneumonia isolates was reported in numerous studies by Hasnawi (2020) and Al-Jubouri (2023) in Al-Najaf (55, 56). As well as, study in Northwest Ethiopia done by Worku et al (2024) (30), who found proportion on MDR K. pneumonia was 57.5% is lower than present study. Infection caused by such isolates frequently results in significant therapeutic difficulties and, in some cases, death of infected individuals (57). Many reasons may lead to the spread of multidrug resistant isolates in Najaf hospitals, including poor infection control measures and antibiotic usage. Multidrug-resistant K. pneumonia is one of the most important growing challenges in bacterial resistance.

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