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Molecular Study of Quinolone Resistance in *Klebsiella pneumonia*e and *Citrobacter freundii* Isolates

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Abstract

A total of 97 burns swab collected from patients treated in burns unit of Al-Sader Hospital, Al-Najaf Province, during the period five months (1/6/2014 to 1/11/2014). Only 75/97(77.31%) were positive bacterial culture on MacConkey agar medium compared with 22/97(22.68%) specimens gave negative bacterial growth. The results of microscopic, morphology culture, biochemical test and Vitek-2 system revealed that 23/75(30.66%) isolates, were obtained as following 21/75(28%) *Klebsiella pneumonia*e ssp *pneumoniae* and 2/75(2.66%) *Citrobacter freundii* were recovered from 75 positive specimens obtained aseptically from admitted patients in the burn unit. Out of these, 15/23 (65.21%) were isolated from female patients and 8/23 (34.78 %) from male patients.

Antimicrobial susceptibility were done using disk diffusion method and minimal inhibitory concentration (MIC) strip test. Results showed high degree resistance to most antibiotic under study and all tested isolates were at least resistant to three or more of antibiotic classes and then consider as multidrug resistant isolates (MDR) , At same time, ertapenem revealed maximum effectiveness against *K. pneumonia*e and *C. freundii* isolates with resistance rate reached to (28.57%) and (0%) respectively. While cloxacillin, oxacillin, and carbenicillin were offered less effective among antibiotic through resistance 100%. 14(66.66%) of *K. pneumoniae* isolates gave high level of resistance to nalidixic acid and ciprofloxacin with MIC ≥ 256 µg/ml and MIC ≥32 µg/ml respectively compared with 1(50%) in *C. freundii* isolates and these were considered as highly resistance. The PCR data showed that *aac(6')-Ib –cr* gene high prevalence 15(71.42%) in *K. pneumoniae* isolates compared to 1(50%) in *C. freundii* no.2. Also *qnr B* gene was positive in 10(47.61%) *K. pneumonia*e, while only 1 (50%) of *C. freundii* isolates was harbored *qnr B* gene. While *qnr*, *qnr C* and *qnr D* genes did not detect in this study.

Keyword: *Klebsiella pneumonia*e**;** *Citrobacter freundii***;** PCR; Quinolone Resistance

Introduction

Drug resistance is a significant problem for treatment of infectious diseases in the hospitals and in the community [1,2]. The burns form a major health concern responsible for morbidity and mortality because of the danger of burn infections due to reduces effectiveness of treatment caused by multidrug resistant nosocomial pathogens [3,4]. The evolution of multidrug resistant isolates in units of the burns , especially in economically underdeveloped and developing countries, form a prime problem in the control of infections moreover have repeatedly been reported as the reason of outbreaks of nosocomial infection in units of the burns. [5,6,7]. Antimicrobial resistance has significantly increased among Enterobacteriaceae (including *K. pneumoniae* and *C. freundii*), which has made it difficult to treat infections. Therefore, there is a need for safe and effective drugs to treat infections resistant to multiple antibiotics [8]. Consistent surveillance of microbial profile and their antimicrobial susceptibilities should be encouraged to help guide first-line therapy for burns infection [3].

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Quinolones are broad spectrum antimicrobial agents, widely used for bacterial infection treatment [9]. The first report for quinolone resistance gene was *qnrA*, located on plasmid pMG252 in *K. pneumoniae* isolates in 1988 from the United States. Other plasmid mediated quinolone resistance genes have been described in Enterobacteriaceae involving *qnr B, qnr C, qnr S qnr D, qepA* and *aac(6)-Ib-cr* [10]. Several recent studies have indicated that quinolone resistance is increasing worldwide. Therefore, the current work was achieved to examine the frequency of quinolone genes and antibiotic susceptibilities among clinical isolates of *K. pneumoniae* and *C. freundii* isolated from patients treated in burns unit of Al-Sader Hospital, Al-Najaf Province, Iraq.

Materials and Methods

The study was done at Laboratories of Bacteriology and Molecular in Biology Department, Faculty of Sciences, University of Kufa, Iraq.

Samples collection and bacterial identification

The study included 97 swabs collected from pus of burn patients suffering from second to third degree who admitted to Al-Sader Medical City in Al-Najaf Province during the period five months (1/6/2014 to 1/11/2014). Each swab was transport quickly using transport media to the laboratory to culture on ,MacConkey agar under sterile conditions, and then incubated aerobically overnight at 37C˚. The grown isolates were diagnosis initially using gram stain and some conventional biochemical tests listed in table 3, and the motility test was also done [11]. Furthermore these tests, all suspected isolates were cultured on CHROMagar Oreintation medium (CHROMagar™, France). Finally the diagnosis was confirmed through automated Vitek-2 system (bioMèrieux France) using ID-GN card. Then all positive isolates were stored at -20˚C until further molecular and microbiological experiment.

Antibiogram profile

Disk diffusion method

Antibiotic susceptibility was carried out for all isolates of *K. pneumoniae* and *C. freundii* according to the guidelines of the Clinical and Laboratory Standards Institute [12], using disk diffusion method (Kirby-Bauer method)[13]. Individual cells were suspending adjusted to a 0.5 McFarland standard tube and spread on surface of Mueller Hinton Agar. The test was conducted using some disks commercially obtainable antibiotics (Bioanalyse, Turkey) including oxacillin (5µg), cloxacillin(10µg), carbenicillin (25µg), (cefoxitin (30µg), ceftazidime (30µg), cefepime (30µg), aztreonam (30µg), gentamicin (10µg), amikacin (10µg), nalidixic acid(30µg), ciprofloxacin (5µg), imipenem (10µg) and Ertapenem (10µg). In this test *Escherichia coli*, ATCC 25922 strain was used as quality negative control.

Measurement of minimal inhibitory concentration (MIC)

All isolates were offered to Antibiotic susceptibility testing regarding minimal inhibitory concentration (MIC) for ciprofloxacin (ranging from 0.002 to 23 µg/ml) and nalidixic acid (ranging from 0.16 to 256 µg/ml) using MIC tests strip that applied onto an incubated Mueller Hinton agar (England) The turbidity of isolates suspension was set to 0.5 McFarland standard depending on manufacturer's recommendations (Liofilchem®, Italy), The MIC is record at the mark where the margin of the inhibition ellipse intersects with the Strip and data were explained by Clinical Laboratory Standards Institute (CLSI) instructions [12]. However, These were set up at the same time with the disk diffusion test, and incubated under the same conditions. *Escherichia coli* ATCC 25922 strain was used as negative quality control.

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Total DNA extract

Total DNA of all isolates were extracted using the boiling method. Bacterial suspension was prepared by a single colony for each isolate was inoculated in sterile tube contain 5 ml of sterile nutrient broth medium (LAB M, UK) and incubated for 24 hours at 37 ºC. then 1.5 ml of the growth culture was harvested using centrifugation at 8000 xg for 5 minutes. Supernatant was discard and the Pellets were resuspended in 300 µl of molecular biology grade water, the cells subjected to lysis using boiling at 100 ºC in a water bath apparatus for 10 minutes. Then directly cooled on ice for 15 minutes and cellular debris was removed by centrifugation at 800 xg for 5 minutes. Supernatant was used as DNA template for PCR amplification and stored at - 20 ºC [14,15].

PCR amplification and gel electrophoresis

DNA of all isolates were subjected to PCR to detect *qnr, qnr B, qnr C, qnr D* and *aac(6')-Ib –cr* genes. The specific primers and reaction conditions that used in the work are shown in tables 1 and 2.

Amplified products were confirmed using 1% agarose gel electrophoresis to estimate the PCR products size. The gel was stained with 4 µL of 10 mg/mL ethidium bromide (Sigma, USA) and it run at 80v for 1.5h. A single band was observed at the desired position on ultraviolet light transillumintor (Cleaver, UK); bands were photographed using gel documentation system (Cleaver, UK). A 100bp ladder (Bioneer, Korea) was used to measure the molecular weights of amplified products [16].

Table1(1): Specific primers used in the present study

Gene	Name	Sequence (5' to 3')	product size(bp)	Reference
aac(6')-Ib -cr	*aac(6')-Ib -cr* -F	TTGCGATGCTCTATGAGTGGCTA	482	[17]
	aac(6')-Ib -cr -R	CTCGAATGCCTGGCGTGTTT		
qnr	*qnr* -F	GATAAAGTTTTTCAGCAAGAGG	593	[18]
	qnr -R	ATCCAGATCCGCAAAGGTTA		
qnr B	*qnr B*-F	ATGACGCCATTACTGTATAA	560	[19]
	qnr B-R	GATCGCAATGTGTGAAGTTT		
qnr C	*qnr C*-F	GGGTTGTACATTTATTGAATC	447	[20]
	qnr C-R	TCCACTTTACGAGGTTCT		
qnr D	*qnr D*-F	TATTCCCCGTAAATTGATCTCG	2200	[21]
	qnr D-R	CAGGCGCTTCAGCTTGTT		

F:Forward primer; R: Reverse primer

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Table (2): The thermal cycler conditions

monoplex gene	Temperature (c) / Time					Cycle number
	Initial denaturation	Cycling condition			Final extension	
		Denaturation	Annealing	Extension		
aac(6')-Ib-cr	94°C/4 min	94°C/45 sec.	55°C /45sec.	72°C/ 45sec.	72°C/5 min	35
qnr	94°C/5 min	94°C/1 min.	57°C/40 sec	72°C/1 min	72°C/7 min	35
qnr B	95°C/5 min	94°C/45 sec	53°C/45 sec	72°C/1 min	72°C/5 min	35
qnr C	94°C/5 min	94°C/40 sec.	50°C/40 sec.	72°C/40 sec	72°C/5 min	35
qnr D	94°C/5 min	94°C/40 sec.	53°C/40 sec.	72°C/3 min	72°C/10 min	35

Statistical Analysis

The electrophoresis results were analyzed size of DNA bands (PCR amplicons) were measured by using gel analyzing program (UV band software version12.14) in comparison with DNA ladder (100 bp).

Results:

Results indicated that among 97 skin swabs 75/97 (77.81%) gave positive culture on MacConkey agar medium compared with 22/97 (22.68%) were negative growth. Also the results showed depending on characteristics of the microscopic , culture morphological and some conventional biochemical tests. Further identified by Vitek 2 system (Biomerieux, France) that, only 23/75(30.66%) isolates obtained from 75 positive specimen as following 21/75(28%) of isolates belong to *K. pneumoniae* ssp *pneumoniae* and only 2/75(2.66%) isolates identified as *C.freundii***.** According to sex the data in this study observed that among 21 isolates of *K. pneumoniae* were 13/21 (61.90%) isolated from female patient compared with 8/21(38.09%) in male. At same time 2/2(100%) *C.freundii* were isolated from female patients resting in burns unit of Al-Sader Hospital, Al-Najaf Province.

The present study revealed that features of *K. pneumoniae* isolates were gram negative bacilli, encapsulated, large spherical , mucoid colonies and pink color on MacConkey agar while metallic blue color on CHROMagar orientation medium. *C.freundii* isolates were also gram negative straight rods, small circular, convex and dark pink color on MacConkey agar while metallic blue with red

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halo that surrounded colony when cultured on CHROMagar orientation medium at 37C for 24 h.(figure 1). The routine biochemical for two genus were described in table (3).

When comparing these results with the results of other local studies, this finding was closer to study which conducted in the city of Medicine of the capital Baghdad, by Alkaabi, [22] who found among 67 specimens recovered from burn patients (males 32, females 35) obtained 54 (80.59%) bacterial growth and 13 specimens were sterile (19.4%), while the percentage of *K. pneumoniae* and *C. freundii* isolates were (31.48%) and (1.85%) respectively.

In another local study reported by Razzaq *et al*.,[23] mention that among 60 specimens from patients suffering postoperative wound infection obtained 50 (83%) gave positive growth compared with 10 (17%) were negative bacterial growth. Which found rate of *K. pneumoniae* and *Citrobacter spp*. isolates amounted (11.42%) and (2.85%) respectively. Elsewhere international study conducted by Ahmed *et al*., [24] found A total of 119 (70%) bacterial isolates were recovered from 170 non-duplicate samples collected aseptically from admitted patients in the burn unit of tertiary care hospital in Pakistan, . among these, 74(62.1%) were isolated from male patients and 45 (37.9%) from female patients. *K. pneumoniae* and *Citrobacter* spp were 5 (4.2%)and 1(0.84%) respectively.

However, isolation of Enterobacteriaceae (including *K. pneumoniae* and *C freundii* isolates) from burn units usually is repeated and become reported by various universal studies, including that Iraq [22,23]. The importance of these agents, especially in burn patients, is coupled with their deficient immune responses as well as their skin damaging effects. Also, the existence of both intrinsic resistance and acquired resistance among these bacteria, especially in *K. pneumoniae* isolates, produce them a large challenge.

Table (3): Identification results of some biochemical tests for *K. pneumoniae* and *C. freundii*

Test / Result	Oxidase	catalase	urease	Citrate	VP	MR	motility	Kliglar iron agar	Indole	Lactose fermentation	H_2S production
***K. pneumonia*e**	-	+	+	+	+	-	-	Acid/Acid	-	+	-
C. freundii	-	+	+	+	-	+	+	Acid/Acid	-	+	+

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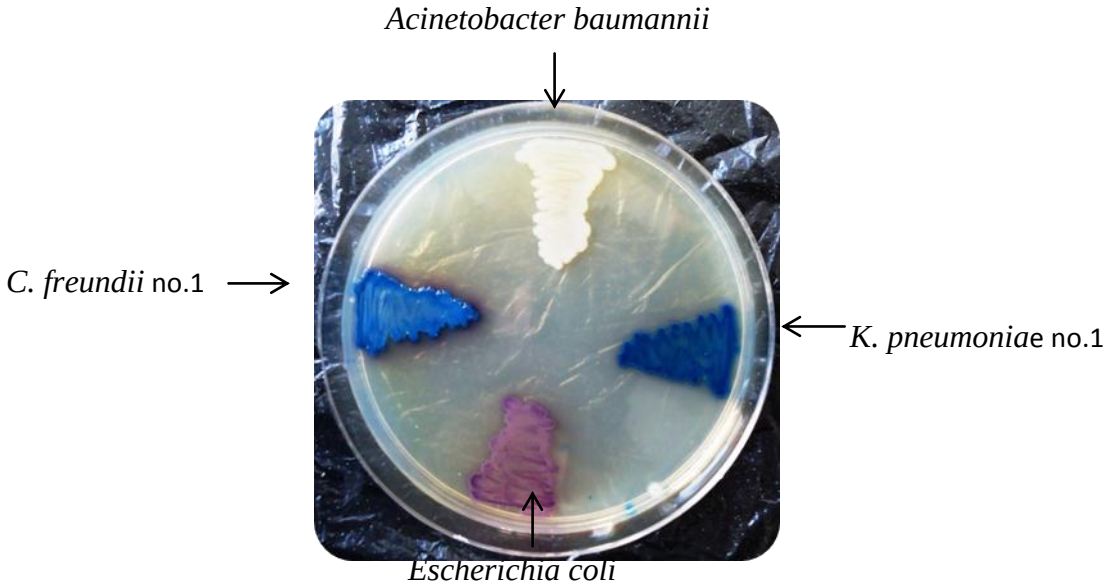

Figure (1): *K. pneumoniae* no.1 and *C. freundii* no.1 on CHROMagar orientation medium after 24 hours (*K. pneumoniae* gave metallic blue, *C. freundii* gave metallic blue with red halo).

Antibiogram profile test
Disk diffusion method

Antimicrobial resistance is a major clinical problem on treating bacterial infection worldwide [1]. All isolates were investigated for their susceptibility toward selective antibiotics agents which recommended by CLSI [12]. In the current study, *K. pneumoniae* and *C. freundii* isolates showed high level of resistance to antibiotic classes under study. However, the data revealed that all the isolates were resistant to three or more classes of antibiotics so considered as multi-drug resistant isolates (MDR).

K. pneumoniae isolates were revealed a varied levels of resistance where started with cloxacillin, oxacillin, and carbenicillin were offered less effective among antibiotic through resistance 100%, then nalidixic acid with percentage 90.47%. While amounted to 85.71% in ceftazidime and cefepime; the resistance to aztreonam, ciprofloxacin and cefoxitin were 76.19%, 71.42% and 47.61% respectively. The study also showed moderate resistance to aminoglycosides antibiotic (gentamicin and amikacin) reached to 57.14% and 61.90% respectively. Finally, 52.38% of isolates were resistance imipenem compared with 28.57% to Ertapenem antibiotic which gave lower rate of resistance among *K. pneumoniae* isolates in this work (table 4). However, This data were closer to a previous local study establishedin Karballa Province by Mohammed [25] who indicated that *K. pneumoniae* isolates which isolated from burns samples were significantly high against antimicrobial agent. Through resistance to piperacillin 100, gentamicin 66.66 , amikacin 27.2, ceftazidime 100, cefotaxime 100 , tobramycin 87.8, ciprofloxacin 45.4 and imipenem 30.3, meropenem 42.4.

C. freundii isolates were 100% resistance to cloxacillin, oxacillin, carbenicillin, cefoxitin and amikacin. While 50% of isolates were resistance to ceftazidime, cefepime aztreonam, nalidixic acid, ciprofloxacin gentamicin and imipenem. Ertapenem was more active against *C. freundii* isolates through *C. freundii* no.2 was appeared intermediate compared with *C. freundii* no.1 that was sensitive by Disk diffusion method according to CLSI guide [12] criteria (table 4).

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The results were not different widely from the results of a former local study carried out in Baghdad by Alkaabi [22] who found the resistance of *K. pneumoniae* isolated from burn swabs to Amoxycillin (100%), Cefotaxime (100%), cefepime (58.82%), ciproflaxocin (70.58%), amikacin (76.47%) and imipenem (5.88%) while it were Amoxycillin (100%), Cefotaxime (100%), cefepime (0%), ciproflaxocin (100%), amikacin (100%) and imipenem (0%) in *C. freundii* isolates.

Measurement of minimal inhibitory concentration (MIC)

Estimation of MIC value for two quinolones antibiotics including nalidixic acid and , ciprofloxacin was done as complementary test to the previous antibiotic susceptibility test to resistance level of all isolates toward their substrates. out of 21 isolates of *K. pneumonia*e; it was notice 19 (90.47) isolates were resistance to nalidixic acid, with MIC ranging from 32 µg/ml to ≥ 256 µg/ml. 14(66.66%) of *K. pneumoniae* isolates gave high level of resistance with MIC ≥ 256 µg/ml compared with 2(9.52%) isolates were sensitive to nalidixic acid with MIC 6 µg/ml and 8 µg/ml. The data of MIC of ciprofloxacin in this study indicated that only 15 (71.42%) isolates of *K. pneumoniae* were resistance to this antibiotic, with MIC ranges of 4 µg/ml to 32 µg/ml (figure, 2). As shown in table (5), 6(28.57%) isolates of *K. pneumoniae* were sensitive with MIC ranged from 0.32 µg/ml to 0.75 µg/ml. However, this data showed 14 (66.66%) of *K. pneumoniae* isolates gave high level of resistance with MIC ≥32 µg/ml and were considered as highly resistance. Two isolates of *C. freundii* were obtained in this work , *C. freundii* no. 1 was sensitive to nalidixic acid and ciprofloxacin with MIC 8 µg/ml and 0.32 µg/ml respectively. Whereas, *C. freundii* no.2 was resistance to nalidixic acid and ciprofloxacin with MIC 256 µg/ml and 32 µg/ml respectively (figure, 3).

However, quinolone drugs is usually widely used in Iraq [26]; the repeated use of these drugs against gram negative bacteria lead to generating resistance to these antimicrobials among these bacteria. In this study, the resistance rates of *K. pneumoniae* and *C freundii* isolates to tested quinolone's antibiotics (nalidixic acid and ciprofloxacin) were moderate to high. However, The resistance to quinolones in Enterobacteriaceae is usually as a result of a change in the alteration in the target enzyme and prevent the engagement of these antibiotics with this enzymes (DNA gyrase and DNA topoisomerase type IV) or by prevent the entry of the drug into the bacterial cell by controlling process in membrane porin proteins permeability, as well as the phenomenon of efflux pump or by plasmids [27].

Table (4): Antibiogram susceptibility profile for *K. pneumoniae* and *C. freundii* isolates

Isolate No.	PY	OX	CX	FOX	CAZ	FEP	ATM	NA	CIP	CN	AK	IMP	ETP
K1	R	R	R	S	R	R	R	R	S	**S**	**S**	**S**	**S**
K2	R	R	R	R	I	S	S	R	S	S	I	R	I
K3	R	R	R	S	S	S	S	S	S	I	R	S	S
K 4	R	R	R	I	R	R	S	R	R	S	S	R	S
K 5	R	R	R	R	R	R	R	R	R	R	R	R	R
K 6	R	R	R	S	R	R	S	R	I	S	I	S	S
K 7	R	R	R	R	S	S	S	R	R	**S**	**S**	**S**	**S**

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K 8	R	R	R	S	R	R	R	S	S	**S**	**S**	**S**	**S**
K 9	R	R	R	R	R	R	R	R	R	R	R	R	R
K 10	R	R	R	S	R	R	R	R	R	S	R	S	S
K 11	R	R	R	R	R	R	R	R	R	R	R	R	I
K 12	R	R	R	S	R	R	R	R	R	R	I	S	S
K 13	R	R	R	S	R	R	R	R	R	R	R	S	S
K 14	R	R	R	S	R	R	R	R	R	R	R	R	S
K 15	R	R	R	S	R	R	R	R	R	S	I	S	S
K 16	R	R	R	R	R	R	R	R	R	R	R	R	R
K 17	R	R	R	R	R	R	R	R	R	R	R	R	I
K 18	R	R	R	R	R	R	R	R	R	R	R	R	R
K 19	R	R	R	R	R	R	R	R	R	R	R	R	R
K 20	R	R	R	S	R	R	R	R	S	R	R	S	S
K 21	R	R	R	R	R	R	R	R	R	R	R	R	R
Cf. 1	R	R	R	R	S	S	S	S	S	I	R	S	S
Cf. 2	R	R	R	R	R	R	R	R	R	R	R	R	I

K, *K. pneumoniae***; Cf.** *C. freundii* ; R, Resistance ; I, Intermediate ; S, Sensitive ; PY, carbenicillin; OX, oxacillin; CX, cloxacillin ; FOX, cefoxitin ; CAZ, ceftazidime ; FEP, cefepime ; ATM, aztreonam ; NA, nalidixic acid; CIP, ciprofloxacin ; CN, gentamicin ; AK, amikacin ; IMP, imipenem and ETP, Ertapenem.

A **B**

Figure (2): MIC determination of tested isolates using MIC test strip (A, sensitive to ciprofloxacin *K. pneumoniae* no.6 ; B, resistance to ciprofloxacin *K. pneumoniae* no.4)

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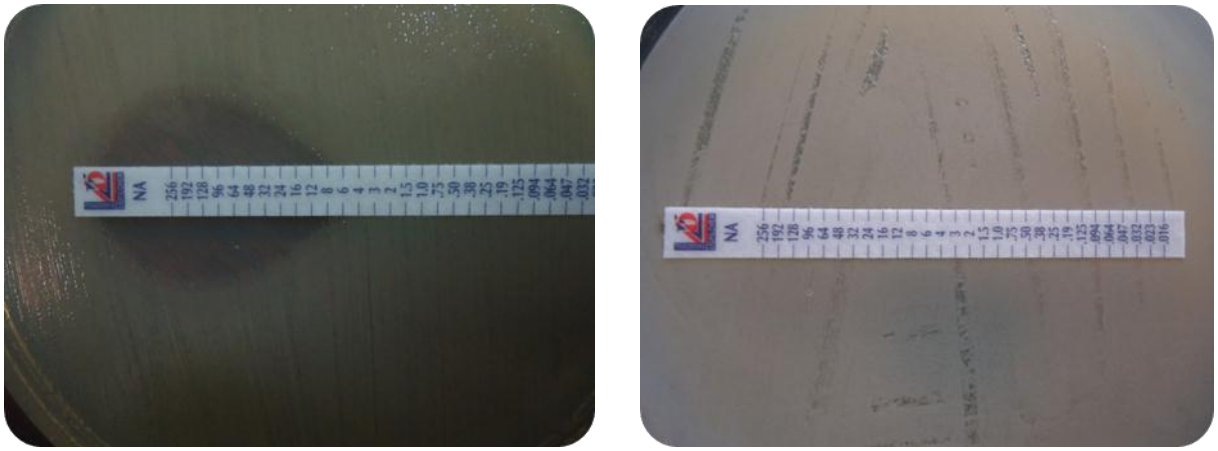

A **B**

Figure (3): MIC determination of tested isolates using MIC test strip (A, sensitive to nalidixic acid *C. freundii* no.1 ; B, resistance to nalidixic acid *C. freundii* no.2)

Molecular detection

Several studies indicated that the resistance to quinolones drugs is associated with different enzymes that are encoded by bacterial genes including *qnr , qnr B, qnr C, qnr D* and *aac(6')-Ib -cr*.

All 23 isolates (21 isolates *K. pneumoniae* and 2 isolates *C. freundii*) were investigated to detect genes; *aac(6')-Ib -cr , qnr , qnr B, qnr C* and *qnr D* which encode for enzymes responsible for catalysis quinolones antibiotics using PCR technique with specific forward and reverse primers . Seen from the results shown in figure 4 of the current study to *aac(6')-Ib –cr* gene high prevalence among tested isolates represented 15(71.42%) in *K. pneumoniae* compared to 1 (50%) in *C. freundii* no.2. Also the results of PCR amplification for *qnr B* gene gave positive bands in 10 (47.61%) *K. pneumoniae*, while only one isolate of *C. freundii* no.2. (50%) was harbored *qnr B* gene (figure, 5). However, The data in this work was revealed that the gene amplicons for *qnr, qnr C and qnr D* genes did not detect in any isolates of *K. pneumoniae* and *C. freundii* as shown in table (5). Several studies indicated that *aac(6')-Ib-cr* gene seems to be more prevalent [28] . In Iraq this gene was previously recorded in clinical isolates of *E. coli* and *Acinetobacter* spp.in Al-Najaf and Al-Qadisiya cities respectively [26, 29].Cruz *et al*., [30] in a study carried out in Argentina state among Enterobacteriaceae found high rates of *aac(6')-Ib-cr* and *qnr B,* genes which reached to 42.4% and 33.3%, respectively. While *qnr C* and *qnr D* genes did not appear in any bacterial isolates. In a study, reported by Jacoby *et al*., [31] they indicated that *qnr B* is more common in *Citrobacter* species than in other Gram-negative bacteria when investigate 71 clinical isolates belonging to the *C. freundii* complex found 37% contained *qnr B* alleles, while none contained *qnr A, qnr C, qnr D, qnr S,* or *aac(6)-Ib-cr.* Park *et al*., [32] reported a quinolones genes prevalence of 38.4% in *C. freundii* isolates from South Korea.The prevalence of isolates that able to catalyze quinolones drugs and their isolation from life-threatening infections such as those found in burn patients is increasing at an alarming rate worldwide. Increased pressure for usage of antimicrobial drugs in the treatment of burns has resulted in the eradication of normal flora, which may be a cause of the substitution of MDR isolates .

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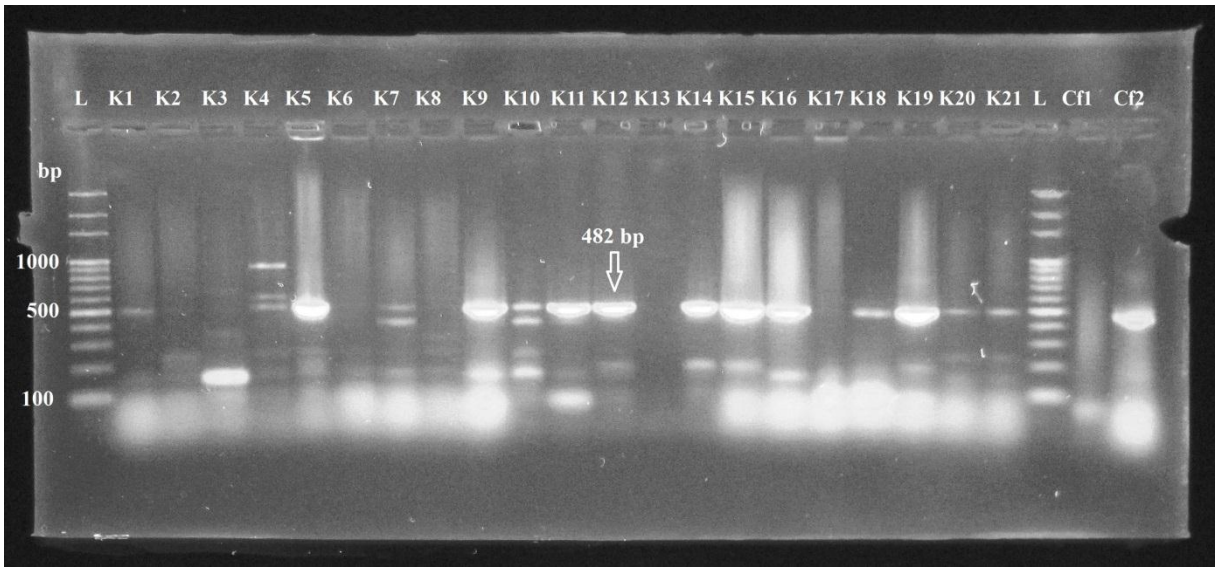

Figure (4): Gel electrophoresis (1% agarose, 80V for 1.5 hour) of PCR product for *aac(6')-Ib -cr* gene (482bp), K, represent *K. pneumoniae*; Cf, represent *C. freundii* ; L, DNA ladder 100bp.

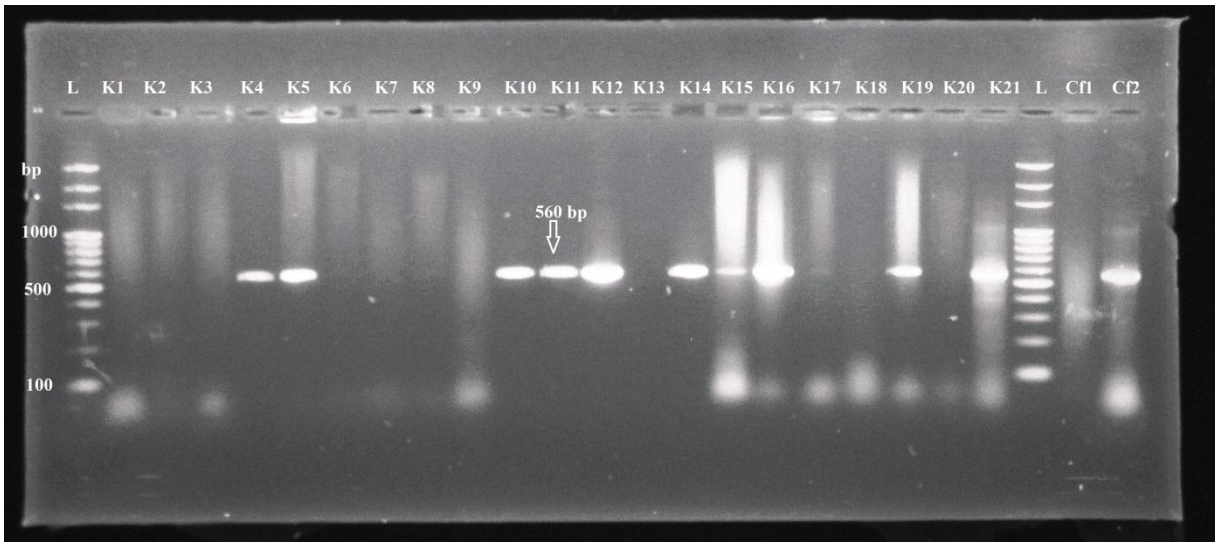

Figure (5): Gel electrophoresis (1% agarose, 80V for 1.5 hour) of PCR product for *qnr B* gene (560bp), K, represent *K. pneumoniae*; Cf, represent *C. freundii* ; L, DNA ladder 100bp.

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Table (5): frequency of phenotypic and genotypic to quinolones antibiotics among *K. pneumoniae* and *C. freundii* isolates

Name and no. of isolate	Phenotypic character				Genotypic character				
	NA by Disk diffusion	NA by MIC strip*	CIP by Disk diffusion	CIP by MIC strip**	*aac(6')-Ib -cr* gene	*qnr* gene	*qnr B* gene	*qnr C* gene	*qnr D* gene
K1	R	32 (R)	S	0.50 (S)	+	-	-	-	-
K2	R	32 (R)	S	0.75 (S)	-	-	-	-	-
K3	S	6 (S)	S	0.32 (S)	-	-	-	-	-
K4	R	256 (R)	R	32 (R)	+	-	+	-	-
K5	R	256 (R)	R	32 (R)	+	-	+	-	-
K6	R	32 (R)	I	0.47 (S)	-	-	-	-	-
K7	R	64 (R)	R	4 (R)	+	-	-	-	-
K8	S	6 (S)	S	0.32 (S)	-	-	-	-	-
K9	R	256 (R)	R	32 (R)	+	-	-	-	-
K10	R	256 (R)	R	32 (R)	+	-	+	-	-
K11	R	256 (R)	R	32 (R)	+	-	+	-	-
K12	R	256 (R)	R	32 (R)	+	-	+	-	-
K13	R	256 (R)	R	32 (R)	-	-	-	-	-
K14	R	256 (R)	R	32 (R)	+	-	+	-	-
K15	R	256 (R)	R	32 (R)	+	-	+	-	-
K16	R	256 (R)	R	32 (R)	+	-	+	-	-
K17	R	256 (R)	R	32 (R)	-	-	-	-	-
K18	R	256 (R)	R	32 (R)	+	-	-	-	-
K19	R	256 (R)	R	32 (R)	+	-	+	-	-
K20	R	48 (R)	S	0.32 (S)	+	-	-	-	-
K21	R	256 (R)	R	32 (R)	+	-	+	-	-
C.f. 1	S	8 (S)	S	0.38 (S)	-	-	-	-	-
Cf. 2	R	256 (R)	R	32 (R)	+	-	+	-	-

K, *K. pneumoniae*; **Cf.** *C. freundii* ; R, Resistance ; I, Intermediate ; S, Sensitive; +, positive result; -,negative result; * resistant breakpoint is 32 µg/mL; ** resistant breakpoint is 4 µg/mL

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