Prevalence of Plasmid-Mediated Quinolone Resistance Genes (*qnr*) in Clinical Isolates of *K. pneumoniae* in Najaf

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الخلاصة:
المقدمة: ان الهدف من الدراسة هو التحري عن وجود جينات المقاومة للكونيلون المحمولة على البلازميدات في بكتري الكلبسيلا الرئوية المعزولة من العينات السريرية في مستشفيات النجف.
طرائق العمل: تم تشخيص بكتريا الكلبسيلا الرئوية بواسطة الاختبارات المظهرية والكيموحيوية التقليدية و فحص API 20E و استخدمة تقنية سلسلة تفاعل انزيم البلمرة PCR لتحري عن جينات *qnr*.
النتائج :تم تشخيص 109عزلة على انها كلبسيلا رئوية من اصل 1590 عينة سريرية مختلفة تم جمعها، وقد كشفت الدراسة ان هنالك 74 عزلة اظهرت انحسار في المقاومة للكونيلين،بينت نتائج اختبار الحساسية لعزلات الكلبسيلا الرئوية ان هنالك 38 عزلة من نوع المتعددة المقاومةMDR وتم اكتشاف 32 عزلة من نوع واسعة المقاومة XDR و 4 عزلة من نوع PDR. اظهرت النتائج تقنية سلسلة تفاعل انزيم البلمرة PCR لتحري عن جينات *qnr* في 74 عزلة ان هنالك20 (27%) عزلة حاملة لجينات qnr ومن بين هذه العزلات وجد ان هنالك 17 (23%) عزلة تحتوي على جينات من نوع *qnrB*و2(2.7%) عزلة تحتوي على جينات *qnrA* و1(1.4%) عزلة تحتوي على *qnrS*.
الخلاصة: الدراسة إلى ان عزلات الكلبسيلا الرئوية التي تحتوي على جينات المقاومة البلازميدية في الوقت الحاضر تنتشر على نحو واسع في مستشفيات النجف.

Abstract:
Introduction: The main purpose of this study was to investigating the presence of qnr-genes among clinical isolates of *K. pneumoniae* recovered from Najaf hospitals.
Material and methods: A total of 1590 clinical specimens were obtained from three main hospitals. The *Klebsiella* spp. were identified by traditional biochemical tests, and confirmed by API 20E system.Theisolates that exhibited reduce susceptibility to quinolones were examined for the presence of PMQR *qnr* (*qnrA*, *qnrB*, and *qnrS qnrC*, and qunD).
Results: The *qnr* genes were detected in 20 (63.5%) isolates *qnrB*, *qnrA* and *qnrS* were identified in 17 (23%), 2 (2.7%) and 1 (1.4%) respectively.
Conclusion: *K. pneumoniae* isolates harboring PMQR are currently widely distributed in Najaf hospitals.
Key words: Plasmid-Mediated Quinolone Resistance Genes PMQR, qnr

Introduction:

Klebsiella pneumoniae is a prominent nosocomial pathogen mainly responsible for bacteraemia, urinary tract, respiratory tract, and wound infections. Most *K. pneumoniae* are hospital associated with a high fatality rate if incorrectly treated. Isolates from hospitals often display antibiotic resistance phenotypes (1), Resistance isolates may also spread into the community settings (2).

Quinolones is group of antimicrobial compounds that are commonly used for the treatment of many bacterial infections. However, several studies have highlighted that, in recent years, resistance to quinolones has increased globally, particularly in members of the *Enterobacteriaceae* such as *Klebsiella* (3; 4; 5). Although quinolone resistance is predominantly caused by chromosomal mutations, it may also result from a plasmid encoded (6). The recent discovery and rapid dissemination of plasmid-mediated quinolone resistance (PMQR) genes has further highlighted the problem of quinolone (7).

Five major *qnr*- PMQR genes with the potential for horizontal transfer opened a novel era in resistance to quinolones have only recently been discovered. The first PMQR discovered, *qnrA* in 1998, and later the *qnr*B, *qnr*S, *qnr*C and *qnr*D were detected, which confer quinolone resistance by binding to DNA gyrase and topoisomerase IV and protect them from quinolones by unknown mechanism (8, 9).
The aim of this study was to investigate the occurrence and diversity of *qnr*-genes in clinical isolates of *K. pneumoniae* in Najaf hospitals.

Material and methods

Collection of Specimens

A cross section study was conducted in three main hospitals in Najaf from November 2012 to June 2013. Clinical specimens were collected from patients attended and/or admitting to these hospitals. The clinical specimens including burn swab, sputum, wound exudate, seminal fluid, throat swabs and urine.

Identification of *Klebsiella* spp.

Suspected *Klebsiella* colonies were isolated and identified through conventional biochemical tests according to standard method described by MacFaddin (10) and Hart (11). *Klebsiella* isolates were identified at the species by using the API 20E.

Antibiotic Susceptibility Testing

Antibiotic susceptibility testing of *Klebsiella* spp. isolates was performed by the Kirby-Bauer disk diffusion method. The selection of antibiotic disks (listed in table 3) was performed according to the guidelines recommended by the CLSI (2013). *E. coli* ATCC 25922 was used as the reference strain for quality control of the antibiotics tested. All susceptibility results were interpreted according to the standard values provided by CLSI (2013).

Screening Test for Quinolones Resistance

Based on CLSI (12) recommendations, disk diffusion test were performed to detected quinolones resistance in all *Klebsiella* spp. isolates by using nalidixic acid (10µg/disk) and ciprofloxacin (5µg/disk). *E. coli* ATCC 25922 was used as control strain

Screening for the *qnr* genes

The isolates exhibited reduce susceptibility to quinolones were screened by multiplex PCR for *qnrA*, *qnrB*, *qnrS* and monplex PCR for *qnrC*, *qunD*, with the primers shown in Table (1) using a DNA template prepared according to the Chang and Jiany (14) method. PCR amplification was performed using 10µ Master mix 2X, 0.5µl Primer forward (10µM), 0.5 µl Primer reverse (10µM), 5 µl DNA template(10-250ng), PCR grade water Up to 20µl. PCR conditions were as shown in Table (2) in a T3000 thermocycler (biometra). Amplicons were separated by electrophoresis in 1.5 % (w/v) agarose gel, stained with ethidium bromid. The positive results were distinguished when the DNA band base pairs of sample equal to the target product size. Finally, the gel was photographed using Biometra gel documentation system.

Table (1): Primers (Bioneer)

Type	Primer name	Gene name	Oligo sequence	Product (bp)	Reference
QnrA	*qnrA*	F	5-ATTTCTCACGCCAGGATTTG-3	516	14
		R	5-GATCGGCAAAGGTTAGGTCA-3		
QnrB	*qnrB*	F	5-GATCGTGAAAGCCAGAAAGG-3	469	
		R	5-ACGATGCCTGGTAGTTGTCC-3		
QnrS	*qnrS*	F	5-ACGACATTCGTCAACTGCAA-3	417	
		R	5-TAAATTGGCACCCTGTAGGC-3		
QnrC	*qnrC*	F	5-GGGTTGTACATTTATTGAATC-3	447	15
		R	5-TCCACTTTACGAGGTTCT-3		
QnrD	*qnrD*	F	5-CGAGATCAATTTACGGGGAATA-3	644	16
		R	5-AACAAGCTGAAGCGCCTG-3		

Only 74 *K. pneumoniae* isolates exhibited resistant or intermediate resistant to at least one quinolone tested were chosen for further studies. The results of antimicrobial susceptibility testing of 74 *K. pneumoniae* isolates are presented in Table (3).

Results

The results revealed that only 109 (6.9%) isolates were confirmed as *K. pneumoniae* and 2 (0.1%) isolates were recognized as *K. oxytoca* based upon colonial characteristics and conventional biochemical tests and API 20E system test.

35(47.3%) were identified as MDR isolates, whereas, 32 (43.2%) isolates was considered as XDR organisms, PDR-producers could be detected among 4(5.4%) the isolates non-susceptibility to all agents in all antimicrobial classes tested.

Susceptibility testing found that 71(95.9%) of the isolates were resistant to at least one antibiotic in ≥ 3 of the 11 antibacterial classes tested in this study, therefore all these isolates were considered as multiple antibiotic resistance. Among which,

Table (3): Antibiotic susceptibility expressed by *K. pneumoniae* isolates (n= 74)

Antibiotic classes	Antibiotic disk	No. (%) of isolates exhibited:		
		Resistance	Intermediate	Susceptible
Quinolones	Nalidixic acid	47 (63.5)	13 (17.6)	14(18.9)
	Ciprofloxacin	51 (68.9)	11 (14.9)	12(16.2)
	Gatifloxacin	19 (25.7)	8 (10.8)	47(63.5)
	Levofloxacin	24 (32.4)	9(12.2)	41(55.4)
	Lomefloxacin	56 (75.7)	8(10.8)	10(13.5)
	Moxifloxacin	49 (66.2)	11(14.8)	15(20.2)
	Norfloxacin	35 (47.3)	4(5.4)	35(47.3)
	Ofloxacin	36 (48.6)	3(4.1)	35(47.3)

The *qnr*-genes were detected in only 20 (27%) of the isolates, of these, 17 (23%) isolates carried *qnrB* (Figure 1), 2 (2.7%) isolates carried *qnrA*, and only1 (14%) isolate carried *qnrS*. Neither *qnrC* nor *qnrD* genes were found in any of the tested isolates.

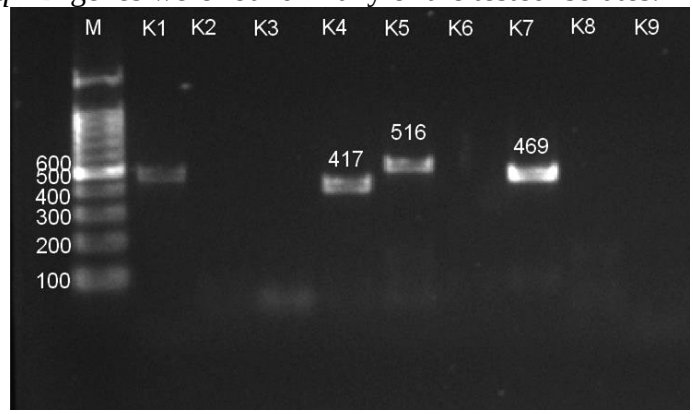

Figure (1): Ethidium bromide-stained agarose gel of multiplex PCR amplified products from extracted DNA of *Klebsiella pneumoniae* isolates and amplified with three genes primers. The electrophoresis was performed at 70 volt for 2 hr. Lane (M), DNA molecular size marker (100 bp ladder, Qiagen), Lanes (K 4) show positive results with *qnrS* (417 bp), Lanes (K 5) show positive results with *qnrA* (516 bp), Lanes (K7) show positive results with *qnrB* (469 bp).

Discussion

Frequency of *Klebsiella* spp. among Clinical Samples

Frequency of *Klebsiella* spp. among clinical samples, *Klebsiella* spp. is increasingly important opportunistic pathogens that cause a variety of communities and hospital-acquired infections (13). Present data found that *K. pneumoniae* is the most frequently isolated pathogenic *Klebsiella* spp. (109/111), can be discriminated confidently between/ against/ in favor of API 20E system which is in agreement with other studies (14,15, 16).

Quinolones Resistance of *K. pneumoniae* Isolates

Resistance to quinolones by family *Enterobacteriacae* became common and widespread shortly after the introduction to these agents (17). The results revealed that 74 (66.6%) of *K. pneumoniae* isolates had displayed reduced susceptibility

(intermediate, or resistant) to nalidixic acid and/or ciprofloxacin, this results resembles with studies from other countries such as Pakistan and Malaysia reported that 72.2% and 71% of *K. pneumoniae* were reduced susceptible to ciprofloxacin (18, 19). However, present results are in agreement with previous study in Najaf performed by Al-Sehlawi (15) who found 51.5% and 50% *K. pneumoniae* clinical isolates were resistance to nalidixic acid and ciprofloxacin respectively.

Prevalence of Qnr Genes.

In this study 20 (27%) isolates carried different type of *qnr* genes. In a related study in Morocco the *qnr* genes were detected in (50%) of ESBL-producing *K. pneumoniae* isolates (21). High prevalence also detected in study performed by Al-Morzooq *et al*. (19) who found *qnr* genes were detected in (65.5%) of *K. pneumoniae* clinical isolates in Malaysia. In contrast, the low prevalence of *qnr* genes has been reported in France and Canada. In France, the prevalence of *qnr* genes was 1.6% (2/125) among ESBL-producing *E. coli* and *Klebsiella* spp. isolate (22, 23). Several reports demonstrating that *qnr* genes alone doing not to confer resistance to fluoroquinolones; however, its presence promotes the selection of additional chromosomally encoded quinolone resistance mechanisms, and *qnr* genes may facilitate further selection to low-level to high-level resistance to the usage of quinolones (24, 25, 4, 26).

Among of the 20 *qnr* genes positive isolates, 17 (23%) isolates carried *qnrB*, 2 (2.7%) *qnrA* and 1(1.3%) *qnrS*. The *qnrB* appear predominant *qnr* gene identified in this study, the data of this study in agreement with study carried out by Saiful *et al*. (27) how found that 15/23 (31.9%) isolates were carried *qnrB* genes. Moreover, another studies in Asian and Southeast Asia, determine the *qnrB* predominant of *qnr* gene in *K. pneumoniae* (28, 29, 5, 30).

The information is not available about the presence of the *qnrA* gene in *K. pneumoniae* clinical isolate in Iraq. In the study reported here, 2 (2.7%) *K. pneumoniae* isolates were positive for *qnrA* gene. Low prevalence of *qnrA* genes has also been reported in previous study in ESBL-producing *Enterobacteriaceae* in Turkey (31).

The present study revealed that the *qnrS* was detected in only one (1.4%) *K. pneumoniae* isolate. This result is in accordant with the results being reported on a study in French performed by Cremet *et al*. (32) in which the *qnrS* was detected in 5 (2.7%) *Enterobacteriaceae* isolates. In another similar study carried out by Al-Mrzooq *et al*. (19) who foun only 2 (4.3%) in *K. pneumoniae* isolates were positive *qnrS* gene. Other *qnr* types including *qnrC*, *qnrD*, were not detected in this study. As conclusion there is a high prevalence of plasmid-mediated quinolones resistance genes among *K. pneumoniae* isolates in Najaf hospitals. Additional studies are necessary to understand the clinical information concerning infections produced by plasmid-mediated quinolones resistance positive isolates and risk factors for their acquisition.

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