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FIRST REPORT OF CLASS 1 AND CLASS 2 INTEGRONS IN QUINOLONES RESISTANT *KLEBSIELLA PNEUMONIAE* ISOLATES FROM NAJAF, IRAQ

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Abstract

Background: Quinolone-resistant *Klebsiella pneumoniae* is considered a serious global threat. However, little is known regarding their multidrug resistance (MDR) and the rule of integron classes in this phenotype. The present study was conducted to investigate the antimicrobial susceptibility and prevalence of class 1and 2 integrons in quinolone resistance clinical isolates of *K. pneumoniae* from Najaf, Iraq patients.

Methods: A total of 109 *K. pneumoniae* were isolated, the quinolone-resistance isolates were selected for antibiotic resistance test as well as presence of class I and II integron assessed by PCR.

Results: Of the 109 *K. pneumoniae* isolates tested, 74 (67.8%) were shown resistant to quinolone and selected for further studies. Among the clinical specimens, a total of 40 (54%) and 30 (40.5%) of quinolones resistant isolates, 47 (63.5%) isolates were MDR, while 23 (31%) were considered as XDR, and PDR isolates were identified in 4 (5.4%) isolates resistant to all agents in all antimicrobial categories tested. Among the 74 quinolone-resistant *K. pneumoniae* isolates the class 1 integron was found in 41(55.4%0), whereas it was detected in only 10 (28.5%) of quinolone susceptible *K. pneumoniae* isolates.

Conclusion: quinolone resistance becomes one of leading concern in global public health. Findings of this study clearly and obviously show that resistance to this antibiotic agent is associated with the presence of class 1 integrons suggesting that integron may assist forward the spread of quinolone-resistance in Najaf. A serious threat to human health may associate with quinolone-resistant bacteria among worldwide.

Keywords:

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Klebsiella pneumoniae is an important opportunistic pathogen associated with nosocomial infections and one of the leading causes of many diseases, and become public health concern especially when characterized as multidrug resistance. due to its responsibility of treatments failure[1]. Transfer of antibiotic resistance genes between different species of bacteria is associated with mobile DNA elements such as transposons and plasmids. recently, a significant part of resistance genes occur in mobile genetic elements of Gram-negative bacilli, have been detected in DNA elements called integrons[2]. Integrons encode the antibiotic resistance genes through site-specific recombination and are capable of capturing, integrating and mobilizing gene cassettes [3]. Various species of Gram negative bacteria that conducted from hospital environments can carry integrons[4]. The definition of integron based on their respective integrase (*intI*) genes, which located in the 5′ conserved segment (5′CS)[3]. This mobile genetic element also contains a 3′ conserved segment that carries the ethidium bromide and quaternary ammonium resistance gene (*qacEΔ1*) and sulfonamide resistance gene (*sul1*) , which confer resistance to ethidium bromide and quaternary ammonium compounds and to sulfonamide, respectively transfer[4]. The nucleotides sequence of integron gene revealed five class this mobile genetic element. Integron class 1 and 2 are frequently detected in clinical isolates of *Enterobacteriaceae*, including *K. pneumoniae*[4]. This study was conducted to investigate the antimicrobial susceptibility and prevalence of class 1and 2 integrons in quinolone resistance of *K. pneumoniae* isolates from Najaf, Iraq patients.

Materials and Methods

Bacterial Isolates

A total of 1590 clinical specimens, including urine, burn wound seminal fluid, wound abscesses and sputum were collected from two teaching hospitals in Najaf (Al-Sader Medical City, Al-Hakeem General Hospital, and Al-Zahra

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Maternity and Children) from December 2012 till June 2013. Among of these 109, non-duplicated *K. pneumoniae* were collected. The isolates were collected from urine (n=59) followed by burn wound (n=46), seminal fluid (n=2), wound abscesses (n=1) and sputum (n=1).

Detection of Quinolones Resistant Phenotype:

All *K. pneumoniae* isolates were classified as quinolones resistant according to susceptibility or resistant to nalidixic acid and ciprofloxacin antibiotics (Cypress, Belgium), and confirmed by MIC Strip (Liofilchem, Italy). According to the CLSI breakpoint criteria, the MIC standerd for ciprofloxacin resistance was ≥ 4 μg/mL, and > 32 μg/mL for nalidixic acid, according to the CLSI breakpoint criteria.

Antibiotic Susceptibility Phenotype:

Antibiotics disks (Cypress, Belgium) were used to test the susceptibility of quinolones resistant *K. pneumoniae* isolates, using the Kirby-Bauer method according to CLSI guidelines [5]: Amoxicillin (25 μg), piperacillin (25 μg), amoxicillin-clavulanic acid (30 μg), ampicillin-sulbactam (20 μg), piperacillin-tazobactam (10 μg), ticarcillin-clavulanic acid (85 μg), cefotaxime (30 μg), ceftazidime (30 μg), ceftriaxone (30 μg), cefepime (30 μg), cefoxitin (30 μg), aztreonam (30 μg), imipenem (10 μg), meropenem (10 μg), nalidixic acid (30 μg), ciprofloxacin (5 μg), gatifloxacin (5 μg), levofloxacin (5 μg), lomefloxacin (10 μg), moxifloxacin (5 μg), norfloxacin (10 μg), ofloxacin (5 μg), amikacin (30 μg), tobramycin (10 μg), gentamicin (10 μg), kanamycin (30 μg), netilmicin (30 μg), chloramphenicol (30 μg), sulfamethoxazole (50 μg), trimethoprim (5 μg). The ATCC standard strain *E. coli* (ATCC 25922) was used as a positive control.

Detection of class 1 and 2 integrons by PCR

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The K. pneumoniae DNA was extracted as described previously by Cheng and Jiang[6], after which the DNA samples were used as a source of templates for the polymerase chain reaction (PCR) amplification. The intI1and intI2 genes were amplified by PCR using primers obtained from bioneer (Daejeon, South Korea) listed in table 1. The PCR amplifications were performed in a 20µl reaction mixture including 10µl KAPA TaqReadyMix mixture (Kapa Biosystems, Massachusetts, US), 25 pmol of each primer of the single gene, 5 µl of genomic DNA, and nuclease-free water to complete the volume. The PCR amplification was performed in Tprofessional thermal cycler (Biometra, Germany) in the following sequence: 5 min at 94ºC, followed by 35 and 30 cycles for integron class 1 and class 2 respectively of 30 s each at 94ºC (30 s min at 55ºC for integron class 1 and 1 min at 62ºC for integron class 2), 1 min at 72ºC, and a final extension step at 72ºC for 10 min.

Using gel electrophoresis (Biometra, Germany), the PCR products were separated in 1.5% agarose gels with ethidium bromide stained and visualized with gel documentation system (Biometra, Germany).

Statistical Analysis:

Chi-square(χ^2) and Fisher's exact test were used to determine the relation between the presence of integrons and antibiotic resistance in SPSS software (SPSS 16, USA). A P value of < 0.05 was considered as statistically significant.

Results:

Of the 109 *K. pneumoniae* isolates tested, 74 (67.8%) were shown resistant to quinolone was chosen for further studies. Among the clinical specimens, a total of 40 (54%) and 30 (40.5%) of quinolones resistant isolates were obtained from urine and burn wound respectively, while the residual isolates were recovered from seminal fluid 2 (2.7%), wound abscesses and sputum 1 (1.3% each) (table 2).

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The 74 *K. pneumoniae* isolate had shown resistance to nalidixic acid and/or ciprofloxacin antibiotics were involved in this study. Among the 31 detected antibiotics, the highest drug resistance rate were ampicillin and amoxicillin (100%) and similarly for amoxicillin-clavulanic acid, while, the lowermost drug resistance rate were 36.4% for imipenem and gatifloxacin, 37.8% Meropenem, Amikacin and 39.1% for Chloramphenicol (table 3).

According to Magiorakos *et al.*[8], of the 74 QRKP isolates, 47 (63.5%) isolates were MDR, while 23 (31%) were considered as XDR, that susceptible to two or fewer antimicrobial categories and PDR isolates were identified in 4 (5.4%) isolates that resistant to all agents Known (Table 4).

The present study indicate that integrons are widespread in *K. pneumoniae* isolates. Among the 109 isolates, 51 (46.7%) were observed to have integrons, whereas, no class 2 integrons were detected. Among the 74 QRKP isolates the class 1 integron present in 41(55.4%0), while present in only 10(28.5%) of quinolone susceptible *K. pneumoniae* isolates, and were found strongly associated with QRKP isolates (p=0.008). class 1 integron was presented in all XDR and PDR and less existent in MDR isolates (Table 5).

Discussion

The emerging quinolone-resistant clinical isolates and multidrug-resistant *K. pneumoniae* strains have been increased among clinical isolates in worldwide and become a serious therapeutic challenge. Integron has been recognized as one of the major sources of genes that responsible for antimicrobial resistance and is suspected to be a source of resistance genes to *Enterobacteriaceae*. Our study analyzed 109 *K. pneumonaie* clinical isolates obtained from hospitals in Najaf, Iraq, comprehensively for possession of integrase genes in quinolone and multidrug resistance isolate, and the association between them.

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The results of this study indicated that 74 (67.8%) of *K. pneumoniae* isolates had displayed quinolone resistance, these results were higher than studies from other countries [9,10]. Among these 74 quinolone resistance isolates, high resistance to penicillins and generations of cephalosporins were observed, being in the range of (97-100%) and (55.4-89.1%) respectively, which is quite high. The present results also revealed the resistant pattern to fluoroquinolone a range of (36-87%), in addition to other antibiotic drugs, which is relatively high. Similar results were revealed previously in United States [11].

The present study demonstrated an unexpectedly high rate of MDR isolates in present antibiotic resistance profile that represents 63.5% in addition 31% where XDR, and extraordinary 5.4% where PDR. Maybe the increased use of antibiotics during recent years in an uncontrolled way could be the cause of this. Continued used of certain antibiotics also supports the selection of certain resistance elements and promotes the perseverance of MDR bacteria (12). Similar result was established in previously in Iran [2].

Till now, there are no published studies estimated the presence of integrons (class 1 and 2) in QRKP isolates from the Najaf, Iraq. Therefore, the present study first reported the class 1 integron were associated with 41(55.4%0) of QRKP. A study from Iran [13] reported integron present in 66.6% of *K. pneumoniae* isolates. Similar finding previously reported revealed this association of integron and QRKP isolates. This association may be due to increasing the rate of mutation of the bacterial cell, and/or the presence of resistance genes on integrons that responsible for decreased the permeability of membrane or improved efflux pump[14]. Coexistence of quinolone resistance with the presence of integron is an important public health problem and requests for incessant surveillance, monitoring, and adjustment of the antibiotic use policies.

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In conclusion, quinolone resistance becomes the one of leading concern in global public health. Findings of this study clearly obviously show that resistance to this antibiotics is associated with the existence of class 1 integrons suggests that integron may be assisting forward the spread of quinolone-resistant in Najaf. A serious threat to human health may associate with quinolone resistance bacteria among worldwide. Additional studies of integrons are required to understand the mechanisms of possessing of MDR genes in quinolone resistance clinical isolates.

Conflict of interest

None to declare.

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TABLES

Table 1: Primers Used to Detect Integron Type I and Type II in this Study

Target	Primer name	Primer sequence (5´-3´)	References
intI1	Int1F	CAGTGGACATAAGCCTGTTC	7
	Int1R	CCCGAGGCATAGACTGTA	
intI2	intI2F	CACGGATATGCGACAAAAAGGT	7
	intI2R	GTAGCAAACGAGTGACGAAATG	

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Table 2: Frequency of quinolone resistant *K. pneumoniae* isolates in clinical specimen.

Clinical specimen	Total No (%) of *K. pneumoniae* isolates	No (%) of isolates had quinolone resistant
Urine (n= 1340)	59 (4.4)	40 (67.8)
Burn wound (n= 225)	46 (20.4)	30 (65.2)
Sputum (n= 7)	1 (14.3)	1 (100)
Wound abscess (n= 7)	1 (14.3)	1 (100)
Seminal fluid (n= 11)	2 (18.2)	2 (100)
Total (n= 1590)	109 (6.9)	74 (67.8)

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

Antibiotic	No. (%) of isolates showed:	
	Resistance	Susceptible
Ampicillin	74 (100)	0(0)
Amoxicillin	74 (100)	0(0)
Piperacillin	72(97.3)	2(2.7)
Amoxicillin-clavulanic acid	74 (100)	0(0)
Ampicillin-sulbactam	70 (94.6)	4 (5.4)
Piperacillin-tazobactam	57(77)	17 (22.9)
Ticarcillin-clavulanic acid	65(87.8)	9 (12.2)
Cefotaxime	66(89.1)	8 (10.8)
Ceftazidime	64(86.4)	10 (13.5)
Ceftriaxone	64(86.4)	10 (13.5)
Cefepime	55(74.3)	19 (25.7)
Cefoxitin	41(55.4)	33 (44.6)
Aztreonam	63(85.1)	11 (14.8)
Imipenem	27(36.4)	47 (63.5)
Meropenem	28(37.8)	46 (62.2)
Nalidixic acid	66(89.1)	9 (12.2)
Ciprofloxacin	65(87.8)	10 (13.5)
Gatifloxacin	27(36.4)	47 (63.5)
Levofloxacin	33(44.5)	41 (55.4)
Lomefloxacin	64(86.4)	10 (13.5)
Moxifloxacin	60(81)	15 (20.2)
Norfloxacin	39(52.7)	35 (47.3)
Ofloxacin	39(52.70	35 (47.3)
Amikacin	28(37.8)	46 (62.1)
Gentamicin	43(58.1)	31 (41.9)
Kanamycin	44(59.4)	30 (40.5)
Netilmicin	30(40.5)	44 (59.4)
Tobromycin	57(77)	17 (22.9)
Chloramphenicol	29(39.1)	45 (60.8)

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Sulfamethoxazole	64(86.4)	10 (13.5)
Trimethoprim	66 (89.2)	8 (10.8)

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Table 4: Multiple antibiotic resistance phenotypes of 74 quinolone resistant *K. pneumoniae* isolates

Resistance Pattern No.(%)	No. of antibiotic categories (n=11)	No. of resistance isolates (%)	Isolate code No.
MDR 47(63.5)	3	5(6.7)	Kp 10, 21, 31, 35, 65
	4	1(1.3)	Kp 33
	5	2(2.7)	Kp 6, 32
	6	4(5.4)	Kp 9, 50, 97, 98
	7	12(16.2)	Kp 4, 14, 20, 36, 47, 68, 91, 94, 105, 117, 118, 123
	8	14(18.9)	Kp 3, 7, 46, 62, 48, 72, 76, 74, 73, 83, 85, 108, 115, 110
	9	9(12.1)	Kp 22, 49, 67, 77, 102, 111, 114, 120, 130
XDR 23(31)	10	12(16.2)	Kp 1, 15, 45, 55, 56, 57, 58, 60, 66, 93, 95, 128
	11	11(14.8)	Kp 12, 18, 23, 37, 38, 69, 84, 87, 100, 103, 113
PDR 4(5.4)	11	4(5.4)	Kp 63, 64, 104, 92

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Table 5: Frequency of class 1 integron gene in 74 quinolone resistance *K. pneumoniae* isolates

Type of Resistance	Quinolone resistant			P Value
	intI +	intI -	Total	
MDR	14	33	47	0.012
XDR	23	0	23	0.00
PDR	4	0	4	0.00
	41	33	74	

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Conflict of interest

- All authors have no conflict of interest

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