qnrA implicated quinolone resistance in *Escherichia coli* and *Klebsiella pneumoniae* clinical isolates from a University Teaching Hospital

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Abstract: Background: Plasmid mediated quinolone resistance is an additional burden consequent to extended spectrum beta lactamase production identified in Enterobactericeae isolates worldwide. The present study aimed at detection of *qnrA* mediated resistance in quinolone resistant *E.coli* and *Klebsiella pneumoniae* isolated in our University Teaching hospital in Northwest Iran. **Materials and Methods**: A total of 63 *E.coli* and 71 *K.pneumoniae* isolates found resistant to ciprofloxacin or nalidixic acid on disk agar diffusion were collected between May and December 2013 for the presence of *qnrA* gene by polymerase chain reaction and their association with ESBL was confirmed on disk diffusion confirmatory test as per clinical and laboratory standards institute (CLSI) guidelines. Presence of resistance to 3 more than three antibiotics was considered as multidrug resistance (MDR). Minimum inhibitory concentration (MICs) of ciprofloxacin and nalidixic acid for the *qnrA* positive isolates were determined according to CLSI. **Results**: Of 71 *E.coli* isolates, 39(54.9%) and 29(40.8%) were resistant to nalidixic acid and ciprofloxacin respectively. *qnrA* gene was detected in 3 (4.2%) ciprofloxacin resistant isolates. Among 63 *K.pneumoniae* isolates, resistance to ciprofloxacin and nalidixic acid was found in 35(55.5%) and 15(23.8%) isolates. *qnrA* gene was detected in 2(3.2%) ciprofloxacin resistant isolates. The ciprofloxacin MIC for all of *qnrA* positive *E.coli* and *K.pneumoniae* isolates was high (≥32 µg/mL). Surprisingly, no nalidixic acid resistant isolate was positive for *qnrA*. Additionally, all *qnrA* positive isolates were found ESBL producers and 80% were MDR. **Discussions:** Our results showed emergence of *qnrA* mediated resistance, however, this resistance was lower compared to other published studies from Iran. ESBL and MDR accompanying quinolone resistance in *E.coli* and *K. pneumoniae* isolates suggests revising the choice of antibiotic therapy.

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1. Introduction:

Quinolones are one of the broad-spectrum antimicrobial agents being prescribed greatly in hospitals. However, their extensive utility in clinical practice has led to an increase in resistance to this group of antibacterial agents(1). The majority of the literature regarding the mechanisms of action and resistance to the quinolones refers to studies done on the *Enterobacteriaceae*, especially *Escherichia coli(2)*. Mutations in type II DNA topoisomerase genes and/or changes in efflux pumps and the expression of outer membrane lead to quinolone resistance, particularly in *Enterobacteriaceae* (2, 3)

In addition to chromosomal mutations incriminated with quinolone resistance, emergence of plasmid-mediated quinolone resistance (PMQR) has been evidenced worldwide (4). For the first time, PMQR was reported in a *K.pneumoniae* clinical isolate from USA in 1998 (5). The first PMQR gene to be reported by Martinez et al, was named *qnrA* which encodes a 219 amino acid pentapeptide repeat protein. It protects DNA gyrase from the activity of quinolones (6). A research study conducted in Shanghai, China earlier reported the prevalence of this gene in about 8% in clinical isolates of *E.coli* (7). On one hand, studies for the presence of *qnr* are few, and on the other hand, remarkably *qnr* has been found seldom in such clinical strains. Nevertheless, the presence the frequency of *qnr*A gene is gradually being increased all over the world including Iran (8, 9). *qnrA* confers resistance to quinolones such as ciprofloxacin and nalidixic acid. It also upsurges MICs of fluoroquinolones, up to 32-fold in *Klebsiella pneumoniae* and *Escherichia coil* (10).

qnr genes, comprising *qnrA* are also frequently associated with Extended Spectrum ß-Lactamase (ESBL) producing isolates. On the other hand, *ESBL* production has been associated with development of Multidrug resistance (MDR). Since genes with other mechanisms of resistance as well as *ESBL* gene may exist within the same plasmid, co- resistance to quinolones, aminoglycosides, and trimethoprim-sulfamethoxazole is revealed in some *ESBL* producing strains (9). Since a lacunae exist in this regard in clinical isolates being obtained from North West Iran, a necessity was felt to determine the prevalence of *qnrA* gene in quinolone resistant *K. pneumoniae* and *E.coli* and its influence on the increase in MIC in these strains. Additionally, we searched for any relation between phenotypic *ESBL* production by phenotypic test and multidrug-resistance in isolates possessing *qnrA*.

2. Materials and Methods

Bacterial isolates

One hundred and thirty four quinolone resistance isolates of *E.coli* and *K. pneumoniae* were collected from out-patients and hospitalized patients in Sina hospital, Tabriz –Iran, during May to December 2013. All isolates were identified by conventional bacteriological tests as previously described (11).The isolates were collected from various clinical specimens including urine; blood, wound and endotracheal tube. The bacterial isolates were kept frozen at -70º C before tested.

Antimicrobial susceptibility test

Antibiotic susceptibility testing was performed as recommended by the Clinical Laboratory Standards Institute (CLSI) (12) using disk diffusion method. Antimicrobial disks (Mast.UK) tested included ciprofloxacin (5µg) and nalidixic acid (30µg), gentamicin (10µg), amikacin (30µg), Ceftriaxone (30µg), ceftazidime (30µg), imipenem (10µg), co-trimoxazole (1.25µg), cefamandole (30µg) and ceftizoxime (30µg).

Determination of Minimum Inhibitory Concentrations (MICs)

Minimum inhibitory concentrations (MICs) of all isolates for ciprofloxacin or /and nalidixic acid was determined by E-test (Liofilchem) according to the manufacturer's instructions and CLSI guidelines.

ESBL screening methods

For determination of an organism to be a potent *ESBL* producer, antibiotic susceptibility testing was performed as recommended by the CLSI (12) using disks containing cefotaxime (30µg) versus cefotaxime/clavulanic acid (30-10µg), ceftazidime (30µg) versus ceftazidime/clavulanic acid (30-10µg), cefpodoxime(30µg) versus cefpodoxime/clavulanic acid (30-10µg), cefepime (30µg) versus cefepime/clavulanic acid (30-10µg) and Aztreonam (30µg). A positive test result was defined as a ≥5mm increase in zone diameter compared with a disk without clavulanic acid (12). ESBL producing strain *K.pneumoniae* ATCC 700603 and non-ESBL producing strain *E. coli* ATCC 25922 were used as positive and negative control respectively.

DNA extraction and PCR amplification

E.coli and *K.pneumoniae* clinical isolates were cultured overnight in Lauria Bertani (LB) broth at 37°C, and plasmid DNA was extracted by using the Plasmid Purification Kit (Bioneer Plasmid Mini Extraction, Korea). The primers used for amplification of *qnrA* gene were as follows: F: 5′ - AGAGGATTTCTCACGCCAGG-3′ and R: 5′ - TGCCAGGCACAGATCTTGAC- 3′ to detect a 580-bp amplicon (13). Amplification was carried out with the following thermal cycling profile: 10 min at 95° C and 35 cycles consisting of denaturation for1 min at 95° C, annealing for 1 min at 54° C and extension for 1 min at 72° C and 10 min at 72° C for the final extension (5).

3. Result

Of the one hundred and thirty four quinolone resistant isolates, 71 (53%) of strains were *E.coli* and 63(47%) were *K.pneumoniae*. of the 134 clinical isolates included in our study (66; 48.9%) were collected from urine, (35; 25, 9%) from wound, (31; 23%) from blood and (2; 1.5%) endotracheal tube.

Antibiotic susceptibility testing

The result of disk agar diffusion test using panel of 10 antibiotics against clinical isolates of *E.coli and K.pneumoniae* is shown in Table 1. Of the total clinical isolates, 47 (74%) *K.pneumoniae* and 59(83%) *E.coli* were observed as MDR (MDR was defined as resistance to 3 or more different groups of antibiotics). Ninety three (69.4%) isolates were observed as *ESBL*; comprising 47(66.2%) *E.coli* and 46 (73%) *K. pneumoniae*. *ESBL* producing *E.coli* showed high resistance to cefpodoxime (92%), aztreonam (78%), cefotaxime (69%) and ceftazidime (66.1%). Similarly *K. pneumonia* were also high resistant to cefpodoxime and aztreonam (90%), ceftazidime (87%) and cefotaxime (85%). Low resistance to cefepime was exhibited by *E.coli* (28%) and *K.pneumoniae* (23%).

Detection of *qnrA:*

Of the 134 quinolone resistant isolates, 104 (78%) were highly resistant to ciprofloxacin (MIC, ≥32 µg/ml) and 43 (65%) to nalidixic acid (MIC, ≥256 µg/ml). The *qnrA* gene was detected in 5 (3.7%) ciprofloxacin resistant isolates, including 3 (4.2%) *E. coli* and 2 (3.2%) *K. pneumoniae.* The ciprofloxacin MIC was >32 µg/mL for all *qnrA* positive *E.coli* and *K.pneumoniae* isolates. All of these *qnrA* positive isolates were found *ESBL* producers and 80% were

MDR. The characteristics of *qnrA* -positive isolates were shown in (Table 2).

4. Discussion

Although β-lactam and quinolones are frequently used for the treatment of *K. pneumonia and E. coli* infections, unfortunately *ESBL*-production and quinolone resistance has hampered their usage. Also, emergence of multiple-drug -resistance is considered a serious problem (1). In this study, isolates with resistance to three or more different antibiotics were common. One hundred six isolates (79.1%) had MDR phenotype, which is near to the rate of multi-drug resistance reported in *E. coli* by Rezaee *et al* from Tabriz, Iran (1). In the present study, majority of the community isolates of *K. pneumoniae and E.coli* were resistant to all antibiotics except for Imipenem and Amikacin.

In this study, among ciprofloxacin and nalidixic acid resistant isolates, 78% and 77.8% of them were *ESBL*-producer respectively, similar to the observation being reported from Taiwan (14). In the present study the highest antibiotic resistances were observed in *ESBL* producing *E.coli* and *K. pneumoniae* and were related to cefpodoxime and aztreonam, with lowest to cefepime. In Concordantly in the representation of Kim et al (14) from Korea, similar finding has been reported with cefepime and cefoxitin presenting with low resistance and cefodaxime and aztreonam with highest antibiotic resistance. However, Pakzad et al from Iran reported highest antibiotic resistance in *E.coli* against ceftazidime and the lowest to cefpodoxime and aztreonam (9). The PMQR gene *qnrA* was found in 3.7% (n= 5) of our isolates. Though Seyedpour et al from Iran and Saiful Anuar et al from Malaysia didn't find *qnrA* in *K. pneumoniae* (15, 16). While Firoozeh et al (17) reported that the frequency of *qnrA* genes among 63 ciprofloxacin-resistant *E. coli* was 14 (22.2%) for *qnrA*. The prevalence of aforementioned gene in *Salmonella spp* from Iran has been reported as 25.8% (18). Most (80%) of *qnrA* positive isolates were detected from blood. The prevalence of *qnrA* gene in *ESBL* producing isolates was 4.6% (5 of 93), while research study of Pakzad et al found as high as 37.5% (n= 9) in *ESBL* producing *E. coli* (9). Plasmid-mediated quinolone resistance mechanisms play an important role in the development of quinolone and fluoroquinolone resistance and the high prevalence of PMQR genes all over the world, is alarming in view that these genes can spread widely via plasmids. Although *qnrA* was the first PMQR gene that discovered and dedicated the highest prevalence among reports, this study shows the low rate of *qnrA* in contrast to previous studies (6, 19).

Table 1. Antibiotic resistant profile on disk agar diffusion

Antibiotics	Resistant No (%)	
	E.coli **(n=71)**	***K. pneumonia*** **(n=63)**
Ciprofloxacin	29(40.8%)	35(55.5%)
Nalidixic acid	39(54.9%)	15(23.8%)
Amikacin	14(19.7%)	12(19%)
Gentamicin	48(67.9%)	41(65%)
Co-trimoxazole	44(61.9)	38 (60.3%)
Cefamandole	61(85.9%)	61(96.8%)
Ceftizoxime	35(49.3%)	33(52.3%)
Ceftazidime	45(63.3%)	56(88.8%)
Ceftriaxone	58(81.6%)	56(88.8%)
Imipenem	9(12.6%)	4(6.3%)

Table 2. Clinical and laboratory characteristics of the *qnrA*-positive isolates

Number of isolates	Specimen	ESBL	MDR	qnr	MIC (µg/ml)	
					CIP	NA
E. coli(N)						
33	Blood	+	+	*qnrA*	≥32	------
116	Wound	+	+	*qnrA*	≥32	------
111	Blood	+	-	*qnrA*	≥32	------
K.pneumoniae						
42	Blood	+	+	*qnrA,*	≥32	-------
32	blood	+	+	*qnrA*	≥32	------

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