Egyptian Journal of Medical Human Genetics

RESEARCH Open Access

Characterization of plasmid-mediated qnrA and qnrB genes among Enterobacteriaceae strains: quinolone resistance and ESBL production in Ismailia, Egypt

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Abstract

Background: Plasmid-mediated quinolone resistance genes (PMQR) are mainly associated with clinical isolates of Enterobacteriaceae and complicate treatment of infections caused by these isolates worldwide. Extended-spectrum-beta-lactamase (ESBL)-producing bacteria are resistant to common antibiotics and also through many mechanisms, ESBL could be disabling other types of antibiotics. This study aimed to assess the prevalence of quinolone resistance and ESBL among Enterobacteriaceae strains and investigated the presence of qnrA and qnrB genes in these strains which were isolated from urinary tract infections in Ismailia, Egypt. Ninety-four Enterobacteriaceae isolates were collected from cases of UTIs admitted to the intensive care unit, Suez-Canal University Hospitals, between October 2017 and January 2018. Antibacterial susceptibility was determined by the disk diffusion method. A polymerase chain reaction assay was used to detect qnrA and qnrB resistance genes in quinolone- and fluoroquinolone-resistant and ESBL strains. Also, ciprofloxacin MIC was determined by the agar dilution method.

Results: Resistance rates were 59.6%, 54.3%, 53.2%, 53.2%, and 53.2% to NA, LEV, NOR, CIP, and FX, respectively. Of 56 NA-resistant isolates, 7 (12.5%) and 6 (10.7%) were positive for qnrA and qnrB, respectively, with only one isolate co-harboring both genes. ESBL-producing bacteria was 66.2% of isolates. The MICs for ciprofloxacin ranged from 32–256 μg/ml in ciprofloxacin-resistant isolates.

Conclusion: Our study shows high resistance rates of Enterobacteriaceae to quinolones and ESBL in our hospital which necessitate appropriate use of these antibiotics to reserve their application for therapy. The prevalence of quinolone-resistant and ESBL-producing Enterobacteriaceae was approximately 60% and 70% respectively.

Keywords: Enterobacteriaceae, Extended-spectrum-beta-lactamase, Fluoroquinolone, Genes, Resistance

Background

Enterobacteriaceae is a family of Gram-negative, non-spore-forming bacteria which could be involved in important critical infection mainly causing the majority of urinary tract infections (UTIs) in both community and healthcare settings [1]. These types of infections are initially treated empirically based on frequency of pathogens, local antimicrobial resistance rates and illness severity [2].

Fluoroquinolones (FQs) are synthetic chemical artificial substance agents that a lot of physicians preferred to use these drugs as initial and first agents for empiric therapy because of their high bacteriological and clinical cure rates among uropathogens [2, 3].

Resistance to quinolones and beta-lactam antibiotics in Enterobacteriaceae is considered as an important common vital issue and widespread [4]. It was mostly acquired as a result of intra- or interspecies exchange of transferable plasmid-encoded antibiotic-resistant genes [5]. It commonly arises as a result of chromosomal mutations which accumulate in the genes encoding DNA

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gyrase and topoisomerase IV in quinolone resistance determining regions leading to target modification [6].

In addition, plasmid-mediated quinolone resistance (PMQR) determinants have been described, namely *qnr* genes, *aac (6')-Ib-cr*, and efflux pumps genes *qepA*, *qepA2*, and *oqxAB* [7]. The first plasmid-mediated quinolone resistance-conferring gene *(qnr)*, now named *qnrA*, was first reported in a *Klebsiella pneumoniae* clinical isolate from the USA in 1998 [8]. The *qnr* genes protect DNA gyrase against the effect of the quinolone [9]. The *qnrB* is another plasmid-mediated quinolone. Resistant gene, which shares 43% amino acid identity with *qnrA* [10]. The horizontal transfer of plasmids which is carrying the quinolone resistance determinants beside and adjacent to the accumulation of chromosomal mutations plays an important role in increasing rates of resistance and therapeutic failure [11].

Extended-spectrum β-lactamase producing *E. coli* and *K. pneumonia* could contribute to the potential risk of antibiotic resistance spreading in clinical situations [12].

Interpretation of minimum inhibitory concentrations (MICs) of ESBL-producing Enterobacteriaceae and clinical outcome are good tools for treatment selection because the microorganisms are continuously and increasingly in developing standard therapy resistance [13].

The prevalence of extended-spectrum-beta-lactamase-producing (ESBL) and quinolone resistance Enterobacteriaceae is increasing all over the world and infection with these bacteria strains were associated with an increase of morbidity, mortality, and health care costs [14, 15]. In the USA, the Centers for Disease Control and Prevention demonstrated in 2013 an increase in drug-resistant microorganisms and approximately 26,000 ESBL-producing Enterobacteriaceae infections and 1700 death s[16].

Little is known about the prevalence of quinolone resistance and frequency of *qnr* genes in *Enterobacteriaceae* isolates recovered from urinary tract infection patients in the intensive care unit from our hospital. Therefore, the aim of this study was to assess the prevalence of quinolone resistance and ESBL and investigate the presence of plasmid-mediated *qnrA* and *qnrB* genes among the Enterobacteriaceae strains isolated from urinary tract infections of patients admitted to the intensive care unit (ICU), Suez-Canal University Hospitals, Ismailia, Egypt.

Methods

Bacterial isolates

From October 2017 to January 2018, a total of 94 non-duplicate Enterobacteriaceae strains were collected from cases of UTIs admitted to the ICU of University Hospitals. These non-duplicate isolates included *Escherichia coli* (*n* = 62), *Klebsiella pneumonia* (*n* = 21), *Klebsiella* oxytoka (*n* = 6), *Enterobacter* (*n* = 3), *Citrobacter* (*n* = 1) and *Klebsiella ozaenae* (*n* = 1).

Bacterial culture

A sample of urine was inoculated using calibrated loops in the blood and MacConkey agars. The plate was incubated aerobically at 37 °C for 24–48 h. Conventional methods were used to identify the isolates eg: colonial morphology, Gram-staining characteristics, and biochemical tests.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was performed in this study, according to the standard Kirby-Bauer disk diffusion method on Mueller Hinton agar and interpreted according to the CLSI guidelines [17]. The MICs by the disk diffusion method were determined by the agar dilution method. All isolates were tested for the following 5 classes of antibiotics: cephalosporins (ceftazidime (CAZ 30 μg), cefotaxime (CTX 30 μg), and cefepime (FEP 30 μg)); monobactams (aztreonam (ATM 30 μg), piperacillin (PRL 100 μg); carbapenems: imipenem (IMP10 μg) and meropenem (MEM 10 μg); aminoglycoside (gentamicin (CN 10 μg), tobramycin (TOB 10 μg), and amikacin (AK 30 μg). Quinolone and fluoroquinolone resistance was tested using nalidixic acid (NA 30 μg), norfloxacin (NOR 10 μg), levofloxacin (LEV 5 μg), ciprofloxacin (CIP 5 μg) and ofloxacin (FX 5 μg).

To confirm the presence of extended-spectrum β-lactamase (ESBL)-producing bacteria, we used ceftazidime disks with and without clavulanic acid [18]

The reference strain *E. coli* ATCC 25922 was used as a control. Results were interpreted as susceptible or resistant according to the criteria recommended by the CLSI and the manufacturer protocols (Mast Group, Bootle, UK).

DNA extraction and *qnr* gene amplification

A polymerase chain reaction (PCR) assay was used to detect *qnrA* and *qnrB* resistance genes in quinolone, fluoroquinolone-resistant and ESBL strains (isolates found to be resistant to at least one antibiotic in the disk diffusion method).

DNA was extracted by using DNA mini-Extraction kit (Quiagen, Germany). The primers used for amplification of *qnrA* genes were qnr A f5′-ATTTCTCACGCCAGGATT TG-3′ and r 5′- GATCGGCAAAGGTTAGGTCA-3′ and *qnrB* gene were f-5′ GATCGTGAAAGCCAGAAAGG-3′- r-5′ - ACGATGCCTGGTAGTTGTCC-3′ [10].

The amplification conditions were 5 min at 95 °C and 35 cycles consisting of 94 °C for 45 s, 51 °C for 45 s and 72 °C for 45 s, and 72 °C for 7 min in the final extension [10]. The electrophoresis of PCR products was performed on 1.2% agarose gel, and then the gels were stained with ethidium bromide for 15 min and visualized by a gel documentation system (Biospectrum UVP, UK).

Statistical analysis

The data was presented as mean and standard deviation or frequency and percentages. Chi-square test was used to compare between two qualitative variables and *t* test was used to compare qualitative variables. *p* value was significant < 0.05. SPSS 16 software (SPSS Inc., Chicago, IL, USA) was used.

Results

Antibacterial Susceptibility

The antibiotic susceptibility results of the 94 Enterobacteriaceae *i*solates showed that all isolates were high resistance to cephalosporins followed by monobactams then the aminoglycosides. Resistance rates to cephalosporins were 80.9%, 76%, and 72.3% to CAZ, CTX, and FEP, respectively. For monobactams, 78.7% to PRL and 64.9% to ATM. The resistance rates to aminoglycosides were 57.4 % to TOB, 45.2% to CN, and 40.4% to AK. Carbapenems were the group with the least resistance as only 14.9% of all isolates were resistant to IPM and 11.7% were resistant to MEM. For quinolone and fluoroquinolone resistance, about half of all the strains showed resistance to at least one antibiotic of this group. Resistance rates were 59.6%, 54.3%, 53.2%, 53.2%, and 53.2% to NA, LEV, NOR, CIP, and FX, respectively.

Among the 56 quinolone- and fluoroquinolone-resistant strains, 40 *E.coli* strains showed resistance to NA only while 37 strains showed resistance to LEV, NOR, CIP, and FX. Thirty *E.coli* strains were XDR, 17 were MDR and only one strain was a PDR strain. For *Klebsiella* spp., 15 strains showed resistance to NA only, 12 strains showed resistance to LEV only while 11 strains were resistant to NOR, CIP, and FX. Nine of the isolated *Klebsiella* spp. were XDR, 7 were MDR and only 5 were PDR strains. Out of the 3 *Enterobacter* strains, only one strain showed resistance to NA and 2 strains were resistant to LEV, NOR, CIP, and FX. Only one *Enterobacter* strain was a PDR strain. The sole isolated *Citrobacter* strain was a MDR strain and it was sensitive to the 5 quinolone and fluoroquinolone antibiotics tested (Table 1).

Of 56 NA-resistant isolates, 7 (12.5%) and 6 (10.7%) were positive for *qnrA* and *qnrB*, respectively, with only one isolate co-harboring both genes. The prevalence of ESBL-producing bacteria was 66.1% and it was found that 6 (12.8%) and 4 (8.6%) were positive for *qnrA* and *qnrB*, respectively (Table 2).

The MICs for CIP ranged from 32– 256 μg/ml in the 50 isolates that showed CIP resistance in the disk diffusion test. Among the 7 isolates carried the *qnrA* gene, 4 isolates were *E.coli* and 3 were *K. pneumoniae* strains. *E. coli* strains showed MICs in the range of 64- 256 μg/ml. While the MICs were 256 μg/mL for *K. pneumoniae* strains (Table 3).

On the other hand, four *E.coli*, one *K. pneumoniae*, and one *Enterobacter* were *qnrB*-positive isolates. Three *E.coli* isolates showed MIC of 128 μg/mL and only one strain co-harboring both genes and showed MIC of 256 μg/mL. The MICs for *K. pneumoniae* and *Enterobacter* isolates were 256 μg/mL and 32 μg/mL, respectively (Table 3).

The relationship of quinolone and fluoroquinolone resistance, drug resistance, and ESBL-producing bacteria by disk diffusion method and *qnrA* gene presence was a statistically insignificant while it revealed statistically significant difference only between quinolone resistant Enterobacteriaceae and *qnrB* gene (Table 4). The majority of CIP-resistant isolates carrying *qnrA* genes showed complete resistance to CIP (MICs > 32 μg/mL) which showed statistically significant difference while it was statistically insignificant for *qnrB* gene (Table 5).

Discussion

Antibiotic resistance is a difficult obstacle in our environment. It is common nowadays. Quinolones and fluoroquinolones are frequently used for the treatment of UTIs. The development of PMQR mechanisms made these infections difficult to treat as *qnr* genes increase resistance to NA and fluoroquinolones by four- to eight-fold [19]. In this study, resistance rates to NA, LEV, NOR, CIP, and FX were indicating a high rate of resistance to quinolones and fluoroquinolones by

Table 1 Percentage of quinolone resistance, MDR, XDR, and PDR among the isolated Enterobacteriaceae strains

Enterobacteriaceae strains % resistance	*E.coli*	*Klebsiella* spp.	*Enterobacter*	*Citrobacter*
NA	40	15	1	0
NOR	37	11	2	0
CIP	37	11	2	0
LEV	37	12	2	0
FX	37	11	2	0
MDR	17	7	0	1
XDR	30	9	0	0
PDR	1	5	1	0

MDR: multidrug resistant, *XDR*: extensively drug resistant, *PDR*: pan drug resistant

Table 2 qnrA and qnrB genes among quinolone-resistant and ESBL Enterobacteriaceae and prevalence of ESBL production

Enterobacteriaceae		Number	Percent (100%)
Quinolone-resistant enterobacteriaceae (*n* = 56)			
qnrA	Negative	49	87.5
	Positive	7	12.5
qnrB	Negative	50	89.3
	Positive	6	10.7
ESBL (*n* = 71)			
	Negative	24	33.8
	Positive	47	66.2
ESBL-resistant Enterobacteriaceae (n = 47)			
qnrA	Negative	41	87.2
	Positive	6	12.8
qnrB	Negative	43	91.4
	Positive	4	8.6

ESBL: extended-spectrum-beta-lactamase

Enterobacteriaceae strains isolated from ICU of University Hospitals. This could be due to the inappropriate use of these drugs in the treatment of UTIs. The resistance rate for NA is the highest as it has been used for the treatment of UTIs for more than five decades since its development in 1962 [20].

High prevalence of quinolone resistance in *Enterobacteriaceae*, particularly in *E. coli*, have been described in Europe, South America, and Asia and other studies in most parts of the world have indicated that it is increasing [19, 21–23].

In Iran and Pakistan, the frequencies of NA and CIP resistance among *E. coli* isolated from UTIs were (82.8 and 45 %) and (84.2% and 36.5%), respectively, which is in agreement with our findings [24, 25]. In addition, a study in China showed the frequency of CIP resistance to have increased to 59.4% [26].

On the other hand, our findings are in contrast to Moreno and his colleagues (2006) whose study was conducted in the USA. Their study showed that 21% and 12% of uropathogenic *E. coli* were resistant to quinolones and fluoroquinolones, respectively [27]. This could be due to many reasons including the situation of drug use as many people in our country take antimicrobial drugs without a prescription and over-the-counter use of quinolones in veterinary medicine, as well as environmental conditions.

Plasmid-mediated quinolone resistance determinants have been mostly identified in clinical isolates of Enterobacteriaceae and shown to play not only an important role in quinolone resistance but also resistance to other antibiotics, particularly β-lactams and aminoglycosides [28]. This would explain our findings that all isolates showed high resistance to cephalosporins followed by monobactams then the aminoglycosides and most of these strains were MDR and XDR strains.

The frequency of *qnrA* and *qnrB* genes in NA-resistant isolates in the present study was 7 (12.5%) and 6 (10.7%), respectively. These findings showed that some *qnrA*-and *qnrB*-negative isolates were also resistant to NA and fluoroquinolones signifies that other *qnr* genes or resistance mechanisms, such as mutations in the target enzyme (e.g., DNA gyrase and topoisomerase IV) and/or activation of efflux pumps, may be involved. In Greece, 10% of CIP-resistant *E. coli* clinical isolates were *qnr*-positive [29]. Also, In Iran, of 116 NA-resistant isolates, 14 (12.1%) and 9 (7.8%) were positive for *qnrA* and *qnrB*, respectively, which is similar to the findings of our study [24]. On the other hand, the frequency of *qnr* genes in the present study was higher than that found in China and Japan, where the rates were only 7.5% and 6.5%, respectively [26, 30].

According to our results, the prevalence of qnr A was 10.8% and qnr B was 8.6% in ESBL-positive bacteria from UTI isolated. Oktem et al. reported the prevalence of qnr A was 6.4% in ESBL-positive blood culture isolates collected from Turkey [5].

In contrary to our results, Shams and his colleagues (2015) investigated the ESBL-producing *K. pneumoniae* isolates. They reported no qnr A gene was detected in their isolates while they found approximately 46% had qnr B gene [31]. However, a previous study done by a

Table 3 Ciprofloxacin MICs values of qnrA- and qnrB-positive quinolone-resistant isolates

Bacterial strain	number	Resistance gene	MIC of CIP (μg/mL)	Total *n* = 56 (%)
E. coli	1	qnrA	46	
	2	qnrA	128	
	1	qnrA	256	7 (12.5%)
K. pneumoniae	3	qnrA	256	
E. coli	3	qnrB	128	
	1	qnrB	256	
K. pneumoniae	1	qnrB	256	6 (10.7%)
Enterobacter	1	qnrB	32	

MICs: minimal inhibitory concentrations

Table 4 Relationship of quinolone- and fluoroquinolone-resistant Enterobacteriaceae, drug resistance and ESBL, and qnrA and qnr B genes

Variables	qnr A gene		*p* value	qnr B		*p* value
	Negative	Positive		Negative	Positive	
Quinolone-resistant microorganism isolated (*n* = 56)						
E. coli	36	4		36	4	
K. pneumonia	12	3	0.374	14	1	0.023*
Enterobacter	1	0		0	1	
Drug resistance (*n* = 71)						
MDR	23	2		24	1	
XDR	34	5	0.586	35	4	0.573
PDR	7	0		6	1	
ESBL (*n* = 71)						
Negative	23	1	0.250	22	2	0.980
Positive	41	6		43	4	

MDR: multidrug resistant, *XDR*: extensively drug resistant, *PDR*: pan drug resistant, *ESBL*: extended-spectrum-beta-lactamase.

number of researchers in their group demonstrated the prevalence of qnr A gene was high among *E. coli* bacteria [24].

The presence of a PMQR determinant increases resistance to NA and fluoroquinolones four to eight times [19]. This could explain that the majority of CIP-resistant isolates carrying *qnr* genes had high MIC values (MICs > 32 μg/mL) in our study.

In the current study, ESBL-producing Enterobacteriaceae was more than half of the isolates. In contrast to our results, Azargun et al. (2018) reported the 34.2% of their isolates were ESBL-producing and *K. pneumoniae* was 53.5% then *E. coli* was 33.8% [32]. However Rao et al. (2014) found 61.4% of ESBL production among E. coli and 46.2% among K. pneumoniae [33]. In South Korea, 30% of isolates were reported positive for ESBL. Karami et al. (2017) investigated the stool samples of Iranian children. They found approximately 80% of the isolates were ESBL-producing enteropathogenic *E. coli* [34]. In addition, in the north of Iran, among the 160 clinical *E. coli* isolates, 83 (51.9%) were ESBL-positive [35]. The difference between these results could have resulted from several factors such as the variety in species, geographical regions, the length of ICU stay and increase using of antibiotics.

Table 5 ciprofloxacin MICs values of qnr A and qnr B genes positive quinolone resistant isolated specimens

Resistant isolated specimens	ciprofloxacin MICs values		*p* value
	mean	± SD	
qnrA			0.002*
Negative	89.9	± 74	
Positive	192	± 82	
qnrB			
Negative	97.7	± 81	0.119
Positive	154.6	± 86	
ESBL			
Negative	84.89	± 85.24	0.203
Positive	116.8	± 81.38	

MICs: minimal inhibitory concentrations;
ESBL: extended-spectrum-beta-lactamase

Conclusion

In conclusion, the resistance rate of Enterobacteriaceae isolated from UTIs to fluoroquinolones and β-lactams is high in ICU in our hospital. The prevalence of quinolone-resistant and ESBL-producing Enterobacteriaceae was approximately 60% and 70% respectively. Our findings showed high frequencies of *qnrA* and *qnrB* genes in UTI isolates of *Enterobacteriaceae*. Therefore, detection of plasmid-mediated qnrA and qnrB genes among quinolone-resistant and ESBL-producing Enterobacteriaceae is important for appropriate empirical treatment and infection control. Moreover, appropriate use of these antibiotics will limit the potential spread of resistant genes and reserve their application for therapeutic uses.

Abbreviations

AK 30 μg: Amikacin; ATM 30 μg: Aztreonam; CAZ 30 μg: Ceftazidime; CIP 5 μg: Ciprofloxacin; CN 10 μg: Gentamicin; CTX 30 μg: Cefotaxime; ESBL: Extended-spectrum-beta-lactamase-producing; FEP 30 μg: Cefepime; FQs: Fluoroquinolones; FX 5 μg: Ofloxacin; ICU: Intensive care unit; IMP10 μg: Imipenem; LEV 5 μg: Levofloxacin; MDR: Multidrug resistant; MEM 10 μg: Meropenem; MICs: Minimal Inhibitory concentrations; NA 30 μg: Nalidixic acid; NOR 10 μg: Norfloxacin; PCR: Polymerase chain reaction; PDR: Pan drug resistant; PMQR: Plasmid-mediated quinolone resistance genes; PRL 100 μg: Piperacillin; qnr: Quinolone resistance-conferring gene; TOB 10 μg: Tobramycin; UTIs: Urinary tract infections; XDR: Extensively drug resistant

Acknowledgements

Not applicable.

Authors' contributions

All authors have seen and approved the manuscript, contributed significantly to the work, and also that the manuscript has not been previously published in or is not being considered for publication elsewhere. ST: design of the study, interpretation of data, drafting the article and final approval HO: design of the study, analysis of the data, editing and final revision WH: acquisition of data, interpretation of data, drafting the article and final revision.

Funding

No funding was obtained for this study. This work was done by the authors' own funding.

Availability of data and materials

Data will not be shared due to the policy of our institute.

Ethics approval and consent to participate

The study was approved by the Ethics Committee at the Faculty of Medicine, Suez Canal University, Egypt.
Informed verbal consent was taken from all cases included in the study because no personal or clinical data were included. The study was done on the microorganisms found in the urine specimens.
Reference number: not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare that they have no competing interests.

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Received: 23 May 2019 Accepted: 21 September 2019
Published online: 25 November 2019

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