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ORIGINAL ARTICLE

Frequency of Quinolone resistant genes in *Escherichia coli* causing Urinary tract infections

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ABSTRACT

***Background**: Quinolone resistance has increased worldwide especially among Gram negative bacteria.* ***Objectives:** This research study was done to determine the frequency of quinolone resistant genes (Qnr) (A, B, and S) and its correlation with extended spectrum beta lactamase (ESBL) production in E. coli (30 isolates) isolated from urinary tract infections (UTIs).* ***Results**: 19 (63.3%) isolates appeared to be resistant to quinolone antibiotics and 21(70%) of the E.coli isolates proved to be ESBL producer. Qnr genes exist with percentage 60% (18 isolates) and qnrS gene showed a significant association with quinolone resistance. There was also a significant association between qnrS and qnrB and ESBL production.* ***Conclusion**: This study proved that resistance to β-lactams and quinolones in E.coli isolated from UTIs is high with high prevalence of Qnr genes especially in ESBL producers and this association may participate in multidrug resistance dissemination and difficulty in patients' treatment.*

INTRODUCTION

Urinary tract infections (UTIs) are the most common infections around the world that has been estimated by 150 million case occurring each year worldwide, with about 70%–80% of uncomplicated UTIs caused by *E.coli* [1]. Quinolone and β-lactam antibiotics have been commonly used for UTIs' treatment, however quinolone resistance and extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae have increased globally [2].

The most important mechanism of quinolone resistance is chromosomal mutations in the quinolone resistance-determining region of genes encoding DNA gyrase and topoisomerase genes [3]. In addition, plasmid-mediated quinolone resistance (PMQR) determinants have been also reported. PMQR include quinolone resistance proteins (Qnr) which protect the DNA gyrase and topoisomerase [4]. Qnr genes such as *qnrA*, *qnrB*, and *qnrS* represent the main classes of Qnr determinants which have been discovered in several species of Enterobacteriaceae family including *E.coli*, however some other classes including *qnrC* and *qnrD* have also been discovered recently [5]. Although the PMQR genes lead to low- quinolone resistance level, they can easily encourage and assist the chromosome-encoded quinolone resistance [6].

PMQR genes have been proved to be on the same plasmid as the ESBL genes, accordingly transferring resistant plasmids by conjugation with genes encoding ESBLs can help dissemination of PMQR genes in different species of Enterobacteriaceae [7]. The coexistence of PMQR and ESBL genes on the same plasmid represents a great threat, as infections caused by these multidrug resistant (MDR) isolates are associated with therapeutic failures, restriction of antimicrobial drugs' choice, increase in public health cost, increased hospitalization interval and rising morbidity and mortality [8].

Qnr genes extend globally especially in hospitalized patients and the level of quinolone resistance in Gram negative bacteria such as *E. coli* is unexpectedly high in Egypt, especially in those strains that produce ESBLs [9]. The aim of this research is to detect the level of resistance to quinolones and its correlation to PMQR genes (A, B, and S) and to determine the frequency of these genes within ESBL-producing strains of *E. coli* isolated from UTI-diagnosed patients from Ain Shams University Hospitals (ASUHs) in Egypt.

METHODOLOGY

Bacterial isolates and culturing:

This study was performed on 145 patients from ASUHs in the period from May 2018 till November 2018, where urine specimens were taken from Inpatients and Outpatients suspected of having a UTI, who had not received antibiotics within the previous 2 months. The method of patient selection was simple random sampling. Written informed consent was taken from all the participants. Approval of the research design was obtained from the ethical committee, Faculty of Medicine, Ain Shams University.

Urine was collected from adult patients by clean-catch midstream. Inoculation of all urine samples was done on blood agar as well as MacConkey's agar. A specimen was considered positive for UTIs if a single microorganism was cultured at a count of 10^5 CFU/mL. Thirty *E.coli* strains have been isolated and were

identified by standard culture methods and conventional biochemical tests.

Antimicrobial susceptibility testing:

Profile of antimicrobial susceptibility was detected on Muller–Hinton agar plates (Merck, Munchen, Germany) by the disk diffusion method as described by the Clinical and Laboratory Standards Institute (CLSI)[10]. The used disks were cefotaxime (30µg), ceftazidime (30µg), ceftrioxone (30µg), cefpdoxime (30 µg), cefoxitin (30 µg), aztreonam (30 µg), ciprofloxacin (5µg), nalidixic acid (30µg), levofloxacin (5µg), norfloxacin (5µg).

ESBLs detection

Detection of ESBL activity was done first using an initial screening test which was carried out by the disk diffusion technique according to the CLSI guidelines. Cefotaxime, ceftriaxone, cefpodoxime, ceftazidime or aztreonam disks were used, resistance or decreased susceptibility to any of the tested agents indicates suspicion for ESBL production, then a confirmatory phenotypic test was done. The confirmatory phenotypic test for ESBL was done by double disk synergy using the five previous cephalosporin antibiotics individually and combined with clavulanic acid (10 µg). Increase in the zone diameter by ≥5 mm around the antibiotic disc combined with clavulanic acid confirms ESBL activity. *E. coli* ATCC 25922 was used as an ESBL-positive control strain while *Klebsiella pneumoniae* ATCC 700603 represented the ESBL- negative control strain.

Detection of PMQR genes

***DNA extraction*:**

DNA from the thirty *E.coli* strains was extracted from bacterial colonies grown on nutrient agar using the Qiagen DNeasy, kit for bacterial suspension (Qiagen, United states) according to the manufacturer's instruction.

Multiplex PCR technique

Pairs of primers were prepared to amplify the internal fragments of the *qnrA*-, *qnrB*- and *qnrS*-like genes based on their sequence alignment (Table 1) and a pair of degenerated primers was specifically manufactured to amplify *qnrB* with its six variants. Total DNA (2 mL) was subjected to multiplex PCR in a 50 mL reaction mix including PCR buffer [10 mM Tris–HCl (pH 8.3), 50 mM KCl], 1.5 mM MgCl2, 20 pmol of each of the six primers (Table 1), 200 mM each deoxynucleotide triphosphate, and 2.5 U of Taq polymerase (Applied Biosystems, Courtaboeuf, France). Amplification was carried with the following thermal cycling profile: initial hold at 95˚ C 2 minutes, 35 cycles of: 95 ˚C 45 seconds, 53 ˚C 45 seconds, 72 ˚C I minutes and final extension: 72 ˚C for two minutes.

Table 1: Primers used in this study [11]

Primer	Sequence (5'- 3')	Gene	position	Size of amplified product (bp)
Qnr Am- F	AGAGGATTTCTCACGCCAGG	qnrA1 to qnrA6	30-49	580
Qnr Am-R	TGCCAGGCACAGATCTTGAC		589-608	
Qnr Bm-F	GGMATHGAAATTCGCCACTG	qnrB1 to qnrB6	283-302	264
Qnr Bm-R	TTTGCYGYYCGCCAGTCGAA		526-545	
Qnr Sm-F	GCAAGTTCATTGAACAGGGT	qnrS1 to qnrS2	137-156	428
Qnr Sm-R	TCTAAACCGTCGAGTTCGGCG		543-563	

Statistical analysis

Statistical analysis was performed with IBM® SPSS® Statistic Version 20 and Qualitative data were presented as frequencies (n) and percentages (%). Chi square test was used to study the correlation between different modalities. The significant level was set at $P \leq 0.05$.

RESULTS

This research study was performed on 30 *E.coli* isolates collected from patients suffering from UTI, from ASUHs in Egypt. According to susceptibility of the isolates to quinolone antibiotics, 19 (63.3%) isolates appeared to be Quinolones resistant with the highest resistance was to Nalidixic acid as 17 (56.7%) isolates were resistant and 3 (10%) were intermediate resistant. However, the lowest resistance was to levofloxacin where 14 (46.7%) isolates appeared to be resistant to the antibiotic. 15(50%) *E.coli* strains were resistant to ciprofloxacin and 3 (10%) were intermediate resistant while 16 (53.3%) isolates showed resistance to norfloxacin and only one (3.3%) isolate was intermediate resistant (Table 2).

Table 2: Susceptibility to Quinolones

		No.	%
Sens. to Nalidixic acid	R	17	56.7%
	S	10	33.3%
	IR	3	10.0%
Sens.to Ciprofloxacin	R	15	50.0%
	S	12	40.0%
	IR	3	10.0%
Sens. to Levofloxacin	R	14	46.7%
	S	16	53.3%
Sens. to Norfloxacin	R	16	53.3%
	S	13	43.4%
	IR	1	3.3%
Sens. To Quinolones*	R	19	63.3%
	S	11	36.7%

R: resistant , S: sensitive, IR: intermediate resistant

Regarding ESBL production, 21(70%) *E.coli* isolates proved to be ESBL producer. Resistance to β lactams was as follows: 22(73.3%) isolates appeared to be resistant to ceftazidime, 20 (66.7%) isolates resistant to cefotaxime , ceftriaxone and cefopodoxine, while 15 (50%) isolates proved to be resistant to Azetreonam (Table 3)

Table 3: screening for ESBL production

Screening test for ESBL production		No.	%
Ciftazidime	R	22	73.3%
	S	7	23.3%
	IR	1	3.3%
Cefotaxime	R	20	66.7%
	S	4	13.3%
	IR	6	20.0%
Ceftrioxone	R	20	66.7%
	S	9	30.0%
	IR	1	3.3%
Aztreonam	R	15	50.0%
	S	11	36.7%
	IR	4	13.3%
Cefpdoxime	R	20	66.7%
	S	8	26.7%
	IR	2	6.7%
Result	Not a possible ESBL	9	30.0%
	Possible ESBL producer	21	70.0%

Upon testing the sensitivity of the *E.coli* isolates against other groups of antibiotics the strains appeared to be most sensitive to imipenem and meropenem antibiotics (96.7%) followed by amikacin with sensitivity reaching (80%) and least sensitive to piperacillin- tazobactam (23.3%) (Table4)

Table 4: susceptibility to other groups of antibiotics

Antimicrobial susceptibility		No.	%
gentamycin	R	8	26.7%
	S	22	73.3%
Amikacin	R	5	16.7%
	S	24	80.0%
	IR	1	3.3%
Imipenem	R	1	3.3%
	S	29	96.7%
Meropenem	R	1	3.3%
	S	29	96.7%
Piperacillin tazobactam	R	23	76.7%
	S	7	23.3%

Concerning the PMQR genes prevalence among the *E.coli* isolates, PMQR genes exist with percentage 60% (18 isolates) either individually or combined. The *qnr A* genes appeared to be present in 2 (6.7%) isolates, while *qnr B* gene was present in 7 (23%)of the isolates, however *qnr S* gene proved to be the most prevalent as it exists in 11 (36%) isolates. (Table 5)

Table 5: prevalence of PMQR genes among the collected *E.coli* isolates

PMQR genes		No.	%
qnr A(516)	Negative	28	93.3%
	Positive	2	6.7%
qnr B(469)	Negative	23	76.7%
	Positive	7	23.3%
qnr S(417)	Negative	19	63.3%
	Positive	11	36.7%
Prevalence of PMQR	Negative	12	40.0%
	Positive	18	60.0%

Upon correlation between susceptibility to quinolones and the existence of PMQR genes, *qnr S* gene proved to be significantly correlated to quinolones resistance with (P value = 0.017) as 10 (52%) quinolones resistant isolates carry the *qnr S* gene. On the other hand, *qnr A* and *qnrB* proved to be non-significantly correlated to quinolones resistance although most of the isolates carrying these genes proved to be resistant to quinolones (Table 6)

Table 6: correlation between Quinolone resistance and PMQR genes

		Sens. To Quinolones				Test value	P-value	Sig.
		R		S				
		No.	%	No.	%			
Qnr A(516)	Negative	17	94.7%	11	100%	1.24	0.265	NS
	Positive	2	5.3%	0	00%			
Qnr B(469)	Negative	13	68.4%	10	90.9%	1.969	0.165	NS
	Positive	6	31.6%	1	9.1%			
Qnr S(417)	Negative	9	47.4%	10	90.9%	5.687	0.017	S
	Positive	10	52.6%	1	9.1%			

P-value >0.05: Non significant (NS); P-value <0.05: Significant (S); P-value< 0.01: highly significant (HS) *: Chi-square test

Regarding the relation between ESBL production and quinolones resistance in the *E.coli* isolates, 16 (76.2%) of the quinolones resistant isolates appeared to be ESBL producer with a significant correlation (P value = 0.0256). There was also a significant association between the presence of *qnr B* gene and *qnr S* gene and ESBL production with P value (0.048, 0.0063) respectively as all the isolates carrying these genes appeared to be ESBL producer. However, there was a non- significant correlation between *qnrA* gene presence and ESBL production, as one of the two isolate carrying the gene proved to ESBL producer while the other one was non ESBL producer. (Table 7)

Table 7: correlation between quinolones resistance and ESBL production

		Not a possible ESBL		Possible ESBL producer		Test value*	P-value	Sig.
		No.	%	No.	%			
Sens. To Quinolones	R	3	33.3%	16	76.2%	4.982	0.0256	S
	S	6	66.7%	5	23.8%			
qnr A(516)	Negative	8	88.9%	20	95.2%	0.408	0.522	NS
	Positive	1	11.1%	1	4.8%			
qnr B(469)	Negative	9	100.0%	14	66.7%	3.913	0.048	S
	Positive	0	0.0%	7	33.3%			
qnr S(417)	Negative	9	100.0 %	10	47.6%	7.443	0.0063	S
	Positive	0	0.0 %	11	52.4%			

DISCUSSION

Over the last decades, *E. coli* has been proved to be one of the leading causes of UTIs in the world. Treatment of *E.coli* infections also becomes a big problem due to resistance to many antibiotics including quinolones and β-lactams which are widely used in treatment of Enterobacteriaceae UTIs [12]. The Qnr genes which are members of PMQR genes are made of pentapeptide repeated proteins which protect the bacteria against the action of quinolone antibiotics. Moreover, PMQR genes have always been reported as being co-existed with genes that encode for ESBL production, the condition which allows the spread of resistance to these antibiotics globally, especially in long interval hospitalized patients [13].

In this study 30 *E.coli* strains were collected from patients with UTIs. High percentage of the isolates 63.3% (19 isolates) were resistant to Quinolones and most of them were resistant to Nalidixic acid as 17 (56.7%) isolates appeared to be resistant and 3 (10%) isolates showed intermediate resistance. On the other side, the lowest resistance was to levofloxacin with percentage 46 (n=14 isolates), accordingly, it appears that the use of nalidixic acid for the treatment UTIs caused by *E. coli* might be ineffective in this center. These results agreed with those proved by [14] whose study showed that 68% of the collected *E.coli* strains showed resistance to Quinolones with the highest resistance was to nalidixic acid (72%) and the least to levofloxacin (58%).

The spread of ESBL-producing bacteria has been obviously increasing worldwide, accordingly continuous screening and monitoring system together with effective infection control measures are absolutely necessary. In this study, the prevalence of ESBL producing *E. coli* was high, about 70% (21 isolates). These results agreed with [15&16] who reported the

prevalence of ESBL in *E.coli* was 64.07% and 69.2% respectively. [17&18] reported lower percentage of ESBL in *E.coli* isolates being 27.3% and 28.6% respectively, the reason of differences can be due to improper and extreme use of antibiotics, duration of hospitalization, invasive diagnostic or therapeutic procedures, type of cephalosporins and test method.

Upon testing of the collected *E.coli* isolates against other groups of antibiotics, the isolates were most sensitive to meropenem and imipenem with sensitivity reaching 96.7% followed by Amikacin with sensitivity (80%) which makes these antibiotics effective as empiric treatment especially for the strains which are quinolones and β lactams resistant.

Regarding the prevalence of PMQR genes among the *E.coli* isolates, this study proved that PMQR genes was present in 60% of the isolates (18 isolates) either individually or combined. The *qnr S* gene was the most common existing gene found in 11 (36.7%) isolates. The second was *qnr B* gene which was found in 7 Isolates (23.3%) and the least common one was *qnr A* gene which was found only in two isolates (6.7%). There was a significant association between the presence of *qnr S* gene and quinolones resistance while the relation between *qnrA* and *qnr B* genes and quinolones resistance proved to be non-significant in this study. Two of the isolates harbor *qnrB* and *qnrS* genes and appeared sensitive to quinolones which indicate silent non expressed genes.

These results highly agreed with those proved by [15] in which the prevalence of PMQR genes in *E.coli* isolates was 59.88% with *qnr S* gene being the most prevalent one with percentage reaching 41% followed by *qnr B* gene with percentage reaching 21% and the least one was *qnr A* gene which was present with percentage reaching 10%. His study also showed a significant association between the presence of *qnrS* gene and quinolones resistance.

The prevalence of PMQR genes in this study was lower than that reported by 14 (89.1%) and more than that reported by 17 (26.1%), these difference may be due to the abuse of antibiotics in different areas and high frequency of transconjugation or transformation mechanisms of the genes occurring in area with high PMQR prevalence.

In the present study, there was a significant association between quinolones resistance and ESBL production as 16 (76.2%) of the quinolones resistant isolates appeared to be ESBL producer. The presence of the Qnr genes and those encoding for ESBL production on the same plasmid explains the co-resistance to β-lactams and quinolones in the same bacterial isolate [19]. There was also a significant correlation between the presence of *qnr B* gene and *qnr S* gene and ESBL production with P value (0.048, 0.0063) respectively as all the isolates carrying these genes appeared to ESBL producer. On the other side, there was a non-significant correlation between *qnrA* gene presence and ESBL production, as one of the two isolate carrying the gene proved to ESBL producer while the other one was non ESBL producer.

These results were in concordant with those reported by 14 and 15 where the prevalence of quinolones among ESBL producer *E.coli* were 58% and 89.3% respectively with a significant association between *qnrS* and *qnrB* genes and ESBL production. On the other hand, [20&21] reported non-significant association between ESBL production and quinolones resistance.

CONCLUSION

The rate of resistance to β-lactams and quinolones in *E.coli* isolated from UTIs is high. Imipenem, meropenem and Amikacin are the most effective and preferred antibiotics for empirical therapy in our setting. The frequency of PMQR genes is high in *E.coli* isolates especially ESBL producers and this association may lead to the spread of multidrug resistance and may contribute to serious therapeutic problems. Therefore, detection of Qnr and ESBL genes among quinolone resistant *E..coli* is important for appropriate empirical treatment and infection control.

Conflicts of interest: The authors declare that they have no financial or non financial conflicts of interest related to the work done in the manuscript.

- Each author listed in the manuscript had seen and approved the submission of this version of the manuscript and takes full responsibility for it.
- This article had not been published anywhere and is not currently under consideration by another journal or a publisher.

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