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Detection of Fluoroquinolones Resistance in *Enterobacteriaceae* and *Pseudomonas* species Using Molecular Techniques

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ABSTRACT

Background: quinolone resistance is traditionally mediated by chromosomal mutations mutation of DNA gyrase and/or topoisomerase IV or by the mutation of genes regulating the expression of efflux pumps, until PMQR was described in a clinical isolate of *Klebsiella pneumoniae* in 1998. PMQR genes generally confer low-level resistance, with their MICs falling below Clinical and Laboratory Standards Institute (CLSI) breakpoints for intermediate resistance; therefore, their contribution to quinolone resistance can be masked in strains also harboring QRDR mutations in gyrA and parC. However, their clinical significance stems from the fact that they greatly facilitate the selection of more highly quinolone-resistant strains. Although the PMQR mechanism only confers low-level resistance to FQs, its association with the occurrence of mutations in QRDR can lead to clinically relevant resistance levels. These PMQR determinants are increasingly being identified worldwide in clinical isolates of *Enterobacteriaceae* and *Pseudomonas* spp. **Aim of the work**: this study aimed to identify different mechanisms of fluoroquinolones resistance and determine fluoroquinolones resistance pattern among the studied isolates. **Material and methods**: this study was carried on 100 non duplicate clinically relevant *Enterobacteriaceae* and *Pseudomonas* spp. recovered from clinical specimens referred to Central Microbiology Laboratory, Ain Shams University Hospital for routine culture and sensitivity, aiming to 1) Determine the occurrence of plasmid-mediated fluoroquinolones resistance (PMQR) determinants by multiplex PCR and chromosomal mutations by PCR-RFLP among *Enterobacteriaceae* and *Pseudomonas* spp. in clinical specimens. 2) Identify different mechanisms of Fluoroquinolones resistance. 3) Determine Fluoroquinolones resistance pattern among the studied isolates. **Results:** in this study we found that 77% of FQs resistant isolates were positive to one or more plasmids, oqxAB was highest recovered PMQR among *Klebsiella*. 78% were positive for gyrA mutations, gyrA gene mutations were higher in *Pseudomonas*, Asp-87mutation was 56/78(72%) higher than Ser-83 mutation 38/78 (49%) isolates.

Keywords: Fluoroquinolones, *Enterobacteriaceae*, *Pseudomonas*, Molecular Techniques.

INTRODUCTION

Quinolones are a family of synthetic broad spectrum antimicrobial drugs, they have been widely used for the treatment of several community and hospital acquired infections, in the food processing industry and in the agricultural field [1]. Resistance has been reported with increasing frequency in clinical isolates of *Enterobacteriaceae* [2]. The targeting of either DNA gyrase or topoisomerase IV varies with bacterial species and specific fluoroquinolone; however, as a broad generalization, the key target in gram-negative bacteria is DNA gyrase, whereas in gram-positive microorganisms topoisomerase IV was preferentially targeted [3]. Plasmid-mediated quinolone resistance (PMQR) genes are alternative mediators of quinolone resistance or reduced susceptibility, they encode DNA gyrase protection proteins, efflux pumps and a variant of a common aminoglycoside-modifying enzyme [4]. Although chromosomal QRDR mutations in topoisomerases play an important role in conferring a high level of quinolone resistance, some of researchers believed that PMQR may contribute to an increase in quinolone resistance in clinical isolates of *Enterobacteriaceae* [5].

The level of resistance of *Enterobacteria* and *Pseudomonas aeruginosa* to fluoroquinolone is alarming and significantly increasing in Europe including the Central European Region. Considerably high fluoroquinolone resistance among *E. coli*, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa* was reported by the European Centre for Disease Prevention and Control (ECDC) in Slovakia reached 41.9%, 70.6% and 58.7%, respectively. Assuming that such a high rate of fluoroquinolone resistance may be

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associated with their frequent use in clinical practice, as Slovakia belongs to the ‘top 10’ European countries with the highest outpatient use of quinolones [6].

AIM OF THE WORK

The aim of this work was to identify different mechanisms of fluoroquinolones resistance and determine fluoroquinolones resistance pattern among the studied isolates.

SUBJECT AND METHODS

This study was conducted on 100 clinical non duplicate isolates which were collected from different clinical samples, whose were submitted to The Central Microbiology Laboratory in Ain Shams University Hospitals for culture and sensitivity, those isolates were including 64 *Enterobacteriaceae* ,32 *klebsiella,* 32 *E.coli* and 36 *Pseudomonas spp*., during the period from February 2016 to February 2017.

All isolates were subjected to:

I- Subculture on MacConky agar media *(Oxoid, Basingstoke, UK)* to obtain fresh isolates.

II- Identification of the isolates using: VITEK® 2 Compact system *(bioMérieux, France).*

III- Antibiotic susceptibility to Fluoroquinolones group by disc diffusion method according to ***CLSI***[7].

IV- Antibiotic susceptibility to Fluoroquinolones by using VITEK® 2 Compact system *(bioMérieux, France).*

V- Detection of PMQR determinants such as (qnrB, qnrS, oqxAB, qepA and aac(6')-Ib-cr) using multiplex PCR[4].

VI- Detection of gyrA mutations for Chromosomal quinolones resistance by PCR restriction fragment length polymorphism (RFLP) [8].

The study was approved by the Ethics Board of Ain Shams University.

RESULTS

The results of this study were summarized in **tables 1-6.**

77% of FQs resistant isolates were positive to one or more plasmids, the frequency of positive plasmids among studied isolates: 20/32 (63%) *E.coli*, 25/32(78%) *Klebsiella* and 32/36(89%) *Pseudomonas*. Plasmids were higher significantly among *Pseudomonas* isolates followed by *Klebsiella* then *E.coli*, shown in **table 1.**

Prevalence of different types of plasmid among positive clinical isolates oqxAB was highest recovered PMQR among *Klebsiella* followed by *Pseudomonas* then *E.coli* P (< 0.05). While, qepA efflex pump gene was the least prevalent PMQR, shown in **table 2.**

Among total 77 positive PMQR, 23/77(30%) isolates were presented with single plasmid, while 54/77(70%) were plasmids coexistent isolates to two or more plasmids: 23/77 (30%) of isolates carried oqxAB and qnrS genes, 14/77 (18%) carried both oqxAB and qnrB, shown in **table 3.**

78% were positive for gyrA mutations, 40/78 (51%) were positive to single Asp-87 mutation, 22/78 (28%) were positive to single Ser-83mutation, while 16/78(21%) isolates were positive to double gyrA mutations, shown in **table 4.** As regards Asp-87mutation, 56/78(72%) isolates were positive, Asp-87mutation was higher among *E.coli* (89%) followed by *klebsiella* (75%) then *Pseudomonas* (55%). Ser-83 mutation 38/78 (49%) isolates were positive. The Ser-83 mutation was higher among *Pseudomonas* (68%) followed by *klebsiella* (45%) then *E.coli* (30%) as shown in **table 5.**

There was a positive association between PMQR and gyrA mutations with no statistical significance (NS) as P was (> 0.05) as shown in **table 6**

Table 1: frequency of PMQR among the studied isoltaes

Plasmid	Isolates						Total
	E.coli		***Klebsiella***		***Pseudomonas***		
	n.	**%**	**n.**	**%**	**n.**	**%**	
Positive	20	63	25	78	32	89	77
Negative	12	37	7	22	4	11	23
Total	32		32		36		100

(P value =0.035) Significant

Table 2: frequency of PMQR among the studied isolates

Plasmids	Isolates						Total	P value
	E.coli		*Klebsiella*		*Pseudomonas*			
	n.	%	n.	%	n.	%		
qnrB	3	15	6	24	7	22	16	0.746
qnrS	13	65	9	36	13	41	35	0.117
oqxAB	13	65	23	92	28	88	64	0.038
qepA	3	15	3	12	3	9	9	0.826
aac (6')	4	16	1	4	7	22	12	0.148
Total	20		25		32		77	

Table 3: **frequency of plasmids coexistence**

Plasmids		Types	Frequency	%
Single plasmid (23)		oqxAB	16	21
		QnrS	4	5
		aac (6')	2	3
		qepA	1	1
existent plasmid (54)	**Double (49)**	oqxAB/qnrS	23	30
		oqxAB/qnrB	14	18
		qnrS/ aac (6')	4	5
		oqxAB/ aac (6')	4	5
		oqxAB/qepA	2	3
		qnrS/qepA	2	3
	Tripple (5)	oqxAB/qnrS/qepA	2	3
		oqxAB/qnrB/qepA	1	1
		oqxAB/qnrB/ aac (6')	1	1
		oqxAB/qepA/ aac (6')	1	1
Total		77		(100%)

Table 4: frequency of gyrA mutations

gyrA mutations	Frequency	%
Single Asp-87	40	51
Single Ser-83	22	28
Double mutations	16	21
Total	78	(100%)

Table 5: frequency of Asp-87mutation among the studied isolates

gyrA mutations	Isolates						Total	P value
	E.coli		*Klebsiella*		*Pseudomonas*			
	n.	%	n.	%	n.	%		
Asp-87	24	89	15	75	17	55	56	0.015
Ser-83	8	30	9	45	21	68	38	0.014
Total	27		20		31		78	

Table 6: association between chromosomal and PMQR

Isolates	gyrA +	gyrA -	Total
Plasmid +	60	17	77
Plasmid -	18	5	23
Total	78	22	100

(P value =1.0) NS

DISCUSSION

This current study included 100 FQs resistant human clinical isolates, 64 *Enterobacteriaceae spp*., 32 *klebsiella,* 32 *E.coli* and 36 *Pseudomonas*, oqxAB gene was prevalent mostly in *Klebsiella* (92%) followed by *Pseudomonas* (88%). This result is in agreement with results of **Yanat *et al*.**[9] who reported that the oqxAB pump is common on the chromosome of *K. pneumoniae* isolates (75% or more). However, this is disagreement with results of **Kulková *et al*.** [5] who reported that the most prevalent PMQR gene in *K. pneumoniae* was aac(6')-Ib-cr gene (68.8%). While, **Ciesielczuk *et al*.** [4] suggested that aac(6')-Ib-cr gene (34.5 %) was the most common PMQR gene detected in uropathogenic *E.coli* isolates and least positivity was oqxAB (0.6 %). As regards *P. aeruginosa*, **Saleh *et al*.** [10] suggested that most recovered plasmids among *P. aeruginosa* were qnr (6.3%) genes (qnrS 4.7 % and qnrB 1.5%), neither other qnr genes, oqxAB, aac(6')-Ib-cr nor qepA gene was detected in their studied isolates.

Finally, in the current study the high prevalence rate of oqxAB gene might reflect inappropriate use of antimicrobial agents and even close contact with domestic animals, with subseqent dissemination of oqxAB gene as regards; these results are in agreement with those of **Kulková *et al*.** [5].

The coexistence of PMQR genes among the isolates increased the levels of resistance to quinolone antibiotics [11].

In the current study, the isolates with coexistent plasmids were (70%), there was no isolate carried two types of qnr gene but the most coexistences presented were oqxAB/qnrS (30%) followed by oqxAB/qnrB (18%) then qnrS/aac(6')-Ib-cr (5%). This result agreed with those of **Majlesi *et al*.** [11] who reported that there was also no isolates carried two types of qnr genes and qnrS/aac(6')-Ib-cr coexistence was present in low rate (6.2%) with a relationship between them as, aac(6')-Ib-cr was detected in qnr positive isolates by higher rate (78.9%) than qnr negative isolates (20.9%) as they can circulate dependently as reported in 2008[12]. or some plasmids can carry both aac(6')-Ib-cr and qnr genes [13]. The results of the current study are in disagreement with those of **El-Mahdy *et al*.**[14] who reported that qnr/aac(6')-Ib-cr (29.9%) was the highest coexistant plasmids.

The most prevalent single mutation was Asp-87 (51%) mostly present in *E.coli* (89%) followed by *Klebsiella* (75%) then *Pseudomonas* (55%), disagreed with [7,15]. While, **CLSI** [7] -87 mutation was high in *K. pneumoniae* (36%), **De Silva *et al*.** [15] reported that reported that Asp Asp-87 was the highest mutation that lead to drug resistance occur among *P. aeruginosa.*

As regards, Ser-83 mutation was detected in the current study by the rate of (28%) and mostly prevalent in *Pseudomonas* (68%) followed by *Klebsiella* (45%). These results disagreed with those of Johnning ***et al*.** [16] who reported that Ser-83 mutation was detected mostly in *E.coli* (86 %).

In the current study, (5%) of FQs resistant studied isolates were negative for PMQR and gyrA mutations. These finding may suggest that other resistant mechanisms may be present and responsible for FQs resistance, but they were not investigated in the current study.

REFERENCES

1-**Álvarez-Hernández DA, Garza-Mayén GS and Vázquez-López R (2015):** Quinolones: Nowadays perspectives and mechanisms of resistance. Rev. Chilena Infectol., 32(5):499-504.

2- **Dalhoff A (2012):** Resistance surveillance studies: A multifaceted problem the fluoroquinolone example. Infection, 40: 239-262.

3- **Redgrave LS, Sutton SB, Webber MA *et al* (2014):** Fluoroquinolone resistance: mechanisms, impact on bacteria and role in evolutionary success. Trends of Microbiol., 22(8):438-445.

4- **Ciesielczuk H, Hornsey M, Choi V *et al* (2013):** Development and evaluation of a multiplex PCR for eight plasmid-mediated quinolone-resistance determinants. J. Med. Microbiol., 62(12):1823-1830.

5- **Kulková N, Babálová M, Brnová J and Krcméry V (2014):** Transferable fluoroquinolone resistance in *Enterobacteriaceae* and *Pseudomonas aeruginosa* isolated from hemocultures. Cent. Eur. J. Public Health, 22(1):60-63.

6- **Goudarzi M, Azad M and Seyedjavadi SS (2015):** Prevalence of plasmid-mediated quinolone resistance determinants and OqxAB efflux pumps among extended spectrum β-Lactamase producing *Klebsiella pneumoniae* isolated from patients with nosocomial urinary tract infection in Tehran, Iran. Scientifica, 2015: 1-7.

7- **Clinical and Laboratory Standards Institute (CLSI 2015):** Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement (M100-S25). Wayne, PA: Clinical and Laboratory Standards Institute.

8- **Nakano R, Okamoto R, Nakano A *et al* (2013):** Rapid assay for detecting gyrA and parC mutations associated with fluoroquinolone resistance in

Enterobacteriaceae. J. Microbiol. Methods., 94(3):213-216.

9- **Yanat B, Rodríguez-Martínez JM and Touati A (2017):** PMQR in *Enterobacteriaceae*: a systematic review withfocus on Mediterranean count ries. Eur.J.Clin.Microbiol. Infect. Dis., 36(3):421-435.

10- **Saleh MA and Balboula MM (2017):** Plasmid mediated quinolone resistance determinants among nosocomial clinical *Pseudomonas aeruginosa* isolates. Int. Curr. Microbiol. App. Sci., 6(1): 42-50.

11- **Majlesi A, Kakhki RK, Nejad AS, Mashouf RY, Roointan A, Abazari M and Alikhani MY (2017):** Detection of plasmid-mediated quinolone resistance in clinical isolates of *Enterobacteriaceae* strains in Hamadan, West of Iran. Saudi J. Biol. Sci., 24: 1-5.

12- **Jiang Y, Zhou Z, Qian Y, Wei Z, Yu Y and Hu S (2008):** Plasmid-mediated quinolone resistance determinants qnr and aac (6')-Ib-cr in extended-spectrum b-lactamase-producing *E.coli* and *Klebsiella pneumoniae* in China. J. Antimicrob. Chemother., 61: 1003–1006.

13- **Robicsek A, Strahilevitz J, Sahm D, Jacoby G and Hooper D (2006):** Qnr prevalence in ceftazidime-resistant *Enterobacteriaceae* isolates from the United States. Antimicrob. Agents Chemother., 50: 2872–2874.

14- **El-Mahdy RH (2015):** Plasmid- mediated quinolone resistance (PMQR) determinants in nosocomial isolates of *Enterobacteriaceae*. Egypt J. Med. Microbiol.,24(1):43-48.

15- **De Silva CJ, Wimalasena S , Hossain S , Pathirana H and Heo GJ (2017):** Characterization of quinolone resistance of *Pseudomonas aeruginosa* isolated from Pet Chinese Stripe-necked Turtles (*Ocadia sinensis*). Asian J. Anim. Vet .Adv., 12 (3): 152-160.

16- **Johnning A, Kristiansson E, Fick J, Weijdegard B and Larsson DG (2015):** Resistance mutations in gyrA and parC are common in *Escherichia* communities of both fluoroquinolone-polluted and uncontaminated aquatic environments. Front Microbiol.,6:1355-1360.