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Investigation of plasmid-mediated quinolone resistance genes in carbapenem resistant Enterobacterales

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Abstract

Fluoroquinolones, are effective agents both against gram-positive and gram-negative bacteria. Quinolones show bactericidal effect as a result of inhibition of DNA gyrase and topoisomerase IV enzymes. Main quinolo resistance mechanisms are chromosomal mutations in these enzymes and decreased intracellular accumulation due to efflux pumps or decreased membrane uptake. Recently a new quinolone resistance mechanism mediated by plasmids has been defined. These plasmids carry genes called as qnr. Qnr genes do not cause quinolone resistance but they cause decreased quinolone susceptibility and lead to higher minimum inhibitory concentrations. Currently there are qnrA, qnrB, qnrC, qnrD and qnrS genes. This study was aimed to investigate the presence of plasmid-mediated quinolone resistance determinants in carbapenem resistant Enterobacterales isolates. A total 154 carbapenem resistant Enterobacterales isolates were included in the study. Presence of qnrA, qnrB, qnrC, qnrD and qnrS genes were investigated by multiplex polymerase chain reaction (PCR) method. The results of the PCR amplification revealed that qnrA was detected in two isolates (E6, E85) (1.29%), qnrB was detected in 12 isolates (8.4%) (E32, E43, E46, E61, E62, E84, E94, E149, E 166, E167, E177, E179) and qnrS was detected in six isolates (E15, E25, E57, E63, E70, E80) (4.54%). And one isolate (E9) was both positive for qnrB and qnrS. QnrC and qnrD were not detected in any isolates. Transferable quinolone resistance due to the dissemination of qnr genes may have important impacts in terms of infection control and treatment problems. Survey of plasmid mediated quinolone resistance will help to determine the size of the issue and guide the measures that should be taken to avoid escalation of resistance and dissemination problem.

Keywords: Qnr, Enterobacterales, quinolone, carbapenem

1. Introduction

Enterobacteriaceae are inhabitants of the intestinal flora and are among the most common human pathogens, causing infections such as cystitis and pyelonephritis with fever, septicemia, pneumonia, peritonitis, meningitis, and device-associated infections (1). Multidrug-resistant organisms are a major public health concern worldwide; of particular concern has been the emergence of resistance to carbapenem antimicrobial drugs among Enterobacteriaceae and quinolones are one of the options for treatment of carbapenem resistant enterobactericeae (CRE) infections (2, 3).

Quinolones are synthetic agents that are opponent to betalactames in clinical usage. They have been using in the treatment of both gram positive, gram negative and anaerobic bacterial infections (4). Quinolones show their bactericidal activity by inhibitng DNA gyrase and topoisomerase IV (5). The resistance development has been inevitable result of the widespread use of quinolones. The main resistance mechanism to quinolones are chromosomal mutations in

DNA gyrase (gyrA and gyr B) and topoisomerase IV (parC and par E). And other resistance mechanims are reduced accumulation of drug in the cell by hiperactivation of efflux pumps and decreased permeability of cell wall and plasmid mediated qnr (A, B, C, D, S), qepA and aac (6')- Ib- cr genes (6).

Plasmid mediated quinolone resistance was first reported in Klebsiella pneumoniae in 1998 and this resistance determinate called 'qnr' (7). Different qnr genes have been identified in time and first determinant named as qnrA. The primary structures of qnrA, qnrB, and qnrS are similar, with nine pentapeptide repeat units connected by a single glycine, followed by a cysteine, with variable numbers of units (8). He proteins that encoded by qnr genes are thought to protect DNA gyrase and topoisomerase IV by binding them from influence of quinolones (6).

The clinical importance of plasmid-mediated quinolone resistance is uncertain, although it is postulated that it may help to stabilize or select for mutations in the quinolone resistance-determining region (QRDR) of DNA gyrase and topoisomerase, which then confers high-level quinolone resistance (9).

In this study we aimed to investigate then prevelance of plasmid-mediated quinolone resistance determinants in carbapenem resistant Enterobactericeae.

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2. Materials and Methods

2.1. Bacterial isolates

Carbapenem resistant Enterobactarales clinical isolates (n=154, K. pneumoniae n=120, E. coli n=14, Klebsiella oxytoca n=10, Enterobacter cloacae n=4, Enterobacter aerogenes n=2, Proteus mirabilis n=2, Proteus vulgaris n=1, Providencia rettgeri n= 1) that isolated from clinical specimens in Microbiology laboratory of Ondokuz Mayıs University Faculty Medicine Hospital was tested in the study. Identification of the isolates was performed on Vitek MS (Biomeriux, France) and antimicrobial susceptibility was studied in Vitek2 Compact (Biomeriux, France) automated systems.

2.2. Polimerase chain reaction

DNA preperation was performed by a boiling technique that includes a heating step at 100˚C of colonies from Mueller-Hinton agar in a 500µl sterile distilled water for 20 min. followed by a centrifugation step of the cell suspension at 15000g for 20min, supernatant was used as template DNA in PCR. For optimisation of multiplex PCR well-characterized qnr-positive strains were used as positive controls. These strains were provided by: Prof. GA Jacoby (Lahey Clinic, Burlington, Massachusetts, USA), Prof. P Nordmann (Service de Bactériologie-Virologie, INSERM U914 "Emerging Resistance to Antibiotics", Hôpital de Bicêtre, Assistance Publique/Hôpitaux de Paris, Faculté de Médecine et Université Paris-Sud, K.-Bicêtre, France) and qnrC plasmid was provided by Prof. M Wang (Institute of Antibiotics, Huashan Hospital, Fudan University, 12 M. Wulumuqi Rd., Shanghai 200040, People's Republic of China).

Qnr A, qnrB, qnrC, qnrD and qnrS determinants were studied in multiplex PCR and primer pairs were used as identified by Kim et al. and Cavaco et al. (Table 1) (10, 11). Amplification was carried out with the following thermal cycling profile: 1 min at 95˚C and 35 cycles of amplification consisting of 1 min at 95˚C, 1 min at 60˚C and 1 min at 72˚C and 10 min at 72˚C for the final extension.

Ethical approve was taken from Ondokuz Mayıs University Clinical Research Ethics committe (B.30.2.ODM.0.20.08/364).

Table 1. Sequence of primers

Gene	Primer	Sequence	Bp	Ref
*qnr*A	Qnra-F QnrA-R	ATTTCTCACGCCAGGATTTG GATCGGCAAAGGTTAGGTCA	516	1017
*qnr*B	QnrB-F QnrB-R	GATCGTGAAAGCCAGAAAGG ATGAGCAACGATGCCTGGTA	476	10
*qnr*C	QnrC-F QnrC-R	GGGTTGTACATTTATTGAATCG CACCTACCCATTTATTTTC	307	10
*qnr*S	QnrS-F QnrS-R	GCAAGTTCATTGAACAGGGT TCTAAACCGTCGAGTTCGGCG	428	10
*qnr*D	QnrD-F QnrD-R	CGAGATCAATTTACGGGGAATA AACAAGCTGAAGCGCCTG	565	11

3. Results

3.1. Bacterial isolates

The distribution of specimens that CRE isolated were presented in Table 2. The most common specimen that CRE isolates were identified was urine (50.0%) and it was followed by blood (22,0%) and respiratory tract (13.6%) samples. Materials were sent from different clinics to the laboratory. Internal medicine clinic was the most frequent clinic (23.37%) that CRE isolates were isolated (Table 3).

Ciprofloxacin resistance was detected in 119 (77.27%) isolates. And 8 (1.29%) isolate were intermediate to ciprofloxacin.

3.2. Polimerase chain reaction

Positive control strains yielded expected bands (Fig. 1). QnrA was detected in two isolates (E6, E85) (1.29%), qnrB was detected in 12 isolates (8.4%) (E32, E43, E46, E61, E62, E84, E94, E149, E 166, E167, E177, E179) and qnrS was detected in six isolates (E15, E25, E57, E63, E70, E80) (4.54%). And one isolate (E9) was both positive for qnrB and qnrS. QnrC and qnrD were not detected in any isolates (Table 4).

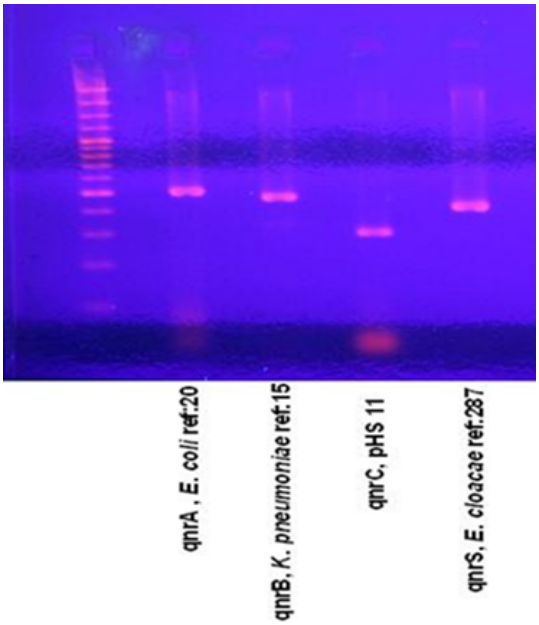

Fig. 1. Gel electrophoresis of positive qnrA, qnrB, qnrS and qnrC

Seventeen of the qnr determinant positive isolates found to be resistant to ciprofloxacin, four of them were intermediate and one of the isolate was susceptible to ciprofloxacin.

Table 2. Distribution of qnr positive isolates

	Isolate	Specimen type	Ciprofloxacin resistance
qnrA positive			
E6	*K.pneumoniae*	Urine	R
E5	*K.oxytoca*	Urine	R
qnrB positive			
E32	*K.pneumoniae*	Urine	I
E43	*K.pneumoniae*	Blood	R
E46	*K.pneumoniae*	Sterile body fluid	R
E61	*K.pneumoniae*	Sterile body fluid	R
E84	*K.pneumoniae*	Urine	R
E94	*E.cloacae*	Blood	S
E149	*K.pneumoniae*	Wound	R
E166	*K.pneumoniae*	Urine	I
E167	*E.aerogenes*	Tracheal aspirate	I
E177	*K.pneumoniae*	Wound	R
E179	*K.pneumoniae*	Urine	R
qnrS positive			
E15	*K. oxytoca*	Urine	R
E25	*E. coli*	Wound	I
E57	*E. cloacae*	Blood	R
E63	*K. oxytoca*	Urine	R
E70	*K. oxytoca*	Urine	R
E80	*K. oxytoca*	Urine	R
qnrB+qnrS positive			
E9	*E. coli*	Wound	R

Table 3. Distribution of clinical wards that samples sent

Clinical wards	N (%)
Internal Medicine	36(23.37%)
Pediatrics	22(14.28%)
Intensive care unit	21 (13.63%)
Neurology	17(11.03%)
Urology	11(7.14%)
Cardiology	10(6.50%)
Infectious Diseaes	8(5.20%)
Emercency	7(4.54%)
Surgery	7(4.54%)
Pulmonology	5(3.24%)
Other*	10(6.50%)

Table 4. Distribution of qnr positive isolates

	Isolate	Specimen type	Ciprofloxacin resistance
qnrA positive			
E6	*K.pneumoniae*	Urine	R
E5	*K.oxytoca*	Urine	R
qnrB positive			
E32	*K.pneumoniae*	Urine	I
E43	*K.pneumoniae*	Blood	R
E46	*K.pneumoniae*	Sterile body fluid	R
E61	*K.pneumoniae*	Sterile body fluid	R
E84	*K.pneumoniae*	Urine	R
E94	*E.cloacae*	Blood	S
E149	*K.pneumoniae*	Wound	R
E166	*K.pneumoniae*	Urine	I
E167	*E.aerogenes*	Tracheal aspirate	I
E177	*K.pneumoniae*	Wound	R
E179	*K.pneumoniae*	Urine	R
qnrS positive			
E15	*K. oxytoca*	Urine	R
E25	*E. coli*	Wound	I
E57	*E. cloacae*	Blood	R
E63	*K. oxytoca*	Urine	R
E70	*K. oxytoca*	Urine	R
E80	*K. oxytoca*	Urine	R
qnrB+qnrS positive			
E9	*E. coli*	Wound	R

4. Discussion

Quinolones are synthetic chemotherapeutic agents that have a bactericidal effect. Nalidixic acid, which was used in the 1960s, has expanded its antimicrobial effect spectrum and changed its pharmacodynamic properties with the changes made in its chemical structures. Today, they are effective on both gram-positive, gram-negative and anaerobes. The use of quinolones has become widespread with these changes over time (12). Widespread use has brought along the problem of resistance. The main development of resistance to quinolone antibiotics occurs through two mechanisms: a change in the target of quinolones and a decrease in membrane permeability or a decrease in drug accumulation within the cell due to the presence of pulse pumps (13). Both of these resistance mechanisms are of chromosomal origin. However, they first identified a new gene region that belongs to the repeating

pentapeptide family consisting of 218 aa from 1998 K. pneumoniae isolates and transferred by the plasmid called qnr (14). Later, this gene region was named qnrA. These qnr genes generally differ in sequence by 35% or more from qnrA and from each other. Furthermore, most of them contain allelic variants differing by 10% or less (qnrA: 8, qnrS: 9, qnrB: 88, qnrC: 1, qnrD: 2, and qnrVC: 7) in which qnrB constitutes the most heterogeneous cluster of the qnr gene family (15).

Qnr genes in the Enterobactericeae family have been previously investigated in different centers in Turkey. In a study conducted in our country in 2005, the qnr A gene region was investigated in 49 isolates and one E. cloacae and one C. freundii qnrA gene were detected14 (16). Later, Öktem et al. qnrA, qnrB and qnrS genes were investigated in 356 Enterobacteriaceae members isolated from blood cultures, and qnrA was detected in 61 isolates and qnrS in 3 isolates. It was observed that two of the isolates with qnrA and one of the isolates with qnrS formed ESBL (17).

In another study conducted in our country, qnrA, qnrB, qnrS genes were investigated in a total of 460 gram negative bacteria isolated from intensive care patients, and 1 qnrB1 and 2 qnrS1 genes were found in three (0.65%) E. cloacae isolates. They investigated the frequency of qnrA, qnrB, qnrS, aac (6 ') - Ib-cr genes in a total of 248 E. coli and K. pneumoniae isolates isolated from different hospitals in Turkey, qnrB1 on different plasmids in 1 K. pneumoniae isolates. and detected the aac (6 ') - Ib-cr genes (18). Coban et al. İnvestigated the qnr genes in total of 647 Enterobactericeae isolate and determined qnrA in two isolates, qnrB in six isolates and qnrS in two isolates (19).

In a multicenter study conducted in Spain, 19010 isolates (18624 Salmonella spp., 285 E. coli, 68 Shigella spp., 29 K. pneumoniae, 2 C. freundii and 2 P. mirabilis) were investigated for quinolone resistance and decreased ciprofloxacin susceptibility (Qnr genes were investigated in 123 isolates showing MIC 0.12-0.5 mg / L) but sensitive to nalidixic acid and 2 Salmonella spp. isolate qnrB, 25 Salmonella spp. and qnrA in 1 E. coli isolate, 4 Salmonella spp. The qnrS gene was detected in the isolate (20). Again, 485 Salmonella spp. isolated from human, animal, food and environment collected from 13 European countries. and in 133 E. coli isolates, the presence of quinolone resistance genes transferred by plasmid was investigated and in 59% of Salmonella spp. isolates (288/485); positivity was detected in 15% (20/133) of E. coli isolates, qnrA 3 Salmonella spp. isolate, qnrB 138 Salmonella spp. and in 1 E. coli isolate, qnrS 125 Salmonella spp. and in 19 E. coli isolates (21).

Majlesi et al. tested total of 100 fluoroquinolone-resistant Enterobacteriaceae and determined two (2%) were positive for qnrS, seventeen (17%) isolates were positive for qnrB (22). In a study total of 155 bacterial strains tested were found harboring at least one qnr gene consisting of 74 (47.74%) qnrB, 73 (47.10%) qnrS and 4 (2.58%) qnrA (23). Amin et al. investigated the presence of qnrA, qnrB and class I integron in Enterobacter spp. isolates and they found that 29% of the isolates were positive for qnrB and none of them positive for qnrA, and 22.4% of the isolates were positive for both qnrB and int1 (24).

Antibiotic resistance is an emerging problem in Enterobactericeae, carbapenem resistance is increasing and other antimicrobials like quinolones are drug of choice. But sometimes resistance to more than one group of antimicrobials can be seen. Therefore identifying ways of resistance development and increasing the number of studies related to them and identifying the worldwide spread can help develop treatment strategies will be.

Conflict of interest

None to declare.

Acknowledgments

None to declare.

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