INVESTIGATION OF OQXAB AND QEP A THE QUINOLONE RESISTANCE DETERMINANTS IN CARBAPENEM RESISTANT ENTEROBACTERICEAE ISOLATES

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ABSTRACT

Aim*: Quinolone resistance is very important among Enterobactericeae isolates. Recently, new plasmid-mediated quinolone resistance were defined. In this study we aimed to investigate the presence of plasmid-mediated efflux pump genes oqxAB and qepA in carbapenem resistant clinical isolates of Enterobactericeae.*

Materials and method*: Total of 204 Enterobacterceae isolates were tested in the study. Identification of the isolates were performed in Vitek MS (Biomeriux, France) and antimicrobial susceptibility was tested in Vitek2 Compakt system (Biomeriux, France). DNA preperation was performed by a boiling technique. The oqxA, oqxB and qepA genes were investigated in polymesrase chain reaction, by using specific primers. The oqxA positive isolates were screened for oqxB.*

Results*: Of the 204 isolates, 102 were carbapenem resistant Enterobactericeae. oqxAB was detected in 20.1% of carbapenem resistant isolates and 11.7% of carbapenem susceptible isolates. Both oqxA and oqxB positivity were determined in carbapenem resistant K. pneumoniae isolates. QepA was detected in any of the isolates.*

Conclusion*: In this study it was found that positivity of oqxAB was higher in carbapenem resistant isolates. This finding may suggest the presence of resistance genes in same plasmid and carried from one bacterium to another.*

Keywords: *Enterobactericeae, oqxAB, qepA.*

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Introduction

Multidrug resistance in bacteria is a significant issue in the treatment of infectious diseases[1]. Betalactam resistance is commonly seen in Enterobactericeae and quinolones are one of the option for treatment of enterobactericeae infections[2].

In 1998, plasmid-mediated quinolone resistance (PMQR) was detected. The qnrA, qnrB, qnrC, qnrD, and qnrS genes have been identified as major groups of qnr[3]. After then, the plasmid-encoded efflux pump, OqxAB, conferring resistance to quinoxaline-di- N-oxide olaquindox which is used as a promoter of growth in pigs, was discovered in *Escherichia coli* isolates of porcine origin in Denmark and Sweden[4-6].

OqxAB is encoded by the oqxA and oqxB genes, located on a 52 kb conjugative plasmid, designated pOLA52, and confers resistance to multiple agents, including fluoroquinolones such as nalidixic acid, ciprofloxacin and norfloxacin, as well as biocides such as triclosan and chlorhexidine[7,8]. Yamane et al.[9] have identified plasmid mediated qepA gene which set out efflux pump activation in 2007.

In this study we aimed to investigate the prevelance of oqxAB and qepA determinants in carbapenem resistant Enterobactericeae in Turkey. In Turkey, oqxAB genes have been reported from Enterobactericeae but there have been no reports about prevelance of oqxAB and qepA in carbapenem resistant isolates.

Material and methods

Bacterial isolates

Enterobactericea clinical isolates (n=204) collected from Ondokuz Mayıs University Medicine School bacteriology subdicipline laboratory were identified in Vitek MS (Biomeriux, France) and antimicrobial susceptibility of them were tested by Vitek2 Compact (Biomeriux, France) automated systems. Antimicrobial susceptibility of the isolates were evaluated according to the EUCAST criteria.

Polimerase chain reaction

DNA preperation was performed by a boiling technique that includes a heating step at 100˚C of a single colony 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 PCR well-characterized oqxAB and qepA positive strains were used as positive controls. The positive isolates for oqxA were screened for oqxB. The primers used for OqxA and OqxB were as follows: OqxA-F (5'-CTCGGCG-CGATGATGCT-3') and oqxA-R (5'- CCACTCT-TCACGGGAGACGA-3') for OqxA; and OqxB-F (5'- TTCTCCCCCGGCGGGAAGTAC-3') and OqxB-R (5'–CTCGGCCATTTTGGCGCGTA -3 ') for OqxB. Amplification was carried out with the following thermal cycling conditions: 1 min at 96°C and 35 cycles of amplification consisting of 1 min at 96°C, 1 min at 58°C, and 1 min at 70°C, with 5 min at 72°C for the final extension. DNA fragments were analysed by electrophoresis in a 1% agarose gel at 120 V for 60 min in 1X TBE containing ethidium bromide.

QepA PCR primer pairs used as Yamane et al.[9] studied as QepA-F (GCAGGTCCAGCAGCGGG-TAG), QepA-R (CTTCCTGCCCGAGTATCGTG). Amplification was carried out with the following thermal cycling profile; 1 min at 96°C and 30 cycles of consisting 1 min at 96°C, 1 min at 60°C, 1 min at 72°C and 5 min at 72°C for the final extention. Amplification was carried out with the following thermal cycling profile; 1 min at 96°C and 30 cycles of consisting 1 min at 96°C, 1 min at 59°C, 1 min at 72°C and 5 min at 72°C for the final extention.

The amplicons obtained were confirmed by sequencing.

Results

Total of 204 Enterobactericeae isolates (*C. koseri* n=1, *E. aerogenes* n=4, *E. cloacae* n=6, *E. coli* n=67, *K. oxytoc*a n=10, *K. pneumoniae* n=97, *M. morgnanni* n=5, *S. liqefacien*s n=1, *S. marcenscens* n=9, *P. mirabilis* n=3, *P. vulgaris* n=1) were tested.

Of these isolates, 102 were carbapenem resistant Enterobactericeae (E. aerogenes n=1, E. cloacae n=2, E. coli n=9, K. oxytoca n=9, K. pneumoniae n=77, P. mirabilis n=3, P. vulgaris n=1). OqxA was detected in 64 of carbapenem resistant and 25 of carbapenem susceptible isolates. These isolates were screened for presence of oqxB. And totally, oqxAB was detected in 20.1% of carbapenem resistant isolates and 11.7% of carbapenem susceptible isolates (Table 1 and 2). In carbapenem resistant isolates both oqxA and oqxB positivity were determined in *K. pneumoniae* isolates (n=29). Therefore in carabepenem susceptible isolates both oqxA and oqxB positivity w*ere determined in E. coli (n=1) and* K. pneumoniae (n=11) isolates.

Ciprofloxacin resistance was determined in 37 (90.2%) of the oqxAB positive isolates.

QepA was not detected at none of the isolates.

Bacteria	*OqxA*	*OqxB*
E. cloacae (n=2)	1	-
K. oxytoca (n=9)	1	-
K. pneumoniae (n=77)	60	29
P. mirabilis (n=2)	2	-

Table 1: *OqxA* ve *oqxB* positivity rates at carbapenem resistant isolates.

Bacteria	*OqxA*	*OqxB*
E. aerogenes (n=4)	1	-
E. coli (n=67)	8	1
K. pnemoniae (n=20)	16	11

Table 2: *OqxA* ve *oqxB* positivity rates at carbapenem susceptible isolates.

Discussion

The member of Enterobacteriaceae family is the leading causative agent of both community-acquired and nosocomial infections. These microorganisms, which are resistant to antibiotics due to the outer membrane structure on the cell walls, have acquired multi-resistance feature by transfer of genetic material and selective stress of antibiotics[10].

Quinolones inhibit topoisomerization by forming drug-enzyme-DNA complexes, thus by creating a barrier in front of replication-competent, RNA polymerase and DNA helicase, they cause events that will result in cell death[11,12].

Quinolones are widely used due to their broad spectrum. In a study, it has been stated that ciprofloxacin is the most commonly used antibacterial agent all over the world[13].

The resistance against quinolones occurs by three main mechanisms; i) the changes in the chromosomal targets of the quinolones, ii) reduced accumulation due to reduced membrane permeability and/or excessive working of the efflux pump systems and iii) plasmid-mediated resistance[14].

Plasmid-mediated nalidixic acid resistance was first reported in the clinical isolate of *Shigella dysenteriae* in 1987 but was subsequently not confirmed and resistance was reported to be due to chromosomal mutation[15-17]. However, quinolone resistance (PMQR- plasmid-mediated quinolone resistance) transferred by plasmid was first reported in the clinical isolate of K. pneumoniae isolated from a urine isolate of a patient in 1994 in the US in 1998. This gene in the plasmid was called 'Qnr'. It was subsequently named as qnrA1 because of the detection of different qnr genes. Over the years, qnrB, qnrS, qnrC, qnrD, qepA leading to efflux pump activation transferred by plasmid and aac(6')-Ib-cr genes, which are variants of aminoglycoside acetyltransferase and reduce ciprofloxacin activity, have also been identified[9,18,19].

Efflux pumps genes are often chromosomally encoded. In Japan, however, qepA gene (quinolone efflux pump), which is a plasma-mediated efflux pump, was first described in the E. coli strain isolated from the urine sample[9]. This gene encodes 511 aa of protein. This protein is significantly similar to the 14-transmembrane segment (14-TMS) in the major facilitator superfamily (MFS) making proton-dependent transport[9,20]. The plasmid that includes QepA gene, cause resistance against quinolones, aminoglycosides and betalactam groups. QepA significantly causes reduction in quinolone susceptibility. Especially it causes 8-12 fold increase in MIC values of the hydrophilic quinolones such as ciprofloxacin and norfloxacin when compared to wild-type strains[9]. Although the natural reservoir of the QepA gene is not known, reservoirs are thought to be these bacteria similar to Actinomycetales family with the membrane carriers and high content of guanine cytosine (72%)[9,20].

The pOLA52 plasmid, which causes resistance to olaquindox (quinoxaline derivative used in agricultural feeds as growth promoter) antibiotics was identified from *E. coli* strains isolated from porcine embryos[21]. OqxAB, a multi-drug efflux pump of RND (resistance nodule cell division superfamily) family, was shown as the reason for this resistance[6]. It has been recently detected in **E. coli** isolates isolated from human. It is also found in *K. pneumoniae* chromosome and causes resistance to olaquindox according to different expression ratios[22]. It was detected that the plasmid pOLA52 caused an 8- to 16-fold increase in the MIC values of nalidixic acid and ciprofloxacin in *E. coli* strain without AcrA gene[4].

Kim et al.[22] investigated oqxA gene in 461 isolates in which the genes that caused plasmid-mediated quinolone resistance was investigated, and then investigated the oqxB gene in isolates that were found to be positive. They found both OqxA and OqxB genes positive in 1 of *E. coli* isolates (0.4%), 3 of *E. cloacae* isolates (4.6%) and 100 of K. pneumoniae isolates (74.1%). This study is the first study to determine the positivity of oqxAB in *E. coli* isolates isolated from human. The presence of the oqxAB gene was investigated in 114 *K. pneumoniae* isolates and found their frequency to be 75%[23].

In a study, found that oqxAB positivity in KPC-positive isolates of *K. pneumoniae* was 71-100% in strains isolated from different areas. They found that the positivity rate was higher in especially ST258 positive *K. pneumoniae* isolates[24]. Similarly, in our study, it was found that oqxAB positivity was higher in carbapenem resistant isolates. In a study from China, oqxAB positivity in *E. col*i and *K. pneumoniae* isolates isolated from clinical specimens was found as 6.6% and 100%, respectively[25]. In their study, Park et al. found oqxA in 11.5% of *E. coli* isolates, but they did not find oqxB[26]. In the same study, they found oqxA in 36.5% (15/41) and oqxB in 24.4% (10/41) of K. pneumoniae isolates. While in a study from Turkey, oqxA was found in 14 and oqxB was found in 12 isolates of 85 Enterobactericeae (E. coli n = 72, K. pneumoniae n = 13) tested; oqxAB positivity reported as 1.4% in *E. coli* isolates and 84.6% in K. pneumoniae isolates[27]. In a study conducted in China, oqxAB positivity was found in 10 of 33 farm workers (30.3%)[28].

In a recent study oqxAB is common and frequent in human, animal, and environment samples

in China in their studies they were investigating plasmid-mediated quinolone resistance determinants. And they indicated that the presence of oqxAB in animal isolates extended to 1994[3]. The oqxAB positivity is reported in a foodborne Salmonella isolate[29]. In a study conducted in Iran, oqxA and oqxB positivity was found to be 60.2% in K. pneumoniae isolates[30].

In our study; as OqxAB was found in 29 of carbapenem resistant isolates (28.4%) and 12 of carbapenem sensitive 102 isolates (11.7%), qepA was not found any of the isolates. Further studies are needed to determine the presence of these plasmid-mediated genes in isolates with different resistance paterns and from regions.

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