Investigation of *qnrD*-harbouring plasmid in *Morganella morganii*

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26th ECCMID Amsterdam, Netherlands 9 – 12 April 2016

P1500

Introduction

Increasing number of fluoroquinolone resistant Enterobacteriaceae clinical isolates are identified (1). Fluoroquinolone resistance develops in Enterobacteriaceae by plasmid-mediated quinolone resistance (PMQR) mechanisms and by chromosomal mutations. PMQR includes Qnr determinants (QnrA, QnrB, QnrC, QnrD, QnrS), Aminoglycoside acetyltransferase (6')-Ib-cr, Qep and OqxAB efflux pumps. PMQRs alone confer low-level resistance with ciprofloxacin MIC values up to 0.25 µg/ml but facilitates the selection to higher level resistance. Chromosomal mutations in *gyrA*, *gyrB*, *parC* and *parE* lead to high-level fluoroquinolone resistance (2).

Materials and methods

In our study **214 Enterobacteriaceae** strains were investigated namely, 99 *Escherichia coli*, 32 *Klebsiella spp.*, 36 *Proteus spp.*, 20 *Enterobacter spp.*, 15 *Serratia spp.*, 6 *Citrobacter spp.*, 5 *Morganella morganii and one Providencia stuartii*. All were identified in urinary tract infections and all exhibited reduced susceptibility or resistance to fluoroquinolones. **MIC values** of strains were determined by broth microdilution method for ciprofloxacin, cefotaxime, ceftazidime, amikacin. **PMQR screen** was performed by PCR. *qnrD*-plasmid was further investigeted by **inverse PCR** with complementer antiparallel strands of *qnrD* fwd and *qnrD* rev (Table 1.) (3). Positive PCRs were sequenced. **Conjugation** experiment was performed by *E. coli* Azid^{R} J53 to check the transferability of *qnrD*-plasmid. **Chromosomal mutations** in *gyrA, gyrB, parC* and *parE* genes of *qnrD* postive *M.morganii* were analyzed by PCR and sequencing.

Inverse PCR and primer walking was performed to investigate *qnrD*-harbouring plasmid (Figure 1). PCR thermal profile was as follows: 95°C for 2 min; 30 cycles of 94°C for 30 sec, 53°C for 30 sec and 72°C for 5 min; a final elongation step 72°C for 10 min and 4°C to close the reaction.

Figure 1. Inverse PCR on *qnrD*-plasmid

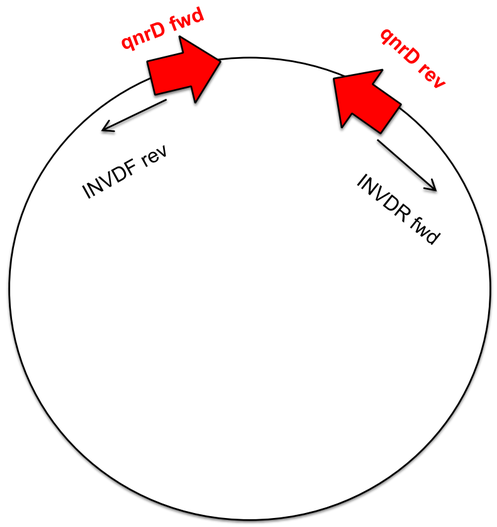


Table 1.: Primers used in *qnrD*-plasmid analysis

Primer	sequence
qnrD fwd	CGAGATCAATTTACGGGGAATA
qnrD rev	AACAAGCTGAAGCGCCTG
INVDR fwd	CAGGCGCTTCAGCTTGTT
INVDF rev	TATTCCCCGTAAATTGATCTCG

Results

Our study detected 44 PMQR positive strains among the tested 214, that represnts 20.5% prevalence (Figure 2). **Two *M. morganii* yielded *qnrD*** resistance determinant, showing 0.9% prevalence in Enterobacteriaceae but 40% among *M. morganii*. Table 2 features *qnrD* positive strains.

The *qnrD*-plasmids were **not transferable** to *E. coli* J53 Azid resistant recipient strain in conjugation experiment.

One *qnrD*-plasmid was entirely sequenced after inverse PCR and primer walking. It resulted in a **2662 bp plasmid**, that contained *qnrD* gene, flanked by invert repeat left and invert repeat right sequences. No other coding genes were on this plasmid. This plasmid seqeunce is submitted to Genbank, on the following **accession number: KU160530.**

Nucleic acid seqeunce analysis of *qnrD*-plasmid showed 95-98% similarity to other *qnrD*-harbouring plasmids sequences present in Genbank.

Table 2.: Characteristics of *qnrD* positive strains

	M. morganii 10	*M. morganii* 71
ciprofloxacin MIC	2 µg/ml	2 µg/ml
cefotaxime MIC	0.06 µg/ml	0.06 µg/ml
ceftazidime MIC	0.06 µg/ml	0.06 µg/ml
amikacin MIC	16 µg/ml	0.25 µg/ml
tobramycin MIC	64 µg/ml	0.25 µg/ml
qnr positivity	*qnrD*	*qnrD*
gyrB	S463A	S463A
parC	S80I	-
aac(6')-Ib-cr	negative	positive

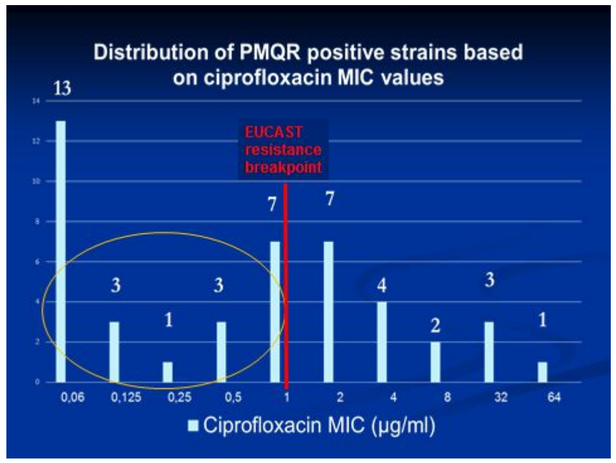
Conclusions

Amog PMQR determinants *qnrD* represents modest 0.9% prevalence in Enterobacteriaceae This resistant determinant is mainly present in Proteeae tribe including *M. morganii*.

Analysis of *qnrD* plasmid sequence revealed promoter sequence of *qnrD* gene. It has a lexA binding site that takes part in the SOS-response system.

Upstream and downstream to the *qnrD* gene mobile insertion cassettes were detected, making the plasmid capable to recombinate. This may foster the dissemination of this resistance determinant in Enterobacteriaceae.

Figure 2.: Distribution of PMQR positive strains



References

1) European Center for Disease Control Database
2) Martinez-Martinez L. et al. Lancet 1998.
3) Mazzariol A. et al. Clin Microb Inf 2012.

Our study was financially supported by OTKA Hungarian Research Fund, Grant: K 108481

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