Prevalence of *qnr* genes in 1032 invasive isolates of *Escherichia coli* in Croatia

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Introduction

Multidrug resistance in *Enterobacteriaceae* including resistance to quinolones is rising worldwide. Quinolone resistance in *Enterobacteriaceae* usually results from mutations in genes carried by chromosomally encoded type II topoisomerases, efflux pumps, or porin-related proteins[1]. Plasmid-mediated quinolone resistances (PMQR) was described in a *Klebsiella pneumoniae* strain from the USA in 1998 with the discovery of *qnr* genes. The Qnr peptides are pentapeptide repeat proteins, which protect DNA gyrase and topoisomerase IV from quinolone inhibition, leading to an 8- to 32-fold increase in MICs of quinolones and are often combined with extended-spectrum beta-lactamases (ESBLs)[2]. Qnr mediated quinolone resistance has been reported worldwide in unrelated enterobacterial species and five major groups of qnr determinants QnrA, QnrB, QnrS, QnrC and QnrD have been identified to this day[3]. The aim of this study was to determine the prevalence of plasmid-mediated quinolone resistance (*qnr*) genes in all invasive isolates of *Escherichia coli* collected in Croatia during 2011.

Materials and Methods

During a 12 month period (1st January 2011 – 31st December 2011) 1032 invasive *E. coli* isolates were collected from inpatients in Croatian hospitals trough EARS-Net. Isolates were screened for presence of all reported alleles of *qnrA, qnrB, qnrC, qnrD* and *qnrS* genes by multiplex PCR, using a combination of primers designed in this study. Determination of *qnr* gene family was based on PCR product size evaluation by gel electrophoresis. Presence of respective genes was confirmed by DNA sequencing. Qnr-positive isolates were screened by PCR for presence of genes for class A β-lactamases, metallo- β-lactamases and plasmid AmpC. Antimicrobial susceptibility testing was performed by disc diffusion method to amoxicillin, gentamicin, amikacin, ciprofloxacin, ceftriaxone, ceftazidime, imipenem and meropenem. The combination-disk test using cefotaxime and ceftazidime alone and in combination with clavulanic acid was performed for detection of ESBL. Clonal relatedness of qnr-positive strains was determined by Pulsed-field-gel electrophoresis of XbaI digested genomic DNA.The assignment of the *E. coli* phylogenetic group was carried out by multiplex PCR assay.

References

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Results

Eighteen *E. coli* (18/1032, 1.74%) isolates were positive for *qnr* determinants (1 *qnrA*, *5 qnrB*, 12 *qnrS*). Seven qnr positive isolates were non-susceptible to ciprofloxacin (1 *qnrA*, 1 *qnrB*, 5 *qnrS*) and only two were non-susceptible to 3rd generation cephalosporins (2 *qnrS*). Bla_{TEM} genes were detected in all isolates and bla_{CTX-M} and bla_{SHV} genes were detected in two isolates. *Qnr* positive isolates did not exhibit any apparent epidemiological relation. Isolates belonged to phylogenetic groups A, B1, B2 and D.

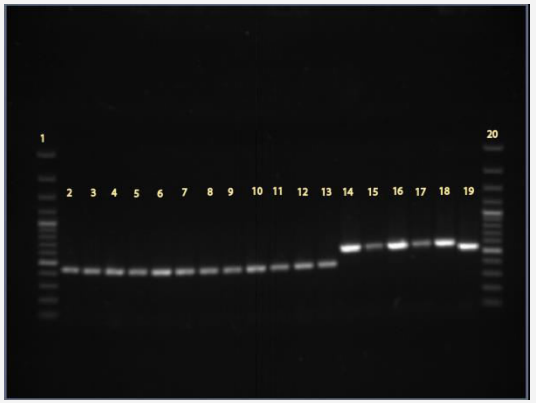

Figure 1: Multiplex PCR products of qnr - positive *E. Coli* isolates. Lanes 1, 20, Size marker; lanes 2 – 13, *qnrS;* lanes 14 – 18, *qnrB;* lane 19, QnrA.

Conclusions

This is the first study of prevalence of *qnr* genes in invasive isolates of *E. Coli* in Croatia. Prevalence of plasmid mediated quinolone resistance in invasive isolates of *E. coli* is still low. Qnr positive isolates are clonally distinct and are detected in various parts of Croatia. Resistance to ciprofloxacin is not uniform for all *qnr*-positive isolates. Most studies so far reported presence of *qnr* genes mostly in ESBL isolates. In this study, only 2 *qnr* positive isolates manifested an ESBL phenotype. This study was performed on all invasive *E. coli* isolates collected during one year. Most *qnr* prevalence studies could potentially show biased data due to short time intervals of isolate collection during an outbreak or when only performed on isolates which are resistant to various antimicrobials, most commonly quinolones or ESBLs[4].