Veterinary Microbiology 170 (2014) 89–96

Contents lists available at ScienceDirect

Veterinary Microbiology

journal homepage: www.elsevier.com/locate/vetmic

The association between occurrence of plasmid-mediated quinolone resistance and ciprofloxacin resistance in *Escherichia coli* isolates of different origins

Tong Yang, Zhenling Zeng, Lili Rao, Xiaojie Chen, Dandan He, Luchao Lv, Jing Wang, Li Zeng, Minsha Feng, Jian-Hua Liu *

College of Veterinary Medicine, National and Regional Joint Engineering Laboratory for Medicament of Zoonosis Prevention and Control, South China Agricultural University, Guangzhou, People's Republic of China

ARTICLE INFO

Article history:
Received 28 September 2013
Received in revised form 23 January 2014
Accepted 24 January 2014

Keywords:
Escherichia coli
Food animals
Resistance gene
Animal reservoirs
Quinolones

ABSTRACT

This study was performed to determine the prevalence of plasmid-mediated quinolone resistance (PMQR) determinants and characterize the ciprofloxacin resistance in *Escherichia coli* isolated from different sources in China. PMQR determinants were detected by PCR amplification and sequencing in 2297 *E. coli* isolates randomly collected from animals, food and humans during 2004 to 2011. MICs of ciprofloxacin were determined by agar dilution method. Of the 2297 *E. coli* isolates, 43.6% harbored at least one PMQR gene. The most common PMQR gene was *oqxAB* (29.3%), followed by *qnr* (13.6%), *aac(6′)-Ib-cr* (11.6%), and *qepA* (3.3%). 12.0% isolates carried two or more PMQR genes. The prevalence of PMQR genes in food animal isolates increased over time, from 38.7% in 2004 to 69.8% in 2011. The prevalence of PMQR/ciprofloxacin resistance among isolates from pig, chicken, duck, companion animals, animal food and human volunteers were 65.2%/69.6%, 42.4%/60.0%, 59.4%/65.0%, 28.6%/57.5%, 29.3%/25.6%, and 14.0/8.7%, respectively. Most isolates carrying *qnr* along showed susceptible to ciprofloxacin, and only 21.6% the isolates exhibited resistance to ciprofloxacin, which was significantly lower than those carrying other PMQR genes (65.2–89.9%) and those that do not (43.1%) ($p < 0.01$). In conclusion, high frequency of ciprofloxacin resistance and PMQR genes was observed among *E. coli* isolates of different origins in China, with *oqxAB* being the most frequent. *qnr*-positive *E. coli* isolates have relatively low ciprofloxacin resistance rate compared with other PMQR determinants-carrying isolates and PMQR-negative isolates.

1. Introduction

In 1998, the emergence of plasmid-mediated quinolone resistance (PMQR) first reported by Martinez-Martinez et al. (1998), stimulated a great deal of interest in the transferable mechanism of quinolone resistance. In the last 15 years, research on PMQR is rapidly expanding and resulted in a host of new findings (Rodriguez-Martinez et al., 2011; Ruiz et al., 2012; Strahilevitz et al., 2009). Currently, three different transfefable quinolone resistance mechanisms have so far been identified: (1) the Qnr families (QnrA, QnrB, QnrS, QnrC, QnrVC and QnrD) of proteins that protect DNA gyrase and topoisomerase IV from quinolones; (2) AAC(6′)-Ib-cr aminoglycoside acetyltransferase that acetylate fluoroquinolones with a

* Corresponding author. Tel.: +86 2085283824/+86 2085280237; fax: +86 2085283824.
E-mail address: jhliu@scau.edu.cn (J.-H. Liu).

0378-1135/$ – see front matter © 2014 Published by Elsevier B.V.
http://dx.doi.org/10.1016/j.vetmic.2014.01.019

piperazinyl substituent, such as ciprofloxacin and norfloxacin; and (3) active efflux pumps QepA and OqxAB (multidrug efflux pump) that confer decreased susceptibility to fluoroquinolones (FQs) (Poirel et al., 2012; Rodriguez-Martinez et al., 2011; Ruiz et al., 2012). Although these PMQR determinants only confer low-level resistance to quinolones, they can be spread horizontally among Enterobacteria and these determinants are believed to be a favorable background for the selection of additional chromosome-encoded quinolone resistance mechanisms (Poirel et al., 2012). However, their actual contribution on selection of QRDR point mutations is not well known.

A recent report has shown that the presence of Qnr determinants may result in a lower selection of mutations in the genes encoding the quinolone targets (Cesaro et al., 2008). It has also been reported that *qnr*-positive isolates contained fewer QRDRs mutations and showed higher level of ciprofloxacin susceptibility, while *qnr*-negative isolates harbored more QRDRs mutations and displayed a high-level of ciprofloxacin resistance (Yang et al., 2011). Other studies showed that the prevalence of *qnr* genes in ciprofloxacin-susceptible isolates was higher than in ciprofloxacin-resistant isolates (Yang et al., 2010; Zhou et al., 2011). We summarized the reported data on PMQR in *Escherichia coli* from 32 published reports (Table S1), and found that of 197 *E. coli* carrying *qnr* alone, 39.1% showed resistance to ciprofloxacin, however, 92.9% of the *aac(6′)-Ib-cr*, 51.7% of the *oqxAB* and 92.5% of the *qepA*-positive *E. coli* isolates showed resistance to ciprofloxacin (Fig. 1). We hypothesize that different PMQR genes may have different role in selecting higher-level quinolone-resistant mutants due to their distinct mechanism of resistance.

To date, many epidemiological surveys have been conducted to identify the prevalence of PMQRs in Enterobacteriaceae strains from humans and animals, and their contribution on quinolones resistance. However, because of the difficulties of phenotypic detection of this type of resistance, its real prevalence is only partially known (Rodriguez-Martinez et al., 2011). Most of these studies have focused on isolates with specific resistance phenotypes, such as reduced susceptibility to fluoroquinolones or resistance to cephalosporins, or on isolates collected in a short period of time and by a limited number (Cattoir and Nordmann, 2009). In addition, the epidemiology of *oqxAB* and its association with quinolones resistance have not been well studied. Hence, it is difficult to compare the contribution of different PMQR determinants on fluoroquinolones resistance based on currently published data.

This study investigated the prevalence of different PMQR genes among randomly selected *E. coli* isolates (irrespective of the resistance background) from different sources, including humans, chicken and pork sold in markets, livestocks and domesticated pet animals, to assess whether significant differences in the distribution of these genes are present between isolates of different sources, and to determine the role of different PMQR determinants in ciprofloxacin resistance.

2. Materials and methods

2.1. Bacterial isolates

During September 2004 to July 2011, 2297 individual *E. coli* strains were obtained from food animals (503 pigs, 493 chickens and 389 ducks), companion animals (353), healthy volunteers (207), and retail meat (352). Of these, 1385 food animal isolates were recovered from liver or fecal samples of diseased or healthy animals from farms located in different geographic areas of China (including Guangdong, Anhui, Guangxi, Henan, Jiangsu, Sichuan, Fujian and Jiangxi provinces and Beijing), during 2004–2011; and 353 pet strains were randomly collected from feces, pus or sneeze samples from diseased or healthy companion animals in Guangdong province from 2007 to 2010. Strains of animal food origins were randomly

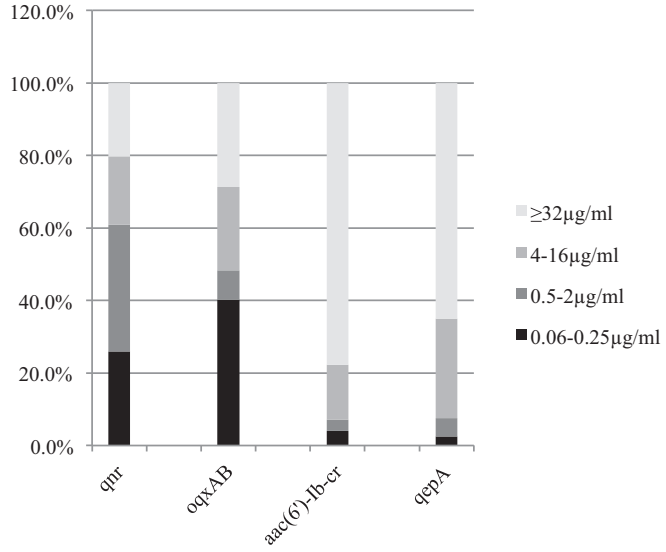

Fig. 1. Ciprofloxacin MIC distributions of PMQR-positive *E. coli* isolates reported in 32 published papers.