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Short communication

Genetic diversity in fluoroquinolone and macrolide-resistant *Campylobacter coli* from pigs

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Abstract

The genetic diversity of 115 *Campylobacter coli* strains, isolated from pigs of 59 geographical distant farms in Switzerland, were characterized on the basis of their DNA fingerprints and resistance to macrolides and fluoroquinolones. Sequence analysis showed that the macrolide-resistant isolates had a point mutation in the 23S ribosomal RNA (rRNA) genes (A2075G) and that the fluoroquinolone-resistant isolates had a point mutation in the gyrase gene *gyrA* (C257T). One fluoroquinolone-resistant strain had an additional transition mutation in the *gyrB* gene (A1471C). The *flaA* restriction fragment length polymorphism (RFLP) genotyping revealed that 57% of the isolates were genetically different. Point mutations in the 23S rRNA and *gyrA* genes could be found in both genetically distant and genetically related isolates. Additionally, isolates with and without point mutations were found within individual farms and on different farms. This study showed that the ciprofloxacin and erythromycin-resistant *C. coli* population present on the pig farms is not issued from a common ancestral clone, but individual *Campylobacter* strains have most likely mutated independently to acquire resistances under the selective pressure of an antibiotic.

Keywords: Campylobacter; Fluoroquinolones; Macrolides; Resistance; Genotyping; Pigs

1. Introduction

Campylobacter coli and *Campylobacter jejuni* are the two most common campylobacter species causing gastroenteritis in humans (Friedman et al., 2004). The disease is commonly associated with consumption of poultry meat or pork (Blaser, 1997; Duim et al., 2000) contaminated at slaughter. In Switzerland, more than 93% of the fecal samples of pigs were tested *Campylobacter* positive (Regula et al., 2003). In 2001, the annual incidence of campylobacter infections in humans in this country was 5% (Ledergerber et al., 2003).

Although the campylobacteriosis is usually self-limiting, some severe cases require antibiotic treatment, macrolides and fluoroquinolones being among the drugs of choice today (Engberg et al., 2001; Butzler, 2004; Moore et al., 2005; Yates, 2005). These

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antibiotics are also widely used for the treatment of infectious diseases in food-producing animals (Arnold et al., 2004). As a consequence, macrolide and fluoroquinolone-resistant *Campylobacter* have been selected for in animals (Perreten, 2005), and today they represent approximately 20% of all the campylobacters isolated from pigs in Switzerland (Schuppers et al., 2005).

In *Campylobacter*, the fluoroquinolone resistance is mainly caused by a single step point mutation in the gyrase gene *gyrA* (C257T) which results in threonine 86 being substituted with a isoleucine (Thr-86-to-Ile) (Engberg et al., 2001; Zhang et al., 2003; Beckmann et al., 2004), while others mutations appear less frequently (Thr-86-to-Lys, Thr-86-to-Ala, Asp-90-to-Asn, Asp-90-to-Tyr, Ala-70-to-Thr, Pro-104-to-Ser) (Wang et al., 1993; Zirnstein et al., 1999; Luo et al., 2003; Piddock et al., 2003; Beckmann et al., 2004). The major mechanism conferring resistance to macrolides consists of an alteration of the target site (A2075G) in the peptidyl transferase region of the 23S ribosomal RNA (rRNA) genes (Gibreel et al., 2005; Mamelli et al., 2005). Another mutation (A2074C) in the 23S rRNA genes was also associated to macrolide resistance (Vacher et al., 2003).

The genetic analysis of epidemiologically unrelated *C. coli* from pigs using *flaA* restriction fragment length polymorphism (RFLP) genotyping (Nachamkin et al., 1996; Wittwer et al., 2005) should demonstrate whether resistance to macrolides and fluoroquinolones occurred by point mutation individually or resulted from the spread of specific clones.

2. Materials and methods

2.1. Bacterial strains, growth conditions and antibiotic resistance testing

In 2001, 269 *C. coli* were isolated from pig feces in Switzerland (Regula et al., 2003). From this isolation campaign, we used for our study all the isolates displaying resistance to erythromycin ($n = 36$), to ciprofloxacin ($n = 38$) or to both erythromycin and ciprofloxacin ($n = 10$) and 31 susceptible strains. They originated from 59 geographically distant farms. *C. jejuni* NCTC 11351 (=ATCC 33560) and *C. coli* NCTC 11366 (=ATCC 33559) were used as control strains for susceptibility testing and sequence analysis. The strains were cultured on Mueller–Hinton agar containing 5% sheep blood under microaerobic conditions (N_2: 85%, O_2: 5%, CO_2: 10%) at 37 °C for 48 h.

The minimal inhibitory concentrations (MICs) of erythromycin and ciprofloxacin were determined in duplicate by agar dilution method according to the CLSI recommendations (Clinical and Laboratory Standards Institute, 2002). The resistance breakpoints were $\geq$8 μg/ml for erythromycin and $\geq$4 μg/ml for ciprofloxacin.

2.2. DNA extraction, PCR and sequencing analysis

Genomic DNA was extracted using Puregene® DNA Purification Kit (Gentra Systems, Inc., Minneapolis, MN). The 233-bp fragment covering the quinolone resistance-determining region of *gyrA* from *C. coli* was amplified by PCR using the forward primer gyrA-campy-F: 5′-GAGTGTTATTATAGGTCGTGC and the reverse primer gyrA-campy-R: 5′-GGCACTATCAC-CATCTATAG, and the 358-bp partial fragment of *gyrB* was amplified using primers gyrB-campy-F: 5′-TAATGGCAGCTAGAGGAAGA and gyrB-campy-R: 5′-ATGAGATCCATCAACATCCG as described previously (Bachoual et al., 2001). The mutations in the 23S rRNA genes were detected by the analysis of a 508-bp fragment as published previously (Vacher et al., 2003). Sequencing was achieved on a Hitachi 3100 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA) using dRhodamine-labeled terminators.

2.3. flaA restriction fragment length polymorphism (RFLP)

The isolates were characterized by *flaA*-RFLP according to the protocol described in Campynet (http://campynet.vetinst.dk/CONTENTS.HTM), but using primers described previously (Wassenaar et al., 1995). The gels were photographed with a Gel Doc 2000 System (Bio-Rad, Hercules, CA) and the pictures were imported into BioNumerics 3.0 (Applied Maths, Kortrijk, Belgium). The similarity matrix was calculated with Pearson correlation algorithm. The unweighted paired group method with arithmetic means (UPGMA) was used for the dendrogram construction.