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Case Report Open Access

Tetracycline Resistance of Chilean Campylobacter Jejuni Strain Bank from Humans, Cattle and Chickens

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Campylobacter spp. are a leading cause of foodborne illness around the world, and poultry products (especially chicken meat), are major sources of *Campylobacter* spp. infection in humans [1]. In recent years, both animal and human *Campylobacter jejuni* isolates have shown high to extremely high antibiotic resistance to several antibiotics such as tetracycline in several European countries [2]. Tetracycline resistance in *Campylobacter* spp. is primarily mediated by a ribosomal protection protein Tet (O) that binds to the bacterial ribosome and displaces tetracycline [3]. The *tet* O gene can be located on both plasmids and chromosomes and is associated with high levels of resistance to tetracycline in *C. jejuni* [4].

Little was known about tetracycline resistance in Chilean *Campylobacter* spp., therefore we investigated this resistance in 153 banked *C. jejuni* strains from humans, cattle, and chickens. Tetracycline resistance and the occurrence of the *tet* (O) gene were determined. The *C. jejuni* isolates (n = 153) were obtained from the strain collection at the Microbiology and Probiotics Laboratory of the Food Technology and Nutrition Institute, University of Chile. All 153 *C. jejuni* isolates were collected in the Metropolitan Region during five years (2006 to 2010). Among the isolates, 55 were from stool specimens of diarrheal patients, the remaining 54 strains were obtained from broiler chicken carcasses and 44 were obtained from cattle rectal swabs. These strains were replicated on selective Skirrow agar [5]. Strain identifications were confirmed by standard microbiological methods and 16S rDNA polymerase chain reaction, PCR [6]. The hippurate hydrolysis test was used for determination of the *C. jejuni* strains. All hippurate-positive isolates were determined as *C. jejuni*.

C. jejuni strains were tested for antimicrobial resistance against tetracycline by agar disk diffusion method and susceptibility categorization was carried out according to Gaudreau et al. [7]. The minimal inhibitory concentration (MIC) of tetracycline was determined using the broth microdilution susceptibility testing according to the Clinical and Laboratory Standards Institute (CLSI) [8]. MIC determination was applied to isolates with a zone diameter of more than 20 mm or of less than 26 mm (intermediate susceptibility), and less than 20mm (resistant).

DNA from 153 *C. jejuni* isolates was extracted by standard molecular biological techniques using the kit: Genomics DNA Purification (Bioingentech, Concepción, Chile). We used as an internal control the amplification of a segment of the 16S rRNA gene by PCR [6]. The presence of the *tet* (O) gene in all isolates was screened by a *tet* (O)-specific PCR [9,10]. For this purpose, primers *tet*(O)-F (5′-GGCGTTTTGTTTATGTGCG-3′) and *tet*(O)-R(5′-ATGGACAACCCGACAGAAGC-3′) were used to amplify a 559 bp region of the *tet*(O) gene as described elsewhere (9) using the following conditions: an initial denaturation at 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 57°C for 30 seconds, and 72°C for 1 minutes; and a final extension of 72°C for 10 minutes [10].

The results showed that *C. jejuni* have high rates of resistance to tetracycline (45/153, 29%, range: 16-256 µg/mL). Resistance to tetracycline was high but the rates varied according to their origin. The most frequently tetracycline resistant *C. jejuni* isolates were detected in chickens broiler 28/54 (51.9%), followed by human strains 14/55 (25.5%), and 3/44 (6.8%) cattle strains ($P<0.05$, Chi-square test). In the screening of tetracycline resistance gene, 37% of *C. jejuni* isolates (56/153) were positive for *tet* (O). The 98% (44/45) tetracycline-resistant isolates, based on phenotype, were also *tet* (O) positive. There was one *C. jejuni* strain from chicken that did not fit this description (i.e. strain PC18). Eleven C. *jejuni* strains were *tet* (O) positive and were found to be not resistant to tetracycline by disk diffusion and microbroth dilution. There was also a significant difference ($P<0.05$, Chi-square test) in the carrying of *tet* (O) gene among *C. jejuni* strains according to their origin, again the higher rates of *tet* (O) detection found in chicken strains (34/54, 63%) followed by human strains 17/55 (31%). Five of the 44 cattle strains were positive for *tet* (O) (7.3%).

A large geographical variation in the susceptibility patterns of *C. jejuni* to tetracycline has been observed in Europe (resistance ranges from 2% to 91 %) (2). In the South of Chile, low percentages (2 %) of tetracycline resistance in *C. jejuni* isolated from hens and human has been described [11,12]. In contrast, in the present study in Metropolitan region, 45 (29 %) out of 153 strains tested were resistant to this antibiotic. Based on the evidence mentioned above, we can conclude that in this geographical region *C. jejuni* tetracycline resistance is a problem as it is in other countries [2,3].

Caution should be taken in the exclusive use of this PCR method for *tet* (O) to evaluate tetracycline resistance in *C. jejuni*, because the strain collection included 11 *tet* (O) PCR positive *C. jejuni* strains that were phenotypically sensitive to tetracycline. On the other hand, these 11 strains could be tested to confirm the reliability of the disk diffusion, the potential resistance to Tetracycline needs to be confirmed in these strains using the agar dilution method.

Tetracycline resistance and *tet* (O) are widespread among *C. jejuni* from chicken broilers. It would be necessary to study the reasons for this finding, and more attention should be addressed to limit the antimicrobial resistance of chicken *C. jejuni* isolates.

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