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Short communication

Campylobacter jejuni strains isolated from chicken meat harbour several virulence factors and represent a potential risk to humans

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ABSTRACT

This study aimed to evaluate the virulence characteristics of 55 *Campylobacter jejuni* strains isolated from chicken carcasses. These characteristics included antibiotic resistance, the presence of virulence genes, and the transcription virulence genes, changes after the inoculation of Caco-2 cells and the phylogenetic relationship between strains. Resistance to amoxicillin and norfloxacin was observed in 34/55–61.8% and 26/55–47.3% respectively, and resistance to tetracycline was also observed (18/55–32.7%). The genes *flaA*, *pldA*, *cadF*, and *ciaB* and the CDT complex were detected in 41/55 (74.5%), 35/55 (63.6%), 37/55 (67.3%), 37/55 (67.3%) and 36/55 (65.5%) strains respectively, and transcripts for the *ciaB* and *dnaJ* genes evaluated in 46 strains were detected in 60.9%. In Caco-2 cells, loss of cell confluence was observed. Genetic heterogeneity among these strains was confirmed by RAPD-PCR. The data indicate the potential role of these *C. jejuni* strains in the pathogenesis of human diseases, emphasising the need for vigilance and strict control during production to protect the health of the consumer.

1. Introduction

Campylobacter jejuni is the most common cause of food-borne bacterial gastroenteritis in humans (EFSA – European Food Safety Authority 2009; Moore et al., 2005). Campylobacteriosis is a self-limiting disease in healthy adults, but in children, elderly individuals, and immunosuppressed individuals, it can become a severe illness requiring antibiotic therapy (Cox, 2002).

Poultry meat is the main disseminator of *C. jejuni*. The Panel on Biological Hazards presented by EFSA (2010) revealed a high prevalence of *Campylobacter* spp. in poultry meat, with alarming rates in Europe (of 10 carcasses, 8 were contaminated). In addition to testing for the presence of *C. jejuni* strains, virulence factor verification is a useful tool to assess the potential risk of chicken meat as a pathogen disseminator. This work evaluated five *C. jejuni* strains isolated from chicken carcasses to determine the levels of antimicrobial resistance, virulence, and damage to Caco-2 cells.

2. Methods

We used 55 *C. jejuni* strains derived from analyses of 420 chilled and frozen chicken carcasses samples for human consumption. The strains were isolated by the technique recommended by the ISO (ISO, 2006), and the species were identified by multiplex-PCR (Harmon, Ramsom, & Wesley, 1997). *C. jejuni* NCTC 11351 was used as a positive control. We evaluated *flaA*, *pldA*, *cadF*, and *ciaB* genes which are important genes for adherence, colonisation, and invasion (Zheng, Meng, Zhao, Singh, & Song, 2006), and the genes *cdtA*, *cdtB*, cdtC, which are related to the production of cytolethal distending toxin (CDT) (Martinez et al., 2006).

The antimicrobial susceptibility testing was performed by the disk diffusion method according to the protocol of the Clinical and Laboratory Standards Institute - CLSI (2010) for the following antimicrobials: amoxicillin (10 μg), erythromycin (15 μg), gentamicin (10 μg), neomycin (30 μg), norfloxacin (10 μg), sulphazotrim (25 μg), and tetracycline (30 μg) (Laborclin®).

Gene transcription was evaluated by rt-PCR (reverse transcriptase polymerase chain reaction). In this assay, the *ciaB* and *dnaJ* genes were used according to the method of Li, Ingmer, Madsen, and Bang (2008). The RNA was extracted and reverse transcribed. Then, we performed the PCR according to the method of Li et al. (2008) with some modifications.

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To examine morphological changes, were selected five strains that had more virulence characteristics. Caco-2 cells were seeded onto coverslips in 24-well plates at a density of 3.5×10^5 cells/ml and then incubated for 14 days. After that period, each coverslip covered with Caco-2 cells was infected with 2×10^6 CFU *C. jejuni* strain per well; infection with each strain was performed in triplicate, and negative and positive controls were run in parallel. The culture method was performed as 67 described by Fonseca et al. (2012). After 72 h, each coverslip was evaluated by computer analysis of digitised images obtained from an Olympus BX 40 microscope with a 100x objective coupled to an Olympus camera (Oly 200) and connected to a PC via the PC card scanner Data Translation 3153.

The genetic diversity among the isolates was determined by RAPD-PCR (*Random Amplification of Polymorphic DNA*) according to the method of Akopyanz, Bukanov, Westblom, Kresovich, and Berg (1992). The results from the RAPD-PCR were evaluated using the GelCompar II programme (*Comparative Analysis of Electrophoresis Patterns*), version 1.50, *Applied Maths Korthrijk, Belgium*. The similarity matrix was obtained by comparing pairs of strains using the Dice similarity coefficient, with a 1% tolerance for each primer separately. We used the UPGMA (*unweighted pair group method with arithmetic mean*) method for the construction of the dendrogram (Madden, Moran, & Scates, 2007).

3. Results and discussion

The PCR showed that the genes *flaA, pldA, cadF, ciaB,* and *cdtABC* were present in 41/55 (74.5%), 35/55 (63.6%), 37/55 (67.3%), 37/55 (67.3%) and 36/55 (65.5%) of the studied strains of *C. jejuni*, respectively (Fig. 1). A total of 46 (83.6%) of these strains had at least one gene and 20 (43.6%) had all studied genes. Similar results to these were also found by Thakur et al. (2010), Biswas, Hannon, Townsend, Potter, and Allan (2011), Rizal, Kumar, and Vidyarthi (2010) and Hanning, Biswas, Herrera, Roesler, and Ricke (2010) that studied the same species.

Virulence potential observed in strains may explain the fact that *C. jejuni* be much more common as a cause of human infections (90%–95%) (Thakur et al., 2010). Moreover, differences in the presence of these genes in *C. jejuni* suggests that not all strains from chickens are capable to cause humans diseases.

FlaA gene, responsible for flagellar motility of the bacteria, is essential for cell adhesion and invasion (Malik-Kale et al., 2007), but its absence indicates severe reduction in motility and intestinal mucosa colonization of humans and chickens (Konkel et al., 2004). *PldA* is related to the phospholipase synthesis outer membrane and, consequently, to cell invasion (Ziprin et al., 2001). *CadF* presence indicates colonization through interaction with the host extracellular matrix (Monteville, Yoon, & Konkel, 2003). Protein encoded by CIAB promotes microtubules destruction that potentiates invasion (Rivera-Amill, Kim, Seshu, & Konkel, 2001). The activity of the toxin CDT occurs by blocking the cell cycle, inducing cell death (Martinez et al., 2006).

Table 1
Antimicrobial resistance 55 strains isolated from chicken carcasses.

Antimicrobial	*C. jejuni* N/55 (%)
Amoxiline	34 (61.8)
Eritromicin	14 (25.5)
Gentamicin	2 (3.6)
Neomicin	0
Norfloxacin	26 (47.3)
Sulphazotrim	12 (21.8)
Tetraciclin	18 (32.7)

N-number of resistant strains. % – Percentage in relation to total isolates.

Several strains were resistant to amoxicillin and norfloxacin (34/55–61.8% and 26/55–47.3%), as well as tetracycline (18/55–32.7%). A total of 14/55 (25.5%) strains exhibited resistance to erythromycin. For the antibiotic neomycin, resistance was not observed (Table 1).

Due to the self-limiting nature of campylobacteriosis, antimicrobials are generally not recommended for treatment except in severe cases, for which fluoroquinolones and macrolides are the treatment options of choice (Yates, 2005). Nevertheless, resistance to norfloxacin (fluoroquinolone) (47.3%) was confirmed in this study (Table 1). Studies conducted in the United States, Poland and other EU countries also confirm this trend (EFSA 2010; Gupta et al., 2004; NARMS 2007; Rozynek et al., 2008). In Brazil, amoxicillin and enrofloxacin are routinely used to treat poultry flocks, and this practice may favour the selection of resistant strains (Borges, 2009). The low levels of resistance to aminoglycosides (gentamicin and neomycin) can be explained by the fact that these antibiotics are not routinely used in chicken production, which reduces the selection pressure (Borges, 2009).

The rt-PCR assay (Fig. 2) and the inoculation of five strains into Caco-2 cells demonstrated that the important virulence-related genes are transcribed into mRNA and that these strains can damage intestinal epithelial cells, respectively (Fig. 3).

The presence of virulence transcripts were made in strains that had at least one of the genes studied (46 strains). There were virulence transcripts in a total of 28/46 (60.9%). Of these, 8/28 (28.6%) with *ciaB* transcripts, 2/28 (7.1%) *dnaJ* and 18/28 (64.3%) for both genes.

The transcription of *ciaB* is involved in the invasion of epithelial cells, and *C. jejuni* requires the *ciaB* protein for efficient

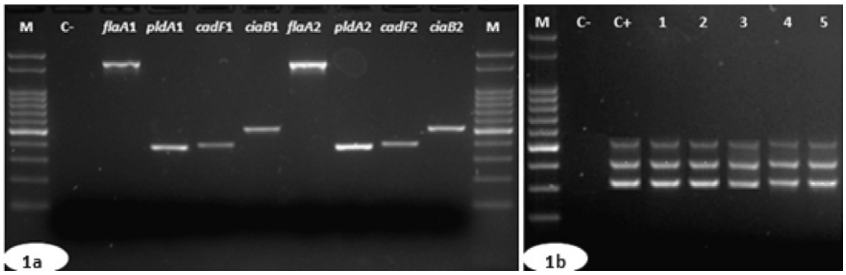

Fig. 1. PCR amplification of the *flaA, pldA, cadF,* and *ciaB* genes (1a) and the CDT complex (1b) from *Campylobacter jejuni* strains isolated from chicken carcasses. M (molecular weight marker, 100 bp), C- (negative control); *flaA*1, *pldA*1, *cadF*1, and *ciaB*1 (positive control, *C. jejuni* NCTC 11351) and *flaA2, pldA2, cadF*2, and *ciaB*2 (one *C. jejuni* strain isolated from a chicken carcass); C+ (positive control, *C. jejuni* NCTC 11351 positive for the CDT complex); 1–5 (*C. jejuni* strains isolated from chickens and positive for the CDT complex).