

Research Paper

Characterization of the virulence, growth temperature and antibiotic resistance of the *Campylobacter jejuni* IAL 2383 strain isolated from humans

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Abstract

The objective of this study was to characterize the *C. jejuni* IAL2383 strain isolated from humans in Brazil. Transcripts for the *racR*, *dnaJ* and *ciaB* genes were found and *flaA*, *plda* and *cadF* genes were present in the genome and bacteria was sensitive to most of the important antimicrobials used to treat humans. *C. jejuni* IAL2383 is a good experimental model to analyze the interactions with cells.

Key words: virulence, experimental model, thermotolerance, *Campylobacter jejuni*.

Introduction

The diversity of *Campylobacter jejuni* reflects this bacterium's ability to colonize and cause disease. Variation in the virulence factors of different strains has been observed in *in vitro* and *in vivo* models, demonstrating different strategies and consequent risks. An understanding of the molecular basis of the virulence patterns of the strains associated with disease and knowledge of the genetic diversity among strains are of paramount importance in disease control (Ahmed *et al.*, 2002).

The *C. jejuni*-host cell interaction mechanisms are not well understood, and there is great disagreement among researchers. Some of the conflicting results may be explained by differences between strains, which have been reported by some authors (Deun *et al.*, 2008; Li *et al.*, 2008). The *C. jejuni* IAL2383 strain was isolated from humans with clinical symptoms in Brazil and is maintained by the Instituto Adolfo Lutz strains. This strain appears to be ideal for understanding the pathogen-host interactions because this strain causes symptoms in humans. However, the virulence factors, degree of thermotolerance and resistance to antibiotics of this strain have not been analyzed. The aim of this study was to characterize the IAL2383 strain based on the presence of certain genes, the expression of transcripts for the major *C. jejuni* virulence factors, the growth temperature and resistance to antibiotics.

Materials and Methods

This study used the *C. jejuni* strain IAL 2383, which was isolated from a human outbreak and is maintained at the Bank of the Instituto Adolfo Lutz, São Paulo (IAL). All experiments were performed with bacteria from the first expansion after receipt from the Bank.

The antibiotic resistance of the IAL2383 strain was analyzed by the Kirby-Bauer disk diffusion method (Bauer *et al.*, 1966) using the protocol recommended by the National Committee for Clinical Laboratory Standards. The following antibiotics were tested: trimethoprim (5 µg), cefoxitin (30 mg), ceftiofur (30 mg), neomycin (30 mg), chloramphenicol (30 mg), trimethoprim-sulfamethoxazole (23 mg), amoxicillin (10 mg), tetracycline (30 mg), gentamicin (10 mg), nalidixic acid (30 mg), norfloxacin (10 mg), enrofloxacin (5 mg), erythromycin (15 mg), and sulfazotrim (300 mg). The assays were performed in triplicate on Mueller Hinton agar (Difco) containing 5% horse blood hemolysate. The plates were incubated at a temperature of 37 °C for 48 h in a microaerophilic atmosphere (Probac Brazil).

To determine the optimum temperature for the growth of *C. jejuni* IAL 2383. Nine serial dilutions were performed. Aliquots of 0.1 mL of the 10⁶ to 10⁹ dilutions were removed and placed on the surface of CCDA agar (Oxoid) and spread using a Drigalski spatula. Each dilution

was streaked in triplicate on plates that were then incubated at temperatures of 37 and 41 °C in parallel. After 30 h of incubation in a microaerophilic atmosphere, the bacteria were quantified.

Primers described in the literature were used to determine if strain IAL2383 harbors the virulence genes *cadF*, *pldA* (Zheng *et al.*, 2006) and *flaA* (Hänel *et al.*, 2004). *C. jejuni* NCTC 11351 was used as a positive control strain. The extraction of DNA was performed described by Guo *et al.* (2000) and Borsoi *et al.* (2009) with modifications. The final amplification reaction was composed of 20 ng of bacterial DNA and others reagent (Invitrogen) to PCR. The microtubes were placed in a thermocycler (Eppendorf) and subjected to annealing temperature at 45 °C. At the end of the reaction, the amplified products were subjected to electrophoresis and visualized with a UV transilluminator (L.PIX Loccus Biotechnology).

Assays were performed to verify that the virulence genes *ciaB*, *dnaJ* and *racR* were expressed in *C. jejuni* IAL2383 (Li *et al.*, 2008). To do this, we performed reverse transcriptase (RT-PCR) and then conventional PCR. We conducted a test with 3 types of bacteria: *C. jejuni* IAL2383; *C. coli* isolated from chicken (expression of virulence factors no know), and a virulent strain of *C. jejuni* NCTC 11351 (positive control). All strains were incubated at a temperature of 41 °C. In addition, to determine the change in the levels of transcripts in the presence of eukaryotic cells, Caco-2 cells (derived from a human colon adenocarcinoma) infected with 2.0×10^7 CFU *C. jejuni* IAL 2383 were used. RNA was extracted using the Trizol Reagent® method (Invitrogen). After RNA extraction, reverse transcription was used to synthesize the cDNA using RNase inhibitor (Invitrogen), MMLV-RT (Amersham Biosciences), MMLV-RT buffer (Amersham Biosciences), 200 mM dNTPs and 126 pmol of random hexamers as primers at 37 °C for 1 hour. Control reactions were used to verify the presence of exogenous contaminants. The final volume of the amplification reaction included 3 µL of the cDNA and PCR reagents (Invitrogen). The microtubes were placed in a thermocycler (Eppendorf) and submitted to annealing temperature 51 °C. At the end of the reaction, the amplified products were subjected to electrophoresis on agarose gels as performed for the presence of genes in the bacterial genome as oversized above.

Results and Discussion

Our results indicate that the *C. jejuni* IAL 2383 strain is susceptible to most antibiotics tested, the exceptions were trimethoprim (5 µg) and cefoxitin (30 mg). Many strains of *C. jejuni* exhibit multidrug resistance to antimicrobial agents (Jonker and Picard, 1999; Chen *et al.*, 2011), and the resistance of pathogenic Enterobacteriaceae to fluoroquinolones and third-generation cephalosporins rep-

resents a major problem because of the possibility of generating superbugs (Pignatari, 2011).

Regarding the various groups of antibiotics, the resistance of *Campylobacter* to macrolides, fluoroquinolones, beta-lactam, trimethoprim and sulphonamides has been high in several studies, while resistance to other antimicrobials agents including tetracycline, aminoglycoside and chloramphenicol is generally low (Aarestrup and Engberg, 2001; Yan *et al.*, 2005). The sensitivity of this strain shows that it can be used as a negative control strain for resistance to most classes of antimicrobials or for the testing of the acquisition of resistance.

The bacterium grew better at 41 °C, and the number of colonies could be counted only after diluting by 10^8 or more. At the temperature of 37 °C, the average growth was 8×10^7 cfu (SD = 0.060), lower than the average of 2×10^8 (SD = 0.242) at 41 °C.

C. jejuni IAL 2383 was found to harbor the virulence genes *cadF*, *pldA* and *flaA* (Figure 1). These genes, in addition to *ciaB*, are used to study the pathogenic mechanisms of *C. jejuni* (Hänel *et al.*, 2004; Zheng *et al.*, 2006). The RT-PCR results showed that the bacterium expressed transcripts for the *ciaB*, *dnaJ* and *racR* proteins even in the absence of contact with eukaryotic cells (Figure 2).

Microorganisms with an optimal growth temperature near the body temperature of the birds, which varies between 40 °C and 42 °C (18), including the strain studied herein, have the greatest ability to survive in and colonize the bodies of animals. Coupled with the growth temperature, the presence of transcripts of the *racR* and *dnaJ* genes indicates that the strain used in this work can colonize the intestinal tracts of poultry. Therefore, this strain could be useful as a model to assess the pathogenesis of *C. jejuni* in these animals because *racR* is a component of the *racR* and RACS (reduced Ability to colonize) regulation system and is involved in the ability of these bacteria to colonize the intestinal tracts of birds (Bras *et al.*, 1999). The *dnaJ* gene encodes a heat shock protein that allows the bacteria to grow at temperatures greater than 40 °C (Konkel *et al.*, 1998).

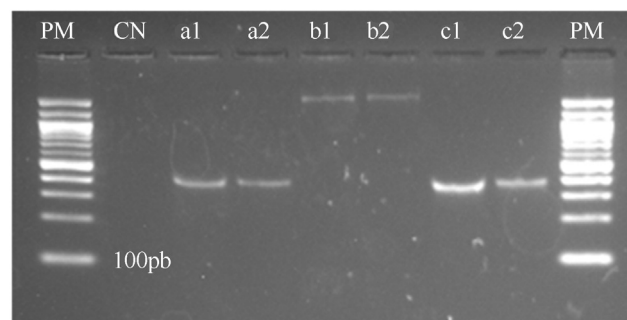


Figure 1 - PCR to presence of genes *cadF*, *flaA* and *pldA* to *C. jejuni* IAL 2383. PM = 100-bp molecular weight marker. CN: negative control, a.1: gene *cadF* *C. jejuni* NCTC 11351, a.2: gene *cadF* *C. jejuni* IAL 2383, b.1: gene *flaA* *C. jejuni* NCTC 11351, b.2: gene *flaA* *C. jejuni* IAL 2383, c.1: gene *pldA* *C. jejuni* NCTC 11351, c.2: gene *pldA* *C. jejuni* IAL 2383.

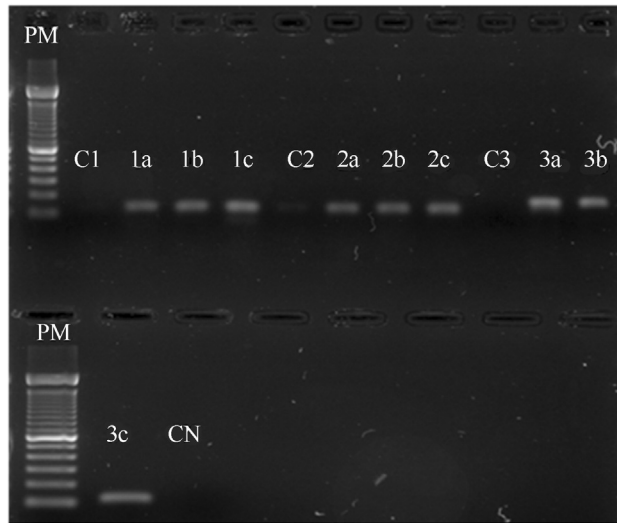


Figure 2 - RT-PCR to presence of transcripts genes *dnaJ*(1), *racR*(2) *ciaB*(3). PM = 100-bp molecular weight marker. 1 (*dnaJ*) C1 = *C. coli* chicken isolated 1a = *C. jejuni* IAL 2383, 1b = Caco-2 cells with *C. jejuni* IAL2383, 1c = *C. jejuni* NCTC 11351. 2 (*racR*) C2 *C. coli* chicken isolated, 2a = *C. jejuni* IAL 2383, 2b = Caco-2 cells with *C. jejuni* IAL 2383, 2c = *C. jejuni* NCTC 11351. 3 (*ciaB*) C3 = *C. coli* chicken isolated, 3a = *C. jejuni* IAL 2383, 3b = Caco-2 cells with *C. jejuni* IAL 2383, 3c = *C. jejuni* NCTC 11351, CN: negative control.

The presence of the *ciaB* transcripts in this strain is important because the secretion of the corresponding protein is of great importance for the invasion of epithelial cells and the colonization of the intestines of birds (Konkel *et al.*, 1999; Ziprin *et al.*, 2001). In previous studies, we found that the strain causes early embryonic mortality in chicken embryos (Fonseca *et al.*, 2011), changes the size of enterocytes in chicken embryos (Santos *et al.*, 2011) and induces the production of inflammatory cytokines in ileum fragments of SPF (specific pathogen free) birds and the production of inflammatory cytokines and chemokines in Caco-2 cells by activating the innate immune system (data not shown). This strain also alters the size of ileum fragments in SPF birds and Caco-2 cells (data not shown).

The presence of these virulence genes reflects the adhesion, invasion and internalization abilities of strain IAL2383. The *flaA* gene is necessary for the adhesion to and invasion of epithelial cells. The *cadF* gene encodes a protein that interacts with fibronectin in the extracellular matrix of the host, thus participating in colonization (Monteville *et al.*, 2003). The *pldA* gene is related to cell invasion and encodes a protein involved in the synthesis of an outer membrane phospholipase (Ziprin *et al.*, 2001).

C. jejuni IAL2383 strain does not show resistance to the primary antibiotics used to treat humans and animals infected by enterobacteria. This strain harbors virulence genes and produces transcripts from the major virulence genes. Because of these characteristics, this strain is a useful model for the *in vitro* analysis of the acquisition of resis-

tance to antibiotics and the pathogenicity in chickens and humans.

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