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High survival rates of *Campylobacter coli* under different stress conditions suggest that more rigorous food control measures might be needed in Brazil

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ABSTRACT

Campylobacter spp. have been the most commonly reported gastrointestinal bacterial pathogen in many countries. Consumption of improperly prepared poultry meat has been the main transmission route of *Campylobacter* spp. Although Brazil is the largest exporter of poultry meat in the world, campylobacteriosis has been a neglected disease in the country. The aim of this study was to characterize 50 *Campylobacter coli* strains isolated from different sources in Brazil regarding the frequency of 16 virulence genes and their survival capability under five different stress conditions. All strains studied presented the *cadF, flaA*, and *sodB* genes that are considered essential for colonization. All strains grew at 4 °C and 37 °C after 24 h. High survival rates were observed when the strains were incubated in BHI with 7.5% NaCl and exposed to acid and oxidative stress. In conclusion, the pathogenic potential of the strains studied was reinforced by the presence of several important virulence genes and by the high growth and survival rates of the majority of those strains under different stress conditions. The results enabled a better understanding of strains circulating in Brazil and suggest that more rigorous control measures may be needed, given the importance of contaminated food as vehicles for *Campylobacter coli*.

1. Introduction

Campylobacter spp. are Gram-negative microaerobic pathogens that have been the most commonly reported gastrointestinal bacterial pathogens in many countries. In 2014, the number of confirmed cases of human campylobacteriosis reported was 236,851 in the Europe Union, while the number of salmonellosis cases was 88,715 (EFSA, 2015). In the United States, the Foodborne Diseases Active Surveillance Network (FoodNet) indicated that *Campylobacter* was the most reported cause of bacterial foodborne illness in 2016 and it was estimated that 1.3 million human illnesses occur every year (CDC, 2017).

Clinical symptoms of campylobacteriosis can range from mild diarrhea to severe, watery to bloody diarrhea, fever and vomiting and can lead to serious medical sequelae, such as Guillain-Barré syndrome, a debilitating and sometimes fatal paralysis (Nachamkin, 2002; Cameron et al., 2012; Xia et al., 2013). The most important potential transmission routes of *Campylobacter* spp., specifically *Campylobacter coli* and *Campylobacter jejuni*, to humans have been considered to be consumption and handling of improperly prepared poultry meat, activities related to recreational waters, contact with farm animals or pets and consumption of unpasteurized milk (Man, 2011; Silva et al., 2011).

Although Brazil is the largest exporter of poultry meat in the world (ABPA, 2016), campylobacteriosis is a neglected disease and there is insufficient data to estimate the incidence of this pathogen in the country. Furthermore, the research conducted in this country was mostly focused in the occurrence and antimicrobial resistance of *C. coli* and *C. jejuni* (Gomes et al., 2006; Freitas and Noronha, 2007; Biasi et al., 2011) and there is a paucity of studies on molecularly characterized *Campylobacter* spp. strains (Aquino et al., 2002; Scarcelli et al., 2005; Gomes et al., 2016; Frazão et al., 2017).

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Despite the high incidence of *Campylobacter* around the world, the microbial factors that lead to the gut colonization and pathogenesis of this species have not been thoroughly characterized to date (Young et al., 2007; Man, 2011; Gao et al., 2017). In general, *Campylobacter* cells present in contaminated food are ingested and reach the stomach, the intestinal tract, and afterwards, the feces, allowing their transmission to a new host (Ketley, 1997; Man, 2011). During the stressful course of infection, *Campylobacter* encounters and must adapt to life-threatening environmental conditions, such as temperature variation, the high osmolality of food products in which preservatives such as sodium chloride (NaCl) dehydrate foodborne microorganisms, the acidic pH of the stomach, reactive oxygen compounds, and changes in nutrient availability, among others (Fordtran and Locklear, 1966; Stintzi et al., 2005; Cameron et al., 2012).

In addition, factors involved in the colonization and pathogenesis of *Campylobacter* include flagella expression, the ability to adhere and invade host cells, intracellular survival and toxin production, which have been associated with the expression of several virulence genes, including *flaA* and *flhA*, which are necessary for motility; *docA, cadF, dnaJ* and *racR*, which are responsible for adherence and colonization; *virB11*, responsible for adherence and invasion; *iamA*, *ciaB* and *pldA,* responsible for invasion and survival in host cells; *cdtA*, *cdtB* and *cdtC,* responsible for toxin production and having additional roles in adherence and invasion. The *sodB* and *csrA* genes provide protection against oxidative stress and the *wlaN* gene was considered to be related to the Guillain–Barré syndrome (Konkel et al., 1999; Hickey et al., 2000; Datta et al., 2003; Rivera-Amill et al., 2001; Wassenaar et al., 2002; Muller et al., 2006; Fields and Thompson, 2008; Biswas et al., 2011; Acik et al., 2013; Klancnik et al., 2015).

Despite the importance of assays that mimic the food processing stages, transmission of the pathogen from the environment and/or food to a human host and the survival of *Campylobacter* spp. during the course of infections in humans, few studies have been conducted with *C. coli*, as the majority of studies are performed with *C. jejuni* (Young et al., 2007; Reid et al., 2008; Birk et al., 2012; Kumar-Phillips et al., 2013). However, the role of *C. coli* in human disease has been increasingly recognized, as *C. coli* accounts for 1–25% of all *Campylobacter* related diarrheal diseases and have been reported to cause bacteremia, sepsis, and meningitis, among other complications (Blaser et al., 1986; Selander et al., 1993; Gillespie et al., 2002; Roux et al., 2013).

Therefore, phenotypic and molecular studies that can help to elucidate the pathogenesis and can provide a better characterization of the *Campylobacter coli* strains isolated in Brazil are of the utmost importance, because there is a paucity of data on this globally important pathogen and the importance of this country as the largest poultry meat exporter (Gomes et al., 2016; ABPA, 2016).

The aims of this study were to assess the frequency of several virulence genes and the survival of *Campylobacter coli* isolates from different sources in Brazil under different stress conditions.

2. Materials and methods

2.1. Bacterial strains

A total of 50 *C. coli* strains were studied. Those strains were isolated from human feces (12 strains), animals (15 strains), the environment (15 strains) and chicken meat (8 strains) from some cities in the Rio de Janeiro, São Paulo and Minas Gerais States in the southeast region of Brazil between 1995 and 2011. These strains were selected from the collections of the *Campylobacter* Reference Laboratory of the Oswald Cruz Institute of Rio de Janeiro (FIOCRUZ) and of the Adolfo Lutz Institute of Ribeirao Preto in Brazil (IAL). The strains were systematically chosen to represent isolates from sporadic cases of the two collections of the reference laboratories mentioned above, which occurred during different years (Table 1).

Moreover, the *C. coli* strains studied were chosen considering previous results from our research group, in which 63 strains were typed by *Pulsed-field gel electrophoresis* (PFGE), sequencing of the small variable region (SVR) of the *flaA* gene and high-resolution melting analysis (HRMA) of the CRISPR *locus* (Gomes et al., 2016). Those methodologies provided information for selecting 50 *C. coli* strains for this study that were the most genetically distinct as possible. Table 1 lists the year, source and State of isolation of the 50 *C. coli* strains studied. *Salmonella* Typhimurium ATCC 14028 and *Campylobacter jejuni* ATCC 33291 were used as controls in all experiments.

Table 1
Source, State and year of isolation of 50 *Campylobacter coli* strains studied isolated from humans (12), animals (15), the environment (15) and food (8) in Brazil.

Strains	Source[b]	State[a]	Year
CCAMP 771	Sewage	RJ	1995
CCAMP 840	Sewage	RJ	1995
CCAMP 821	Monkey	RJ	1995
CCAMP 820	Monkey	RJ	1995
CCAMP 787	Sewage	RJ	1996
CCAMP 819	Sewage	RJ	1996
CCAMP 765	Sewage	RJ	1996
CCAMP 767	Sewage	RJ	1996
CCAMP 761	Sewage	RJ	1996
CCAMP 764	Sewage	RJ	1996
CCAMP 791	Monkey	RJ	1997
CCAMP 818	Sewage	RJ	1997
CCAMP 775	Sewage	RJ	1997
CCAMP 495	Human	RJ	1998
CCAMP 494	Human	RJ	1998
CCAMP 490	Human	RJ	1998
CCAMP 841	Monkey	RJ	1998
CCAMP 502	Human	RJ	1999
CCAMP 498	Human	RJ	1999
CCAMP 975	Monkey	RJ	1999
CCAMP 726	Monkey	RJ	2000
CCAMP 503	Human	RJ	2000
CCAMP 834	Water	RJ	2000
CCAMP 595	Human	RJ	2001
CCAMP 667	Monkey	RJ	2002
Cc 01	Human	SP	2002
Cc 10	Human	SP	2003
Cc 04	Human	SP	2003
CCAMP 182	Monkey	RJ	2003
CCAMP 165	Monkey	RJ	2003
Cc03	Human	SP	2003
Cc05	Human	SP	2003
CCAMP 446	Monkey	RJ	2004
CCAMP 463	Potable Water	MG	2004
CCAMP 469	Potable Water	MG	2004
CCAMP 464	Potable Water	MG	2004
CCAMP 394	Monkey	RJ	2004
CCAMP 769	Sewage	MG	2004
CCAMP 392	Monkey	RJ	2007
CCAMP 1010	Monkey	RJ	2007
CCAMP 1000	Monkey	RJ	2007
CCAMP 1117	Monkey	RJ	2009
CCAMP 1062	Chicken wing	RJ	2010
CCAMP 1067	Chicken liver	RJ	2010
CCAMP 1063	Chicken gizzard	RJ	2010
CCAMP 1064	Chicken wing	RJ	2010
CCAMP 1066	Chicken liver	RJ	2010
CCAMP 1071	Chicken wing	RJ	2011
CCAMP 1075	Chicken liver	RJ	2011
CCAMP 1073	Chicken wing	RJ	2011

[a] MG, Minas Gerais; RJ, Rio de Janeiro; SP, São Paulo.
[b] Human-diarrheal stools; Monkey-non-diarrheal stools.