Survey of *Campylobacter* in foods implicated in foodborne diseases in Southern Brazil

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ABSTRACT

Campylobacter is not routinely tested in foodborne disease investigations in Brazil. Here, we studied the occurrence of *Campylobacter* among other food-related bacteria commonly found in foodborne disease outbreaks reported in Rio Grande do Sul State, Southern Brazil. Seventy-two food samples were analyzed by using culture-based detection methods during the 18-month investigation of 36 foodborne disease outbreaks. The sampled foods from the foodborne disease outbreaks were all negative for *Campylobacter*. However, at least one of other routinely investigated foodborne-related bacteria was detected in 29.17% (21/72) of the samples. Taken together, these results suggest the need to monitor *Campylobacter* in foodborne diseases to detect sporadic cases caused by *Campylobacter* that might go unnoticed in Rio Grande do Sul.

KEYWORDS: *Campylobacter*. Food safety. Outbreak.

INTRODUCTION

Campylobacter is the most prevalent pathogen in foodborne gastroenteritis infections in developed countries[1,2]. *Campylobacter jejuni* is mostly associated with human infections and is more frequent in laboratory-confirmed cases, followed by *C. coli* and *C. lari*[1]. Epidemiological studies have demonstrated that campylobacteriosis primarily results from the consumption or mishandling of contaminated food of animal origin, especially from chicken[1,3].

In Brazil, *Campylobacter* has been reported in chicken meat and other foods of animal origin[3,4], as well as in human feces[4]. However, only eight foodborne disease outbreaks involving *Campylobacter* spp. were reported to the Brazilian Ministry of Health among the approximately 14,000 outbreaks occurring in the period 2000 to 2019[5]. From 2009 to 2018, 6,903 foodborne disease outbreaks were reported in Brazil, and *Escherichia coli* (24.0%), *Salmonella* spp. (11.2%), and *Staphylococcus aureus* (9.5%) were the most frequently identified microorganisms in laboratory-confirmed cases[6]. The Central Public Health Laboratories (LACENs) network is structured to provide, among other attributes, the laboratory analyses to support the Brazilian epidemiological surveillance system for foodborne diseases. However, *Campylobacter* is not routinely tested in foodborne disease investigations. Hence, the incidence of foodborne campylobacteriosis may be underestimated in Brazil. This study aimed to analyze the occurrence of *Campylobacter* in foods implicated in foodborne diseases reported in Rio Grande do Sul State, Southern Brazil.

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MATERIALS AND METHODS

Food samples

In the period from March 2015 to September 2016, the LACEN-RS (Porto Alegre, Rio Grande do Sul State) provided 72 subsamples of foodborne disease-associated foods for *Campylobacter* analysis. Samples were of dairy products; sausages, eggs, meals prepared with eggs, chicken, pork, beef and fish, and foods of other origins. Samples were collected by the municipal health surveillance services as part of the investigation of 36 foodborne disease outbreaks to determine their etiological agents. These outbreaks comprised cases of a similar disease with similar incubation period distribution, deriving from common foods sources, and patients presented with at least one of the following symptoms: nausea; vomiting; stomach cramps; diarrhea and headache. The foods came from nine of the 19 Regional Health Coordinating Units (CRS) in Rio Grande do Sul, covering different municipalities (Figure 1). Samples were transported chilled to the laboratory and analyzed within 24 h. A descriptive analysis was carried out to determine the relationship between the categories of foods analyzed, the bacteria identified and the CRS of origin.

Routine bacteriological analysis

Foods were submitted to conventional bacteriological analyses at LACEN-RS to detect pathogenic (*Salmonella* spp., coagulase-positive staphylococci, *Listeria monocytogenes*, *Bacillus cereus* and sulphite-reducing *Clostridium* at 46 °C) and non-pathogenic (*Escherichia coli*) bacteria[7]. Samples were tested according to the clinical presentation and food microbiological criteria established in Brazil[8].

Qualitative analysis of thermotolerant *Campylobacter*

Subsamples of either cooked or raw foods were subjected to *Campylobacter* qualitative analysis using a protocol applicable to products for human consumption[9]. Briefly, the samples were homogenized in Bolton broth (Fluka, Darmstadt, Hesse, Germany) and incubated in a microaerobic atmosphere (5% O_2, 10% CO_2, with balance in N_2) at 37 °C for 4 h, followed by incubation at 41.5 °C ($\pm$ 1 °C) for 44 h ($\pm$ 4 h). Enriched samples were plated on modified Charcoal Cefoperazone Deoxycholate agar (mCCD, Fluka, Darmstadt, Hesse, Germany) and on Preston agar (Fluka, Darmstadt, Hesse, Germany), and incubated in microaerobic atmosphere at 41.5 °C ($\pm$ 1 °C) for 44 h ($\pm$ 4 h). Typical or putative *Campylobacter* colonies were subcultured on blood agar base n°. 2 (Fluka, Darmstadt, Hesse, Germany) to be further analyzed by Gram staining, motility, microaerobic growth at 25 °C, aerobic growth at 41.5 °C and oxidase. *C. jejuni* (BRM 34342) and *C. coli* (BRM 34343) from the microbial culture collection (CMISEA) of Embrapa (Empresa Brasileira de Pesquisa Agropecuaria) Swine and Poultry were used as controls strains.

RESULTS AND DISCUSSION

In the period studied, the most common analyzed food samples were from the category of beef meals (14/72, 19.44%), followed by fish meals (11/72, 15.28%) and sausages (11/72, 15.28%). Samples were primarily collected by the 2nd CRS (27/72, 37.50%) and the 5th

Figure 1 - Provenance (number of samples/total analyzed number) of the foods according to the Regional Health Coordinating Unit (CRS) in Rio Grande do Sul, Southern Brazil. 1: 1st CRS Porto Alegre (6/72); 2: 2nd CRS Porto Alegre (27/72); 4: 4th CRS Santa Maria (1/72); 5: 5th CRS Caxias do Sul (23/72); 6: 6th CRS Passo Fundo (7/72); 9: 9th CRS Cruz Alta (1/72); 11: 11th CRS Erechim (4/72); 13: 13th CRS Santa Cruz do Sul (2/72); 16: 16th CRS Lajeado (1/72).

CRS (23/72, 31.94%). Contrary to the known high rates of *Campylobacter* in chicken and poultry samples[3,4] in Brazil, thermotolerant *Campylobacter* species were not detected in the foods analyzed in this study. Differences in campylobacteriosis incidence vary among countries or regions within the same country due to the sensitivity of laboratory procedures, population or surveillance bias[10].

Notably, campylobacteriosis generally occurs as sporadic cases[1]. Sporadic campylobacteriosis might be part of the underreported foodborne diseases in Brazil as they are seldom identified. In the USA, *Campylobacter* accounts for the highest incidence of bacterial foodborne infections per 100,000 inhabitants[2]. However, when considering bacterial foodborne disease outbreaks, it is the third most common (4%), after *Salmonella* spp. (19%) and *C. perfringens* (6%)[11]. The campylobacteriosis foodborne outbreaks in Brazil occurred in 2001, 2003, 2005, 2014, 2016 and 2019 and involved fruits, desserts, water, mixed foods, milk, dairy products and poultry meat. In total 299 people fell ill[5].

On the other hand, the competing microbiota could have hindered the detection of low levels of *Campylobacter* in the foods, since a relatively low dose (500 CFU) is able to cause intestinal colonization and trigger acute enteritis[12]. *Campylobacter*-negative samples may reflect the effect of several intrinsic and extrinsic factors to which the foods were exposed to. For example, *Campylobacter* cells can suffer sublethal injuries caused by oxidative stress[13], acidic conditions[14], low temperatures[15] or excessive use of disinfectants and preservative additives[16], which might trigger the viable but non-culturable (VBNC) state. It has been hypothesized that VBNC *Campylobacter* cells may proliferate in the human intestine and cause infection[17-19]. Despite the presence of VBNC forms that were not evaluated in this study and the lack of evidence that their presence in foods could trigger campylobacteriosis in consumers, the public health risk should not be ignored.

At least one of the other pathogenic and non-pathogenic bacteria tested was detected in 29.17% (21/72) of the analyzed samples (Figure 2), involved in 17 of the 36 investigated outbreaks. Five samples presented contamination by more than one bacterial agent (data not shown). Among the microorganisms that are not normally harmful but may be used as an indirect indicator of health hazard in food, non-pathogenic *E. coli* was detected in 20.83% of the analyzed samples (15/72, Figure 2). *E. coli* was the second most frequently reported microorganism in foodborne disease outbreak investigations in Brazil from 2000 to 2019[5]. Such a number includes non-pathogenic *E. coli* and Shiga toxin-producing *E. coli*[5]. Most of the samples evaluated in this study (51/72, 70.83%) were negative for the routinely tested bacteria. Other foodborne microorganisms, such as zoonotic viruses, may be related to outbreak-associated foodborne diseases, which, however, were not focused on in this study. Similar frequency of food-related bacteria has been reported in foodborne disease outbreaks between 2009 and 2018 in Brazil, whose etiological agent was laboratory-confirmed in approximately 30% of the cases[5]. Despite the number of cases in which the involved food was not identified, the closure of foodborne disease outbreaks investigations has been mostly achieved based on epidemiological criteria without considering the laboratory result[20]. Moreover, the attribution of outbreaks to any of the agents detected during this study depends on the establishment of a clear epidemiological relationship.

In conclusion, the foods analyzed in the studied period tested negative for thermotolerant *Campylobacter*. Nevertheless, other bacteria were laboratory-confirmed in 29.17% of the samples. Taken together, the results suggest the need to monitor *Campylobacter* in foodborne diseases to detect sporadic Campylobacter-associated cases that might go unnoticed in Rio Grande do Sul.
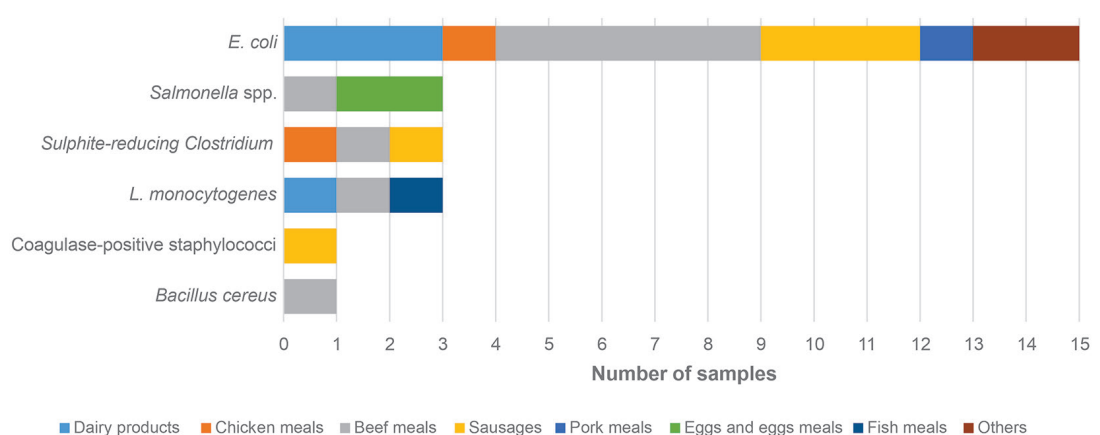

Figure 2 - Distribution of the pathogenic and non-pathogenic bacteria identified in the foods involved in foodborne disease outbreaks in Rio Grande do Sul, Southern Brazil, in the period 2015-2016.

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AUTHORS' CONTRIBUTIONS

MCB, CSLV and AC conceptualized the study design; MCB, SH, RCR, RR, RR and SCD carried out formal analysis and investigation; MCB, DVR and CSLV prepared the original draft; MCB, SH, RCR, RR, DVR, RR, SCD, AC and CSLV reviewed and edited the manuscript; MCB supervisioned the work.

CONFLICT OF INTERESTS

None.

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