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Brief communication

Clostridium difficile contamination in retail meat products in Brazil



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ABSTRACT

Introduction: *Clostridium difficile* is an important cause of diarrhoea, particularly in patients receiving antibiotic therapy. Recent studies have shown that a substantial proportion of *C. difficile* infections are acquired in the community, as a zoonotic disease. Brazil is a large exporter of meat and so far no study has evaluated meat contamination with *C. difficile* spores.

Methods: Here we analysed 80 retail meat products purchased from local supermarkets in a Brazilian metropolis (Porto Alegre, Southern Brazil). Samples from these products were grown in anaerobic conditions, and tested with a real time polymerase chain reaction test. **Results:** Contamination with *C. difficile* spores was not found in the study. Bacteria isolated from meat included *Streptococcus gallolyticus*, *Lactobacillus plantarum*, *Enterococcus gallinarum* and *Pediococcus acidilactici*.

Discussion: Close vigilance is required in order to guarantee the quality of Brazilian retail meat in the long term.

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Clostridium difficile is a spore-forming anaerobic intestinal pathogen and a major cause of hospital-acquired diarrhoea. The view of *C. difficile* infection (CDI) as a nosocomial infection transmitted only by symptomatic patients is changing, due an apparent increasing incidence of CDI in the community.^{1,2}

There are reports of genetic overlap between toxigenic *C. difficile* strains isolated from animal and humans, suggesting that the transmission of the pathogen may occur as a zoonotic

disease process. Reported prevalence ranges from 2% to 20% in different countries and meat categories. Brazil is the largest beef exporter in the world, with approximately US\$ 500 million per month in global trade exports. The per capita meat consumption in Brazil is 95.9 kilograms per inhabitant/year, including consumption of poultry, pork, beef and veal.³ The Southern region of the country accounts for more than 70% of the total beef exported and has *C. difficile* as an emerging pathogen in hospitalized patients. Therefore, an evaluation of this potential reservoir is needed at short notice.

Here we aimed to estimate the frequency of contamination with *C. difficile* in different types of retail meats ready for consumption in a large metropolis in Southern Brazil.

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To assess *C. difficile* contamination in retail meat products, ready-for-consumption packed meat was purchased in four large markets in Porto Alegre, Brazil, in May 2017. Beef, pork, chicken, and hamburger products were analyzed, totalling 80 meat samples. These meats were purchased in local super markets and random samples were obtained from several points of each of the meat samples. Meat products were stored in a refrigerated thermal box and processed on the same day of purchase at the Molecular Biology Laboratory of Santa Casa de Misericórdia de Porto Alegre.

Molecular tests were performed using a GeneXpert modified protocol for tissue. In summary, 5 g of meat were obtained from each of the samples. Tissue was ground and homogenized in PBS, placed in 4% NaOH (1:1 ratio), kept at room temperature for 15 min and centrifuged at $1300 \times g$ for 15 min. The supernatant was then discarded, resuspended with 1 ml of PBS and the remainder transferred to the Elution reagent from the GeneXpert Kit and then this solution was transferred to the GeneXpert cartridge (Xpert[®] *C. difficile* test, Cepheid, Sunnyvale, CA). *C. difficile* laboratory detection was based on real-time polymerase chain reaction (qPCR) testing for *C. difficile* toxin genes. In addition, samples were also submitted to *C. difficile* culture, as follows⁴: 2 g of each sample were incubated in thioglycolate broth for seven days under anaerobic atmosphere. The thioglycolate test was performed with the ATCC strain and with faeces samples known to be *C. difficile* contaminated. The growth was very satisfactory. No germination factor but sporulation was used, which was the alcohol shock.

After incubation, an aliquot of the broth was removed and the absolute alcohol shock treatment (1:2 proportion) was carried out for 30 min at room temperature. After centrifugation, the supernatant was discarded. The sediment was subcultured in CM0601 *C. difficile* agar (Oxoid), enriched with 7% blood horse, D-cycloserine and cefoxitin for 48 h in anaerobic atmosphere. The growth was very satisfactory (the microbiological load was high). For validation of protocols, meat samples were contaminated with *C. difficile* strains and tested by culture and PCR. Both protocols were validated and used as positive control.

We analyzed 80 retail meat products. No contamination by *C. difficile* was detected in neither culture or PCR in the study. Six samples showed suspicious growth in the culture and were identified by MALDI-TOF MS as *Streptococcus gallolyticus*, *Lactobacillus plantarum*, *Enterococcus gallinarum*, and *Pediococcus acidilactici*.

Community-acquired CDI has emerged in developed countries, causing concerns of bacterial transmission through food, animals, and environmental sources. In fact, the presence of the pathogen in different meats, seafood, fruits, and vegetables endorse the risk of foodborne transmission. A major

concern was the finding of binary toxin-producing hypervirulent strains as 027 and 078 in food, both strains has emerged in CDI in hospitals and in the community.^{5,6}

In a study performed in the USA using uncooked (ground beef, ground pork, ground turkey, pork sausage, and pork chorizo) and ready to eat (beef summer sausage, pork braunschweiger) meat products, prevalence rates were striking at 40.0%–62.5% (depending on product category).⁵ Another study analyzed 1755 retail meats and found results similar to ours, with *C. difficile* not being detected in any of the meats.⁷ The profound contrast in these results occurred possibly because of differences in methods, type of meats sampled, or even national or geographic variations. Since CDI is an emerging (and neglected) problem in Latin America,⁸ this work can contribute to enhance focus in hospital transmission and other potentially environmental sources. Even though our study evaluated a relatively small number of meat samples, total absence of *C. difficile* is an important result regarding food safety.

Conflicts of interest

The authors declare no conflicts of interest.

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