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Heat survival of *Clostridium difficile* spores in ground meat during cooking process

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BACKGROUND

Clostridium difficile is a spore-forming pathogen considered as a major cause of enteric disease in humans, with faecal-oral route as the primary mode of transmission. However, recent studies have reported the occurrence of *C. difficile* in ground meats at retail stores, indicating that foods could be an additional source of infection in the community.

OBJECTIVES

The objective of this study was to quantify and compare the resistance of *C. difficile* spores in contaminated ground meat during cooking process at different temperatures, including the minimum temperature recommended (70°C) for adequate cooking of meats.

MATERIALS AND METHODS

Spore production	To enhance heterogeneity, spores of two strains were produced in two nutritious broths (BHI), anaerobically at 37°C for 15 days . Spore count was conducted in triplicate. Genotypes tested included toxigenic strain VPI 10463 (the highest toxin producer) and PCR-ribotype 027 (hypervirulent strain isolated from a human patient).
Inoculation	*C. difficile* spores were inoculated in 45 g of ground beef and pork with a final contamination of 4,500 ufc g^{-1}. Six heating temperatures (70, 75, 80, 85, 90 and 95°C) were tested. Experiments were conducted in duplicate.
Thermal inhibition	Samples were heating in a water bath with an integrated program for time-temperature. One sample without inoculum was used as control with a temperature probe placed inside. Once the desired temperature was reached in the core of the sample, the heat treatment was prolonged for 10 min. All the samples were placed in the chilling room (4°C) before analyse.
Culture	A total of 10 g of miced meat was added to 90 ml of cycloserine cefotoxin fructose taurocholate (CCFT) and both were homogenaised in the Pulsifier[R]. A total of 10 µl of broth was spread directly on CCFT and incubate. Another 10 µl of this broth were also cultured after 3 days of anaerobically enrichment at 37°C.

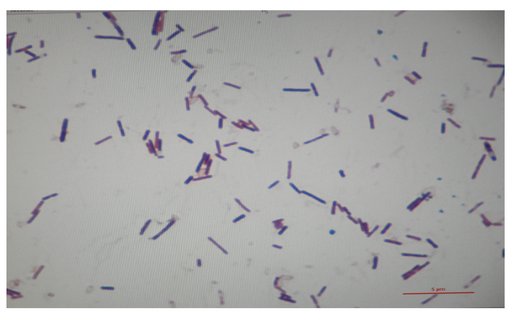
RESULTS

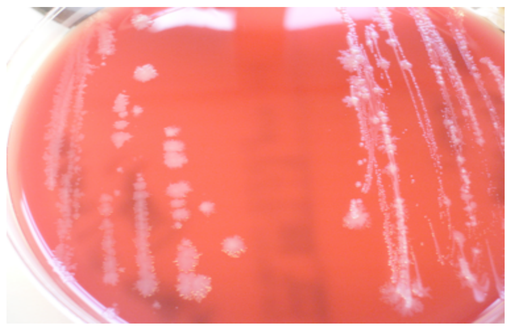
Table 1. Positive detection of *C. difficile* after heat treatment

***C. difficile* detection**

Strain	Heat treatment	Culture	
		Direct	Enrichment
VPI 10463	70°C	+	+
027	70°C	+	+
VPI 10463	75°C	+	+
027	75°C	+	+
VPI 10463	80°C	+	+
027	80°C	-	+
VPI 10463	85°C	-	-
027	85°C	-	-
VPI 10463	90°C	-	-
027	90°C	-	-
VPI 10463	95°C	-	-

Heating contaminated ground meat at 70 or 75°C for 10 min was not effective to prevent *C. difficile* germination. Heat treatment 10 min at 80°C was the lower temperature that showed a reduction in the number of countable colonies. Heating at 85°C (or more) inhibits the germination of both of the strains tested.





SIGNIFICANCE

Numbers of *C. difficile* spores in food are low and in most of the cases have been detected only after enrichment. While most of the meats are cooked before eating, *C. difficile* survives over 70°C. Therefore, strategies such as proper cooking and heading are important interventions to control food-borne exposure. This study shows that ground meat, like burgers or sausages, must be heated to minimum 85°C during 10 minutes in the core to reduce the risk of *C. difficile* food-borne transmission.