

Behavior of *Clostridium difficile* in culture media at different temperatures

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Clostridium difficile is an anaerobic Gram-positive bacillus, causing diarrhea and pseudomembranous colitis due to the action of toxins A, B, and binary. Recently, research suggests that transmission of the disease may be associated with ingestion of contaminated food of animal origin. The optimal conditions of time and temperature for multiplication and toxin production in food products are not described in the literature, thus, we studied the multiplication capacity of *C. difficile* in culture media stored at different temperatures. The culture media (BHI - brain heart infusion and PYG - Peptone yeast extract glucose) with meat inoculated with *C. difficile* VPI 10463 (10^4 - 10^5 CFU / mL) were stored at 4 and $7 \pm 1^\circ\text{C}$ for 28 days and $15 \pm 1^\circ\text{C}$ for 10 days. The counts were carried out in RCM agar (Reinforced Clostridial Media supplemented with 1.5% agar) incubated at 37°C for 48 hours in anaerobic conditions and evaluated every 7 days in samples stored at 4 and 7°C and on days 3, 5, 7 and 10 when stored at 15°C . At 15°C , an increase of 2.75 log CFU/mL count was observed after 5 day and a slight decrease was observed after 10 days (0.7 log CFU/mL) in both media. Toxins A and B were detected in the culture media at the end of the storage time at 15°C by "RIDASCREEN *Clostridium difficile* toxin A/B" kit. At 15°C significant difference between PYG and BHI broths was observed, BHI was better than the PYG broth ($p < 0.05$). *C. difficile* did not develop at low temperatures (4 and 7°C), however, it was possible to detect after 28 days of storage. At 7°C , reductions of 1.5 log CFU/mL and 1.7 log CFU/mL were observed in PYG and BHI broth, respectively, after 28 days. At 4°C , the reduction was < 1 log for both media. The temperatures of 4 and 7°C , usually used in the storage of food products, can prevent the development of *C. difficile*. However, a temperature abuse can provide multiplication and toxins production in meats, making the product unsafe to consumer.

Key- words: *Clostridium difficile*, toxins, diarrhea, cooling.

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