TITLE: EVALUATION OF CYTOTOXIC PROFILE OF *Cronobacter* spp. ISOLATED FROM FOODS AND CLINICAL SPECIMENS

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ABSTRACT:

Cronobacter is considered an opportunistic pathogen that causes infections in humans. Previous studies have reported cases of infections in several countries, including Brazil. Clinical syndromes of *Cronobacter* infections in neonates include meningitis, necrotizing enterocolitis, and bacteremia. The objective of this study was to evaluate the production of proteases and the cytotoxic activity of five strains *Cronobacter* species isolated from foods (n=50) and clinical samples (n=6) in Brazil. Protease activity was evaluated into modified milk agar plates incubated at (36 ± 1) ºC for 3 to 12 days. The cytotoxic activity was tested against RK13 (ATCC® CCL-37™). For the preparation of bacteria filtrates, strains were grown in Tryptic Soy Broth (TSB), harvested by centrifugation and the supernatant was sterilized through a 0.22 µm filter, and divided in two parts, and one half received thermal treatment (100ºC/20min). Cells monolayers were cultivated in Minimal Essential Medium (MEM) with fetal bovine serum, in 96 wells microplates. The bacterial filtrates, Triton-X (positive control), and TSB (negative control) were transferred to three wells and the microplates were incubated at (36 ± 1) °C/48h. The quantification was based on Coomassie Brilliant Blue adsorption and measurement by spectrophotometry at 595 nm. Values were statistically assessed using *t*-test and significance was defined as $p<0.05$. All strains showed proteolytic activity after incubation for 3 to 12 days indicating that the use of milk agar test is not a good virulence marker for cytotoxicity. The untreated bacteria filtrates percentage of death ranged from 0 to 86%, and these values were not significantly reduced after thermal-treated ($p= 0,62$). The cytotoxicity effect among clinicals isolates and food samples strains isolated did not showed significant difference. Among the five *Cronobacte*r species evaluated, the greatest cytotoxic activity average was found in *C. sakazakii*. The results of this study showed that *Cronobacter* strains can produce cytotoxic compounds in cell sobrenadant, providing insights into the pathogenesis of *Cronobacter*.

Keywords: *Cronobacter spp.,* cytotoxic, protease activity

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