

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

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ABSTRACT:

Cronobacter are considered opportunistic pathogens that cause infections in humans. The literature presents reports of 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 species of *Cronobacter* strains 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)°C for 3-12 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), harvest by centrifugation and the supernatant was sterilized through a 0.22 µm filter and divided into halves, and one half received thermal treatment (100°C/20min). The cells monolayers were grown in Minimal essential medium 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 3-12 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 0-86%, and these values were not significantly reduced after thermal-treated ($p = 0,62$). Clinical isolates did not showed significant difference ($p = X$) compared to strains isolated from food samples in cytotoxicity effect. Comparing the five *Cronobacter* species evaluated, the major cytotoxic activity average was found in *C. sakazakii*. The results obtained show 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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