TITLE: ASSESSMENT OF CELL VIABILITY OF GRAM-NEGATIVE BACILLI ISOLATED FROM FECES AFTER ENRICHMENT, REFRIGERATION, AND FREEZING.

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ABSTRACT:

Antimicrobial-resistant gram-negative bacilli (AMR-GNB) are part of a critical heterogeneous group with opportunistic and pathogenic species distributed in the most diverse environmental compartments and hosts. In addition, it is one of the leading causes of hospital-acquired and community infections. The WHO classifies these bacteria as priority threats for developing new drugs, epidemiological and surveillance studies, and understanding of their dissemination routes. Therefore, it is essential to improve methodologies that aim to investigate and detect the occurrence of them. Here, we investigated the cell viability of GNB isolated from feces after refrigeration and freezing. To assess the effect of refrigeration, fresh feces were solubilized in 0.85% saline followed by dilutions at 10^{-3}, 10^{-4}, 10^{-5}, $10^{-6,}$ and 10^{-7} CFU/mL. Dilutions were seeded confluently on MacConkey (MC) agar in triplicate and incubated. The CFU count was performed for the dilution that showed isolated colonies homogeneously arranged. This process was repeated after 24, 48, 72, 95 and 190h of stool refrigeration at -4 °C. To assess the effect of freezing, fresh feces were solubilized in MC broth and incubated. The suspension was filtered in sterile gases and centrifuged. The pellet was solubilized in saline, centrifuged under the same conditions and resuspended in STGG medium. Three aliquots were subjected to UFC count at zero time (t0) with and without enrichment, and aliquots stored at -70ºC for subsequent UFC count in the time intervals t1=1, t2=2, t3=3, t4=5, t5=7, t6=9, t7=15 and t8=28 months from freezing. Five dilutions (from 10^{-3} to 10^{-7}) from each sample tube were performed to determine the best three for counting and in the subsequent months only these were used. In triplicate, aliquot from each dilution was seeded on MC agar and the count was performed under conditions defined above. Pearson correlation were performed with GraphPad Prism. After refrigeration, it was observed a tendency for cell viability to drop over the days (sample 1 $p<0.05$) and a sharp drop on the sixth day. The analysis of the samples after freezing revealed that the bacterial cells gradually decay over the months, probably due to the cryoprotective action of the STGG (Sample 1, aliquit 3 $p<0.05$; Sample 2, aliquit 1 $p<0.005$; Sample 3, aliquit 1 $p<0.05$). It is known that the enrichment step favors some species over others, however, the absence of it means subjecting fewer cells to freezing and therefore reducing the possibility of recovery.

Keywords: gram-negative bacilli, cell viability, feces, effect of freezing

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