TITLE: IDENTIFICATION OF LISTERIA SPP. BY MALDI-TOF MASS SPECTROMETRY IN FECES AND FOOD.

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ABSTRACT:
Listeria monocytogenes is a food-borne pathogen agent of human listeriosis, an opportunistic infection that primarily infects pregnant, neonates and immunologically compromised individuals. Rapid and accurate discrimination among *Listeria* strains is essential for appropriate therapeutic and timely intervention for infection control. Matrix Associated Laser Desorption Ionization–Time of Flight Mass Spectrometry (MALDI-TOF MS) is a rapid and simple microbial identification method, which is a promise for identification of *Listeria* spp. In the study, the performance of MALDI TOF MS in detection and identification of *Listeria* spp. directly from the broth for enrichment of fecal material and food samples was evaluated. In order to do that, 34 standard strains of different *Listeria* species belonging to the Collection of *Listeria* (CLIST/LABZOO-FIOCRUZ), as well as the broth for enrichment of 12 samples of pregnant feces and 15 samples of *sashimi* and *temaki*, all positive for *Listeria*, were analyzed by MALDI-TOF MS. Following a short cell extraction procedure with ethanol, formic acid and acetonitrile, the cell material from the broth was deposited on a sample target, dried, overlaid with a matrix necessary for the MALDI process and analyzed. The method was also confirmed using the conventional methodology and PCR with 23S rDNA and *hly* primers in the detection and identification of this pathogen, respectively. This study showed that 97% (n=33) of the standard strains were identified correctly. Except for one *L. innocua* that was identified only on the genus level, all other isolates were identified in both. Results revealed that was possible to detect *Listeria* in 40% (n=6) of the food enrichment broths, among them 27% (n=4) were identified at the genus and 13% (n=2) at the genus and species. From the fecal broth it was possible to identify the presence of *Listeria* in 25% (n=3), being 17% (n=2) identified at the level of genus and species and 8% (n=1) at the genus. The remaining 58% (n=7) of the total broth of fecal material weren´t analyzed because it didn´t remain viable until the end of the study. This was probably due to the long storage time, even under refrigeration (4°C), since these samples were collected in previous studies. This study indicates the possibility of identifying *Listeria* in samples contaminated directly from the enrichment stage, which can contribute to the reduction of costs of laboratory tests and provide a faster diagnosis of listeriosis.

Keywords: *Listeria* spp., MALDI-TOF MS, Enrichment broth, Listeria monocytogenes, Diagnosis.

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