

**TITLE:** EVALUATION OF THE CLASSICAL METHODOLOGY, MALDI-TOF, VITEK 2 AND PCR IN THE IDENTIFICATION OF ATYPICAL STRAINS OF THE GENUS *LISTERIA*

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**ABSTRACT:**

*Listeria monocytogenes* is a human pathogen responsible for listeriosis, a foodborne disease with high mortality rate (20-30%) that affects mainly elderly, neonates and immunocompromised individuals. *L. monocytogenes* and *L. innocua* are closely related species, widely distributed in the environment and in food processing plants. Atypical *Listeria* strains, which cannot be distinguished from other species of *Listeria* using only conventional phenotypical methods, has been reported. In recent years, several alternatives have been proposed for a faster identification of microorganisms, such as Matrix-Assisted Laser Desorption Ionization (MALDI-TOF / MS) and automated method. The objective of this study was to evaluate the efficacy of phenotypical tests, PCR with primers specific for the most common species, and the automated methods VITEK 2 and MALDI-TOF/MS in the identification of 94 strains of the genus *Listeria* with atypical identification profile, previously characterized as *L. innocua* non typeable. These strains were isolated from food sources from different regions of Brazil and deposited in the Collection of *Listeria* - CLIST/LABZOO/IOC/Fiocruz. The identification resulted from the PCR was considered the correct identification, which presented a total of 31 strains identified as *L. innocua*, 54 as *L. monocytogenes*, 8 as *L. welshimeri* and 1 as *L. grayi*. The phenotypical test correctly identified 64,81% of the *L. monocytogenes* strains, 100% of *L. innocua* and *L. grayi* strains and misidentified all *L. welshimeri* strains. The VITEK 2 automated system correctly identified 83,87% of the *L. innocua* strains, 18,52% of the *L. monocytogenes* strains and all *L. welshimeri* strains. In the analysis by MALDI-TOF/MS 74,07% of the *L. monocytogenes* strains and 25% of *L. welshimeri* strains were correctly identified, however all *L. innocua* strains were misidentified. Both VITEK2 and MALDI-TOF/MS correctly identified the *L. grayi* strain. Our preliminary results demonstrate that the automated methodologies were not able to discriminate the atypical strains of the genus and point to the necessity of the use of other methods, such as PCR or chromogenic media, for the correct identification of these species.

**Keywords:** *Listeria* spp., MALDI-TOF MS, Vitek 2, *Listeria monocytogenes*, Diagnosis, Foodborne pathogen.

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