TITLE: MATRIX-ASSISTED LASER DESORPTION IONIZATION-TIME OF FLIGHT MASS SPECTROMETRY (MALDI-TOF MS) AS A USEFUL TOOL FOR IDENTIFICATION OF ENTEROCOCCAL STRAINS ISOLATED FROM WILD BIRDS

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ABSTRACT:
Timely and accurate identification of microorganisms is the underlying function of any clinical microbiology laboratory, including in veterinary medicine. MALDI-TOF MS is a high throughput technology based on the comparison of protein fingerprints obtained from microbial cells with a database of reference spectra by using algorithms integrated into commercial systems. The aim of this study was to explore the accuracy and feasibility of MALDI-TOF MS for the identification of enterococcal strains isolated from the fecal microbiota of wild birds, as compared to phenotypic methods and PCR. In addition, MALDI-TOF spectra were analyzed with the software Bionumerics 7.5, by constructing a *Neighbor Joining* tree and determining species-specific biomarkers. This analysis took into account both the presence/absence of biomarkers (m/z) and their mean intensity (u.a). Biomarkers with averages of less than 1000 u.a were considered non-significant. A total of 260 enterococcal strains were isolated from 16 species of wild birds. Using MALDI-TOF MS, all strains were accurately identified at the genus level (scores 2.0 to 2.299) and 96.9% of them were also accurately identified at the species level (scores≥2.3). Phenotypic atypical reactions in different biochemical tests were not associated with lower scores. *E. faecalis* was the most prevalent species (63.8%), followed by *E. hirae* (16.2%), *E. faecium* (11.5%), *E. gallinarum* (5.4%), *E. casseliflavus* (0.8%), *E. avium* (1.5%), *E. raffinosus* (0.4%) and *E. cecorum* (0.4%). Enterococcal strains were distributed and clustered according to the species in a *Neighbor Joining* tree. A number of 51 biomarkers (3000 to 10000m/z) were selected to compose species-specific profiles, being some of the biomarkers unique to certain species. *E. raffinosus* presented the highest number of exclusive biomarkers (n=9), followed by *E. avium* (n=6), *E. cecorum* (n=5), *E. hirae* (n=4), *E. casseliflavus* (n=3), *E. faecium* (n=2) and *E. faecalis* (n=1). The results indicate that MALDI-TOF MS is an accurate and rapid method for the identification of enterococcal strains isolated from animals, even when they show atypical phenotypic characteristics in conventional tests or belong to some of the rarely found species.
Key words: Wild birds, microbial identification, *Enterococcus*, MALDI-TOF MS