TITLE: EVALUATION OF THE MALDI-TOF TECHNOLOGY MS AS A TOOL FOR CHARACTERIZING *emm* TYPES AND IDENTIFY CIRCULATING CLONES OF *Streptococcus dysgalactiae* subspecies *equisimilis*

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ABSTRACT:

Group C streptococci (GCS) are etiological agents of many diseases in humans, especially those caused by *Streptococcus dysgalactiae* subspecies *equisimilis* (SDSE). This species has been implicated in causing severe invasive infections more recently. In a collection of SDSE isolates, previously studied by us, two main PFGE clones (A and B) corresponded to 83.5% of the isolates. These predominant clones showed higher virulence potential in *Caenorhabditis. elegans* models compared with the sporadic (rare) clones. The M protein is a major virulence factor found in *Streptococcus pyogenes* that is also present in SDSE. The protein M typing allows an epidemiological characterization of these streptococci, distinguishing them in *emm* types. The MALDI-TOF MS (Matrix-assisted Laser Desorption /Ionization - Time of Flight Mass Spectrometry) is an easy methodology that enables analysis of biomolecules and identification of microorganisms by the comparison of generated spectra with those that have already been stored in a database. In order to develop a simple method to characterize SDSE isolates we molecularly typed the M protein and analyzed these isolates by MALDI-TOF MS aiming to find potential biomarkers that could be used to quickly characterize the different *emm* types, and also to differentiate the two major PFGE clones (A and B) found in the collection studied. Of the 62 SDSE isolates evaluated (A clone: n=21; B clone: n=23; and sporadic clone: n=18), 18 *emm* types were found, with the most prevalent types being stC6979 (24.2%), stC839 (22.6%) and stC74a (11,3%). While the analysis of the spectra found no differential biomarkers for stC6979, specific biomarkers were identified to the *emm* type stC839 (3421.35 m/z and 3954.69 m/z) and stC74a (2085.37, 2432.56, 3366.73 and 5957.98 m/z). More importantly, exclusive markers were detected to identify isolates belonging to the most predominant SDSE clones in Brazil: clone A (3421.35 m/z; n=21, 100%) and clone B (6731.07 m/z; n=23, 100%). Concluding, the use of biomarkers in association with a rapid identification of the SDSE by MALDI-TOF MS could greatly facilitate the characterization of SDSE isolates from infections, contributing to easily track more virulent SDSE clones and thus to a better understanding of the epidemiology route of these microorganisms.

Key-words: *Streptococcus dysgalactiae* subsp**.** *equisimilis*, *emm* typing, PFGE clones, MALDI-TOF MS, biomarkers.

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