

TITLE: Phenotypic and molecular characterization of *Streptococcus iniae* isolated from animal and human sources

AUTHORS: SOUZA, S.S.R.^{1,2}; FARIA, A.R.^{1,2}; MERQUIOR, V.L.C.¹; TEIXEIRA, L.M.²

INSTITUTIONS: ¹Universidade do Estado do Rio de Janeiro, Faculdade de Ciências Médicas, Rio de Janeiro, RJ (Av. 28 de Setembro, 87 – Fundos. 3º andar, CEP 20551-030, Rio de Janeiro - RJ, Brazil)

²Universidade Federal do Rio de Janeiro, Instituto de Microbiologia Paulo de Góes, Departamento de Microbiologia Médica, Rio de Janeiro, RJ (Av. Carlos Chagas Filho, 373 – CCS/Blcco I, CEP 21941-590, Rio de Janeiro – RJ, Brazil)

ABSTRACT:

Streptococcus iniae is considered an important fish pathogen leading to significant economic losses in fish farming. In recent decades, this bacterial species has also been recognized as a zoonotic agent causing infections in humans. However, precise identification of *S. iniae* isolates remains problematic due to phenotypic similarities with other members of the genus. Consequently, several biological aspects of this species remain largely unknown. In this study, biochemical tests and molecular methods were used to characterize *S. iniae* isolates recovered from infections in both fish and humans. A total of 33 *S. iniae* strains were included in this study. Their phenotypic characteristics were tested by conventional tests and by the rapid ID 32 STREP system. Molecular characterization was performed by MALDI-TOF MS and by PCR for detection of the 16S rRNA. Susceptibility to 12 antimicrobials was determined by the disk diffusion method and tetracycline resistance genes were detected by PCR. *Sma*I restriction profiles of the genomic DNA were analyzed by pulsed-field gel electrophoresis (PFGE). Most strains showed the expected phenotypic profiles, except for 13 strains showing susceptibility to bacitracin. The rapid ID 32 STREP system was not able to correctly identify the strains, a result that was expected, since *S. iniae* is not present in the database of the system. The PCR technique allowed the identification of all the 33 strains as *S. iniae*, discriminating them from other species of *Streptococcus* that are phylogenetically related. As *S. iniae* is still not included in the Bruker MALDI-TOF MS database, a local *S. iniae* database was created and a phyloproteomic tree was constructed with the spectra, by using the software Bionumerics v7.1. The 33 strains were separated from the other species tested, suggesting this technique may also be an available tool to identify *S. iniae* isolates. The isolates were susceptible to most of the 12 antimicrobials tested, except for three strains that were resistant to tetracycline and carried the *tet(M)* gene. PFGE analysis revealed 3 clonal complexes and 7 unique profiles. In conclusion, the results obtained in our study contribute to a more accurate identification and suggest the occurrence of variants of *S. iniae* adapted to different hosts. The use of more accurate procedures to properly identify and characterize members of this species will help in clarifying their role as causes of different infections.

Keywords: *Streptococcus iniae*, zoonotic pathogen, phenotypic identification, molecular characterization, antimicrobial susceptibility

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