

TITLE: EVALUATION OF VIRULENCE FACTORS BY PATHOGENIC *Leptospira* spp. ISOLATED FROM HUMAN CASES OF LEPTOSPIROSIS

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ABSTRACT:

Leptospirosis is a worldwide distribution zoonosis caused by pathogenic serovars of *Leptospira* species. The infection is considered a public health concern in many countries, including Brazil. Even though the mechanisms of virulence of this pathogen are not completely understood, biofilm formation and proteins involved in pathogenesis are considered to be important factors in the establishment of the disease. The aim of this study was to evaluate the biofilm production and the expression of whole proteins of different strains of *Leptospira* spp. isolated from human clinical cases. Initially, 18 strains were selected (one saprophytic and 17 pathogenic), all belonging to the Leptospira Collection (CLEP/IOC-Fiocruz). The pathogenic strains were obtained from different clinical cases of patients with leptospirosis presenting anicteric, icteric or severe pulmonary (SPFL) forms of the disease. Previously, a growth curve was established for all strains for ten days in order to define the phase of biofilm production. Subsequently, a crystal violet assay to quantify the biofilm was evaluated after seven days of incubation by using 24-wells polystyrene plates. Statistical analysis was made by the Graphpad Prism software by using the One-way ANOVA test. The scanning electron microscopy was used for observing the production of biofilm by selected strains. A comparative protein profile was evaluated by SDS-PAGE by using the whole protein extract from planktonic and biofilm adherent cells and characterization and identification of the proteins were performed by a immunoprecipitation assay, using sera from human patients previously diagnosed with leptospirosis, followed by mass spectrometry (Nano-LC ESI-MS/MS coupled to Orbitrap LTQ.). Until now, our results show that one clinical isolate, involved in SPFL (CLEP 00047), was a strong biofilm producer, compared to other clinical strains, and to the saprophytic (Patoc) strain. Approximately 268 proteins were identified for both strains after analysis by immunoprecipitation, being associated with several conditions of stress and virulence, such as LipL45, LipL71, GroEL and OmpA. All proteins were identified by using the PEAKS software (database *Leptospira* spp.). The elucidation of the virulence potential by pathogenic leptospires might reveal mechanisms of survival, colonization and virulence associated with clinical isolates and a better understanding of the infectious disease.

Keywords: virulence factors, biofilm, whole proteins, Orbitrap.

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