

TITLE: EVALUATION OF VIRULENCE FACTORS BY PATHOGENIC *LEPTOSPIRA* SPP. ISOLATED FROM ICTERIC AND ANICTERIC 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. Although 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 during 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 were made by the Graphpad Prism software by using the One-way ANOVA test. The scanning electron microscopy will be used for observing the production of biofilm by selected strains. A comparative protein profile will be evaluated by SDS-PAGE by using the whole protein extract from planktonic and biofilm adherent cells. The characterization and identification of the proteins will be performed by a Western blotting assay, using sera from human patients previously diagnosed with leptospirosis, followed by mass spectrometry (MALDI TOF-TOF MS). Until now, our results show that three clinical isolates, two obtained from anicteric cases and one involved in SPFL, were strong biofilm producers, compared to other clinical strains, and to the saprophytic strain. Among them, the strain isolated from a fulminant case of leptospirosis associated with massive pulmonary hemorrhage and renal failure, presented a higher level of biofilm formation. The elucidation of the virulence potential by pathogenic leptospirae 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, MALDI TOF-TOF

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