

Isolation of *Leptospira* strains from Carioca River in Rio de Janeiro city

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Abstract

Leptospirosis is a global zoonotic disease caused by spirochetes of the genus *Leptospira*. Human infection usually occurs by contact with water and soil contaminated with urine of chronically infected animals. The environment becomes a contamination source that needs to be investigated regarding the etiological agent. In this study, we evaluated the presence of *Leptospira* spp. in five points of Carioca River, located in the metropolitan region of Rio de Janeiro city. A total of 5 samples were collected in different points of the river, using sterile tubes and passed through 0.45 µm and 0.22 µm pore size membranes filters, respectively. A volume of 0.5mL of each sample was inoculated into both semisolid EMJH and Fletcher mediums containing 5-fluorouracil (100µg/mL). The cultures were examined by dark-field microscopy and those presenting cells compatible with *Leptospira* morphology were selected for further investigation. Microscopic Agglutination Test (MAT), using a panel of 18 rabbit anti-*Leptospira* sera, was applied for serotyping of isolated strains. Genotyping was performed using polymerase chain reaction (PCR) and sequencing to confirm the genus *Leptospira* and to detect the pathogenic status of the isolates. Five *Leptospira* sp. strains could be recovered from the water samples tested, corresponding to isolations in 100% of the collection points. The presence of the genus was confirmed by cell morphology under microscopy and sequencing of the *rrs* gene, which encodes the ribosomal RNA 16S. Only one strain presented reaction against serogroup Icterohaemorrhagiae sera at MAT, indicating a possible pathogenic strain among the isolates. Although no amplification was observed for *flaB* and *lipL41* genes, which encode virulence determinants, the pathogenic status of the strains will be confirmed by sequencing of *ppk* gene. This marker, which encodes a polyphosphate kinase, was recently described as an important candidate for phylogenetic classification in leptospire. Considering the impact of leptospirosis in the approach of one health, our results confirm the importance of investigate the presence of *Leptospira* spp. in the environment, which represents the main source of contamination for man and animals.

Key words: leptospirosis, leptospire, environment.

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