TITLE: CORRELATION BETWEEN MOLECULAR IDENTIFICATION DATABASES FOR EF-1α AND RPB2 GENES OF PATIENTS WITH FUNGAL KERATITIS BY FUSARIUM

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ABSTRACT

Fungal keratitis (FK) is an opportunistic ocular mycosis that causes inflammation of the cornea and, in severe cases, can lead to total blindness or even loss of the eyeball. Identification of the pathogen is essential for appropriate therapy, so our objective was to identify to the species level *Fusarium* isolates that cause FK, since this is one of the main fungi that causes the disease. Therefore, a study was carried out in the database at the Hospital of Clinics of Porto Alegre (HCPA) from 2008 to 2016, and 6 FK cases diagnosed in the HCPA Microbiology Laboratory were screened as a positive culture for *Fusarium* spp. DNA was extracted using the Mini Kit-Qiagen and amplified by the Polymerase Chain Reaction (PCR) using the EF-1α and RPB2 genes, according to O'Donnell et al, 2007. The amplified products were separated on 1,5% agarose gel and quantified comparing to Low Mass (Invitrogen). Subsequently, they were purified using the enzyme EXOSAP and sequenced in the Molecular and Protein Analysis Unit (UAMP, HCPA) using the ABI 3500 Genetic Analyzer (Applied Biosystems). Reactions were performed on the Veriti® 96-Well Thermal Cycler thermal cycler (Applied Biosystems). Purification in sequencing is performed with BigDye XTerminator Purification Kit (Applied Biosystems) and injected in the automatic sequencer. The sequences obtained were edited in the CHROMAS PRO program and then compared to the MLST database, BLAST, BLAST type strain and *Fusarium* ID, comparing the results of those bases. As a result, for both genes the BLAST bases compared to MLST obtained the best results, presenting 83% agreement in the samples. The RPB2 gene also obtained 83% agreement when comparing the BLAST base with *Fusarium* ID. In general, the EF1α gene obtained the best results in our research, and this agrees with the literature, which recently has been bringing this gene as marker of *Fusarium* species. Identification to the species level allows a better treatment for the patient, so it is extremely relevant for the correct clinical management of FK the identification of the pathogen, thus avoiding the severe complications of the disease.

Key-words: *Fusarium* spp., MLST, ITS.

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