Antifungal susceptibility of clinical strains of C*ryptococcus neoformans* and *Cryptococcus gattii* using the broth microdilution

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Abstract:
Cryptococcosis is an opportunist mycosis which has as etiological agent *Cryptococcus neoformans* and *Cryptococcus gattii* yeasts which belong to the phylum Basidiomycota. Therefore, that mycosis has a primary infection in lungs that can affect other organs particularly developing central nervous system cryptococcosis. Northern Brazil shows a lack of studies about the issue of resistance of *Cryptococcus* spp. to antifungal drugs used to treat this mycosis of medical importance. The disease treatment consists in use three antifungals Amphotericin B in the induction phase, the consolidation phase Fluconazole and Itraconazole or Fluconazole with lower doses during the maintenance phase. Faced with the limited data available with isolated in our region, this study aims to evaluate the antifungal susceptibility of clinical isolates of *C. neoformans* and *C. gattii*. A number of 10 clinical samples from Fundação de Medicina Tropical Doutor Heitor Vieira Dourado were used in this study. Minimum inhibitory concentration (MIC) assays were performed with broth microdilution method as described by the CLSI (Clinical and Laboratory Standards Institute) in documents M27-A3/CLSI. Following the CLSI, 100 µL of each evaluated compound diluted in RPMI 1640 broth was added to 96-well microplates with the final concentrations of the compounds ranging from 64 to 0.125 µg/mL (fluconazole) and 0.0313 to 16 µg/mL (amphotericin and itraconazole). Next, 100 µL of inoculums containing 2.5×10^3 cells/mL of opportunistic yeasts was added to the microplate. The microdilution plates were incubated at room temperature (35°C) from 24 to 48 hours. Finally, visual readings were performed after 24 hours. As a result, all clinical isolates of *C. neoformans* and *C. gattii* showed sensitivity to amphotericin (< 0.0313 µg /mL). However, the MIC for *C. neoformans* against to fluconazole and itraconazole drug concentration was ≤ 4 µg/ mL and ≤ 0.06 µg/ mL, respectively. Moreover, MICs to *C. gattii* using fluconazole and itraconazole drugs showed growth in high concentrations ≤ 16 µg /mL and ≤ 4 µg /mL. Thus, this susceptibility data showed a different response to antifungal drugs used in Cryptococosis therapy, so we need more studies to evaluate the reasons behind these results.

Key words: Cryptococosis, In vitro test**,** *C. neoformans/ C. gattii* specie complex.

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