

Title: MICs distribution and epidemiological cutoff values of triazoles against *Trichosporon asahii* isolates: a preliminary study

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Abstract:

Trichosporon asahii has been isolated as an opportunistic pathogen of invasive infections in neutropenic and critically ill patients, with mortality rates up to 80%. *T. asahii* isolates have limited sensitivity against different antifungal agents, including some of the triazoles, which are the first line of choice for treatment of infections caused by this agent. Due to the lack of internationally accepted protocols to perform antifungal susceptibility tests for *Trichosporon* spp. as well as clinical trials with patients infected by this emergent pathogen, clinical breakpoints of antifungal drugs for this genus remain unclear. The aim of this study was to perform susceptibility tests according to the CLSI protocol to evaluate the wild-type distribution of minimal inhibitory concentrations (MIC) of triazoles and determine the epidemiological cutoffs (E-coffs) for *T. asahii*. As a preliminary study, we evaluated 100 clinical isolates from different isolation sources: urine (57), blood (21), deep sterile sites (12), superficial mycoses (7) and feces (3). As quality control strains we used two *T. asahii* reference strains (CBS2479 and CBS7631), the *C. parapsilosis* ATCC22019 and *C. krusei* ATCC6258. All *T. asahii* isolates were identified by sequencing of the IGS1 rDNA region and BLASTn comparisons in the NCBI-GenBank. Susceptibility tests for fluconazole (FLC), itraconazole (ITC), posaconazole (POS), and voriconazole (VRC) were performed using the microdilution broth method (CLSI M27-A3 S4), with readings after 24, 48 and 72 hours incubation. E-coff values were determined based on MICs distribution, according to Arendrup *et al.* (2010). By sequencing analysis, all isolates were identified as *T. asahii* with scores $\geq 99\%$ of identity and coverage between sequences. MIC readings at 24h were not reliable due to the lack of robust growth by most isolates. Readings at 48h and 72h of incubation demonstrated high reproducibility with MIC₅₀ values as follows: FLC (2 and 4 μ g/ml), ITC (0.125 and 0.25 μ g/ml), POS (0.25 and 0.25 μ g/ml), and VRC (0.03 and 0.06 μ g/ml). The E-coffs at 48h and 72h were, respectively: FLC (16 and 32 μ g/ml), ITC (1 and 2 μ g/ml), POS (1 and 4 μ g/ml), and VRC (0.25 and 0.5 μ g/ml). At 48h readings, 9 isolates from blood and urine were considered non-wild types, whereas at 72h, 2 were classified as non-wild types, exhibiting high MICs against one or multiple triazoles. We concluded that VRC exhibited the best antifungal activity against *T. asahii* isolates.

Key words: epidemiological cutoff of triazoles, antifungal resistance, *Trichosporon asahii*

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