R520
Publication Only
Mycology: Resistance and mechanisms of action of antifungals

Development of antifungal resistance in *Candida glabrata* and its relationship with phenotypic switching

A. Silva-Dias[1], I.M. Miranda[1], J. Branco[1], S. Costa-de-Oliveira[1], C. Costa[2], M.C. Teixeira[2], C. Pina-Vaz[1], A.G. Rodrigues[1]

[1]Microbiology, Faculty of Medicine University of Porto, Porto, Portugal ; [2]Biological Sciences Research Group, IBB – Institute for Biotechnology and Bioengineering Instituto Superior Técnico Universidade de Lisboa, Lisboa, Portugal

Objectives: *C. glabrata* is the second most prevalent *Candida* species colonizing humans and its incidence in systemic infection is increasing dramatically, possible due to the extensive use of antifungal therapy. Our goal was to study the mechanisms underlying acquisition of antifungal resistance in *C. glabrata*. Moreover *C. glabrata* possesses the ability to switch between different phenotypes, so we also investigate the relationship between resistance and phenotypic switching.

Methods: *In vitro* induction of resistance assays were initiated with a bloodstream isolate of *C. glabrata* O44, susceptible to all antifungal drugs used in this assay. The strain was incubated daily in fresh YPD containing one of the following antifungal drugs: fluconazole, voriconazole, posaconazole, clotrimazole, amphotericin B and caspofungin. Every 5 days of incubation, Minimal Inhibitory Concentrations (MIC) values were re-determined, according to the CLSI microdilution reference protocol M27-A3 S4. Reversion of the resistance was assessed by the incubation of the resistant strains in medium without antifungal for 30 days.

Relation between antifungal exposure and phenotypic switching was investigated using two media supplemented either with $CuSO_4$ or phloxine B. Induced strains were allowed to grow for 7 days at 25°C. Distinct phenotypes were quantified accordingly with Lachke et al., and the MIC for each colony phenotype determined according to the CLSI microdilution reference protocol M27-A3 S4.

Results: *C. glabrata* resistance was successfully induced to all antifungals tested. Respecting to azoles, clotrimazole was the antifungal which triggered resistance more promptly, at day 5 of induction. The second was posaconazole at day 20, followed by fluconazole, at day 45, and voriconazole, at day 55. No reversion of resistance was noticed after 30 days of incubation without antifungal pressure. The acquisition of resistance to one azole always generate cross resistance to all other tested azoles. Interestingly, along induction time, azole strains gradually became browner in the $CuSO_4$ medium and whiter in the phloxine B medium.

Resistance to caspofungin was developed at day 5 and to amphotericin B at day 50. The amphotericin B and caspofungin induced strains displayed distinct phenotypes over induction ranging from white to dark brown, in the $CuSO_4$ medium, and dark pink to white in the phloxine B medium. White, light brown and dark brown colonies isolated from $CuSO_4$ agar showed different susceptibility patterns to these antifungals: white-susceptible, light brown-susceptible and dark brown-resistant.
Conclusions: *C. glabrata* acquired resistance to all tested antifungals in the following order: caspofungin, clotrimazole, posaconazole, amphotericin B, fluconazole and voriconazole. Antifungal stress triggered phenotypic switching whose role in antifungal resistance is yet to uncover. Nevertheless and for the first time, a relation between antifungal resistance and phenotypic switching was established in *C. glabrata*.