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Genotypic Characterization of C. glabrata After Exposure to Fluconazole

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Candida glabrata, has emerged as a major pathogen in HIV-infected patients with oropharyngeal candidiasis. This is mainly due to drug resistance acquired with prophylactic use of fluconazole. However, mechanisms for fluconazole resistance in *C. glabrata* are not properly understood. Objective: To determine genotypic changes associated with fluconazole exposure in a *C. glabrata* strain. Methods: *C. glabrata* ATCC2001 was grown on SDA and resuspended in PBS to reach a cell concentration of 10^8 cells/ml. The cells were pelleted and grown in 20 ml RPMI broth overnight at 37°C, and subsequently exposed to fluconazole MIC concentrations (x1 and x2) for 60 min. Control tests were carried out without the drug. The karyotipic changes in *C. glabrata* were analyzed with randomly amplified polymorphic DNA (RAPD), restriction fragment length polymorphism (RFLP) and, contour-clamped homogenous electric field electrophoresis (CHEF) techniques. Results: 1. *C. glabrata* treated with (x2) MIC fluconazole demonstrated varying electrophoretic patterns with 6 of 8 primers used with RAPD whereas no changes were seen with (x1) MIC of the drug; 2. RFLP analysis showed changes in the genomic profile (around 700-2500 bp) with 2 of 4 restriction enzymes (*Dde* I and *Hae* III) and, 3. CHEF analysis failed to show any positive results. Conclusion: Considering the changes noted in the genotypic profile of the yeast isolate pre-treated with fluconazole, it could be said that long term prophylaxis with this drug may lead to chromosomal instability in *C. glabrata*. (Supported by the Research Grants Council and the Committee of Research and Conference grants, University of Hong Kong, Hong Kong SAR, and the Outstanding Researcher Award of LPS).

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