Investigation of mutations in *ERG*11 gene of two *in vitro* fluconazole-resistant *Candida albicans* clinical isolates

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ABSTRACT. *Candida* species are present in the human body normal microbiota. When there is an imbalance in the relationship between microbiota and host, these yeasts can become opportunistic pathogens. *Candida albicans* is one of the major pathogenic fungi causing systemic infection among immunocompromised hosts – cancer and transplant patients, and those with HIV infections. Azole antifungal agents (specially fluconazole) are commonly prescribed to treat systemic and mucocutaneous candidiasis, i.e. esophageal infection. However, emergence of fluconazole-resistant *C.albicans* strains in patients receiving azole treatment has become a serious problem. In all fungal species, *ERG*11 is the gene encoding ERG11p, an essential enzyme for ergosterol synthesis. Resistance to fluconazole has been associated with *ERG*11 gene point mutations and also alterations in the ergosterol biosynthetic pathway. These mutations result in ERG11p conformational changes that reduce effective binding between fluconazole and this target. **OBJECTIVES.** This study aimed to investigate mutations in *ERG*11 gene in two clinical isolates of fluconazole-resistant *C.albicans* using the methods of PCR amplification and gene sequencing. The two fluconazole-resistant *C.albicans* were isolated, respectively, from oral cavity (fluconazole Minimal Inhibitory Concentration value: 8µg/mL) and esophageal cavity (fluconazole MIC value: 64µg/mL) of two different patients treated with oral fluconazole at Hospital and Clinics of the State University of Campinas. **METHODS.** The *ERG*11 gene was amplified by PCR with specific primers ErgSec1A/1B, ErgSec2A/2B and ErgSec3A/3B (Sigma-Aldrich), and analyzed by automated sequencing on ABI Prism®3100 Genetic Analyzer (Applied Biosystems). The sequencing products were compared to the fluconazole-sensible standard *C.albicans* ATCC 90028. **RESULTS.** Nucleotide sequence analysis of the *ERG*11 gene of both *C.albicans* isolates revealed 6 nucleotide mutations encoding six distinct amino acid substitutions: E116D, T128K, E266D, A298V, G448V and G464S. All of them have been reported previously as associated with fluconazole resistance. **CONCLUSION.** Resistance to fluconazole is becoming a major problem in *C.albicans* infections treatment. In this cases, development of resistance was observed after a long oral treatment, which allows greater antifungal/pathogen contact. This fact, coupled with fluconazole fungistatic action, may have contributed to the selection of resistant strains.

Keywords: *Candida albicans*; candidiasis; *ERG*11 gene mutations; fluconazole resistance.

Grant #2014/08693-8, São Paulo Research Foundation (FAPESP).