Investigation of mutations in ERG11 gene of two in vitro fluconazole-resistant Candida albicans clinical isolates


1 Clinical Pathology Department - Faculty of Medical Sciences. State University of Campinas - UNICAMP. Campinas/SP, Brazil.
2 Internal Medicine Department - Faculty of Medical Sciences. State University of Campinas - UNICAMP. Campinas/SP, Brazil.
3 Digestive Diseases Surgical Unit and Gastro Center - Hospital and Clinics. State University of Campinas - UNICAMP. Campinas/SP, Brazil.

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, ERG11 is the gene encoding ERG11p, an essential enzyme for ergosterol synthesis. Resistance to fluconazole has been associated with ERG11 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 ERG11 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 ERG11 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 ERG11 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; ERG11 gene mutations; fluconazole resistance.

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

The opinions, assumptions, conclusions or recommendations expressed in this material are those of the author(s) and do not necessarily reflect the views of FAPESP.