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Letter to the Editor

Effects of Aspirin as an Anti-inflammatory Drug on Azole-resistant *Candida glabrata* In Vitro

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Dear Editor-in-Chief

Candida glabrata previously considered as a nonpathogenic commensal microorganism of human mucosal tissues, is now mentioned as the second or third cause of nosocomial candidiasis and have significantly increased recently due to immunosuppressive therapies (1). Among the *Candida* species, *C. glabrata* is inherently resistant to azole anti-fungal agents. The mutations occurred in several ergosterol biosynthesis genes including *ERG1*, *ERG3*, *ERG6*, *ERG7*, *ERG9* and *ERG11* confer the resistance to azoles (2). The nonessential gene *ERG6*, undergoes mutations can develop multiple phenotypes, including, decreased ergosterol content, increased resistance to polyenes and increased cycloheximide sensitivity (3). The non-steroidal anti-inflammatory drugs (NSAID) especially ibuprofen diclofenac and aspirin have been indicated to have inhibitory effect on biofilm formation and decrease of drug resistance in *C. albicans* (4).

The aim of this investigation was to assess the effects of aspirin as an anti-inflammatory drug on the *C. glabrata* species in vitro.

A resistance isolate of *C. glabrata* obtained from patients with vulvo vaginal candidiasis was selected for further analysis in this study. The antibiotic susceptibility test of *C. glabrata* isolates for fluconazole (Pfizer Central Research, Sandwich, United Kingdom) according to the CLSI M27 A3 standard protocol.

The MICs endpoints of fluconazole as the level, induced a prominent reduction of growth (50% inhibition), compared to drug-free growth control. Based on this method, strains considered as susceptible when the MIC was ≤ 8 µg/ml, considered as susceptible dose dependent when MIC was =16–32 µg/ml, and considered as resistant when MIC was ≥64 µg/ml. One fluconazole-resistant isolate has been chosen for further investigation.

The effect of aspirin (Bayern, Germany) on the growth as well as gene regulation of the mentioned fluconazole-resistant isolate was performed. Briefly, according to a serial dilution process, an initial dilution of aspirin (250 mg/ml) was used for serial dilutions (125, 62.5, 31.25, 15.62, 7.81 mg/ml).

Inoculum suspensions were prepared from 24 h of *Candida* cultures ranged from 2.5×10^3 0.5×10^3 to CFU/ml by spectrophotometry. The plates were then incubated at 35 °C for 48 h. The plates were incubated in 35 °C for 48 h then visual readings were performed. For the confirmation of growth of isolates, 10 µl of each plate were inoculated to the sabouraud's dextrose agar plates.

The revealed MIC was used in order to culture the resistant *C. glabrata* and the obtained micro-organism was further analyzed for *ERG6* gene regulation assessment. Total RNA molecules from *C. glabrata* isolates were extracted using RNA isolation kit (Gene JET RNA purification kit, fermentase, Germany). The extractions were performed in isolates grown in the dilution of 15/62 mg of aspirin as well as non-treated sample. For each test 6×10^8 cells was used. Isolated RNA was preserved in -20 °C.

First-strand cDNA was synthesized from 0.1 ng-5 µg of total RNA in a 20 µl reaction volume using a Life Science kit (fermentase) according to the manufacturer's instructions. The primers were designed using the Oligo Explorer program. PCR was performed on the Rotor Gene 6000 system (Corbett Life Sciences, Sydney, Australia). To quantify possible changes in ERG6 genes expression in *C. glabrata*, gene regulations were measured by Relative real-time -PCR.

ERG6 genes expression were normalized to the housekeeping β-actin gene and analyzed by using REST (2009 V2.0.13) software. The MIC was indicated in 15.62 mg of aspirin after incubation in 35 °C for 48 h.

Considering RT-PCR reaction, the *ERG6* was compared in comparison to control sample (untreated *C. glabrata*). The *ERG6* gene expression was significantly increased when using aspirin at the concentration of 15.62 mg/ml. for the growth inhibition of *C. glabrata*. The amplification of *ERG6* was detected at 15.62 mg/ml and *C. glabrata* were capable of growth at this concentration (MIC=15.62 mg/ml). However, aspirin caused a fatal effect at the concentration of 31.25 mg/ml which no growth was observed.

We obtained 15.62 and 31.25 mg/ml as MIC and MFC respectively. This result showed inhibition effect of aspirin similar to Sharma et al. investigation that mentioned non-steroidal anti-inflammatory drugs to have anti-fungal activity (5). Regarding the Real Time PCR test, the *ERG6* gene was amplified before the cycle of 38. The amplification of *ERG6* was detected in *C. glabrata* growth at 15.62 mg/ml concentration of aspirin. However, aspirin conferred a fatal effect at the concentration of 31.25 mg/ml. we tried to find the role of ERG6 gene in azole resistant *C. glabrata* treated with aspirin. A nonsense mutation in this gene has been indicated to make reduction in susceptibility to azole compounds (6).

The present study exhibited that aspirin induced the anti-fungal effect of fluconazole at higher concentrations which conferring a synergistic effect.

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