The role of antifungals agents on *Candida glabrata* biofilms matrix composition

Sónia Silva, Tatiana Lourenço, Melyssa Negri, Mariana Henriques , Joana Azeredo and Rosário Oliveira

Institute for Biotechnology and Bioengineering, Centre of Biological Engineering, Universidade do Minho, Campus de Gualtar, 4710-057 Braga, Portugal

Candida glabrata was considered, for years, a relatively non-pathogenic saprophyte of the normal flora of healthy individuals and as no causative agent of serious infection in humans. However, its high mortality rate and its quick spread confirm the opposite. In fact, due to the widespread and increased use of immunosuppressive therapy together with broad-spectrum antifungal treatments, the frequency of mucosal and systemic infections caused by *C. glabrata* has increased significantly. Furthermore, biofilms are described as surface associated communities of microorganisms within an extracellular matrix, generally composed of carbohydrate and proteins. Biofilm formation is an important virulence factor for a number of *Candida* species, as it confers significant resistance to antifungal therapy by limiting the penetration of substances through the matrix and protecting cells from host immune responses. Moreover, little is known about the role of antifungals on *C. glabrata* biofilms. Thus, the aim of this work was to study the role of fluconazole, itraconazole and amphotericin B on 24 h pre-formed *C. glabrata* biofilms and specially on their matrix composition.

A total of 3 *C. glabrata* strains isolated from oral, urinary and vaginal tract were used, as well as a reference strain from ATCC (*C. glabrata* 2001). Biofilms were formed on 12-well plates on RPMI 1640, during 24h at 37ºC and 120 rpm. Then, the antifungal agents (fluconazole, amphotericin B and itraconazole) were added to the previously formed biofilms. After 48 h of action of each antifungal agent, the biofilms were evaluated in terms of total biomass by crystal violet staining and number of viable cells by colony forming units (CFUs). The role of itraconazole on biofilms of the clinical vaginal isolate (*C. glabrata* 534784) was also examined in terms of matrix composition. For this, biofilms were formed in 6-well plates during 24h and, after 48h of exposure to itraconazole, were scraped from the wells and the extracellular matrix was extracted by sonication. Biofilm matrix contents in proteins and carbohydrates were determined using the BCA kit and the Dubois method, respectively.

The results showed that, amphotericin B and fluconazole were able to cause a significant decreased on total biomass and CFUs of *C. glabrata*. However, itraconazole was not able to affect biofilms, except for the clinical vaginal isolate (*C. glabrata* 534784) at 256 µg/mL point concentration, which presented an increase in total biofilm biomass. *C*andida *glabrata* 534784 biofilms matrix exposed to itraconazole (256 µg/mL) presented an increase in proteins content but not in carbohydrate comparatively to the control.

In summary, fluconazole and amphotericin B were able to significantly decrease the pre-formed biofilms of *C. glabrata* strains. Furthermore, the highest amount of total biofilm biomass of the vaginal isolate seems to be due to the increased protein content in its matrix.

Key words: *Candida glabrata*, Biofilms, antifungals agents; resistance