TITLE: VIRULENCE OF CLINICAL ISOLATES OF CANDIDA *SPP*. SUSCEPTIBLE AND RESISTANT TO FLUCONAZOLE IN *GALLERIA MELLONELLA* MODEL

AUTHORS: FERNANDA WALT MENDES DA SILVA and KELLY ISHIDA

INSTITUTION: DEPARTMENT OF MICROBIOLOGY, INSTITUTE OF BIOMEDICAL SCIENCES, UNIVERSITY OF SÃO PAULO, SÃO PAULO, SP (AVENIDA PROFESSOR LINEU PRESTES, 1374, LAB 150, CEP 05508-000, SÃO PAULO-SP, BRAZIL)

ABSTRACT: Yeasts of the genus *Candida* are part of the human microbiota, however they can cause both superficial and systemic mycoses. Many *Candida* species are responsible for candidiasis, but *Candida albicans* accounting for 50-70% of cases of invasive candidiasis; presenting a high mortality rate. Invertebrate animal models have been increasingly used as alternatives to investigate fungal virulence; among them the larval stage of the insect *Galleria mellonella* presents advantages such as easy maintenance and low cost. Thus, this study evaluated the virulence of *Candida* spp. susceptible and resistant to fluconazole (S-FLZ and R-FLZ, respectively). Minimum Inhibitory Concentration (MIC) values were obtained by the broth microdilution assay and the susceptibility/resistance profile was determined according to the breakpoint values established in the document M27-S4 (CLSI, 2012); and 2 strains of each species of *Candida* was selected (*C. albicans*, *C. parapsilosis*, *C. tropicalis*, *C. glabrata* and *C. krusei*), being one S-FLZ and another R-FLZ. For the virulence study, larvae of *G. mellonella*, in the last instar (2.0 - 2.5 cm), were infected with yeasts of *Candida* spp. (5 x 10^5 CFU/larvae), incubated at 37 °C and observed for 5 days. The isolates *C. albicans* IAL-40 (R-FLZ) and SC5314 (S-FLZ) were the most virulent leading to the death of 100% of the larvae (P<0.0001 compared to the uninfected control), followed by *C. tropicalis* IAL-01 with 55% of mortality (P=0.002), *C. krusei* ATCC 6258 (R-FLZ) with 44.5% mortality (P=0.0016), *C. parapsilosis* IAL-17 (R- FLZ) with 25% of mortality (P=0.0182). On the other hand, the survival curve of larvae infected by *C. parapsilosis* ATCC 22019 (S-FLZ), *C. krusei* IAL-30 (R-FLZ), *C. tropicalis* ATCC 200956 (R-FLZ), *C. glabrata* ATCC 2001 (SDD-FLZ), and *C. glabrata* IAL-23 (R-FLZ) were the less virulent isolates and similar to the uninfected control group (P> 0.05). In conclusion, among the five species evaluated in this study *C. albicans* was the most virulent followed by *C. tropicalis*, *C. krusei*, *C. parapsilosis* and, finally, *C. glabrata*.

Key words: *Galleria mellonella*, *Candida*, Virulence, Fluconazole, Resistance.

Agency: Fundação de Amparo a Pesquisa do Estado de São Paulo (FAPESP, 2015/07993-0)