TITLE: RESISTANT DEVELOPMENT TO ECHINOCANDINS IN *CANDIDA HAEMULONII* SELECTED *IN VITRO*

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The widespread use of echinocandin to prevent or treat invasive fungal infections has led to increased appearance of breakthrough infections due to resistant *Candida* species. *Candida haemulonii* complex and closely related species like *C. auris* are some of the rare multidrug-resistant (MDR) species that are often mistakenly identified but have raised as notorious healthcare-associated yeasts causing invasive infections with significant clinical treatment failures. In this context, we aimed to determine the evolutionary trails of echinocandin resistance in *Candida haemulonii*, and the impact of resistance mutations in the *FKS1* gene on fitness. Following direct selection of mutants on caspofungin increased concentrations, we obtained an echinocandin-resistant *C. haemulonii* isolate (*Ch4*'r) that showed high levels of resistance with a minimal inhibitory concentration (MIC) of >16 mg/L to all echinocandin tested (caspofungin, micafungin and anidulafungin). The evolved strains harbored a mutation at position 481 (H481F/Y), in the *HS1* region of *FKS1*. Compared with the corresponding original isolate, the variant isolate had growth, biofilm formation and phagocytosis ratio significantly reduced evidencing the decreased fitness. To confirm the attenuated virulence of the resistant strain, we tested the parental type isolate *Ch4* and the resistant *Ch4*'r in *Galleria mellonella* as a model of disseminated candidiasis. Moreover, the mutant strain showed attenuated virulence in *in vivo* model and a fungal burden decreased in infected larvae along time. The resistant strain *Ch4*'r maintained its *in vitro* resistance profile, since treatment with caspofungin and micafungin did not increase larvae survival nor was it able to reduce the fungal burden. Our findings suggested that the acquisition of pan-echinocandin resistance raised promptly after drug exposure and was associated with a significant fitness cost in *C. haemulonii*.

Keywords: Non-*albicans Candida*, virulence, *Galleria mellonella*, caspofungin, biofilm

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