TITLE: DECIPHERING THE AMPHOTERICIN B RESISTANCE MECHANISMS IN *Candida haemulonii* SPECIES COMPLEX

AUTHORS: SILVA, L.N.[1]; OLIVEIRA, S.S.C.[1]; MAGALHÃES, L.B.[1]; ANDRADE-NETO, V.V.[2]; TORRES-SOUZA, E.C[2].; CARVALHO, M.D.C.[1]; PEREIRA, M.D.[1]; BRANQUINHA, M.H.[1]; SANTOS, A.L.S[1].

INSTITUTION: [1]UNIVERSIDADE FEDERAL DO RIO DE JANEIRO, RIO DE JANEIRO, BRAZIL; [2] FUNDAÇÃO OSWALDO CRUZ , RIO DE JANEIRO, BRAZIL

ABSTRACT:
The polyene antifungal amphotericin B (AMB) exerts a powerful and broad activity against a vast array of fungi and, in general, it displays a remarkably low rate of antimicrobial resistance. AMB acts via ergosterol binding, thereby inducing pores formation at the cell membrane pursuant to ion leakage. Additionally, AMB exerts its effects by inducing the intracellular accumulation of reactive oxygen species (ROS). In this work, we have demonstrated primary evidences of AMB resistance mechanisms in clinical isolates of *Candida haemulonii* species complex comparing with other non-*albicans Candida* species (*C. tropicalis*, *C. krusei* and *C. lusitaneae*) displaying different resistance phenotypes. Our results showed that the AMB-resistant *C. haemulonii* complex isolates presented altered respiratory status as revealed by their poor growth in non-fermented carbon sources, low consumption of oxygen and derisive mitochondrial membrane potential. The use of specific inhibitors of mitochondrial respiratory chain components (complex I-IV) had no effect on yeast growth, indicating that *C. haemulonii* species complex preferably display fermentative metabolism. We also characterized the production of ROS in response to AMB treatment. AMB induces the formation of ROS in all species tested. Moreover, this phenomenon was slightly seen in strains that displayed AMB-resistance phenotype. *C. haemulonii* species complex proved to be highly resistant to oxidative burst agents, which can be correlated with a high activity of antioxidant enzymes. Consistently, AMB failed to induce lipid peroxidation in these resistant strains. GC-MS analysis revealed a marked increase of ergosterol in response to AMB treatment in *C. haemulonii* species complex. Our data reveal that, mitochondrial function, fungal redox homeostasis and ergosterol content are crucial to AMB exerts its fungicidal effects and might explain the resistance presented in this fungal complex. Finally, these data provide evidence to expend novel therapeutic proposals against the multidrug-resistant opportunistic yeast *C. haemulonii*.

Keywords: AMB intrinsic resistance, non-*albicans Candida* species, oxidative stress response, ergosterol, catalase, reactive oxygen species.

Development Agency: CNPq, CAPES, FAPERJ