In vitro and *in vivo* experimental evolution expose a broad diversity of acquired amphotericin B resistance mechanisms and fitness trade-off compensation in *Candida auris*

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ABSTRACT

Candida auris is a recently emerged human fungal pathogen of growing concern due to common (multi)drug resistance. Although it is the first fungal pathogen to be officially considered an urgent antimicrobial resistance threat by CDC, resistance mechanisms have only begun to be studied. Notably resistance to amphotericin B, present in 40 to 60% of all clinical isolates of *C. auris*, remains largely uncharacterized.

This is the first large scale investigation of amphotericin B resistance in *C. auris*, in which we have typed 441 *in vitro* (serial transfer) and *in vivo* (systemic mouse model) evolved *C. auris* lineages from four parental strains of genetically diverse clades. We show a great diversity of acquired resistance responses with resistance magnitude- and clade dependent fitness trade-offs. Genotyping and membrane sterol analyses of selected lineages show membrane sterol alterations due to mutations in genes involved in sterol biosynthesis including *ERG6, NCP1, ERG3, ERG11, HMG1, ERG10* and *ERG12.* Whole genome sequencing identified additional novel resistance mechanisms, including chromosomal aneuploidies, alterations in oxidative stress tolerance, iron homeostasis, sphingolipid and sterol biosynthesis regulation. Furthermore, fitness characterization revealed mechanisms of fitness trade-off compensation, a phenomenon unexplored in pathogenic fungi.

A comparative genome analysis of 773 clinical isolates of *C. auris* showed that a number of strains show allelic variability in genes of interest identified in our study. Overall, the novel mechanisms of resistance and fitness compensation as identified here can pose a significant clinical threat to be taken into consideration in the fight against *Candida auris*.