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The G458S aminoacid substitution in the lanosterol 14-alpha demethylase (Erg11p) is involved in azole resistance in Candida orthopsilosis

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Background: Azole drugs, together with echinocandins represent the main antifungals for the treatment of invasive candidiasis. As shown in previous studies on *C. albicans* and *C. glabrata*, acquired azole resistance relies on various molecular mechanisms, sometimes combined in a single isolate including mutation(s) in the *ERG11* gene or drug efflux. Although *C. orthopsilosis* has been recognized in 2008, conventional phenotypic methods, still used in many clinical laboratories worldwide, do not display enough discriminatory power to identify this species. Additionally, molecular mechanisms leading to azole resistance in this *C. parapsilosis* sibling species have not been yet explored. Here we investigated the mechanisms leading to azole resistance in a clinical strain of *C. orthopsilosis* isolated from a patient with hematological malignancy receiving azole therapy for probable invasive pulmonary aspergillosis.

Material/methods: The clinical isolate *C. orthopsilosis* CAOR2 was isolated from bronchial secretions, in a mixed culture with *Aspergillus fumigatus*, from an autogenic stem cell transplant recipient. The isolate, identified by ITS rDNA barcoding was initially erroneously identified as *C. parapsilosis* by MALDI-TOF (VITEK MS, Biomérieux). Antifungal susceptibility testing was performed using the Etest® method against fluconazole (FLC), voriconazole (VRC), posaconazole (POS), micafungin (MIC),

amphotericin B (AMB) and flucytosine (5FC). FLC resistance was further confirmed by the CLSI reference method. To gain further insights into the mechanisms leading to azole resistance, different primer pairs were designed to sequence the *CoERG11* gene (identified from the available complete genome of the *C. orthopsilosis* Co 90-125 by homology with the *C. albicans* X13296 reference sequence). Three additional isolates, collected from two unrelated patients were included as controls. *In vivo* resistance against FLC (1, 5 or 50µg) was investigated in the larval model *Galleria mellonella.*

Results: Compared to control isolates, CAOR2 exhibited high MICs for FLC (64µg/mL), VRC (1µg/mL) and POS (0.38µg/mL) (although no breakpoints are yet available for this species) but low MICs for AMB and MIC. FLU resistance was confirmed by the CLSI (CMI=32µg/mL). Three heterozygous aminoacid substitutions were identified in the CoErg11p among which, G458S could explain the azole-resistant phenotype of CAOR2, corresponding to G464S, previously confirmed to be involved in azole resistance in *C. albicans* by site-directed mutagenesis. As expected, no improvement in larval survival was noted for CAOR2 when treated with either 1 µg or 5 µg of FLC, confirming *in vivo* resistance. The impact of the G458S aminoacid substitutions in the tridimensional conformation of the CoCyp51A is ongoing.

Conclusions: The present study highlights that azole resistance mechanisms are conserved across *Candida* species and illustrates the utility of invertebrate models, here *Galleria mellonella*, to evaluate *in vivo* resistance of human pathogenic fungi.