Abstract
Introduction: The African continent has the highest burden of HIV infection, accounting for approximately 65% of new infections globally. Oral candidiasis is a major cause of increased morbidity in HIV-infected individuals and is usually treated with fluconazole, an antifungal drug which interferes with ergosterol synthesis in the fungal cell membrane. The increased resistance of Candida species to antifungal drugs leads to treatment failure and an increase in untreatable Candida infections.

Objectives/Methods: Drug susceptibility patterns were determined for Candida species isolated from oral swabs of 254 patients from two HIV-positive African populations. Forty cell membrane fractions isolated from the clinical isolates were analyzed using High Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS) and the results compared to the UniProt protein database for the identification of Candida peptides known to be involved in colonization and drug resistance strategies within the host.

Results: Fluconazole-susceptible and –resistant C. albicans isolates were found to express oxireductases that bind mammalian estrogens with high affinity. Peptides that confer resistance to formaldehyde were found in C. albicans, C. glabrata and C. dubliniensis in both susceptible and resistant cell fractions. Multidrug resistance proteins (CDR1 and CDR2) were seen in C. albicans cell fractions.

Conclusion: The combination of different drug resistance mechanisms and binding abilities to salivary histatins and estrogen seem to be instrumental in the ability of these organisms to colonize and retain antifungal drugs.

Materials and Methods
254 oral Candida isolates were collected from HIV-infected South African and Cameroonian patients presenting with white pseudomembranous plaques on the tongue or other visible oral candidiasis. Approval from the Ethical Committee at the University of the Western Cape, South Africa was obtained.

Confirmation of Candida species was achieved using microscopy, Gram staining and the germ tube test. Growth on Oxoid and Fluka chromogenic media [2] allowed for Candida species identification. Presumptive C. albicans and C. dubliniensis isolates were differentiated by growth at 37°C for 48 hours in Tomato (V8) agar [3]; at 28°C for 48-72 hours in Tobacco agar [4] and at 45°C for 24-48 hours in Sambouraud dextrose agar [5]. Drug susceptibility testing of the isolates was done using the TREK Sensititre YeastOne 9 (Y09) system (Thermo Scientific, USA), a CLSI-approved broth microdilution method.

Forty (40) crude cell membrane fractions isolated from the clinical isolates were prepared using a protein isolation method first described by Niimi et al [6]. The samples were prepared for mass spectrometry analysis using Filter Aided Sample Preparation (FASP). These were subsequently analyzed using High Performance Liquid Chromatography – Mass Spectrometry (HPLC-MS), using a Thermo Scientific EASY-nLC II connected to a LTQ Orbitrap Velos mass spectrometer (Thermo Scientific, Bremen, Germany) equipped with a nano-electrospray source.

The HPLC-MS data results (acquired using the Xcalibur software package) were compared to the UniProt protein database for the identification of Candida peptides known to be involved in colonization and drug resistance strategies within the host.

Results
Identified proteins of interest included SSA1, SSA2 and 90 homolog salivary histatin-binding heat-shock proteins, which affect the fungicidal activity of these human antimicrobial proteins (seen across all tested isolates), estrogen-binding NADPH dehydrogenase oxireductases in C. albicans isolates, pH responsive protein 2 and multi drug resistance transporter proteins CDR1 and CDR2 in C. albicans isolates (table 1).

Discussion
It is known that Candida species express estrogen-binding proteins, which result in a higher predisposition of females to Candida infection [7]. High performance liquid chromatography-mass spectrometry analysis of cell surface fractions obtained from African Candida isolates in this study revealed the presence of oxireductase (probable NADPH dehydrogenase) proteins that bind to mammalian estrogen with high affinity. These findings further elucidate the predisposition of females to Candida colonization.

The expression of Candida proteins that are related to colonization and pathogenicity mechanisms were found in different Candida species, in both drug-susceptible and – resistant isolates. The combination of different drug resistance mechanisms and binding abilities to salivary histatins and estrogen found in Candida species through HPLC-MS analysis seem to be instrumental in the ability of these organisms to colonize immunocompromised patients and resist the action of different chemicals and antifungal drugs.

The presence of a multidrug efflux transporter protein (CDR2) known to confer resistance to azole drugs, which was seen only on a fluconazole-resistant C. albicans cell fraction, appears to demonstrate differences in the protein expression of fluconazole-susceptible and –resistant isolates in the HIV population.

References

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