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Proteomics of drug-resistant HIV-associated candidiasis

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Abstract

Introduction: Candidiasis and HIV co-infection may cause increased patient morbidity and mortality due to oropharyngeal or systemic dissemination. Limited information exists on the prevalence, antifungal susceptibility profiles and drug resistance mechanisms of *Candida* species on the African continent, the highest HIV-affected region globally and home to new and emerging drug resistant *Candida* species.

Methods: *Candida* species isolated from the oral mucosa of HIV-positive African patients were found to be resistant to many of the antifungals routinely used in HIV-associated candidiasis. *Candida* cell membrane fractions were examined using sodium dodecyl sulphate polyacrylamide gel electrophoresis (SDS-PAGE) and high performance liquid chromatography mass spectrometry (HPLC/MS) in order to elucidate the cell membrane proteins specifically expressed by antifungal drug resistant isolates.

Results: SDS-PAGE and HPLC/MS allowed for the identification of multi-drug resistance efflux transporter CDR2 proteins and the elucidation of *Candida* colonization mechanisms and pH-responsive proteins, with significant associations observed between specific drug resistance and the duration of antiretroviral (ARV) therapy.

Conclusion: This study provided useful information on the mechanisms of antifungal resistance in *Candida* species. It also formed the basis for further studies to address the transfer of resistance between *Candida* species in an oral microbial biofilm.

Introduction

According to the WHO [1], Sub-Saharan Africa has the highest rate of HIV infection globally (71%), with *Candida* co-infection contributing to the increased morbidity and mortality seen in immunocompromised individuals.

Access and availability of antimicrobial drugs is often limited in healthcare facilities in most parts of Sub-Saharan Africa while in many parts of Africa, the sale of prescription medications is largely unregulated thereby exacerbating the problem of indiscriminate use and emerging resistance.

Methods

The study sample comprised of 254 African HIV-infected patients who presented at HIV clinics with pseudomembranous plaque on the tongue or visible oral candidiasis. Ethics approval for the study was obtained from the Research Committee at the University of the Western Cape and the study complied with the Declaration of Helsinki (2013). Confirmation of *Candida* species was achieved using conventional routine methods [2] and *C. albicans* and *C. dubliniensis* isolates differentiated as previously described [3-5].

Drug susceptibility testing of the isolates was done using the TREK Sensititre® YeastOne™ platform (Thermo Scientific, USA), a CLSI-approved broth micro-dilution method that provides the minimal inhibitory concentration of nine antifungal drugs, including azoles and echinocandins [6]. Proteomic analysis was performed using gel electrophoresis (SDS-PAGE) and liquid chromatography – mass spectrometry (HPLC/MS) [7] and proteins profiles compared between drug-susceptible and -resistant isolates using the Uniprot protein database search.

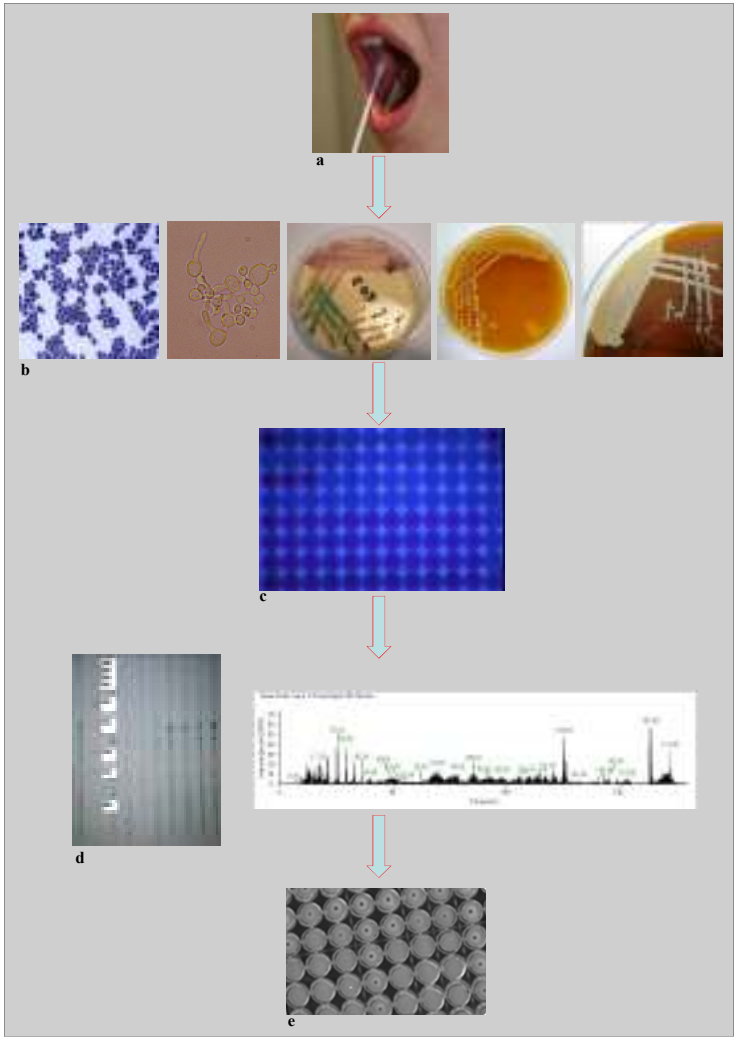

Fig.1. Sample collection (a), *Candida* species identification and differentiation by microscopy, germ tube test and growth on chromogenic and differential media (b), VersaTREK MIC determination (c), proteomic analysis using SDS-PAGE and HPLC (d), *Candida* biofilm formation (e).

Results

The isolated species included *C. albicans* (n=198), *C. glabrata* (n=36), *C. dubliniensis* (n=11), *Candida tropicalis* (n=4), *Candida krusei* (n=3) and either *Candida kefyr*, *Candida parapsilopsis*, or *Candida lusitaneae* (n=2), with Cameroonian patients harbouring double the species of their South African counterparts.

Drug resistance to different classes of antifungals was found to be widespread, especially in the case of azole drugs.

Fluconazole-resistant *Candida* isolates run on SDS-PAGE expressed proteins with molecular weights consistent with that of tropiase (a 24kDa acid proteinase enzyme associated with increased *Candida* virulence) and exoglucanase (a 44kDa protein involved in host cell surface adhesion). In the case of HPLC/MS, a much more detailed spectrum of fluconazole-resistance related proteins was identified, ranging from multi-drug resistance efflux transporter CDR1 and CDR2 proteins and the elucidation of *Candida* colonization mechanisms through the action of oxidoreductases that bind to mammalian estrogen, heat shock proteins that bind to human salivary peptides and mediate their fungicidal activity, pH-responsive proteins which may increase the pathogenic ability of the organisms and S-(hydroxymethyl) glutathione dehydrogenase.

Conclusion

The expression of *Candida* proteins that are related to colonization and pathogenicity mechanisms were found in most *Candida* species, with resistant species expressing further mechanisms of resistance that allow for their survival when exposed to antifungals. This has serious implications for HIV-associated candidiasis in patients on prolonged antiretroviral therapy.

Studies on the formation of *Candida* biofilms and the use of natural alternatives in combating candidiasis and/or restoring the actions of antifungal drugs rendered ineffective due to drug resistance are currently underway in our laboratories.

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