Drug susceptibility profiles of *Candida* species isolated from the oral mucosa of HIV-positive West African patients using the TREK Sensititre system

[1]McArthur C, [2]Abrantes PM, [3]Awasom C, [3]Ayuk L, [2]Africa CWJ

[1]Department of Oral and Craniofacial Science, School of Dentistry, University of Missouri-Kansas City, Kansas City, USA, [2]Oral Microbiology Group, Department of Medical Biosciences, University of the Western Cape, Cape Town, South Africa, [3]Regional Hospital, Bamenda, Cameroon

Abstract

Background: *Candida* infections are a common cause of death in immunocompromised patients. The prevalence and anti-mycotic drug susceptibility profiles of *Candida* species from Cameroon in Africa are unavailable. This study was prompted by an increasing incidence of treatment failure. Drug susceptibility profiles, necessary to improve treatment outcomes, is particularly important in countries where the sale of antimicrobials and antifungals is uncontrolled and resistance may emerge due to the indiscriminate use.

Objective: The goal of this study was to characterize and determine drug susceptibility of oral *Candida* species in Cameroonian patients with HIV/AIDS.

Materials and Methods: *Candida* species were isolated from the oral cavity of 126 HIV-positive patients attending a local HIV/AIDS clinic in the Cameroon. Drug susceptibility to azoles and echinocandins was determined using the commercial TREK Sensititre® YeastOne™ platform that provides the minimal inhibitory concentration of amphotericin B, 5-flucytosine, anidulafungin, caspofungin, micafungin, fluconazole, itraconazole, posaconazole, and voriconazole.

Results: Ninety two isolates identified were *Candida albicans*. Remaining isolates were *C. glabrata* (24), *C. tropicalis* (4), *C. krusei* (3), *C. parapsilopsis/lusitanreae/keyfr* (2), and one isolate was *C. dubliniensis.* More than 50% of *C. albicans* isolated were resistant to azoles but 115 *Candida* species (87%) were susceptible to amphotericin B. Twenty one of the twenty four *C.glabrata* identified (88%) were resistant to micafungin. The majority of Cameroonian *Candida* species were sensitive to flucytosine (5-FC) (95%) and echinocandins (79%).

Conclusions: The report of azole resistance in all *Candida* species isolated from immunocompromised patients in Cameroon is a new and important observation. We found the approach using a broad screening platform an effective means to obtain data rapidly. We propose confirmation of these data and regional surveillance of *Candida* species in other areas in Cameroon and surrounding countries to develop an effective public health management and treatment strategy.

Introduction

HIV-infected individuals are prone to *Candida* species infections, which can result in increased patient morbidity and mortality due to oropharyngeal or systemic dissemination. The need for antifungal drug susceptibility in the African continent is imperative, due to high infection rates, the lack of surveillance studies and the uncontrolled distribution of medications, which can result in increased drug resistance. This is especially true in resource-poor countries, where very few related studies have been done.

The TREK Sensititre YeastOne 9 (YO9) system is a broth microdilution method that provides multiple antifungal drug susceptibility testing. This methodology has the advantage of being standardized to the Clinical and Laboratory Standards Institute (CLSI) standards (Eraso *et al*, 2008, Pfaller *et al*, 2012). The technology consists of microtiter plates embedded with nine different drugs (anidulafungin, micafungin, caspofungin, 5-flucytosine, posaconazole, voriconazole, itraconazole, fluconazole and amphotericin B) in ascending concentrations. The wells are also coated with a colorimetric agent, allowing for the minimum inhibitory concentration (MIC) of each drug to be easily detected both with the naked eye and with the supplied Vizion computer-assisted plate reading system.

The objective of this study was to investigate the drug susceptibility profiles of different *Candida* species isolated from HIV-infected Cameroonian patients using the TREK Sensititre system.

Materials and Methods

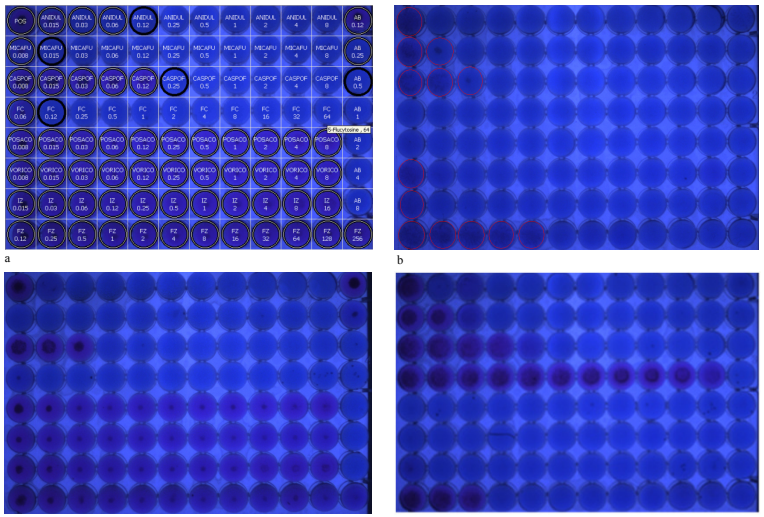
Oral *Candida* samples were collected from 126 HIV-positive patients presenting with white pseudomembranous plaque in the tongue or visible oral candidiasis at the Regional Hospital in Bamenda, Cameroon, by scraping the patient's oral mucosa and tongue using a sterile mouth swab. Ethical clearance for this project was granted by the Ethics Committee of the University of the Western Cape.

The samples were transported to a private laboratory in Bamenda for microscopic examination and inoculation onto *Candida* selective media. The samples were then sent to the Medical Microbiology laboratories at the University of the Western Cape, South Africa, for species confirmation.

Running of the samples on the TREK Sensititre system was done by diluting second-generation *Candida* strains onto sterile phosphate buffered saline tubes to a 0.5 McFarland standard, using the supplied TREK nephelometer. This was followed by vortexing the suspension according to protocol, dispensing 100µl of the solution into the YeastOne broth, dispensing the inoculated broth onto the YO9 plate using an Ovation 25-1250µl multichannel pipette (VistaLab, NY, USA, cat. no. 1160-1250) and incubating for 24 hours at 37°C. The plates were then read using the Vizion plate reader and the TREK SWIN software.

Type strains of *C. albicans* (ATCC 90028 and NCPF 3281), *C. krusei* (ATCC 2159), *C. glabrata* (ATCC 26512), *C. tropicalis* (ATCC 950) and *C. dubliniensis* (NCPF 3949a) served as positive controls for the species differentiation and drug susceptibility testing.

Newly developed species-specific clinical breakpoints were used for the determination of echinocandin drug resistance (anidulafungin, caspofungin and micafungin) for *C. albicans*, *C. tropicalis* and *C. krusei* (Pfaller *et al*, 2012), while CLSI breakpoint categories were used for 5-flucytosine, itraconazole, fluconazole and amphotericin B (Eraso *et al*, 2008) and proposed breakpoints were used for voriconazole (Pfaller *et al*, 2006). Statistical analysis was done by means of Chi-square tests using SPSS.



a b c d

Fig.1.: Drug panel and different results seen on the TREK Sensititre plates.
a: Different drugs and concentrations of the TREK panel
b: Susceptible strain (growth only in red circled wells)
c: Azole drug resistance
d: 5-Flucytosine drug resistance

Results

Of the 126 samples, 92 were identified as *C. albicans*, 24 as *C. glabrata*, 4 as *C. tropicalis*, 3 as *C. krusei*, 2 as *C. parapsilopsis/lusitaneae/kefyr*, and 1 as *C. dubliniensis*.

The results from Table 1 demonstrate that *C. albicans*, *C. tropicalis* and *C. krusei* strains showed no resistance to echinocandin drugs, while *C. glabrata* strains showed high resistance levels against micafungin.

In the case of the azole drugs, the reverse was seen: *C. albicans* strains were more resistant to azoles (greater than or equal to 50% resistance in all azoles for *C. albicans*), with *C. glabrata* responding better to this class of drugs. The *C. dubliniensis* strain and two species identified as *C. parapsilopsis/lusitaneae/kefyr* showed no resistance to azole drugs, with *C. tropicalis* strains showing susceptibility to both fluconazole and voriconazole.

Posaconazole results were not included in this study, as there are no breakpoints for this drug. However, this antifungal showed very similar results to voriconazole. 5-flucytosine showed very promising results against most species tested. The exceptions were *C. krusei*, with only one isolate (33.3% of total) showing intermediate resistance, and *C. albicans*, where 5.4% of isolates were totally resistant. All *Candida* species contained isolates that were completely resistant to amphotericin B.

		C. albicans	*C. glabrata*	*C. tropicalis*	*C. krusei*	*C. para/lusi/kefyr*	*C. dubliniensis*	Spp/resistance associations
Amphotericin B	Susceptible	88	23	2	1	1	0	p=0.001
	Intermediate	0	0	0	0	0	0	
	Resistant	4	1	2	2	1	1	
5-Flucytosine	Susceptible	86	24	4	2	1	1	p=0.265
	Intermediate	0	0	0	1	0	0	
	Resistant	6	0	0	0	0	0	
Anidulafungin	Susceptible	92	16	4	3	-	-	p=0.000
	Intermediate	0	5	0	0	-	-	
	Resistant	0	3	0	0	-	-	
Caspofungin	Susceptible	92	16	4	3	-	-	p=0.000
	Intermediate	0	7	0	0	-	-	
	Resistant	0	1	0	0	-	-	
Micafungin	Susceptible	92	3	4	3	-	-	p=0.000
	Intermediate	0	5	0	0	-	-	
	Resistant	0	16	0	0	-	-	
Fluconazole	Susceptible	45	16	4	1	2	1	p=0.041
	Intermediate	1	7	0	0	0	0	
	Resistant	46	1	0	2	0	0	
Itraconazole	Susceptible	44	5	1	1	2	1	p=0.044
	Intermediate	1	15	3	2	0	0	
	Resistant	47	4	0	0	0	0	
Voriconazole	Susceptible	46	23	4	2	2	1	p=0.000
	Intermediate	0	0	0	0	0	0	
	Resistant	46	1	0	1	0	0	

Table 1: Overall drug susceptibility results according to *Candida* species (- : no breakpoint available for the organism/drug). The different *Candida* species identified in the study are seen in the x-axis, with the different drugs on the YO9 plate and respective susceptibility breakpoints shown in the y-axis.

Discussion

The need for a simple antifungal drug susceptibility platform for use in clinical laboratories is imperative, as the emergence of resistant *Candida* species is a cause of concern. The use of the TREK Sensititre platform for drug susceptibility testing can be done rapidly and with minimal training and reagents and is therefore a promising method for use in resource-limited laboratories in Africa.

This study points out that fluconazole, the most widely available medication for *C. albicans* infections in the African continent, is only working on half of the patients or less. The same occurs in the case of the other azole drugs tested. It is suggested that the dispensing of these antibiotics should not be arbitrary, as this procedure promotes antibiotic resistance. Dispensing should be based upon known antibiotic susceptibility profiles of the *Candida* species present in the local population.

We emphasize that there is a need for regional surveillance of *Candida* species, as this study has shown that certain species, such as the prevalent *C. albicans* do not respond to specific antifungal drugs that might be dispensed empirically.

References

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We wish to thank Dr. Nadine Sullivan and Cynthia Knapp at Thermo Fisher Scientific for their advice and expertise in conducting this project.