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Multi-drug resistant (MDR) oral *Candida* species isolated from HIV-positive patients in South Africa and Cameroon

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Abstract

Candida species are a common cause of infection in immune-compromised HIV-positive individuals, who are usually treated with the antifungal drug, fluconazole in public hospitals in Africa. However, information about the prevalence of drug resistance to fluconazole and other antifungal agents on *Candida* species is very limited. This study examined 128 *Candida* isolates from South Africa and 126 Cameroonian *Candida* isolates for determination of species prevalence and antifungal drug susceptibility. The isolates were characterized by growth on chromogenic and selective media and by their susceptibility to nine antifungal drugs tested using the TREK™ YeastOne9 drug panel (Thermo Scientific). Eighty three percent (82.8%) of South African isolates were *C. albicans* (106 isolates), 9.4% were *C. glabrata* (12 isolates) and 7.8% were *C. dubliniensis* (10 isolates). Of the Cameroonian isolates, 73.02% were *C. albicans* (92 isolates); 19.05% *C. glabrata* (24 isolates); 3.2% *C. tropicalis* (4 isolates); 2.4% *C. krusei* (3 isolates); 1.59% either *C. kefyr*, *C. parapsilopsis* or *C. lusitaneae* (2 isolates); and 0.79% *C. dubliniensis* (1 isolate). Widespread *C. albicans* resistance to azoles was detected phenotypically in both populations. Differences in drug resistance were seen within *C. glabrata* found in both populations. Echinocandin drugs were more effective on isolates obtained from the Cameroon than in South Africa. A multiple drug resistant (MDR) *C. dubliniensis* strain isolated from the South African samples was inhibited only by 5-flucytosine *in vitro* on the YO9 panel. Drug resistance among oral *Candida* species is common among African HIV patients in these two countries. Regional surveillance of *Candida* species drug susceptibility should be undertaken to ensure effective treatment for HIV-positive patients.

1. Introduction

The chronic nature of HIV infection and the increased incidence of mucosal and disseminated forms of *Candida* infections have necessitated the systemic use of antifungal agents, notably, the azole drugs, fluconazole and itraconazole. Fluconazole is routinely administered for candidiasis in healthcare facilities on the African continent and is also used to treat cases unresponsive to topical antifungal treatment (Powderly et al., 1999).

Widespread and repeated use of azole drugs (Jia et al., 2008) has led to resistance to antifungal therapies; a problem that is apparently spreading widely (Manzano-Gayosso et al., 2008; Luque et al., 2009). Thus, there is an urgent need to determine the extent of this problem on the African continent. High HIV infection rates, the lack of surveillance and the uncontrolled distribution of medications have all contributed to drug resistance that has emerged unchecked. This is especially important in resource-poor countries, where little information and limited resources by which to obtain it, seriously complicates the issue.

Various methods are available for the determination of antifungal drug susceptibility, employing either broth dilution or disk diffusion. These include the use of Yeast Nitrogen Base agar (May et al., 1997) and the methylene-blue and glucose-enriched Mueller-Hinton agar diffusion test, the antifungal disk diffusion medium recommended by the Clinical and Laboratory Standards Institute (2009). However, these time- and resource-consuming methods are being replaced by more modern techniques such as the TREK Vision diagnostic system. The TREK Sensititre YeastOne 9 (YO9) system (Thermo Scientific, USA) is a broth micro-dilution method that provides antifungal drug susceptibility testing for multiple drugs simultaneously and relatively inexpensively. This method has the advantage of being standardized to CLSI standards (Eraso et al., 2008; Pfaller et al., 2012) and consists of microtiter plates coated with nine different drugs in ascending concentrations which provide for the determination of a minimal inhibitory concentration (MIC). The drugs are as follows: the echinocandins (anidulafungin, micafungin and caspofungin), which inhibit β1-3 glucan synthesis in the fungal cell wall; the fluorinated pyrimidine analogue (5-flucytosine), that inhibits protein and DNA synthesis; triazole drugs (posaconazole, voriconazole, itraconazole and fluconazole), which block ergosterol synthesis thereby affecting the fungal cytoplasmic membrane, and a polyene (amphotericin B), which interferes with ergosterol synthesis, leading to cell membrane leakage. The wells are also coated with a colorimetric agent with the advantage that the MIC of each drug can be easily detected with the naked eye and with the supplied Vizion computer-assisted plate reading system (Thermo Scientific, USA). In resource-limited environments the plates can also be read manually with the aid of a simple inexpensive light box.

The objective of this study was to determine the species prevalence and phenotypic drug susceptibility profiles of *Candida* species isolated from HIV-infected African populations in South Africa and Cameroon, using chromogenic and selective media and the TREK Sensititre diagnostic system. This study was prompted by an increasing number of patients being lost to follow-up or failing conventional therapy.

2. Materials and Methods

Approval from the Ministry of Health Regional Hospital Institutional Review Board (IRB) in Cameroon and from the Ethics Committee at the University of the Western Cape in Cape Town, South Africa, was obtained. A total of 212 HIV-positive patients

attending clinics in Khayelitsha (n=18) and Delft (n=204) in the Western Cape, South Africa, and 262 HIV-positive patients receiving routine care from the HIV clinic at the Bamenda Hospital in Cameroon, participated in the study. Samples were collected over a period of 6 months.

Prior to sample collection, the reasons for, and nature of the study were explained to the patients who willingly consented to participate. Only HIV-positive patients presenting with white pseudomembranous plaque on the tongue or other visible oral candidiasis were selected. Included in the study were two South African patients (male and female) who reported that they had started fluconazole therapy at the time of sample collection. Another South African patient had started taking Amphotericin B lozenges at the time of sample collection and two females from Cameroon reported recent fluconazole therapy. The application of adequate exclusion criteria was limited by the fact that we were unable to collect accurate patient history of previous *Candida* infection or antifungal treatment due to incomplete patient records and patients' lack of knowledge of drug names and usage.

Oral swabs were used to collect samples from the affected areas and swabs were plated onto Sabouraud's agar and incubated for 24 hours at 37 ˚C followed by 24-72 hours of growth at 30 ˚C on Fluka chromogenic *Candida* identification agar, (Cat. no. 94382, Sigma-Aldrich, USA) and Oxoid chromogenic *Candida* agar (Cat. no. CM1002A, Oxoid, UK). Confirmation of *Candida* species was achieved using microscopy, Gram staining and the germ tube test.

Presumptive *C. albicans* and *C. dubliniensis* cultures were incubated at 37 ˚C for 2-3 hours in fetal bovine serum to stimulate germ tube production, and the two species further differentiated by growth at 37 ˚C for 48 hours in Tomato (V8) agar (Alves, Linares et al. 2006) at 28 ˚C for 48-72 hours in Tobacco agar (Khan, Ahmad et al. 2004) and at 45 ˚C for 24-48 hours in Sabouraud dextrose agar (Pinjon, Sullivan et al. 1998). Differences in growth, colony morphology and pseudohyphae/chlamydospore expression, allowed for species identification (Messeir et al., 2012).

Type strains of *C. albicans* (ATCC 90028 and NCPF 3281) and *C. dubliniensis* (NCPF 3949a) served as positive controls for the germ tube test, while *C. tropicalis* (ATCC 950) served as a negative control. Type strains of *C. albicans* (ATCC 90028 and NCPF 3281), *C. tropicalis* (ATCC 950), *C. dubliniensis* (NCPF 3949a), *C. glabrata* (ATCC 26512) and *C. krusei* (ATCC 2159) served as quality control organisms for the chromogenic species differentiation and drug susceptibility testing.

Second-generation *Candida* strains were diluted with sterile phosphate buffered saline to concentrations of $1x10^6$ to $5x10^6$ cells per ml, corresponding to a 0.5 McFarland standard, measured using the supplied TREK nephelometer. This was followed by vortexing of the suspension and the addition of 100µl of the vortexed

solution to YeastOne broth (Product code Y3462, Thermo Scientific). The diluted broth was dispensed into the YO9 plate using an automated 25-1250µl multichannel pipette and incubated for 24 hours at 37 °C.
The plates were then read using the Vizion plate reader and analyzed using the TREK SWIN software (Thermo Scientific, USA).

Recently developed species-specific clinical breakpoints were used (Pfaller et al., 2012) to categorise *C. albicans* and *C. tropicalis* as susceptible, intermediate or resistant to echinocandin drugs (anidulafungin, caspofungin and micafungin). CLSI breakpoint categories were used for 5-flucytosine, itraconazole, fluconazole and amphotericin B (Eraso, et al., 2008) and the breakpoints proposed by Pfaller *et al.* (2006) were used for voriconazole. In the case of posaconazole, for which no clinical breakpoints have been established, wild-type MIC values were used as previously proposed (Pfaller et al., 2011). MICs were defined as the lowest concentrations that inhibited growth at 100%. The MIC breakpoints of the different drugs for different *Candida* species are listed in Table 1.

Statistical analysis to demonstrate the association between *Candida* species and drug susceptibility was calculated by means of Chi-square tests using the SPSS 21.0 statistics software ($p<0.05$).

Table 1
Drug susceptibility clinical breakpoints used in this study

	C. albicans			*C. glabrata*		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Anidulafungin	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL	≤0.12 µg/mL	0.25 µg/mL	≥0.5 µg/mL
Caspofungin	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL	≤0.12 µg/mL	0.25 µg/mL	≥0.5 µg/mL
Micafungin	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL	≤0.06 µg/mL	0.12 µg/mL	≥0.25 µg/mL
5-Flucytosine	≤4 µg/mL	8-16 µg/mL	≥32 µg/mL	≤4 µg/mL	8-16 µg/mL	≥32 µg/mL
Itraconazole	≤0.12 µg/mL	0.25-0.5 µg/mL	≥1 µg/mL	≤0.12 µg/mL	0.25-0.5 µg/mL	≥1 µg/mL
Fluconazole	≤8 µg/mL	16-32 µg/mL	≥64 µg/mL	≤8 µg/mL	16-32 µg/mL	≥64 µg/mL
Amphotericin B	<1 µg/mL	_	≥1 µg/mL	<1 µg/mL	_	≥1 µg/mL
Posaconazole	<0.016µg/mL	_	≥0.016µg/mL	<0.5µg/mL	_	≥0.5µg/mL
Voriconazole	≤1 µg/mL	2µg/mL	≥4 µg/mL	≤1 µg/mL	2µg/mL	≥4 µg/mL

	C. tropicalis			*C. krusei*		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Non-susceptible
Anidulafungin	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL
Caspofungin	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL
Micafungin	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL	≤0.25 µg/mL	0.5 µg/mL	≥1 µg/mL
5-Flucytosine	≤4 µg/mL	8-16 µg/mL	≥32 µg/mL	≤4 µg/mL	8-16 µg/mL	≥32 µg/mL
Itraconazole	≤0.12 µg/mL	0.25-0.5 µg/mL	≥1 µg/mL	≤0.12 µg/mL	0.25-0.5 µg/mL	≥1 µg/mL
Fluconazole	≤8 µg/mL	16-32 µg/mL	≥64 µg/mL	≤8 µg/mL	16-32 µg/mL	≥64 µg/mL
Amphoteri	<1 µg/mL	_	≥1 µg/mL	<1	_	≥1

cin B				µg/mL		µg/mL
Posaconazole	<0.03µg/mL	_	≥0.03µg/mL	<0.25µg/mL	_	≥0.25µg/mL
Voriconazole	≤1 µg/mL	2µg/mL	≥4 µg/mL	≤1 µg/mL	2µg/mL	≥4 µg/mL

	C. dubliniensis			*C. kefyr/para/lusi*		
	Susceptible	Intermediate	Resistant	Susceptible	Intermediate	Resistant
Anidulafungin	_	_	_	_	_	_
Caspofungin	_	_	_	_	_	_
Micafungin	_	_	_	_	_	_
5-Flucytosine	≤4 µg/mL	8-16 µg/mL	≥32 µg/mL	≤4 µg/mL	8-16 µg/mL	≥32 µg/mL
Itraconazole	≤0.12 µg/mL	0.25-0.5 µg/mL	≥1 µg/mL	≤0.12 µg/mL	0.25-0.5 µg/mL	≥1 µg/mL
Fluconazole	≤8 µg/mL	16-32 µg/mL	≥64 µg/mL	≤8 µg/mL	16-32 µg/mL	≥64 µg/mL
Amphotericin B	<1 µg/mL	_	≥1 µg/mL	<1 µg/mL	_	≥1 µg/mL
Posaconazole	_	_	_	_	_	_
Voriconazole	≤1 µg/mL	2µg/mL	≥4 µg/mL	≤1 µg/mL	2µg/mL	≥4 µg/mL

"-" No clinical breakpoint available for this drug.

3. Results

3.1 Frequency of species

Of the 212 South African samples, 128 (60%) were positive for *Candida* of which 82.8% were identified as *C. albicans* (106 isolates), 9.4% as *C. glabrata* (12 isolates) and 7.8% as *C. dubliniensis* (10 isolates). *Candida albicans* was the most frequently isolated species from both regions. A greater diversity of species was observed among the Cameroonian isolates. One hundred and twenty-six of the 262 Cameroonian samples (48%) were positive for *Candida*, of which 73.02% were *C. albicans* (92 isolates); 19.05% were *C. glabrata* (24 isolates); 3.2% were *C. tropicalis* (4 isolates); 2.4% were *C. krusei* (3 isolates); 1.59% were either *C. kefyr*, *C. parapsilopsis* or *C. lusitaneae* (2 isolates); and 0.79% were *C. dubliniensis* (1 isolate).

3.2. Susceptibility profiles of isolates

All *C. albicans* and *C. glabrata* species isolated from South Africa were susceptible to micafungin and exhibited intermediate resistance to caspofungin and complete resistance to anidulafungin. While more than 50% of the South African *C. albicans* strains were found to demonstrate resistance to all azoles tested (Table 2), *C. glabrata* and *C. dubliniensis* strains demonstrated good overall susceptibility to azoles. A *C. dubliniensis* strain was remarkable in that it was resistant to all the antifungal drugs on the panel except 5-flucytosine in concentrations above 2µg/ml (Table 2).

Table 2
Antifungal susceptibility for South African *Candida* isolates

		C. albicans n=106	*C. glabrata* n=12	*C. dubliniensis* n=10	Spp/resistance associations
Amphotericin B	Susceptible	97	7	9	p=0.01
	Intermediate	0	0	0	
	Resistant	9	5	1	
5-Flucytosine	Susceptible	101	11	10	
	Intermediate	0	0	0	
	Resistant	5	1	0	
Anidulafungin	Susceptible	101	11	_	p=0.000
	Intermediate	3	0	_	
	Resistant	2	1	_	
Caspofungin	Susceptible	98	9	_	p=0.000
	Intermediate	8	3	_	
	Resistant	0	0	_	
Micafungin	Susceptible	106	12	_	p=0.000
	Intermediate	0	0	_	
	Resistant	0	0	_	
Fluconazole	Susceptible	53	8	9	p=0.032
	Intermediate	1	4	0	
	Resistant	52	0	1	
Itraconazole	Susceptible	43	4	9	p=0.008
	Intermediate	1	6	0	
	Resistant	62	2	1	
Voriconazole	Susceptible	49	12	9	p=0.000
	Intermediate	0	0	0	
	Resistant	57	0	1	

"-" No clinical breakpoint available for the organism/drug.

The results from Cameroonian strains showed that with the exception of *C. glabrata*, other *Candida* species, namely, *C. albicans*, *C. tropicalis* and *C. krusei* strains were susceptible to echinocandin drugs (Table 3). In the case of the azole drugs, the

reverse of this pattern was observed: *C. albicans* strains were resistant to azoles (greater than or equal to 50% resistance to all azoles tested). *C. glabrata* was better inhibited by this class of drugs. *Candida dubliniensis* and two species identified as *C. parapsilopsis/lusitaneae/kefyr* were susceptible to all azole drugs tested, while *C. tropicalis* strains were susceptible to both fluconazole and voriconazole.

Table 3
Antifungal susceptibility for Cameroonian *Candida* isolates

		***C. albicans* n=92**	***C. glabrata* n=24**	***C. tropicalis* n=4**	***C. krusei* n=3**	***C. para/lusi/ke* n=2**
Amphotericin B	Susceptible	88	23	2	1	1
	Intermediate	0	0	0	0	0
	Resistant/non-susceptible	4	1	2	2	1
5-Flucytosine	Susceptible	86	24	4	2	1
	Intermediate	0	0	0	1	0
	Resistant/non-susceptible	6	0	0	0	0
Anidulafungin	Susceptible	92	16	4	3	-
	Intermediate	0	5	0	0	-
	Resistant/non-susceptible	0	3	0	0	-
Caspofungin	Susceptible	92	16	4	3	-
	Intermediate	0	7	0	0	-
	Resistant/non-susceptible	0	1	0	0	-
Micafungin	Susceptible	92	3	4	3	-
	Intermediate	0	5	0	0	-
	Resistant/non-susceptible	0	16	0	0	-
Fluconazole	Susceptible	45	16	4	1	2
	Intermediate	1	7	0	0	0
	Resistant/non-susceptible	46	1	0	2	0
Itraconazole	Susceptible	44	5	1	1	2
	Intermediate	1	15	3	2	0
	Resistant/non-susceptible	47	4	0	0	0
Voriconazole	Susceptible	46	23	4	2	2
	Intermediate	0	0	0	0	0
	Resistant/non-susceptible	46	1	0	1	0

"-" No clinical breakpoint available for the organism/drug.

Six South African isolates, 5(4.7%) *C. albicans* and 1(8.3%) *C. glabrata* were resistant to 5-flucytosine, while most of the species isolated in Cameroon were sensitive to 5-flucytosine. The exceptions among the Cameroonian samples were *C. krusei*, where only one isolate (33.3% of total) showed intermediate non-susceptibility and 6 (6.52%) *C. albicans* were totally resistant.

Notably, although isolates were frequently susceptible to amphotericin B, *Candida* species from both populations contained isolates that were resistant (Tables 2 and 3).

Wild-type MIC determinations with respect to both South African and Cameroonian isolates tested, demonstrated resistance / non-susceptibility to posaconazole (84% for *C. albicans* and 41.7% for *C. glabrata in* South Africa and 80.4%, 62.5%, 100% and 66.7% for *C. albicans*, *C. glabrata*, *C. tropicalis* and *C. krusei*, in Cameroon respectively). *C. krusei* is intrinsically resistant to fluconazole and there is a general lack of certainty about the interpretive categories with regard to other azoles. We therefore elected to use the term "non-susceptible" rather than "resistant" to the azoles when referring to *C. krusei* in our results.

Of the 106 *C. albicans* isolated from the South African population, 13 (12.3%) were resistant to 2 classes of antifungals, while 6/92 (6.5%) *C. albicans* isolates from Cameroon were resistant to 2 classes of antifungals. In Cameroon, the *C. albicans* resistance was either to Amphotericin B and the azoles or to 5-flucytocine and the azoles, while in South Africa, *C. albicans* demonstrated resistance to Amphotericin B, 5-Flucytosine, anidulafungin and the azoles in different combinations.

C. glabrata demonstrated resistance to 2 or more antifungals in 3/12 (25%) of the South African isolates and 10/24 (41.7%) isolates from Cameroon. In Cameroon, the antifungal combinations were anidulafungin, micafungin and azoles, while in South Africa, the antifungal combinations were either Amphotericin B and the azoles or anidulafungin and the azoles.

Statistical analysis showed significant associations between *Candida* species and antifungal drug resistance.

4. Discussion

Oropharyngeal candidiasis (OPC) continues to be a common opportunistic infection in patients infected by HIV. It occurs in up to 90% of HIV/AIDS patients during the prolonged course of HIV disease and greatly reduces their quality of life. They are predisposed to recurrent episodes of OPC that can increase in frequency and severity, resulting in increased morbidity and mortality. This is especially true in Africa, where high incidences of mucosal and deep seated forms of candidiasis have resulted in the use of systemic antifungal agents, especially fluconazole and itraconazole. As in other African countries, the present guidelines for South Africa and Cameroon for the management of HIV-positive patients include fluconazole as a first choice drug

for systemic infection (Guidelines, 2009). The widespread use of these antifungal agents have been followed by an increase in antifungal resistance and by a shift from *C. albicans* to non-albicans species prevalence such as those described in this study.

As reported in previous studies from Africa (Blignaut et al., 2002; Hamza et al., 2008), *C. albicans, C. glabrata* and *C. krusei* were the most frequently isolated species from these South African and Cameroonian patients. *Candida dubliniensis* was isolated more frequently from South African patients.

Two South African patients (one male and one female) and two Cameroonian patients (both females) who had recently started fluconazole therapy had visible oropharyngeal thrush at the time of sample collection and their samples yielded the growth of *C. albicans* highly susceptible to all the drugs tested. Another South African patient who had started taking Amphotericin B lozenges at the time of sample collection, also yielded an isolate of *C. albicans* highly susceptible to all the drugs tested except posaconazole,

However, this study reports that over 50% of *C. albicans* isolated from South Africa and Cameroon have developed resistance to fluconazole. A previous report of baseline data from South Africa demonstrated 100% susceptibility of *C. albicans* to fluconazole indicating a marked change in susceptibility. However, and of importance, is that the study was done before the introduction of fluconazole to patients attending HIV-AIDS clinics (Blignaut et al., 2002) and that the patient population was from another province. High resistance levels were observed to other azole drugs tested. Although lower resistance levels have been previously reported from South Africa (Molepo et al., 2006), compared with the current data, other African studies have reported frequent resistance of *C. albicans* and non-albicans species to azoles (Njunda et al., 2012; Mulu et al., 2013). Cross-resistance to fluconazole has been observed in patients receiving itraconazole prophylaxis (Goldman et al., 2000) and other previously administered azole therapies such as ketoconazole and miconazole (Pelletier et al., 2000; Rautemaa et al., 2008). These observations are a cause for concern as fluconazole is the most widely used antifungal available to treat *Candida* infections in HIV patients in South Africa. The same applies in Cameroon and the rest of the African continent. Second choice is itraconazole. Ketoconazole is also used, but has been superseded by fluconazole and itraconazole.

Resistance to amphotericin B was seen with respect to all *Candida* species, with non-albicans species demonstrating especially high resistance levels, particularly *C. glabrata* isolated from the South African population. The South African *C. dubliniensis* isolate which showed resistance to all eight of the nine drugs on the YO9 panel may indicate a serious public health problem, since it suggests the emergence of multiple-drug resistant *Candida* species in the HIV population.

Another finding presenting a clinical challenge was the demonstration of *C. albicans* and *C. glabrata* resistance to 2 or more classes of antifungals in this study, with *C. albicans* being the predominant resistant species in the South African population and *C. glabrata* in the Cameroonian population. The dispensing of these antifungal drugs should therefore be carefully monitored and should be based upon established epidemiological data (Blignaut, Pujol et al. 2002). In this way, *in vitro* resistance may be related to treatment failure (Rogers 2006) and aid in the assessment prevalence of multiple drug resistance in the population.

The rate of relapse and clinical response to therapy varies in different populations. Some HIV-positive patients experience recurrent *Candida* infections with shorter disease-free episodes. They are therefore subjected to numerous courses of antifungals which may ultimately result in antifungal resistance. There is a possibility that the repeated exposure of *Candida* species to antifungal drugs, particularly in the Cameroon, might have led to increased variability in the distribution of *Candida* species with more non-albicans species reported. Species-specific azole resistance has been documented in Brazil (Colombo, Da Matta et al. 2002) and resistance to a specific antifungal drug has been shown to result in cross-resistance to other drugs of the same class (Muller, Weig et al. 2000). This was clearly evident in the present study.

As in many other African countries, street access to antifungals and other drugs without prescription is common in Cameroon. This factor, along with a lack of patient knowledge of antifungal drugs, made it difficult to ascertain whether patients were exposed to these drugs for a long period of time. The possibility exists that specific *Candida* consortia play a role in the acquisition of resistance and with most patients not having access to adequate medical treatment, combined with limited health care resources and the inability of health care workers to monitor the acquisition of antifungals on the street, this problem will escalate leading to an increase in HIV-positive patient morbidity and mortality.

This study demonstrates a need for regional surveillance of *Candida* species on the African continent and improved control over the sale of medications. This study has shown that the prevalent *C. albicans* does not respond to specific antifungal drugs that might be dispensed empirically. *C. glabrata* from Cameroon was resistant to micafungin while South African isolates were susceptible, demonstrating significant regional differences. The reverse of this pattern was seen in the case of 5-flucytosine, thereby re-emphasizing the need for more epidemiological studies.

Limitations of this study include the paucity of patient information (whether early or late presenters of HIV-AIDS), and the lack of patient history of previous episodes of *Candida* infection and treatment. Inconsistencies occur with species differentiation and drug susceptibility techniques due to lack of resources in many African

countries, thus complicating a direct comparison of our results with other studies done in Africa.

Finally, the use of the TREK Sensititre platform in this study for drug susceptibility testing proved a rapid and reliable method requiring minimal training and utilizing available reagents. We suggest this approach may be a promising method for use in resource-limited laboratories in Africa. The TREK system avoids the limitations of current drug susceptibility testing protocols for fungi, as multiple drugs can be tested simultaneously on a single plate using a simple protocol. Furthermore, an inexpensive light box can be used to screen the plates manually.

Standardized methods available from the Clinical and Laboratory Standards Institute (CLSI) are useful for the calculation of clinical breakpoints and epidemiologic cutoff values for reliable in vitro antifungal susceptibility testing. These results indicate that not only does resistance differ from country to country, but also in different regions of the same country.

Programmes on species prevalence and antifungal use and resistance pattern surveillance have been successfully developed and introduced in Europe, Asia-Pacific, Latin America and North America (Cuenca-Estrella et al., 2008; Adriaenssens et al., 2010; Pfaller et al., 2011). The high HIV prevalence and accompanying immunodeficiency in sub-Saharan Africa are strong driving factors emphasizing the need for regional *Candida* surveillance programmes. Changes in drug susceptibility over time serve as a reminder for the need to test clinical *Candida* isolates for sensitivity to antifungal drugs in the effort to improve patient care and reduce patient morbidity and mortality.

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