Idiosyncrasy of oropharyngeal yeast response to antifungal agents used as prophylaxis by HIV infected patients in South-Western Uganda

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The idiosyncrasy of oropharyngeal yeast response to antifungal agents used as prophylaxis by HIV infected patients in South-Western Uganda.

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Abstract

Background: Although cutting edge technologies have advanced overall knowledge of microbial infections, what microorganisms will do when challenged with different surface-active agents is still incompletely understood, in resource-limited settings.
Objective: This study was therefore designed to determine the idiosyncratic in-vitro response of yeast strains, challenged with selected routine antifungal agents used for routine prophylactic management of HIV infected

Methods: A total of 304 (235 females and 69 males) patients with different conditions were recruited and oral lesions collected and analyzed using standard phenotypic methods. The MIC) of the isolates were determined by the E-test stable agar gradient method (AB BIODISK, Solna, Sweden)

Results: All *Candida albicans* strains were sensitive to the azoles except in Masaka District where C albicans showed a sensitive-dose dependent MIC of (0.14 µg/ml) to Itraconazole. District of isolation of yeast strains significantly (p<0.05) impacted on MIC results as different MIC values were obtained after testing similar yeast strains from different districts against the same anti-fungal agents. Thus, intra-species variations in average MIC values were noted among the yeast strains from different Districts. Itraconazoles tested against *C. albicans* showed varying MICs values of 0.05 µg/ml in Mbarara District; 0.03 µg/ml in Rukungiri District and 0.14 µg/ml in Masaka District. Fluconazole tested against *C. albican*s also showed varying MICs of 0.5 in Mbarara District; 0.99 µg/ml in Rukungiri District and 0.14 µg/ml in Masaka District.

Conclusion: Observed intra-species variations in average MIC values noted among the yeast strains from different Districts in the same region of the country signifies idiosyncrasy in yeast response to antifungal agents used in the management of yeast infections in a given locality. Sustained yeast MIC surveillance as panacea to impact treatment outcome is recommended

Background

Oropharyngeal Candidiasis (OC) is one of the Acquired immunodeficiency syndrome (AIDS) defining opportunistic infections caused by Human Immunodeficiency viruses (HIV). Recurrent oropharyngeal Candidiasis common among HIV seropositive patients may be associated with: drug-resistance-related treatment failure (1); non-compliance to drug prescription guidelines (2); use of sub-lethal drug concentration (3-4); re-infection of microbial disease etiology in disease Endemic regions (5) or unruffled progression of HIV disease to AIDS (1-2). To succeed in oropharyngeal lesion management in the immune-suppressed HIV disease, the intervention must be based on what is known about the population structure of the oral microecology and yeast resistance to the treatment regimen (6-9). This should then give rise to bases for new drug development plans, in answer to identified challenges and unmet therapeutic needs so that sustainable intervention for today and tomorrow outbreaks can be contained (10)

To reduce rates of treatment failure, systematic treatment should take place during the fungal growth phase when constant breakdown and re-synthesis of cell walls constituent giving polyenes more access to the sensitive targets of host cells (11). Polyenes and Azoles remain the antifungal agents most commonly used to treat *Candida* infections in immunocompromised patients living in Uganda. The polyenes like amphotericin B and nystatin are fungicidal agents that bind preferentially to the ergosterol found in fungal

membranes (12-13). This disrupts the integrity of the cellular membrane, causing cell death. Resistance to polyenes may develop by modifying the ergosterol molecule or its membrane content and mechanisms of yeast cells resistance to polyenes include replacement of the polyene-binding ergosterol, cholesterol, or stigmasterol with polyene-less binding 3-hydroxy or 3-oxo sterols (1, 3-4). Fluconazole and ketoconazole are fungistatic agents that inhibit cytochrome P450 14a-demethylase, a component of the sterol biosynthesis pathway (11, 14-16). New azole and triazole derivatives [voriconazole, ER-30346, and D0870] and SCH-56592, which is a hydroxylated analog of Itraconazole may be available but they are not reachable to the population that needs them in resource-limited counties like Uganda.

Accepting to live with HIV (17) may be understandable because HIV infections have no clear routine cure yet, but no one would accept to live with recurrent oropharyngeal lesions associated with yeast strains when presumed cures are available (18). Management of HIV-infected patients with recurrent oropharyngeal lesions remains a challenge because of the inherent dilemma associated with the persistence of infection. Thus, It is not easy to determine the cause attribution factors to either HIV disease progression (19) or treatment failure associated with resistance of microbial oral pathogens found in the microecology of the oral cavity (1, 8-9, 20). There is no anti-fungal medication widely available for the majority of HIV-infected patients with oral lesions in Uganda. The majority of HIV patients in Uganda are on prophylactic drugs depending on the disease staging status. They are either routinely given antibacterial, antifungal, or antiviral either separately or together.

There is no organized surveillance to update healthcare managers with the performance of antimicrobial agents used in the prophylactic management of HIV-associated diseases. While in vivo assessment of drug performance when administered to sick people to treat associated infections remain relevant, such an experiment depends on enough representative in-vitro data. This study was therefore designed to determine the idiosyncratic in-vitro response of yeast strains, challenged with selected routine antifungal agents used for routine prophylactic management of HIV-infected TASO patients in Uganda, with the ultimate goal of ascertaining the performance of the prophylactic medications and impacting the outcome of future intervention.

Methods

Three TASO centers in the Masaka, Rukunguri, and Mbarara districts were used for this investigation. The patients sampled were TASO HIV patients with clinically diagnosed oral lesions and receiving co-trimoxazole, Nystatin, and clotrimazole prophylaxis. Approvals were obtained from the Uganda National Council of Sciences and Technology, The AIDS Support Organizations (TASO), TASO clients (patients) through their informed consent. A total of 304 (235 females and 69 males) patients with different oral lesions consented to participate in this study and were therefore recruited. This 304

sample size surveyed was guided by the upper limit required to give a 95% level of confidence at an expected prevalence of about 74% (6) using the precise prevalence formula: Sample size (N) = Z^2P (100-P)/D2 giving 296 samples, (Epi-info version 3.2 database; 1995), where Z is a constant given as (1.96), P is expected prevalence (74%), and D is an acceptable error (5%). Eight (8) more samples were added arbitrarily just in case of drop out and unseen error making a total of 304 samples. The mouth examination of the TASO clients was done by previously trained and calibrated oral clinicians according to WHO standards (21).

All analyses for yeast identification were done at the Microbiology Department, Kampala International University Western Campus. The analysis performed included: cultivation on Sabouraud Dextrose Agar, Corn Meal Agar, Potato Dextrose Agar, germ-tube test, chlamydospore production, test for the ability to grow at 45°C. For further morphologic identification of yeasts, they were first grown on CHROMagar *Candida* medium at 37 ± 2^0C for 24-48 hours and sub-cultured on Pal's agar. (22). Suspect colonies of yeast strains were further identified and differentiated by a biochemical test using sugar assimilation test pattern (ID32 C) for *Candida* species provided by BioMereux(R) Paris, France.

Determination of MIC of fungal Isolates by E-test

The minimum inhibitory concentration (MIC) of the isolates were determined by the E-test stable agar gradient method (AB BIODISK, Solna, Sweden) which

has been standardized and comparable with Clinical Laboratory Standard Institute (CLSI) and European Committee for Antimicrobial Susceptibility Testing (EUCAST) (23-24). This involved placing a plastic strip containing a gradient of an antifungal agent on the surface of an inoculated RPMI(R) agar plate. The drug diffuses into the RPMI(R) agar and establishes a stable concentration gradient. Inhibition of fungal growth produces an ellipse, and the MIC is read where the ellipse intersects the test strip.

The Randomized Block Design (RBD) and Completely Randomized Design (CRD) (α = 0.1 or 99% level of confidence) summarized as [Total sum of squares (SS_T) = Sum of squares due to different effects (SS_D) + Error sum of effects (SS_E)] (25), for one observation per treatment were used to test for the relationship between each yeast strains isolated and the district of isolation and between each yeast strains isolated and antifungal agents in different districts studied. The relation between each yeast response to antifungal used was compared to responses from other yeasts within the same district. Fisher's least significant difference (FLSD; α = 0.05) and Duncan's Multiple Range test (DMRT; α = 0.01) were used to testing for levels of significance of any relationship identified after computation with CRD and RBD.

Results

From (Table 1) below, one hundred and forty-four (144) yeast isolates, obtained from the oropharyngeal lesions of consenting 304 HIV patients on routine co-trimoxazole, Nystatine, and clotrimazole prophylaxis comprised of 100 Candida albicans (34 from Mbarara, 33 were from Masaka and 33 from Rukungiri); 10 C glabrata (4 from

Mbarara, 3 were from Masaka and 3 from Rukungiri), 3 C sake (2 from Mbarara, 1 were from Masaka and 0 from Rukungiri), 6 C parapsilosis (2 from Mbarara, 2 were from Masaka and 2 from Rukungiri), 10 C norevegiensis (4 from Mbarara, 3 were from Masaka and 3 from Rukungiri), 5 C tropicalis (3 from Mbarara, 1 was from Masaka and 1 from Rukungiri), 3 Saccharomyces kluverii (1 from Mbarara, 0 were from Masaka and 2 from Rukungiri), 5 Saccharomyces cerevesie (3 from Mbarara, 2 were from Masaka and 0 from Rukungiri), and 2 Zygosaccharomyces species (1 from Mbarara, 0 were from Masaka and 1 from Rukungiri) (Table 1).

MIC Result interpretation, based on CLSI formerly (NCCLS) guideline for antifungals were as follows: Fluconazole**:** MIC<8µg/ml =sensitive; Fluconazole**:** MIC>8µg/ml and ≤32µg/ml = sensitive dose dependent (SDD) and MIC>32µg/ml = resistant. For Itraconazole: MIC ≤ 0.125µg/ml = sensitive; MIC> 0.125µg/ml and ≤ 0.5 µg/ml = sensitive dose dependent and MIC > 0.5 µg/ml = Resistant. For Amphotericin B: *Candida* species isolates with Amphotericin B with MIC > 0.5µg/ml should be considered resistant because specific breakpoints have not been proposed and intra-laboratory variations mandates the use of reference isolates with known resistance.

Figures 1-6 shows representative pictures of yeast response pattern to tested antifungals and so formed the basis for which different yeast strains were declared either sensitive, sensitive dose-dependent, or resistant to fluconazole, Itraconazole, and amphotericin B respectively. The average MIC results across the Districts surveyed, show that all *Candida albicans* strains were sensitive to the azoles except in Masaka District where C albicans showed a sensitive-dose dependent MIC of (0.14 µg/ml) to Itraconazole. In selection, Amphotericin B in Masaka showed a sensitive MIC of 0.125 µg/ml while *C. glabrata* was: resistant to Itraconazole (MIC>32.0 µg/ml); and its sensitivity to Fluconazole (MIC = 12.0 µg/ml) was dose-dependent in all districts surveyed. (Table 1)

Candida sake showed a uniform dose dependent sensitivity to Itraconazole (MIC = 0.5 µg/ml), fluconazole (MIC = 0.9 µg/ml) and Amphotericin B (0.25 µg/ml). *C. parapsilosis* showed uniform sensitivities to Itraconazole and Fluconazole (MICs = 0.01 µg/ml and 0.38 µg/ml). *C. norevegensis* showed uniform resistance to both Amphotericin B (MIC=0.75 µg/ml) and Itraconazole (mean MIC = 2.0 µg/ml). However, *C. norvegensis* sensitivity to fluconazole (MIC=8.0) was dose-dependent. *C. tropicalis* and *Saccharomyces kluyverii* was sensitive to all the antifungals used in all 4 Districts with mean MICs ranging from 0.05 µg/ml to 1.0 µg/ml. *Saccharomyces cerevesiae* was sensitive to Fluconazole (MIC = 2.0 µg/ml) and resistant to Amphotericin B (mean MIC = 0.9 µg/ml) in all the Districts. Finally, *Zygosaccharomyces species* was uniformly resistant to Itraconazole (MIC = >32 µg/ml) and sensitive to Fluconazole (MIC = 0.75 µg/ml) in all Districts surveyed (Table 1)

When MIC results within each District and among the 9 representative yeast strains tested were compared, *C.*

parapsilosis in Mbarara District, showed more sensitivity to Itraconazole, (MIC = 0.01 µg/ml). This sensitivity was followed by *C. albicans* and *C. tropicalis* (MIC = 0.05 µg/ml each). *C. sake* (MIC = 0.5 µg/ml) showed dose-dependent sensitivity.*C. glabrata, C. norvegensis* and *Zygosaccharomyces sp* were all resistant to Itraconazole with MIC values of >32.0 µg/ml, 2.0 µg/ml and >32.0 µg/ml respectively.

In Mbarara District, all the representative 9 yeast strains were sensitive to Fluconazole with *Candida sake* being the most sensitive (0.09 µg/ml) followed by: *C. parapsilosis* (0.38 µg/ml); *C. albicans* (0.5 µg/ml); *Zygosaccharomyces species* (0.75 µg/ml); *C. tropicalis* (1.0 µg/ml); *Saccharomyces cerevasiae* (2.0 µg/ml) and *Saccharomyces kluyverii* (3.0 µg/ml). *C. glabrata* and *C. norevegensis* had dose dependent sensitivities of (MICs 12.0 µg/ml and 8.0µg/ml). *C. albicans* showed sensitivities of (MICs = 0.99 µg/ml; 1.06 µg/ml) in Rukungiri and Masaka Districts.   In Mbarara District the most sensitive yeast strain to Amphotericin B were *C. glabrata* and *C. sake* (MIC = 0.25 µg/ml), followed by *C. tropicalis* and *Saccharomyces*

kluyverii (MIC = 0.38 µg/ml). *C. norvegensis* and *Saccharomyces cerevesiae* were resistant to Amphotericin B (MICs = 0.75 µg/ml & 9.0 µg/ml).

District of isolation of yeast strains significantly (p<0.05) impacted on MIC results as different MIC values were obtained after testing similar yeast strains from different districts against the same anti-fungal agents. Thus, intra-species variations in average MIC values were noted among the yeast strains from different Districts. Itraconazoles tested against *C. albicans* showed varying MICs values of 0.05 µg/ml in Mbarara District; 0.03 µg/ml in Rukungiri District and 0.14 µg/ml in Masaka District. Fluconazole tested against *C. albican*s also showed varying MICs of 0.5 in Mbarara District; 0.99 µg/ml in Rukungiri District and 0.14 µg/ml in Masaka District. Itraconazole MIC tested against *C. norvegensis* was 2.0 µg/ml in Mbarara and Rukungiri Districts but was 0.125 µg/ml in Masaka District. Fluconazole tested against *C. tropicalis* showed different MICs of 1.0µg/ml in Mbarara District and 0.38 µg/ml in Rukungiri and Masaka Districts.

Table 1: Break-point mean Minimal Inhibitory Concentration (MIC) of Azoles and Polyene against selected representative *Candida* strains from the population surveyed **n=144**

Yeast strains	Mean MIC (µg/ml) in Districts									
	Mbarara			Rukungiri				Masaka		
	NT(IT	FL	AM)	NT(IT	FL	AM)	NT(IT	FL	AM)	No (%)
C. albicans (**n=100**)	34[0.05 ^{S}	0.5^S	Nil]	33[0.03 ^{S}	0.99 ^{S}	Nil]	33[0.14^{SDD}	1.06 ^{S}	0.125 ^{S}]	100(69.4)
C. glabrata (**n=10**)	4[>32 ^{R}	12 ^{SDD}	0.25 ^{S}]	3[>32 ^{R}	12 ^{SDD}	0.25 ^{S}]	3[>32 ^{R}	12 ^{SDD}	0.25 ^{S}]	10(6.9)
C. sake (**n=3**)	2[0.5^{SDD}	0.09^S	0.25 ^{S}]	1[0.5^{SDD}	0.09 ^{S}	0.25 ^{S}]	0[Nil	Nil	Nil]	3(2.1)
C. parapsilosis (**n=6**)	2[0.01^S	0.38 ^{S}	Nil]	2[0.01 ^{S}	0.38 ^{S}	Nil]	2[0.01 ^{S}	0.38 ^{S}	Nil]	6(4.2)
C. norevegen (**n=10**)	4[2.0 ^{R}	8.0 ^{SDD}	0.75 ^{R}]	3[2.0 ^{R}	8.0 ^{SDD}	0.75 ^{R}]	3[0.125 ^{S}	8.0^{SDD}	0.75^R]	10 (6.9)
C. tropicalis (**n=5**)	3[0.05 ^{S}	1.0 ^{S}	0.38 ^{S}]	1[0.05 ^{S}	0.38 ^{S}	0.38 ^{S}]	1[0.05 ^{S}	0.38 ^{S}	0.38^S]	5 (3.5)
Sacch. kluyverii (**n=3**)	1[Nil	3.0 ^{S}	0.38 ^{S}]	0[Nil	Nil	Nil]	2[Nil	3.0 ^{S}	0.38 ^{S}]	3 (2.1)
Sacch. cereves (**n=5**)	3[Nil	2.0 ^{S}	0.9 ^{R}]	2[Nil	2.0 ^{S}	0.9 ^{R}]	0[Nil	Nil	Nil]	5 (3.5)
Zygosacch. sp (**n=2**)	1[>32 ^{R}	0.75 ^{S}	Nil]	0[Nil	Nil	Nil]	1[>32 ^{R}	0.75 ^{S}	Nil]	2 (1.4)

IT= Itraconazole, **NT**= No of isolates tested, **FL**= Fluconazole, **AM**= Amphotericin B, **Sacch**. = Saccharomyces, **S**= Sensitive, **SDD**= Sensitivity is Dose Dependent, **R**=Resistant, **Nil**= Antibiotics not tested against the corresponding fungi. Using Randomized Block Design (α=0.05), no significant difference (p>0.05) in MIC observed between district **(a)**; there was a significant difference (p<0.05) in MIC between yeast strains **(b)** and no significant difference (p>0.05) in the interaction of different antifungal agents used against different yeasts in the districts surveyed **(c)**. n= total number tested. a= 0.00, b= 3.905, c= 0.001. MIC 90 is the MIC at which 90% of tested isolates are inhibited by agents
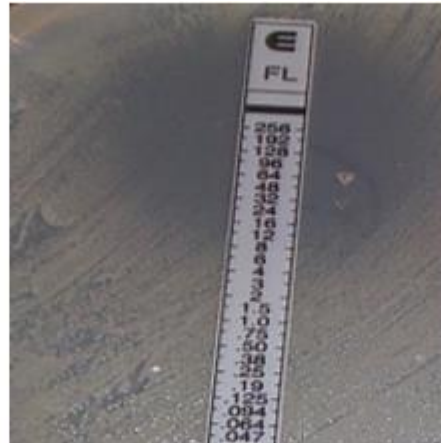
Fig 1: Fluconazole= sensitive   0.5   Fig 2: Fluconazole = 12 SDD,   Fig 3: Itracon MIC = 0 is resist

Fig 4: Itracon MIC = 0.064 sense   Fig 5: Amphot B MIC = 0.1 Sens Fig 6 AP: MIC = 2 Resistant

Included in these manuscripts are the following figures which depict representative pictures of E test results of Fluconazole, Itraconazole, and Amphotericin B showing resistance, sensitive dose dependence, and sensitive results respectively

Discussions

Although cutting-edge modern technologies have advanced overall knowledge of microbial infections with a consequent better outcome, what microorganisms will do when challenged with different surface-active agents is still vague and incompletely understood. Disease diagnostic precision remains far below the minimum threshold needed to yield a meaningful and reliable outcome, especially in resource-poor settings. Understanding the idiosyncrasy or characteristic uniqueness of an organism when challenged with antimicrobial agents is a huge challenge to many antibiotic stewardship programs in resource-limited settings. Perhaps the unpredictability of microbial response to surface-active agents' emphasis the need to embrace the use of tests that are more sensitive and specific for a more result-oriented outcome.

Oropharyngeal lesions remain a significant health challenge (26) that HIV infected population must overcome for a better life

because many questions need hard-to-get answers about possible disease comorbidities that could delay or prolong the healing of such oropharyngeal lesions. Confirmatory investigations are needed to determine if prolong or delayed healing of oropharyngeal lesions could be linked to drug-resistance (1), non-adherence to prescription guidelines (2)), abuse of antibiotics with sub-lethal doses (3-4), re-infection (5), the progression of HIV disease to AIDS (1-2) or resistant opportunistic infections (27). With the increasingly notorious nature of yeast as predominant opportunistic pathogens in oropharyngeal lesions of HIV/AIDS patients, it becomes necessary to determine the characteristic vulnerability of yeasts to routine prophylactic antifungal agents to achieve better intervention.

In this study, *Candida albicans* were sensitive to fluconazole, Itraconazole, and Amphotericin B respectively (Table 1; Fig 1, 4-5). Dose-dependent sensitivity to various antifungal agents used was also shown by *Candida glabrata* (MIC=12.0), *Candida sake* (MIC=0.5), and *Candida norevegensis* (MIC=8.0) respectively (Fig 2). This may indicate the possible emergence of antifungal drug resistance. Again, it is not clear why yeast strains' development of dose-dependent sensitivity was different in different TASO centers because disease management policies are presumed to be the same in these centers. The observation that same yeast species from different centers under the same prescription and TASO management policy yielded different MIC, shows the unique idiosyncratic nature of yeasts response to the treatment regimen and this makes it hard to extrapolate results across centers. Thus inter-regional surveillance to monitor the emergence and re-emergence of yeast susceptibility in a locale antimicrobial stewardship program is urgently needed.

While *C. albicans* remain the leading aetiology of candidiasis (28-30), reports that infections caused by other nonalbicans yeast species are globally gaining more research attention with species varying between different geographic regions. This is problematic, because resistance to fluconazole may also mean resistance to other azoles, and resistance is commonly controlled by point mutation and efflux pumps (31-32). The rates of azole resistance may differ between institutions due to prescribing patterns of clinicians for both the treatment of and prophylaxis against pathogenic candidiasis (33). In India, *C. tropicalis* is the predominant species, and rates of fluconazole resistance may vary significantly (34). The prevalence of *C. parapsilosis* was similar to *C. albicans* in some Chinese hospitals (35). Fluconazole susceptibility is also highly variable between institutions, with some reporting as high as 50% in intensive care units (35).

In Table 1 and Fig 1-5, all yeast isolates showed dose-dependent sensitivity (DDS), susceptibility to both azoles and polyene antifungals, while *C. glabrate, C. norvegensis* and *Zygosaccharomyces* species exhibited reduced susceptibility (SDD) to fluconazole and were resistant to Itraconazole in all studied districts. While the advantages of prophylaxis in the HIV-infected population cannot be denied, the emergence of sub-population showing reduced sensitivity may be announcing the imperatives of new prophylactic algorithms in immunocompromised HIV-infected population living in resource-limited countries like Uganda. Again, while prophylactic medication usually has a visible entry plan for infected patients, exit plans are often not stated, omitted, or ignored by drug prescribers in Uganda. Patients on prophylactic drugs, therefore, continue to use the drugs even when the efficacy of the drugs decreases (36).

Non-albicans species, including *C. glabrata,* have known innate resistance to antifungal drugs (4, 37-38). The discrepancy between this report (Table 1) of reduced susceptibility and known innate resistance of *C. glabrata* may be explained by a possibility of misdiagnoses with other phenotypically similar yeast strains. Thus, the phenotypic similarity between *C. glabrata, C. nivariensis* , and *C. bracarensis,* could be misdiagnosed as *C. glabrata* in the absence of good confirmatory typing methods (39). There may be the emerging generation of *Candida glabrata* which may not be highly resistant to conventional antifungals as previously anticipated because sensitivity to *Candida glabrata* reports is beginning to emerge from different geographical settings (37-38, 40-42). Reports of *C. glabrata* resistant from some African countries include Cameroon (43), Ethiopia (44), and Tanzania (45), while reduced or dose-dependent sensitivity to *C. glabrata* has been reported in South Africa (43) and Nigeria (42). The result of these studies is similar to most reported studies reporting that C. globate resistance/dose-dependent sensitivity to fluconazole but selectively sensitive to other antifungal agents such as nystatin, and amphotericin B, which are similar to the result of this study. Despite this window of hope for non-albicans C glabrata susceptibility, resistant strains have increased in patients presenting with candidiasis in recent years (46) with increased mortality rates (39).

To understand the emergence of this dose-dependent sensitivity in Candida albicans and non-Candida albicans species cohabiting the microecology of the oropharyngeal cavity of HIV patients, Melo *et al.* (47), found the high-frequency transfer of plasmid DNA when pathogenic and food-borne yeasts were grown together even though many pathogenic yeast species are asexual and therefore not involved in intra- or inter-species mating. This high-frequency transfer of plasmid DNA could define the observed emergence of dose-dependent sensitivity by oropharyngeal *Candida albicans, Candida glabrata, Candida sake,* and *Candida norevegensis* respectively (Tables 1).

Micro evolutionary high-frequency transfer of plasmid DNA (through cell lyses, or cytoduction) has been proved between *C. glabrata* and *Saccharomyces cerevesiae* (48). Although factors that govern the evolutionary revolution of micro-organisms are not yet completely understood, it is thought that environmental and nutritional factors are key to this evolutionary revolution of micro-organisms that can make them change from the original characteristics of their ancestral genealogy (the wild type) to something new (48-49). Microbial evolution tends to remove with time what will not assist its bid to survive in a particular environment and acquire new characters that will assist the organism to manipulate its environment (50) thus accounting for the suggested emergence of dose-dependent sensitivity in the reported yeast strains (51). Besides, despite the "asexual" nature of *C. glabrata*, a potential cryptic sexual life cycle could promote rare interspecies mating with C albicans and other non albicans species of Candida (52).

From (Table 1, fig 1-6), *C. norvegensis, C glabrata, Saccharomyces cerevisiae* and *Zygosaccharomyces* species isolated from different districts showed a resistant MIC to Itraconazole, and Amphotericin B respectively. Immunosuppression, previous exposure to oral azoles, Clinical, cellular and molecular factors are known to contribute to the development of antifungal drug resistance (53-54). Clinical factors leading to treatment failure of the refractory disease may be associated with

the patient's immune status (55), pharmacology of the drugs (13), or type of fungal infection (8). Cellular factors could be the result of the replacement of a susceptible strain with a more resistant species or the alteration of an endogenous strain (by mutation or gene expression) to a resistant phenotype.

Molecular factors responsible for the resistance phenotype in the cell identified have been reported to include overexpression of two types of efflux pumps, mutation of the target enzyme, and alteration of other enzymes in the same biosynthetic pathway as the target enzyme (53, 11). Generally, azoles and polyenes' actions are mainly directed against ergosterol, the major sterol of the fungal plasma membrane, which is analogous to cholesterol in mammalian cells, (13-14). The dosing schedule may affect the development of resistance (4) since patients treated with intermittent therapy were more likely to develop resistance than those treated continuously. Primary resistance and secondary resistance have been documented in HIV-infected patients (56). Although symptomatic candidiasis develops more commonly due to *C. albicans*, candidiasis is known to be caused also by non-*albicans Candida* sp. Fluconazole-resistant species, *Candida norvegensis,* have recently been reported as pathogens in patients receiving fluconazole (41).

The polyenes are a known class of amphipathic antifungal drugs that target membranes containing ergosterol forming a channel through which cellular potassium ions, leak and thereby destroying the proton gradient within the membrane (11, 57). Amphotericin B has an antioxidant effect in vivo, protecting fungal cells against oxidative attack from the host (11, 58). Reports of Amphotericin B resistance are limited, but it appears

prolonged pre-exposure to azole antifungal drugs and severely immunocompromised patients, especially patients with cancer, are at the highest risk perhaps explained by an alteration of cellular membrane components (59). Amphotericin B-resistant clinical isolates of *C. tropicalis* have also been found (11, 58). Minimum inhibitory concentration (MIC) of tested antifungal agents (Table 1) was significantly ($p < 0.05$) dependent on strains of Candida species isolated from oropharyngeal lesions of the studied population. Thus, the types of oropharyngeal yeast strains present in the oral lesions significantly ($p < 0.05$) impacted the MIC values as well as the efficacy of antifungal agents tested.

There is evidence to suggest that intermittent versus continuous dosing, the amount of drug administered, the length of treatment (27), the immune status of the patient, and alterations in the membrane structure or the sterol-to-phospholipid ratio in the membrane may be associated with resistance (53, 11, 58). The success or failure of antifungal drug therapy is dependent not only on the drug but also on the immune status of the host and a combination of azoles and cytokines may be an important therapeutic strategy for resistant fungal infections in immunocompromised individuals.

The susceptibility of *C. norvegensis* was significantly ($p < 0.05$) dependent on the types of antifungals used. The depicted different levels of exposure to various antifungal agents tested may be a pointer to the emergence of resistant clones of oropharyngeal yeast strains among the HIV-infected patients studied. The frequency of diagnosed fungal infections is known to have risen due to an increase in the number of immunosuppressed patients resulting from the AIDS epidemic and increase in the frequency of recalcitrant infections to standard antifungal therapy

(13-14), Susceptibility of isolates to antifungals varied and MIC values were significantly (p<0.05) dependent on the district of sample collection. Thus, despite being treated with the same guidelines by clinicians in the same TASO centers, patient's uptake and compliance to treatment guidelines were different, thus depicting different levels of exposure to antifungals agents and probable prescription patterns. Fluconazole-resistant *Candida norvegensis,* have been reported as pathogens in patients receiving fluconazole (41, 24, 60). It has been suggested that selection for resistant strains occurs with azole treatment but nosocomial transmission and transmission of resistant strains between partners can also occur (53). The polyene is an amphipathic drug that intercalates into membranes containing ergosterol, forming a channel through which cellular components, especially potassium ions, leak and thereby destroying the proton gradient within the membrane (57, 24, 60). Amphotericin B resistance in *Candida* spp. can develop in patients previously exposed to azoles (59). Several strains of fluconazole- and amphotericin B-resistant *C. albicans* have been found in HIV-infected patients who have received prolonged courses of azole developed resistance with azoles (59).

Underlying medical conditions of the immunocompromised HIV-infected population studied together with the type of antifungal used, district of yeast isolation, and strains of yeast isolate significantly (p<0.05) impacted on the MIC values obtained in this investigation (Table 1). To justify the role of underlying medical conditions in the healing of oral lesions in the immunocompromised HIV infected population (60), Nube et al (61) summarised specific risk factors of delayed wound healing to include but not limited to: infection, ischemia, ulcer size,

depth, and duration as well as probing to bone (or osteomyelitis), location of the ulcer, sensory loss, deformity (and high plantar pressure), advanced age, number of ulcers present and renal disease with ulcer depth (or size), infection, and ischemia listed as the most common risk factors. When there are high-quality treatment protocols and guidelines, 66%–77% of foot ulcers will heal and the remaining group is unlikely to heal, thus supporting the fact that quality of treatment and care impacts on wound healing rate (61). Nonhealing wounds are prevalent in resource-poor settings due to poor treatment protocol while healing or delayed healing wounds are more prevalent in adequate resource settings (62).

Conclusion: In conclusion, Candida albicans remain significantly susceptible to tested antifungal agents. Emerging dose-dependent susceptibility in non albicans oropharyngeal yeast strains is interesting in this era of increasing treatment failures due to drug resistance. The pattern of resistance recorded may suggest a significant contribution of yeast in the recurrent or delayed oropharyngeal lesions of the HIV population studied. Sustained surveillance with MIC which impacts treatment outcome is recommended

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Apendix

1. List of Abbreviations

MIC = Minimum inhibitory concentration, TASO = The AIDS Support Organizations , HIV = Human Immunodeficiency Virus , AIDS = Acquired Immunodeficiency syndrome, OC = Oropharyngeal Candidiasis , CLSI = Clinical Laboratory Standard Institute , EUCAST = EUropean Committee for Antimicrobial Susceptibility Testing , RBD = Randomized Block esign ,CRD = Completely Randomized Design ,FLSD = Fisher's least significant difference , SDD = sensitive dose dependent , AP = Amphotericin B, Flu = Fluconazole, IT = Itraconazole

Ethics approval and consent to participate

This study was approved by Kampala International University, Western Campus, The AIDS Support Organization, Uganda National Council of Science and Technology, Kampala, and research participants' informed consent. All consent was written and there was no verbal consent. Those who could not read or write were allowed to thumbprint to show consent after the research was interpreted in the local language. All participants and stakeholders consented to participate in this study after informed consent as required by the law of Uganda.

Consent for publication

All figures included are original pictures taken by authors during the study. It does not need any consent from anybody to publish. However, the single author of this manuscript gave full consent for this manuscript to be published including others were acknowledged in this work.

Availability of data and materials

All data generated or analyzed during this study are included in the body of the text while figures have been attached as a separate document for your perusal

Authors contributions:

This is not applicable as this is a single-author manuscript. All other state holders who are not authors are listed in the acknowledgment section

Competing interests

Authors' contributions

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