



The impact of polyene, azole, and DNA analogue antimycotics on the cell surface hydrophobicity of *Candida albicans* and *Candida tropicalis* in HIV infection

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Abstract

Oral candidiasis is the most common opportunistic infection in individuals infected with the human immunodeficiency virus. Though *Candida albicans* is the major aetiological agent, non-*albicans* species such *Candida tropicalis* are now emerging as important agents of such infection. The *Candida* cell surface hydrophobicity (CSH) is considered a critical factor contributing to its colonization potential and virulence. It is also known that brief exposure to sub-cidal concentrations of antifungal agents is a likely scenario in the oral environment where the administered drugs are diluted continuously due to the flushing action of saliva. Hence the objective of the present study was to compare the CSH of 10 isolates each of *C. albicans* and *C. tropicalis* from HIV-infected individuals following brief exposure (1hour) of isolates to sub-therapeutic concentrations of nystatin, amphotericin B, ketoconazole, fluconazole and 5-fluorocytosine. The CSH was assessed by a previously described biphasic aqueous-hydrocarbon assay. The mean percentage reduction of CSH of *C. albicans* following brief exposure to nystatin, amphotericin B, ketoconazole, fluconazole and 5-fluorocytosine was 27.33 ($p < 0.001$), 21.34 ($p < 0.05$), 11.74 ($p > 0.05$), 18.4 ($p > 0.05$) and 14.64 ($p > 0.05$) respectively. The mean percentage reduction of CSH of *C. tropicalis* following brief exposure to nystatin, amphotericin B, ketoconazole, fluconazole and 5-fluorocytosine was 33.81 ($p < 0.01$), 28.88 ($p < 0.01$), 12.6 ($p > 0.05$), 21.53 ($p > 0.05$) and 17.68 ($p > 0.05$) respectively. A significant inter-species variation in CSH was observed for nystatin and amphoterecin B. Overall the results reveal that the CSH of *C. albicans* is affected to a significantly lesser degree compared with *C. tropicalis* when exposed to the antifungals. These data further illustrate another mode of action of antifungals on *Candida* leading to a reduction in the CSH and thereby the yeast adherence to host tissues.

Key words: antifungal agents, *Candida*, HIV infection, hydrophobicity

Introduction

Oropharyngeal candidosis is the most common opportunistic infection seen in patients with the human immunodeficiency virus (HIV) infection [1]. As HIV disease progresses and immunosuppression worsens, the incidence and severity of oropharyngeal candidosis increases. This disease is also considered as the commonest oral manifestation in such patients [2]. Although *Candida albicans* is the predominant pathogen, isolated non-*albicans* *Candida* species

such as *Candida tropicalis* is infrequently but consistently isolated from these patients either singly or in combination with *C. albicans* [3, 4].

Multiple factors have been implicated in potentiating *Candida albicans* pathogenicity [5]. Among these candidal adhesion is considered an important prerequisite for successful microbial colonization and infection, and its critical role in the pathogenesis of many fungal infections is widely recognized [6]. Further, increasing recognition of the ecological and medical significance of candidal adhesion to mucosal and inert surfaces has resulted in a vast escalation

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of research effort in this area. Cell surface hydrophobicity (CSH) of *Candida* is considered a physical force that modulates yeast adhesion and also correlates with *Candida* germ tube formation, adhesion to epithelial cells and extracellular matrix proteins, cell wall surface fibril organization and phagocytosis [2, 8, 7]. Furthermore hydrophobic cells demonstrate greater resistance than hydrophilic cells to phagocytic killing. Thus CSH appears to be an important factor in the pathogenesis of *Candida*-induced diseases [8]. Oral *Candida* responds to a variety of antimycotic agents ranging from the classic polyenes (nystatin and amphotericin B) the imidazoles such as ketoconazole, the newer triazoles such as fluconazole [1] to the less commonly used DNA analogue 5-fluorocytosine. Despite the availability of such a spectrum of antimycotics for treatment of oropharyngeal candidosis failure of therapy is not uncommon. In the mouth the diluent effect of saliva and cleansing action of the oral musculature tend to reduce the availability of the agent to below that of the effective therapeutic concentration [9]. Hence the organisms are only suboptimally exposed to the antimycotics and the concentration is likely to vary in different niches of the mouth. Further the development of *Candida* (both *albicans* and non-*albicans*) resistance to antifungal agents in general, and to azoles in particular also contributes to drug failure in HIV-infected patients with oral candidosis [1, 3, 10].

Though it is recognized that CSH is an important attribute contributing to candidal adhesion [11], there are only three reports on the effect of antifungal agents on the CSH of oral *Candida albicans* isolates [12, 13]. However, the impact of these drugs on *C. albicans* and *C. tropicalis* isolates obtained from HIV infected patients has not been studied hithertofore. Hence the main aim of this study was to compare the CSH of 10 isolates each of oral *C. albicans* and *C. tropicalis* obtained from HIV infected patients after their brief (1 hour) exposure to sub therapeutic concentrations of five different antifungals, namely nystatin, amphotericin B, ketoconazole, fluconazole and 5-fluorocytosine.

Materials and methods

Fungi and culture conditions

A total of 10 oral isolates each of *C. albicans* and *C. tropicalis* from HIV- infected subjects with oral candidiasis were used in the study. All isolates of were

from patients attending the Queen Elisabeth Hospital, Hong Kong and were obtained by the oral rinse technique [14]. The procedure involves the patient holding and rinsing 10 ml of sterile phosphate-buffered saline (0.01 M, pH 7.2) in the mouth for 60 s. The solution is then expectorated into a chilled container, immediately transported to the laboratory, 50 μ l of concentrate inoculated on Sabouraud's dextrose agar using a spiral plating system. After 24–48 hrs incubation at 37 °C, the growth is sub-cultured for purity and identified using the germ-tube test and the commercially available API-20C AUX (bioM,rioux, Basingstoke, UK) [15]. Stock cultures were maintained at –20 °C.

After recovery these were maintained on Sabouraud's dextrose agar, stored at 4–6 °C, during the experimental period. *C. albicans* ATCC 90028 and *C. tropicalis* ATCC 13808 obtained from the American Type Culture Collection, USA, were used as the reference strains for the determination of MIC.

Antifungal agents

Five antifungal agents obtained as reagent grade powders were tested: nystatin and amphotericin B (Sigma Chemicals Co. St.Louis,USA) were dissolved in dimethylsulphoxide (DMSO) and absolute ethanol (3:2 ratio), respectively. 5-fluorocytosine (Sigma) was dissolved in sterile distilled water. Ketoconazole (Janssen, Beerse, Belgium) was dissolved in dimethylsulphoxide, and fluconazole (Pfizer Inc. New York, USA) in absolute methanol. All agents were prepared initially as 10,000 μ g/ml solutions and stored at –20 °C until used.

MIC determinations

Broth microdilution method

Antifungal susceptibilities to nystatin were determined as per National Committee for Clinical Laboratory Standards (NCCLS) guidelines [16]. The inoculum was prepared from 24 hours cultures of the yeasts. Cell suspensions were prepared in RPMI-1640 medium and adjusted to field a final inoculum of 10⁵ cfu/ml. Testing was performed in 96-well round-bottomed microtitre plates. The plates were incubated at 35 °C and read at 24 hours. The MIC of nystatin was defined as the lowest concentration at which there was 100% inhibition of yeast growth.

E-test method

The MIC determinations of amphotericin-B, fluconazole, ketoconazole and 5-fluorocytosine were

performed using the E-test. The E-test (AB BIODISK, Solna, Sweden) is a patented commercial method for the quantitative determination of MICs of antimicrobial drugs. Comparisons of the E-test method with the NCCLS broth dilution method have demonstrated high levels of agreement [17].

The inoculum for the E-test was prepared from 24 hours cultures of *Candida* species. Cell suspensions were prepared in sterile distilled water and adjusted to a concentration corresponding to a 0.5 McFarland standard using a spectrophotometer set at 520 nm. The medium used was RPMI-1640 agar (1.5%) with 2% glucose buffered with MOPS (pH 7.0). The plates were inoculated by dipping a sterile swab into the appropriate cell suspension and streaking it across the entire surface of the agar in three directions, and dried at room temperature for 15 min before the E-test strips were applied. Afterwards plates were incubated at 35 °C and read at 24 h. The E-test MIC was read as the drug concentration at which the border of the elliptical inhibition zone intersected the scale on the antifungal test strip.

Preparation of the yeast suspension for the hydrophobicity assays

Yeast cells, maintained on Sabouraud's dextrose agar (SDA), were inoculated onto fresh plates and incubated overnight for 24 hr prior to use. The organisms were harvested and a cell suspension prepared in sterile phosphate buffered saline (PBS) of pH 7.4, at 520nm to an optical density of 1.5. From this cell suspension, 1 ml was added to tubes containing 4ml of RPMI broth (control) and 4ml of RPMI/drug solution (test) in which the drug concentrations were twice the MIC. This gave a cell suspension of 10^6 – 10^7 cells/ml in each assay tube.

The tubes were then incubated at 37 °C for a period of 1 hr in a rotary incubator. Following this limited exposure, the drugs were removed by two cycles of dilution with sterile PBS and centrifugation for 10 minutes at $3000 \times g$. Afterwards the supernatant was completely decanted and the pellets were resuspended in 5 ml of sterile PBS. This washing procedure was then repeated and the pellets resuspended in 2.5 ml of sterile PBS. It has been found by previous investigators that removal of 90% of the supernatant with two washings reduces antimicrobial concentration 100-fold, while complete decanting of the supernatant with two washings (as carried out in the current study) reduces the concentration 10,000-

fold [18]. Hence this method virtually eliminates any "carry-over effect" of the drug following its removal.

Viable counts of the control and test were done by spiral plating after drug removal and control suspensions were reconstituted as needed to obtain a cell concentration comparable to the test.

Hydrophobicity assay

The biphasic hydrocarbon / aqueous method of Sweet et al. [19] was used for the assessment of CSH with slight modifications. In brief, 5 ml of the control and test yeast suspension was vortex mixed and the absorbance measured at 520 nm (Beckman, DU 530 spectrophotometer, Life Science, CA, USA). For each organism tested, 2.5 ml volumes of suspension were added to two sterile glass test tubes, representing one test and one control. 0.5 ml of xylene was added to each test suspension. The test and the controls were placed in a water bath at 37 °C for 10 min to equilibrate, then taken in turn and vortex-mixed for 30 s and returned to the water bath for further 30 min to allow the immiscible xylene and aqueous phases to separate. The lower aqueous phase of the sample was carefully removed using a pipette and transferred to a clean test tube. Any traces of contaminating xylene that may have been carried over in the pipette or bound to the yeast was removed by bubbling air through the suspension at a rate of 180 ml/min, for 2 min. The absorbance was measured as before at 520 nm (Beckman, DU 530 spectrophotometer, Life Science, CA, USA) following vortex mixing for 5 s to disrupt and resuspend any aggregates that may have formed. The hydrophobicity was expressed as the percentage reduction in optical density of the test suspension compared with the control. Thus, the greater the change in absorbance, the greater the shift in yeasts from the bulk medium to the interface, i.e., the more hydrophobic the yeast strain.

Each assay procedure was performed on three separate occasions with duplicate determinations each time.

Results

*Effect of absolute methanol and DMSO/absolute ethanol on *C. albicans* and *C. tropicalis**

Since the antifungal agents used were dissolved in either absolute methanol or DMSO/absolute ethanol, equivalent amounts of the latter chemicals were tested initially to ascertain whether they had an effect on the

isolates tested. The minute volumes of the chemicals used did not have any effect on yeast survival/growth when compared with the controls.

Minimum inhibitory concentrations (MIC)

The MIC values of the ten isolates of *C. albicans* to nystatin, amphotericin B, fluconazole, ketoconazole and 5-fluorocytosine were 0.78–1.56 µg/ml, 0.19–0.38 µg/ml, 0.12–0.38 µg/ml, 0.012–0.016 µg/ml and 0.09–0.12 µg/ml, respectively, whereas the MIC values of the ten isolates of *C. tropicalis* to nystatin, amphotericin B, fluconazole, ketoconazole and 5-fluorocytosine were 0.78 µg/ml, 0.25–0.38 µg/ml, 0.25–0.50 mg/ml, 0.064–0.12 µg/ml and 0.09–0.12 µg/ml, respectively.

Alterations in CSH of C. albicans isolates following limited exposure to antifungal agents

The relative CSH of the unexposed controls ranged from 12.09 to 22.74 (mean 18.58), whereas the CSH of nystatin, amphotericin B, fluconazole, ketoconazole and 5-fluorocytosine exposed isolates ranged from 9.13 to 15.57 (mean 13.48), 9.94 to 17.47 (mean 14.56), 11.02 to 19.88 (mean 16.38), 9.91 to 18.19 (mean 15.16) and 10.50 to 19.32 (mean 15.81), respectively. The percentage reduction in the CSH following brief exposure to these antimycotics is shown in Table 1. Compared with the control, an overall significant reduction (27.33%) in CSH ($p < 0.05$ for 50% of the isolates) was seen following exposure to nystatin.

Though the mean percentage reduction was lower than that of nystatin (27.33% vs. 21.34%), amphotericin B too elicited an overall significant reduction ($p < 0.05$ for 30% of the isolates) in CSH when compared with the unexposed controls. In contrast the overall reduction in CSH following exposure to the two azoles and the DNA analogue was not significant (Table 1). However the CSH of two isolates (HK9TB and HK36SC) exposed to ketoconazole and a single isolate (HK36SC) exposed to 5-fluorocytosine was significantly reduced by these drugs.

Alterations in CSH of C. tropicalis isolates following limited exposure to antifungal agents

The relative CSH of the unexposed controls ranged from 29.45 to 51.14 (mean 39.71), whereas the CSH of nystatin, amphotericin B, fluconazole, ketoconazole and 5-fluorocytosine exposed isolates ranged from

22.57 to 34.85 (mean 26.03), 23.54 to 34.89 (mean 27.69), 26.44 to 43.21 (mean 34.34), 24.86 to 39.29 (mean 31.10) and 26.20 to 39.99 (mean 32.61), respectively. The percentage reduction in the CSH following brief exposure to these antimycotics is shown in Table 2. Compared with the control, an overall 33.81% ($p < 0.05$ for 70% of the isolates) reduction in CSH was seen following *C. tropicalis* exposed to nystatin. Although this value was lower than that for nystatin (33.81% vs 28.88%), amphotericin B too elicited an overall significant reduction ($p < 0.05$ for 60% of the isolates) in CSH when compared with the unexposed controls. In contrast the overall reduction in CSH following exposure to the two azoles and the DNA analogue was not significant. However one isolate (HK5LF) exposed to ketoconazole was significantly affected by the drug.

Inter-species variation in CSH of C. albicans and C. tropicalis exposed to antimycotics

Statistical analysis of inter-species variation in CSH due to antimycotics exposure of *C. albicans* and *C. tropicalis* isolates revealed that the both polyene drugs had a significantly greater impact on suppressing the CSH of the latter species than that of the former ($P < 0.01$ /Tukey-Kramer Test). However exposure to the azoles and the DNA analogue did not elicit significant interspecies differences akin to the polyene antifungals.

Discussion

Infections due to *Candida* species are increasingly evident as the numbers of HIV infected patients in the community increase, and oral candidosis is considered the most common oral opportunistic infection seen in such patients [20, 21].

Adhesion of *Candida* to host surfaces is considered the initial step in its pathogenicity. This process is rather complex which involves both biological and non-biological factors, and the relative CSH is considered an important pathogenic attribute of *Candida* species pertaining to its adhesion and retention on host surfaces. It is also known that hydrophobic yeasts are more virulent than their hydrophilic counterparts [7].

Brief exposure to sub-therapeutic concentrations of nystatin induced a significant suppression of CSH with 50% of the *C. albicans* and 70% of *C. tropicalis* isolates affected, yielding an overall 27.33%

Table 1. The relative and percentage reduction in cell surface hydrophobicity (Mean and SD) of *Candida albicans* isolates following brief exposure (1 hour) to $\times 2$ MIC of antifungal drugs *in vitro*

| Isolate | Control CSH | NYS | | AMB | | FLU | | KETO | | 5-FC | |
|---------|----------------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|
| | | CSH | %Redn | CSH | %Redn | CSH | %Redn | CSH | %Redn | CSH | %Redn |
| HK1KD | 12.09* | 9.15 | 24.35 | 9.94 | 17.37 | 11.02 | 9.02 | 9.91 | 18.04 | 10.50 | 13.80 |
| HK2OB | 13.70 | 9.13 | 32.80 | 10.84 | 20.89 | 12.02 | 12.37 | 10.65 | 23.96 | 12.22 | 10.96 |
| HK4RB | 16.58 | 12.83 | 23.82 | 13.79 | 16.87 | 14.66 | 12.05 | 13.94 | 16.04 | 14.33 | 14.35 |
| HK5SD | 19.79 | 14.78 | 25.52 | 15.39 | 22.88 | 17.80 | 10.49 | 16.81 | 15.35 | 16.96 | 14.33 |
| HK6SC | 22.74 | 15.56 | 30.73 | 17.47 | 22.16 | 19.88 | 12.27 | 18.19 | 18.86 | 19.32 | 15.16 |
| HK8CA | 21.57 | 15.56 | 26.64 | 17.06 | 20.15 | 19.88 | 7.19 | 18.19 | 14.06 | 19.32 | 10.22 |
| HK9TB | 20.53 | 15.57 | 24.35 | 16.00 | 22.59 | 17.51 | 14.54 | 16.29 | 20.85 | 17.07 | 16.73 |
| HK10OD | 20.88 | 14.86 | 29.10 | 16.45 | 21.33 | 17.68 | 14.55 | 16.20 | 22.51 | 16.43 | 20.63 |
| HK36SC | 17.84 | 13.39 | 25.21 | 13.87 | 22.09 | 15.76 | 11.95 | 14.66 | 17.52 | 15.50 | 12.94 |
| HK39RE | 20.03 | 13.97 | 30.76 | 14.77 | 27.07 | 17.58 | 12.93 | 16.72 | 16.80 | 16.47 | 17.29 |
| Mean | 18.58 | 13.48 | 27.33 | 14.56 | 21.34 | 16.38 | 11.74 | 15.16 | 18.4 | 15.81 | 14.64 |
| SD | 3.63 | 2.90 | 7.5 | 3.09 | 7.13 | 3.32 | 5.48 | 3.10 | 7.72 | 3.20 | 6.06 |
| | | $p < 0.01$ | | $p < 0.05$ | | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | |

* Mean value of three different experiments in duplicate. SD – standard deviation.

Table 2. The relative and percentage reduction in cell surface hydrophobicity (Mean and SD) of *Candida tropicalis* isolates following brief exposure (1 hour) to $\times 2$ MIC of antifungal drugs *in vitro*

| Isolate | Control CSH | NYS | | AMB | | FLU | | KETO | | 5-FC | |
|---------|----------------|------------|-------|------------|-------|------------|-------|------------|-------|------------|-------|
| | | CSH | %Redn | CSH | %Redn | CSH | %Redn | CSH | %Redn | CSH | %Redn |
| HK1KA | 51.14* | 25.94 | 50.59 | 29.27 | 40.98 | 43.21 | 16.02 | 36.99 | 28.24 | 39.95 | 23.96 |
| HK1KE | 39.68 | 25.18 | 36.50 | 26.03 | 30.59 | 33.71 | 14.46 | 29.31 | 26.00 | 32.27 | 17.98 |
| HK4 LA | 37.30 | 25.28 | 32.35 | 26.85 | 27.80 | 31.77 | 8.03 | 30.12 | 20.35 | 30.40 | 18.44 |
| HK5LG | 37.11 | 25.27 | 30.99 | 27.63 | 25.52 | 31.87 | 13.64 | 28.91 | 21.94 | 30.40 | 18.10 |
| HK5LF | 38.82 | 25.27 | 33.84 | 27.63 | 28.40 | 31.87 | 17.55 | 28.91 | 25.22 | 30.40 | 21.71 |
| HK9LF | 41.26 | 25.63 | 37.50 | 26.65 | 34.23 | 37.02 | 10.33 | 33.22 | 19.79 | 34.18 | 17.11 |
| HK9LG | 48.34 | 34.85 | 27.57 | 34.89 | 27.33 | 42.56 | 11.50 | 39.29 | 19.23 | 39.99 | 17.50 |
| HK36LA | 41.76 | 27.20 | 34.95 | 30.75 | 26.82 | 37.31 | 11.11 | 33.66 | 19.50 | 35.28 | 15.56 |
| HK44TD | 29.45 | 22.57 | 24.04 | 23.54 | 21.27 | 26.44 | 9.72 | 24.86 | 15.53 | 26.20 | 10.33 |
| HK44LF | 32.28 | 23.06 | 29.75 | 23.61 | 25.83 | 27.60 | 13.68 | 25.75 | 19.45 | 27.02 | 16.11 |
| Mean | 39.71 | 26.03 | 33.81 | 27.69 | 28.88 | 34.34 | 12.6 | 31.10 | 21.53 | 32.61 | 17.68 |
| SD | 9.00 | 6.86 | 9.12 | 5.81 | 8.96 | 7.68 | 5.84 | 7.78 | 6.86 | 7.92 | 6.66 |
| | | $p < 0.01$ | | $p < 0.01$ | | $p > 0.05$ | | $p > 0.05$ | | $p > 0.05$ | |

* Mean value of three different experiments in duplicate. SD – standard deviation.

and 33.81%, reduction in CSH respectively. The other tested polyene; amphotericin B also induced a significant suppression of CSH but to a lesser extent with 30% of the *C. albicans* and 60% of *C. tropicalis* isolates affected with an overall CSH reduction of 21.34% and 28.88%, respectively. In a previous study our group has demonstrated that a single brief exposure of *C. albicans* to similar concentrations of nystatin and amphotericin B leads to a reduction in CSH that ranges from 14.26–27.14%. [12,22]. However, CSH

data on *C. tropicalis* exposed to antifungals is not available and the current study is the first to evaluate this phenomenon in *C. tropicalis* isolates.

Polyenes, bind avidly to sterol components in the cell wall of *C. albicans*, and prevents the biosynthesis of ergosterol making it more permeable leading to impairment of barrier function, leakage of cellular components and metabolic disruption. Further, the function of many membrane-bound enzymes, including chitin synthetase, which is necessary for proper

cell growth, is also affected by the polyenes [10]. It is known that CSH of *C. albicans* correlates well with concentrations of fibrils as well as other physico-chemical properties of the cell wall [23]. Further ultrastructural studies are needed to confirm or refute this contention. Therefore it is tempting to speculate that the reduction of candidal CSH by limited exposure to polyenes may be related to the action of these drugs on possibly the fibrillar, floccular layer of the yeast cell wall. Further it is noteworthy that the percentage tested as the CSH of some of the isolates in the current study were not significantly affected by exposure to polyenes and the ultrastructural variations in these and the affected isolates may prove rewarding. Indeed in previous studies we have demonstrated that the cell surface undergoes profound changes due to the collapse of the internal structures, leaving an intact cell wall with "puckering" of cell surface giving rise to a mulberry-like appearance or, total deflation of the cell, possibly due to leakage of cellular constituents [24, 25].

Limited exposure of the yeasts to the two azole drugs, fluconazole and ketoconazole did not result in a significant reduction in CSH of both *Candida* species tested. Nevertheless, two isolates of *C. albicans* and a single *C. tropicalis* demonstrated significant reduction in CSH and overall the azoles suppressed the CSH of the yeasts to a considerable extent. For instance fluconazole and ketoconazole induced 11.74% and 12.60%, and 18.40% and 21.53% suppression in CSH of *C. albicans* and *C. tropicalis* isolates, respectively (Table 1 and 2). We also reported similar findings in a previous study where fluconazole failed to produce a significant reduction in CSH (6.16%) in *C. albicans* isolates [12]. Others have also noted that the CSH of *C. albicans* isolates exposed to sub-inhibitory concentrations of fluconazole for a prolonged period (26 hours) is not significantly affected as compared with the controls [13]. In contrast there is a single study, which reports that ketoconazole, elicits a 19.47% ($p = 0.04$) reduction in CSH of *C. albicans* isolates [12].

The azole antifungals act by inhibition of cytochrome p-450 enzyme that is involved in cell membrane synthesis in fungi. The principal target is 14 α -demethylase, which converts 14 α -methylsterols to ergosterol in the fungal cell membrane. Therefore, they block the 14 α -demethylation step in the synthesis of ergosterol, (depletion of ergosterol and accumulation of 14 α methylsterols) an important constituent of fungal cell membrane which thus become permeable

to intracellular constituents and leads to alterations in a number of membrane associated functions. Recent studies have also shown that prolonged exposure to sub-therapeutic concentrations of fluconazole could affect the cell wall and membrane structure of *Candida albicans* isolates [13]. As in the case of polyenes these changes may be responsible for the observed reduction in CSH on oral *Candida* following exposure to the azoles.

The DNA analogue 5-fluorocytosine is transported into the fungal cell by the action of cytosine permease. Within the cell it is converted to 5-fluorouracil which is incorporated into RNA with resulting abnormalities of protein synthesis. In addition it blocks thymidylate synthetase causing inhibition of DNA synthesis. Even though this drug elicited a slight reduction in *Candida* CSH (14.64% for *C. albicans* and 17.68% for *C. tropicalis*), it was not significantly different from that of the controls. In a previous study too exposure to the DNA analogue failed to significantly suppress the CSH of *C. albicans* [12].

Analysis of inter-species variation on the impact of antimycotics on the CSH on the two *Candida* species revealed a significant variation between *C. albicans* and *C. tropicalis*, where the former were less affected by all the antimycotics tested as compared to the latter. Hence, it seems that *C. albicans* is by far the more robust species in comparison to *C. tropicalis*, having the ability to sustain its cell wall characteristics, despite drug exposure (For instance 70% of the *C. tropicalis* isolates were affected by nystatin whereas the percentage of *C. albicans* isolates affected were only 50%). Previous studies have also reported that *C. albicans* was the least affected compared to other non-*albicans* species when the yeast's were exposed to nystatin for a brief period [25, 26]. This, together with the current findings, further substantiate the fact that *C. albicans* is the most virulent of all *Candida* species, and the reason for its pre-eminent position in the hierarchy of virulence amongst common pathogenic yeasts [27].

To our knowledge there have been only one report on the effect of a wide range of antimycotics on the CSH of oral *C. albicans* isolates [12]. Further, there have been no previous similar reports on oral *C. tropicalis* isolates and no information from isolates obtained from HIV-infected patients. The current data reveal that exposure to commonly used antifungals modifies the CSH of not only *C. albicans* but also *C. tropicalis* isolates to varying degrees. This appears to be a poorly understood salutary feature of these drugs and illustrates further mechanisms by which

these antimycotics operate *in vivo* to suppress candidal pathogenicity.

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