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In vitro Impact of Limited Exposure to Subtherapeutic Concentrations of Chlorhexidine Gluconate on the Adhesion-Associated Attributes of Oral *Candida* Species

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Key Words

Candida · Adhesion · Buccal epithelial cells · Denture acrylic · Cell surface hydrophobicity · Chlorhexidine gluconate

Abstract

Objective: *Candida albicans* and its non-*albicans* counterparts, such as *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. dubliniensis*, are the major etiological agents of oral candidosis. Their adherence to buccal epithelial cells (BEC), denture acrylic surfaces (DAS) and cell surface hydrophobicity (CSH) are attributes associated with yeast colonization and infection. Chlorhexidine gluconate (CG) is a widely used antiseptic in dentistry. When administered, the diluent effect of saliva and the cleansing effect of the oral musculature reduce its bioavailability, compromising its efficacy. Hence, intraorally, *Candida* undergoes a transient exposure to high CG concentrations, and thereafter it is likely to be subtherapeutic. Therefore, the impact of CG on adhesion to BEC, DAS and CSH of different oral *Candida* species was investigated following brief exposure to three subtherapeutic concentrations of CG. ***Materials and Methods:*** Ten oral isolates of each of the above five *Candida* species obtained in Kuwait from oral rinse samples were exposed to 0.00125, 0.0025 and 0.005% CG for 30 min. Subsequently, the yeast adhesion to BEC, DAS and CSH was determined. The data were analyzed using ANOVA Dunnett's t tests. ***Results:*** Exposure to the lowest dilution (0.00125%) of CG did not elicit a noteworthy collective suppression on all three adhesion traits evaluated. Exposure to 0.0025% CG curtailed the adhesion to BEC, DAS and CSH of *Candida* species by 50.89, 40.79 and 24.58%, respectively ($p < 0.001$). Exposure to the highest concentration (0.005%) of CG reduced the adhesion to BEC, DAS and CSH of *Candida* species by 64.68, 54.59 and 50%, respectively ($p < 0.001$). ***Conclusions:*** Brief exposure to subtherapeutic concentrations of CG suppressed the adhesion to BEC, DAS and CSH of oral *Candida* species, indicating probable pharmacodynamics that may potentiate its antiseptic properties.

Introduction

Oral yeast infections due to *Candida albicans* and non-*albicans Candida* species are common in compromised patient population groups [1]. Such oral yeast infections and the level of salivary *Candida* closely correlate with the degree of host immunosuppression, and are predictive of the underlying disease progression, especially in HIV disease [1]. Globally, *C. albicans* is by far the most prevalent of all the *Candida* species [1]. However, infections due to non-*albicans Candida* species, such as *C. tropicalis*, *C.*

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krusei, *C. glabrata* and *C. dubliniensis*, are becoming increasingly common [1]. These organisms are thought to be emerging as pathogens due to their varied virulent attributes. For instance, *C. krusei* is intrinsically azole resistant, whilst *C. glabrata* had been reported to acquire azole resistance, causing serious and persistent infections in immunocompromised patients [2, 3]. Similarly, *C. tropicalis* is frequently acquired from oral niches of HIV-infected patients [1]. Furthermore, *C. dubliniensis* is now universally recognized as a close relative of *C. albicans* with almost similar virulent attributes [1, 3]. Fluconazole resistance has been witnessed in this yeast acquired from HIV-infected individuals, as well as resistance to the newer antifungals, voriconazole and itraconazole [4–6]. In general, resistance to nearly all antifungal agents has been reported in clinical *Candida* isolates, signifying the urgent necessity for a substitute or adjunct antifungals [7].

The adherence of *Candida* to human oral mucosal surfaces is an essential prerequisite for the colonization process and infection, and a direct correlation between the enhanced yeast adhesion to the mucosa and their infectivity has been demonstrated [8]. It is also believed that the ability of *Candida* species to adhere to denture acrylic surfaces (DAS) is important in the pathogenesis of *Candida*-induced denture stomatitis [8, 9]. The relative cell surface hydrophobicity (CSH) of *Candida*, which modifies the initial encounter between the fungus and the host, is considered a significant nonbiological trait allied to candidal adherence to either biotic or abiotic surfaces, such as oral prostheses [10]. It has also been noted that hydrophobic *Candida* are more pathogenic than their relatively more hydrophilic counterparts [10]. Studies have also shown positive correlations between the relative CSH of *Candida* and its adhesion to oral buccal epithelial cells (BEC) as well as DAS [11, 12].

Chlorhexidine gluconate (CG) is prescribed as an antiseptic mouthwash in routine dentistry because of its wide antimicrobial spectrum, which also includes *Candida* species [13]. CG is used as an adjunct for traditional antimycotic agents in the management of oral yeast infections, including *Candid*a-associated denture stomatitis [14]. The antimycotic activity of CG has been shown both in vivo and in vitro, although most of these tests were focused on *C. albicans* species [14]. For instance, the exposure of either *C. albicans* isolates or BEC to 0.2% CG has been shown to suppress candidal adherence to BEC from healthy individuals or diabetics [15]. Others have shown that with *C. albicans* and its close phenotypic relative *C. dubliniensis*, CG is effective in suppressing CSH and adhesion to DAS [16, 17]. Therefore, by suppressing *Candida* adhesion to BEC, DAS and their CSH, mouthwashes containing CG may reduce the pathogenic potential of the yeast.

Although the pharmacodynamics of oral rinse agents have not been studied extensively, it is known that after an oral rinse with CG this antiseptic will be almost totally removed from the oral cavity during the first hour due to the diluent effect of saliva and the cleansing effect of the oral musculature, thus compromising its therapeutic efficacy [18]. As a result, *Candida* is likely to be briefly exposed to high concentrations of CG immediately after administration, and eventually to residual low subtherapeutic concentrations. The impact of such low concentrations of CG on the adhesion of different oral *Candida* species obtained from a single geographic locale to BEC, DAS and CSH has not been reported previously. Hence, the main aim of the current investigation was to evaluate the adhesion to five different oral *Candida* species *(C. albicans*, *C. tropicalis*, *C. krusei*, *C. glabrata* and *C. dubliniensis)* obtained from Kuwait University Dental Clinic to BEC, DAS and the relative CSH following limited exposure to three different subtherapeutic concentrations of CG (i.e. 0.00125, 0.0025 and 0.005%).

Materials and Methods

Organisms

Oral *Candida* isolates from patients attending the Kuwait University Dental Clinic that were obtained in a previous study were used here [19]. Ten isolates each of *C. albicans* and the non-*albicans* species of *Candida* studied – *C. krusei*, *C. tropicalis*, *C. glabrata* and *C. dubliniensis* – were used (i.e. a total of 50 *Candida* isolates). The identification of the isolates was reconfirmed by observing colony colors on CHROMagar Candida medium (Becton Dickinson Microbiology Systems, Cockeysville, Md., USA) and detecting the carbohydrate assimilation profiles using API 20C AUX *Candida* identifications kits (bioMérieux Vitek Inc., Hazelwood, Mo., USA). The formation of rough colonies with hyphal fringes and chlamydospores by *Candida* species on simplified sunflower seed agar was also observed, as done previously [19].

Antimycotic Drugs and Media

The 0.2% CG mouthwash (Corsodyl, GlaxoSmithKline, Brentford, UK) was dissolved in sterile phosphate-buffered saline (PBS) at pH 7.2. Thereafter, three different concentrations of the antiseptic (0.00125, 0.0025 and 0.005%) were prepared as solutions immediately before each experiment, as described previously [16, 17].

Candidal Cell Suspension Preparation for the Adhesion to BEC Assay, DAS Assay and CSH Assay

For the preparation of the cell suspension, a previously described method was employed with slight modifications [16, 17]. In brief, *Candida* cells preserved on Sabouraud's dextrose agar were inoculated onto fresh plates and incubated overnight at 37°C

for 24 h. The organisms were harvested and thereafter a cell suspension was prepared using sterile PBS at 520 nm to obtain an optical density of 1.5. Thereafter, 0.5 ml of this cell suspension was mixed with 2 ml of sterile PBS alone (control) and 2 ml of PBS/CG (test), thereby producing a cell suspension of 10^6 cells ml^{-1} in each assay tube. The tubes were then incubated at 37°C for a period of 30 min. Following this limited exposure, the drugs were removed by dilution using sterile PBS. For this purpose three cycles of dilution with sterile PBS followed by centrifugation of the whole solution for 10 min at 3,000 *g* was carried out. Afterwards, the supernatant was completely removed and the remaining pellets of cells were resuspended in 15 ml of sterile PBS. This procedure of drug removal by dilution was carried out in previous studies and was shown to reduce the concentration of CG by as much as 10,000-fold, resulting in the eradication of any carryover effect of CG after its removal [16, 17, 20, 21]. Viable counts of the control and the test groups were also obtained subsequent to drug removal by evaluating colony-forming unit counts.

Adhesion to BEC Assay

For the adhesion to BEC assay, a previously used method [20] was performed with slight modifications. Briefly, human BEC from 4 adults (laboratory personnel) were obtained using sterile cotton swabs by softly rubbing the inner side of the right and left sides of the buccal mucosa of the mouth. Thereafter, the BEC were disseminated in sterile PBS. The suspension of pooled BEC from the four volunteers was washed in PBS and any attached organisms were removed by centrifugation at 3,500 *g* for 10 min. The BEC were resuspended in sterile PBS to obtain a concentration of 1×10^5 cells/ml by hemocytometer counting. To execute the adhesion procedure, 0.75 ml of BEC and 0.75 ml of *Candida* cell suspension following brief exposure to CG were gently mixed within plastic tubes and incubated at 37°C for a period of 1 h. The *Candida*/BEC suspension was diluted using 5 ml of sterile PBS. The BEC were harvested onto polycarbonate filters and gently washed with sterile PBS to remove any *Candida* cells unattached to BEC. Each polycarbonate filter was subsequently placed on a glass slide and removed gently after 10 s. The glass slide preparation was air dried, Gram stained and prepared for BEC counting. The quantification of the number of adherent *Candida* cells was done under light microscopy at a magnification of ×400. Fifty sequential BEC were perceived for the purpose of counting. *Candida* cells adhered to BEC were expressed as the number of yeasts per 50 BEC. *Candida* attached to folded or overlapping and clumped BEC were not counted.

Adhesion to DAS Assay

The acrylic strips for the adhesion assay was prepared as described previously [11, 12]. Transparent self-polymerizing acrylic powder (1.5 g polymethyl methacrylate powder) was spread on an aluminum foil-covered glass slide (2.5 × 7.5 cm). One milliliter of monomer liquid (Dentsply Ltd., Weybridge, UK) was poured onto the surface of the slide and immediately a second, similar slide was placed on top of the polymerizing mixture and the slides were firmly secured at both ends with two binder clips. After bench curing for 30 min, the glass slides were separated. The resultant acrylic strips were cut into 5 × 5 mm squares, then immersed in distilled water for 1 week to leach excess monomer and washed in running water for 3 h. The strips were then disinfected by dipping in 70% alcohol and washed with sterile distilled water. Next they were ultrasonicated for 20 min to remove any contaminants and artifacts from the surfaces, washed again in sterile distilled water, dried and used for the adhesion assay. The ensuing adhesion assay was executed as described previously [11, 12]. In brief, using aseptic techniques, the acrylic strips were placed vertically in the wells of a sterile serological plate. Thereafter, following brief exposure to CG, 400 µl of *Candida* cell suspension was added to each well, completely covering the acrylic strips. The whole assembly was thereafter placed in an incubator for 1 h at 37°C with gentle agitation at 120 rpm. The strips were then recovered aseptically from the wells and washed three times by dipping gently in sterile PBS, which helped to dislodge the loosely attached *Candida* cells. The strips were then dried and stained using modified Gram stain without the counterstain. After air drying at room temperature they were mounted on glass slides with glycerol and the adherent *Candida* were quantified. Adherent *Candida* cells in 20 fields of view for each strip (0.25 mm^2 per field) were determined using a light microscope at ×400 magnification, and the results were expressed as *Candida* cells/mm^2, as performed previously [21]. The majority of the attached *Candida* cells were in the blastospore stage (cells with a rounded format), some with daughter cells and only very few with hyphae or pseudohyphae. The following previously used parameters were used to standardize the counts: a budding yeast was considered to be a unit cell if the daughter was smaller than the mother cell, and a hypha was counted as a single cell [21].

Relative CSH Assay

For the assessment CSH on oral *Candida* species an aqueous-hydrocarbon assay based on the biphasic separation of solutions was used as previously described [16, 17]. In brief, 5 ml of *Candida* cell suspension following exposure to CG was mixed in a vortex. Thereafter, its absorbance was measured at 520 nm. Subsequently, 1 ml of xylene was added to the cell suspension. The test tubes were placed in an incubator at 37°C for 10 min to equilibrate. Thereafter, it was mixed in a vortex for 30 s and placed again in the incubator for a further 30 min to allow the aqueous phases and xylene to separate. The bottom aqueous phase of the sample was meticulously taken out with a pipette and placed in a sterile test tube. By bubbling air through the aqueous suspension at a rate of 180 ml per minute for 2 min, traces of contaminating xylene that may have been carried over in the pipette or bound to *Candida* cells was removed. The optical density (absorbance) was measured at 520 nm after mixing in a vortex for 5 s to disrupt and resuspend any aggregates that might have formed. The relative CSH was expressed as the reduction in the percentage of the optical density of the suspension, as done in previous studies [16, 17]. All tests were performed in duplicate on three separate occasions.

Statistical Analysis

The data obtained from the three different concentrations of CG on adhesion to BEC, DAS and CSH assays were analyzed using ANOVA Dunnett's t tests, with one group treated as a control (that unexposed to CG) against which all the other groups (exposed to CG) were compared. In addition, the mean percentage reduction of the two concentrations (i.e. 0.005 and 0.0025%) of the *Candida* isolates tested, which had an overall significant effect in suppressing adhesion to BEC, DAS and CSH, was analyzed between *C. albicans* and the non-*albicans* species of *Candida*.

Table 1. Adhesion of different oral *Candida* species to BEC (*Candida*/50 BEC) following brief exposure to three concentrations of CG

Oral *Candida* species	Control	0.00125%	0.0025%	0.005%
C. albicans (n = 10)				
Mean	237.3	201.6	129.3	98.7
SEM	4.10	3.71	2.52	4.22
Mean percentage reduction		15.04	45.51	58.41
p value		0.004	<0.001	<0.001
C. krusei (n = 10)				
Mean	120.4	99.1	55.8	37.8
SEM	4.27	4.50	2.75	2.44
Mean percentage reduction		17.69	53.65	68.60
p value		0.003	<0.001	<0.001
C. tropicalis (n = 10)				
Mean	191.2	159.2	95.4	65.9
SEM	6.18	2.90	3.46	5.69
Mean percentage reduction		16.74	50.10	65.53
p value		<0.001	<0.001	<0.001
C. glabrata (n = 10)				
Mean	138.8	118.7	61.7	45.8
SEM	2.47	3.52	3.08	3.54
Mean percentage reduction		14.48	55.55	67.00
p value		<0.001	<0.001	<0.001
C. dubliniensis (n = 10)				
Mean	209.3	181.4	98.3	68.6
SEM	5.44	5.54	2.04	1.94
Mean percentage reduction		13.33	53.03	67.22
p value		0.002	<0.001	<0.001
Candida species				
Mean	179.4	152.00	88.10	63.36
SEM	21.81	19.08	13.41	10.59
Mean percentage reduction		15.27	50.89	64.68
p value		0.004	<0.001	<0.001

Results

The mean adhesion to BEC of the 50 *Candida* isolates unexposed to CG was 179.4 ± 21.81 (yeasts/50 BEC), whereas following limited exposure to 0.00125, 0.0025 and 0.005% concentrations of CG there was a lessening in the adhesion to 152, 88.10 and 63.36, respectively (table 1). Hence, compared to the controls, a marked diminution in the adhesion to BEC of all the isolates was seen following exposure to 0.005% CG, with a percentage reduction of 64.68% ($p < 0.001$). The suppressive effect on the adhesion to BEC following exposure to the 0.0025% dilution of the antiseptic was also significant ($p < 0.001$), although lower than for the higher concentration (50.89%; table 1). Overall, although there was a significant reduction in the adhesion to BEC by the yeasts exposed to 0.00125% CG, this effect was only 15.27%.

The mean adhesion to DAS of the 50 *Candida* isolates unexposed to CG was 46.80 ± 0.39 (yeasts/mm^2), whereas following brief exposure to 0.00125, 0.0025 and 0.005% concentrations of CG there was a decline in the adhesion to 43.34, 27.71 and 21.25, respectively (table 2). Therefore, compared to the controls, a distinct reduction of 54.59% in adhesion to BEC of all the isolates was seen following exposure to 0.005% CG ($p < 0.001$). Likewise, the suppressive impact on the adhesion to DAS following exposure to the 0.0025% dilution of the antiseptic was also significant ($p < 0.001$), although lower than for the higher concentration (40.79%; table 2). Even though there was a significant 7.39% reduction in adhesion to DAS of the yeasts exposed to 0.00125% CG, it was not very prominent compared to the higher concentrations.

The mean CSH of the *Candida* isolates unexposed CG was 27.67 ± 4.06, whereas following limited exposure to

Table 2. Adhesion of different oral *Candida* species to DAS (cells/mm^2) following brief exposure to three concentrations of CG

Oral *Candida* species	Control	0.00125%	0.0025%	0.005%
C. albicans (n = 10)				
Mean	46.03	43.07	32.36	26.89
SEM	0.71	0.4	0.33	0.41
Mean percentage reduction		6.43	29.7	41.6
p value		0.003	<0.001	<0.001
C. krusei (n = 10)				
Mean	47.21	43.6	26.83	19.43
SEM	0.61	0.38	0.41	0.4
Mean percentage reduction		7.65	43.17	58.84
p value		<0.001	<0.001	<0.001
C. tropicalis (n = 10)				
Mean	46.1	43.22	25.9	19.63
SEM	0.48	0.43	0.7	0.57
Mean percentage reduction		6.25	43.82	57.42
p value		<0.001	<0.001	<0.001
C. glabrata (n = 10)				
Mean	46.65	43.75	27.36	19.74
SEM	0.57	0.29	0.38	0.46
Mean percentage reduction		6.21	41.35	57.68
p value		0.001	<0.001	<0.001
C. dubliniensis (n = 10)				
Mean	46.58	43.06	26.12	20.55
SEM	0.59	0.44	0.47	0.52
Mean percentage reduction		7.56	43.92	55.88
p value		<0.001	<0.001	<0.001
Candida species				
Mean	46.80	43.34	27.71	21.25
SEM	0.39	0.14	1.19	1.42
Mean percentage reduction		7.39	40.79	54.59
p value		<0.001	<0.001	<0.001

0.00125, 0.0025 and 0.005% concentrations of CG there was a reduction in the CSH values to 26.51, 20.83 and 13.81, respectively (table 3). Hence, compared to the controls, a striking reduction of CSH of all the isolates was seen following exposure to 0.005% CG, with a percentage reduction of 50% ($p < 0.001$). The suppressive effect on the CSH following exposure to the 0.0025% dilution of the antiseptic was also significant ($p < 0.001$) in comparison with that of the unexposed control, although substantially lower than for the higher concentration (24.58%; table 3). Although there was a very slight reduction in the CSH of all the yeasts exposed to 0.00125% CG (4.02%; table 3), the suppressive outcome was not significant.

When the percentage reduction of the two concentrations (i.e. 0.005 and 0.0025%) which had a significant effect in subduing adhesion attributes of all the *Candida* isolates was considered, it was noted that the suppressive effect on *C. albicans* isolates was the least compared to the non-*albicans* counterparts (tables 1–3). For instance, the reduction on adhesion to BEC of *C. albicans* isolates was 58.41% compared to 65.53–68.60% for the non-*albicans* species of *Candida* following exposure to 0.005% CG. Likewise, the reduction in DAS adhesion of *C. albicans* isolates was 41.6% compared to 55.88–58.84% for the non-*albicans* species of *Candida* following exposure to 0.005% CG. Similarly, the reduction in CSH of *C. albicans* isolates was 41.47% compared to 50.54–53.57% for the non-*albicans* species of *Candida* with this concentration. This difference was significant between *C. albicans* and non-*albicans* species of *Candida* ($p < 0.05$ to $p < 0.001$). The reduction in adhesion to BEC by *C. albicans* isolates was 45.51% compared to 50.10–55.55% for the non-*albicans* species of *Candida* following exposure to 0.0025% CG. Likewise, the diminution of *C. albicans* isolates on

Table 3. Relative CSH of different oral *Candida* species following brief exposure to three concentrations of CG

Oral *Candida* species	Control	0.00125%	0.0025%	0.005%
C. albicans (n = 10)				
Mean	22.28	21.68	17.91	13.04
SEM	0.28	0.31	0.26	0.43
Mean percentage reduction		2.69	19.61	41.47
p value		0.167	<0.001	<0.001
C. krusei (n = 10)				
Mean	30.24	28.80	22.53	14.92
SEM	0.51	0.28	0.35	0.31
Mean percentage reduction		4.76	25.50	50.66
p value		0.025	<0.001	<0.001
C. tropicalis (n = 10)				
Mean	37.79	36.39	27.91	18.69
SEM	0.54	0.35	0.51	1.19
Mean percentage reduction		3.70	26.14	50.54
p value		0.042	<0.001	<0.001
C. glabrata (n = 10)				
Mean	32.95	31.72	25.16	15.30
SEM	0.67	0.41	0.68	1.05
Mean percentage reduction		3.73	23.64	53.57
p value		0.134	<0.001	<0.001
C. dubliniensis (n = 10)				
Mean for *Candida* species	14.86	13.95	10.66	7.09
SEM	0.93	0.87	0.52	0.36
Mean percentage reduction		6.12	28.26	52.29
p value		0.484	<0.001	<0.001
Candida species				
Mean	27.62	26.51	20.83	13.81
SEM	4.06	3.94	3.03	1.91
Mean percentage reduction		4.02	24.58	50.00
p value		0.537	<0.001	<0.001

adhesion to DAS was 29.7% compared to 41.35–43.92% for the non-*albicans* species of *Candida* following exposure to 0.0025% CG. Similarly, the reduction in CSH of *C. albicans* isolates was 19.61% compared to 23.64–28.26% for the non-*albicans* species of *Candida* with this concentration. This difference was also significant between *C. albicans* and non-*albicans* species of *Candida* ($p < 0.001$).

Discussion

In this study brief exposure to subtherapeutic concentrations of CG suppressed the candidal adherence to BEC and DAS of all the *Candida* species tested. The discerned overall significant suppression of *Candida* adhesion to BEC and DAS due to CG is related to the pharmacodynamic interactions between the antiseptic and the *Candida* cell wall. Scanning and transmission electron micrographic observations showed that the antifungal effect of CG was most likely due to a loss of cytoplasmic components along with the coagulation of nucleoproteins and associated morphological changes in the cell wall structure [23]. Furthermore, a decreased budding or germinating of *Candida* cells was also observed [23]. Interestingly, other chlorhexidine derivatives, such as chlorhexidine diacetate, have also generated cytological changes in yeast species such as *Saccharomyces cerevisiae*, involving dense and granular cytoplasmic constituents, withdrawal of the interior constituents from the cell wall and a general loss of the typical cellular organization [24]. Moreover, chlorhexidine-induced leakage of K^+ and pentose material from *S. cerevisiae* and protoplast lysis has also been documented [25]. Considering these probable effects of CG and other chlorhexidine derivatives on yeast species, it is reasonable to speculate that, by affecting both the cell

wall structure as well as other cellular events, CG could explain the suppression of the adhesion of *Candida* to BEC as well as DAS.

Microbial structures that contribute to the CSH include outer membrane proteins, lipoproteins, phospholipids, lipopolysaccharides and fimbriae [26, 27]. Hence, drugs that perturb these structural features have been shown to reduce the CSH of microbes [16, 17]. In the case of *C. albicans*, it has been shown that CSH correlates well with the concentration of 'fibrils' in the exterior layer of the cell wall [26, 27]. In addition, the antifungal effect of this antiseptic is most likely a result of a loss of cytoplasmic components and coagulation of nucleo-proteins and associated morphological changes in the cell wall structure of *Candida* [23]. Therefore, it is tempting to speculate that even low, subtherapeutic concentrations of CG may affect the cell wall structure to some extent, and suppress the CSH of *Candida* species.

When the relative interspecies variation of the impact of CG was compared, it was evident that the antiseptic had the least impact on the adhesion to BEC, DAS and relative CSH of *C. albicans* when exposed to two concentrations (i.e. 0.005 and 0.0025%) of CG. Hence, it appears that of all the *Candida* species studied, *C. albicans* to be the most resilient compared to the non-*albicans* species of *Candida*. This observation adds further credence to the fact that *C. albicans* is the most virulent and pervasive of all the *Candida* species [28].

The current study revealed that the exposure of oral *Candida* species to CG even at subtherapeutic levels suppressed three major virulent attributes of the yeast that dictates its mucosal colonization. Of importance to the geographical locale, a recent report indicates that *C. dubliniensis* was the most prevalent of the non-*albicans* oral *Candida* species isolated from Kuwait [19]. There are also reports of the emergence of resistance to 5-fluorocytosine, a potent DNA-analogue antifungal, in *Candida* isolates from Kuwait and contiguous locales of the Middle East [29, 30]. In addition, resistance to nearly all antifungal agents has been reported in virtually all clinical *Candida* species [2–7, 28–30]. The emergence of such resistance has important therapeutic implications and indicate the need for possible alternative antifungal strategies. In this context our results seem to add further credence to the use of CG in vivo as an adjunct in the management of oral candidosis.

Conclusion

In this study CG induced a suppression of the adhesion to BEC, DAS and CSH in five different *Candida* species obtained from a single geographic location in the Middle East.

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