ABSTRACT: Oral candidiasis is an opportunistic infection caused primarily by *Candida albicans*. However, in recent years, species of non-albicans *Candida* have been implicated more frequently in mucosal infection. *Candida* species usually reside as commensal organisms and are part of normal oral microflora. Determining exactly how transformation from commensal to pathogen takes place and how it can be prevented is continuous challenge for clinical doctors. Candidal adherence to mucosal surfaces is considered as a critical initial step in the pathogenesis of oral candidiasis. Acrylic dentures, acting as reservoirs, play an important role in increasing the risk from *Candida* colonisation. Thus, this review discusses what is currently known about the adhesion of non-albicans *Candida* species of oral origin to buccal epithelial cells and denture acrylics.

KEY WORDS: Adhesion, antifungals, buccal epithelial cells, *Candida* carriage, denture acrylic surface, non-albicans *Candida* spp.

INTRODUCTION

Oral candidiasis is a common opportunistic infection both in immuno-compromised and otherwise healthy individuals. *Candida albicans* is the most frequently isolated pathogenic member of the genus *Candida* (Meyer et al., 1998). However, in recent years, as a consequence of the extensive use of azole drugs, such as fluconazole, species of non-albicans *Candida*, such as *C. glabrata*, *C. krusei* and *C. parapsilosis*, have been implicated more frequently in mucosal and sistemic infections (Kremery and Barnes, 2002). *Candida glabrata* has emerged as a notable pathogenic agent in the oral mucosa, frequently being coisolated with *C. albicans* or the only detectable species. This is particularly important because *C. glabrata* isolated from oral lesions is much more resistant to antifungal treatment than *C. albicans* (Redding, 2001). An additional recent development is the recognition of new species associated with human pathology, such as *C. dubliniensis* (Gutierrez et al.,

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2002). Candida dubliniensis is a recently described species first isolated from oral lesions in HIV-infected individuals (Schorling, 2000).

CANDIDA CARRIAGE

Candida spp. form a part of the normal oral flora and is present in at least 50% of the population. Reports of oral carriage of C. albicans vary greatly in the literature, although most investigators agree that yeast are commonly found in the mouths of healthy persons and that a significant percentage of the species found are C. albicans. A compilation of data from a number of reports showed that oral carriage rate in healthy individuals was from 35 to 80% (Ben-Aryeh et al., 1995). It is difficult to give a precise oral carriage rate for C. albicans, since this depends on the age and health of the studied population and used sampling methods (White et al., 2004). Candida carriage is more frequent in women, persons of blood group 0, denture wearers, smokers, immunocompromised persons and hospitalized patients. Also, the high carbohydrate diet, xerostomia and use of broad-spectrum antibiotics increase the possibility of Candida carriage (Scully, 2004). Mucosa of the tongue dorsum may represent a site of residual colonization and a reservoir of organisms. In healthy individuals, C. albicans is most commonly isolated from the mid-line of the middle and posterior thirds of the tongue (Epstein et al., 2001).

HOST DEFENCES

In the mouth, epithelial physical barrier, indiginous of saliva, salivary IgA, lysozyme, histidine-rich polypeptides, lactoferrin, lactoperoxidase seem to play an important role in keeping Candida under control (Jorge et al., 1993). Candida elicits both humoral and cell mediated immune response (CMI) in a mammalian host. CMI is a predominant defence system against Candida during infection, as well as under asymptomatic carriage, and can be detected by in vitro and in vivo assays. Cytokines, such as interleukin (IL) IL-2, IL-12, THF-alpha and IFN-gamma seem to be of importance in the defence system (for more extensive discussion see review by Dongari-Bogtzoglou and Fidel, 2005). Anti-Candida antibodies of all immunoglobulin types can be detected in experimental and natural infections and in healthy humans carrying Candida (Segal, 2005). Anti-Candida sIgA can be detected in saliva, and its concentration is increased in whole or parotid saliva from HIV-positive individuals, but reduced in AIDS patients, suggesting that a compensatory response is overcome with progressive immunodeficiency (Challacombe and Sweet, 1997).

PREDISPOSING FACTORS

The transition from harmless commensal to unrelenting pathogen is dependent not only on virulance factor of the organisms but also equally, or even more, on host factors. The presence of such predisposing factors, both local
and systemic (Table 1), is important since it has been extremely rare to find a
case of oral candidiasis in which one or more of these factors cannot be identi-
fied (reviewed in Scully et al., 1994).

Table 1. Predisposing factors for oral candidiasis

<table>
<thead>
<tr>
<th>Factor</th>
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<tr>
<td>Xerostomia (irradiation, Sjögren’s syndrome, xerogenic drugs, cytotoxic drugs)</td>
</tr>
<tr>
<td>Smoking</td>
</tr>
<tr>
<td>Changes in oral microbial flora (broad-spectrum antibiotics, corticosteroids, dentures)</td>
</tr>
<tr>
<td>Physiological (infancy, pregnancy, old age)</td>
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<tr>
<td>Endocrine disorders (Diabetes mellitus, Addison’s disease, hypothyroidism)</td>
</tr>
<tr>
<td>Malnutrition (high-carbohydrate diet, iron, folate, vitamin B12 deficiencies)</td>
</tr>
<tr>
<td>Malignancies (leukemia, agranulocytosis)</td>
</tr>
<tr>
<td>Immune defects (HIV infection, AIDS, transplantation)</td>
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</tbody>
</table>

THE ADHESION OF NON-ALBICANS CANDIDA SPECIES

Candida species have developed an effective battery of virulence factors and
specific strategies to assist in their ability to colonize host tissues, cause
disease and overcome host defences. The virulence factors expressed by Can-
dida species causing infections may well vary depending on the type of infec-
tion, the site and stage of infection, and the nature of the host response.

The adhesion of Candida to host mucosal surfaces is a vital prerequisite
for successful colonisation and infection. Attachment enables the organisms to
avoid dislodgement by cleansing action of mucosal secretions, and it facilitates
infection. It has been shown that the yeast cell wall components, capable of in-
teracting with a variety of ligands on the host cell surface, including proteins
are carbohydrates, are important constituents of the adhesion process (reviewed
by Chaffin et al., 1998, Cannon and Chaffin, 1999). Oral cavity
presents a number of surfaces for candidal adhesion. The adhesion to buccal
epithelial cells (BECs) and denture acrylic surfaces will be discussed in this
review.

THE ADHESION TO BUCCAL EPITHELIAL CELLS

There are numerous reports concerning the adherence ability of C. albi-
cans, and the readers are referred to review articles which summarize the most
relevant data available (Cannon and Chaffin, 1999). However, only a
few studies evaluate the adherence ability of other Candida species. Sama-
rawanyak et al. (1995) demonstrated a positive correlation between the sur-
face hydrophobicity of C. kruzei and C. albicans and their adherence to BECs.
Elliopoulos et al. (1999) did not find significant differences in the adherence
ability between C. albicans, C. glabrata, C. parapsilosis and C. tropicalis, and
Lyman et al. (1999) demonstrated a hierarchy in adherence in which C. gla-
brata and C. krusei adhered in greater number to rabbit esophageal mucosa
than fluconazole-susceptible species. Differently, in a study made by Bia-
s o l i et al. (2002), *C. albicans* was significantly more adherent to BECs than *C. glabrata, C. krusei* and *C. lusitaneae*. In the recent study, *C. albicans* adhered to BECs in a greater number, followed by *C. tropicalis, C. glabrata* and *C. parapsilosis* with a significant difference in adhesion between the species, except for *C. glabrata* and *C. parapsilosis*. *Candida glabrata, C tropicalis* and *C. parapsilosis* strains obtained from the oral cavity of denture wearers with signs of denture stomatitis were able to adhere to BECs in a higher intensity than the isolates obtained from patients with normal palatal mucosa (L y o n and d e R e s e n d a, 2006). T o b g i (1989) used five isolates of *C. parapsilosis* to demonstrate the hierarchy of adherence to BECs among six species of *Candida*. He found *C. albicans* to be the most adherent, followed by *C. tropicalis, C. parapsilosis, C. glabrata, C. guilliermondii* and *C. krusei*. However, P a n a g o d a et al. (2001) investigated *in vitro* adherence of 24 isolates of *C. parapsilosis* and 12 isolates of *C. albicans*, and found no significant intraspecies difference in the adhesion of both species to BEC, although the former demonstrated a tendency for increased adherence. Analysis of the data reveals that such apparent differences do not reach significant levels due to large spread of the adhesion values. The apparent increase in the number of adherent *C. parapsilosis* noted may also be a reflection not only of yeast cell to epithelial cell adherence, but also of high co-adherence between organisms. The latter type of interaction results in the formation of yeast aggregates on the epithelial cell surface, which was more often observed with *C. parapsilosis* to BECs that could be attributed to variation in the strains and culture condition. In the same study, P a n a g o d a et al. (2001) observed a significant intraspecies variation in adherence to BECs of *C. parapsilosis* isolates. An analogous phenomenon was documented by S a m a r a n a y a k e et al. (1995), where a significant intraspecies variation in adherence to BECs by 20 isolates of *C. krusei* was demonstrated. There is only a single study indicating the relationship between the source of the isolate and adherence of *C. parapsilosis* to BECs. On investigation of the adherence results of superficial and systemic isolates of *C. parapsilosis*, the former demonstrated a tendency for higher adherence to BECs than the systemic isolates. Although no significant difference was not noticed between these two groups, the superficial isolates demonstrated 51,5% more avidity for BECs than the systemic counterparts (P a n a g o d a et al., 2001).

Despite the availability of a spectrum of antifungals for the treatment of oral candidiasis, therapy failure is observed frequently. The diluent effect of saliva and cleansing effect of the oral musculature may reduce the level of antifungals below their effective therapeutic concentrations (M a r t i n, 1990). Thus, during topical treatment, the yeast undergoes exposure to a relatively brief antifungal agent and the drug concentration is likely to vary in different niches of the mouth. Moreover, the formation of *Candida* biofilms on oral surfaces may also contribute to a failure of drug therapy (H a w s e r and D o u - g l a s, 1995). Nystatin belongs to the polyene group of antymycotic agents and is widely used as a topical agent in the management of oral candidiasis. There is only a single report on the adhesion of 30 oral isolates of *Candida* belonging to six different species (comprising *C. albicans, C. tropicalis, G. glabrata,*
C. guilliermondii, C. krusei and C. parapsilosis), to human BECs, following their brief exposure (1h) to a minimum inhibitory concentration of nystatin. Nystatin induced suppression of adhesion was the least for C. albicans (53.85%) compared with the other five species (64.09—67.74%). However, such significant intraspecies difference could not be elicited amongst the other five Candida species (Ellepola et al., 1999). In clinical terms these results demonstrated that exposure to nystatin significantly reduces candidal adherence to BECs irrespective of the Candida species concerned. The subtherapeutic levels of antifungal likely to persist in the oral cavity during dosing intervals may be beneficial in reducing candidal colonisation, though possibly ineffective in their total elimination. Ellepola and Samarana (1999) measured the post-antifungal effect (PAFE) of 30 oral isolates of six different Candida species, and found that nystatin-elicited PAFE was lowest for C. albicans and greatest for C. parapsilosis, while C. krusei, C. tropicalis, C. glabrata and C. guilliermondii elicited intermediate values. These findings clarify another possibility for the persistent, chronic recurrence of oral C. albicans infection despite apparently adequate antifungal drug regimens. It seems that even a limited exposure to the minimum inhibitory concentration of nystatin would confirm the growth suppression of non-albicans species.

There are only a few studies evaluating the action of fluconazole in the adherence ability of other Candida yeast. Darwazeh et al. (1991), in a study involving four dentate healthy subjects, found a significant reduction in C. albicans adhesion to BECs after a week of fluconazole intake. Braga et al. (1996) found that fluconazole in subinhibitory concentration was inactive to interfere in the adherence ability of C. glabrata. Furthermore, a reduction in the adherence ability of C. glabrata, C. tropicalis and C. parapsilosis to BECs was found after exposure to fluconazole, both among the isolates obtained from the denture wearers with sign of oral candidiasis, and the isolates obtained from the denture wearers with normal palatal mucosa, even considering that C. glabrata frequently shows high minimum inhibitory concentrations to fluconazole (Lyon and de Resende, 2006). These results suggest that the adherence, even of non-albicans species, could be factor that, along with predisposing conditions related to the host, determines whether an individual will develop disease or remain as a healthy carrier and confirm that fluconazole has an impact on the adherence ability of Candida spp. Dorocka-Bozkowska et al. (2003), using C. albicans and C. glabrata isolates obtained from the oral cavity with denture stomatitis, found that the incubation of human epithelial cell and human squamous cell carcinoma HSC-3 cells with both Candida spp., in the presence of amphotericin B, nystatin or natamycin, reduced the candidal adherence to these cells. When compared to amphotericin B, nystatin and natamycin suppressed the adherence less effectively, and these differences were statistically significant. Also, candidal adherence was significantly reduced when the tested polyenes were present during the “adherence phase”. These findings suggest that subtherapeutic levels of polyenes, that are likely to persist in the oral cavity following topical treatment may modulate candidal colonisation when present during the “adherence phase”.

73
THE ADHESION TO DENTURE ACRYLIC SURFACES

The adhesion of *C. albicans* to denture acrylic surfaces, and the ability to promote colonisation and infection in the oral cavity have been investigated in a number of studies (reviewed in Chaffin et al., 1998). However, there have been a few studies on the adhesion of non-albicans *Candida* species, and all have used *C. albicans* as the test organisms. In one study, the adhesion of *C. albicans* and *C. tropicalis* to 21 denture base material was investigated, and the adherence of *C. albicans* in general was far inferior to that of *C. tropicalis* (Minagi et al., 1985). Furthermore, the isolates of *C. krusei*, and emerging pathogen, showed variable but greater hydrophobicity than *C. albicans* isolates, and there was no correlation between hydrophobicity and adherence to denture acrylic (Samaranayake et al., 1995).

There have been few studies on the adhesion of *C. glabrata* isolates to acrylic surfaces with contradictory findings (Miyake et al., 1986, Hazen et al., 1986, Minagi et al., 1986, Klotz et al., 1985). The reason for these contradictory findings could be that the above studies used limited number of *C. glabrata* (up to 6 species). Therefore, Luo and Samaranayake (2002) studied a battery of 34 oral isolates of *C. glabrata* and 15 oral isolates of *C. albicans*, with respect to their relative cell surface hydrophobicity (CSH) and adhesion to denture acrylic surfaces. Their results indicated a remarkable intraspecies differences in both CSH and the adhesive ability of *C. glabrata* strains. Compared with *C. albicans*, *C. glabrata* demonstrated a four-fold greater CSH value and a two-fold greater tendency to adhere to denture acrylic surfaces. They have also noted a highly significant positive correlation between the relative CSH and adhesion of *C. glabrata*. This implies that the higher hydrophobicity of isolates, the greater tendency to adhere to acrylic surfaces. A significant positive correlation was also noted between the relative CSH and adhesion of 24 isolates of *C. parapsilosis* to acrylic surfaces confirming the interrelationship between these pathogenic attributes (Panagota et al., 2001). These data substantiated the close relationship between the relative CSH and adhesion of *Candida* spp. In this relationship there may exist some yet unrealized changes in the surface free energy which entail the process of attachment (Minagi et al., 1986, Gerson and Akita, 1980). It is also likely that the phenomenon of co-adhesion between closely apposed blastoconidia, particularly of *C. glabrata*, may contribute, since hydrophobic cells exhibit a higher tendency to co-adhere than their hydrophobic counterparts.

The adhesion of *C. albicans*, *C. glabrata*, *C. krusei* and *C. dubliniensis* to heat-cured acrylics (Vetex™ Rapid Simplified and ProBase™ Hot) and cold-cured acrylics (Paladur® A and Paladur® B) was investigated and the most important finding was the difference in yeast adherence between Vetex™ and the other acrylics. Only *C. glabrata* species attached to Vertex. All four species tested attached to all the remainder of the tested acrylics, except ProBase™ Hot, which could not sustain the adherence of *C. krusei*. Also, there were significant differences in the adhesion of *C. albicans*, *C. glabrata* and *C. krusei* between heat-cured acrylics and cold-cured acrylics (He et al., 2006). These data indicate that candidal adhesion to denture base acrylics differ de-
pending on the quality of the acrylic used as well as the Candida species in question. Heat-cured acrylics in general tended to have significantly lesser number of yeast attached than cold-cured acrylics. Thus, heat-cured materials, such as Vertex, might be clinically the best choice. Moreover, candidal adhesion to denture acrylics is reduced to a great extent following pre-treatment of acrylic strips with nystatin (Ell ep o l a and S a m a r a n a y a k e, 1998). Further, it has been documented that pre-treatment of BECs with nystatin resulted in reduction in candidal adhesion (D a r w a z e h et al., 1997). These studies indicate that pre-exposure of either the target surface or the yeast to nystatin results in reduced adhesion, which in clinical terms may prevent Candida adhesion and colonisation in the oral cavity.

CONCLUSION

From this review, it is evident that there are only a few in vitro studies on the adhesion of non-albicans Candida species with a limited number of isolates. As there are significant intra-species variations in Candida adhesion, it is important to evaluate a large number of isolates in order to elicit differences in relative adhesion among Candida species. It is not known to what extent the relative CSH contributes to colonization in vivo as other factors, such as saliva and the presence of bacteria on host surfaces, may confound this association. Further studies simulating the in vivo environment are required to confirm whether the observed phenomena operate intraorally.

ACKNOWLEDGMENTS

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REFERENCES


ОРАЛНА КАНДИДИЈАЗА — АДХЕЗИЈА NON-ALBICANS CANDIDA SPP.

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Резиме

Инфекција гљивом рода Candida представља проблем од све већег клиничког значаја. У последње две деценије, преваленција оралне гљивичне инфекције је енормно повећана, вероватно због повећања популације имунокомпетентних пацијената. Орална кандидијаза је опортунистичка инфекција примарно изазвана C. albicans. Међутим, у последњих неколико година уочено је да је ова инфекција много чешће изазвана врстама рода non-albicans Candida. Candida као комензал чини део нормалне микрофлоре усне дупље. Познавање начина трансформације из комензала у патогену форму, и како се она може спречити, је непрекидан изазов за клиничке лекаре. Алхезија гљиве рода Candida за површину слузкове предстavlja критичан, први корак за насељавање и настанак инфекције, као и у патогенези оралне кандидијазе. Акрилатне протезе, делујући као резервоари гљиве Candida, играју важну улогу у повећању ризика од насељавања усне дупље. Због тога је у овом раду приказан преглед литератури који се односи на алхезију оралних изолата non-albicans Candida spp. за епителне ћелије усне дупље и акрилатне протезе.