Oral Candida carriage and immune status in Thai human immunodeficiency virus-infected individuals

Panida Thanyasrisung,1 Piyanate Kesakomol,2 Patchara Pipattanagovit,1 Pornpan Youngnak-Piboonratankit,3 Waranuch Pitiphat4 and Oranart Matangkasombut1

1Department of Microbiology and DRU on Oral Microbiology, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
2Interdepartmental Program in Medical Microbiology, Graduate School, Chulalongkorn University, Bangkok, Thailand
3Department of Oral Medicine, Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand
4Department of Community Dentistry, Faculty of Dentistry, Khon Kaen University, Khon Kaen, Thailand

Oral candidiasis is a common opportunistic infection among human immunodeficiency virus (HIV)-infected individuals, with growing concerns about the emergence of non-albicans species with resistance to antifungal agents. This cross-sectional study determined the prevalence of oral Candida species in Thai HIV-infected adults and factors affecting their colonization. Candida species were identified from oral rinse samples of 60 HIV-infected participants of the MTCT-Plus initiative and 49 healthy controls by culture-based and molecular assays. The prevalence of oral Candida carriage was similar in HIV-infected patients (56.6 %) and in controls (55.1 %, \( P = 0.87 \)).

Candida albicans was the most predominant species in both groups (94.1 % of Candida carriers in HIV, 88.9 % in control). Interestingly, Candida dubliniensis was the second most common species in controls (29.6 %) and the third in HIV-infected patients (11.8 %, \( P = 0.08 \)). Multivariate analysis showed that, amongst HIV-infected individuals, CD4 count \( < 200 \) cells mm \(^{-3} \) was associated with increased prevalence of oral carriage of both C. albicans \( (P=0.03) \) and non-albicans species \( (P=0.03) \). Moreover, patients with tuberculosis infection had a higher prevalence of the non-albicans species than those without \( (P=0.03) \). Intriguingly, contraceptive use was also associated positively with non-albicans and multi-species carriage \( (P=0.04 \) for both). However, use of antiretroviral drugs protected the patients from Candida carriage \( (P=0.03) \), especially from C. albicans \( (P=0.02) \). In conclusion, while HIV-infected individuals had a similar prevalence of oral Candida carriage to that of the control group, host immune status, tuberculosis infection, and contraceptive use may influence oral colonization of Candida, especially of the non-albicans species.

INTRODUCTION

Candida infection, in both the mucocutaneous and systemic forms, has become increasingly prevalent as the number of populations with immunocompromising conditions increases (Miceli et al., 2011; Sardi et al., 2013). These include patients with human immunodeficiency virus (HIV) infection, cancers and transplantation receiving immuno-suppressive therapy. Oropharyngeal candidiasis is one of the most common opportunistic infections associated with HIV disease progression (Patton et al., 2002). The high incidence of recurrent oropharyngeal candidiasis in HIV-infected and candidaemia in immunocompromised individuals has led to the need for repeated administration and prophylactic use of antifungal agents. Importantly, the emergence of drug resistance has become problematic in several clinical settings (Sardi et al., 2013).

Whilst Candida albicans remains the predominant species isolated from the oral cavity, a number of studies have shown that non-albicans species have been detected more frequently, especially in immunosuppressed patients (Redding, 2001; Miceli et al., 2011; Sardi et al., 2013). The non-albicans species most commonly recovered from the oral cavity of HIV-infected and immunocompromised
patients include *Candida parapsilosis*, *Candida glabrata*, *Candida krusei*, *Candida tropicalis* and *Candida dubliniensis* (Sardi et al., 2013). In the majority of cases, non-*albicans* species are found in mixed infection with *C. albicans*, although they have occasionally been found as the sole pathogenic species. As several non-*albicans* species are intrinsically less susceptible to the widely used antifungal agent, fluconazole, accurate species identification is important for treatment choice. Thus, great care is required in the isolation of different *Candida* species; for instance, the use of a chromogenic differential medium is recommended (Williams & Lewis, 2000). Furthermore, the identification of *C. dubliniensis* requires the use of commercial kits or molecular methods, such as PCR, to distinguish it from *C. albicans* (Loreto et al., 2010). These techniques had not been employed in previous studies conducted in the Thai population (Itharatana et al., 1997; Teanpaisan & Nittayanananta, 1998; Pongsiriwet et al., 2004), except in a few recent studies (Reichart et al., 2007; Santiwongkarn et al., 2012). To date, there has not yet been a formal report on the prevalence of *C. dubliniensis* in the Thai population, particularly in HIV-infected patients. Therefore, currently available data may not fully reflect the prevalence of non-*albicans* species, especially in instances of co-colonization. With approximately 500,000 living HIV-infected individuals in Thailand, accurate data on oral *Candida* colonization in this population are highly relevant (UNAIDS, 2011).

Host immune status of HIV-infected patients is a known risk factor for oral candidiasis; however, various studies have yielded different results with regard to its relationship with oral *Candida* colonization (Campisi et al., 2002; Vargas & Joly, 2002; Hung et al., 2005; Sánchez-Vargas et al., 2005; Liu et al., 2006; Yang et al., 2006; Erköse & Erturun, 2007; Back-Brito et al., 2009; Delgado et al., 2009; Cerqueira et al., 2010; Domaneschi et al., 2011; Wu et al., 2012). Moreover, host factors that affect oral colonization of non-*albicans* and multi-species *Candida* are not well understood. Therefore, this study was conducted to gain epidemiological data on oral carriage of *Candida* species in a group of Thai patients with HIV infection in comparison with healthy subjects, and to examine the relationship between host immune status and oral *Candida* carriage in HIV-infected individuals.

**METHODS**

**Study population.** Individuals aged 20–60 years without current oral candidiasis were included in the study. HIV-infected patients (*N=60*) were sampled from participants of the MTCT-Plus initiative at King Chulalongkorn Memorial Hospital of the Thai Red Cross (Abrams et al., 2007). Control subjects were healthy individuals without any immunocompromising condition (*N=49*) attending the oral diagnostic clinic at the Faculty of Dentistry, Chulalongkorn University, Bangkok, Thailand.

The study protocol was approved by the ethical review boards of the Faculties of Dentistry and Medicine, Chulalongkorn University. All participants were informed of the objectives and study protocol, and gave written consent prior to sample collection.

**Data collection.** Study subjects were interviewed for data on medical history and routine oral hygiene practice. Current health status of HIV-infected patients was obtained from medical records of the MTCT-Plus initiative program. Oral examination was given to all subjects, and those with oral candidiasis or wearing dentures were excluded from the study.

Oral rinse samples were collected by having each subject hold 10 ml sterile PBS (0.01 M, pH 7.2) in the mouth for 60 s (Samaranayake et al., 1987). After collection, the samples were placed on ice and transported to the laboratory for microbiological analysis.

**Quantification and identification of Candida species.** Oral rinse samples were concentrated 10-fold by centrifugation at 3000 r.p.m. for 5 min. Aliquots of 10 and 100 µl were used for inoculation on Sabourau’s dextrose agar (ingredients from Oxoid) and chromogenic *Candida* agar (Oxoid) media. After 48 h of incubation at 37 °C, yeast colonies grown on each plate were counted and representative colonies of different colours on chromogenic *Candida* agar were isolated for further analyses. *Candida* species were identified using chlamydospore formation tests on gluttonous rice agar, carbohydrate fermentation tests (substrates from Oxoid) and carbohydrate assimilation tests (API 20C AUX; bioMerieux). Isolates with ambiguous results from biochemical tests were analysed by PCR using species-specific primers as described previously for *C. albicans* (CAL5-NL4CAL), *C. glabrata* (CGL1-NL4CGGL1), *C. parapsilosis* (CPA4-NL4LELI), *C. tropicalis* (CTR22-NL4CTR1) and *C. dubliniensis* (CDU2-NL4CAL), and PCR-RFLP using MspI digestion of ITS1–ITS4 PCR products as described (Mannarelli & Kurtzman, 1998; Mirhendi et al., 2006). All isolates preliminarily identified as *C. albicans* and *C. dubliniensis* were distinguished by testing for growth at 45 °C and PCR analysis as described previously (Mannarelli & Kurtzman, 1998; Pinjon et al., 1998).

**Statistical analysis.** Characteristics of the two groups were compared using t-tests for continuous data and χ2 or Fisher’s exact tests for categorical data. Differences in species distribution were analysed using χ2 and Fisher’s exact tests, whilst the quantity of *Candida* carriage in the two groups was compared using the Mann–Whitney *U* test. Multiple logistic regression analyses were used to identify predictors of *Candida* carriage among HIV-infected patients. Variables with *P*≤0.25 in bivariate analyses were considered for inclusion in multivariable analyses (Hosmer & Lemeshow, 2000). Odds ratios (OR) and 95% confidence intervals (CIs) were calculated for the variables that were statistically significant. All statistical tests were two-sided with a significance level of 5%.

**RESULTS**

A total of 60 HIV-infected patients and 49 healthy control subjects were enrolled in this study. Characteristics of the study population are shown in Table 1. There was no difference between the HIV-positive group and the control group in terms of age, gender, smoking habit, xerostomia, oral hygiene status, hospitalization and pregnancy status. However, the percentages of people using mouthwash and contraceptives were significantly higher in the HIV-positive group than in the control group (*P*=0.007 each).

Prevalence of oral *Candida* carriage is shown in Table 2. Overall *Candida* carriage rates were similar: 56.6 and 55.1% in the HIV-positive and the control groups, respectively. Moreover, among *Candida* carriers, there was no difference in the quantity of *Candida* carriage between HIV-infected
patients (median = 36.3 c.f.u. ml⁻¹, interquartile range = 5.8–119.7) and controls (median = 52.2 c.f.u. ml⁻¹; interquartile range = 17.4–200.3; P = 0.23). No statistically significant difference was found in the distribution of Candida spp. among Candida carriers of the HIV-positive and the control groups (Table 2). C. albicans was the most predominant species found in Candida carriers of both groups (94.1% of HIV, 88.9% of control), followed by multi-species (41.2% of HIV, 55.6% of control) and non-albicans Candida species (41.2% of HIV, 44.4% of control). Amongst non-albicans species, C. dubliniensis and C. parapsilosis were the major species isolated from both groups. Interestingly, C. dubliniensis was quite common, and was isolated from both HIV-infected patients (11.8%) and the controls (29.6%, P = 0.08).

Amongst HIV-infected patients, the prevalence of oral Candida carriage was compared between groups with different medical characteristics (Table 3). Current immune status of the HIV-infected patients, as reflected by CD4 T-lymphocyte count and per cent CD4, appears to be an important factor for the prevalence of oral Candida. In multivariate logistic regression analysis, the prevalence of Candida species in patients with a CD4 cell count < 200 cells mm⁻³ was significantly higher than those with a CD4 cell count ≥ 200 cells mm⁻³ (adjusted OR = 7.86; 95% CI = 1.50–41.29). Patients who used antiretroviral drugs were less likely to have Candida compared with those who did not (adjusted OR = 0.16; 95% CI = 0.03–0.86). Similar results were observed for C. albicans species. A CD4 cell count < 200 cell mm⁻³ was associated with increased prevalence

### Table 1. Characteristics of study population

<table>
<thead>
<tr>
<th>Characteristic</th>
<th>HIV-positive (N=60)</th>
<th>Control (N=49)</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years, mean ± SD)</td>
<td>30.5 ± 5.4</td>
<td>29.5 ± 9.5</td>
<td>0.54*</td>
</tr>
<tr>
<td>Female gender (%)</td>
<td>75.0</td>
<td>69.4</td>
<td>0.51†</td>
</tr>
<tr>
<td>Smoking (%)</td>
<td>13.3</td>
<td>8.2</td>
<td>0.39†</td>
</tr>
<tr>
<td>Xerostomia (%)</td>
<td>3.3</td>
<td>4.1</td>
<td>1.00‡</td>
</tr>
<tr>
<td>Poor oral hygiene (%)</td>
<td>13.3</td>
<td>14.3</td>
<td>0.89‡</td>
</tr>
<tr>
<td>Use of mouthwash (%)</td>
<td>18.3</td>
<td>2.0</td>
<td>0.0007†</td>
</tr>
<tr>
<td>Recent hospitalization (%)</td>
<td>3.3</td>
<td>2.0</td>
<td>1.00‡</td>
</tr>
<tr>
<td>Pregnancy (%)</td>
<td>6.7</td>
<td>0.0</td>
<td>0.13‡</td>
</tr>
<tr>
<td>Use of contraceptive (%)</td>
<td>18.3</td>
<td>2.0</td>
<td>0.0007†</td>
</tr>
</tbody>
</table>

For P-values, values in italics indicate statistically significant difference.
*By t-test.
†By χ² test.
‡By Fisher’s exact test.

### Table 2. Prevalence of oral Candida carriage and Candida species in the study population and amongst Candida carriers

<table>
<thead>
<tr>
<th>Yeast</th>
<th>Study population</th>
<th>Candida carriers</th>
<th>P-value</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>HIV-positive (N=60) [% (n)]</td>
<td>Control (N=49) [% (n)]</td>
<td>P-value</td>
</tr>
<tr>
<td><strong>Candida</strong></td>
<td>56.7 (34)</td>
<td>55.1 (27)</td>
<td>0.87*</td>
</tr>
<tr>
<td>Multi-species</td>
<td>23.3 (14)</td>
<td>30.6 (15)</td>
<td>0.39*</td>
</tr>
<tr>
<td>C. albicans</td>
<td>53.3 (32)</td>
<td>49.0 (24)</td>
<td>0.65*</td>
</tr>
<tr>
<td>Non-albicans Candida</td>
<td>23.3 (14)</td>
<td>24.5 (12)</td>
<td>0.89*</td>
</tr>
<tr>
<td>C. dubliniensis</td>
<td>6.7 (4)</td>
<td>16.3 (8)</td>
<td>0.11*</td>
</tr>
<tr>
<td>C. parapsilosis</td>
<td>13.3 (8)</td>
<td>10.2 (5)</td>
<td>0.62*</td>
</tr>
<tr>
<td>C. lusitaniae</td>
<td>6.7 (4)</td>
<td>6.1 (3)</td>
<td>1.00†</td>
</tr>
<tr>
<td>C. krusei</td>
<td>6.7 (4)</td>
<td>6.1 (3)</td>
<td>1.00†</td>
</tr>
<tr>
<td>C. tropicalis</td>
<td>1.7 (1)</td>
<td>4.1 (2)</td>
<td>0.59†</td>
</tr>
<tr>
<td>C. glabrata</td>
<td>0.0 (0)</td>
<td>4.1 (2)</td>
<td>0.20†</td>
</tr>
<tr>
<td>Other fungi‡</td>
<td>1.7 (1)</td>
<td>4.1 (2)</td>
<td>0.59†</td>
</tr>
</tbody>
</table>

*By χ² test.
†By Fisher’s exact test.
‡Other fungi were identified as Rhodotorula rubra, Trichosporon cutaneum and Trichosporon inkin.
Table 3. Association of Candida carriage with clinical status and medications in the HIV-positive group

<table>
<thead>
<tr>
<th>Total (N=60)</th>
<th>Candida spp.</th>
<th></th>
<th></th>
<th>C. albicans</th>
<th>Non-albicans</th>
<th>Multi-species</th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>%</td>
<td>Crude P-value</td>
<td>Adjusted P-value</td>
<td>%</td>
<td>Crude P-value</td>
<td>Adjusted P-value</td>
<td>%</td>
<td>Crude P-value</td>
<td>Adjusted P-value</td>
<td>%</td>
<td>Crude P-value</td>
<td>Adjusted P-value</td>
</tr>
<tr>
<td>CD4 count (cells mm(^{-3})) (N=59)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥200 (n=47)</td>
<td>48.9</td>
<td>0.03</td>
<td>0.02†</td>
<td>46.8</td>
<td>0.08</td>
<td>0.03†</td>
<td>17.0</td>
<td>0.03*</td>
<td>0.03†</td>
<td>19.2</td>
<td>0.13*</td>
<td>41.7</td>
</tr>
<tr>
<td>&lt;200 (n=12)</td>
<td>75.0</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Per cent CD4 (N=57)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>≥15 (n=33)</td>
<td>48.5</td>
<td>0.09</td>
<td></td>
<td>45.5</td>
<td>0.11</td>
<td></td>
<td>15.2</td>
<td>0.05</td>
<td></td>
<td>12.1</td>
<td>0.01</td>
<td>0.01†</td>
</tr>
<tr>
<td>&lt;15 (n=24)</td>
<td>66.7</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Antiretroviral drugs (N=59)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=48)</td>
<td>50.0</td>
<td>0.09*</td>
<td>0.03†</td>
<td>45.8</td>
<td>0.03</td>
<td>0.02†</td>
<td>25.0</td>
<td>1.00*</td>
<td></td>
<td>22.9</td>
<td>0.71*</td>
<td></td>
</tr>
<tr>
<td>No (n=11)</td>
<td>81.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of opportunistic infection (within 2 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=28)</td>
<td>57.1</td>
<td>0.94</td>
<td></td>
<td>50.0</td>
<td>0.63</td>
<td></td>
<td>32.1</td>
<td>0.13</td>
<td></td>
<td>28.6</td>
<td>0.37</td>
<td></td>
</tr>
<tr>
<td>No (n=32)</td>
<td>56.3</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tuberculosis infection (within 2 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=17)</td>
<td>76.5</td>
<td>0.05</td>
<td></td>
<td>64.7</td>
<td>0.27</td>
<td></td>
<td>47.1</td>
<td>0.01*</td>
<td>0.03†</td>
<td>41.2</td>
<td>0.09*</td>
<td></td>
</tr>
<tr>
<td>No (n=43)</td>
<td>48.8</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Current prophylaxis</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=26)</td>
<td>69.2</td>
<td>0.09</td>
<td></td>
<td>61.5</td>
<td>0.27</td>
<td></td>
<td>30.8</td>
<td>0.23</td>
<td></td>
<td>26.9</td>
<td>0.57</td>
<td></td>
</tr>
<tr>
<td>No (n=34)</td>
<td>47.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>History of antifungal therapy (within 2 years)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=16)</td>
<td>62.5</td>
<td>0.58</td>
<td></td>
<td>62.5</td>
<td>0.39</td>
<td></td>
<td>37.5</td>
<td>0.17*</td>
<td></td>
<td>37.5</td>
<td>0.17*</td>
<td></td>
</tr>
<tr>
<td>No (n=44)</td>
<td>54.6</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of mouthwash</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=11)</td>
<td>45.5</td>
<td>0.51*</td>
<td></td>
<td>36.4</td>
<td>0.21</td>
<td></td>
<td>27.3</td>
<td>0.71*</td>
<td></td>
<td>18.2</td>
<td>1.0*</td>
<td></td>
</tr>
<tr>
<td>No (n=49)</td>
<td>57.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Use of contraceptive</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Yes (n=11)</td>
<td>63.6</td>
<td>0.74*</td>
<td></td>
<td>54.6</td>
<td>0.93</td>
<td></td>
<td>54.6</td>
<td>0.01*</td>
<td>0.04†</td>
<td>45.5</td>
<td>0.11*</td>
<td>0.04†</td>
</tr>
<tr>
<td>No (n=49)</td>
<td>55.1</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

*By Fisher’s exact test, otherwise by \( \chi^2 \) test.
†By multivariate logistic regression.
For P-values, values in italics indicate statistically significant difference.
of *C. albicans* (adjusted OR=5.31; 95% CI=1.22–23.16), whilst antiretroviral drug use was associated with decreased prevalence (adjusted OR=0.14; 95% CI=0.03–0.77). Colonization of non-*albicans* species was influenced by a CD4 cell count <200 cells mm\(^{-3}\) (OR=6.10; 95% CI=1.20–31.07), tuberculosis infection (adjusted OR=5.48; 95% CI=1.24–24.11) and contraceptive use (adjusted OR=5.71; 95% CI=1.11–29.46). Factors significantly associated with the mixed species of *Candida* included per cent CD4 <15% (adjusted OR=6.53; 95% CI=1.52–28.12) and contraceptive use (OR=5.94; 95% CI=1.14–31.01).

**DISCUSSION**

This study aims to obtain epidemiological data on oral *Candida* carriage in Thai HIV-infected individuals compared with healthy controls. The use of chromogenic *Candida* agar as an isolation medium is significant in facilitating isolation of non-*albicans* species in samples with more than one species of *Candida*. Furthermore, to distinguish *C. dubliniensis* from *C. albicans*, proper techniques including molecular analysis are critical. Therefore, the dataset could provide a more accurate estimate of the prevalence of non-*albicans* *Candida* species in the study population.

When compared with the healthy controls, a higher proportion of subjects in the HIV group used contraceptive drugs (18.3% in HIV versus 2.0% in controls, *P*<0.01) and were pregnant (6.7% in HIV versus 0% in controls, *P*=0.13) (Table 1). This is due to the fact that the subjects recruited were participants of the MTCT-Plus initiative, which aims to prevent mother-to-child transmission of HIV infection, and mainly enrolls mothers and expecting women along with their families into the program (Abrams et al., 2007). A higher proportion of HIV-infected patients (18.3%) compared with controls (2.0%, *P*<0.01) regularly used antiseptic mouthwash. This could possibly be explained by the likelihood that the HIV-infected patients were more concerned about their oral infection.

When the prevalence of total *Candida* carriage was compared, similar rates were observed in the HIV (56.6%) and control groups (55.1%; *P*=0.87). This is not surprising considering the well-maintained health status of this group of HIV-infected patients. Ninety-five per cent of the subjects were on highly active antiretroviral therapy or had CD4 counts of >350 cells mm\(^{-3}\) at the time of sample collection.

The prevalence of non-*albicans* species in healthy controls in this study (24.5%) is higher than that found in previous studies in the Thai population (0–19.2%) (Itharatana et al., 1997; Teanpaisan & Nittayananta, 1998; Pongsiriwet et al., 2004; Santiwongkarn et al., 2012), although one study had isolated up to 30% (Itharatana et al., 1997). In this study, we frequently found that at least one species constituted only a small percentage of the yeast population (0.5–10%); 68% of cases with mixed colonization) and cases with one dominant species representing >90% of total yeast colonies (48% of cases with mixed colonization, data not shown). These observations emphasize the importance of careful isolation with the use of chromogenic differential medium (Ghelardi et al., 2008). Of particular interest are the isolation and identification of *C. dubliniensis*. This is, to our knowledge, the first formal report of the prevalence of *C. dubliniensis* in the Thai population, especially in HIV-infected individuals. Our results suggested that *C. dubliniensis* can be found quite frequently even in the healthy population. It is the second most common species isolated in healthy controls and the third in HIV-infected patients in this study. Therefore, our data indicated that *C. dubliniensis* should not be considered prevalent only in HIV-infected individuals (Loreto et al., 2010; Sardi et al., 2013).

The prevalence of non-*albicans* *Candida* species is of concern as certain *Candida* species are intrinsically less susceptible to common antifungal agents. For example, *C. krusei* and *C. glabrata* (11.5 and 3.3% of *Candida* carriers in this study, respectively) are less susceptible to fluconazole and voriconazole, whilst *C. parapsilosis* and *C. tropicalis* (21.3 and 4.9% of *Candida* carriers, respectively) are as susceptible as *C. albicans* (Pfaller, 2012). The identification of causative species is thus important for physicians when choosing appropriate antifungal agents for patients with candidiasis. Nevertheless, the data on the prevalence of *Candida* species in the oral cavity could be useful for initial treatment choice.

Our findings suggested that CD4 T-lymphocyte count and per cent CD4 are important factors associated with oral *Candida* carriage even in this group of relatively healthy HIV-infected patients. There was a significant increase in the prevalence of oral *Candida*, especially of the non-*albicans* species, in patients with a lower CD4 count (Table 3). This is in concordance with earlier studies that observed a higher rate of *Candida* colonization in patients with low CD4 counts (Vargas & Joly, 2002; Hung et al., 2005; Yang et al., 2006; Wu et al., 2012); however, other reports did not find the association (Campisi et al., 2002; Sánchez-Vargas et al., 2005; Liu et al., 2006; Erköse & Erturan, 2007; Back-Brito et al., 2009; Delgado et al., 2009; Cerqueira et al., 2010; Domaneschi et al., 2011), although none looked specifically at the relationship with non-*albicans* species. Interestingly, different populations and geographical locations may affect the outcome, as most studies that observed the association were from Asia, whilst several studies from Europe and South America frequently found otherwise. Another factor that reflects the host immune status of HIV-infected patients is the presence of opportunistic infection. We found a marginally significant association of tuberculosis infection (within 2 years) with *Candida* colonization (*P*=0.05) and a strong association with the non-*albicans* species (adjusted OR=5.48; 95% CI=1.24–24.11). These findings are consistent with the study of Owotade et al. (2013) who showed a significantly higher rate of tuberculosis infection in the *Candida*-colonized
group compared with the non-colonized group, although the association with non-
*albicans* species was not investigated. Furthermore, we found a significant association
between contraceptive use and oral colonization of non-
*albicans* *Candida* species and of multi-species colonization.
Interestingly, oral contraceptive use is considered as a
predisposing factor for vulvovaginal candidiasis as it could
alter hormonal conditions and may promote growth of
*Candida* species (Sobel, 2007). However, the effect of
contraceptive use on colonization of *Candida* species in the
oral cavity has not been well investigated. One study
suggested that certain species of *Candida* were detected
more frequently in periodontal pockets of women who
used contraceptives (Brusca et al., 2010). This finding and
our results suggest that contraceptive use could also
influence oral *Candida* colonization and should be further
investigated.

When compared among HIV-infected patients, the use of
antiviral drugs was associated with less *Candida*, especially of
*C. albicans*, colonization (adjusted *P*=0.03 and 0.02,
respectively). This is in agreement with previous reports
(Hung et al., 2005; Yang et al., 2006). However, earlier
studies suggested that long-term use of antibiotics for
prophylaxis may increase oral *Candida* carriage, which
may in turn increase the risk for future *Candida* infection
(Hung et al., 2005; Wu et al., 2012). We did not observe a
significant association between the use of antibiotics for the
prophylaxis of opportunistic infection (mostly trim-
ethoprim/sulfamethoxazole) and oral *Candida* carriage
(*P*=0.09).

History of antifungal drug use for the treatment or
prophylaxis of oral candidiasis and other opportunistic
fungal infection therapy, especially the use of fluconazole,
could lead to a higher prevalence of non-*albicans* species
(Sardi et al., 2013). Although we did not find a significant
difference between the groups of patients with and without
a history of antifungal therapy (within 2 years), those with
such a history showed a higher percentage carrying non-*albicans*
species than those without (37.5 versus 18.2%, *P*=0.17).
As the numbers of patients with a history of antifungal
therapy and those with non-*albicans* species were quite
small, the data did not possess sufficient power to detect
the difference in this situation. Therefore, a larger number
of participants would be required in a future study to
address this question.

Furthermore, our findings showed that antiretroviral therapy
was associated with a decreased risk of oral *Candida* carriage.
However, we were not able to analyse the effect of different
antiretroviral regimens since most of the patients were on
similar regimens without the use of protease inhibitors (only
one patient received protease inhibitors).

In conclusion, our results suggested that the prevalence of oral
*Candida* carriage was similar between HIV-infected individ-
uals and healthy controls. Host immune status of HIV-
infected patients and contraceptive use could influence the
colonization of *Candida*, especially the non-*albicans* species.

ACKNOWLEDGEMENTS

The authors are sincerely grateful to all the participants of this study.
We also deeply appreciate the assistance of Dr Nittaya Phanupak-
Pungpapong of the MTCT-Plus initiative and the program staff
members. This work is supported by the Special Task Force for
Activating Research (STAR) under the Chulalongkorn University
Centenary Fund and the Faculty Research Grant and DRU Fund from
the Faculty of Dentistry, Chulalongkorn University.

REFERENCES

Prevention of mother-to-child transmission services as a gateway to
family-based human immunodeficiency virus care and treatment in
resource-limited settings: rationale and international experiences.

Back-Brito, G. N., Mota, A. J., Vascconcellos, T. C., Querido, S. M.,
Frequency of *Candida* spp. in the oral cavity of Brazilian HIV-positive
patients and correlation with CD4 cell counts and viral load.
*Mykopathologia* 167, 81–87.

Brusca, M. I., Rosa, A., Albaina, O., Moragues, M. D., Verdugo, F. &
Pontón, J. (2010). The impact of oral contraceptives on women’s
periodontal health and the subgingival occurrence of aggressive perio-
dontopathogens and *Candida* species. *J Periodontol* 81, 1010–1018.

Campisi, G., Pizzo, G., Milici, M. E., Mancuso, S. & Margiotta, V.
(2002). Candidal carriage in the oral cavity of human immunodefi-
Radiol Endod* 93, 281–286.

Cerqueira, D. F., Portela, M. B., Pominrico, L., de Araújo Soares, R. M.,
relation with predisposing factors in HIV-infected children and their
uninfected siblings in Brazil: the era of highly active antire-

Delgado, A. C., de Jesus Pedro, R., Aoki, F. H., Resende, M. R.,
Trabasso, P., Colombo, A. L., de Oliveira, M. S., Kimoki, Y. & Moretti,
M. L. (2009). Clinical and microbiological assessment of patients with
a long-term diagnosis of human immunodeficiency virus infection and

Domaneschi, C., Massarente, D. B., de Freitas, R. S., de Sousa
Oral colonization by *Candida* species in AIDS pediatric patients.
*Oral Dis* 17, 393–398.

immunodeficiency virus infected subjects in Turkey and its relation with

Ghelardi, E., Pichierri, G., Castagna, B., Barnini, S., Tavanti, A. &
and presumptive identification of pathogenic yeast species. *Clin
Microbiol Infect* 14, 141–147.


Hung, C. C., Yang, Y. L., Lauderdale, T. L., McDonald, L. C., Hsiao,
human immunodeficiency virus-infected outpatients in Taiwan with


oral candidal carriage, oral candidiasis and CD4 lymphocyte count in


