Salivary carriage of candida species in relation to dental caries among primary school children in Jazan City, KSA

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Running title: Salivary candida in Saudi children
Abstract

**Background:** salivary candida carriage patterns seem to significantly vary by geographical location. The aim of this study was to assess carriage of candida species in saliva of primary school children in a Saudi population, and correlate it to their dental caries activity.

**Methods:** a total of 270 children of both gender were recruited from six primary schools. Oral hygiene and dental caries were assessed using the simplified oral hygiene and dft/DMFT indices, respectively. Chromagar Candida medium was used to identify and quantify candida species in unstimulated saliva samples.

**Results:** seven percent of the subjects were caries free while 74% had high caries activity. Candida was detected in 63.3% of the children with a mean count of 1076±1888 CFU/ml. Significantly higher carriage was observed in the males (70% vs 56%; P=0.02). *C. albicans* accounted for 69 % of the isolates, while *C. tropicalis, C. glabrata, C. kruzie* and unidentified species represented 11.8 %, 5.5 %, 2.3 % and 11.4 %, respectively. Twenty-five percent of the carriers harbored 2 or more species. Twenty carriers clusters were identified as described previously. Carriage status showed the strongest association with both d and D scores (P<0.01), caries status (OR= 5.9; P=0.003) and caries extent (OR=5.5; P=0.001). Carriage at > 1000 CFU was only seen in caries active subjects.

**Conclusions:** asymptomatic oral carriage of candida at high counts seems to be common among children from Arabia. Further evidence is provided here for the potential use of candida counts for prediction of caries risk.

**Key words:** candida; dental caries; etiology; saliva
**Background**

Genus candida comprises more than 500 species [1], of which around twenty species are commonly recovered from human samples. These are usually part of the commensal microflora, but can cause opportunistic infections in susceptible hosts. *Candida albicans* is the most frequently isolated species; however, non-albicans species (NAC) including *C. parapsilosis*, *C. krusie*, *C. tropicalis*, *C. glabrata*, and *C. dubliniensis* are increasingly being detected in clinical samples [2], and seem to dominate in certain populations [3]. Differentiation among these species has become a routine laboratory procedure due to differences among them in virulence factors and susceptibility to antifungal agents [4].

Candida are asymptotically present in the oral cavity of many individuals with the posterior part of the dorsum of the tongue being the favorite habitat [5]. Carriage patterns seem to be influenced by demographic factors and oral habits as well as the sampling and analysis method used [5-7]. Significant geographical variation has been particularly observed. For example, carriage rates as low as 7.7% have been reported in Asian children, compared to as high as 70% in children from the West [7]. Species-wise, *C. albicans* is the most frequently isolated species from Europeans and Americans, while NAC species seem to predominate among Chinese [3]. Obviously, further investigations to elucidate geographical variation in candida carriage patterns are warranted.

Whether or not candida plays a role in the etiology of dental caries is controversial. Candida carriage has been shown to be associated with caries in cross-sectional [8, 9], case-control [10] and risk assessment studies [11, 12]. Candida has also been detected in dentinal caries [13, 14]. In addition, *C. albicans* has been demonstrated in vitro to enhance colonization of dental biofilm by *Streptococcus mutans* [15], possess much greater ability than *S. mutans* to dissolve
hydroxyapatite [16] and even induce dental caries in rats experimentally [17]. Therefore, the
cariogenic potential of candida should not be underestimated in view of the existing evidence.

Data on oral candida carriage in the Arab population is limited to one very recent study, in which
some unique aspects of salivary candida carriage and its relation to dental caries were described
in Yemeni primary school children [18]. The purpose of the current study was to take a step
further by exploring the same parameters in a second Arab population, this time primary school
children in Jazan City, South of Saudi Arabia.

**Methods**

**Study subjects and ethical considerations**

A total of 270, 6-12 years old children (128 females and 142 males), were recruited from six
randomly selected primary schools in Jazan City. Children with signs of oral candidiosis or
wearing orthodontic or other intraoral appliances were not included.

The study was approved by the research committee at the faculty. Permission to conduct the study
in the selected schools was obtained from the Office of Education in Jazan. Written informed
consent was obtained from parents of all of the children included in the study.

**Clinical examination and sample collection**

The children were assessed for oral hygiene and dental caries using the soft debris component of
the simplified oral hygiene index (SD-OHI; [19] and the dft/DMFT index according to WHOs’
methodology for oral health surveys [20] respectively. Children with a zero dft/DMFT score were
defined as caries-free; having 4 or more carious teeth was used to define high caries activity.
Microbiological analysis

Chromagar Candida selective and differential agar (Chromagar, France) was used for detection and quantification of candida species in the samples. After brief vortexing, 100 µl of each sample were used to inoculate the plates followed by incubation at 37°C for 48-72 hours. The plates were then checked for growth (carriage) and, when present, colony counting was performed and recorded as CFUs per ml of unstimulated whole saliva (carriage counts). A cutoff of 1000 CFU/ml was used to categorize subjects into non/low-level carries and high-level carriers.

Species were identified based on colony color as described previously [21, 22]: light green colonies as *Candida albicans*; metallic blue to dark blue colonies with or without a purple halo as *Candida tropicalis*; pink and rough spreading colonies with pale edges as *Candida krusie*, dark pink/mauve colonies with pale edge as *Candida glabrata*, and white or gray colonies as unidentified species (Figure 1). Colony counts for each species were then obtained.

Statistical analysis

Clinical data included caries status (caries-free/caries-active), caries scores (dft, DMFT and their components) and caries extent (low-caries/high-caries). Microbiological variables comprised carriage status (carrier/non-carrier), carriage counts in CFU/ml, species detected, and dichotomous carriage level (non/low-level carriage and high-level carriage). Data were summarized as means±SD or percentages as appropriate. Significance of association of the microbiological variables with caries status and caries extent was sought using multiple logistic regression. Correlation with caries scores (dft, DMFT and their components) was tested with
multiple linear regression. Age, gender and oral hygiene status were included in both models as covariates. Odds ratios (OR) or correlation coefficients (r) were obtained as appropriate. Differences with a P-value of ≤ 0.05 were considered as significant. Statistical analyses were performed using SPSS and LogXact software (Cytel Corporation, USA).

Results

Clinical characteristics

The mean age of the study population was 9.51±1.92. The difference in age by gender was not statistically significant. However, the males had significantly worse oral hygiene compared to the females (mean SD-OHI of 1.14±0.53 vs. 0.82±0.51; P<0.001). SD-OHI scores positively correlated with age (r= 0.22; P<0.001); i.e. the older the age the worse the oral hygiene.

Seventeen (7%) of the children were caries-free while 70 (26%) had high caries activity (4 or more carious teeth). Worse oral hygiene (higher SD-OHI scores) was associated with high caries activity (OR= 2.5; CI= 1.3-4.9). The caries scores are presented in Table 1. High mean D and d scores were noted (1.89 and 4.14); the mean F and f scores represented only 4% and 3% of mean DMFT and dft scores, respectively. Two children presented with missing permanent teeth. As expected, age negatively correlated with d scores while positively correlated with D scores (r= -0.41 and 0.48, respectively; p < 0.001). Being a female was significantly associated with lower d scores (r= -0.12; P= 0.048), but with higher D (r= 0.26; P< 0.001) and F (r= 0.15; P= 0.022) scores. Worse oral hygiene significantly correlated with both d and D scores (r= 0.16 & 0.21, respectively; P< 0.01).
Candida species carriage patterns

One-hundred seventy-one children (60.3%) harbored candida with a mean carriage count of 1067±1888 CFU/ml unstimulated saliva. Compared to the females, the males had significantly higher carriage prevalence (70% vs. 56%; P=0.02) and counts (0.45 log difference; P= 0.01) independent of age and oral hygiene, which did not show any association with either parameter. Dichotomizing by carriage level (1000 CFU/ml cutoff), 228 children (84.4%) were non/low-level carriers and 42 (15.6%) were high-level carriers. Carriage counts as high as 10,000 CFU/ml were observed. One-hundred twenty-eight of the carriers (74.8%) harbored only one candida species, 39 (22.8%) carried two species, 3 (1.8%) had three species and one (0.6%) harbored four species.

Two-hundred nineteen species isolates were recovered. The detection frequencies and mean and maximum counts of each species in the carriers are presented in Table 2. C. albicans accounted for 69 % of the isolates, while C. tropicalis, C. glabrata, C. krusie and unidentified species represented 11.8 %, 5.5 %, 2.3 % and 11.4 %, respectively. No species was detected above 1000 CFU/ml except C. albicans and, in one case, C. krusie. C. glabrata was almost exclusively seen in the males (11 out of 12 cases).

Carriers clusters

Two-step clustering by carriage data revealed the presence of 4 clusters of carriers (Figure 3). The largest cluster (65.5%) included carriers of C. albicans alone. Carriers of C. tropicalis alone or combined with one or two non-glabrata species formed the second cluster (16.4%). The third cluster (11.1%) comprised of those who harbored both C. albicans and unidentified species. The fourth cluster was characterized by carriage of both C. glabrata and C. albicans (7%).
Dental caries in relation to candida carriage

The carriage status (carriers vs. non-carriers) significantly correlated with both the d and D scores (Table 3). It also showed strong association with caries status and caries extent as shown in Figure 3. Log-transformed carriage counts also significantly correlated with the d and D scores (r= 0.37 & 0.2, respectively; P< 0.001) as well as caries status (OR= 1.9; P= 0.009) and extent (OR= 1.9; P < 0.001).

All high-level carriers were caries-active (OR= 6.9; P= 0.04) however, they showed significant association with only the d scores compared to the non/low-level carriers group, after adjusting for other variables.

Discussion

This study represent a continuation of previous work [18] aimed at describing the oral candida carriage patterns and assessing their relation to caries experience in children from Saudi Arabia. It is driven by the need for such information from different parts of the world to improve our understanding of the geographical variation in carriage of this genus [3], and substantiate evidence on its association with dental caries. To the best of our knowledge, this is probably the first such report for children from Kingdom of Saudi Arabia.

Sensitive and accurate quantification of oral candida has been reported with the use of whole saliva samples [4]. Un-stimulated, rather than stimulated, saliva was used in the current study to avoid dilution effect and subsequent reduction of detection sensitivity. Candida species were identified and quantified using Chromagar candida. The reported specificity for this medium is
high (95-100%) and it is, therefore, recommended for rapid screening of clinical samples [22, 23]. A few studies have used it in assessment of oral candida carriage, though.

The carriage rate in this study (63.3%) falls within the upper part of the range reported for similarly aged children in other studies. It is comparable to carriage prevalence recently found in children from Yemen [18] and Israel [24] as well as UK [10], North America [25] and Brazil [26]. The higher prevalence observed in the males (70%) is very close to that shown in Scottish children [27], which is the highest rate reported so far (71%). Rates in the lower part of the range (as low as 7.7%) come from studies on Chinese children [28]. While some previous studies do show that children can asymptotically harbor high counts of [8, 10, 29], this is the first study to demonstrate asymptomatic carriage of as high as 10,000 CFU. In contradiction with this, Epstein et al. [30] showed that a cutoff of 400 CFU per ml unstimulated saliva can be used to differentiate carriers from infected patients. While these differences can be attributed, at least in part, to variations in methodologies used, genuine geographical variations do seem to exist [3].

C. albicans has been reported to account for as high as 95% of isolates in studies from the West (Moreira et al., 2001) and as low as 9.4% in studies from China [3]; the proportion reported in this study (69%) thus falls midway and is comparable to that found in our recent study in Yemeni children [18]. In this latter study, C. albicans was found to account for 100% of carriage at ≥1000 CFU/ml. With the exception of one case, in which C. krusie was detected at 6400 CFU/ml, similar findings were seen in the current study. In line with this, Kleinegger et al. [6] showed that only C. albicans could be detected at ≥500 CFU in buccal swabs. The mean counts of NAC species reported in this study, except for unidentified species, are higher than those found in Yemeni children. Compared to the same study, C. tropicalis was here detected more frequently, while C. krusie and unidentified species were recovered much less frequently; C. glabrata had
almost the same prevalence. Notably, this latter species has not been detected in several previous reports [6, 10, 31, 32].

Co-carriage of two or more species was seen in 25% of the carriers, which is less than that found in Yemeni children (38%) [18], but remains far higher than the range (2-5%) reported by other investigators [6, 33]. The 4 carrier clusters based on co-carriage patterns identified in this study are largely the same as the clusters described in our previous study [18], although the relative sizes of the clusters were different. Findings from both studies strongly suggest that there may be certain interactions among candida species that favor co-existence of two or more species (synergistic) or render presence of particular species together unlikely (antagonistic). It is evident, for example, that carriage of \textit{C. tropicalis} and that of \textit{C. glabrata} are mutually exclusive, while carriage of \textit{C. albicans} favors the presence of \textit{C. glabrata} or unidentified species. More studies are required to explore this further.

Of the three microbiological parameters assessed, carriage status (carriers vs. non carriers) showed the strongest association with all caries parameters. Log-transformed counts also maintained significant associations, but dichotomization by carriage level resulted in losing the association with caries scores in permanent teeth as well as caries extent after adjustment for other variables. Nevertheless, carriage at $\geq$ 1000 CFU/ml was only seen in the caries-active group (OR=6.9) which is identical to the finding from our previous study in Yemen. It is also consistent with the results by Raja et al. [10] and Signoretto [29] who reported mean counts $> 1000$ CFU/ml in their caries-active groups. This cutoff could, therefore, be validated for use in predicting caries risk. The study thus substantiates evidence on the association between carriage of candida and dental caries although the level of evidence it provides may be lower than that provided by previous case-control [10, 29] and longitudinal risk-assessment studies [11, 12, 34].
In conclusion, the current study supports the view that asymptomatic oral carriage of relatively high counts of candida species may be typical for children from Arabia. The study also substantiates evidence for the existence of specific species co-carriage patterns among carriers, and also for the possible use of specific candida carriage counts for predicting caries risk.

**Competing interests**

The authors declare that they have no competing interests.

**Authors' contributions**

NNH designed the study, contributed to the laboratory work, performed the statistical analysis of data and drafted the manuscript. MFQ was responsible of the field work, collection of samples, and data entry. AA carried out the laboratory work. FT coordinated the study and helped with drafting of the manuscript. All co-authors read and approved the final version of the manuscript.

**Acknowledgement**

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References


Figure Legends

**Figure 1.** The four candida species isolated on Chromagar Candida.

**Figure 2.** Four carriers’ clusters identified based on species co-carrigae patterns; similar cluster were described in a previous study [18].

**Figure 3.** Candida carriage by caries status (left) and extent (right). Significance of association was sought using logistic regression analysis, adjusting for age, oral hygiene and gender.
<table>
<thead>
<tr>
<th>Component</th>
<th>Overall (N=270)</th>
<th>Gender</th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>DT</td>
<td>1.89 ± 2.10</td>
<td>1.65 ± 2.07</td>
<td>2.16 ± 2.07</td>
</tr>
<tr>
<td>MT</td>
<td>0.02 ± 0.31</td>
<td>0.01 ± 0.08</td>
<td>0.04 ± 0.44</td>
</tr>
<tr>
<td>FT</td>
<td>0.09 ± 0.45</td>
<td>0.03 ± 0.17</td>
<td>0.16 ± 0.62</td>
</tr>
<tr>
<td>DMFT</td>
<td>1.98 ± 2.10</td>
<td>1.69 ± 2.07</td>
<td>2.30 ± 2.17</td>
</tr>
<tr>
<td>dt</td>
<td>4.14 ± 3.41</td>
<td>4.60 ± 3.54</td>
<td>3.63 ± 3.20</td>
</tr>
<tr>
<td>ft</td>
<td>0.14 ± 0.52</td>
<td>0.11 ± 0.45</td>
<td>0.16 ± 0.59</td>
</tr>
<tr>
<td>dft</td>
<td>4.26 ± 3.42</td>
<td>4.67 ± 3.61</td>
<td>3.80 ± 3.17</td>
</tr>
<tr>
<td>dft/DMFT</td>
<td>6.23 ± 3.73</td>
<td>6.36 ± 4.04</td>
<td>6.09 ± 3.37</td>
</tr>
</tbody>
</table>

* Stepwise multiple linear regression model, adjusting for age, oral hygiene and candida carriage. NS: not significant
<table>
<thead>
<tr>
<th>Species</th>
<th>Frequency No. (%)</th>
<th>CFU/ml (Mean±SD)</th>
<th>Maximum</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>C. albicans</em></td>
<td>148 (86.5)</td>
<td>1100 ± 1927</td>
<td>10000</td>
</tr>
<tr>
<td><em>C. glabrata</em></td>
<td>12 (7.0)</td>
<td>115 ± 176</td>
<td>530</td>
</tr>
<tr>
<td><em>C. tropicalis</em></td>
<td>26 (15.2)</td>
<td>242 ± 268</td>
<td>930</td>
</tr>
<tr>
<td><em>C. kruzie</em></td>
<td>5 (2.9)</td>
<td>1306 ± 2847</td>
<td>6400</td>
</tr>
<tr>
<td>Unidentified</td>
<td>25 (14.6)</td>
<td>68 ± 86</td>
<td>280</td>
</tr>
</tbody>
</table>

Table 2. Candida species detection frequencies and counts among the carriers.
Table 3. Caries scores (mean± standard deviation) by candida carriage status

<table>
<thead>
<tr>
<th></th>
<th>(Non-carriers)</th>
<th>(Carriers)</th>
<th>r*</th>
<th>P*</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n= 99</td>
<td>n= 171</td>
<td></td>
<td></td>
</tr>
<tr>
<td>DT</td>
<td>1.59 ± 1.90</td>
<td>2.14 ± 2.20</td>
<td>0.19</td>
<td>0.003</td>
</tr>
<tr>
<td>MT</td>
<td>0.00 ± 0.00</td>
<td>0.04 ± 0.42</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>FT</td>
<td>0.08 ± 0.51</td>
<td>0.09 ± 0.39</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>DMFT</td>
<td>0.88 ± 1.24</td>
<td>1.15 ± 1.62</td>
<td>0.19</td>
<td>0.003</td>
</tr>
<tr>
<td>dt</td>
<td>3.02 ± 3.10</td>
<td>5.06 ± 3.39</td>
<td>0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>ft</td>
<td>0.17 ± 0.65</td>
<td>0.11 ± 0.37</td>
<td>-</td>
<td>NS</td>
</tr>
<tr>
<td>dft</td>
<td>3.16 ± 3.12</td>
<td>5.16 ± 3.41</td>
<td>0.33</td>
<td>&lt;0.001</td>
</tr>
<tr>
<td>DMFT/dft</td>
<td>4.83 ± 3.51</td>
<td>7.39 ± 3.52</td>
<td>0.37</td>
<td>&lt;0.001</td>
</tr>
</tbody>
</table>

* Stepwise multiple linear regression model, adjusting for gender, oral hygiene and age. r: correlation coefficient; NS: not significant
Cluster 1: *C. albicans* alone.

Cluster 2: *C. tropicalis* alone or combined with one or two non-*glabrata* species.

Cluster 3: *C. albicans* and unidentified species.

Cluster 4: *C. glabrata* and *C. albicans.*
Figure 3

- Caries free: OR = 5.9, P = 0.003
- Caries active:
- Low caries: OR = 5.5, P > 0.001
- High caries