Oral Candida carriage among individuals chewing betel-quid with and without tobacco

Fawad Javed, BDS, PhD, a Maha Yakob, RDH, PhD, b Hameeda Bashir Ahmed, BDS, C Orth, c Khalid Al-Hezaimi, BDS, MSc, FRCD (C), d and Lakshman P. Samaranayake, BDS, DDS, FRCPath d
College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia; School of Dentistry, University of California and Los Angeles, Los Angeles, CA, USA; Al-Farabi Dental College, Riyadh, Saudi Arabia; and Prince Philip Dental Hospital, University of Hong Kong

Objective. The aim was to assess oral Candida carriage among individuals chewing betel-quid (BQ) with and without tobacco.

Study design. A retrospective and comparative study of oral Candida carriage among individuals chewing BQ with and without tobacco. Oral yeast samples were collected from 103 BQ-chewers (52 chewing BQ with tobacco and 51 chewing BQ without tobacco) and 100 non-chewers. Candida strains were cultured in Sabouraud dextrose agar and identified using the API 32-C System and polymerase chain reaction-DNA sequencing. Tongue lesions were clinically identified and numbers of missing teeth were recorded. Unstimulated whole salivary flow rate was recorded.

Results. Oral Candida species were isolated from 72.7% BQ-chewers (73.1% in individuals chewing BQ with tobacco and 72.4% in individuals chewing BQ without tobacco) and 61% non-chewers.


Betel-quid (BQ)-chewing habit has a high prevalence in several Asian and South-Asian countries including Bangladesh, China, India, Pakistan, Sri Lanka, Taiwan, and Thailand. 1-3 It has been reported that BQ-chewing is practiced by nearly 600 million individuals worldwide. 4 The classical components of a BQ include areca-nut, slaked lime (aqueous calcium hydroxide paste), catechu, menthol, and artificial sweeteners wrapped in a betel-leaf (Piper betle leaf). However, addition of smokeless powdered tobacco to the quid may make BQ-chewing more enjoyable for some individuals. The BQ is initially placed in the buccal vestibule and gently chewed and sucked. It is then held against the buccal mucosa over long durations and continued to be gently chewed and sucked intermittently. When desired, the contents are either swallowed or expectorated.

Oral Candida species (particularly Candida albicans [C. albicans]) are an integral component of the normal human oral flora. It has been reported that the prevalence of oral Candida carriage in healthy human oral cavities ranges from 40% to 60%. 5 Risk factors, have been reported to influence oral Candida carriage, include increasing age, female gender, orthodontic treatment, immunocompromised conditions (such as poorly-controlled diabetes mellitus and infection with human immunodeficiency virus [HIV]) and tobacco smoking. 6-11 It has been suggested that tobacco contents (such as nicotine, polycyclic aromatic hydrocarbons, polonium, and nitrosoprolin) act as nutrients for Candida species and facilitate their proliferation. 12 To our knowledge from indexed literature, 2 studies have assessed oral Candida carriage and species prevalence in BQ-chewers and non-chewers. 2,3 The results demonstrated no significant difference in carriage of Candida species isolated from BQ-chewers and non-chewers. 2,3 However, it is notable that in these studies 2,3 it remained unclear whether the study participants were chewing BQ with or without tobacco. Since nicotine may act as a nutrient for Candida species 12; we hypothesize that oral Candida carriage and species prevalence is increased in individuals chewing BQ with tobacco as compared to those chewing BQ without tobacco and non-chewers.

The aim of the present study was to assess the oral Candida carriage among individuals chewing BQ with and without tobacco.

Statement of Clinical Relevance
Oral Candida carriage is similar in individuals chewing betel-quid either with or without tobacco. This suggests that individuals in either group are equally susceptible to oral Candida infections compared to non-chewers.
**METHODS**

**Ethical approval**
The present study was approved by the research ethics review committee of the Engineer Abdullah Bugshan Research Chair for Growth Factors and Bone Regeneration, College of Applied Medical Sciences, King Saud University, Riyadh, Saudi Arabia. The study was performed in accordance with the Declaration of Helsinki as revised in 2000. It was mandatory for subjects to have read and signed the consent form before being included in the present study.

**Inclusion and exclusion criteria**
Exclusively BQ-chewers were included in the present study. The exclusion criteria comprised of the following: (a) tobacco smoking; (b) alcohol consumption; (c) exclusive areca-nut and gutka chewing; (d) individuals currently using or those who had used antibiotics, antifungal agents, steroids and/or non-steroidal anti-inflammatory drugs within the past 12-weeks; (e) self-perceived systemic disorders such as diabetes mellitus, Hepatitis B, Hepatitis C, infection with HIV and acquired immunodeficiency syndrome; and (f) individuals who reported to be wearing partial and/or complete dentures.

**Study participants**
Individuals who reported to have been chewing BQ with or without tobacco at least once daily were defined as “individuals chewing BQ with tobacco” and “individuals chewing BQ without tobacco” respectively. Individuals reported to have never used BQ, gutka, and/or areca-nut either with or without tobacco were designated as “non-chewers.”

**Questionnaire**
A standardized questionnaire was used to gather information regarding age, gender, duration of BQ-chewing habit, daily frequency of BQ consumption, duration of placement of BQ in the mouth (in minutes), daily frequency of tooth brushing, tongue brushing, and oral rinsing after BQ-chewing.

**Collection of unstimulated whole saliva and measurement of unstimulated whole salivary flow rate (UWSFR)**
Unstimulated whole saliva (UWS) samples were collected early mornings (between 8:00 AM and 8:30 AM). Participants were requested to refrain from eating and/or drinking at least 2 h before the UWS samples were collected. For collection of UWS samples, participants (BQ-chewers and non-chewers) were seated comfortably on a chair in a “coach-man” position and requested to spit (without swallowing) for 5 continuous minutes into a gaged measuring cylinder. UWSFR was recorded in milliliters per minute (mL/min).

**Collection of oral Candida samples and culture**
Oral Candida samples were collected as described elsewhere, immediately after collection of the unstimulated whole saliva samples. Briefly, participants were instructed to avoid eating and drinking at least 2 h before collection of microbiological samples. Each sample was collected by scraping the dorsum of the tongue and bilateral buccal mucosa with a sterile cotton swab (Biomérieux SA, Montalieu-Vercieu, France). The swabs were immediately returned to the containment tube after sampling.

*Candida* strains were cultured in Sabouraud dextrose agar (Becton, Dickinson and Company, Sparks, MD, USA) at 37 °C to quantify the colony-forming units in the oral cavities of *gutka*-chewers and controls. The cultures were inspected after 24-h and until 7 days of incubation for yeast growth. The cultures were subjected to speciation.

**Identification of oral yeast samples**
Oral yeast species were identified using a yeast identification system (API 32-C System bioMérieux yeast identification programme, Lyon, France). If identification was not possible with the API 32-C system, the yeast isolate was subjected to molecular identification.

For DNA isolation, yeast cells were suspended in 200 µL sterile polymerase chain reaction (PCR)-grade water and genomic DNA was prepared using MagNA pure (Roche Diagnostics GmbH, Mannheim, Germany) a DNA preparation robot. A region (about 500-bp) of 18S ribosomal ribonucleic acid gene was amplified by PCR using universal primers and ampliTag Gold DNA polymerase for DNA sequencing and PCR analysis. Primers and free nucleotides from the PCR products were removed using the QIAquick PCR purification kit (250) (Qiagen, GmbH, Hilden, Germany). The purified PCR products were then processed for DNA sequencing by BigDye Terminator Cycle Sequencing using capillary electrophoresis technology in ABI 310 Genetic Analyzer (Applied Biosystems, Foster City, CA, USA). To avoid error of sequencing, both strands of PCR amplified DNA fragments were sequenced. For yeast identification and typing, the DNA sequence was analyzed and searched in the Blast DNA database.

**Lesions on the tongue and numbers of missing teeth**
After a complete clinical oral examination, clinical diagnosis of tongue lesions (fissured tongue, geographic tongue, coated tongue, hairy tongue, and median
rhomboid glossitis) was made using standardized World Health Organization criteria.\textsuperscript{26-30} Numbers of missing teeth were also recorded.

### Statistical analysis

Statistical analysis was performed using a software program (SPSS Version 18, Chicago, IL, USA). Level of significance between the groups (individuals chewing BQ with and without tobacco and non-chewers) was assessed using Mann–Whitney U test. For multiple comparisons, the Bonferroni post hoc test was used. \( P \) values <0.05 were considered statistically significant. A multiple logistic regression model was applied to adjust for confounding variables (age, gender, BQ-chewing with and without tobacco, daily frequency of chewing, duration of chewing, oral hygiene measures, saliva flow rate, number of missing teeth, presence of tongue lesions, and culture/PCR results).

### RESULTS

#### Characteristics of the study population

In total, 103 BQ-chewers (52 individuals chewing BQ with tobacco and 51 individuals chewing BQ without tobacco) and 100 non-chewers (controls) were included. Among BQ-chewers and non-chewers, most of the participants were male. The mean age of BQ-chewers and non-chewers was 37.2 years (31-42 years) and 36.2 years (30-40 years) respectively (Table I).

Among individuals chewing BQ with and without tobacco, the mean duration of chewing habit was 11.1 years (4-15 years) and 8.5 years (3-12 years) respectively. The mean numbers of BQs consumed daily by individuals chewing BQ with and without tobacco was 5.4 quids (2-6 quids) and 2.5 quids (1-4 quids) respectively. The mean duration of BQ placement in the mouth was significantly higher in individuals chewing BQ with tobacco (23.6 min) compared to those chewing BQ without tobacco (8.2 min) (\( P < .05 \)) (Table I).

#### UWSFR among BQ-chewers and non-chewers

The mean UWSFR among individuals chewing BQ with and without tobacco and non-chewers was 0.57 mL/min (range 0.5-0.65 mL/min), 0.55 mL/min (range 0.5-0.6 mL/min) and 0.61 mL/min (range 0.5-0.7 mL/min) respectively.

#### Oral Candida species isolated from BQ-chewers and non-chewers

All Candida species were isolated via culture method except for Candida kruasei, Candida luscitanea, and Candida parapsilosis that were identified using PCR-DNA sequencing. Overall, oral Candida species were isolated from 72.7% BQ-chewers (73.1% in individuals chewing BQ with tobacco and 72.4% in individuals chewing BQ without tobacco) and 61% non-chewers. The most common oral yeast species isolated from individuals chewing BQ with and without tobacco and

### Table I. Characteristics of the study cohort

<table>
<thead>
<tr>
<th>Parameters</th>
<th>All betel-quid chewers</th>
<th>Individuals chewing betel-quid with tobacco</th>
<th>Individuals chewing betel-quid without tobacco</th>
<th>Controls</th>
</tr>
</thead>
<tbody>
<tr>
<td>No. of individuals</td>
<td>103</td>
<td>52</td>
<td>51</td>
<td>100</td>
</tr>
<tr>
<td>Gender</td>
<td>92 male</td>
<td>45 male</td>
<td>47 male</td>
<td>88 male</td>
</tr>
<tr>
<td>Mean age in years (range)</td>
<td>37.2 (31-42)</td>
<td>40.7 (33-42)</td>
<td>35.4 (31-38)</td>
<td>36.2 (30-40)</td>
</tr>
<tr>
<td>Mean duration of betel-quid chewing habit in years (range)</td>
<td>10.5 (3-15)</td>
<td>11.1 (4-15)</td>
<td>8.5 (3-12)</td>
<td>–</td>
</tr>
<tr>
<td>Mean numbers of betel-quids consumed daily (range)</td>
<td>4.3 (1-6)</td>
<td>5.4 (2-6)</td>
<td>2.5 (1-4)</td>
<td>–</td>
</tr>
<tr>
<td>Mean duration of placement of betel-quid in the mouth in minutes (range)</td>
<td>16.4 (5-30)</td>
<td>23.6* (10-30)</td>
<td>8.2* (5-10)</td>
<td>–</td>
</tr>
</tbody>
</table>

#### Daily oral hygiene maintenance

| Tooth brushing (once daily) (%) | 61 (59.2) | 29 (55.7) | 32 (62.7) | 58 (58) |
| Tooth brushing (twice daily) (%) | 42 (40.8) | 23 (44.3) | 19 (37.3) | 42 (42) |
| Tongue brushing after tooth brushing (%) | 14 (13.5) | 6 (11.5) | 8 (15.6) | 13 (12) |
| Rinsing the mouth with water after betel-quid consumption (%) | 73 (70.9) | 33 (63.5) | 39 (76.5) | – |

\*\( P < .05 \).
non-chewers was *C. albicans* (26.9%, 23.5%, and 24% respectively). *Candida tropicalis* was isolated from 19.2% of individuals chewing BQ with tobacco, 17.6% of individuals chewing BQ without tobacco and 16% non-chewers. Among individuals chewing BQ with and without tobacco and non-chewers, *C. parapsilosis* was isolated from 5.8%, 7.8%, and 3% individuals respectively. Among non-chewers, *C. krusei* and *C. luscitanie* were isolated from 1% and 1% individuals respectively. *C. albicans* and *C. tropicalis* as mixed species were isolated from 17.5% BQ-chewers (15.4% in individuals chewing BQ with tobacco and 19.6% individuals chewing BQ without tobacco) and 16% non-chewers. As mixed species, *C. albicans* and *C. parapsilosis* were isolated from 4.8% BQ-chewers (5.8% individuals chewing BQ with tobacco and 3.9% individuals chewing BQ without tobacco) and 2% non-chewers (Table II).

**Lesions on the tongue and numbers of missing teeth**
Tongue lesions were not detected upon clinical examination among individuals chewing BQ with and without tobacco and non-chewers. The mean numbers of missing teeth between individuals chewing BQ with and without tobacco and non-chewers were 6.5 teeth (range 3-8 teeth), 5.7 teeth (range 4-9), and 5.5 teeth (range 4-8 teeth), respectively.

**Relationship between confounding variables and oral Candida carriage in the study population**
The multiple logistic regression analysis did not show significant relationships between age, gender, BQ-chewing with and without tobacco, daily frequency of chewing, duration of chewing, oral hygiene measures, saliva flow rate, number of missing teeth, presence of tongue lesions and culture/PCR results and oral **Candida** carriage in the study population.

**DISCUSSION**
The present results failed to show significant difference in oral **Candida** carriage among individuals chewing BQ with and without tobacco and non-chewers. In addition, individuals in all study groups were relatively young and there were no significant differences in UWSFR between the groups. These factors could possibly explain why significant differences in oral **Candida** carriage among BQ-chewers and non-chewers were not observed.

In the present study, oral **Candida** was isolated from ~70% BQ-chewers and 61% non-chewers. These results are in accordance with earlier studies. In studies by Javed et al. and Torres et al. oral **Candida** was isolated from nearly 70% of the study participants. It is known that oral **Candida** carriage rates are higher in tobacco smokers and among patients with systemic diseases (such as poorly-controlled diabetes). Although we excluded individuals who self-reported to be tobacco smokers and/or having systemic disorders, it is possible that there might have been participants with impaired glucose tolerance and/or undiagnosed diabetes in the study group. Furthermore, it is possible that some of the participants who reported to be non-smokers may have either preferred to veil their tobacco habits had recently quit smoking.

Mixed **Candida** species have been associated with oral infections including oral candidiasis and denture stomatitis. Studies using the chromogenic medium CHROMagar have reported rates of mixed **Candida** carriage to vary between 26.3% and 60%. Therefore, regardless of the population studied, the method for processing the samples may also influence the frequency

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**Table II. Oral Candida species isolated from individuals chewing betel-quid with and without tobacco and non-chewers**

<table>
<thead>
<tr>
<th>Oral Candida species</th>
<th>All betel-quid chewers (N = 103) n (%)</th>
<th>Individuals chewing betel-quid with tobacco (N = 52) n (%)</th>
<th>Individuals chewing betel-quid without tobacco (N = 51) n (%)</th>
<th>Controls* (N = 100) n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><em>Candida albicans</em></td>
<td>26 (25.2)</td>
<td>14 (26.9)</td>
<td>12 (23.5)</td>
<td>24 (24)</td>
</tr>
<tr>
<td><em>Candida tropicalis</em></td>
<td>19 (18.4)</td>
<td>10 (19.2)</td>
<td>9 (17.6)</td>
<td>16 (16)</td>
</tr>
<tr>
<td><em>Candida albicans</em> + <em>Candida tropicalis</em></td>
<td>18 (17.5)</td>
<td>8 (15.4)</td>
<td>10 (19.6)</td>
<td>14 (14)</td>
</tr>
<tr>
<td><em>Candida parapsilosis</em></td>
<td>7 (6.8)</td>
<td>3 (5.8)</td>
<td>4 (7.8)</td>
<td>3 (3)</td>
</tr>
<tr>
<td><em>Candida albicans</em> + <em>Candida parapsilosis</em></td>
<td>5 (4.8)</td>
<td>3 (5.8)</td>
<td>2 (3.9)</td>
<td>2 (2)</td>
</tr>
<tr>
<td><em>Candida krusei</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Candida luscitanie</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>1 (1)</td>
</tr>
<tr>
<td><em>Candida glabrata</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td><em>Candida gullerimoni</em></td>
<td>—</td>
<td>—</td>
<td>—</td>
<td>—</td>
</tr>
<tr>
<td>No. Candida species isolated</td>
<td>28 (27.2)</td>
<td>14 (26.9)</td>
<td>14 (27.5)</td>
<td>39 (39)</td>
</tr>
</tbody>
</table>

*These Candida species were identified using polymerase chain reaction.*
of detection of mixed Candida species. In the present study, mixed Candida species were isolated from nearly 22% BQ-chewers and 16% non-chewers. It is notable that participants in the present study were relatively young, had salivary flow rates similar to those of the controls and were not placing BQ in the mouth for long durations. This may possibly explain why oral mucosal infections were not observed in patients with mixed Candida species. Nevertheless, it is tempting to speculate that elderly individuals placing BQ in the mouth for prolonged durations harbor high percentages of mixed Candida species that could make them more susceptible to oral infections compared to younger BQ-chewers and controls. Further studies are warranted in this regard.

It has been reported that lesions of the tongue lesions (such as median rhomboid glossitis [MRG]) have a direct association with oral Candidiasis, tobacco smoking, denture wearing, and systemic conditions including diabetes mellitus and acquired immune deficiency syndrome. In the present study, no tongue lesions (such as MRG) were observed in the study groups upon clinical examination. It is noteworthy that there was no significant difference in the mean duration of the chewing habit among individuals chewing BQ either with or without tobacco. Although individuals chewing BQ with tobacco were placing the quid in the buccal vestibule for significantly longer duration than those chewing BQ without tobacco; individuals in both groups reported to rinse their mouth with water once the quid was either swallowed or expectorated. This could have possibly prevented tobacco and nicotinic compounds present in the BQ from stagnating the tongue surfaces of BQ-chewers. This may in turn have prevented the formation of tongue lesions by preventing oral Candida species from colonizing the dorsum of the tongue. It is however tempting to speculate that oral health awareness and regular oral hygiene maintenance practiced by the participants of the present study prevented tongue lesions as well as Candida carriage regardless of the BQ (either with or without tobacco) chewing habit. The above notwithstanding, there is a possibility that BQ-chewers (either with or without tobacco) and non-chewers with poor oral health might demonstrate a variation in oral Candida carriage and tongue lesions, which requires further investigations. Furthermore, it cannot be over-emphasized that other methods such as cytologic smears, for quantification of clinically meaningful carriage with oral Candida species should be used for sample analyses.

Limitations of the present study include retrospective study design, self-rated assessment of systemic health status and tobacco habits, and limited geographic distribution and age group of the study population. It is therefore tempting to speculate that oral Candida carriage could be higher in elderly individuals and among patients with and without systemic diseases and among habitual tobacco product users compared to the population assessed in the present study.

CONCLUSION

Within the limits of the present investigation, a significant difference in oral Candida carriage and species prevalence in individuals chewing BQ with and without tobacco and non-chewers was not observed.

REFERENCES


Reprint requests:
Fawad Javed, BDS, PhD
Engineer Abdullah Bugshan Research Chair for Growth Factors and Bone Regeneration
3D Imaging and Biomechanical Laboratory
College of Applied Medical Sciences
King Saud University
P.O.Box 60169, Riyadh 11545
Saudi Arabia
fawjav@gmail.com