Topographic distribution of bacteria associated with oral malodour on the tongue


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ARTICLE INFO

Keywords:
Tongue
Oral malodour
Lingual tonsil
Volatile sulphur compounds

ABSTRACT

Objective: To investigate the topographic distribution of bacterial types and loads associated with mid-morning oral malodour on the tongue surface.

Design: Fifty subjects with good oral health and at least 20 natural uncrowned teeth were included. Samples were taken with sterile brushes from the dorsal anterior (DA), dorsal middle (DM), dorsal posterior (DP), dorsal posterior to the circumvallate papillae (DPCP), lateral posterior (LP) and ventral posterior (VP) tongue surfaces. Samples were cultured on appropriate media for anaerobic bacteria, aerobic bacteria, Gram-negative anaerobic bacteria, volatile sulphur compound (VSC)-producing bacteria and Streptococcus salivarius. Malodour was assessed by trained judges on an intensity basis.

Results: The counts of all bacterial groups were consistently highest at the DPCP surface. Mean VSC-producing bacterial counts (colony forming units/brush × 10^5) were 1.45, 5.67, 32.52, 88.94, 6.46 and 0.33 at DA, DM, DP, DPCP, LP and VP surfaces, respectively. Anaerobic, Gram-negative and VSC counts at DPCP surfaces increased with malodour intensity, whereas aerobic and S. salivarius counts decreased; however these differences were not statistically significant.

Conclusion: It is concluded that the DPCP area consistently carries the highest load of bacteria capable of contributing to oral malodour. The study demonstrates that tongue surfaces not accessible to routine oral hygiene procedures can significantly contribute to oral malodour.

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1. Introduction

Oral malodour, or halitosis, is a common complaint that can affect up to half of the population at any one time. Approximately 90% of the cases of oral malodour are associated with the production of malodorous compounds by oral bacteria. Other causes include gastrointestinal disorders, hepatic diseases, ingestion of certain foods and smoking.

Bacterial products that contribute to oral malodour result from the breakdown of proteins, peptides and mucins found in saliva, gingival crevicular fluid, host cells and residual food. The sulphur-containing amino acids namely cysteine, cystine and methionine, found...
either free in gingival crevicular fluid, in saliva or produced from proteolytic breakdown are the key precursor molecules for malodorous volatile sulphur compounds (VSCs). VSCs are considered to be the most significant products as regards oral malodour and include hydrogen sulphide, methyl mercaptan and dimethyl sulphide as the main contributors. Many Gram-negative anaerobic species of bacteria, found in the oral cavity, produce malodorous compounds including VSCs, short-chain organic acids (butyrate, propionate, valerate), diamines (cadaverine, putrescine) and phenyl compounds (indole, skatole, pyridine). These species include Treponema denticola, Porphyromonas gingivalis, Prevotella intermedia, Tannerella forsythensis, Porphyromonas endodontalis and Eubacterium species. In contrast, Streptococcus salivarius and other Gram-positive bacteria are more commonly found in individuals with low/no malodour.

The dorsum of the tongue, with a papillary structure that allows the retention of considerable quantities of food and other debris, supports a large population of bacteria including Gram-negative anaerobes. Most published studies on the microbiota of the tongue have treated the dorsal surface as a single habitat for sampling purposes and have reported an association between bacterial numbers and oral malodour. The objective of this study was to more closely examine the topographic distribution and loads of those bacterial types associated with oral malodour on the tongue surface, including the dorsal area posterior to the circumvallate papillae which has received little attention to date. Possible associations between bacterial counts and mid-morning oral malodour intensity scores were also investigated. Such information has particular relevance to the successful control of oral malodour.

2. Materials and methods

The study was carried out at a single centre (Institute of Dentistry, London) and did not involve any treatment intervention. Consent was sought and ethical clearance for the study was obtained from the East London and the City Health Authority, UK. Eligible subjects, with good general oral health, periodontally healthy and at least 20 natural uncrowned teeth, attended two visits. At the first visit subjects underwent an oral soft tissue examination. After a further phase of between 7 and 14 days, subjects attended a second time (mid-morning) for oral soft tissue examination, oral malodour intensity assessment and an oral biofilm sampling from various areas of the tongue.

The tongue was sampled by gently pressing a Wisdom® baby toothbrush (surface area 84 mm²) onto the surface and oscillating it slightly without actual lateral movement. Randomized sampling of subjects on either the left or right hand side of the tongue was undertaken. Samples were taken from the dorsal anterior (DA), dorsal middle (DM), dorsal posterior (DP) and dorsal posterior to the circumvallate papillae (DFCP), lateral posterior (LP) and ventral posterior (VP) tongue surfaces. Samples were placed into a reduced transport fluid, stored at 4°C for no more than 2h, and then vortexed for 30s to disaggregate bacteria from the brush. Samples were cultured on appropriate media using a spiral plater (Don Whitley Instruments, Shipley, UK). Blood agar base No.2. (Oxoid CM0271) with defibrinated horse blood (5% v/v) was used to culture aerobic bacteria. Anaerobe basal agar (Oxoid CM0972) with defibrinated horse blood (5% v/v) was used to culture anaerobic bacteria. This medium was supplemented with vancomycin (5 μg/ml) to enumerate Gram-negative anaerobic bacteria. A differential medium was used to enumerate VSC-producing bacteria. This medium contained Columbia Agar Base (CM0331) supplemented with haemin (0.0001% w/v), glutathione (0.12% w/v) and lead acetate (0.02% w/v). VSC-producing colonies exhibit a grey or black colour following incubation. This medium was validated with known VSC-producing species including F. nucleatum, P. gingivalis and Prev. intermedia. TYC medium (Lab M) was used to enumerate S. salivarius. Biochemical tests were used on representative colonies to ensure correct identification to species level. Aerobic and anaerobic cultures were incubated in air with 5% CO₂ at 37°C for 24h, and 80% N₂, 10% CO₂, 10% H₂ at 37°C for 48h, respectively.

Malodour was assessed by trained judges on an intensity basis and scored as 0 (no malodour), 1 (very slight), 2 (slight), 3 (moderate), 4 (strong) and 5 (very strong).

2.1. Statistical methods

Raw data were converted to colony forming units/sample (CFU/sample). Duplicate counts were averaged and then log₁₀ transformed for statistical analysis. Counts recorded as <10² were artificially transformed to 50 prior to log₁₀ transformation to account for the limit of detection of the system. For the comparison between tongue sites, a mixed model was fitted, with tongue site and malodour group as fixed effects and subject as random effect. Results were adjusted for multiple testing using the Tukey-Kramer method. For the comparison between malodour groups, a linear model was fitted for each tongue site with malodour group as the fixed effect. Malodour was assessed by trained judges on an intensity basis and scored as 0 (no malodour), 1 (very slight), 2 (slight), 3 (moderate), 4 (strong) and 5 (very strong).

3. Results

19 men and 32 women were recruited to the study. The ethnicity mix comprised 21 Caucasian, 10 Asian, 15 Afro/Caribbean and 5 Others with a mean age of 34.72 years (minimum 18; maximum 59; median 31.95 years). Final data was available for 50 subjects. 22 subjects fell within the strong malodour category (scores of 4 or 5).
Table 1 – Summary of mean bacterial counts by area of tongue

<table>
<thead>
<tr>
<th>Bacterial type</th>
<th>DA</th>
<th>DM</th>
<th>DP</th>
<th>DPCP</th>
<th>LP</th>
<th>VP</th>
</tr>
</thead>
<tbody>
<tr>
<td>Total anaerobes</td>
<td>10.57±1.08</td>
<td>27.38±2.64</td>
<td>175.93±20.00</td>
<td>327.97±27.49</td>
<td>32.02±4.55</td>
<td>5.83±0.88</td>
</tr>
<tr>
<td>Total aerobes</td>
<td>9.80±1.17</td>
<td>17.72±1.53</td>
<td>77.51±9.15</td>
<td>165.52±16.95</td>
<td>22.54±2.44</td>
<td>5.03±0.67</td>
</tr>
<tr>
<td>Gram-negative anaerobes</td>
<td>2.80±0.41</td>
<td>11.20±1.68</td>
<td>87.78±12.56</td>
<td>175.92±20.14</td>
<td>10.70±1.91</td>
<td>0.85±0.22</td>
</tr>
<tr>
<td>VSC-producing bacteria</td>
<td>1.45±0.19</td>
<td>5.67±0.90</td>
<td>32.52±4.68</td>
<td>88.94±14.03</td>
<td>6.46±1.56</td>
<td>0.33±0.13</td>
</tr>
<tr>
<td>Streptococcus salivarius</td>
<td>0.50±0.09</td>
<td>2.21±0.33</td>
<td>24.01±5.81</td>
<td>54.62±10.23</td>
<td>1.79±0.38</td>
<td>0.09±0.06</td>
</tr>
</tbody>
</table>

Data are presented as mean±SE (n = 50).

DA = Dorsal anterior; DM = Dorsal middle; DP = Dorsal posterior; DPCP = Dorsal posterior to the circumvallate papillae; LP = Lateral posterior; VP = Ventral posterior.

and 28 subjects fell within the slight/moderate malodour category (scores of 1, 2 or 3).

The mean bacterial counts for each tongue sampling location are shown in Table 1. Considering the dorsum of the tongue, there was a general trend for increasing bacterial load moving from the anterior to the posterior of the tongue. The most heavily colonized tongue site was DPCP, with approximately twice as many of each of the five bacterial types assessed than the DP site, the next most heavily colonized. A large increase in numbers, observed across all five bacterial types, was observed between DM and DP (4–12 fold increase). The site with fewest recovered bacteria was the VP surface with 33 (total aerobes) to 270 (VSC-producing bacteria) fold fewer bacteria than DPCP. At each tongue site sampled, the relative proportions of each of the five bacterial types remained broadly similar. Thus, total anaerobes were consistently the most numerous bacterial group at every site, with S. salivarius numbers consistently the lowest.

In common with total anaerobes, the number of VSC-producing bacteria was highest at posterior sites. In addition, recovered aerobe numbers were higher than Gram-negative anaerobes at DA, DM, LP and VP, Gram-negative anaerobes outnumbered aerobes at the posterior of the tongue dorsum (DP, DPCP). The ratio of total aerobes to total anaerobes declined from 0.93 at the anterior of the tongue dorsum to 0.44 and 0.5 at the dorsal posterior (DP and DPCP, respectively).

Comparison between tongue sites within subjects indicated significant differences for each bacterial type (data not shown). Exceptions were LP versus DM for all 5 bacterial types and additionally for S. salivarius comparisons DP versus DPCP and LP versus DA.

Subjects were subdivided into those with slight/moderate (intensity score of 1, 2 or 3) or strong (intensity score of 4 or 5) malodour. Those with strong malodour had higher levels of total anaerobes, Gram-negative anaerobes and VSC-producing bacteria recovered from DPCP compared with those with slight/moderate malodour (Figures 1A, 1C and 1D). Conversely, total aerobe and S. salivarius numbers were higher at this site in the slight/moderate malodour group (Figures 1B and 1E). However, the mean differences in bacterial

Fig. 1 – Mean bacterial counts (±SE) of: (A) total anaerobes, (B) total aerobes, (C) Gram-negative anaerobes, (D) VSC-producing bacteria, (E) Streptococcus salivarius, at tongue sites sampled for subjects with slight/moderate (■, n = 28) or strong (●, n = 22) oral malodour.
numbers on the DM, DP or DPCP tongue dorsum sites between the two malodour groups (slight/moderate malodour and strong malodour) were not statistically different.

4. Discussion

It is well recognised that individual species of bacteria differ in their ability to adhere to different attachment sites within the oral cavity and also to other species. Although an overall load difference between the different groups of bacteria at each tongue site was demonstrated with this study, no indication of site-specificity can be ascertained. However, within each of the four broad categories (aerobes, anaerobes, Gram-negative anaerobes and VSC-producing bacteria) specificities may well exist. Paster et al. attempted to define the bacterial diversity of the oral cavity by examining nine different oral sites, including the lateral side of the tongue and the tongue dorsum. Some species were found to be common to all sites, while many bacteria were site-specific. For example Neisseria spp. were found not to substantially colonise the teeth or subgingival plaque but were commonly found on soft tissues. *S. salivarius* was detected generally on the surface of the tongue dorsum, as was shown in this study.

Several investigators have identified the dorsal posterior surface of the tongue as making the primary contribution to oral malodour, however little attention has been paid to the area posterior to the circumvallate papillae (DPCP). In this study, this area consistently showed the highest count for all bacterial groups; while the ventral posterior area consistently showed the lowest count for all bacterial groups examined. It was observed that counts of total anaerobic, total Gram-negative anaerobic and VSC-producing bacteria were higher at the DPCP surface of subjects with strong malodour whereas counts of total aerobes and *S. salivarius* were higher in the slight/moderate malodour subjects. Further studies, with a greater number of subjects in each malodour category, and including subjects with no oral malodour, may help to confirm the relationship between bacterial counts and oral malodour at the DPCP site. In support of these findings, DeBover and Loesche showed that both total and anaerobic bacterial loads on the dorsal surface of the tongue correlated with organoleptic score. More specifically, the findings of Washio et al. suggest that an increase in the load of H$_2$S-producing bacteria found within the tongue biofilm is largely responsible for the intensity of oral malodour.

Donaldson et al. showed no association between halitosis and any specific genus of anaerobic bacteria. However, they did demonstrate an increase in species diversity in samples from individuals with halitosis. They suggest that interactions between several species, including uncultivable bacteria, may be important in contributing to malodour. Another study, based upon 16S rRNA gene sequencing, demonstrated that certain oral bacterial species are more prevalent in subjects with no malodour, whereas other species tended to predominate in subjects with halitosis. *Streptococcus salivarius* was among those found to be more common in individuals with low/no malodour. The potential of this species to control oral malodour has been explored using the administration of a bacteriocin-producing strain; which was shown to reduce oral VSC levels. The dorsal surface of the tongue offers a large surface area to support a higher bacterial density (estimated at 100 bacteria per tongue epithelial cell) than any other mucosal surface in the oral cavity, for example the ventral and lateral tongue surfaces. The papillary structure of the tongue represents an ecological niche that is unique in nature, and acts as a reservoir for both oral debris and microorganisms, most notably the anaerobic species of bacteria, including *Veillonella* spp. and *Actinomyces* spp. The dorsal surface of the tongue may help to confirm the relationship between bacterial species and any specific genus of anaerobic bacteria. This study describes the first detailed mapping of the tongue surface in relation to the bacteria associated with oral malodour and has demonstrated that the dorsal area posterior to the circumvallate papillae consistently carries the highest load of bacteria capable of contributing to malodour through the production of volatile sulphur compounds.

5. Acknowledgements

This study was financially supported by GSK. GSK were involved in the study design, interpretation of data and writing of the manuscript.
6. Conflict of interest statement

Co-authors MN, RM and MPB were employed by GSK at the time of the study.

REFERENCES