PATHOGENIC MICROBIOTA IN THE ORAL AND NASAL CAVITY OF HEALTHY INDIVIDUALS AS A POTENTIAL SOURCE OF INFECTIONS

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ABSTRACT

Oral microbiota is personalized and varied among human habitats. Detection and identification of pathogenic bacteria and fungi are considered as a strategy for the prevention and control of infectious diseases. To date, no studies have investigated the prevalence of bacterial pathogens and fungi in healthy individuals in Vietnam, particularly the ethnic minorities.

This study aimed to evaluate the presence of aerobic bacteria and fungi in the oral and nasal cavities of Jrai healthy individuals in the Central Highland. Oral and nasal swab samples of 140 healthy Vietnamese were collected. Microbiological procedures were performed using standard techniques. A total of 220 bacterial isolates were identified. Of which, 10% (22/220) were potentially pathogenic species including Klebsiella pneumoniae, Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, Neisseria meningitidis, and Staphylococcus aureus. The most predominant bacteria family was Moraxellaceae (40%, 88/220), followed by Streptococcaceae (36.82%, 81/220).

Fungi were not detected in all samples. The oral and nasal cavity of healthy individuals harbors high frequencies of bacterial pathogens, suggesting its potential role as a source for these species. These pathogenic bacteria constitute the threat of their spread and the development of general infections. Infectious microbiota from the oral and nasal cavity should be examined as a preventive screening to control infectious diseases.

Keywords: Microbiota, Biodiversity, Jrai people, Nasal Cavity, Oral Cavity

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1. INTRODUCTION

Microorganisms can be found everywhere and are related to human life [1]. Healthcare-associated infections (HAIs) are transmissible and result from the interaction of multiple different factors in the infection chain. In this context, humans have been indicated as possible disseminators of pathogenic microorganisms in the environment [2]. A healthy individual’s microbiology has been associated with many factors, such as age, gender, environment, and diet. The healthy human microbiota is personalized, varied systematically across human habitats [3]. Oral and nasal cavity microbiota play an important role in human health [1]. Both oral and nasal cavities are colonized by a system of microorganisms, including bacteria, fungi, and viruses [4]. This microbiota presents significant risk factors to human health, such as oral and body systematic diseases [1]. The oral microbiome can cause many diseases such as caries, periodontal and mucosal diseases and oral cancer [5], peri-implantitis, gastrointestinal system diseases, nervous system diseases [6], endocrine system diseases [7].

Tobacco smoking has significant adverse effects on human health. Smokers cause many diseases, such as organ system diseases and cancers [8]. The oral and nasal cavity microbiota have direct contact with cigarette smoke and may be significantly affected. Numerous toxicants in cigarette smoke can contribute to modifications of the oral and nasal cavity microbial ecology [9]. Although the microorganisms were infrequently detected in human oral and nasal cavity samples, few studies reported microorganisms that may be causing serious systemic infections by conventional culture methods [10]. This study aimed to examine the presence of potentially pathogenic aerobic bacteria and fungi in the oral and nasal cavity of healthy individuals by conventional culture methods to prevent the spread of infectious microorganisms that are risk factors for human health. Furthermore, we also investigated whether smokers modified the oral and nasal cavity microbiota diversity.

2. MATERIALS AND METHODS

Ethics statement

All the healthy individuals agreed to participate in the study after they were explained in detail about the study and written informed consent was obtained from all participants before sampling. All oral samples collected were anonymized after the completion of the sampling. The study was approved by the institutional review board of the Vietnam Military Medical University (VMMU), Hanoi, Vietnam.

Sample collection and processing

This was a cross-sectional, descriptive epidemiological study, performed in July 2020 on 140 Jrai people in the Central Highland region of Vietnam. All the participants had no symptoms of periodontitis or sore throat or any systemic diseases. The participants were classified into three groups of age: the first group included forty-two individuals aged from 1 to 12 years (Children, n = 42), the second group included thirty-nine individuals aged from 13 to 18 years (Adolescents, n = 39), and the third group included fifty-nine individuals over 18 years (Adults, n = 59). The oral and nasal swab samples were
collected from each participant and each sample was placed in sterile tubes containing 3 ml of Brain Heart Infusion (BHI) broth (Merck, Kenilworth, New Jersey, USA). Totaling 280 swab samples (140 oral and 140 nasal specimens) were collected from 140 participants. Samples were collected immediately after the application of routine oral hygiene. Participants did not use any antibiotics before collection.

**Identification of bacteria and fungi**

The determination of Gram-positive and Gram-negative bacteria strains using the Gram stain method. Standard conventional culture methods were also applied to isolate aerobic bacterial and fungi. Briefly, samples were used to grow aerobically on Blood agar, Chocolate agar, and Sabouraud Dextrose agar (Merck, Kenilworth, New Jersey, USA) and then tested for further specific determination. Once identified, the colonies were selected to run an identification analysis by the VITEK MS system (BioMérieux).

**Statistical analysis**

The species composition of microbiota detected in oral cavities and the prevalence of particular species were compared between the three groups and analyzed statistically. Continuous variables were compared using the Mann-Whitney U test or Kruskal-Wallis test, categorical variables were compared using the Chi-square or Fisher exact test. p-values less than 0.05 were considered significant.

### 3. RESULTS

**Characteristics of Vietnamese participants**

<table>
<thead>
<tr>
<th>Characteristics</th>
<th>Total (n = 140)</th>
<th>Children (n = 42)</th>
<th>Adolescents (n = 39)</th>
<th>Adults (n = 59)</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td>Age (years), mean (SD)</td>
<td>21.06 (14.83)</td>
<td>5.93 (0.5)</td>
<td>17.44 (0.2)</td>
<td>33.73 (1.76)</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Gender (male/female)</td>
<td>67/73</td>
<td>17/25</td>
<td>31/8</td>
<td>19/40</td>
<td>&lt; 0.001</td>
</tr>
<tr>
<td>Smoking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>Current-smoker</td>
<td>14</td>
<td>0</td>
<td>2</td>
<td>12</td>
<td></td>
</tr>
<tr>
<td>None-smoker</td>
<td>126</td>
<td>42</td>
<td>37</td>
<td>47</td>
<td></td>
</tr>
<tr>
<td>Drinking status</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.014</td>
</tr>
<tr>
<td>Current-drinker</td>
<td>6</td>
<td>0</td>
<td>0</td>
<td>6</td>
<td></td>
</tr>
<tr>
<td>Non-drinker</td>
<td>134</td>
<td>42</td>
<td>39</td>
<td>53</td>
<td></td>
</tr>
</tbody>
</table>

Age was compared using the Kruskal – Wallis test; smoking and drinking status were compared using the Pearson Chi-Square test.
The characteristics of the Vietnamese participants were shown in Table 1 there was a significant difference among groups on age ($p < 0.001$), gender ($p < 0.001$), smoking status ($p = 0.002$), and alcohol consuming ($p = 0.014$).

Colonized participants

Microscopic examinations of the oral and nasal swab cultures and laboratory tests showed the presence of various microorganisms belonging to different genera, species, and strains of bacteria in participant groups analyzed. The oral and nasal cavity of 140 (100%) participants were colonized by bacteria; of these participants, 42.85% (60/140) carried only one species of bacteria, and 57.15% (80/140) carried two species simultaneously. Thus, the participants were colonized by multiple species of aerobic bacteria.

Bacterial and fungi isolates in the oral and nasal cavity

Table 3.2. Species of aerobic bacteria (n = 15) isolated from the oral and nasal cavity of Vietnamese

<table>
<thead>
<tr>
<th>Bacteria species (n = 15)</th>
<th>Number of isolates: n (%)</th>
<th>Group</th>
<th>p</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td>Children</td>
<td>Adolescents</td>
</tr>
<tr>
<td>Gram-negative</td>
<td>112 (50.9)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Moraxella catarhalis</td>
<td>86 (39.1)</td>
<td>28</td>
<td>24</td>
</tr>
<tr>
<td>Klebsiella pneumoniae</td>
<td>10 (4.55)</td>
<td>2</td>
<td>3</td>
</tr>
<tr>
<td>Pseudomonas aeruginosa</td>
<td>7 (3.2)</td>
<td>2</td>
<td>2</td>
</tr>
<tr>
<td>Raistantia pickettii</td>
<td>2 (0.9)</td>
<td>1</td>
<td>1</td>
</tr>
<tr>
<td>Acinobacter baumanii</td>
<td>2 (0.9)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Herbaspirillum huttense</td>
<td>2 (0.9)</td>
<td>1</td>
<td>0</td>
</tr>
<tr>
<td>Escherichia coli</td>
<td>1 (0.45)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Neisseria meningitidis</td>
<td>1 (0.45)</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Neisseria mucosa</td>
<td>1 (0.45)</td>
<td>0</td>
<td>1</td>
</tr>
<tr>
<td>Total: n (%)</td>
<td>220 (100)</td>
<td>65</td>
<td>63</td>
</tr>
</tbody>
</table>
Table 3.3. Families of aerobic bacteria (n = 8) isolated from the oral and nasal cavity of Vietnamese

<table>
<thead>
<tr>
<th>Bacteria families</th>
<th>Number of isolates: n (%)</th>
<th>Percent (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moraxellaceae</td>
<td>88</td>
<td>40.00</td>
</tr>
<tr>
<td>Streptococcaceae</td>
<td>81</td>
<td>36.82</td>
</tr>
<tr>
<td>Staphylococcaceae</td>
<td>27</td>
<td>12.27</td>
</tr>
<tr>
<td>Enterobacteriaceae</td>
<td>11</td>
<td>5.00</td>
</tr>
<tr>
<td>Pseudomonadaceae</td>
<td>7</td>
<td>3.18</td>
</tr>
<tr>
<td>Burkholderiaceae</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td>Neisseriaceae</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td>Oxalobacteraceae</td>
<td>2</td>
<td>0.91</td>
</tr>
<tr>
<td><strong>Total</strong></td>
<td><strong>220</strong></td>
<td><strong>100</strong></td>
</tr>
</tbody>
</table>

A total of 220 bacterial strains were isolated belonged to 15 different species. The most prevalent species was *Moraxella catarrhalis* (39.1%, 86/220), followed by *Streptococcus* sp. (29.1%, 64/220), and *Staphylococcus epidermidis* (11.4%, 25/220) (Table 3.2) The most common families were *Moraxellaceae* (40%, 88/220), followed by *Streptococcaceae* (36.82%, 81/220), and *Staphylococcaceae* (12.3%, 27/220) (Table 3.3). Potentially pathogenic bacteria were isolated including *Klebsiella pneumoniae* (4.55%), *Pseudomonas aeruginosa* (3.2%), *Acinetobacter baumannii* (0.9%), and *Escherichia coli, Neisseria meningitidis, Staphylococcus aureus* (0.45% each) (Table 3.2). However, there was no significant difference in the distribution of bacterial species among the three groups ($p > 0.05$). Furthermore, fungi were not detected in any of the samples.

A total of 140 participants, including 14 current smokers and 126 non-smokers, were investigated specifically for the presence and diversity of aerobic bacteria using a routine culture method. A total of 15 different bacterial species were detected in non-smokers compared, of which 5 species (*Moraxella catarrhalis, Streptococcus* sp., *Staphylococcus epidermidis, Streptococcus mitis, and Herbaspirillum huttoniense*) were observed in current smokers. All the bacterial species isolated from the current-smokers were also found in non-smokers.

4. DISCUSSION

A large number of healthcare-associated infections is difficult to prevent, and thus making the problem of colonization for healthy individuals by pathogenic bacteria [11]. The individuals who carried the pathogenic bacteria can spread among the community, and the identification of potential sources of infection is a vital strategy to prevent and control infectious diseases. The present study has identified important pathogenic bacteria such as *Klebsiella pneumoniae*,
Pseudomonas aeruginosa, Acinetobacter baumannii, Escherichia coli, Neisseria meningitidis, and Staphylococcus aureus in the oral and nasal cavity of local Vietnamese individuals. Therefore, the oral and nasal cavity can be a potential reservoir of pathogenic bacteria that can spread to the environment and susceptible individuals.

The six most common genera in the oral and nasal cavity including Moraxella, Staphylococcus, Streptococcus, Haemophilus, Dolosigranulum, and Corynebacterium have been recognized [12-14]. However, only three genera Moraxella, Staphylococcus, Streptococcus have been identified in this study, which is in line with a previous study [15]. Streptococcaceae is the second most common and this observation is also in concordance with the human oral microbiome database (HOMD) and another study reported that Streptococcus is higher abundant compared to other genera [16, 17]. Besides, the most frequent species isolated from the oral cavity are S. salivarius, S. sanguis, S. mitis, and Streptococci [18], of which, S. mitis is associated with oral cancer [19].

The oral microbiota is associated with many diseases [20], and the prevalence of colonization in the oral and nasal cavity by aerobic potentially pathogenic bacteria was 10%. These bacteria can enter the bloodstream from periodontitis, untreated carious lesions, or wound healing and cause infections [21, 22]. In this study, except S. epidermidis was isolated in all groups, S. aureus, and Staphylococcus sp. were only isolated only from children. Another study showed that S. aureus is frequent in the oral cavity. Therefore, it should be considered as a source of S. aureus which can spread and infect other individuals [23].

K. pneumoniae was found in all three groups of age and has emerged as a major source of antibiotics resistance genes. Surfaces contaminated with Enterobacteriaceae are well-documented sources of outbreaks of drug-resistant bacteria [24]. Therefore, K. pneumoniae can survive persistently in the oral and nasal cavity and is particularly noteworthy. P. aeruginosa and Acinetobacter spp. are important pathogens involved in hospital-acquired pneumonia. The oral and nasal cavity may be a major source of these respiratory pathogens [25].

In this study, seven P. aeruginosa isolates were identified in all three groups and two A. baumannii were found only in adults. Studies of the oral and nasal cavity colonization by P. aeruginosa and A. baumannii are scant and the relevance of these carriers should be enlightened [26, 27]. Asymptomatic oropharyngeal carriage of N. meningitidis is common in adolescence and young adults, corresponding to an increased risk of disease in these groups [28]. In the present study, N. meningitidis was isolated in a 22-year-old participant. N. meningitidis is a transient commensal of the human pharynx that causes severe diseases such as meningitis and bacteremia.

However, the mechanism of how N. meningitidis interacts with the pharyngeal microbiome to cause diseases is not clearly understood [29]. Similar to other previous studies [30, 31], we observed that smoking is related to the reduced diversity of oral and nasal microbiota and the potentially
pathogenic bacteria were uniquely found in non-smokers. The mechanisms by which tobacco smoking reduces the oral and nasal cavity microbiota diversity include acidity of saliva, toxicants, and depleting oxygen [32]. Thus, further studies are needed to explore whether tobacco smoke inhibits the growth of the oral and nasal bacteria species which is unique in non-smokers.

5. CONCLUSIONS
Potentially pathogenic bacteria are detected in the oral and nasal cavity, suggesting the adoption of strict hygienic actions should be applied to decrease the risk of cross-infection, or at least delay the occurrence of infections caused by pathogenic bacteria in local rural regions of Vietnam.

Conflict of interest
The authors declare that they have no conflict of interest.

REFERENCES


