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*

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 3.1. Characteristics of Vietnamese participants.

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

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

Bacteria species (n = 15)	Number of isolates: n (%)	Group			p
		Children	Adolescents	Adults	
Gram-negative	112 (50.9)				
Moraxella catarhalis	86 (39.1)	28	24	34	0.655
Klebsiella pneumoniae	10 (4.55)	2	3	5	0.765
Pseudomonas aeruginosa	7 (3.2)	2	2	3	0.996
Ralstonia pickettii	2 (0.9)	1	1	0	0.476
Acinobacter baumanii	2 (0.9)	0	0	2	0.248
Herbaspirillum huttiense	2 (0.9)	1	0	1	0.649
Escherichia coli	1 (0.45)	0	1	0	0.271
Neisseria meningitidis	1 (0.45)	0	0	1	0.501
Neisseria mucosa	1 (0.45)	0	1	0	0.271
Gram-positive	108 (49.1)				
Streptococcus sp	64 (29.1)	17	19	28	0.712
Staphyloccocus epidermidis	25 (11.4)	7	9	9	0.595
Streptococcus mitis	16 (7.3)	5	2	9	0.302
Staphyloccocus aureus	1 (0.45)	1	0	0	0.309
Staphylococcus sp	1 (0.45)	1	0	0	0.309
Streptococcus salivarius	1 (0.45)	0	1	0	0.271
Total: n (%)	220 (100)	65	63	92	

**Table 3.3. Families of aerobic bacteria (n = 8) isolated from the oral
and nasal cavity of Vietnamese**

Bacteria families	Number of isolates: n (%)	Percent (%)
Moraxellaceae	88	40.00
Streptococcaceae	81	36.82
Staphylococcaceae	27	12.27
Enterobacteriaceae	11	5.00
Pseudomonadaceae	7	3.18
Burkholderiaceae	2	0.91
Neisseriaceae	2	0.91
Oxalobacteraceae	2	0.91
Total	220	100

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 huttiense*) 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

1. **Gao L, Xu T, Huang G, Jiang S, Gu Y, Chen F. 2018.** Oral microbiomes: more and more importance in oral cavity and whole body. *Protein Cell* 9: 488-500.

2. **Leao-Vasconcelos LS, Lima AB, Costa Dde M, Rocha-Vilefort LO, Oliveira AC, Goncalves NF, Vieira JD, Prado-Palos MA. 2015.** Enterobacteriaceae isolates from the oral cavity of workers in a Brazilian oncology hospital. *Rev Inst Med Trop Sao Paulo* 57: 121-7.

3. **Costello EK, Relman DA. 2014.** Population health: immaturity in the gut microbial community. *Nature* 510: 344-5.

4. **He J, Li Y, Cao Y, Xue J, Zhou X. 2015.** The oral microbiome diversity and its relation to human diseases. *Folia Microbiol (Praha)* 60: 69-80.

5. **Gholizadeh P, Eslami H, Yousefi M, Asgharzadeh M, Aghazadeh M, Kafil HS. 2016.** Role of oral microbiome on oral cancers, a review. *Biomed Pharmacother* 84: 552-8.

6. **Alzheimer's A. 2016.** 2016 Alzheimer's disease facts and figures. *Alzheimers Dement* 12: 459-509.

7. **Ussar S, Fujisaka S, Kahn CR. 2016.** Interactions between host genetics and gut microbiome in diabetes and metabolic syndrome. *Mol Metab* 5: 795-803.

8. **Onor IO, Stirling DL, Williams SR, Bediako D, Borghol A, Harris MB, Darensburg TB, Clay SD, Okpechi SC, Sarpong DF. 2017.** Clinical Effects of Cigarette Smoking: Epidemiologic Impact and Review of Pharmacotherapy Options. *Int J Environ Res Public Health* 14.

9. **Huang C, Shi G. 2019.** Smoking and microbiome in oral, airway, gut and some systemic diseases. *Journal of Translational Medicine* 17: 225.

10. **Tsuzukibashi O, Uchibori S, Kobayashi T, Saito M, Umezawa K, Ohta M, Shinozaki-Kuwahara N. 2015.** A selective medium for the isolation of Microbacterium species in oral cavities. *J Microbiol Methods* 116: 60-5.

11. **De Moura JP, Pimenta FC, Hayashida M, Cruz ED, Canini SR, Gir E. 2011.** Colonization of nursing professionals by Staphylococcus aureus. *Rev Lat Am Enfermagem* 19: 325-31.

12. **Shilts MH, Rosas-Salazar C, Tovchigrechko A, Larkin EK, Torralba M, Akopov A, Halpin R, Peebles RS, Moore ML, Anderson LJ, Nelson KE, Hartert TV, Das SR. 2016.** Minimally Invasive Sampling Method Identifies Differences in Taxonomic Richness of Nasal Microbiomes in Young Infants Associated with Mode of Delivery. *Microb Ecol* 71: 233-42.

13. **Frayman KB, Armstrong DS, Grimwood K, Ranganathan SC. 2017.** The airway microbiota in early cystic fibrosis lung disease. *Pediatr Pulmonol* 52: 1384-404.

14. **Teo SM, Mok D, Pham K, Kusel M, Serralha M, Troy N, Holt BJ, Hales BJ, Walker ML, Hollams E, Bochkov YA, Grindle K, Johnston SL, Gern JE, Sly PD, Holt PG, Holt KE, Inouye M. 2015.** The infant nasopharyngeal microbiome impacts severity of lower respiratory infection and risk of asthma development. *Cell Host Microbe* 17: 704-15.

15. **Kumpitsch C, Koskinen K, Schopf V, Moissl-Eichinger C. 2019.** The microbiome of the upper respiratory tract in health and disease. *BMC Biol* 17: 87.

16. **Butler RR, 3rd, Soomer-James JTA, Frenette M, Pombert JF. 2017.** Complete Genome Sequences of Two Human Oral Microbiome Commensals, Streptococcus salivarius ATCC 25975 and S. salivarius ATCC 27945. *Genome Announc* 5.

17. **Cho I, Blaser MJ. 2012.** The human microbiome: at the interface of health and disease. *Nat Rev Genet* 13: 260-70.

18. **Patricia A, Fernando AA, Célia MOG. 2015.** Prevalence of different streptococci species in the oral cavity of children and adolescents. *Brazilian Journal of Oral Sciences* 2.

19. **Mager DL, Haffajee AD, Devlin PM, Norris CM, Posner MR, Goodson JM. 2005.** The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. *J Transl Med* 3: 27.

20. **Morita E, Narikiyo M, Nishimura E, Yano A, Tanabe C, Sasaki H, Hanada N. 2004.** Molecular analysis of age-related changes of Streptococcus anginosus group and Streptococcus mitis in saliva. *Oral Microbiol Immunol* 19: 386-9.

21. **Le Bars P, Matamoros S, Montassier E, Le Vacon F, Potel G, Soueidan A, Jordana F, de La Cochetiere MF. 2017.** The oral cavity microbiota: Between health, oral disease, and cancers of the aerodigestive tract. *Can J Microbiol* 63: 475-92.

22. **Su H, Yan X, Dong Z, Chen W, Lin ZT, Hu QG. 2015.** Differential roles of Porphyromonas gingivalis lipopolysaccharide and Escherichia coli lipopolysaccharide in maturation and antigen-presenting functions of dentritic cells. *Eur Rev Med Pharmacol Sci* 19: 2482-92.

23. **McCormack MG, Smith AJ, Akram AN, Jackson M, Robertson D, Edwards G. 2015.** *Staphylococcus aureus* and the oral cavity: An overlooked source of carriage and infection? *American Journal of Infection Control* 43: 35-7.

24. **Baker JL, Hendrickson EL, Tang X, Lux R, He X, Edlund A, McLean JS, Shi W. 2019.** Klebsiella and Providencia emerge as lone survivors following long-term starvation of oral microbiota. *Proceedings of the National Academy of Sciences* 116: 8499-504.

25. **Souto R, Silva-Boghossian CM, Colombo AP. 2014.** Prevalence of *Pseudomonas aeruginosa* and Acinetobacter spp. in subgingival biofilm and saliva of subjects with chronic periodontal infection. *Braz J Microbiol* 45: 495-501.

26. **Conti S, dos Santos SS, Koga-Ito CY, Jorge AO. 2009.** Enterobacteriaceae and p*seudomonadaceae* on the dorsum of the human tongue. *J Appl Oral Sci* 17: 375-80.

27. **Ramos MM, Gaetti-Jardim EC, Gaetti-Jardim Junior E. 2009.** Resistance to tetracycline and beta-lactams and distribution of resistance markers in enteric microorganisms and pseudomonads isolated from the oral cavity. *J Appl Oral Sci* 17 Suppl: 13-8.

28. **Retchless AC, Kretz CB, Rodriguez-Rivera LD, Chen A, Soeters HM, Whaley MJ, Wang X. 2020.** Oropharyngeal microbiome of a college population following a meningococcal disease outbreak. *Sci Rep* 10: 632.

29. **Proctor DM, Relman DA. 2017.** The Landscape Ecology and Microbiota of the Human Nose, Mouth, and Throat. *Cell Host Microbe* 21: 421-32.

30. **Yu G, Phillips S, Gail MH, Goedert JJ, Humphrys MS, Ravel J, Ren Y, Caporaso NE. 2017.** The effect of cigarette smoking on the oral and nasal microbiota. *Microbiome* 5: 3.

31. **Grine G, Royer A, Terrer E, Diallo OO, Drancourt M, Aboudharam G. 2019.** Tobacco Smoking Affects the Salivary Gram-Positive Bacterial Population. *Front Public Health* 7: 196.

32. **Wu J, Peters BA, Dominianni C, Zhang Y, Pei Z, Yang L, Ma Y, Purdue MP, Jacobs EJ, Gapstur SM, Li H, Alekseyenko AV, Hayes RB, Ahn J. 2016.** Cigarette smoking and the oral microbiome in a large study of American adults. *The ISME Journal* 10: 2435-46.