Clinical Research Report

Illumina-based sequencing analysis of pathogenic microorganisms in dental caries patients of different Chinese ethnic groups

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Abstract

Objective: To analyze the pathogenic community diversity of dental caries patients from Tu, Hui, Tibetan, and Han Chinese ethnic groups.

Methods: Forty saliva samples were collected from the following patients with dental caries: Tu from Huzhu County (n = 10), Hui from Ping'an County (n = 10), Han from Xining city (n = 10), and Tibetan from Yushu (n = 10). High-throughput sequencing of bacterial 16S rRNA genes (V3-V4) was performed using the Illumina MiSeq sequencing platform.

Results: Based on 97% similarity clustering, operational taxonomic units of Tu, Hui, Tibetan, and Han ethnic groups were 181, 210, 38, and 67, respectively. In Tu patients, 11 phyla, 19 classes, and 89 genera were identified, compared with 13 phyla, 21 classes, and 113 genera in Hui patients, two phyla, four classes, and 21 genera in Tibetan patients, five phyla, nine classes, and 34 genera in Han patients, and four phyla, five classes, and 12 genera from the control group. The main pathogens of dental caries included *Veillonella*, *Aggregatibacter*, *Leptotrichia*, *Bacteroides*, *Granulicatella*, *Streptococcus*, and *Prevotella*.

Conclusion: The pathogenic microorganisms of dental caries differ greatly among Tu, Hui, Tibetan, and Han ethnic groups. These findings provide a theoretical basis for the effective prevention and treatment of dental caries in different Chinese populations.

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Introduction

Dental caries is an infectious disease caused by a variety of factors, including the interaction between oral microbes, dietary habits, poor oral hygiene, low immunity, and host reactions. Among these, interactions between microorganisms such as acidic bacteria (*Streptococcus mutans* and *Lactobacillus*) adhering to or growing on dental plaque to form biofilm play a dominant role in tooth damage and dental caries.[1] Dental caries can occur at different ages, causing dental calcification damage, oral infections, and toothache, and even systemic infections.[2–4] It affects approximately 80% to 90% of the global population.

High-throughput sequencing technology is a current research hotspot in microbial diversity, metagenomics, and metabolomics of microbiology to explore correlations between oral microbes and clinical diseases.[5] Schulze-Schweifing et al.[6] compared traditional culture methods with high-throughput sequencing, and demonstrated that the latter has a faster sequencing speed and larger sequencing volume, and can determine the entire microbiological structure of oral samples, which accurately measures the proportion of dominant bacteria and identifies novel bacterial species. Previous studies indicated that high-throughput sequencing technology can be used to detect oral salivary microbes in patients with dental caries, and analyze the community structure and microbial diversity.[7–9]

The climate in high-altitude areas of China is extremely harsh, and this environment poses a threat to the health of local residents. Indeed, the incidence of dental caries is these regions has elevated in recent years. Domestic research in China mainly focuses on low-altitude residents in the plains, and epidemiological studies on oral diseases in high-altitude residents have been rarely reported. Additionally, there have been few studies on oral microorganisms in the plateau region. In the high-altitude Qinghai Province, different ethnic groups with major lifestyle differences reside in compact communities.

In the present study, we used high-throughput sequencing of 16S rRNA genes to investigate the diversity, community structure, and pathogenic bacteria of oral microorganisms in Tu, Hui, Tibetan, and Han populations in Qinghai Province. Our findings revealed the influence of environmental and host factors of dental caries upon the diversity of the oral microbial community structure in dental caries patients of different ethnic groups, which provides a theoretical basis and novel research direction for the effective prevention and treatment of dental caries.

Materials and methods

Demographic data

Ten individuals were included in the study per ethnic group. In the Tibetan group, four individuals were men and six were women. They were aged 49 to 79 years with a mean age of 67.1 years. Among the Hui population of four men and six women, the age range was 47 to 80 years with an average of 74.4 years. In Tu individuals, two were men and eight were women, with an age range of 47 to 80 years and a mean age of 58.7 years. In the Han group, five individuals were men and five were women. The age range was 25 to 72 years, with an average of 56.6 years.

Sampling collection

Forty saliva samples were collected from patients with dental caries from the Tu ethnic group from Huzhu County (n = 10), the Hui ethnic group from Ping'an County (n = 10), the Han ethnic group from Xining city (n = 10), and the Tibetan ethnic group

from Yushu (n = 10). Saliva samples from 10 healthy subjects were used as controls. All enrolled patients, aged 25 to 80 years, had resided in Qinghai Province for more than 10 years. The diagnostic criteria of dental caries referred to the guideline proposed by the 3rd National Oral Health Epidemiological Survey in the Mainland of China in 2005.[10] Patients were included if they had a decayed, missing, filled tooth (DMFT) index ≥4. None of the enrolled patients had filled teeth. Average DMFT scores were 12.2, 13.3, 8.7, and 11.5 in Tibetan, Hui, Tu, and Han ethnic groups, respectively.

Patient clinical data were collected and retrospectively analyzed. No participants had systemic diseases such as diabetes mellitus, cardiovascular disease, or hyperthyroidism. There was also no history of tobacco addiction, antibiotic use within the past 3 months, oral mucosal disease, ulceration, or pericoronitis. Patients had not received an oral examination within 6 months before enrollment, did not have invasive periodontitis, and were not pregnant or lactating.

Prior to salivary sample collection, subjects gently rinsed away food residues from their mouth with boiled warm water, then saliva was collected in a sterile container such as a test tube. A portion of saliva (0.5–1.0 mL) was mixed with an equivalent quantity of 50% sterilized glycerol, then immediately frozen at −4°C and stored at −20°C until required. Written informed consent was obtained from all participants. Study procedures were approved by the ethics committee of the Affiliated Hospital of Qinghai University.

Extraction and quality control of total DNA

Saliva samples were filtered using a 0.22-μm bacterial filter, then the filter was crushed and placed into the QIAamp Fast DNA Stool Mini Kit (Qiagen Inc., Valencia, CA, USA). Genomic DNA was extracted according to the manufacturer's instructions and stored at −80°C. The DNA integrity was analyzed by 2.0% agarose gel electrophoresis, and a quality purity assay was performed using a microplate reader (Molecular Devices, Sunnyvale, CA, USA).

PCR amplification of 16S rRNA and sequencing analysis

The V3-V4 target gene was amplified using 16S rRNA gene universal primers F341 (5′-CCTACGGGAGGCAGCAG-3′) and R518 (5′-ATTACCGCGGCTG CTGG-3′). PCR reaction conditions were: 95°C for 5 minutes, then 35 cycles of 94°C for 45 seconds, 55°C for 30 seconds, and 72°C for 90 seconds, followed by a final extension at 72°C for 5 minutes. PCR products were purified using the Axyprep DNA gel extraction kit (Axygen Biosciences, Union City, CA, USA), and the Qubit® 2.0 Fluorometer (Invitrogen Corp., Carlsbad, CA, USA) was used for accurate quantitation. Quantified PCR products were subjected to 16S rRNA gene sequencing using the MiSeq system (Illumina, San Diego, CA, USA), 454 GS FLX DNA sequencer (Roche, Basel, Switzerland), and ABI 3730xl Sanger sequencing methods (Applied Biosystems, Foster City, CA, USA) by Shanghai Huiyan Biotech Co., Ltd. (Shanghai, China). Double-ended sequencing data of 20,0000 to 30,000 reads were obtained for each sample. A merging experiment was performed according to the overlap relationship between different reads. Quality control of read qualities and merging effects was evaluated (Q30 >80%). Valid sequences were optimized based on barcodes and primer sequences at both ends of the sequence.

Sequencing analysis

QIIME 1.8.0 software (Minnesota Supercomputing Institute, Minneapolis,

MN, USA) was used to process the raw data. An operational taxonomic unit (OTU) was clustered at the similarity level of 97% to perform OTU picking and species annotation analysis. An alpha rarefaction curve was used to calculate bacterial diversity using the Shannon index, phylogenetic diversity (PD_whole_tree), observed species, and species richness based on the Chao1 index. Based on the results of taxonomic analysis (genera), the community structure, species composition, percentage, and abundance index of the microorganisms were analyzed. Venn diagrams were delineated to identify the common genus of each ethnic group.

Statistical analysis

SPSS 19.0 statistical software was used for data analysis (IBM Corp., Armonk, NY, USA). Data are expressed as means ± standard deviations. Group comparisons were conducted using one-way analysis of variance. Two-paired comparisons were carried out by the Tamhane method.[6] A value of P < 0.05 was considered statistically significant.

Results

Optimization analysis of sequencing data

The Illumina MiSeq sequencing platform was used to determine the bacterial diversity of the 16S rRNA gene (V3-V4) in oral saliva samples. OTUs were clustered at the similarity level of 97%, and OTU picking obtained 181 OTUs from the Tu ethnic group, 210 from the Hui group, 38 from the Tibetan group, and 67 from the Han group. The number of OTUs in the Tu group was significantly higher than in the control group and Tibetan group, while the number in the Han group was significantly higher than in the control (all P < 0.05). There were 46 observed species for the Tu

group, 35 in the Hui group, 11 in the Tibetan group, and 14 in the Han group. The Shannon index was significantly higher for dental caries patients (0.16–3.23, 0.35–3.45, 0.73–2.28, and 0.22–2.93 for Tu, Hui, Tibetan, and Han ethnic groups, respectively) than the control group (0–1.62), as were the numbers of observable species (22–122, 17–94, 7–15, and 9–20, respectively) versus 1 to 3 for the control group, reflecting a much higher oral microbial community diversity. The Shannon index and Chao1 index indicated that the microbial diversity of Tu and Hui populations was relatively high, whereas it was relatively low in Tibetan and Han populations. PD_whole_tree differed significantly among ethnic groups. In patients with dental caries, the overall distance of phylogenetic development ranged from 0.74 to 3.66, which was significantly longer than the 0.24 in the control group (P < 0.05) as illustrated in Table 1.

Analysis of differences in community structure

Using data from the Ribosomal Database Project,[11] we performed a taxonomic analysis at the 97% similarity level of representative OTU sequences, and conducted statistical analysis of species abundance at the levels of phyla, classes, and genera. Based on species annotation screening, 11 phyla, 19 classes, and 89 genera were obtained from the Tu group, 13 phyla, 21 classes, and 113 genera from the Hui group, two phyla, four classes, and 21 genera from the Tibetan group, and five phyla, nine classes, and 34 genera from the Han group. Four phyla, five classes, and 12 genera were obtained from the control group. As shown in Figure 1, the dominant bacterial species differed significantly among ethnic groups. Dominant bacteria of the Tu group were *Lactococcus*, *Enterobacter* no rank of the Enterobacteriaceae, *Aeromonas*

Table 1. Index of microbial diversity of dental caries and control groups from all samples ($\bar{x} \pm s$).

Group	N	OTU	Chao1	Shannon indext	PD_whole_tree	Observed species
Tu with dental caries	10	$58.1 \pm 34.6^{\#,\Delta}$	$69.76 \pm 30.45^{\#,\S,\Delta}$	2.10 ± 1.63	$3.66 \pm 1.74^{\#,\S,\Delta}$	$50.69 \pm 30.26^{\#,\S,\Delta}$
Hui with dental caries	10	$51.1 \pm 28.7^{\#,\Delta}$	$50.54 \pm 25.53^{\#,\S,\Delta}$	1.62 ± 0.99	$2.34 \pm 1.28^{\#,\S,\Delta}$	$35.32 \pm 22.09^{\#}$
Tibetan with dental caries	10	$15.0 \pm 3.7^{\#}$	$13.72 \pm 6.03^{\#}$	$1.54 \pm 0.51^{\#}$	$0.74 \pm 0.1^{\#}$	$11.09 \pm 2.58^{\#}$
Han with dental caries	10	$18.6 \pm 4.9^{\#}$	$16.44 \pm 3.78^{\#}$	1.54 ± 0.82	$0.69 \pm 0.14^{\#}$	$11.87 \pm 3.07^{\#}$
Control group	10	1.9 ± 1.4	1.59 ± 0.82	0.67 ± 0.62	0.24 ± 0.19	1.99 ± 1.26
F value		14.428	24.981	2.683	21.573	14.269
P value		<0.001	<0.001	0.043	<0.001	<0.001

Note: # indicates $P<0.05$ compared with the control group; § represents $P<0.05$ compared with the Han group with dental caries; Δ denotes $P<0.05$ compared with the Tibetan group with dental caries. OTU, operational taxonomic unit.
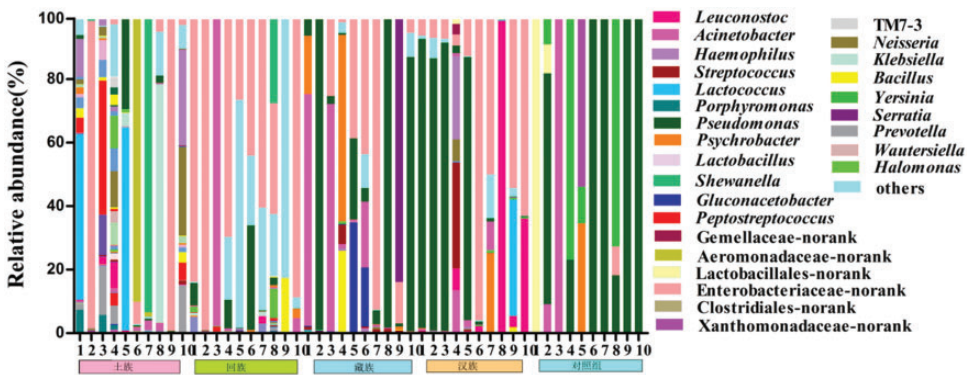

Figure 1. Statistical analysis of relative abundance of dental caries in different ethnic groups.

no rank of the Aeromonadaceae, *Shewanella* and *Klebsiella*. Dominant bacteria of the Hui group were Enterobacteriaceae no rank and *Acinetobacter*, those of the Tibetan group were *Pseudomonas*, *Acinetobacter*, *Psychrobacter*, and *Serratia*, and those of the Han group were *Pseudomonas*, *Streptococcus*, *Leuconostoc*, and *Lactococcus*. In the control group, the dominant bacteria were mainly Lactobacillales no rank, *Pseudomonas*, *Acinetobacter*, *Yersinia*, and Xanthomonadaceae no rank. The microbial community structure of Hui and Han groups was relatively stable, whereas the microorganisms of Tu and Tibetan groups were complex and diverse. In the control group, the microbial community was simple and small.

Analysis of differences in pathogenic microorganisms

The relative abundance of microorganisms and pathogenic bacteria in dental caries and control groups was analyzed statistically (Table 2). Tu, Hui, and Han ethnic groups had a complex mix of microorganisms, whereas those of the Tibetan group were more homogeneous. *Veillonella* was detected in Tu and Han populations, *Bacillus*, *Aggregatibacter*, and *Leptotrichia* were only detected in the Tu group, *Bacteroides* was identified only in the Hui group, *Prevotella* was detected in Tu, Hui, and Han groups, while *Granulicatella* and *Streptococcus* were found in all four groups. Comparative analysis demonstrated that the suspected

Table 2. Statistical analysis of oral microorganisms in dental caries and control groups (%).

Bacterial species	Dental caries group				Control group
	Tu group	Hui group	Tibetan group	Han group	
Acholeplasma	0.05	0	0	0	0
Acinetobacter	0.46	10.39	18.5	1.33	0
Aeromonadaceae*	9.11	0	0	0.1	0
Aggregatibacter	0.23	0	0	0	0
Anaerovorax	0.03	0	0	0	0
Arthrobacter	0.03	0	0	0	0
Atopobium	0.04	0	0	0	0
Bacillus	0.01	1.73	2.36	0.2	0
Bacteroides	0	0.01	0	0	0
Bulleidia	0.32	0	0	0	0
Campylobacter	0.77	0.04	0	0	0
Capnocytophaga	0.39	0	0	0	0
Carnobacterium	0.01	0	0.2	0	0
Catonella	0.01	0	0	0	0
Clostridiales*	0	0	0	0.1	0
Clostridium	1.27	0	0	0	0
Coprococcus	0.04	0	0	0	0
Dialister	0.77	0	0	0	0
Eikenella	0.1	0	0	0	0
Enterobacter	0.25	0	0	0	0
Enterobacteriaceae*	21.2	50.48	21.32	29.92	0
Enterococcus	0.02	0	0	0	0
Erwinia	0.04	0.04	0	2.53	0
Filifactor	0.02	0.01	0	0	0
Fusobacterium	1.09	0.05	0	0.01	0
Garciella	0.01	0	0	0	0
Gemella	0.05	0	0	0.02	0
Gemellaceae-norank*	0	0	0	0.38	0
Gluconacetobacter	0.02	0.06	5.22	0	0
Granulicatella	0.27	0.01	0.15	1.43	0
Haemophilus	4.59	0.08	0.07	2.72	0
Halomonas	1.18	1.12	0.1	0.21	0
Klebsiella	8.88	0.39	0	0	0
Others	4.37	25.13	2.17	2.56	0
Lactobacillales*	0	0	0	0.16	0
Lactobacillus	0	0	0	0.03	0
Lactococcus	11.6	0.05	0.05	3.7	0
Leptotrichia	0.36	0.23	0	0	0
Leuconostoc	1.17	0	0	14.7	0
Moryella	0.01	0	0	0	0
Neisseria	4.39	0.05	0.07	0.74	0
Nesterenkonia	0	0.02	0	0	0
Oribacterium	0.26	0.01	0	0	0
Paludibacter	0.09	0.01	0	0	0
Parvimonas	0.91	0	0	0	0
Peptostreptococcus	5.29	0	0	0	0
Porphyromonas	1.47	0.02	0	0	0
Prevotella	3.83	0.12	0	0	0
Proteus	0.02	0	0	0.01	10.91
Providencia	0.02	0	0.03	0	3.46
Pseudomonas	3.38	5.33	32.46	35.61	51.4
Psychrobacter	0.04	0.49	7.86	0.04	0
Rothia	0.01	0	0	0	0
Selenomonas	0.05	0	0	0	0
Serratia	0	0	8.24	0	0
Shewanella	9.11	2.79	0	0	0
SR1*	0.33	0.03	0	0.91	0.91
Staphylococcus	0.01	0.8	0	0.03	0
Streptococcus	0.23	0.05	1.2	3.43	0
Tannerella	0.02	0.02	0	0	0
TM7*	0.31	0.05	0	0	0
Treponema	0.25	0	0	0	10.9
Veillonella	1.2	0	0	0.03	0
Wautersiella	0	0	0	0.01	0
Weissella	0.05	0.05	0	0	0
Xanthomonadaceae*	0	0	0	0	17.03
Yersinia	0	0	0	0	5.38
Total	100	100	100	100	100

Note: *unclassified bacteria.

pathogens shared by dental caries patients of all four ethnic groups were *Acinetobacter*, *Bacillus*, *Granulicatella*, *Haemophilus*, *Halomonas*, *Lactococcus*, *Neisseria*, and *Psychrobacter*. Suspected pathogenic bacteria common to Tu and Hui groups were *Campylobacter*, *Erwinia*, *Filifactor*, *Weissella*, *Oribacterium*, *Paludibacter*, and *Porphyromonas*. *Gluconacetobacter* was common to Tu, Hui, and Tibetan groups, while *Serratia* was unique to the Tibetan group. There were significant differences between pathogenic microorganisms and suspected pathogenic microorganisms among ethnic groups.

Analysis of common bacterial species in patients with dental caries

A Venn diagram was delineated based on the OTUs at 97% similarity. Different and common bacterial species are illustrated in Figure 2. The total number of unique species was 126 in the Tu group (32.3%), 140 in the Hui group (35.9%), 34 in the Tibetan group (8.7%), and 42 in the Han group (10.8%). Two species of microbes (0.5%) were shared by Tu, Hui, and Tibetan groups. Only one species (0.3%) was

Figure 2. Venn diagram of the number of OTUs common to different ethnic groups.
Overlapping areas represent the number of OTUs shared by different samples.

common to Tibetan, Tu, and Han groups, two (0.5%) were common to Tu, Hui, and Han groups, three (0.8%) were common to Han and Tu groups, and 25 (6.4%) were common to Tu and Hui groups. Fourteen species (3.6%) were common to both the Hui and Han groups, whereas only *Pseudomonas* (0.3%) was common to all four groups. These findings indicated likely pathogenic or suspected pathogenic microorganisms among the dental caries subgroups.

Discussion

Previous investigations analyzed oral salivary microorganisms in dental caries patients using high-throughput sequencing,[11–13] which detected 5738 OTUs belonging to 27 phyla and 218 genera,[11] 80 OTUs belonging to five phyla and 19 genera,[11] and 23 OTUs in the dental caries group and 9223 OTUs in the control group belonging to 26 phyla and 374 genera.[13] In the present study, we conducted a preliminary analysis of the microbial community and structural diversity in dental caries and control groups of individuals in Qinghai Province, which detected a high quantity of pathogenic and suspected pathogenic bacteria. Species annotated screening suggested that the microbial community structure of Tu and Hui groups was more complex and diverse than that of Tibetan and Han groups and healthy controls.

Dental caries not only causes tooth damage, but also leads to the development of infections of dental pulp and periapical tissues. High-throughput sequencing helps unravel the complete community structure of oral microorganisms and the relevance of microbial diversity to dental caries, as well as exploring suspected pathogenic microorganisms that potentially cause disease. Previous studies discovered that *Atopobium*, *Cryptobacterium*, *Lactobacillus*, *Mogibacterium*, *Ochrobactrum*, *Pseudomonas*, *Rhizobium*, *Alloprevotella*, *Bacteroides*,
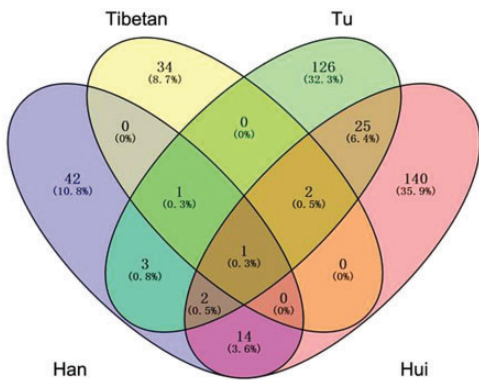
Centipeda, *Campylobacter*, *Megasphaera*, and *Mycoplasma* were the suspected pathogens of dental caries.[14,15] Additionally, Xiao et al.[16] identified *Catonella*, *Centipeda*, *Fretibacterium*, *Rhizobium*, *Ochrobactrum*, and *Mogibacterium* as novel pathogenic species. Li et al.[12] investigated differences in oral salivary microbiological structure among dental caries patients and healthy controls in the Han population, and identified the main pathogens as *Streptococcus* (45%), *Prevotella* (28%), *Neisseria* (14%), *Granulicatella* (9%), *Veillonlla* (8%), and *Porphyromonas* (8%), while Tanner et al.[17] detected *Actinomyces*, *Streptococcus*, *Rothia*, *Selenomonas*, and *Veillonella* as major dental caries pathogens. *Actinobacillus* was identified as an additional pathogen in other studies.[18,19] Here, we detected multiple suspected pathogenic bacteria, including *Acinetobacter*, *Bacillus*, *Haemophilus*, *Halomonas*, *Lactococcus*, *Neisseria*, *Psychrobacter*, *Campylobacter*, *Erwinia*, *Filifactor*, *Weissella*, *Oribacterium*, *Paludibacter*, *Porphyromonas*, *Gluconacetobacter*, *Serratia.*, *Veillonella*, *Aggregatibacter*, *Leptotrichia*, *Bacteroides*, *Granulicatella*, *Streptococcus*, *Prevotella*, and *Staphylococcus*. These show some similarities with previous studies, but also major differences that probably reflect changes associated with living at high altitudes as well as lifestyle variations of various ethnic groups.

Staphylococcus (except *Streptococcus*) is considered the major bacterium causative of dental caries, although the specific disease mechanism remains elusive.[20] Kaur et al.[20] isolated *Staphylococcus aureus*, *Klebsiella*, *Pseudomonas aeruginosa*, and yeast from the saliva of 75 patients with dental caries, while Daniyan[21] and Ouremi et al.[22] independently found that *Staphylococcus aureus* (except *Streptococcus*) plays a key role in the development and progression of dental caries. We identified *Staphylococcus* as a pathogenic bacterium in the saliva samples of Tu, Hui, and Han dental caries patients,

where we observed a relatively low level of bacterial abundance. Li et al.[12] showed that the main pathogens of Han individuals from the Yugur autonomous county of Gansu Province were *Streptococcus parasanguinis*, *Streptococcus pseudopneumoniae*, *Porphyromonas gingivalis*, and *Anaeroglobus geminatus*. Conversely, we identified *Veillonella*, *Granulicatella*, *Streptococcus*, and *Prevotella* in Han individuals, which reflects the differences in pathogenic bacteria in members of the same ethnic group living in different geological regions. Thus, variations in living habits, diet, and the environment cause major changes in microbial community structures.

The unique environment of Qinghai Province appears to allow for the growth and proliferation of specific oral bacteria in dental caries patients, which is intimately correlated with their living environment and dietary habits. These include the long-term consumption of milk and yogurt, which is associated with a relatively high detection rate of *Lactococcus*, *Leuconostoc*, and *Weissella*. *Weissella* belongs to the family Lactobacillaceae and is a Gram-positive selective anaerobe that grows rapidly under microaerophilic conditions. It is similar in microscopic and macroscopic form to other representative strains of lactic acid bacteria such as *Leuconostoc* and *Lactobacillus*, which are readily confused with probiotics.[23] Qinghai Province also has a typical saline–alkaline environment, so halophilic bacteria such as *Halomonas* and *Nesterenkonia* are detected. *Nesterenkonia* belongs to the Micrococcaceae family which also includes *Micrococcus* and Actinobacteria class bacteria. These aerobic, moderately halophilic bacteria are primarily spherical or short-barreled in shape, and occur in salt lakes, saline–alkaline environments, and seawater.[24] The presence of these bacteria reflects the correlation between environmental characteristics and oral microorganisms.

Epidemiological studies have demonstrated significant variation in the incidence of dental caries among different populations; this may partially result from genetic factors, but dietary habit is likely to be the primary cause.[25] In recent decades, the prevalence of dental caries has dramatically increased in the Tibetan population. This is thought to reflect dietary habits of new-generation Tibetans, especially primary and secondary school students, which resemble those of Han individuals. Medical resources for oral healthcare are extremely limited in the Tibetan population and some individuals do not pay attention to oral hygiene, which increases the incidence of dental caries.[26] In contrast, the more varied diet and other environmental factors of Tu, Hui, and Han populations result in a more complex microbial community structure.[18,27,28]

She et al.[29] previously reported a dental caries prevalence of 22.93% in a high-altitude region, which was significantly higher than the 16.04% reported in the plains. They also documented a significantly higher incidence of oral disease in the immigrant Han population compared with local Tibetan residents. The incidence and severity of dental caries are aggravated in line with living conditions,[26] which likely results from the unique environment of Qinghai with its low atmospheric pressure, low oxygen partial pressure, low temperature, high temperature difference between day and night, high number of sandstorms, strong ultraviolet radiation, and a diet based on beef, mutton, pasta, and yogurt. Kianoush et al.[30] found that the pH of saliva is key to the development of dental caries; a lower pH caused by eating kimchi and yogurt promotes the growth of acidic bacteria, which leads to dental caries. In-depth studies into the relationship between low oxygen partial pressure and diet with the incidence of dental caries remain to be performed.

Conclusion

In this study, high-throughput sequencing was used to demonstrate a significantly higher oral microbial community diversity and more complex structure in Tu, Hui, Tibetan, and Han individuals with dental caries than the control group, with significantly different dominant bacteria. Differences in pathogenic bacteria were also documented among the four ethnic groups, but it remains to be determined whether these bacteria are pathogenic. Unique bacteria associated with the consumption of dairy products included *Lactococcus*, *Leuconostoc*, and *Weissella*, while those associated with the environment of Qinghai Province include *Halomonas* and *Nesterenkonia*. Thus, the living environment, dietary habits, and lifestyle are the main causes of bacterial variation. Future studies should elucidate the specific mechanism by which pathogenic bacteria cause dental caries in these populations.

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References

1. Palmer C and Wolfe SH. Position of the American Dietetic Association: the impact

of fluoride on health. *J Am Diet Assoc* 2005; 105: 1620–1628.

2. Shrihari TG. Potential correlation between periodontitis and coronary heart disease-an overview. *Gen Dent* 2012; 60: 20–24.

3. Moses J, Rangeeth BN and Gurunathan D. Prevalence of dental caries, socioeconomic status and treatment needs among 5-15 year old school going children of Chidambaram. *J Clin Diagn Res* 2011; 5: 146–151.

4. Featherstone JD. The science and practice of caries prevention. *J Am Dent Assoc* 2000; 131: 887–899.

5. Park S, Saito-Adachi M, Komiyama Y, et al. Advances, practice and clinical perspectives in high-throughput sequencing. *Oral Dis* 2016; 22: 353–364.

6. Schulze-Schweifing K, Banerjee A and Wade WG. Comparison of bacterial culture and 16S rRNA community profiling by clonal analysis and pyrosequencing for the characterization of the dentine caries-associated microbiome. *Front Cell Infect Microbiol* 2014; 4: 164.

7. Camelo-Castillo AJ, Mira A, Pico A, et al. Subgingival microbiota in health compared to periodontitis and the influence of smoking. *Front Microbiol* 2015; 6: 119.

8. Nyvad B, Crielaard W, Mira, A, et al. Dental caries from a molecular microbiological perspective. *Caries Res* 2013; 47: 89–102.

9. Wade WG. The oral microbiome in health and disease. *Pharmacol Res* 2013; 69: 137–143.

10. Qi X. *The third national oral health epidemiological survey*. Beijing: People's Medical Publishing House(PMPH), 2008, pp.16–44.

11. Li JP, Wu F, Wang ZZ, et al. Analysis on the diversity of salivary microbial of adult dental caries based on 16S ribosomal RNA clone library. *J Oral Sci Res* 2016: 32: 272–277.

12. Wang S, You Xi, Che C, et al. Study of caries microbial community structure and diversity by 16S RNA high-throughput sequencing. *J Oral Sci Res* 2015: 31: 461–465.

13. Wang ZZ, Wang YB, Wu F, et al. Diversity of salivary microbiology in healthy children and adult. *J Oral Sci Res* 2016: 32: 352–355.

14. Jiang W, Ling Z, Lin X, et al. Pyrosequencing analysis of oral microbiota shifting in various caries states in childhood. *Microb Ecol* 2014; 67: 962–969.

15. Obata J, Takeshita T, Shibata Y, et al. Identification of the microbiota in carious dentin lesions using 16S rRNA gene sequencing. *PLoS One* 2014; 9: e103712.

16. Xiao C, Ran S, Huang Z, et al. Bacterial diversity and community structure of supra-gingival plaques in adults with dental health or caries revealed by16S pyrosequencing. *Front Microbiol* 2016; 7: 1145.

17. Tanner AC, Mathney JM, Kent RL, et al. Cultivable anaerobic microbiota of severe early childhood caries. *J Clin Microbiol* 2011; 49: 1464–1474.

18. Ye YL, Jia YH, Huang WMM, et al. Main cariogenic microorganisms: a meta-analysis. *Chinese Journal of Tissue Engineering Research* 2015: 5559–5565.

19. Mason MR, Nagaraja HN, Camerlengo T, et al. Deep sequencing identifies ethnicity-specific bacterial signatures in the oral microbiome. *PLoS One* 2013; 8: e77287.

20. Kaur N, Sahni P, Singhvi A, et al. Screening the drug resistance property among aerobic pathogenic microorganisms of dental caries in North-Western Indian population: a preliminary study. *J Clin Diagn Res* 2015; 9: ZC05–ZC08.

21. Daniyan SY and Abalaka ME. Prevalance and susceptibility pattern of bacterial isolates of dental caries in a secondary health care institution Nigeria. *Shiraz EMed J* 2011; 12: 1–7.

22. Oluremi BB, Osungunna MO, Idowu OA, et al. Evaluation of anticaries activity of selected mouthwash marketed in Nigeria. *Trop J Pharm Res* 2010; 9: 581–586.

23. Wang HJ, Dai YK and Pan Q. Current status of research on Weissell. *Journal of Chengdu Medical College* 2014: 747–750.

24. Chen HH, Li WJ, Zhang YQ, et al. Study on isolation and systematic taxonomy of strains of genus Nesterenkonia. *Acta Microbiologica Sinica* 2004: 811–815.

25. Aerken O. *Analysis of microbiota associated with Yu Gu dental caries and periodontitis using 16SrRNA gene clone library*. Lanzhou: Lanzhou Jiaotong University, 2014.

26. Du Q. Oral disease of Tibetan population and the high altitude-interrelated.

Chinese Journal of Practical Stomatology 2011: 564–566.

27. Jiao K. *A study on oral microbial community structure of periodontitis between Dongxiang and Yugurs in Gansu province*. Lanzhou: Lanzhou Jiaotong University, 2015.

28. Wang X, Willing MC, Marazita ML, et al. Genetic and environmental factors associated with dental caries in children: the Iowa Fluoride Study. *Caries Res* 2012; 46: 177–184.

29. She J, Feng F, Zhou XT, et al. Oral health status of air force personnel on plateau and on plain. *Journal of Military Surgeon in Southwest China* 2016; 2: 101–103.

30. Kianoush N, Adler CJ, Nguyen KA, et al. Bacterial profile of dentine caries and the impact of pH on bacterial population diversity. *PLoS One* 2014; 9: e92940.