JOURNAL OF ORAL MICROBIOLOGY
2025, VOL. 17, 2489603
https://doi.org/10.1080/20002297.2025.2489603

Taylor & Francis
Taylor & Francis Group

RESEARCH ARTICLE

OPEN ACCESS Check for updates

Investigation of oral microbiome composition in elderly Chinese patients with hypertension: a cross-sectional study

Xin Chen[a], Wen-Yong Lin[b], Feng-Wei Zhang[a], Li-Qiang Guo[a], Han Ge[a], Ding-Zuo Ge[a], Juan-Juan Tan[c,d], Bao-Cheng Liu[a,e], Rui-Rui Wang[a,e] and Lei Zhang[a,e]

[a]Shanghai Innovation Center of Traditional Chinese Medicine Health Service, Shanghai University of Traditional Chinese Medicine, Shanghai, China; [b]Cardiovascular Department of Traditional Chinese Medicine, Shuguang Hospital Affiliated to Shanghai University of Traditional Chinese Medicine, Shanghai, China; [c]Institute of Integrative Medicine, Shaanxi University of Chinese Medicine, Xianyang, China; [d]Institute of Integrative Medicine, Shaanxi Key Laboratory of Integrated Traditional and Western Medicine for Prevention and Treatment of Cardiovascular Diseases, Shaanxi University of Chinese Medicine, Xianyang, China; [e]State Key Laboratory of Integration and Innovation of Classic Formula and Modern Chinese Medicine, Shanghai University of Traditional Chinese Medicine, Shanghai, China

ABSTRACT

Background: Hypertension is a prevalent metabolic disorder in the elderly, with its pathogenesis linked to gut microbiota dysbiosis. Recent studies suggested that oral microbiota may also play a role in hypertension development, yet its relationship with hypertension in the elderly remains underexplored.

Objective: This cross-sectional study aimed to examine the structure of the oral microbiota and its association with hypertension in elderly patients, providing insights into hypertension prevention and treatment.

Methods: A total of 206 subjects (60–89 years) were categorized into normal (CON) and hypertensive (HTN) groups, based on the Chinese Hypertension Guidelines. Saliva samples were analyzed using 16S rRNA gene sequencing.

Results: Oral microbiota composition was significantly influenced by blood pressure. At the phylum level, Synergistetes and Spirochaetes were more significantly abundant in the HTN group, while at the genus level Treponema and Leptothrix was higher, Actinomyces and Capnocytophaga were lower in HTN. Random Forest analysis identified 15 key microbiota as strong discriminators of HTN (AUC 0.74). Blood pressure was negatively correlated with Actinomycetes and positively correlated with Leptothrix. PICRUST2 analysis revealed elevated chlorinated compound degradation in HTN patients.

Conclusions: This study identified distinct oral microbiota in elderly hypertensive patients, highlighting the role of the oral microbiome in hypertension pathogenesis.

ARTICLE HISTORY
Received 15 October 2024
Revised 10 February 2025
Accepted 1 April 2025

KEYWORDS
Hypertension; oral microbiome; 16S rRNA gene sequencing; elderly; biomarker

Introduction

Hypertension is one of the most popular metabolic diseases in the elderly population and is characterized by elevated systemic systolic and diastolic blood pressure [1], which is often accompanied by multiple organ dysfunction. It has been reported that there are over 1.1 billion patients suffered with hypertension all over the world [2], of which over 270 million are in China [3]. Furthermore, hypertension is a risk factor for cardiovascular, cerebrovascular and kidney diseases, which will lead to the decline of patients' quality of life and the decline of life span [4,5]. The pathogenesis of hypertension is related to complex mechanisms including the enhancement of sympathetic nerve excitability, abnormal ion transport of cell membrane, vasoconstriction and so on. Screening specific biomarkers of early hypertension will be helpful for disease prevention and control [6–8].

Accumulating studies have involved the human microbiome with the development of various metabolic diseases. Many correlation analyses have revealed that the gut microbiota is closely related to the pathogenesis of hypertension. Compared with the healthy population, patients with hypertension show significantly reduced richness and diversity of intestinal microbiota, accompanied with the increased ratio of the Firmicutes to Bacteroidetes [9]. Researchers have also found that specific bacteria are significantly correlated with blood pressure, including *Christensenella*, *Olsenella*, and *Macellibacteroides* [10]. Furthermore, it has been reported that gut microbiota could promote Ang II-induced vascular dysfunction and hypertension through vascular immune cell infiltration and inflammation [11]. The intestinal microbiota is a crucial factor involved in blood pressure control, which may be related to the influence of steroid hormone levels and activation of neuroinflammation and the sympathetic nervous system [11–13].

CONTACT Rui-Rui Wang wangruirui@shutcm.edu.cn; Lei Zhang zhanglei37@sina.com Shanghai Innovation Center of Traditional Chinese Medicine Health Service, Shanghai University of Traditional Chinese Medicine, 1200 Cailun Road, Pudong New Area, Shanghai 201203, China

Oral microbiota is the second largest biological community in the human body after intestinal microbiota. The oral cavity hosts the second largest and most diverse microbiota, following the gut, with more than 700 bacterial species, consisting of a variety of bacteria, fungi, viruses, and archaea that inhabit the human oral cavity [14–16], mainly including Actinobacteria, Bacteroidetes, Firmicutes, Proteobacteria, and TM7, etc [17–19]. It is reported that most intestinal bacteria originated from the oral cavity. Oral microbiota and intestinal microbiota are highly consistent in species, showing the intimate relationship of microbiota between oral and intestinal. Considering the sampling of oral microbiota is more immediate and convenient, the profiling of oral microbiome and the screening of oral microbial markers of related diseases is of great meaning. For example, it is reported that oral microbiota is not only related to oral and periodontal diseases such as dental caries and oral ulcers, but also related to systemic diseases like diabetes [20–22].

The preliminary population studies have also revealed the correlation between hypertension and oral microbiota. Compared to women with normal blood pressure (BP), Gordon et al found significantly higher amounts of *Fusobacterium, Leptotrichia hofstadii*, and *Prevotella shahii* among women with hypertension. Moreover, *Prevotella*, and *Actinomyces massiliensis* relative abundances were significantly lower for patients with hypertension than for those with normal blood pressure [23]. In the research of Chih-Yuan Ko [24], the abundance of *Porphyromonas* and *Aggregatibacter* is higher in patients with obstructive sleep apnea-associated hypertension when compared with normal individuals. In addition, some studies have found that after treatment with antibacterial mouthwash, the down-regulation effect of L-arginine and nitrate supplementation on systemic blood pressure is weakened [25,26]. The oral microbiota can reduce nitrate to nitrite, which will be further converted to nitric oxide, which has the effect of relaxing vascular smooth muscle and stabilizing blood pressure. Therefore, it is speculated that the initiation and development of hypertension may be relevant to changes in the number and composition of oral microbiome.

In this study, we hoped to identify biomarkers that can be used as diagnostic or therapeutic markers for hypertension in Chinese elderly population. The relationship between oral microbiota and hypertension was explored by comprehensive analysis of the sequencing data of oral microbiota in elderly hypertensive population, which laid a foundation for further research on potential bacterial markers and new methods of hypertension prevention and treatment.

Method

Cohort information

Each participant provided written informed consent before participating in the study at Shanghai Zhangjiang Community Hospital. In line with the Guidelines for Hypertension Prevention and Treatment in China, participants were classified based on their blood pressure measurements. The control group (CON) included normotensive individuals with normal blood pressure and no history of hypertension, while the hypertensive group (HTN) consisted of patients with a systolic blood pressure of ≥140 mmHg or a diastolic blood pressure of ≥90 mmHg. The exclusion criteria were: incomplete medical records, mental disorders, malignant tumors, or serious diseases; antibiotic use within the past three months; and failure to provide written informed consent to participate in the study. Based on the exclusion criteria, a total of 206 subjects were ultimately included in this study Figure 1. The study was conducted in accordance with the

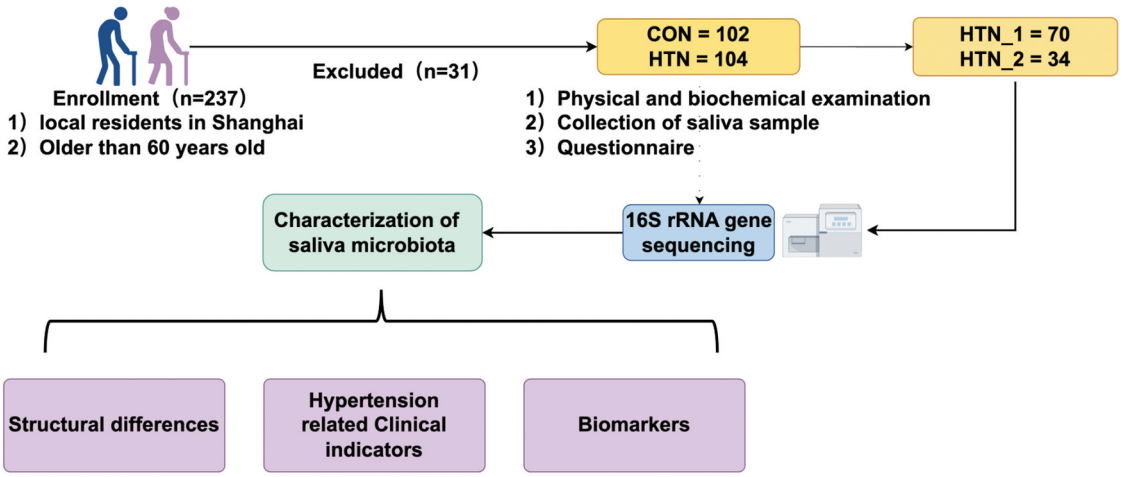

Figure 1.Flowchart of this study.

Declaration of Helsinki and followed the standard protocol established by the Synergistic Innovation Center of Shanghai University of Traditional Chinese Medicine, receiving approval from the Ethics Committee of the same institution.

Clinical investigation

An electronic device was used (Shengyuan, Zhengzhou, China) to calculate the body mass index (BMI). Blood samples were taken after overnight fasting. In the study, the levels of fasting plasma glucose (FBG), triglycerides, total cholesterol, high density lipoprotein (HDL), low density lipoprotein (LDL), alanine aminotransferase (ALT), aspartate aminotransferase (AST), white blood cells (WBC), and monocytes were measured using an automatic biochemistry analyzer (Hitachi, Tokyo, Japan). Blood tests were performed using the Mindray BC-2800 blood cell analyzer (Shenzhen, China). Participants were asked to refrain from brushing their teeth, eating, or smoking for 12 hours before saliva samples were collected. In order to ensure that all samples were preserved until further analysis, they were immediately frozen on dry ice after collection and stored at −80°C. Information on the subjects' lifestyle and diabetes history was gathered via a questionnaire.

DNA extraction and 16S rRNA gene sequencing

Genomic DNA was extracted from samples using the OMEGA Soil DNA Kit (M5635–02) (Omega Bio-Tek, Norcross, GA, USA) according to the manufacturer's instructions and stored at −20°C until further analysis. DNA concentration and purity were evaluated using a NanoDrop NC2000 spectrophotometer (Thermo Fisher Scientific, Waltham, MA, USA) and agarose gel electrophoresis. The V3–V4 region of the bacterial 16S rRNA gene was amplified with specific primers, incorporating 7-bp sample-specific barcodes for multiplex sequencing. The thermal cycling protocol included an initial denaturation step, followed by multiple cycles of denaturation, annealing, and extension, concluding with a final extension. PCR amplicons were purified using Vazyme VAHTSTM DNA Clean Beads (Vazyme, Nanjing, China) and quantified with the Quant-iT PicoGreen dsDNA Assay Kit (Invitrogen, Carlsbad, CA, USA). Equal amounts of purified amplicons were pooled, and paired-end sequencing (2×250 bp) was conducted on the Illumina NovaSeq platform using the NovaSeq 6000 SP Reagent Kit (500 cycles) at Shanghai Personal Biotechnology Co., Ltd (Shanghai, China).

Oral microbiota data analysis

Microbiome bioinformatics analyses were conducted using QIIME2 2022.11 [27], with slight modifications based on the official tutorials. Briefly, raw sequencing data were first demultiplexed using the demux plugin, followed by primer trimming with the cutadapt plugin [28]. Quality filtering, denoising, merging, and chimera removal were performed using the DADA2 plugin [29]. Non-singleton amplicon sequence variants (ASVs) were aligned with mafft [30] and subsequently used to construct a phylogenetic tree with fasttree2 [31]. Taxonomy was assigned to ASVs using the classify-sklearn naive Bayes taxonomy classifier in feature-classifier plugin [32] against the SILVA Release 132. The alpha diversity indices of Shannon index were calculated using the ASV table in QIIME2. Beta diversity analysis was performed to investigate the structural variation of microbial communities across samples using Bray-Curtis metrics [33] and visualized via principal coordinate analysis (PCoA), The significance of differentiation of microbiota structure among groups was assessed by ANOSIM (Analysis of similarities) [34,35]. The Wilcoxon rank-sum test was used to assess the differential abundance of bacterial taxa between groups at various taxonomic levels, including phylum, class, order, family, and genus. Linear discriminant analysis effect size (LEfSe) was applied to identify significantly different genera among groups, with features showing LDA scores above 2 considered significant. A Random Forest model using the AUC-RF algorithm was applied to optimize feature selection and improve model accuracy. Redundancy analysis (RDA) was performed to examine the relationship between the oral microbiome and clinical variables, with statistical significance assessed using permutation tests. Functional predictions of microbial communities were performed using PICRUSt, with functional categories from the Kyoto Encyclopedia of Genes and Genomes (KEGG) and MetaCyc databases.

Statistical analyses

Statistical tests were conducted using GraphPad Prism (CA). The Chi-square test or Mann-Whitney test was applied based on data distribution. Chi-square was used to assess group differences in sex, smoking, and drinking habits. Statistical significance was set at $^{*}p < 0.05$ and $^{**}p < 0.01$.

Results

Hypertension and abnormal cholesterol metabolism

We enrolled 104 patients with primary hypertension (SBP ≥140 mmHg and/or DBP ≥90 mmHg) and 102 control participants. As presented in Table 1, there were no significant differences in age, gender, waist-to-hip ratio (WHR), or fasting blood glucose (FBG)

Table 1. Basic characteristics of the study population.

	NC	HT	
Number subjects	102	104	P value
Age, years	69.07 ± 6.31	69.20 ± 7.06	0.93[b]
Gender(F/M), %	57/45(55.88/44.12)	56/48(53.85/46.15)	0.74[a]
SBP, mmHg	122.55 ± 11.89	155.48 ± 16.19	<0.001[b]
DBP, mmHg	73.44 ± 8.93	89.90 ± 9.80	<0.001[b]
BW, kg	60.66 ± 11.96	64.77 ± 13.13	0.009[b]
BMI, kg/m2	23.59 ± 3.60	25.03 ± 4.57	0.006[b]
WHR, cm/cm	0.88 ± 0.07	0.89 ± 0.06	0.30[b]
TG, mmol/L	1.23 ± 0.56	1.33 ± 0.61	0.209[b]
TC, mmol/L	4.98 ± 0.93	5.21 ± 0.90	0.079[b]
LDL, mmol/L	3.06 ± 0.83	3.36 ± 0.83	0.011[b]
HDL, mmol/L	1.3 ± 0.3	1.22 ± 0.25	0.049[b]
ALT, U/L	22.5 ± 5.44	24.05 ± 9.12	0.361[b]
AST, U/L	21.33 ± 7.86	23.31 ± 13.39	0.772[b]
FBG, mmol/L	5.82 ± 1.18	6.25 ± 2.05	0.406[b]

The data were presented as mean ± SD. [a]*p* values were calculated using Chi-square test; [b]*p* values were calculated using Mann-Whitney U test. SBP, systolic blood pressure; DBP, diastolic blood pressure; BW, Body weight; BMI, body mass index; WHR, Waist-to-Hip Ratio; TG, total triglyceride; TC, total cholesterol; LDL, low density lipoprotein; HDL, high density lipoprotein; ALT, alanine aminotransferase; AST, aspartate aminotransferase; and FBG, fasting blood glucose.

between the two groups. Liver injury markers, including ALT and AST, also showed no notable differences. However, the hypertension group exhibited significantly higher body weight and BMI compared to the control group. Clinical parameter comparisons revealed that individuals with hypertension had elevated LDL levels and reduced HDL (Table 1). Overall, these findings confirm that older adults with hypertension exhibit abnormalities in cholesterol metabolism.

Altered salivary microbiome composition and hypertension

The oral microbiome was investigated by sequencing the V3-V4 region of the bacterial 16S rRNA gene. Following the removal of low-quality sequences, we obtained 6,977,220 valid sequences, with an average length of 395 bp. Each sample yielded a high-quality reading of 33,093.34 ± 7,664.25 (mean ± SD). Utilizing the Greengenes database, we identified a total of 34 classes, 62 orders, 117 families, 233 genera, and 15 phyla within the oral microbiota, characterized by their relative taxonomic abundance. We assessed the relative taxonomic abundance of oral microorganisms in both the control (CON) and hypertension (HTN) groups. Our analysis revealed that there were 4 genera specific to the CON group and 24 genera unique to the HTN group (Figure 2(a)). There was no significant difference in α-diversity between the two groups (Figure 2(b,c)). To further explore similarities in bacterial community structures between the two groups (elderly hypertensive patients and healthy controls), a PCoA of β-diversity based on weighted UniFrac distances was performed (Figure 2(d)). The results showed that the community structures of the two groups were similar. The distribution of oral microbiota in the two groups largely overlapped. ANOSIM analysis indicated no significant separation between groups, (p value = 0.342), suggesting that the overall bacterial structures in the elderly hypertensive patients and healthy controls were comparable. Using the relative abundance data across different taxonomic levels, we selected the top 13 phyla and 20 genera, along with their corresponding abundance information, to create a stacked column chart. At the phylum level, the CON and HTN groups were similar in composition, with Firmicutes, Bacteroidetes, and Actinobacteria being the three most dominant phyla in both groups (Figure 2(e)). *Prevotella, Streptococcus, Rothia, Neisseria, Veillonella, Leptotrichia, Porphyromonas, Actinomyces, Gemella, Granulicatella, Fusobacterium, Peptostrep tococcus, Haemophilus, Leptothrix, Saccharibacteria, Peptostreptococcaceae, Capnocytophaga, Selenomonas, Absconditabacteria* and *Treponema* were the 20 most abundant genera (Figure 2(f)). To further determine the relationship between bacteria and hypertension, we analyzed the difference in the relative abundance of phyla and genera between the two groups (Figure 2(g,h)). The relative abundance of the Synergistetes (p value = 0.002) and the Spirochaetes) p value = 0.024) was significantly higher in the HTN group when compared to the CON group (Mann-Whitney test). Among the abundant genera, the relative abundance of *Treponema* (p value = 0.024) and *Leptothrix* (p value = 0.014) was significantly higher in the HTN group than in the CON group whereas the relative abundance of *Actinomyces* (p value = 0.049) and *Capnocytophaga* (p value = 0.043) were significantly lower than in the CON group (Mann-Whitney test). *S (lenomonas)* p value = 0.053) showed increasing trend in the HTN group. *Neisseria* (p value = 0.058) and *Haemophilus* (p value = 0.099) also showed a trend to be lower in the HTN group when compared to the CON group (Mann-Whitney test).

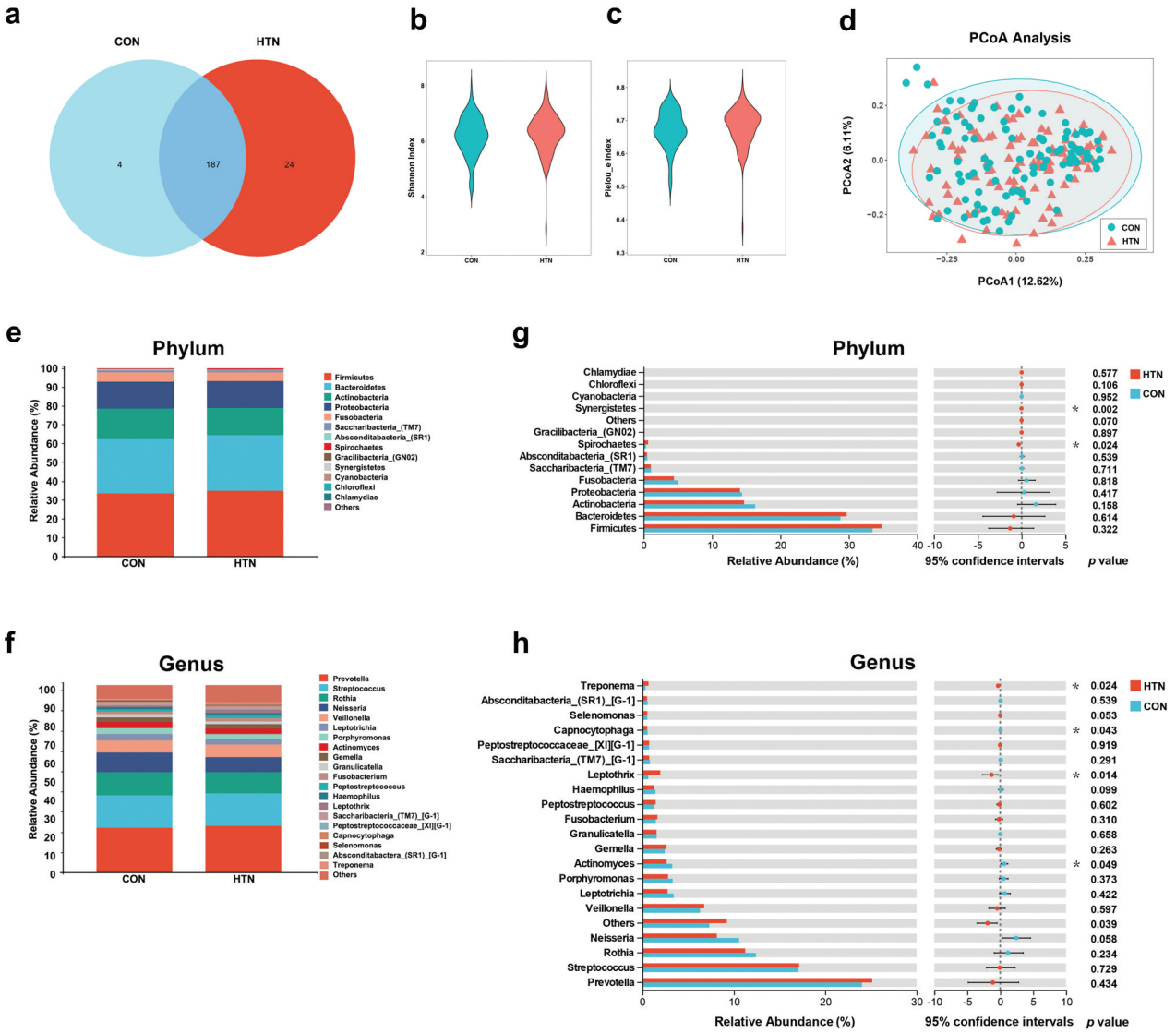

Figure 2.Structural changes of the oral microbiome in HTN group. (a) Venn diagram showing that 187 genera were shared between the two groups, while 24 genera were htn-specific. (b and c) Alpha diversity indices for oral bacteria in each group at 97% identity. (d) PCoA based on the weighted UniFrac distances at the ASV level at 97% identity. Each sample is represented by a dot. PCoA1 explained 12.62% of the variation observed, and PCoA2 explained 6.11% of the variation. (e) Average composition and relative abundance at the phylum level of two bacterial communities. (f) The average composition and relative abundance of the two bacterial communities at the genus level. (g and h) Relative abundance of differential microbial taxa at the phylum and genus levels. Mann – Whitney test. *$p < 0.05$, **$p < 0.01$, the value represented by each dot corresponds to the difference in the average relative abundance of the species between the two groups. The color of the dot indicates the group with the higher species abundance. The I-type interval on the dot shows the upper and lower limits of the difference, and the P-value is displayed on the far right. ASV: amplicon sequence variation, CON: control, HTN: hypertension.

Oral microbiota-based biomarker for HTN

To further identify bacterial taxa that were significantly different between groups, Linear discriminant analysis effect size (LEfSe) analysis was performed. The contribution to group discrimination was represented by the LDA effect size in. A total of 30 taxa with significantly differential abundance between the CON and HTN group were identified (LDA >2, $p < 0.05$). Two phyla, Spirochaetes and Synergistetes and and five classes, *Sphingobacteriia*, *Mollicutes*, *Alphaproteobacteria*, *Spirochaetia* and *Synergistia were* significantly enriched in the HTN group. Five orders, including *Rhizobiales*, *Pseudomonadales*, *Spirochaetales* and *Synergistales* were significantly enriched in the HTN group. The bacteria family *Paenibacillaceae* and genera *Actinomyces*, *Capnocytophaga*, *Paenibacillus* were significantly enriched in the CON group. Higher proportions of *Dermabacter*, *Lactobacillus*, *Filifactor*, *Mogibacterium*, *Anaeroglobus*, *Dialister*, *Cupriavidus*, *Ralstonia*, *Leptothrix*, *Pseudomonas*, *Treponema*, *Fretibacterium* were observed in the HTN group (Figure 3(a)). The differential species identified through both the Mann-Whitney U test and LEfSe analysis methods were largely consistent, indicating the robustness of the oral microbiome profiling data.

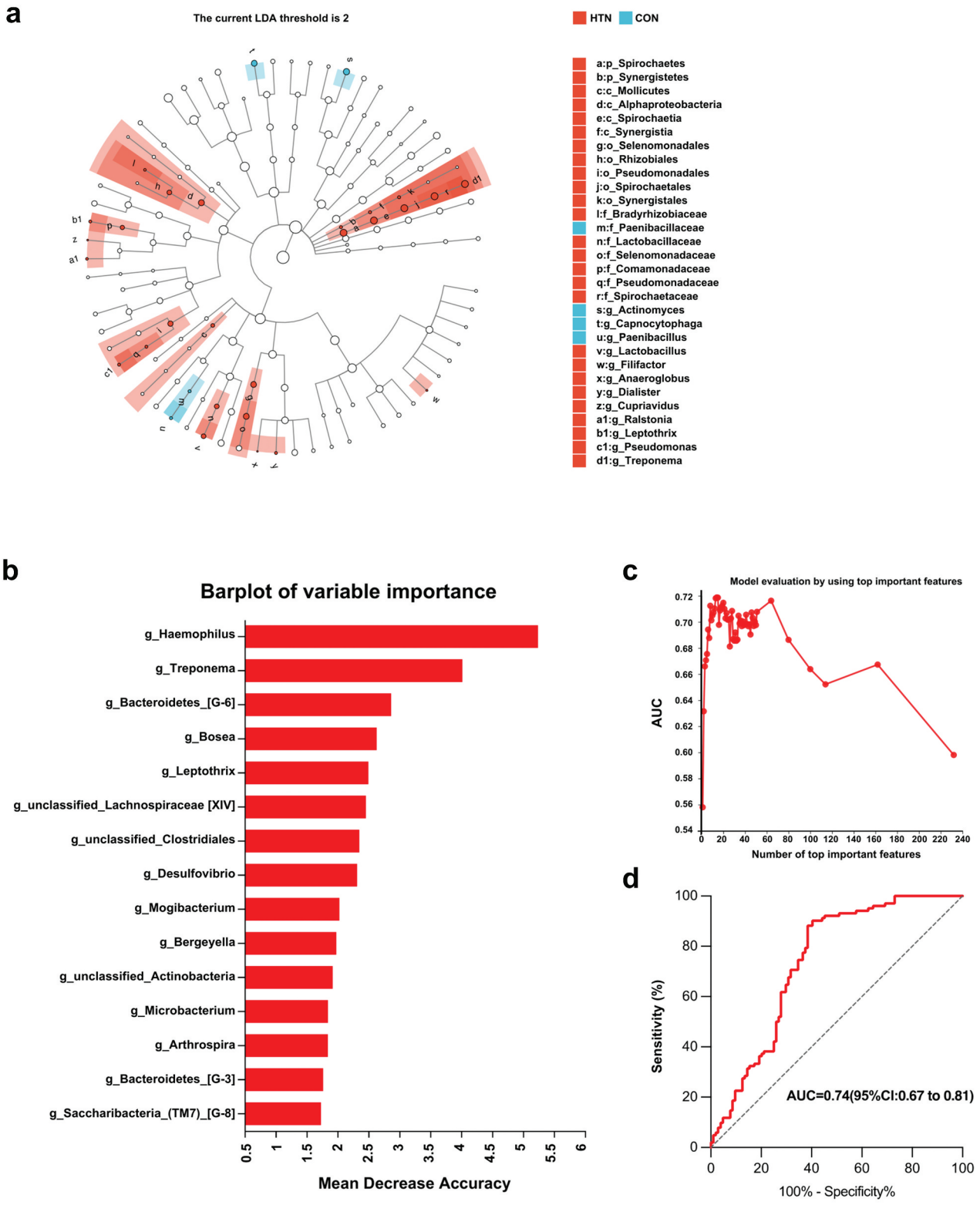

Figure 3.Biomarkers based on oral microbiomes identified using random forest and LEfSe. (a) Map of LEfSe taxonomic cladograms generated from 16S rRNA gene sequences. (b) Taxonomic performance of the 15 most discriminatory genera in the HTN cluster based on the relative abundance of the random forest. (c, d) Area under the curve (AUC) as determined by cross-validating the random forest.

Additionally, to investigate the predictive capability of the oral microbiome in distinguishing hypertension (HTN) status, we employed a Random Forest model based on genus-level relative abundance (Figure 3(b)). Cross-validation was performed to assess the generalization capability of these models (Figure 3(c)). A total of 15 genera were identified as predictive of hypertension (HTN), with *Haemophilus, Treponema, Bacteroidetes_[G-6]*, and *Bosea* among the top-ranked. To evaluate the clinical relevance of these key microbiota, Receiver Operating Characteristic (ROC) curves were generated, and Area Under the Curve (AUC) values were calculated

(Figure 3(d)). These findings demonstrate that the oral microbiome-based classifier can effectively differentiate HTN patients from controls.

Correlation of oral microbiota with clinical indicators

Our study showed that the composition of the oral microbiota was altered in hypertensive patients. To assess whether clinical indicators of hypertension were correlated with oral microbiota, we performed Spearman rank correlation analysis (Figure 4(a)). The results showed that systolic and diastolic blood pressure were significantly negatively correlated with *Actinomycetes* and significantly positively correlated with *Leptothrix*, both of which had significantly lower relative abundance in the HTN group. Body weight and body mass index were positively correlated with the genus with the highest relative abundance, *Prevotella*, in both groups. While BMI was significantly negatively correlated with *Porphyromonas*, body weight was significantly positively correlated with *Actinomyces*. On the other hand, triglycerides, an indicator of lipid metabolism, was significantly negatively correlated with *Streptococcus, Rothia*, and *Veillonella*, genera with increased relative abundance in the HTN group, LDL was significantly negatively correlated with *Streptococcus*, and HDL was significantly positively correlated with *Neisseria*, while FBG was significantly positively correlated with *Neisseria*, a genus with increased relative abundance in the HTN group. WBC, an indicator of systemic inflammation, were positively correlated with *Treponema*, which was increased in the HTN group. ALT and AST were both significantly negatively correlated with *Peptostreptococcaceae*, and AST was negatively correlated with *Veillonella*, *Absconditabacteria*, which were lower in the HTN group. Redundancy analysis (RDA) further revealed correlations between clinical features and genus-level bacterial communities (Figure 4(b)). The correlation of metabolic and inflammatory parameters with key bacteria suggests

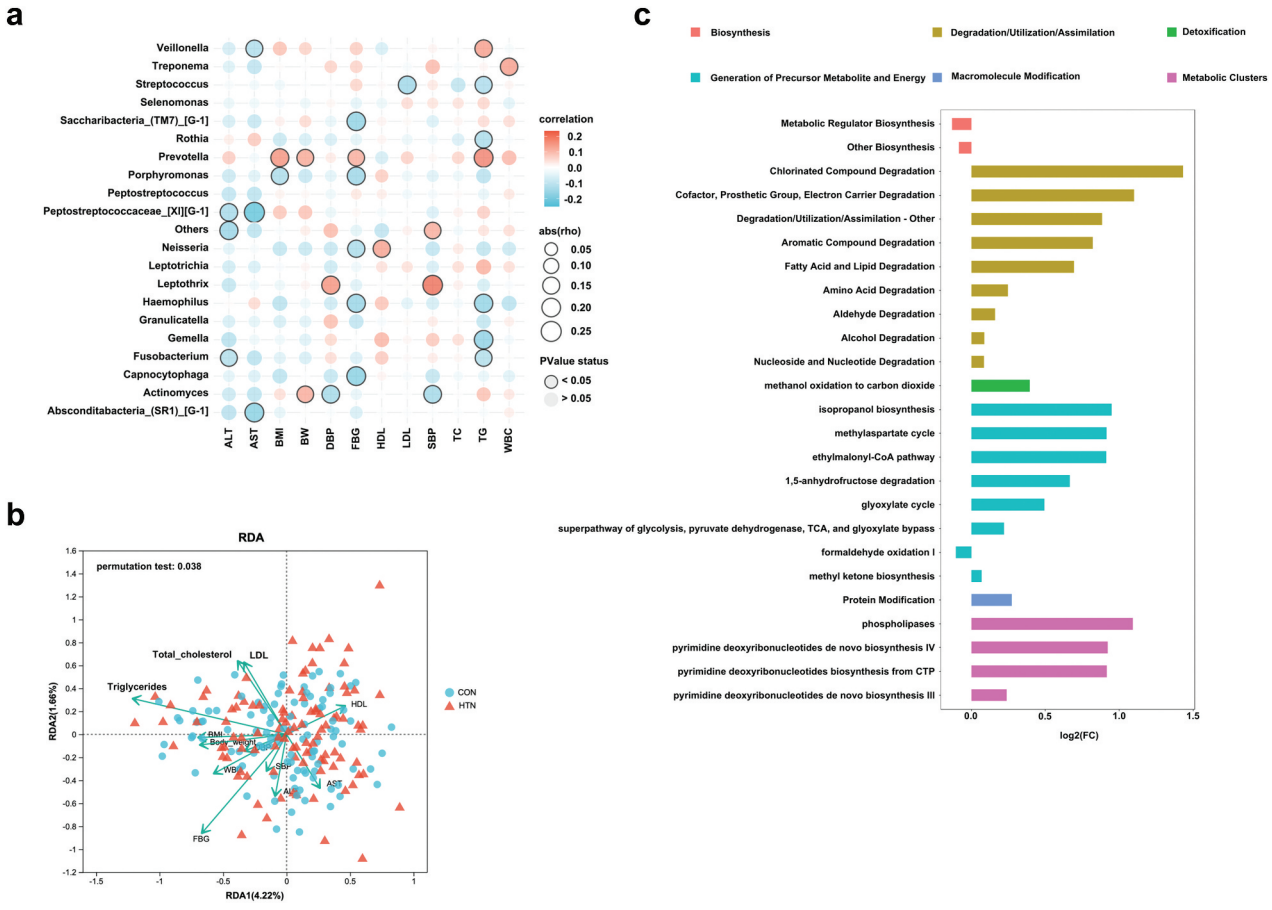

Figure 4.Relationship between oral microbial genera and clinical indicators. (a) Bubble diagram of Spearman's correlation between clinical indicators and genera. (b) Redundancy analysis (RDA) examining the relationship between clinical indicators and genus-level composition. permutation test. p value = 0.038. (c) PICRUSt predicts functional classification between CON and HTN groups. Folding changes of the top 25 pathways with significant differences (FC > 1) in KEGG are shown. ALT, alanine aminotransferase; AST, aspartate aminotransferase; BMI, body mass index; BW, body weight; BMI, body mass index; DBP, diastolic blood pressure; FBG, fasting blood glucose; HDL, high density lipoprotein; LDL, low density lipoprotein; SBP, systolic blood pressure; TC, total cholesterol; TG, total triglyceride; WBC: white blood cell.

that the oral microbiome may be associated with glucose metabolism, lipid metabolism, and inflammatory responses in hypertensive patients.

Functional predictive analysis of oral microorganisms in HTN

To characterize the predicted functions of the oral microbes, a phylogenetic study of the community was conducted using the unobserved state reconstruction (PICRUSt) approach to predict the functions and pathways between the oral microbiomes of the CON and HTN groups (Figure 4(c)). At KEGG, 34 up-regulated and 26 down-regulated pathways were detected in the HTN group compared to the CON group, with the Chlorinated Compound Degradation, Cofactor, Prosthetic Group, Electron Carrier Degradation, phospholipases metabolism pathways being significantly up-regulated compared to the control group.

Composition changes in the oral microbiota of patients with different levels of HTN

To characterize the oral microbiome of patients with different levels of hypertension and provide evidence for grading criteria. Oral microbiota samples from 104 hypertensive patients were categorized into HTN_1 group ($n = 70$, SBP < 160/100 mmHg) and HTN_2 group ($n = 34$, SBP ≥ 160/100 mmHg). Alpha diversity results showed a significant difference in oral microbial diversity between the two groups (Figure 5(a)). In addition, 7659 ASVs and 3218 ASVs were specific to the HTN_1 and HTN_2 groups, respectively. Notably, *Fretibacterium* and *unclassified_Bacteria* were two genera specific to the HTN_1 group (Figure 5(b)). At the genus level, we observed a significant increase in the abundance of *Saccharibacteria_(TM7)* in the HTN_2 group with elevated blood pressure (Figure 5(c,d)), and based on the LDA analyses, it was found that *Peptidiphaga* was significantly enriched in the HTN_1 group, whereas *Atopobium*, *Solobacterium*, and *Megasphaera* were significantly enriched in the HTN_2 group (Figure 5(e)).

Discussion

The oral microbiota is the second most diverse microbial community to colonize the human body after gut microbes [15]. Oral microbes coexist harmoniously with the host, usually in a symbiotic state in which the host provides a stable ecological environment for the oral microbiota, and oral microbes can support host health by participating in host physiological processes through metabolism and the immune system [36]. When oral microbes and body homeostasis are no longer in balance, oral microbes can in turn affect overall host health and distal tissues, and it has been reported that microbes of oral origin colonize the gut of patients with a variety of diseases, creating a translocation of oral microbes to the gut and driving the TH1 cell-induced inflammatory response [37]. These studies suggest that oral microbial dysbiosis may play a role in promoting systemic consequences such as autoimmune and neurodegenerative diseases. This, in turn, provides an opportunity to identify predictive disease biomarkers in oral microbes and to develop intervention strategies to promote oral and general health. Therefore, in this cross-sectional study, we explored the landscape of oral microorganisms in hypertensive patients and identified key oral flora in hypertensive patients, and the identified key microbiota demonstrated significant discriminatory ability in distinguishing HTN patients from controls.

This study reveals the structure and characteristics of the oral microbiota in the HTN group and compares the differences in the structure of the oral microbiota between HTN and CON group. The structure of oral microbiota was disturbed in HTN, and there was a tendency for the oral microbiota of HTN and CON to segregate, but there was no significant difference, probably because the oral microbiota tends to be stabilized in adulthood [19], and the perturbation of oral microbiota by exogenous factors is low. In the HTN group, there was a significant increase in the relative abundance of differential phyla, including Synergistetes and the Spirochaetes. members of the Synergistetes phyla are some of the most recently identified bacteria associated with periodontal disease. the Synergistetes phylum is proinflammatory in the oral cavity and is capable of stimulating the host microbiota by skewing the oral microbiota toward a more pathogenic dysbiotic population [38], thereby stimulating an inflammatory response in the host. And as early as 2006, Ryan T et al. proposed periodontal disease as a potential risk factor for cardiovascular diseases such as atherosclerosis [39]. A significant increase in the relative abundance of Synergistetes was also found in the gut of patients with heart failure [40]. Among the genera with higher abundance, Treponema, a group of bacteria linked to poor oral health [41], was found to be significantly more abundant in the hypertension (HTN) group compared to the control (CON) group, and its levels correlated with the severity and progression of heart failure [42]. *Leptothrix* was also found to have an increased abundance in the HTN group. Our findings revealed a decrease in the relative abundance of *Actinomycetes and Capnocytophaga* in the HTN group. *Sèlenomonas* showed increasing trend in the HTN group, *Haemophilus* and *Neisseria* also showed a trend to be lower in the HTN group. *Actinomyces* is a gram-positive, anaerobic, filamentous bacterium commonly present in the normal oral microbiota [43]. There is growing

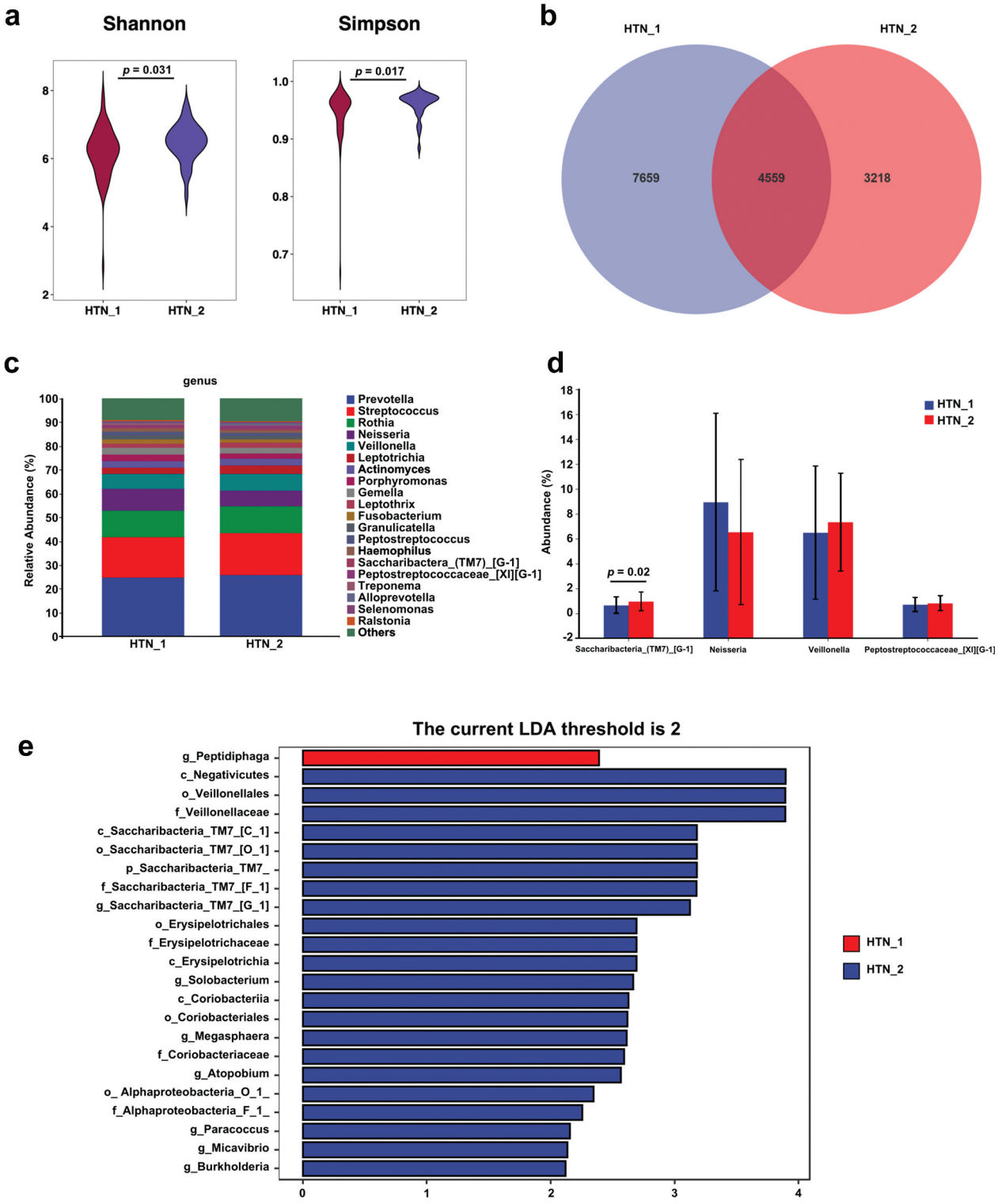

Figure 5. Hypertensive patients with SBP >160 mmHg have increased oral microbial diversity and changes in microbial structure. (a) Alpha diversity of oral microbes was significantly increased in HTN_2 ($n = 34$) compared to HTN_1 ($n = 70$) as measured by Shannon's index and Simpson's index. (b) Venn diagram showing that 7651 ASVs were specific to HTN_1, while 3218 ASVs were specific to HTN_2. (c) Composition and relative abundance of bacterial communities at the genus level for both groups. (d) *Saccharibacteria_(TM7)_[G-1]* showed a significant increase in relative abundance in the HTN_2 group. Bars represent the mean relative abundance of taxa, and error bars indicate the standard deviation. (e) Based on LDA, the two groups were enriched with 22 and 1 oral microbial taxa, respectively. Statistical significance was determined using the Wilcoxon rank-sum test, with an asterisk (*) indicating a significant difference between HTN_1 and HTN_2.

evidence suggesting that *Actinomyces* may contribute to biofilm formation, particularly in the oral cavity [44]. Under normal conditions, *Actinomyces* have low pathogenicity, but poor oral hygiene or complications from maxillofacial trauma can lead to infections [45]. *Capnocytophaga* is not only associated with oral health but may also affect systemic health through the bloodstream, with a bidirectional association between it and

systemic diseases such as diabetes and cardiovascular diseases [46]. *Selenomonas* has been found to be useful in distinguishing diabetic patients from healthy individuals, indicating a potential link between *Selenomonas* and systemic diseases [47]. The relative abundance of Haemophilus species in the oral and gut microbiota of rheumatoid arthritis (RA) patients is relatively low. It is also associated with a decrease in levels of rheumatoid arthritis-specific autoantibodies (anti-citrullinated protein antibodies) and rheumatoid factor [48]. Nitrates can be actively transported from blood to saliva by the salivary glands through sialin, which is encoded by the *SLC17A5*. Oral microbes, including *Veillonella*, *Actinomyces*, *Rothia*, *Haemophilus*, and *Neisseria*, convert these nitrates to nitrite. Once saliva enters the stomach, it is degraded to nitric oxide (NO) in the acidic environment, which is then released into the bloodstream [36,49,50]. As a potent vasodilator and anti-inflammatory signaling molecule, NO plays a critical role in maintaining vascular homeostasis [51]. This may contribute to the elevated blood pressure observed in HTN patients.

The oral cavity is a richly vascularized organ, allowing oral microbes and microbial metabolites to directly enter the bloodstream and contribute to the pathogenesis of systemic diseases. Correlational analyses have indicated a strong association between oral microbes and blood markers related to inflammation and metabolism. Prior research has proposed that low-grade systemic inflammation and redox imbalance may be key mechanisms linking hypertension and periodontitis [52]. Oral-gut microbial transmission is an intermediate mechanism by which diabetes affects myocardial damage and induces cardiovascular events [53], and a cross-sectional study focusing on Chinese individuals revealed that dysbiosis of the oral microbiome may be associated with impaired fasting glucose in Chinese older adults [54]. Our results are consistent with them. Our functional annotation of KEGG revealed significant differences in the predicted biological function of the oral microbiome in HTN, characterized by a significantly elevated chlorinated compound degradation pathway. In HTN group, blood chloride levels are recognized as an independent predictor of mortality.

The Chinese Guidelines for the Prevention and Control of Hypertension (2024 Revision) state that hypertensive patients with blood pressure levels ≥ 160/100 mmHg should also be immediately initiated on antihypertensive drug therapy in the absence of other risk factors. To characterize the oral microbiome in patients with different levels of BP, we further analyzed the HTN samples. The results showed that the Alpha diversity index of the oral microbiome in the HTN_2 group was overall higher than that in the HTN_1 group, indicating that the microbial community was more heterogeneous in HTN_2. And the abundance of *Saccharibacteria_(TM7)* was significantly increased in the HTN_2 group, which is a core microbiome group in periodontitis [55]. In addition, the presence of *Saccharibacteria* in the gut has been associated with type 2 diabetes and impaired glucose metabolism [56], and type 2 diabetes is an important risk factor for the onset, progression, and prognosis of cardiovascular disease. Schulz et al's study in 2021 patients with coronary artery bypass grafting found that *Saccharibacteria_(TM7)* may serve as a secondary predictor of cardiovascular events [57].

The oral microbiota is particularly valuable in identifying taxonomic and molecular biomarkers for both oral and systemic diseases, owing to its ease of sampling and compositional stability [58]. Previous studies have shown that blood pressure is higher in patients with periodontitis than in those without, and that ectopic colonization of the gut by saliva-derived *Veillonella* can exacerbate HTN [59]. In our study, among the genera with variable abundance, a combination of 15 genera such as *Haemophilus, Treponema, Bacteroidetes_[G-6]* and *Bosea* had effective discriminatory ability in HTN, and the AUC was 0.74.

In conclusion, to the best of our knowledge, for the first time, we have graded patients with HTN and analyzed the role of oral microbiota for elevated blood pressure. HTN is characterized by a structural disruption of oral microbes, with a significant increase in the relative abundance of pathogenic bacteria, such as *Treponema*. and a significant increase in the abundance of *Saccharibacteria_(TM7)* in the HTN_2 group. The identified key oral microbiota showed good discriminatory ability in distinguishing HTN patients from controls, and here we comprehensively characterize the oral microbiome of HTN. Hypertension can cause arterial endothelial injury and promote the progression of atherosclerosis, and atherosclerosis, when it occurs in the arteries supplying blood to the heart, can lead to coronary artery disease, in which patients may suffer from angina pectoris or even myocardial infarction, which can lead to the development of heart failure. Our results showed that microorganisms abundant in the oral cavity and gut of patients with heart failure were significantly elevated in the oral microbiome of hypertensive patients, suggesting that prevention and treatment of cardiovascular events should begin with early prevention and treatment of hypertension. However, this study is limited by the resolution of 16S rRNA sequencing, which only allows for analysis at the genus level, potentially limiting the depth of microbial characterization. the physiological and pathophysiological functions of the oral microbiome in HTN require further investigation.

Conclusions

Our study identified specific oral microbial features in elderly hypertensive patients, revealing relationship

between oral microbiota and hypertension. This enhances our understanding of the important role of oral microbiota in the pathogenesis of hypertension and accumulates more evidence for microbial involvement in the development of hypertension.

Acknowledgments

We thank the participants for providing oral and blood samples. Oral microbiome sequencing data are available in the NCBI Sequence Read Archive (SRA) under accession number GSE277296.

Disclosure statement

No potential conflict of interest was reported by the author(s).

Funding

This work has been funded by Shanghai Science and Technology Planning Program "Shanghai Key Laboratory of Health Identification and Assessment" (No. 21DZ2271000); Open Program of Shaanxi Key Laboratory of Integrative Traditional and Western Medicine for Prevention and Treatment of Cardiovascular Diseases, Shaanxi University of Chinese Medicine (KF2023); Natural Science Foundation of Shaanxi Province (2023-JC-QN-0853); Local Colleges Faculty Constitution of Shanghai MSTC 2022 (22010504300); and Shanghai Collaborative Innovation Center for Chronic Disease Prevention and Health Services (2021 Science and Technology 02-37).

Author contributions statement

LZ designed the study and obtained funds for the project. RW reviewed the data and finalized the manuscript. XC sequenced the samples, performed the data analysis, and wrote the manuscript. YL, ZW and QG recruited subjects and collected samples. HG, ZG, JT, and CL processed and sequenced the samples. All the authors have reviewed and approved the final manuscript and consented for publication.

Data availability statement

The dataset supporting the conclusions of this article is available in the NCBI Sequence Read Archive database, the accession number is GSE277296.

Ethics approval and consent to participate

The studies were conducted in accordance with the Declaration of Helsinki and Good Clinical Practice guidelines as defined by the International Conference on Harmonization; all patients provided written informed consent for study participation; and the institutional review board approved the study.

References

[1] Oparil S, Acelajado MC, Bakris GL, et al. Hypertension. Nat Rev Dis Primers. 2018;4(1):18014. doi: 10.1038/nrdp.2018.14

[2] McEvoy JW, McCarthy CP, Bruno RM, et al. 2024 ESC guidelines for the management of elevated blood pressure and hypertension. Eur Heart J. 2024;11(1):7–9. doi: 10.1093/ehjcvp/pvae084

[3] Wang JG, Zhang W, Li Y, et al. Hypertension in China: epidemiology and treatment initiatives. Nat Rev Cardiol. 2023;20(8):531–545. doi: 10.1038/s41569-022-00829-z

[4] Kelly DM, Ademi Z, Doehner W, et al. Chronic kidney disease and cerebrovascular disease: consensus and guidance from a KDIGO controversies conference. Stroke. 2021;52(7):e328–e46. doi: 10.1161/STROKEAHA.120.029680

[5] Burnier M, Damianaki A. Hypertension as cardiovascular risk factor in chronic kidney disease. Circ Res. 2023;132(8):1050–1063.

[6] Tan ZY, Lu Y, Whiteis CA, et al. Chemoreceptor hypersensitivity, sympathetic excitation, and overexpression of ASIC and TASK channels before the onset of hypertension in SHR. Circ Res. 2010;106(3):536–545.

[7] Bing RF, Heagerty AM, Thurston H, et al. Ion transport in hypertension: are changes in the cell membrane responsible? Clin Sci (Lond). 1986;71(3):225–230. doi: 10.1042/cs0710225

[8] Takefuji M, Yura Y, Kaibuchi K, et al. RhoGEF-mediated vasoconstriction in hypertension. Hypertens Res. 2013;36(11):930–931. doi: 10.1038/hr.2013.101

[9] Magne F, Gotteland M, Gauthier L, et al. The Firmicutes/Bacteroidetes ratio: a relevant marker of gut dysbiosis in obese patients? Nutrients. 2020;12(5):1474. doi: 10.3390/nu12051474

[10] Dan X, Mushi Z, Baili W, et al. Differential analysis of hypertension-associated intestinal microbiota. Int J Med Sci. 2019;16(6):872–881. doi: 10.7150/ijms.29322

[11] Karbach SH, Schönfelder T, Brandão I, et al. Gut microbiota promote angiotensin II-Induced arterial hypertension and vascular dysfunction. J Am Heart Assoc. 2016;5(9):e003698.

[12] Toral M, Robles-Vera I, de la Visitación N, et al. Critical role of the interaction gut microbiota – sympathetic nervous system in the regulation of blood pressure. Front Physiol. 2019;10:231. doi: 10.3389/fphys.2019.00231

[13] Robles-Vera I, Toral M, Duarte J. Microbiota and hypertension: role of the sympathetic nervous system and the immune system. Am J Hypertens. 2020;33(10):890–901.

[14] Aas JA, Paster BJ, Stokes LN, et al. Defining the normal bacterial flora of the oral cavity. J Clin Microbiol. 2005;43(11):5721–5732. doi: 10.1128/JCM.43.11.5721-5732.2005

[15] Deo PN, Deshmukh R. Oral microbiome: unveiling the fundamentals. J Oral Maxillofac Pathol. 2019;23(1):122–128. doi: 10.4103/jomfp.JOMFP_304_18

[16] Human Microbiome Project C. Structure, function and diversity of the healthy human microbiome. Nature. 2012;486(7402):207–214.

[17] Dewhirst FE, Chen T, Izard J, et al. The human oral microbiome. J Bacteriol. 2010;192(19):5002–5017.

[18] Dong L, Yin J, Zhao J, et al. Microbial similarity and preference for specific sites in healthy oral cavity and esophagus. Front Microbiol. 2018;9:1603.
[19] Lazarevic V, Whiteson K, Hernandez D, et al. Study of inter- and intra-individual variations in the salivary microbiota. BMC Genomics. 2010;11(1):523. doi: 10.1186/1471-2164-11-523
[20] Xu Y, Zhang M, Zhang J, et al. Differential intestinal and oral microbiota features associated with gestational diabetes and maternal inflammation. Am J Physiol Endocrinol Metab. 2020;319(2):E247–e53. doi: 10.1152/ajpendo.00266.2019
[21] Graves DT, Corrêa JD, Silva TA. The oral microbiota is modified by systemic diseases. J Dent Res. 2019;98(2):148–156. doi: 10.1177/0022034518805739
[22] Zomorodian K, Kavoosi F, Pishdad GR, et al. Prevalence of oral Candida colonization in patients with diabetes mellitus. J Mycol Med. 2016;26(2):103–110.
[23] Gordon JH, LaMonte MJ, Genco RJ, et al. Is the oral microbiome associated with blood pressure in older women? High Blood Press Cardiovasc Prev. 2019;26(3):217–225.
[24] Ko CY, Hu AK, Chou D, et al. Analysis of oral microbiota in patients with obstructive sleep apnea-associated hypertension. Hypertens Res. 2019;42(11):1692–1700. doi: 10.1038/s41440-019-0260-4
[25] Batista RIM, Nogueira RC, Ferreira GC, et al. Antiseptic mouthwash inhibits antihypertensive and vascular protective effects of L-arginine. Eur J Pharmacol. 2021;907:174314. doi: 10.1016/j.ejphar.2021.174314
[26] Petersson J, Carlström M, Schreiber O, et al. Gastroprotective and blood pressure lowering effects of dietary nitrate are abolished by an antiseptic mouthwash. Free Radic Biol Med. 2009;46(8):1068–1075.
[27] Bolyen E, Rideout JR, Dillon MR, et al. Reproducible, interactive, scalable and extensible microbiome data science using QIIME 2. Nat Biotechnol. 2019;37(8):852–857.
[28] Kechin A, Boyarskikh U, Kel A, et al. cutPrimers: a new tool for accurate cutting of primers from reads of targeted next generation sequencing. J Comput Biol. 2017;24(11):1138–1143. doi: 10.1089/cmb.2017.0096
[29] Callahan BJ, McMurdie PJ, Rosen MJ, et al. DADA2: high-resolution sample inference from Illumina amplicon data. Nat Methods. 2016;13(7):581–583.
[30] Katoh K, Misawa K, Kuma K, et al. MAFFT: a novel method for rapid multiple sequence alignment based on fast Fourier transform. Nucleic Acids Res. 2002;30(14):3059–3066.
[31] Price MN, Dehal PS, Arkin AP. FastTree: computing large minimum evolution trees with profiles instead of a distance matrix. Mol Biol Evol. 2009;26(7):1641–1650.
[32] Bokulich NA, Subramanian S, Faith JJ, et al. Quality-filtering vastly improves diversity estimates from Illumina amplicon sequencing. Nat Methods. 2013;10(1):57–59.
[33] Bray JR, Curtis JT. An ordination of the upland forest communities of southern Wisconsin. Ecol Monogr. 1957;27(4):325–349. doi: 10.2307/1942268
[34] Clarke KR. Non-parametric multivariate analyses of changes in community structure. Aust J Ecol. 1993;18(1):117–143.
[35] Warton DI, Wright ST, Wang Y. Distance-based multivariate analyses confound location and dispersion effects. Methods Ecol Evol. 2012;3(1):89–101.
[36] Wade WG. The oral microbiome in health and disease. Pharmacol Res. 2013;69(1):137–143. doi: 10.1016/j.phrs.2012.11.006
[37] Atarashi K, Suda W, Luo C, et al. Ectopic colonization of oral bacteria in the intestine drives T(H)1 cell induction and inflammation. Science. 2017;358(6361):359–365.
[38] McCracken BA, Nathalia Garcia M. Phylum synergistetes in the oral cavity: a possible contributor to periodontal disease. Anaerobe. 2021;68:102250.
[39] Humagain M, Nayak DG, Uppoor AS. Periodontal infections and cardiovascular disease: is it a mere association? Kathmandu Univ Med J (KUMJ). 2006;4(3):379–382.
[40] Simadibrata DM, Auliani S, Widyastuti PA, et al. The gut microbiota profile in heart failure patients: a systematic review. J Gastrointestin Liver Dis. 2023;32(3):393–401.
[41] Darveau RP. Periodontitis: a polymicrobial disruption of host homeostasis. Nat Rev Microbiol. 2010;8(7):481–490.
[42] Bohn B, Pinsino A, Braghieri L, et al. Association between oral microbiome, inflammation, endotoxemia, and heart failure severity. J Heart Lung Transplant. 2022;41(4, Supplement):S203.
[43] Haggerty CJ, Tender GC. Actinomycotic brain abscess and subdural empyema of odontogenic origin: case report and review of the literature. J Oral Maxillofac Surg. 2012;70(3):e210–3.
[44] Jafari-Ashkavandi Z, Kamali F. Actinomyces colonization in keratocystic odontogenic tumor: a case report. J Dent (Shiraz). 2015;16(2):134–137.
[45] D'Amore F, Franchini R, Moneghini L, et al. Actinomycosis of the tongue: a case report and review of literature. Antibiotics (Basel). 2020;9(3):124. doi: 10.3390/antibiotics9030124
[46] Kamer AR, Sirois DA, Huhmann M. Bidirectional impact of oral health and general health. In: Touger-Decker R, Sirois D Mobley C, editors. Nutrition and oral medicine. Totowa, NJ: Humana Press; 2005. p. 63–85.
[47] Yang Y, Liu S, Wang Y, et al. Changes of saliva microbiota in the onset and after the treatment of diabetes in patients with periodontitis. Aging (Albany NY). 2020;12(13):13090–13114.
[48] Zhang X, Zhang D, Jia H, et al. The oral and gut microbiomes are perturbed in rheumatoid arthritis and partly normalized after treatment. Nat Med. 2015;21(8):895–905.
[49] Carpenter GH. Salivary factors that maintain the normal oral commensal microflora. J Dent Res. 2020;99(6):644–649.
[50] Pignatelli P, Fabietti G, Ricci A, et al. How periodontal disease and presence of nitric oxide reducing oral bacteria can affect blood pressure. Int J Mol Sci. 2020;21(20):7538.

[51] Cyr AR, Huckaby LV, Shiva SS, et al. Nitric oxide and endothelial dysfunction. Crit Care Clin. 2020;36 (2):307–321. doi: 10.1016/j.ccc.2019.12.009

[52] Del Pinto R, Pietropaoli D, Munoz-Aguilera E, et al. Periodontitis and hypertension: Is the association causal? High Blood Press Cardiovasc Prev. 2020;27 (4):281–289.

[53] Li Y, Liu Y, Cui J, et al. Oral-gut microbial transmission promotes diabetic coronary heart disease. Cardiovasc Diabetol. 2024;23(1):123. doi: 10.1186/s12933-024-02217-y

[54] Wang RR, Xu YS, Ji MM, et al. Association of the oral microbiome with the progression of impaired fasting glucose in a Chinese elderly population. J Oral Microbiol. 2019;11(1):1605789. doi: 10.1080/20002297.2019.1605789

[55] Abusleme L, Dupuy AK, Dutzan N, et al. The subgingival microbiome in health and periodontitis and its relationship with community biomass and inflammation. ISME J. 2013;7(5):1016–1025.

[56] Nuli R, Cai J, Kadeer A, et al. Integrative analysis toward different glucose tolerance-related gut microbiota and diet. Front Endocrinol (Lausanne). 2019;10:295.

[57] Schulz S, Reichert S, Grollmitz J, et al. The role of Saccharibacteria (TM7) in the subginival microbiome as a predictor for secondary cardiovascular events. Int J Cardiol. 2021;331:255–261.

[58] Tuganbaev T, Yoshida K, Honda K. The effects of oral microbiota on health. Science. 2022;376(6596):934–936.

[59] Chen BY, Lin WZ, Li YL, et al. Roles of oral microbiota and oral-gut microbial transmission in hypertension. J Adv Res. 2023;43:147–161.