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Investigation of dental health and salivary microbiota characteristics of children with visual impairment in Guangzhou, China

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Abstract

Background The prevalence of visual impairment (VS) among children in China is increasing. The oral microbiome is crucial for maintaining homeostasis and health. This study aimed to investigate the oral health and hygiene habits of children with VS in Guangzhou and explore the differences in salivary microbiota (SM) between children with VS and healthy vision (HS).

Method This study included oral health examinations and surveys of oral hygiene habits among 101 children with VS. Saliva samples from 20 children with VS and 20 with HS were analysed for oral microbiota. The 16s rRNA V3-V4 regions were sequenced using the Illumina MiSeq platform and operational taxonomic units were clustered using QIIME for statistical analysis.

Results Inadequate oral hygiene was observed among 101 children with VS, aged 6–16, who displayed a high caries rate of 92.1%. There was no significant difference in the overall composition of the salivary microbiota between the two groups. HS group had a higher abundance of *Bacillota*, *Patescibacteria*, and *Spirochaetota* at the phylum level; *Bacilli*, *Negativicutes*, and *Saccharimonadia* at the class level; and *Streptococcus* at the genus level. In contrast, VS group showed a greater abundance of *Actinomycetota*, *Bacteroidota*, *Pseudomonadota*, and *Fusobacteriota* (at the phylum level) and *Actinomycetia*, *Bacteroidia*, *Gammaproteobacteria*, *Fusobacteriia*, and *Clostridia* (at the class level), along with *Rothia*, *Neisseria*, *Veillonella*, *Prevotella_7*, *Actinomyces*, *Leptotrichia*, and *Lactobacillales* (at the genus level). *Actinomycetota* was significantly and positively correlated with gingivitis and dental caries, and *Streptococcus salivarius* was more abundant in children with VS.

Conclusion This study underscores the importance of improving oral healthcare for schoolchildren with VS in Guangzhou, China and provides valuable insights into the characteristics of the salivary microbiota of this population, identifying potential targets for interventions aimed at enhancing oral health.

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Keywords Visually impaired children, Oral health, Oral microecology, Questionnaire survey, Caries

Background

Visual impairment (VS) is a condition in which an individual's vision cannot be corrected to achieve normal visual acuity [1]. It substantially affects a large population globally, with over 1.4 million children affected worldwide. Notably, 75% of these children reside in the most impoverished regions of Asia and Africa [2]. In low-income countries, the prevalence of VS in children is approximately 0.15%. As of 1 April, 2006 China, the most populous developing country, had 0.14 million schoolchildren affected by VS, with 79.07% of them attending either regular or special education institutions [3].

Oral health challenges faced by individuals with VS. Adolescents with VS tend to have poorer oral health than their sighted peers [1, 4–8]. Research on oral health and related behaviours of children in South China revealed that 54.7% of 12-year-olds had dental caries in their permanent teeth [8]. Visually impaired subjects demonstrated poorer oral hygiene compared to their sighted counterparts, and our findings in the salivary microbiome reflect characteristics typical of poor hygiene conditions. Visual impairment can indeed have a significant impact on oral hygiene practices, as it may hinder effective use of oral care tools and reduce the visual feedback essential for thorough cleaning. This, in turn, can lead to an imbalance in the oral microbiome, favoring the growth of bacteria associated with dental caries and gingival diseases [9]. Qahtani et al. [10, 11] revealed that only 29.4% of 11–12 year-old children with visual impairment exhibited good oral hygiene. Dental caries is a multifactorial and dynamic condition that is typically associated with bacterial proliferation in the oral cavity and excessive sugar consumption [1]. One significant consequence of VS is the difficulty in maintaining proper oral hygiene [1]. Diagnosing oral diseases in children with disabilities can be challenging, and treatment adherence may be suboptimal [1, 12–14] Additionally, lack of hand-eye coordination, inadequate parental supervision, and limited peer influence exacerbate the neglect of oral health [13]. As poor oral hygiene persists, dysbiosis can occur, leading to the demineralisation of teeth and the development of caries [13].

Additionally, disruptions in the oral microbiome may affect the functions of the central nervous system (CNS) [13]. Retinal ganglion cells process and transmit visual information from the eyes to the brain within the CNS [13]. The microbiota can regulate brain and CNS functions, suggesting that VS due to disease or medication may impair cognition and potentially affect oral health [13, 15]. Notably, there is significant evidence linking dental caries with systemic diseases, such as cardiovascular conditions and diabetes, suggesting a broader systemic impact of oral health issues [16, 17]. This suggests that changes in the oral microbiota, which are associated with dental caries (a widespread oral health concern), could reflect broader systemic health implications. Understanding these relationships might offer valuable insights into the interconnectedness of oral and systemic health, providing further support for the oral-gut-brain axis theory [18–20].

The human microbiome is a complex and dynamic ecosystem with the microbial community composition varying in response to various factors [21]. The oral microbiome plays a critical role in maintaining both oral and systemic health, and the advent of 16 S rRNA next-generation sequencing has significantly advanced our understanding of the complexity of bacterial communities [20]. Oral microorganisms have systemic implications and serve as indicators of numerous diseases [20]. Certain oral microbial species may increase the risk of cardiovascular diseases, diabetes, and other conditions [20]. Moreover, oral microbes contribute to disease prevention and treatment and maintain overall health. Oral microbiology research has also enhanced our understanding of human genetics and disease transmission [22].

In China, there has been no specific research on the oral microbiota of children with VS, making it an unexplored area. Additionally, there is limited knowledge regarding the oral microbial diversity and oral hygiene practices of residents with VS in Guangzhou, Guangdong Province. This study aimed to identify differential microbes by analysing the oral microbial diversity of students with VS and compare them with those with children having healthy sight (HS) in Guangzhou, China. These findings will contribute to the existing knowledge regarding the significance of dental caries and oral microbiota in children with VS. These results will also lay the groundwork for future research on oral gut-brain axis communication, potentially leading to more effective preventive and therapeutic strategies. By establishing baseline data, this study will contribute to developing oral health programmes for children with VS, ultimately aiming to promote sustained and consistent oral healthcare.

Methods

Sample selection

This study was approved by the Research Ethics Committee of the Guangzhou Centre for Disease Control and Prevention (Approval number: GZCDC2018035). Informed consent was obtained from each child's primary caregiver. Students aged 6–16 years were eligible to

participate in the study. The inclusion criteria were: (1) enrolment in the same blind school in Guangzhou, (2) identified as double-blind (defined as the inability to correct vision to normal levels), and (3) absence of any other systemic diseases. Exclusion criteria included: (1) systemic illnesses unrelated to ocular conditions, (2) mental disorders that could impede cooperation during the examination process, (3) recent use of antibiotics within the past three months, which could significantly alter the oral and gut microbiota composition, and (4) parental refusal to participate in the study [2, 23]. From September to December 2020, students from a blind school in Guangzhou were selected based on these criteria. The participants ages were determined according to the month of the survey conducted in December 2020. Birth dates corresponding to each age group were categorised; for example, six-year-olds were born between 1 December 2013 and 30 November 2014.

Dental caries examination

The oral examination followed the criteria and procedures outlined in the Fourth National Oral Health Epidemiological Survey in China [24]. Two trained paediatric dentists performed all examinations. To ensure inter-examiner reliability, standard consistency testing was performed on 15 randomly selected children from school before starting the examinations, with a Kappa value of 0.85. Additionally, 5% of the children from each checkpoint were re-examined to maintain consistency and accuracy, thereby avoiding examiner bias.

Disposable dental mirrors and ball-ended WHO Community Periodontal Index probes were used. The prevalence of dental caries was assessed using the Decayed, Missing, and Filled Teeth (DMFT) index for adults and children. According to the WHO Health Organization diagnostic criteria, caries are identified as smooth or soft areas or potential enamel damage [25]. Caries were not diagnosed based on the following conditions unless accompanied by additional positive signs: (1) only chalky or white spots, (2) no signs of softening when probed, (3) pigmentation in pits and fissures without underlying enamel damage, (4) moderate-to-severe fluorosis, and (5) wear-related damage.

Dental caries risk assessment by activity test

Caries activity was evaluated using a Cariostat-based kit (Gonaunt Medical, Hebei, China). To conduct the assessment, the buccal necks of the upper and posterior teeth as well as the lower labial necks of the assessed teeth were swabbed five times with a sterile sampling swab. The swab was placed in a tube containing the test medium and stirred five times. Within 4 h of sampling, the test tubes were incubated at 37 °C for 48 h. After the incubation period, the colour of the culture medium was compared to the standard colour chart provided by the manufacturer and scored on a scale of 0–3 [26]. Subsequently, the test tube was analysed using a specialised chromatograph (Gaunt Medical, Hebei, China) to interpret the results, which were classified as 0, 0.5, 1, 1.5, 2, 2.5, and 3. Based on these readings, caries risk was categorised as low (< 1), medium (1–1.5), or high (≥ 2).

Assessment of oral health knowledge

The assessment also included a section on oral hygiene practices and healthcare behaviours, which comprise: (1) the frequency of tooth brushing, categorised as twice daily, once daily, less than once daily, or never; (2) the level of independence in brushing, ranging from independent to completely dependent; (3) fluoride usage, Fluoride usage, with options of none, once yearly, or twice yearly; (4) sugar consumption habits, including sweet drinks, cakes, and candies, categorised as occurring at least twice daily, once daily, less than once daily, or never; and (5) healthcare behaviour, specifically the frequency of dental check-ups over the past year, categorised as once, twice, three times, or never.

Furthermore, the questionnaire assessed participants' knowledge and attitudes regarding oral healthcare (agree, disagree, or unknown). Key areas included: (1) the belief in the necessity of treating deciduous tooth caries; (2) perceptions of fluoride's effectiveness in dental protection; (3) opinions on the preventive benefits of pit and fissure sealants for dental caries in children; (4) beliefs regarding the innate quality of teeth and its correlation with oral hygiene practices; and (5) the importance of regular dental check-ups. These questions were adapted from the Fourth China National Oral Health Survey [4].

To ensure that children with VS could effectively participate in the survey, we provided schoolteachers with a thorough explanation of the intent and significance of the survey. Questionnaires were distributed uniformly by the school staff to the primary caregivers of the children and collected the following day. Each caregiver received clear instructions on how to complete the questionnaire, which was filled out and collected the following morning. After completion, the questionnaires were reviewed by the designated individual responsible for their administration. Any incomplete questionnaires were returned to the caregivers, and the collection process continued until all questionnaires were properly completed.

Saliva sampling and microbial composition analysis

Twenty children with VS were randomly selected for the 16 S rRNA test from the 94 children who participated in the questionnaire survey to form the experimental group. A control group of 20 typically developing, children with HS from grades 1 to 6, matched for sex and age with the experimental group, was also included. The dental caries

status of all participants was documented and oral examinations were conducted.

All 40 participants were instructed to abstain from eating, drinking, and any oral hygiene practice for at least 3 h before sample collection [27]. Approximately 1 mL of non-stimulated saliva was collected from each participant using a sterile disposable pipette. The saliva samples were then transferred to sterile, labeled centrifuge tubes, which were quickly placed in an insulated container with dry ice. The samples were stored in a cryogenic refrigerator at -80 °C for 2 h.

The collected saliva samples were subjected to a comparative analysis using high-throughput 16 S rRNA sequencing to study the composition of the oral microbiota and changes between the two groups. The saliva samples were sent to Shanghai Baimke Co., Ltd. for total DNA extraction, amplification, purification, and sequencing. High-purity DNA was extracted using a standard phenol-chloroform method. The 16 S rRNA gene regions were amplified using specific primers targeting the V3-V4 variable regions, with the forward primer sequence 341 F (5′-CCTACGGGNGGCWGCAG-3′) and the reverse primer sequence 805R (5′-GACTACHVGGGTATCTAATCC-3′), to capture diverse bacterial taxa. The amplified products were purified using AMPure XP beads to remove the primers and impurities. The Illumina NovaSeq 6000 platform was used to create PCR-free libraries, followed by paired-end sequencing of the purified DNA. Raw sequencing data were processed through quality control steps, including filtering low-quality sequences, trimming by length, merging paired-end sequences, and removing chimeric sequences. The high-quality sequences were then grouped into operational taxonomic units (OTUs) based on 97% similarity using USEARCH (version 10.0) with a 0.005% threshold for sequence assignment.

Species classification for each feature was performed using the SILVA database and a naïve Bayesian classifier. The community composition of each sample was evaluated at several taxonomic levels, including the phylum, class, order, family, genus, and species. The alpha and beta diversities of the samples were assessed using QIIME2 software. Following the initial analysis on the QIIME2 platform, the data were exported for further analysis, particularly linear discriminant analysis (LDA) effect size (LEfSe). LEfSe analysis identified significant differences in the microbial distribution between the groups and highlighted the differential abundance of specific microorganisms. Python was used to conduct co-occurrence analysis among the genera, focusing on the 80 most abundant genera in each group.

Table 1 Status of dental caries in students with VS (mean ± standard deviation)

Sex	dmft/DMFT	Caries prevalence rate (%)	Caries filling rate (%)
Male	5.75 ± 4.0	94.2	17.4
Female	5.8 ± 4.0	87.5	15.6

DMFT (Permanent teeth) Decayed, Missing, Filled Teeth; *dmft (Primary teeth)* decayed, missing, filled teeth

Statistical analysis

GraphPad Prism and SPSS 23.0 software were used for image generation and statistical data analysis, respectively. Proportions were specified, and count data were analysed using either the chi-square or Fisher's exact test. The relationship between caries activity risk and DMFT was investigated using the Kruskal–Wallis test. T-tests were conducted to compare two independent sample groups, assuming normal distribution, which was verified using the Shapiro-Wilk test. For data not meeting normality assumptions, the Mann–Whitney U test was utilized to assess differences. In microbial diversity analyses, alpha diversity indices such as Shannon and Simpson were computed to gauge within-sample diversity. For between-sample comparisons, beta diversity was examined using Bray-Curtis and UniFrac distance metrics. Visualization of microbial community differences employed Principal Coordinates Analysis (PCoA) and Non-Metric Multidimensional Scaling (NMDS) to capture the complexity of community structures. Differential abundance of taxa between groups was assessed using DESeq2 or ANCOM, depending on the dataset characteristics and distribution assumptions. The significance threshold was set at $P < 0.05$, with adjustments for multiple comparisons applied where necessary.

Results

High prevalence of dental caries in children with VS

This study included 101 children with VS, consisting of 69 boys and 32 girls aged 6–16 years, with an average age of 10.13 ± 2.09 years. The overall prevalence of dental caries in these children was 92.1%. The proportion of fillings, primary tooth caries, and permanent tooth caries were 16.8, 60.4, and 76.24%, respectively. The prevalence and severity of dental caries among participants are presented in Table 1. Fisher's exact probability analysis indicated no significant difference between the caries and caries-filling rates between boys and girls ($P > 0.05$).

Caries activity score was associated with the severity of caries

A total of 101 samples were collected for caries activity testing. In this study, both boys and girls had a caries activity score of 1.5. The Mann–Whitney U test did not reveal any statistically significant difference in caries activity grades between boys and girls (Z = -0.73, $P > 0.05$, Table 2). The VS children were categorised into three

groups based on the prevalence of dental caries: low (3.16 ± 1.7), mid- (4.68 ± 3.51), and high (8.1 ± 3.97) caries. The Kruskal–Wallis test showed a statistically significant positive correlation between the severity of caries and caries activity (Z = 18.73, $P < 0.05$, Table 3).

Children with VS showed poor oral hygiene habits

A total of 101 questionnaires were collected, of which 94 were valid, yielding an effectiveness rate of 93.07%. Among the 94 children, 52.1% (49/94) brushed their teeth daily and 84% (79/94) brushed independently. Additionally, 13.8% (13/94) of the participants began brushing after the age of two years. Children who brushed their teeth at least twice a day constituted 52.1% (49/94) and 61.7% (58/94) brushed both in the morning and evening. Furthermore, 39.4% (37/94) used fluoride-containing toothpaste, Out of the total sample size of 94 individuals, 45.7% (43/94) reported consuming sugary food before bedtime. Bedtime consumption revealed a statistically significant difference between sexes ($P < 0.05$) (Table 4).

The caries activity was further analysed in relation to the influencing factors, as outlined in Table 4. Children who brushed independently had higher caries activity scores than those who needed assistance, although this difference was not statistically significant ($P > 0.05$). Additionally, no statistically significant differences in oral hygiene practices were observed between the three caries activity groups ($P > 0.05$).

Table 2 Results of caries activity in children with visual impairment [N (%)]

Sex	Caries activity test score		
	< 1	1–1.5	≥ 2
Male	3 (4.3%)	44 (63.8%)	22 (31.9%)
Female	3 (9.4%)	15 (46.9%)	14 (43.8%)

Low-caries, Caries activity test score < 1; *Medium-caries*, Caries activity test score 1–1.5; *High-caries*, Caries activity test score ≥ 2

Table 3 Relationship between caries risk and severity

Caries risk	*N*	dmft/DMFT (Mean ± standard deviation)
Low-caries	6	3.16 ± 1.77
Mid-caries	59	4.68 ± 3.51
High-caries	36	8.1 ± 4.0

Low-caries, Caries activity test score < 1; *Medium-caries*, Caries activity test score 1 – 1.5; *High-caries* Caries activity test score ≥ 2

Sample characteristics and sequencing data

A total of 20 saliva samples were collected from children with HS, consisting of nine girls and 11 boys, aged 7–12 years. The mean dmft/DMFT score for five children without caries, 11 with moderate caries and four with high caries was 3.15. Similarly, saliva samples were collected from 20 children with VS, consisting of three girls and

Table 4 The oral hygiene habits of children with visual impairment ($n = 94$)

	Male	Female	Total	*P* value	The number of individuals			Total	H cost	*P* value
					Low caries	Mid caries	High caries			
Does the child brush their teeth?				0.48					0.22	0.63
Brush teeth	63	29	92		6	53	33	92		
Brush infrequently or never brush	2	0	2		0	2	0	2		
Degree of independence in brushing				0.25					0	1
Independent	54	25	79		5	46	28	79		
Partially dependent	11	3	14		1	8	5	14		
Completely dependent	0	1	1		0	1	0	1		
Frequency of tooth brushing				0.57					0.31	0.58
Twice daily	31	18	49		4	29	16	49		
Once daily	31	11	42		2	24	16	42		
Not every day	1	0	1		0	0	1	1		
When does your child brush his teeth?				0.23					1.23	0.27
Typically, once in the morning or before going to bed at night	27	9	36		1	22	13	36		
Every day in the morning and evening	38	20	58		5	33	20	58		
Should you use fluoride toothpaste when you brush your teeth?				0.06					2.09	0.15
Yes	21	16	37		3	21	13	37		
Deny	10	4	14		2	9	3	14		
No idea	31	7	38		0	22	16	38		
Sugary food intake before bedtime				< 0.01					0.22	0.64
Often	1	0	1		0	0	1	1		
Every now and then	35	7	42		3	22	17	42		
Never	29	22	51		3	33	15	51		

17 boys, aged 7–12 years. Of these children, two had no caries, eight had moderate caries, and 10 had high caries, resulting in a mean dmft/DMFT score of 6.75. High-throughput sequencing of 16 S rRNA from the 40 saliva samples yielded 3,199,305 raw sequences and 3,189,730 high-quality sequences (Fig. 1A). OTU clustering of these high-quality sequences with 97% similarity produced 10,133 OTUs, with 5,119 in the HS group and 5,014 in the VS group. The rarefaction curves for both groups plateaued once they reached a certain range (Fig. 1B), indicating that the sample size was sufficient to reflect the diversity of the microbial community and for further data analysis. Figure 1C illustrates that the HS group exhibited greater microbial diversity and species uniformity than the VS group.

Comparison of saliva microbiota from children with VS and HS

Regarding the abundance-based coverage estimator index (Fig. 2A) and Chao1 index (Fig. 2B), the number of species in the VS group was higher than that in the HS group ($P > 0.05$), indicating a consistent baseline microbial abundance between the groups. The Shannon (Fig. 2C) and Simpson indices (Fig. 2D) revealed no significant differences in microbial species diversity among the groups.

To assess the differences in beta diversity between samples, analysis of similarities (ANOSIM; Fig. 3A) revealed no significant differences between or within groups. The data points of both the HS and VS groups were concentrated. NMDS (Non-metric Multidimensional

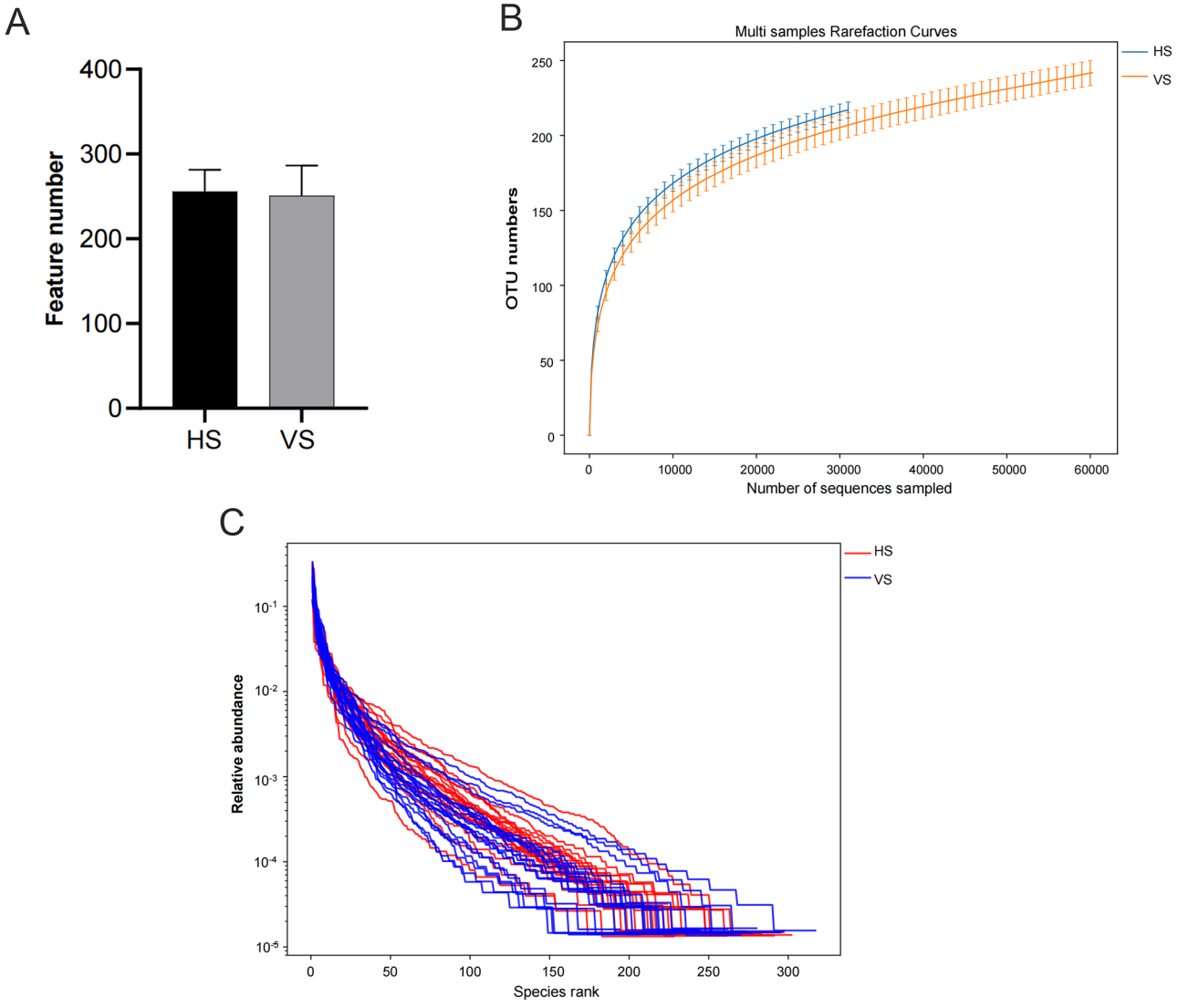

Fig. 1 Sample characteristics and sequencing data. **A**) Distinction of OTUs number in each group; **B**) The rarefaction curves plateaued after rising in a certain range, indicating that the sequencing volume of samples was sufficient for the following data analysis to reflect the diversity of the microbial community; **C**) The richness and uniformity of microbial species in the HS group was greater than those in the VS group. *HS* healthy vision; *VS* visual impairment

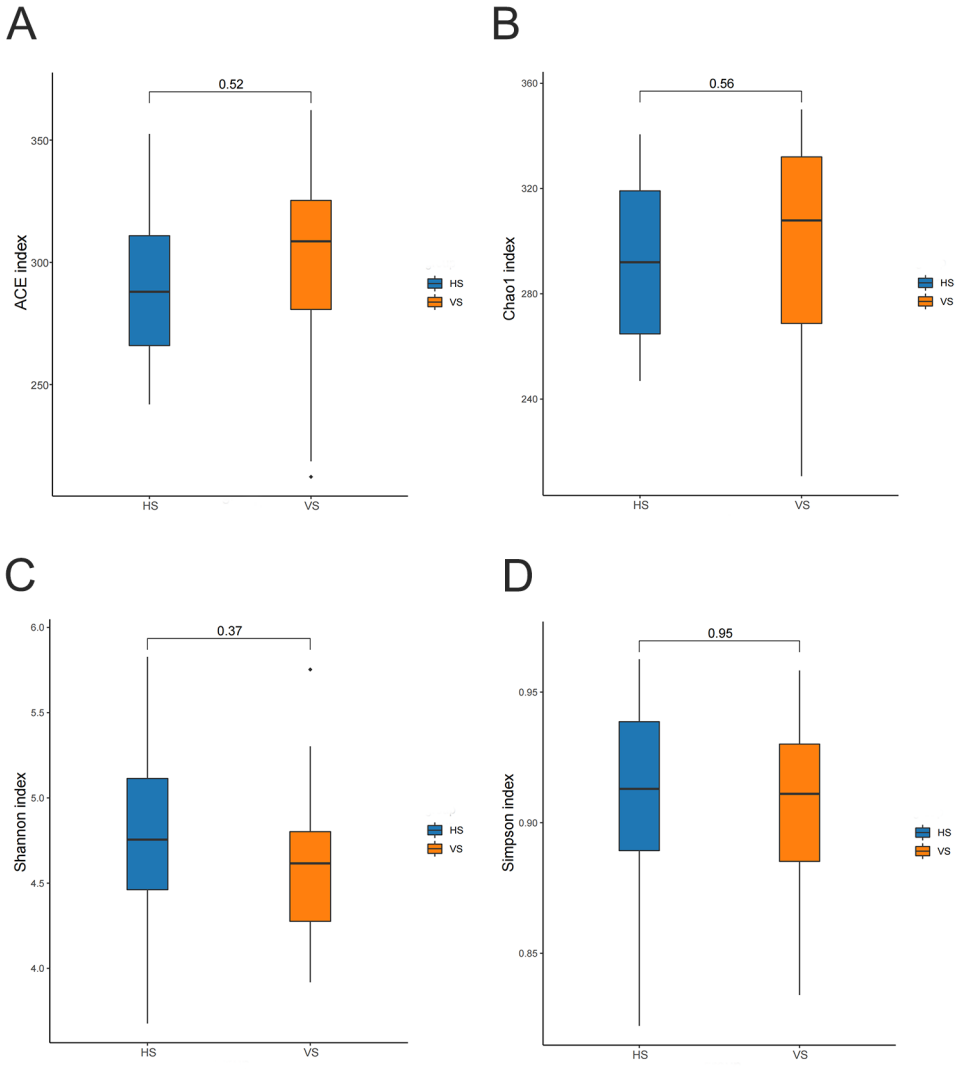

Fig. 2 The alpha diversity of the saliva sample microbiota. **A** ACE index; **B** Chao1 index; **C** Shannon index; **D** Simpson index. HS healthy vision; VS visual impairment; $^{*}P<0.05$, $^{**}P<0.01$

Scaling) analysis further revealed no significant differences between the saliva samples from the HS and VS groups (Fig. 3B). The Unweighted Pair Group Method with Arithmetic Mean (UPGMA) analysis (Fig. 3C) demonstrated that the microbial community composition was similar between the HS and VS groups. The concentrations of the data points between the two groups indicated no statistically significant differences in the composition of the salivary microbial communities ($P>0.05$).

No significant differences in the distribution of predominant microorganisms between the two groups

The salivary microbial communities comprised 17 phyla, 26 classes, 48 orders, 80 families, 143 genera, and 183 species (Table 5). At the phylum level (Fig. 4A and D), the top 10 dominant phyla in terms of relative abundance were *Bacillota* (45.4%), *Actinobacteria* (21.6%), *Bacteroidota* (11.5%), *Pseudomonadota* (10.1%), *Fusobacteriota*(5.6%), *Patescibacteria* (3.9%), *e-proteobacteria* (0.6%), *Proteobacteria* (0.17%), *Verrucomicrobia* (0.04%), and *Mycoplasmatota* (0.04%). Although the VS group had a lower abundance of *Bacillota* (37.8%), the difference was not statistically significant ($P=0.27>0.05$). The VS group also exhibited a higher abundance of *Actinomycetota*, *Bacteroidota*, *Pseudomonadota* and *Bacillota*, although these differences were not statistically significant ($P>0.05$).

At the class level (Fig. 4B and E), we identified 26 classes within the salivary microbial communities in both groups. The top ten dominant classes in relative abundance were *Bacilli* (32%), *Actinomycetota* (20.7%), *Bacteroidua* (11.5%), *Gammaproteobacteria* (10.7%), *Negativicutes* (8.1%), *Fusobacteria* (5.4%), *Clostridia* (4.5%), *Saccharimonadia* (3.2%), *Coriobacteriia* (0.89%),

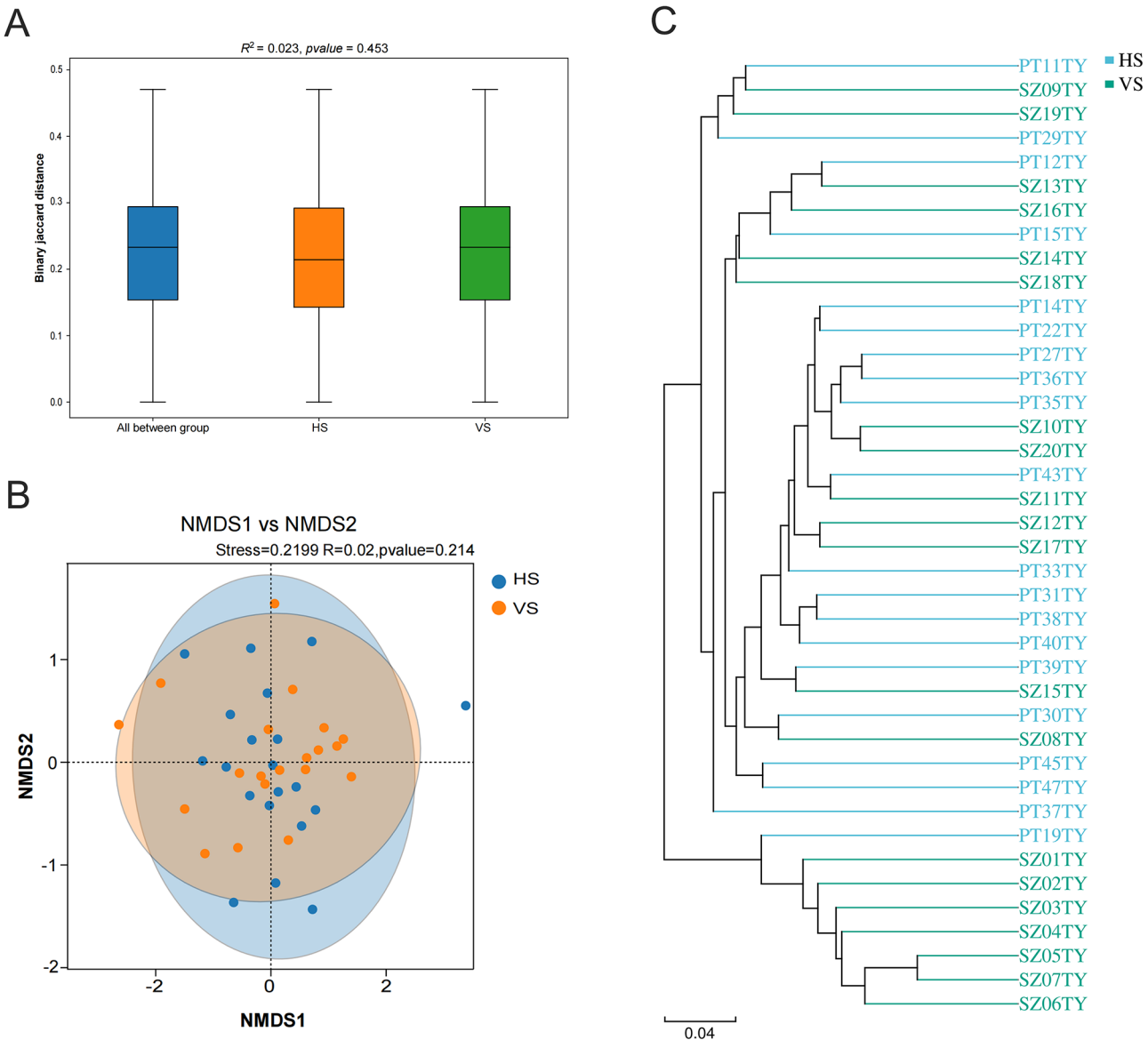

Fig. 3 Comparison of beta diversity among saliva sample groups. **A**) The ANOSIM analysis of saliva samples. The ordinate represents the beta distance. **B**) Plot of NMDS analysis of saliva samples. Each point represents a different sample. When the stress is less than 0.2, the NMDS analysis has a certain reliability, and the closer the samples are on the coordinate map, the higher the species community similarity. **C**) The UPGMA cluster tree of saliva samples. Different colours represent different groups. The closer the samples in the sample hierarchy cluster tree, the shorter the branch length, and the more similar the species composition of the two Samples. HS healthy vision; VS visual impairment. ANOSIM, analysis of similarities; UPGMA, Unweighted Pair Group Method with Arithmetic Mean

Table 5 Number of annotations in each of the two taxonomic groups

Groups	Phylum	Class	Order	Family	Genus	Species
HS	16	24	42	73	132	173
VS	17	26	48	80	143	183

HS healthy vision; *VS* visual impairment

and *Erysipelotrichia* (0.59%). In the VS group, the abundance of *Bacilli* and *Negativicutes* was lower, whereas that of *Actinomycetota*, *Bacteroidia*, and *Gammaproteo* bacteria was higher. However, these differences were not statistically significant ($P > 0.05$).

At the genus level (Fig. 4C and F), 143 genera were identified in the salivary microbial communities of both groups. The 10 most abundant genera were *Bacillota* (22.93%), *Rothia* (15.56%), *Neisseria* (6.27%), *Veillonella* (6.21%), *Prevotella_7* (3.57%), *Actinomyces*

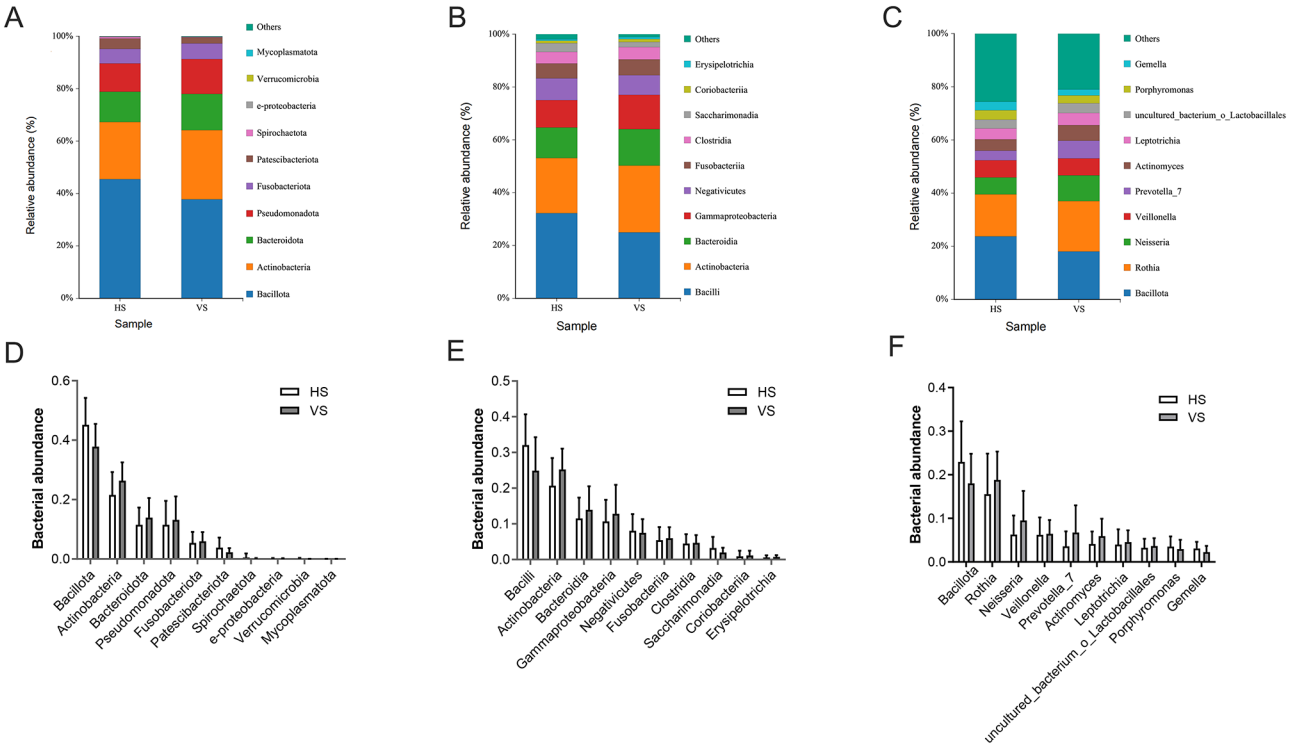

Fig. 4 Predominant microbe in the saliva sample at the phylum, order and genus level

(4.13%), *Leptotrichia* (3.99%), *Porphyromonas* (3.52%), and *Gemella* (3.07%). In the VS group, the abundances of *Bacillota*, *Porphyromonas* and *Gemella* were lower, whereas the abundances of *Rothia*, *Neisseria*, *Veillonella*, and *Prevotella_7* were higher, although these differences were not statistically significant ($P > 0.05$).

Comparative analysis of the effects of dental caries on oral microbiota composition

To exclude the effect of caries-free children, we compared the oral microbiota differences between caries-affected children with VS (VSC, $N = 15$) and healthy children with caries (HSC, $N = 18$). At the genus level (Fig. 5A and B), *Rothia*, *Neisseria*, *Prevotella_7*, *Haemophilus*, *Fusobacterium*, and *Actinomyces* were significantly more abundant in VSC than in HSC. This analysis highlighted the distinct microbiota profile in caries-affected children with VS compared to that of their healthy counterparts.

We then grouped the 18 children with VS into a severe caries group (VSCS, $N = 10$) and a mild-moderate caries group (VSCM, $N = 8$) to analyse microbiota differences based on caries severity within the population with VS. Individuals in VSCS group had higher levels of *Rothia*, *Streptococcus*, *Prevotella_7*, *Actinomyces*, and *Gemella*. In contrast, children in the VSCM group had a higher abundance of *Porphyromonas* and *Fusobacteriia* (Fig. 5C and D). This comparison underscores the variations in oral microbiota composition linked to the severity of caries among children with VS.

Finally, we selected eight children with VS (VSMC) and 11 with HS (HSMC) with comparable levels of caries severity to further isolate the impact of VS on oral microbiota. At the genus level (Fig. 5E and F), the HSMC group had a higher presence of microbiota, such as *Streptococcus* and *Gemella*, whereas those with moderate caries (VSMC) had a higher presence of microbiota such as *Rothia* and *Neisseria*. This analysis demonstrated that despite similar severity of caries, the microbiota composition varied between children with VS and HS, implying a potential influence of VS on the oral microbiome. Collectively, these horizontal comparisons robustly illustrated the unique microbiota compositions attributable to caries severity and VS, thereby providing valuable insights into the interplay between these factors.

Core biomarkers of saliva microbiota among groups

LEfSe analysis revealed differences in the core microbial distribution between the groups, by observing species with significant differences between groups and choosing the microorganisms whose abundance significantly differed between the groups. As illustrated in Fig. 6A and B, through comparative analysis of the microorganisms with significant differences and LDA scores above 4.0, based on LEfSe analysis, the core biomarker in the VS group was *Actinomycetota*. The core biomarkers in the

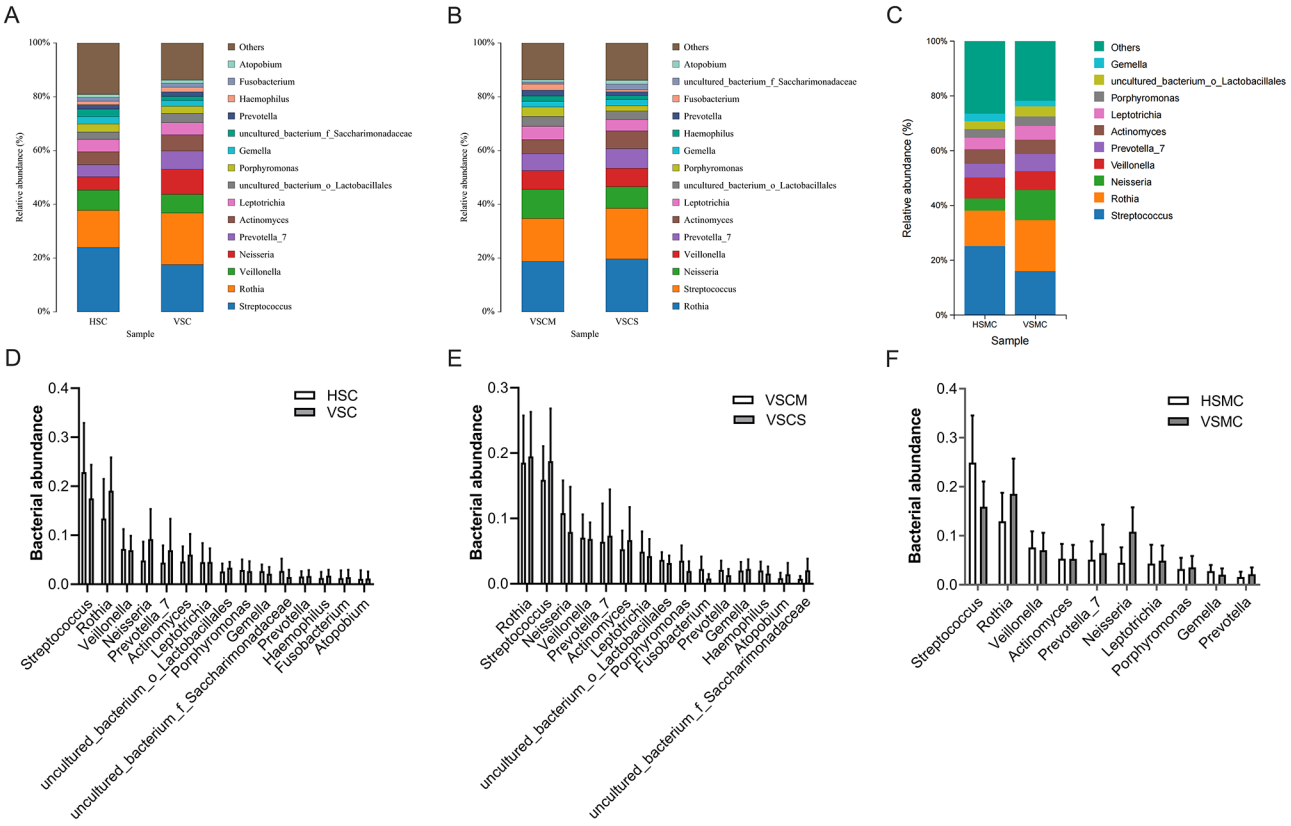

Fig. 5 The distribution of microbial genus levels within saliva sample. (**A**, **D**) HSC and VSC group; (**B**, **E**) VSCM and VSCS group; (**C**, **F**) HSMC and VSMC group. HSC children with HS with caries; VSC children with VS with caries; VSCM children with VS with mild to moderate caries; VSCS children with VS with severe caries; HSMC children with HS with moderate caries; VSMC children with VS with moderate caries

HS group were *Bacillota*, *Bacilli*, *Lactobacillales*, *Streptococcaceae*, and *Streptococcus*. In Fig. 6C and D, the core microbial biomarkers in the VSMC group were *Proteobacteria*, *Burkholderiales*, *Neisseriaceae*, and *Neisseria*, whereas the core microbial markers of the HSMC group were *Saccharimonadia*, *Patescibacteria*, *Lactobacillales*, *Streptococcaceae*, *Bacilli*, and *Bacillota*.

Predominant bacteria at the phylum, class or family level

The 15–16 predominant bacteria at the phylum, class or family level. The 16 phyla with the highest relative abundances demonstrated complex interactions within each group (Fig. 7). Distinct bacterial correlations were observed across the different groups, with the HS group exhibiting seven negative correlations and the VS group showing six negative correlations. In the VS group, *Actinomycetota* was positively correlated with *Bacillota* and *Spirochaetes* and negatively correlated with *Fusobacteria* and *Tenericutes*. In contrast, *Actinomycetota* in the HS group revealed a negative correlation. Overall, the correlation networks in the VS group were simpler than those in the HS group.

Discussion

Dental caries are commonly acknowledged as a prevalent chronic condition in children [28]. Our study results revealed that children with VS exhibited a high incidence of dental caries. China's Fourth National Oral Health Epidemiological survey and other related studies reported that the prevalence of dental caries and the average number of cavities among students aged 6–12 years were significantly higher (95.4% and 6.14 ± 3.35, respectively) compared to the values reported for a control group of the same age [29–32]. The DMFT results of children with VS in this study were higher than in other studies, such as in Sudan (Khartoum State: 0.4 ± 0.7), India (New Delhi: 2.08 ± 1.86; Bengaluru: 1.32 ± 1.36), Iran (Tehran: 0.81 ± 1.15), and Saudi Arabia (Riyadh: 2.13 ± 2.63) [32–34], while it was similar to the study conducted in Northeast China (Shenyang: 2.57 ± 2.83) [35].

The caries activity test is more effective than the standard oral examination because it identifies the need for preventive treatment before carious lesions appear [36]. However, caries risk assessments have not been used in children with VS. We observed a positive correlation between the number of carious teeth and the caries activity. Similarly, Xuan et al. reported a substantial

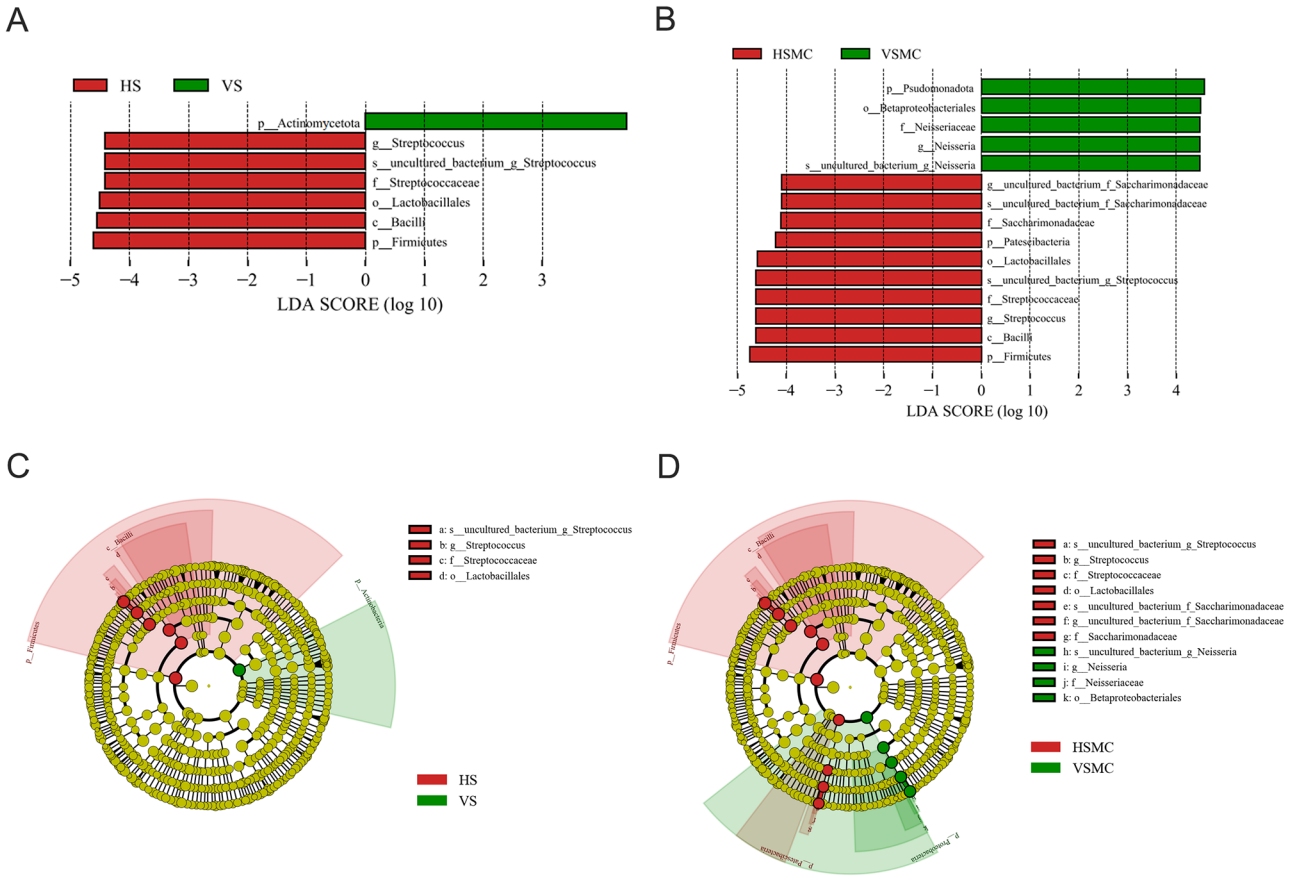

Fig. 6 The comparative analysis for the microorganisms with significant differences and with linear discriminant analysis (LDA) scores above 4.0 based on LEfSe analysis. (**A**, **C**) HS and VS group; (**B**, **D**) HSMC and VSMC group. HS children with healthy sight; VS children with visual impairments; HSMC children with HS with moderate caries; VSMC children with moderate caries

correlation between caries activity test values and early childhood caries incidence [35]. Children with VS, who are at high risk for caries, should undergo regular caries activity testing and receive personalised caries control programs to improve their oral health management. Maintaining oral hygiene remains a considerable challenge for students with VS [35]. Our findings revealed a strong correlation between bedtime eating habits and the incidence of dental caries in these students, suggesting that the poor oral hygiene considerably increases the risk of caries [37]. Sugars are fermented by oral bacteria to produce acids that demineralise tooth enamel and promote caries formation. Owing to their visual limitations, these students may find it difficult to maintain effective oral hygiene, leading to inadequate cleaning after consuming sugary foods, which further exacerbates the development of caries. Therefore, developing personalised oral health programs for students with VS, emphasising the reduction in sweet food consumption before bedtime and improving oral hygiene education is crucial. These measures will enhance dental health, and improve their quality of life.

Caries are complex infectious diseases arising from a combination of factors. Its occurrence is primarily influenced by the disruption in the microecological balance caused by microorganisms that produce and are resistant to acidic conditions [34]. Existing literature indicates a substantial prevalence and severity of dental caries among individuals with VS. However, there is a notable lack of research on the composition of salivary microorganisms, especially in children with VS. Previous studies have identified that key microorganisms, such as *Rothia*, *Neisseria*, *Prevotella_7*, and *Fusobacterium* are strongly associated with dental caries in children [38]. The abundance of *Rothia*, *Prevotella_7*, and *Actinomyces* increased from healthy schoolchildren (HSC) to visually impaired schoolchildren (VSC), and further increased in the mixed dentition stage (VSCM) compared with those at the school-age stage (VSCS). This finding suggests their role in caries development and their potential as predictive markers in children with VS. Notably, owing to its high activity in producing acid from carbohydrates, *Prevotella* may serve as a key microbial predictor of caries, possibly even more than *Streptococcus mutans* [39]. Individuals

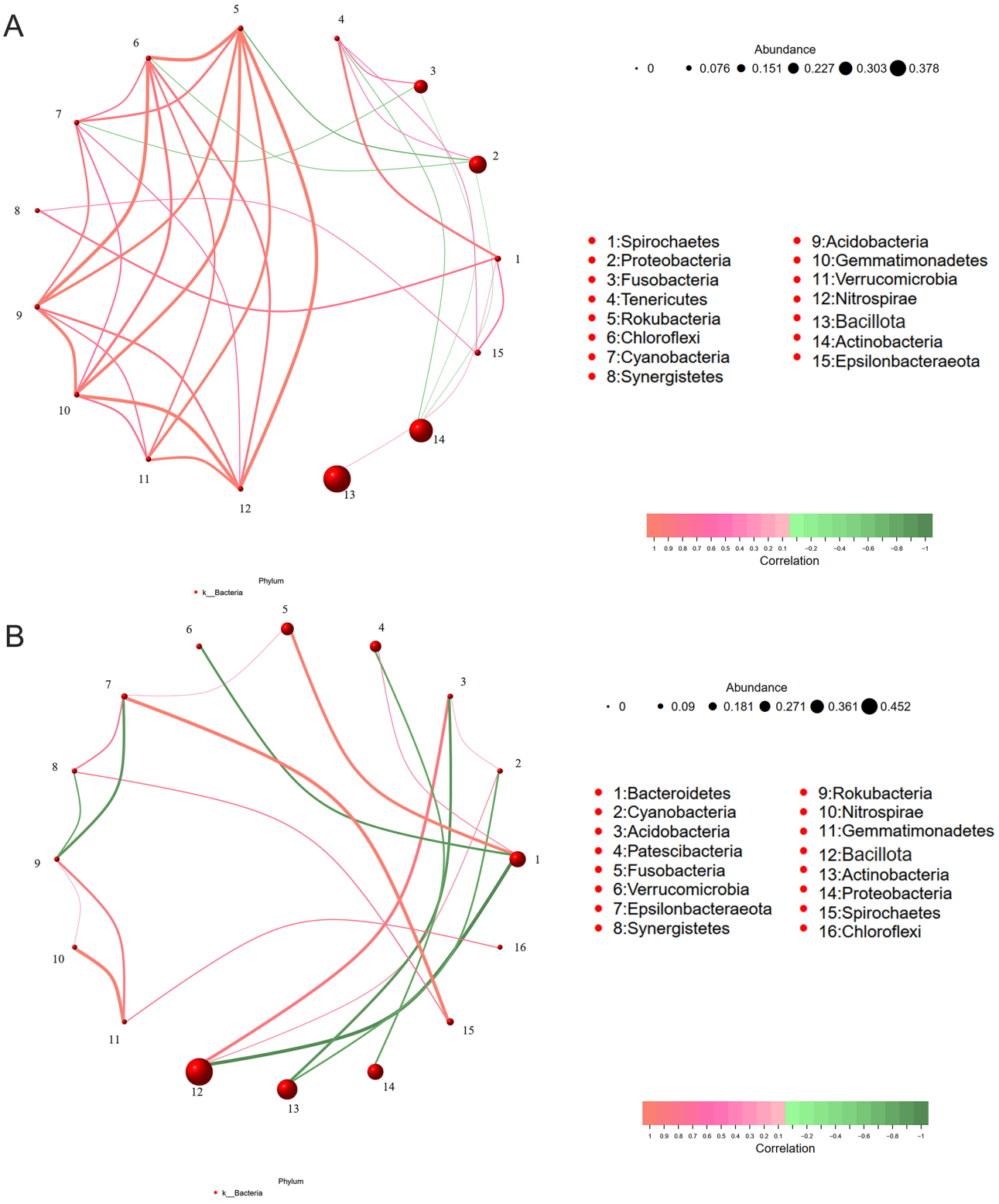

Fig. 7 Network analysis showing the interactions among predominant bacteria at the phylum, class and family level. (|SpearmanCoef| > 0.1 and $P < 0.05$). Bacterial interactions of the two different groups (the 15–16 richest bacteria). The size of the node is proportional to the bacterial abundance. Node color corresponds to bacterial taxonomic classification. Edge color represents positive (red) and negative (green) correlations. (**A**, **B**) HS and VS group

with severe caries have lower levels of *Porphyromonas* and *Fusobacteria*. *Porphyromonas gingivalis*, a key pathogen in periodontal disease, is predominant in individuals without dental caries and effectively co-aggregates with other oral health-related bacteria. *P. gingivalis* is primarily associated with chronic periodontitis, and it can destroy periodontal tissue by secreting proteases and other virulence factors. This bacterium produces lipopolysaccharides that can inhibit certain cytokines involved in insulin regulation, potentially leading to insulin resistance [40]. Similarly, *Fusobacterium nucleatum* is associated with periodontal diseases and systemic conditions, such as colorectal cancer. Therefore, the primary genera associated with caries in children with VS may include *Rothia*, *Neisseria*, *Leptotrichia*, *Actinomyces*, and *Prevotella_7*. Additionally, the presence of *Porphyromonas* and *Fusobacteria* in the oral microbiome may suggest poor periodontal status in these children.

The 16 S rRNA high-throughput sequencing results revealed no significant shifts in the dominant microbial communities. However, alpha diversity analysis indicated that children with VS exhibited an increased variety of bacterial species, but a reduced overall quantity, as reflected by their lower Shannon index compared to those with HS. This suggests a potentially lower microbial diversity in the VS group, possibly because of environmental factors. LEfSe analysis of saliva identified *Actinomycetota* as the dominant microorganism in the VS group. These Gram-positive bacteria, including *Actinomyces*, are significantly linked to childhood caries [41] and crucial for preventing biofilm formation and maintaining microbial stability. Reduced *Actinomycetota* populations may disrupt intestinal stability and allow toxins to affect the brain through the neuroimmune-endocrine pathway [42, 43]. The role of *Actinomyces* in dental caries is well-documented [44–46], and *Actinomyces* can enter oral tissues through dental procedures [47, 48]. Furthermore, *Actinomyces* species have been implicated in ocular infections, such as endophthalmitis and keratitis [49], suggesting a connection between oral *Actinomycetota* and VS. Future research is warranted to further explore this relationship.

In this study, we investigated the effects of VS on oral health by comparing the oral microbiota of children with VS and HS in Guangzhou, China. Despite similar caries severity, we identified significant differences in the microbiota composition, suggesting that VS influences the oral microbial environment. Specifically, the abundance of *Streptococcus* and *Bacillus* was higher in children with HS, whereas *Rothia* and *Neisseria* were more prevalent in children with moderate caries. This suggests that VS may indirectly alter the microbiota distribution through changes in oral hygiene practices, such as challenges in brushing and flossing. These findings underscore the need for personalised caries management, particularly in children with specific needs. Future research should explore how VS affects the oral microbiota composition and its implications for dental caries and other oral diseases. In conclusion, our study offers new insights into the effects of VS on the oral ecosystem and highlights the potential for personalised interventions based on microbiota analyses. This approach could improve the prevention and management of oral diseases and ultimately enhance the quality of life of this population.

A limitation of the current study is the inability to obtain radiographs for the detection of dental caries, primarily because of the constraints imposed by fieldwork settings and potential risks associated with radiation exposure. Furthermore, there is a lack of research on the classification of VS. Nevertheless, it is possible to augment the sample size in subsequent studies and conduct subgroup analyses according to various factors, such as caries risk levels, dentition, sex, and other relevant variables. Hence, it may be necessary to obtain supplementary samples to ascertain the comprehensive scope of microbial diversity in saliva. Caries has an impact on the microbiota at specific lesion sites and on healthy teeth, suggesting that the overall oral microbiome may undergo substantial alterations [50–53]. A significant portion of this diversity remains unaccounted for, despite the fact that various factors, such as diet, environment, host genetics, and early microbial exposure have been suggested as potential explanations. Consequently, it is imperative to conduct dynamic research that spans multiple time points.

Another limitations of our study is the relatively small sample size, consisting of 20 visually impaired children and 20 sighted children. While this exploratory analysis provides valuable preliminary insights into the microbiota profiles of visually impaired individuals, the sample size limits the generalizability and statistical power of our findings [54, 55]. Small samples can lead to variability and may not fully represent the microbiota diversity of the broader population. Consequently, this study serves as a preliminary exploration, highlighting the need for larger sample sizes in future research to verify and expand on these initial observations [56]. Expanding the study sample would strengthen statistical analyses and allow for a more detailed examination of how visual impairment affects the microbiota. This would also enable exploration of other influencing factors, providing a more comprehensive understanding. Overall, while this study offers valuable preliminary insights, future research with larger cohorts is essential for confirming these findings and enhancing their applicability [57].

Conclusion

This study highlights the high prevalence of dental caries among schoolchildren with VS in Guangzhou, underscoring the urgent need for targeted oral health interventions. Although there were no significant differences in the overall salivary microbiota composition between children with VS sand HS, notable changes in microbial abundance cannot be neglected. The association of *Actinomycetota spp*. with caries in children with VS suggests their potential use as diagnostic markers. There are complex interactions between oral health, VS, and microbial balance. Future research should focus on longitudinal studies to monitor changes in oral microbiota and their relationship with the oral health of visually impaired individuals.

Recommendations

Therefore, relevant oral health promotion and treatment programs should be established at the earliest. Oral health authorities must pay more attention to establishing school-based dental-care programs for children with disabilities that are comparable to those in elementary schools.

Abbreviations

VS	Visual impairment
HS	Healthy vision
SM	Saliva sample microbiota
OTUs	Operational taxonomic units
rRNA	ribosomal RNA

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Author contributions

Qiong feng. Wengyan Huang and Xuedan Zhao worte the main manuscript text.Ting Sheng and Bo Peng: Formal analysis, Investigation, Writing – original draft.Lijing Wang: Formal analysis, Investigation, Methodology, Writing – original draft. Lihong Ge: Formal analysis, Investigation, Methodology, Writing – original draft. Qianzhou Jiang: Formal analysis, Investigation, Methodology, Writing – original draft. Janak Lal Pathak: Formal analysis, Investigation, Writing – original draft.Rong lin and Weijia Liu: Conceptualization, Supervision, Writing – original draft. Sujuan Zeng: Conceptualization, Investigation, Project administration, Super – vision, Writing – original draft.

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Data availability

Sequence data that support the findings of this study have been deposited in the National Library of Medicine with the primary accession code PRJNA1131183.

Declarations

Ethics approval and consent to participate

This study was conducted in strict accordance with the ethical principles outlined in the Declaration of Helsinki. Detailed information regarding the study was provided to all participants prior to their involvement, and informed consent was obtained in writing. The study protocol, including all procedures and methodologies, was thoroughly reviewed and received approval from the Research Ethics Committee of the Guangzhou Centre for Disease Control and Prevention. The approval number for this study is GZCDC2018035. We are committed to upholding the highest standards of ethics in research and ensuring the welfare and rights of participants.

Consent for publication

Not applicable.

Competing interests

The authors declare no competing interests.

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