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ORIGINAL RESEARCH

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Temporal variation in the oral microbiome and the prediction of early childhood caries in different ethnicities

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Abstract

Globally, early childhood caries (ECC) is a significant public health concern, necessitating effective prediction and prevention strategies. This study aimed to explore variations in the oral microbiome of saliva from pre-school Han and Uyghur children during ECC development and establish a predictive model based on temporal oral microbiome changes. Saliva samples were collected from a single kindergarten every three months over six months. Forty-four pre-school children provided 132 samples, categorized into six groups: (1) HEF (healthy pre-school Han children), (2) HEO (Han children with caries), (3) HEP (Han children with progressive caries), (4) WEF (healthy pre-school Uyghur children), (5) WEO (Uyghur children with caries), and (6) WEP (Uyghur children with progressive caries). Illumina Miseq sequencing identified oral microbiome differences between groups and time points. The Random Forest (RF) algorithm established ECC prediction models. The T1HEO group exhibited significantly higher Chaol index, observed species index, PD whole tree index, and Shannon index than the T2HEO group ($p < 0.01$). Similarly, the T1WEO group had significantly higher Chaol index, observed species index, and PD whole tree index than the T2WEO group ($p < 0.05$). The AUROC value for the ECC prediction model based on temporal oral flora changes was 0.517 (95% CI: 0.275–0.759) for pre-school Han children and 0.896 (95% CI: 0.78–1.00) for pre-school Uyghur children. In the onset of caries in pre-school Han children, bacterial species richness and community diversity in saliva declined, paralleled by a decrease in bacterial species richness in pre-school Uyghur children's oral saliva. The ECC prediction model grounded on temporal oral microflora changes exhibited robust predictive power, particularly for pre-school Uyghur children, potentially leading to more effective ECC prevention measures.

Keywords

Early childhood caries; Salivary microbiome; Sequencing analysis; Prediction model

1. Introduction

Early childhood caries (ECC) is a complex and multifactorial disease that exerts significant impact on the health of children. ECC is defined as the presence of one or more decayed, missing, or filled teeth in the primary ("milk") teeth of children younger than 72 months-of-age [1, 2]. Although stomatology has advanced significantly over recent years, ECC remains one of the most prevalent diseases affecting children's health, especially in impoverished, remote, socially disadvantaged, and minority groups [3, 4]. The latest survey revealed that almost 573 million children were estimated to have untreated dental caries in their primary teeth [5]. Moreover, ECC affects almost half of preschool children globally [3]. In young children, caries can lead to mouth pain, dental abscesses, eating difficulties, weight loss, and low body mass index (BMI) when compared to children without caries [6–9]. In addition, the early extraction of primary teeth due to ECC can result in malocclusions [10, 11]. Children with ECC are also more likely to develop caries in their permanent teeth [12].

The causes of ECC are multifaceted and involve many different factors. Several elements have been identified that contribute to the onset and progression of ECC, including susceptible host teeth, microorganisms, diet and time [13–15]. It is widely understood that the bacteria that are present in dental biofilms play a crucial role in initiating and advancing caries [16]. The group of bacteria most commonly associated with ECC is *Mutans streptococci* (MS) [17, 18]. Furthermore, environmental factors can significantly influence the progression of ECC, including a low socio-economic status, limited literacy, inadequate oral hygiene, and the lack of fluoride use [19]. Ethnicity can also be an influential environmental factor in the onset and progression of ECC. While many studies

have focused on the oral microbiome in adults from various ethnicities, few have centered on the oral microbiomes of children from different ethnic backgrounds [20].

The human mouth is home to a vast array of microbes, including bacteria, fungi, viruses and bacteriophages. These microbes are also acknowledged as a significant microbiological reservoir in the human body [21]. Approximately 700 species make up the bacterial component of the oral microbiome, and each individual typically hosts between 50–200 species [22]. The structure and diversity of the microbiome in the human oral cavity increase during the first few years after birth, continually evolving with the development of teeth until stabilization [23]. Although there are compositional differences among samples from various sites within the same oral cavity, a common microbe in healthy individuals has already been identified [24]. Previous research indicated that the onset of oral diseases is not attributed to a single bacteria type, such as MS leading to caries, but rather, the collective influence of multiple microbes [25].

Nowadays, predicting the status of human health based on the analysis of microbiota has become increasingly important in human microbiome projects worldwide. Furthermore, an increasing number of researchers concur that competitive interactions dominate oral ecology [26]. In this context, next generation sequencing technology (NGS) has been used to study the oral microbial community. Saliva, representative of the oral cavity, carries the complex genetic information of individuals and oral microbes [27, 28]. and can also act as a diagnostic tool, thus providing a non-invasive, straightforward, and cost-effective method for disease detection and screening [29, 30].

Han and Uyghur are the two most predominant ethnic groups spread throughout Xinjiang. The Uyghur population has distinct diets, living environments, and genetic characteristics, resulting in a different prevalence of ECC when compared to other ethnicities [31]. Previous research indicated that the Han population exhibited a higher prevalence of myopia and high myopia than the Uyghur population [32]. Significant disparities were also noted between the Han and Uyghur populations in the detection rate of peptic ulcers (PUs), the population affected by *Helicobacter pylori* (Hp), morbidity season, age, complications and the proportion of complex ulcers [33]. In addition, the incidence of several diseases, including cholecystolithiasis, diabetes and hepatitis C, is known to differ between these groups [34–36]. Variations have also been identified between the Han and Uyghur ethnicities at the genetic level [37]. Xinjiang, situated in southwest China and comprising numerous rural areas, underscores the importance of identifying differences in the oral microbiomes between pre-school Han and Uyghur children. At present, parents in many areas, especially remote and under-developed areas, lack comprehensive training and information with regards to managing the oral hygiene of their children. As such, assessing and understanding variations in the oral microbiome of pre-school Han and Uyghur children during the onset of ECC is crucial [38].

Thus far, several studies have investigated the diversity of oral microbiota during the onset and progression of ECC. However, few have examined this from a longitudinal point-of-view or considered the impact of different ethnicities on the onset of ECC. It is essential to identify changes in the oral microbiota at the initial stage of ECC and ascertain if the onset of ECC indicates a specific infection. Understanding these factors can guide timely and effective preventive measures and help us to monitor and predict the progression of ECC after its initial phase.

Consequently, our research team undertook a longitudinal study that investigated the role of the salivary microbiome in the development of ECC in pre-school children. We used next-generation sequencing (NGS) to determine variations in the salivary microbiota during the onset and progression of ECC. Concurrently, we aimed to identify potential microbial indicators of the onset of ECC in various ethnicities. By using this approach, we hoped to determine if a particular baseline of microbiota can predict children at a higher risk of developing ECC, thus enhancing prevention strategies for ECC and facilitating the generation of an ECC prediction model tailored to different ethnicities.

2. Materials and methods

2.1 Study design

The sample in this study was taken from a previous study conducted by our research team. A total of 44 participants, aged 3 to 5 years, were enrolled in this study, including 25 pre-school Han children and 19 pre-school Uyghur children. The sample size in this study was consistent with previous studies [18, 39, 40]. All children participating in this study attended the same kindergarten in Shihezi, Xinjiang Province.

Children were excluded from this study if they met any of the following criteria: (1) taking antibiotics over the three weeks prior to sampling; (2) inability to cooperate with treatment and follow-up; (3) the use of dental appliances; (4) voluntary withdrawal or inability to comply with the oral examination and follow-up. Participants in this study were examined by clinical pediatric dentists from the Department of Stomatology at the First Affiliated Hospital of Shihezi University. The dmft (decayed, missing of filled teeth) index was used to assess the status of ECC. The baseline for this study was the first oral examination conducted at the kindergarten. All participating children were then followed up every three months for a duration of six months. The three-time points were: time point 1 (T1, baseline), time point 2 (T2, 3 months), and time point 3 (T3, 6 months). At the end of the 6-month follow-up (T3), children were grouped by ECC status as follows: in the Han ethnic group, four of the 14 ECC-free children who remained healthy (dmft = 0) were categorized into the HEF group. Ten of the 14 ECC-free children who developed new caries (dmft >0) were categorized into the HEO group. Eleven of the 11 ECC-affected children (dmft ≥ 6) who either maintained the same dmft index or developed more caries were categorized into the HEP group. In the Uyghur ethnic group, two of the 10 ECC-free children who remained healthy (dmft = 0) were categorized into the WEF group. Eight of the 10 ECC-free children who developed new caries (dmft >0) were categorized into the WEO group. Nine of the nine ECC-affected children (dmft ≥ 6) who either maintained the same dmft index or developed more caries were categorized into the

HEP group.

2.2 Saliva sample collection

After the oral examination, the same trained pediatric dentist collected the saliva samples. Children were instructed not to brush their teeth the night before and the morning of the saliva collection and to refrain from eating breakfast on the day of the oral examination. In addition, 5 mL sterile cryogenic vials were used to collect unstimulated saliva from children who were seated and asked to tilt their heads down to spit into a tube. After collection, cryogenic vials were transported in a −20 °C Drikold and ultimately stored in a −80 °C refrigerator at the Department of Stomatology at the First Affiliated Hospital of Shihezi University.

2.3 DNA extraction

Microbial DNA was extracted from the saliva samples using the E.Z.N.A. Stool DNA Kit (00D4015040000C01U001, Omega Bio-tek, Inc., Norcross, GA, USA). The quality and purity of the genomic DNA were assessed using a 1% agarose gel and a NanoDrop2000 spectrophotometer (ThermoFisher Scientific, Inc., Waltham, MA, USA).

2.4 PCR amplification and high throughput sequencing

Primers 806R (5′-GGACTACNNGGGTATCTAAT-3′) and 338F (5′-ACTCCTACGGGAGGCAGCAG-3′) were designed to amplify the V3-4 hypervariable region of the bacterial 16S rRNA gene. An 8-digit barcode sequence was added to the 5′ end of both reverse and forward primers (Beijing Allwegene Tech, Ltd., Beijing, China) for each saliva sample. The PCR (Polymerase Chain Reaction) was performed using the ABI 9700 PCR instrument (Applied Biosystems, Inc., Waltham, MA, USA) with a reaction volume of 25 μL, including 5.5 μL of ddH_2O, 2 μL of template DNA, 1 μL of reverse primer (5 μM), 1 μL of forward primer (5 μM), 3 μL of BSA (Bovine Serum Albumin) (2 ng/μL), and 12.5 μL of 2× Taq PCR MasterMix. The cycle parameters were 95 °C for 5 min, followed by 28 cycles of 72 °C for 45 s, 55 °C for 50 s, and 95 °C for 45 s, as well as 72 °C for 10 min for the final extension. The PCR products were purified using the Agencourt AMPure XP Kit (ABI 9700 PCR instrument (Applied Biosystems, Inc., Waltham, MA, USA)). Deep sequencing was performed on the Miseq PE300 platform (Beijing Allwegene Tech, Ltd., Beijing, China). Subsequently, error estimation, base calling, and image analysis, were performed with the Illumina Analysis Pipeline (Illumina, Inc., San Diego, CA, USA) Version 2.6.

2.5 Bioinformatic analysis

Initially, raw data were screened. Sequences were removed and separated based on the sample-specific barcode sequences if they had a low-quality score ($\leq$20), were less than 120 bp, contained unclear bases, or lacked a complete match to the barcode tags and primer sequences. Qualified sequences were denoised to amplicon sequence variants (ASVs) using the unoise3 algorithm of Usearch (v10.0.240). All sequences were classified into various taxonomic groups against the Silva138 database using the BLAST (Basic Local Alignment Search Tool) tool. By applying OUT (Operational Taxonomic Units) information, rarefaction curves were produced with QIIME (v1.8.0), followed by calculations of diversity and richness indices. Heatmaps were generated using the top 20 OTUs via Mothur to compare the structure and membership of communities across sample groups. Data from different groups were compared with the student's *t*-test. Bar plot analyses were conducted with R (v3.6.0) (Ross Ihaka, Robert Gentleman, New Zealand) software based on the results of relative abundance and taxonomic annotation. The relative abundance of the dominant bacteria between groups was compared with the Wilcoxon rank-sum test. Next, we used R (v3.6.0) software and OTU information, to perform PCA (principal components analysis) and clustering analyses to determine similarity between samples. Using the Bray-Curtis algorithm, the evolutionary distance between microbial communities in each sample was computed; this allowed us to generate a clustering tree with the Unweighted Pair Group Method with Arithmetic Mean (UPGMA) to depict differences between samples. Based on the unweighted UniFrac distance, similarity analysis (ANOSIM) was used to compare the different groups. The resultant tree file was then saved in Newick format. Differences were deemed highly significant at $p < 0.01$ and significant at $p < 0.05$. All statistical analyses were conducted with SPSS 25.0 software (SPSS Inc, Chicago, IL, USA).

2.6 Establishing an ECC prediction model by machine learning technology

Random forest models were employed to determine disease status (ECC status). In this study, the dataset was divided into a training set (70%) and a testing set (30%) using random sampling to ensure balance. To better understand the occurrence of ECC in Han and Uyghur ethnicities, we developed a prediction model that focused exclusively on temporal variations in the oral microbiome between different ethnic groups. Data were then assessed by the a 10-fold cross-validation method; this was repeated five times. The mean probability was then determined. The performance of the prediction model was evaluated by the area under the receiver operating characteristic (AUROC) curve; a large area under the curve indicates that the prediction model exhibits both high recall and precision.

3. Results

3.1 Caries status

A total of 44 pre-school children from Han and Uyghur ethnicities were enrolled in this study by the end of the follow-up period (Table 1). Children who participated in this study were divided into six groups based on their dmft index results from oral examinations: (1) HEF group (healthy pre-school Han children with no ECC lesions observed from T1 to T3); (2) HEO group (pre-school Han children with caries; no ECC lesions were observed at T1, but ECC lesions appeared at T2/T3); (3) HEP group (pre-school Han children with the progression of caries; ECC lesions were observed at T1); (4)

WEF group (healthy pre-school Uyghur children with no ECC lesions observed from T1 to T3); (5) WEO group (pre-school Uyghur children with caries; no ECC lesions were observed at T1, but ECC lesions appeared at T2/T3), and (6) WEP group (pre-school Uyghur children with the progression of caries; ECC lesions were observed at T1). The caries status of the children involved in this study are given in Table 1.

3.2 The diversity of salivary microbiota

A total of 132 saliva samples were collected from 44 preschool children. Illumina Miseq technology was used to sequence 16s rRNA gene amplicons from the samples across the six groups. In total, 2824 operational taxonomic units (OTUs) were identified. As shown in Fig. 1A, the HEF, HEO, HEP, WEF, WEO and WEP groups shared 571 OTUs. In addition, 150, 416, 30, 62, 29 and 16 OTUs were unique to the HEO, HEP, HEF, WEP, WEO and WEF group, respectively. Species accumulation curves (spectra) were used to evaluate the current sampling status (Fig. 1B). The specaccum curve for the 132 samples reached a saturation point, thus indicating that the number of samples in this study was adequate from a sequencing perspective.

3.3 Variation of the salivary microbiome during the follow-up period

Analysis of the salivary microbiota revealed a total of 27 phyla and 357 genera in this study. The main structure of the oral microbiota at both the phylum and genus levels for each group is illustrated in Fig. 1C,D. Based on mean relative abundance, only the top 20 taxa are displayed in the diagram.

The α-diversity indices (including the Chao1 index, observed species index, PD whole tree index, and Shannon index) for the six groups are shown in Fig. 2A. For pre-school Han children, no significant differences were observed in the Chao1 index, observed species index, PD whole tree index, and Shannon index between the HEO and HEF groups ($p > 0.05$). Similarly, for pre-school Uyghur children, no significant differences were observed for these α indices ($p > 0.05$). To better understand the variation of the salivary microbiome during the occurrence of ECC, we evaluated the α index for the HEO and WEO groups at three different time points (Fig. 2B,C). As shown in Fig. 2B, the Chaol index for the T1HEO group was significantly higher than that of the T2HEO group ($p = 0.007$). The observed species index in the T1HEO group was significantly higher than that in the T2HEO group ($p = 0.007$). The PD whole tree index in the T1HEO group was significantly higher than that in the T2HEO group ($p = 0.005$). The Shannon index in the T1HEO group was significantly higher than that in T2HEO ($p = 0.007$). As displayed in Fig. 2C, the Chaol index in the T1WEO group was significantly higher than that in the T2WEO group ($p = 0.012$). The observed species index in the T1WEO group was significantly higher than that in the T2WEO group ($p = 0.012$). The PD whole tree index in the T1WEO group was significantly higher than that in the T2WEO group ($p = 0.012$). No significant differences were observed in the Shannon index when compared between the T1WEO and T2WEO groups ($p > 0.05$).

TABLE 1. Caries status of participants at three-time points (dmft index).

Group	Host ID	Ethnic	dmft		
			T1	T2	T3
HEF	1	Han	0	0	0
HEF	68	Han	0	0	0
HEF	86	Han	0	0	0
HEF	57	Han	0	0	0
HEO	20	Han	0	2	3
HEO	46	Han	0	2	2
HEO	64	Han	0	1	1
HEO	85	Han	0	0	3
HEO	103	Han	0	1	1
HEO	146	Han	0	0	3
HEO	6	Han	0	2	2
HEO	21	Han	0	2	2
HEO	23	Han	0	1	1
HEO	87	Han	0	2	2
HEP	2	Han	8	8	8
HEP	3	Han	6	7	7
HEP	11	Han	8	8	9
HEP	12	Han	6	7	7
HEP	17	Han	13	14	14
HEP	43	Han	6	9	9
HEP	73	Han	9	9	9
HEP	74	Han	6	7	7
HEP	100	Han	11	11	11
HEP	101	Han	7	8	8
HEP	119	Han	8	8	8
WEF	139	Uyghur	0	0	0
WEF	132	Uyghur	0	0	0
WEO	93	Uyghur	0	1	6
WEO	88	Uyghur	0	8	8
WEO	26	Uyghur	0	4	5
WEO	29	Uyghur	0	4	4
WEO	38	Uyghur	0	3	3
WEO	50	Uyghur	0	0	2
WEO	111	Uyghur	0	2	2
WEO	141	Uyghur	0	5	5
WEP	36	Uyghur	7	7	7
WEP	71	Uyghur	6	6	7
WEP	104	Uyghur	6	8	8
WEP	109	Uyghur	6	6	6
WEP	117	Uyghur	7	7	7
WEP	125	Uyghur	9	9	10
WEP	127	Uyghur	6	6	6
WEP	130	Uyghur	6	7	7
WEP	142	Uyghur	6	6	7

T1: for baseline; T2: for the time point of 3 months; T3: for the time point of 6 months.
dmft: decayed, missing of filled teeth; HEF: healthy group of pre-school Han children; HEO: a group of Han pre-school children with caries; HEP: pre-school Han children with the progressive caries; WEF: healthy pre-school Uyghur children; WEO: pre-school Uyghur children with caries; WEP: pre-school Uyghur children with progressive caries.

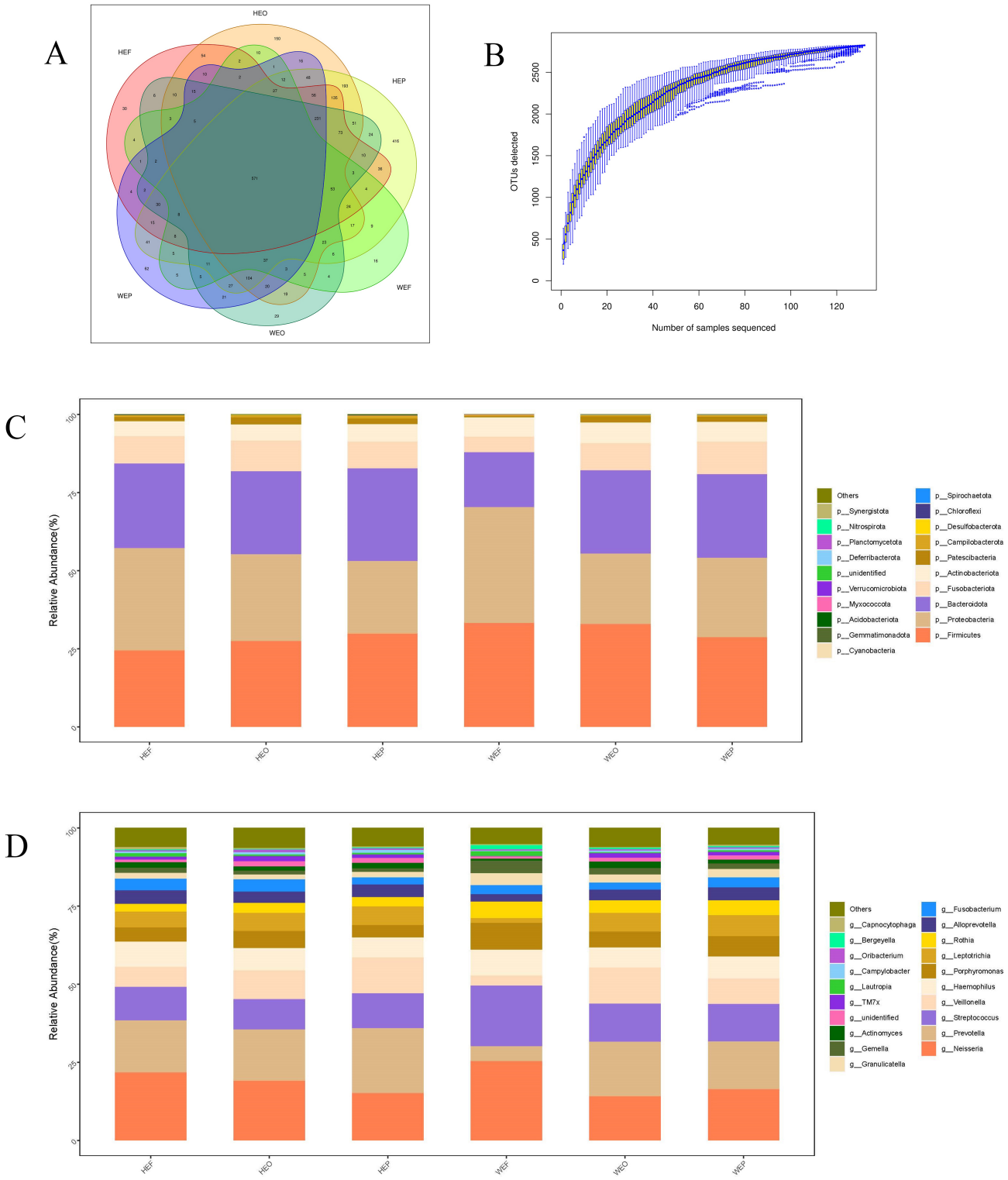

FIGURE 1. The diversity of salivary microbiota. (A) Venn diagram for the six groups. (B) Specaccum curve. (C) Phylum relative abundance in the six groups, and (D) the relative abundance of genera in the six groups. HEF: healthy group of pre-school Han children; HEO: a group of Han pre-school children with caries; HEP: pre-school Han children with the progressive caries; WEF: healthy pre-school Uyghur children; WEO: pre-school Uyghur children with caries; WEP: pre-school Uyghur children with progressive caries.

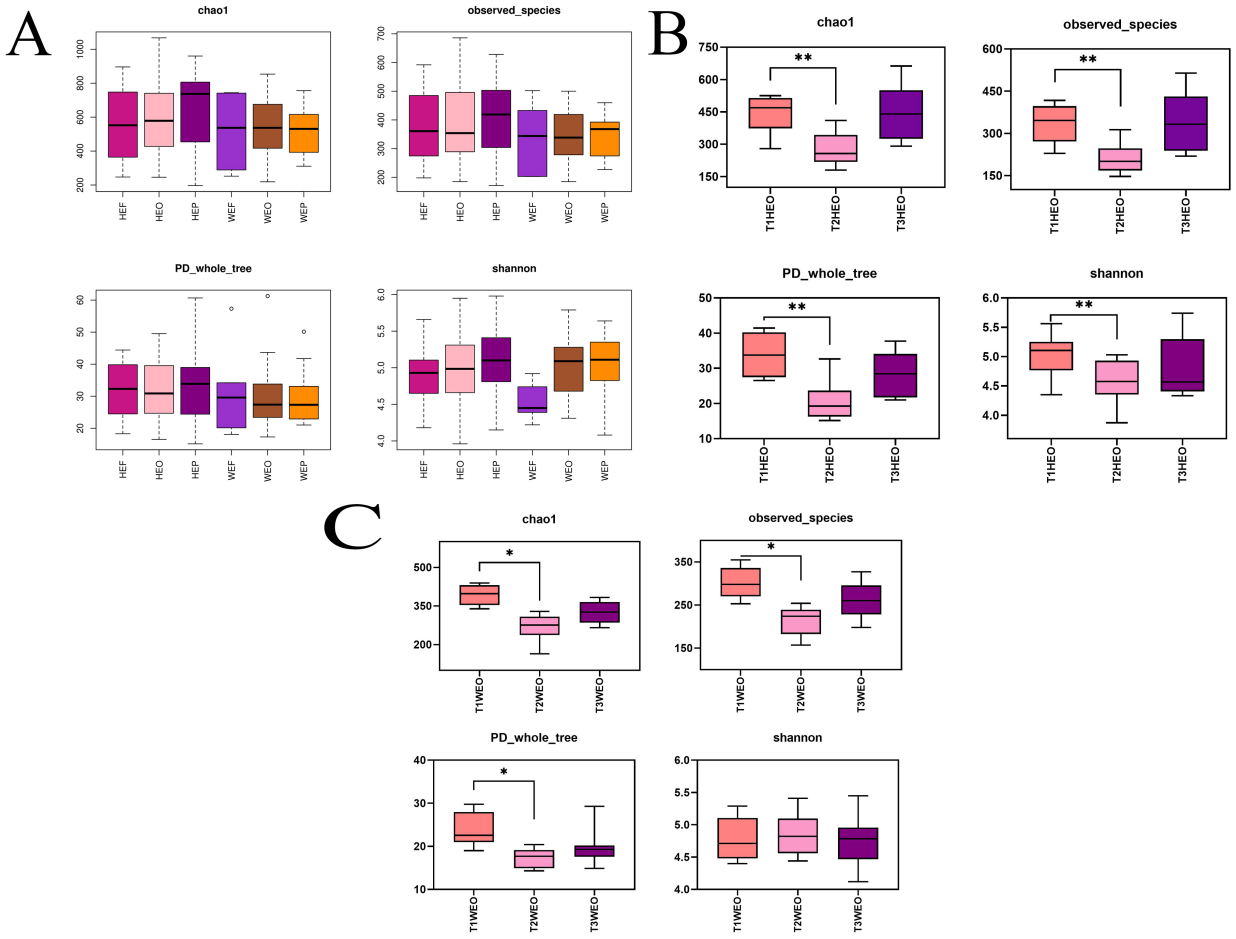

FIGURE 2. Variation of the salivary microbiome during the follow-up period. (A) Box plot of Alpha diversity indices of saliva samples from the six groups. (B) Box plot of Alpha diversity indices of saliva samples from the HEO group at three-time points. (C) Box plot of Alpha diversity indices of saliva samples from the WEO group at three-time points (*$p < 0.05$, **$p < 0.01$).

Principal coordinate analysis (PCoA) is a common method used to evaluate β-diversity in microbiology. In this study, we employed PCoA to investigate β-diversity. As shown in Fig. 3, samples from the six groups were clustered by PCoA based on the Unifrac distance at three different time points. Analysis indicated that regardless of whether the samples belonged to the same group, the oral microbiome community in the saliva samples could be similar.

3.4 ECC prediction model for pre-school Han and Uyghur children

Analysis showed that species diversity and richness varied during the occurrence of ECC. This led us to several questions: (1) could we establish a prediction model for ECC occurrence in pre-school children based on temporal variation of the oral microbiome? (2) If so, would there be a difference between the prediction models for pre-school Han and Uyghur children? (3) If there was a difference, which prediction model would be the best? To address these questions, we trained a classifier using the random forests (RF) algorithm to predict the status of ECC. This classifier used features from the oral microbiota of both pre-school Han and Uyghur children to distinguish between ECC and healthy conditions. the performance of the model was evaluated using the area under the receiver operator curve (AUROC) by applying a 10-fold cross-validation method.

For the salivary microbiome data, we trained one RF classifier based on the genus-level profile. In pre-school Han children, the prediction model performed poorly (AUROC = 51.7%; 95% confidence interval (CI): 27.5%–75.9%, Fig. 4A). However, the performance improved when using the top eight most discriminatory genera: *Selenomonas*, *Capnocytophaga*, *Lautropia*, *Butyrivibrio*, *Eubacterium_nodatum_group*, *Kingella*, *Oribacterium* and *Atopobium* (Fig. 4B). The lowest error rate was 0.30 when these top-eight bacteria were included (Fig. 4C). For pre-school Uyghur children, the prediction model performed well (AUROC = 89.6%; 95% CI: 78.0%–100.0%, Fig. 4D). Maximum performance was achieved with the top six most discriminatory genera: *Mogibacterium*, *Atopobium*, *Megasphaera*, *Butyrivibrio*, *Prevotella* and *Stomatobaculum* (Fig. 4E). The lowest error rate was 0.01 when these top-six bacteria were selected (Fig. 4F).

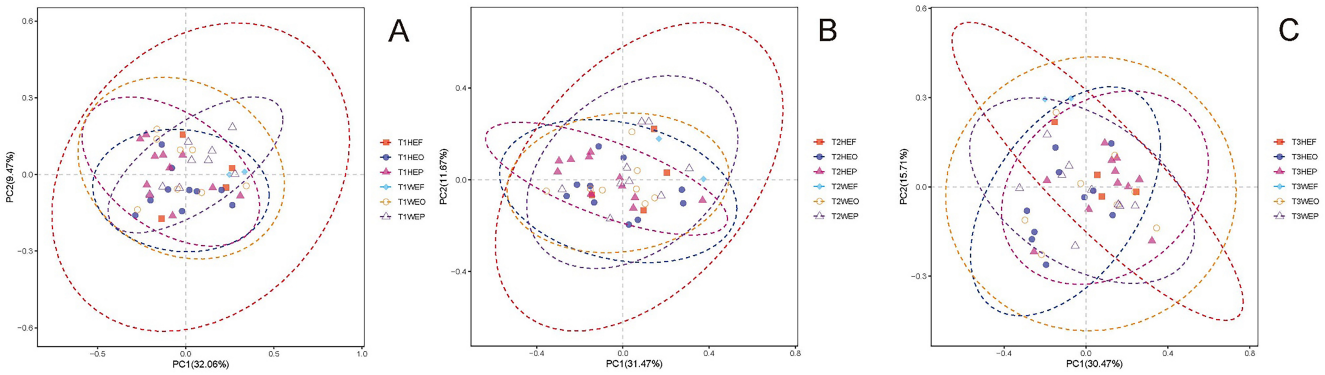

FIGURE 3. Principal coordinate analysis (PCoA) at three different time points (six groups). (A) PCoA at T1 time point. (B) PCoA at T2 time point. (C) PCoA at T3 time point.

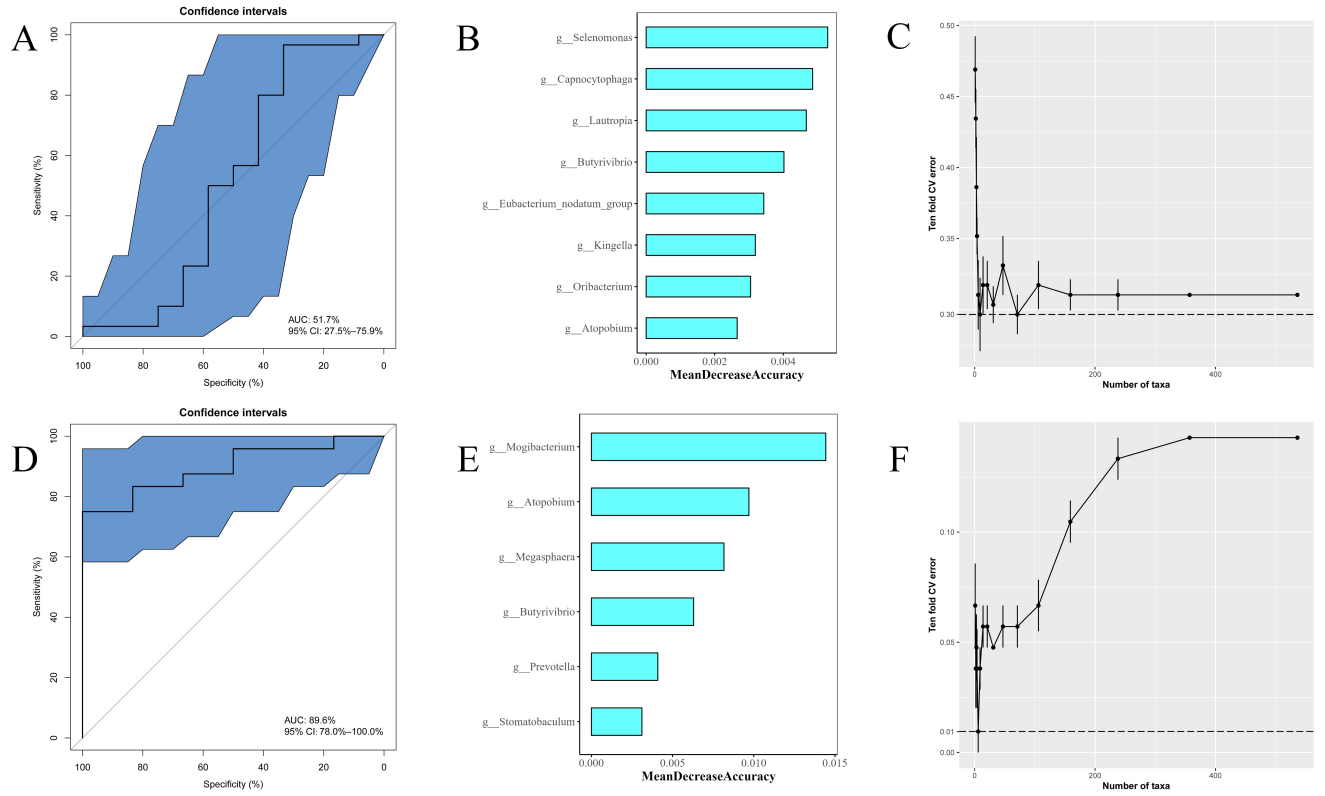

FIGURE 4. ECC prediction model. (A) ECC prediction model based on the characteristics of the oral salivary flora (Han Nationality). (B) Rank of the contribution of different bacteria in saliva from pre-school Han children (genus level). (C) Ten-fold cross-validation error rate curve (Han Nationality). (D) ECC prediction model based on the characteristics of the oral flora (Uyghur Nationality). (E) Rank of the contribution of different bacteria in the saliva of pre-school Uyghur children (genus level). (F) Ten-fold cross-validation error rate curve (Uyghur Nationality). ECC: early childhood caries.

4. Discussion

Early childhood caries (ECC) is one of the most critical public health problems affecting the daily lives of children. ECC impacts not only the diet, growth and development of children, it also presents a significant challenge to society. As such, authorities need to develop risk assessment and early prediction technology for ECC. While several studies have identified traditional predictors for ECC, the findings of these studies remain controversial [2, 11]. Existing methods are associated with certain limitations and disadvantages, thus necessitating innovative approaches to prevent ECC.

The rise of the ecological plaque hypothesis for dental caries has turned the composition and characteristics of the oral microorganism group into focal points for researchers. Recent studies related to the role of oral microorganisms in human health and disease suggest that changes in the oral flora of

pre-school children are driven by fluctuations in the relative abundance of various species rather than the emergence or disappearance of specific species [41, 42]. This implies that dynamic changes in the oral microbial composition of pre-school children may relate to the risk, occurrence, and progression of ECC [43, 44]. To identify if the microbial composition of saliva in pre-school Han and Uyghur children can predict the risk of ECC, we designed a prediction method based on bacterial flora in the saliva. This method aims to identify high-risk individuals, detect variations in salivary microbes, and establish preventive strategies, ultimately reducing the incidence of ECC.

High-throughput sequencing analysis, including the Shannon-Wiener curve and the species accumulation curve (specaccum), confirmed that the sequencing depth and sample size of our study were both adequate and reasonable. α diversity analysis further indicated that neither pre-school Han or Uyghur children showed significant differences between those with caries and those without. Results for pre-school Han children in our study align with previous findings [45]. However, during the onset of ECC in pre-school Han children, we noted a reduction in both species richness and community diversity in the saliva. This contrasts with some previous research findings [42], but resonates with the findings of other researchers [46]. Given these discrepancies, future research should consider larger sample sizes and employ more sophisticated techniques to analyze variations in the salivary flora during the onset of ECC in pre-school Han children. For pre-school Uyghur children, the occurrence of ECC was marked by a decline in the species richness of saliva. Since limited research has been conducted on pre-school Uyghur children, there was a lack of data to compare with our current findings. Overall, irrespective of whether the children were Han or Uyghur, the species composition in the saliva of children with caries did not differ significantly from that of healthy children. In Han children of the same group, as ECC developed, there was a reduction in the species richness and community diversity in the oral saliva. In contrast, for Uyghur children of the same group, only species richness decreased with the progression of ECC. Considering that our small sample size included only Han and Uyghur children, more extensive research and detailed analysis are needed to gain more conclusive results. In addition, β diversity analysis revealed that the microbial community structure in the saliva of children remained constant, irrespective of their caries status or whether they were Han or Uyghur.

In this study, we used the random forest (RF) algorithm to analyze and evaluate the ability of microbial indicators in the saliva to predict the ECC status of preschool children from different ethnicities. We observed differences in the performance of the prediction model based solely on the variation of oral flora in saliva when compared between pre-school Han and Uyghur children. For pre-school Han children, the predictive effect of the ECC model based on temporal changes in the oral flora was poor (AUROC = 51.7%); this was lower that reported by other researchers (AUROC = 69.5%) [47]. These results suggest that the ECC prediction model, based only on changes in the oral flora, cannot predict the occurrence of ECC effectively in pre-school Han children. Although the sample size in this study was deemed sufficient according to the species accumulation curve, a larger sample is necessary to refine the ECC prediction model and enhance its predictive accuracy. Furthermore, even if two children belong to the same ethnicity (Han ethnicity), they might have different diets, living environments, and genetic characteristics. In future studies, researchers should explore other factors, such as salivary protein content and the number of electrolytes in the saliva. Using these factors to establish an ECC prediction model may provide a highly novel approach.

In pre-school Uyghur children, the prediction effect of the ECC model based on temporal changes in the oral flora was good (AUROC = 89.6%). This suggests that for pre-school Uyghur children, an ECC prediction model based solely on variations in the oral flora was able to predict the occurrence of ECC more effectively. Intriguingly, compared to pre-school Han children, pre-school Uyghur children typically had simpler diets, living environments, and genetic characteristics. This might explain why the ECC prediction model based solely on changes in the saliva performed better for this group of children. Future studies should focus on national customs and genetic characteristics among pre-school Uyghur children, possibly leading to the development of a more refined prediction model for ECC.

However, this study has several limitations that need to be considered. Firstly, only 44 preschool children participated in the study; some analysis groups contained very few children. This sample size might be insufficient for further sub-group analyses for ECC. Future studies should include a more diverse group of children to analyze differences in the microflora of the saliva among more nationalities. Second, the Illumina Miseq platform used for high-throughput sequencing could not effectively annotate species below the genus level. Thus, our results were limited to genus-level annotations. To investigate oral microbes in a more advanced manner, it would be better to use more advanced techniques to understand the etiology of caries in young children. Third, ECC is a multifaceted disease that is not just influenced by microbial infection; host and environmental factors are also involved. Although we did not consider all of these factors, the selected pre-school children came from the same full-day kindergarten, shared a diet, received identical oral health instructions, and lived in similar environments. Confounding factors were controlled to minimize contamination. Fourth, based on the findings of other researchers, we know that the oral microbiome is susceptible to genetic or environmental factors [48]. Therefore, the results arising from this study might only be relevant when considering populations from specific regions or ethnicities. The ECC prediction model devised in this study originated from a kindergarten in Shihezi, Xinjiang. Future verification with pre-school children from other Xinjiang kindergartens would be beneficial. Finally, the clear microbial pathogenic mechanism associated with the onset of ECC has yet to be elucidated. The specific pathogenesis and signal transduction pathways involved in the occurrence and progression of ECC warrant further investigation.

5. Conclusions

In conclusion, during the occurrence of caries in preschool children, the bacterial species richness and community diversity in the oral saliva of pre-school Han children decreased. Moreover, the richness of bacterial species in the oral saliva of pre-school Uyghur children also decreased. In addition, the prediction model of ECC, based on temporal changes in the oral microflora, effectively predicted ECC for pre-school Uyghur children. These findings may lead to more effective measures for preventing ECC.

AVAILABILITY OF DATA AND MATERIALS

The raw sequencing data of this study have been submitted to the NCBI Sequence Read Archive. If you need the raw data and code, please contact the corresponding author of this manuscript.

AUTHOR CONTRIBUTIONS

CQY, DHL, CDZ and DC—conceived and designed the experiments. CQY, DHL, YQ, XDQ and XYZ—performed the experiments. CQY, DC, YQ, CDZ, ZZ and XDQ—analyzed the data. DHL, DC, ZZ, CDZ, YFS and XRG—contributed reagents/materials/analysis tools. CQY, DHL, YFS and ZZ—wrote the manuscript.

ETHICS APPROVAL AND CONSENT TO PARTICIPATE

This study was approved by the Medical Ethics Committee of the First Affiliated Hospital of Shihezi University (Issuing number: KJX-2021-084-01). The study was performed in accordance with the Declaration of Helsinki. Informed consent was obtained from the parents or primary caregivers of the participants in all cases.

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CONFLICT OF INTEREST

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

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