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Original article

Study of oral microbiota diversity among groups of families originally from different countries

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ABSTRACT

The diversity of oral microbiota is affected by diets habits, gender, age, ethnic group, and environment. The acquisition of oral microbiota and the role of family on oral microbiota development is poorly understood. This study aims to characterize and compare the oral bacterial microbiota among families using 16S rRNA gene sequencing. This work was conducted in Jeddah city from 2020 to 2021, in which four families composed of 20 members of different ethnicity and lifestyle were recruited. After the collection of saliva samples, the DNA was extracted and processed for 16S rRNA gene metagenomics sequencing. Among 378 OUTs generated, 39 (10.3%) were unique in group A, 13 (3.4%) unique in group B, and 11 (2.9%) were unique in groups C and D. We observed a significant variation at the level of top abundance phylum (14), families (23), genera (24), and species (22) of bacteria among family members. Within family groups, different bacterial species were reported to be more dominant among certain family members than the other; *Prevotella melaninogenica, Prevotella histicola* and *Haemophilus parainfluenzae, Veillonella atypica, Porphyromonas pasteri* and *Haemophilus pittmaniae* were more dominant in parents of some families than the other family member. In summary, this study highlights the precise and perceptible association of oral microbial between family members. Our findings documented the clustering of certain bacterial species in family groups, supporting the role of community in the development of oral microbiota.

1. Introduction

The oral microbiota is acquired very early. Various microorganisms inhabit the oral cavity and colonize teeth surfaces and mucosal membranes (Griffen et al., 2012). Following birth, the microbiota is gradually developing into a miscellaneous ecosystem with age.

Human microbiota offers a barrier against pathogens through colonization resistance and the manufacture of antimicrobial substances. It also plays a fundamental role in the induction and the maintenance of immune functions (Belkaid and Hand, 2014).

About 700 bacterial species inhabit the human mouth (Bik et al., 2010), and some of them remain unculturable due to their specific nutrient requirement, and oxygen sensitivity (Wade, 2013). Different studies have linked the composition and development of the oral microbiome of children to parents and other family members (Gomez and Nelson, 2017; Jo et al., 2021; Mukherjee et al., 2021). Moreover, diet, mouth hygiene, health status, genetics, and lifestyle are biological and cultural factors that strongly affect oral microbiota diversity (Weyrich, 2021). Additionally, tobacco, alcohol, catechol, and reactive oxygen species contribute to the variation in the composition of oral bacterial communities (Lee et al., 2017). More recently, Mukherjee et al. (Mukherjee et al., 2021) reported that the acquisition of oral microbiota is highly influenced by environmental parameters and not by host genetics.

The composition of the microbiota is influenced by bacterial diversity and the immune system's ability to manage the host's state (Cho et al., 2014). The host immune system's activation, training, and functionality are all influenced by the microbiota. The

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interaction of the immune system and microbiota allows the activation of immune responses against pathogens (Belkaid and Hand, 2014). Outside the mouth cavity, oral cavity-associated microorganisms can impact immune responses and pathogenicity, and their capacity to inhibit aberrant sites is consistent with the current state of health in that region (Sedghi et al., 2021).

The mouth constitutes an entrance to the digestive and respiratory systems, which provide evidence about the potential implications of the oral microbiota in other systemic illnesses (Willis and Gabaldón, 2020). Oral microbiota imbalances can lead to oral and other systemic diseases such as oral squamous cell carcinoma (Zhang et al., 2020), severe early-childhood caries (Li et al., 2007), Halitosis (Zhang et al., 2021), and inflammatory bowel disease (Qi et al., 2021). Multiple studies had shown the links between oral microbiota dysbiosis and infections, which suggested that oral microflora could provide possible biomarkers in the investigation of sicknesses (Deo and Deshmukh, 2019; Bartlett et al., 2020; Wingfield et al., 2021). Seerangaiyan et al. (Seerangaiyan et al., 2017) discovered that the microbiome composition between halitosis and healthy adults differed significantly.

A recent study (Hosgood et al., 2021) analyzed the oral microbiome and reported that the risk of lung cancer is increased in individuals with lower microbiota alpha diversity. Additionally, they demonstrated that a greater abundance of the Bacilli class and Lactobacillales order in the oral microbiome was associated with an increased risk of lung cancer (Hosgood et al., 2021). A distinct oral microbial community has not been fully identified. However, *Streptococcus, Actinomyces, Fusobacterium, Lactobacterium, Leptotrichia*, and *Propionibacterium* were formerly reported in the oral cavity of healthy individuals (Park and Yaacob, 1994; Marsh and Zaura, 2017).

Identifying the healthy oral microbiota is significant in the prediction, diagnosis, and management of numerous conditions. The present study was planned to characterize and compare the oral bacterial microbiota in four families from different countries using 16S rRNA gene sequencing.

2. Materials and methods

2.1. Study design, area, duration, and ethical approval

This work was a comparative study conducted at Jeddah city. The study was performed from 2020 to 2021. Jeddah is the largest city in the Makkah Province of Saudi Arabia. It is lies between an attitude of 21.543333 and longitudes of 39.172779 and covers an area of 1500 km^2. Approximately it has 4,697,000 people since 2021. Jeddah humidity ranged between 57% in July to 73% in January. The population is composed of heterogeneous ethnic groups of both Saudi Arabia and foreign origin. Jeddah exhibits a cross-cultural environment, a city where people of many nationalities and cultures live together and interact with each other daily. It is reported that millions of people from around the world visit Jeddah every year. The study was conducted following the Declaration of Helsinki, and the study was approved by the ethics committee of King Abdulaziz University. Participant agreement was granted, and written consent had provided by all participants or children's parents following explaining the protocol and significance of the research. Coding of information was applied to save the contributor's privacy.

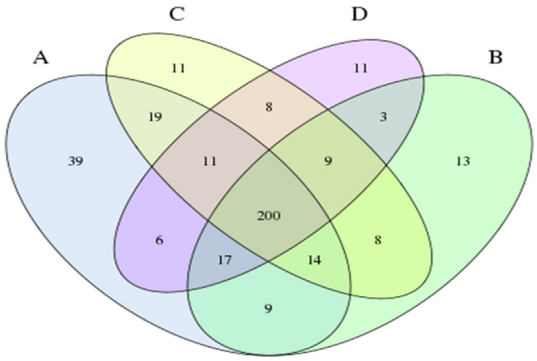

Fig. 1. Shared and unique OTUs (378) across groups. Between the four groups A: Sudanese, B: Yamen, C: Saudi Arabia, D: Indian. 200 OTUs shared among all groups, while 39 (10.3%) were unique in group A, 13 (3.4%) unique in group B, and 11 (2.9%) were unique in groups C and D.

2.2. Study subjects

The study included members of four families who had no apparent sign of acute or chronic oral illnesses. Excluded families include those who had members with a current history of oral or other infection, antibiotics use before less than two weeks, radio or chemotherapy, oral surgery or cancer, immune deficiency or autoimmune disease, chronic illnesses such as diabetes and hypertension, alcoholic or tobacco addiction, or smoking. A structured questionnaire was used to gather the socio-demographic features of four family members. The study subjects were categorized into

Table 1
Study subjects characteristics.

No	Group	Patient ID	Gender	Family rank	Nationality	Age
1	A	1H	M	Father	Sudanese	40
2		2H	F	Mother	Sudanese	31
3		3H	F	Daughter	Sudanese	9
4		4H	F	Daughter	Sudanese	6
5		5H	F	Daughter	Sudanese	4
6	B	1M	M	Father	Saudi	56
7		2M	F	Mother	Saudi	45
8		3M	M1	Son	Saudi	23
9		4M	F	Daughter	Saudi	15
10		5M	M	Son	Saudi	12
11	C	1S	M	Father	Yemen	50
12		2S	F	Mother	Yemen	47
13		3S	F	Daughter	Yemen	18
14		4S	M	Son	Yemen	12
15		5S	F	Daughter	Yemen	10
16		6S	M	Son	Yemen	7
17	D	2A	M	Father	Indian	40
18		3A	F	Mother	Indian	35
19		4A	M	Son	Indian	7
20		5A	M	Son	Indian	6

four groups according to nationality, Sudanese (group A), Saudi (group B), Yemen (group C), and Indian (group D) (Table 1).

2.3. Saliva samples collection, DNA extraction, and 16S rRNA gene sequencing

All families' members were provided sterile containers and informed to collect saliva samples. A tola of 2–3 mL of chewing-induced saliva samples were collected in front of the researcher and processed immediately. DNA was extracted by QIAamp DNA Microbiome Kit-QIAGEN according to the kit protocol. The quality of nucleic acid was evaluated by a Nanodrop spectrophotometer. DNA was saved at −80 °C before sequencing. V3 and V4 region of 16S rRNA gene was sequenced by using Illumina HiSeq/MiSeq platform (BGI, Hong Kong). Chimeras were filtered using UCHIME (v4.2.40) then the filtered tags were clustered into OTU (Operational Taxonomic Units) at 97% similarity.

2.4. Data analysis

To display the number of shared and unique OTUs, a Venn diagram was drawn by VennDiagram of software R (v3.1.1). The

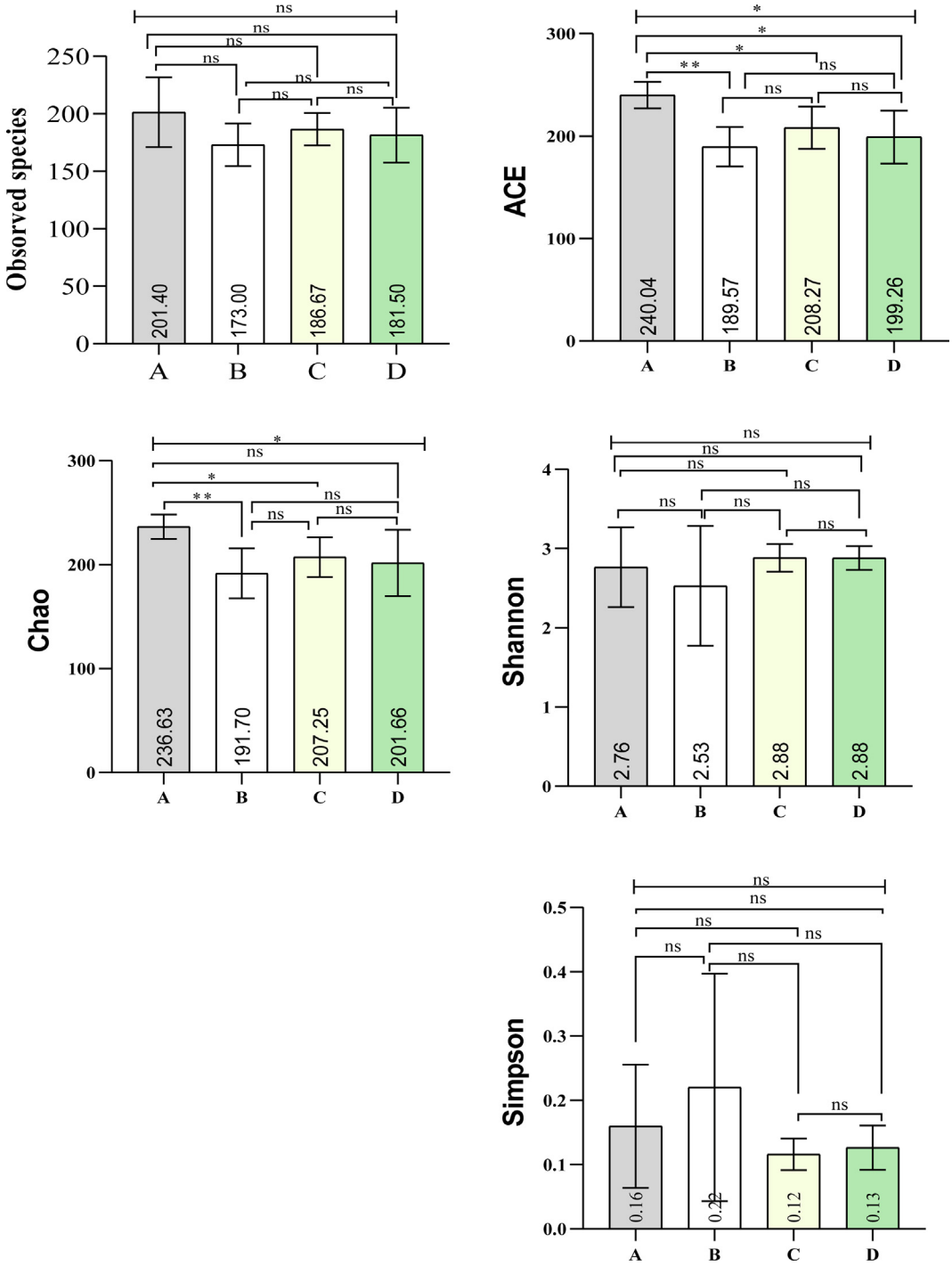

Fig. 2. Variation in alpha diversity between families. Kruskal-Wallis Test was used to assess the variation between the four groups and Wilcoxon Rank-Sum Test to compare and test the distinction between two groups. ns: P>0.05, *: P < 0.05, **: P < 0.01. A: Sudanese, B: Yemen, C:Saudi Arabia, D:Indian.

microbiota abundance was presented in a histogram with the software R(v3.1.1). In all samples, the species, genera, and phylum of which the abundance is<0.5% were classified into 'others'. Alpha diversity indices (i.e., ACE, Chao1, Shannon, and Simpson) were analyzed by Mothur (v1.31.2). Principal component analysis (PCA) was done by QIIME software (v1.80) and presented by software R (v3.1.1). Multi-groups comparison was done by Kruskal-Wallis Test and Bi-groups by Wilcoxon Rank-Sum Test for numerical data. Fisher exact test was used to evaluate the categorical data. P < 0.05 was designated for significant variation. Additionally, NCBI SRA Taxonomy Analysis Tool (Katz et al., 2021), was used to analyze sequencing reads to their taxonomic OTUs.

3. Results

Twenty members of four families groups (A = 5, B = 5, C = 6, D = 4) have been recruited. Most members of families were females (P = 0.002) and age range 1–18 years, P = 0.031 (Table 1). Venn diagram (Fig. 1A-G) displays the number of shared and unique OTUs. Notably, group A had 240 (Fig. 1B), 244 (Fig. 1C), and 234 (Fig. 1D) shared OTUs with groups B, C, and D, respectively. Group B revealed 231 (Fig. 1E) and 229 (Fig. 1F) shared OUTs with groups C and D, respectively. Moreover, group C showed 228 shared OUTs with group D (Fig. 1G). The highest numbers of unique OUTs were observed in group A compared to other groups (Fig. 1B-F). To evaluate the salivary bacterial community variation between the groups of families, alpha diversity indices and PCA were analyzed. We found that Chao (236.62) and ACE (240.03) were significantly higher in Sudanese (A) than others, but the diversity (Shannon and Simpson) indices were non-significantly different between families P>0.05 (Fig. 2A-E). PCA (Fig. 3A-E) displays the degree of variation among groups. Overall, sequences representing 14 phyla, 75 families, 113 genera, and 55 species of salivary bacterial microbiota were identified (Figs. 1, 2, 3, and 4). Between the study families, we observed multiple variations at the level of top abundance phylum (14), families (23), genera (24), and species (22) of bacteria (Fig. 4A-D). The major abundance phylum among groups was Firmicutes, followed by Bacteroidetes and Proteobacteria (Fig. 1). Thus, the four groups shared the same top three genera; however, there is a slight difference in abundance of bacteria between groups (P>0.05). Moreover, the most abundant bacterial family was Streptococcaceae (Fig. 2). *Streptococcus, Haemophilus, Prevotella, Actinomyces*, and *Lactobacillus* were the most abundant genera (Fig. 3). *Streptococcus infantis*, *Prevotella melaninogenica*, *Haemophilus parainfluenzae*, and *Veillonella dispar* were members of major abundance bacteria at the species level (SF4).

Kruskal-Wallis Test was performed to evaluate the degree of variation between the four groups in relative abundance bacteria at phylum, family, genera, and species (Table 2). The relative abundance of one phylum out of 14, 6 families out of 75, 24 genera out of 114, and nine species of bacteria out of 56 were significantly varied between groups. Indeed, Cyanobacteria phylum was highest in group D and lowest in group B, P = 0.029. Acetobacteraceae and Enterobacteriaceae were also significantly higher in group D than others. Group A showed a higher abundance (P < 0.05) of Nocardiaceae, Oxalobacteraceae, and Staphylococcaceae than other groups.

Additionally, Group C was characterized by a higher abundance of Rhodocyclaceae (P < 0.05). Notably, multiple genera were displayed significant variation between the four groups. For example, the relative abundance of *Staphylococcus*, *Johnsonella, Nevskia,* and *Moraxella* was more in groups A, B, C, and D when compared with other groups, respectively. At the species level, nine bacteria were exhibited significant distinction between the groups. An example, *Actinobacillus parahaemolyticus* was highest (P < 0.05) in group D (2.324286) compared to other groups (Table 2).

For further understanding of the evenness and divergence of saliva microbiota between groups, Wilcoxon Rank-Sum Test was also done to analyze the difference in the relative abundance of bacteria at the level of the two group's comparison. Variations in the relative abundance of phyla are presented in Table 3. Markedly, Enterobacteriaceae were significantly higher in group A than B, A than C, and D than C. Staphylococcaceae was also more (P < 0.05) in group A compared to B and A than D. Furthermore, the relative abundance of Staphylococcaceae and Micrococcaceae were higher

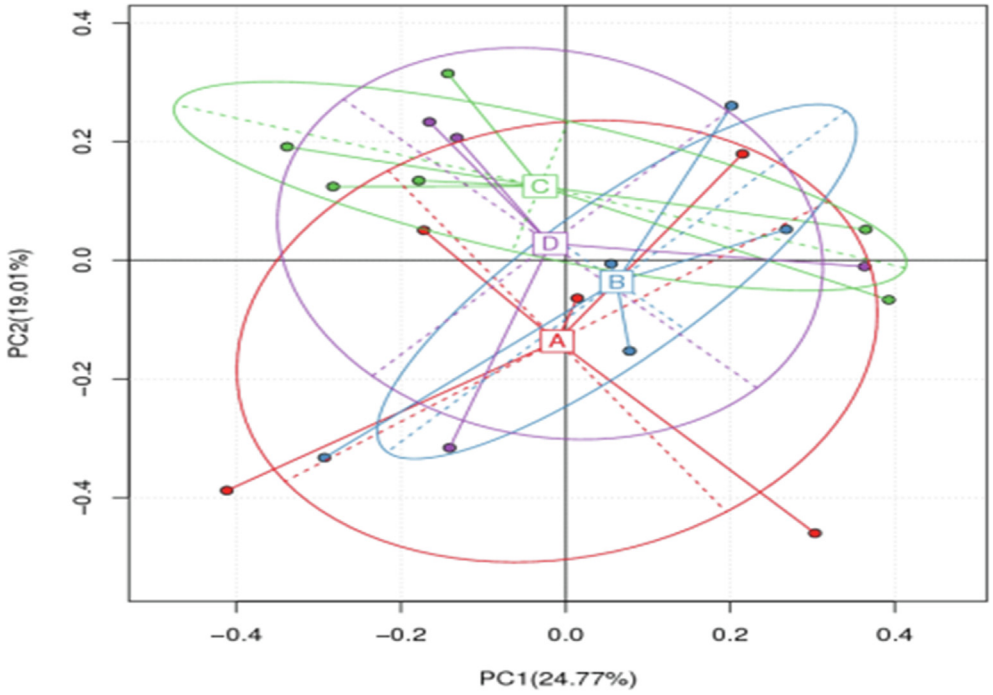

Fig. 3. Principal component analysis (PCA) of the OTU composition in four family groups, displays the degree of variation between the four family groups.

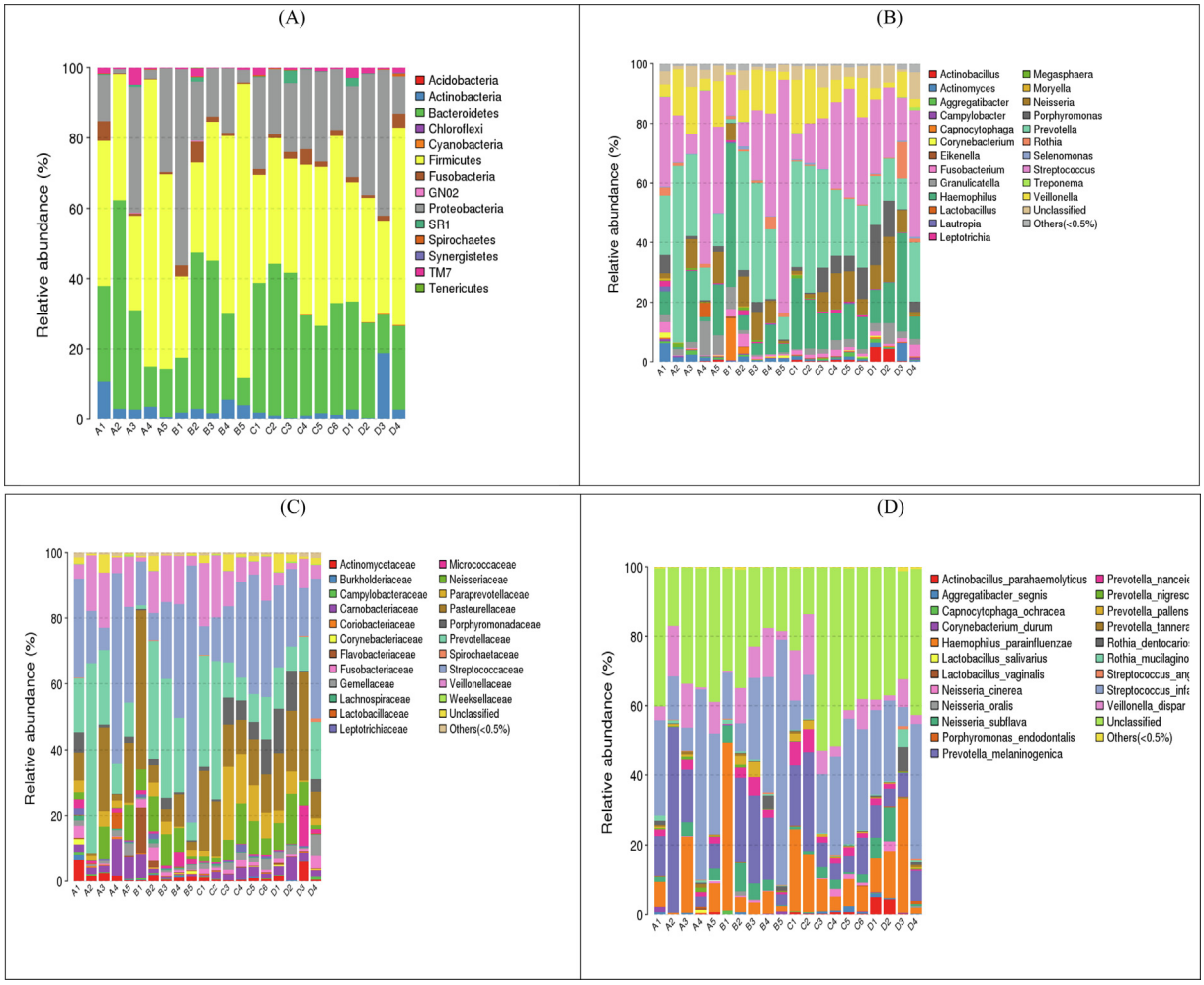

Fig. 4. (A-D). The relative abundance of bacteria at phylum (A), family (B), genera (C), and (D) species level. The aundance of bacteria is shown in coulmns with different colors.

Table 2
Bacteria exhibit significant distinction between the four groups at phylum, family, genera, and species level.

Significant higher abundance of bacteria		
Taxonomy level	Name	Group
Phylum	Cyanobacteria	D
Family	Nocardiaceae, Oxalobacteraceae, and Staphylococcaceae	A
	Rhodocyclaceae	C
	Acetobacteraceae and Enterobacteriaceae	D
Genera	Anaerococcus, Curvibacter, Delftia, Escherichia, Moryella, Rhodococcus, and Staphylococcus	A
	Atopobium and Johnsonella	B
	Methyloversatilis, Nevskia, Ramlibacter, and Sphingomonas	C
	Acetobacter Actinobacillus, Erwinia, Gluconobacter, Klebsiella, Moraxella, Pyramidobacter, and Tatumella	D
Species	*Acinetobacter lwoffii, Bacillus cereus, Escherichia coli, and Staphylococcus epidermidis*	A
	Johnsonella ignava	B
	Nevskia ramosa and Sphingomonas_yabuuchiae	C
	Actinobacillus parahaemolyticus and Pyramidobacter piscolens	D

Kruskal-Wallis Test was used to evaluate the variation between groups.

Table 3
Wilcoxon Rank-Sum Test findings regarding microbiota displayed significant variation among groups at phylum and family level.

Significant higher abundance of bacteria		
Taxonomy level	Name	Groups comparison
Phylum	Cyanobacteria	↑ A than B, ↑ D than B
	Actinobacteria	↑ B than C
Family	Acetobacteraceae, Enterobacteriaceae, Rikenellaceae, and Staphylococcaceae	↑ A than B
	Enterobacteriaceae, Mycoplasmataceae, Nocardiaceae, Oxalobacteraceae, Rikenellaceae, and Xanthomonadaceae	↑ A than C
	Erythrobacteraceae, Pseudomonadaceae, Rhodocyclaceae, and Sinobacteraceae	↑ C than A
	Rs_045 and Staphylococcaceae	↑ A than D
	Coriobacteriaceae, Micrococcaceae, Mycoplasmataceae, Peptococcaceae, and Staphylococcaceae	↑ B than C
	Gemellaceae and Rhodocyclaceae	↑ C than B
	Burkholderiaceae	↑ B than D
	Enterobacteriaceae and Mycoplasmataceae	↑ D than C
	Erythrobacteraceae and Veillonellaceae	↑ C than D

Variation between the groups was evaluated by Wilcoxon Rank-Sum Test.

Table 4
Wilcoxon Rank-Sum Test findings regarding microbiota displayed significant variation among groups at genera and species level.

Significant higher abundance of bacteria		
Taxonomy level	Name	Groups comparison
Genera	Acetobacter, Blvii28, Curvibacter, Escherichia, Gluconobacter, Staphylococcus, and Tatumella	↑ A than B
	Johnsonella	↑ B than A
	Acetobacter, Alloscardovia, Anaerococcus, Blvii28, Brevundimonas,Comamonas, Curvibacter, Delftia, Erwinia, Escherichia, Massilia, Mycoplasma, Rhodococcus, Staphylococcus, Stenotrophomonas, Tatumella	↑ A than C
	Actinobacillus, Dechloromonas, Erythromicrobium, Methyloversatilis, Nevskia, Paludibacter, Pseudomonas, Ramlibacter, Sphingomonas	↑ C than A
	Butyrivibrio and Staphylococcus	↑ A than D
	Klebsiella and Nevskia	↑ D than A
	Alloscardovia, Atopobium, Erwinia, Johnsonella, Moryella, Mycoplasma, Peptococcus, Rothia, Staphylococcus	↑B than C
	Actinobacillus, Burkholderia, Methyloversatilis, Ramlibacter	↑ C than B
	Johnsonella, Lautropia, Moryella	↑ B than D
	Erwinia	↑ D than B
	Butyrivibrio, Erythromicrobium, Peptostreptococcus,Prevotella, Veillonella	↑ C than D
	Curvibacter, Erwinia, Klebsiella, Mycoplasma, Scardovia, Slackia	↑ D than C
Species	*Acinetobacter lwoffii, Bacillus cereus, Escherichia coli, Staphylococcus epidermidis*	↑ A than B
	Johnsonella ignava, Nevskia_ramosa	↑ B than A
	Acinetobacter lwoffii, Bacillus cereus, Brevundimonas diminuta, Escherichia coli, Olsenella uli, Prevotella copri, Prevotella nigrescens, Staphylococcus epidermidis, Stenotrophomonas geniculata	↑ A than C
	Actinobacillus parahaemolyticus, Neisseria oralis, Nevskia ramosa, Sphingomonas yabuuchiae	↑ C than A
	Bacillus cereus, Staphylococcus epidermidis	↑ A than D
	Nevskia ramosa, Sphingomonas yabuuchiae	↑ D than A
	Rothia mucilaginosa, Staphylococcus epidermidis	↑ B than C
	Actinobacillus parahaemolyticus	↑ C than B
	Johnsonella ignava	↑ B than D
	Unclassified	↑ D than B

Wilcoxon Rank-Sum Test was performed to assess the variation between the groups.

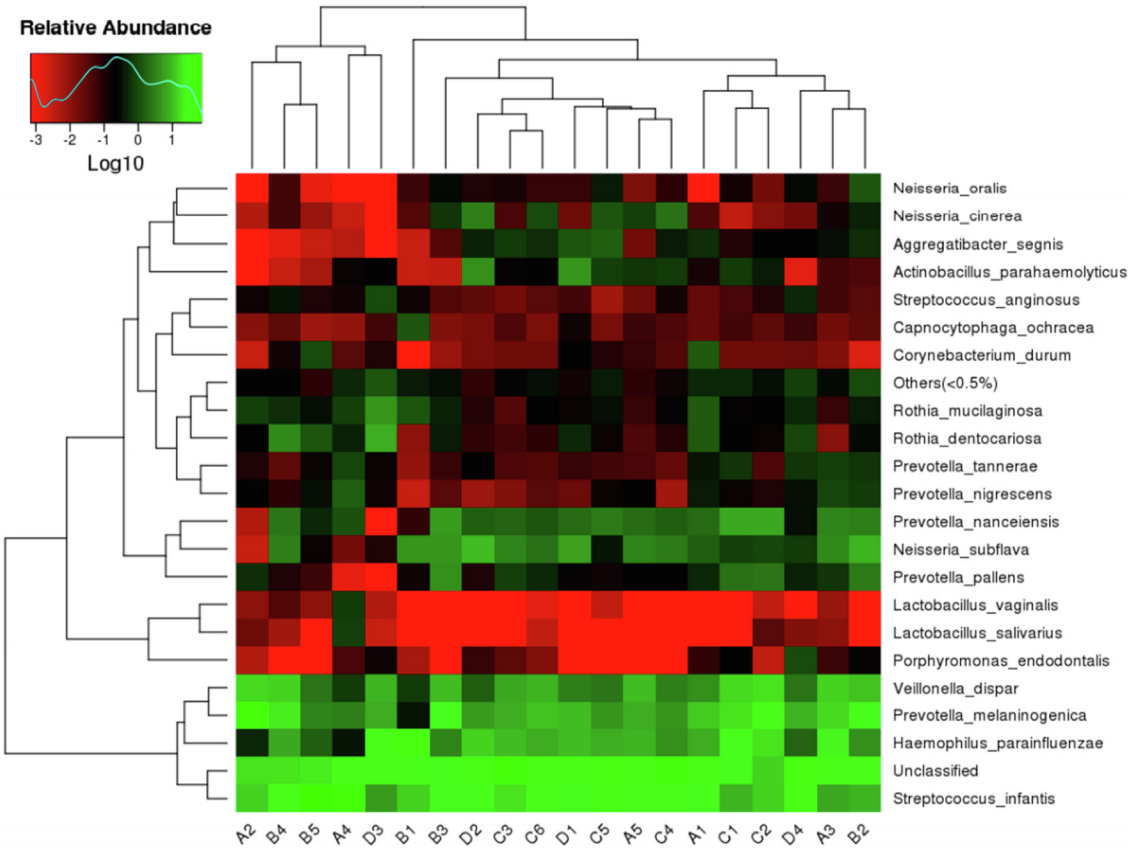

Fig. 5. Heat map showing the relative abundance of bacterial strains among different family groups. Red color signifies that the genus is either absent or present in low abundance, whereas the green color signifies that it is highly abundant.

(P < 0.05) in group B than C (Table 3). At the genus level, Acetobacter, Escherichia, and Staphylococcus were significantly greater in group A than B or C. In contrast, *Johnsonella* was more in group B than A, and *Actinobacillus* was higher in C than A (P < 0.05). Group A was also characterized by a significantly higher abundance of Butyrivibrio and Staphylococcus compared to group D. Whereas group D showed higher abundance (P < 0.05) of *Klebsiella* and Nevskia compared to A. Comparatively, *Johnsonella* and *Moryella* were more in group B than C or D (P < 0.05). *Actinobacillus* and *Erwinia* were significantly higher in group C than B and D than B, respectively. *Erwinia* was also a characteristic bacterium of group B compared with C. While, Butyrivibrio, Peptostreptococcus, and Prevotella were greater in group C than D (P < 0.05). At the species level, group A showed higher (P < 0.05) abundance of *Bacillus cereus* and *Staphylococcus epidermidis* compared to group B, C, or D. In group A, the relative abundance of *Acinetobacter lwoffii* was significantly more than group B or C. Whereas, *Nevskia ramosa* was lower in group A compared to B, C, or D (P < 0.05). Moreover, *Johnsonella ignava and Actinobacillus parahaemolyticus,* and *Neisseria oralis* were significantly higher in groups B and C than A, respectively. Compared to group B, *Rothia mucilaginosa* and *Staphylococcus epidermidis* were lower in group C and *Johnsonella ignava* was lesser in family D (P < 0.05). Analysis at the species level was also showed that the relative abundance of *Actinobacillus parahaemolyticus* and unclassified bacteria was significantly greater in group C than B and D than B, respectively (Table 4) (Fig. 5).

Within family groups, different bacterial species were reported to be more dominant among certain family members than the other; *Prevotella melaninogenica, Prevotella histicola* and *Haemophilus parainfluenzae, Veillonella atypica,* and *Haemophilus pittmaniae* were more prevalent in parents of one family (1S and 2S), than the other family member. *Porphyromonas pasteri* was more commonplace in the parent of one family (2A, and 3A). Among one family of group C, the mother and her oldest son were closer than another family member, and they were commonly shared *Prevotella melaninogenica, Prevotella nanceiensis Prevotella histicola, Streptococcus sanguinis, Streptococcus pneumoniae, Veillonella atypica,* and *Haemophilus parainfluenzae* (Table 5) (Figs. S1-S20).

4. Discussion

Human oral microbiota is gradually developing into the oral cavity with age, and more than 600 species are usually seen in the adult population as a whole (Sulyanto et al., 2019). Several studies link the development of the oral microbiota with the parents, ethnicity, and environment (Schloss et al., 2014; Drell et al., 2017; Premaraj et al., 2020; Blaustein et al., 2021). In this study, we noticed that the relative abundance of some phylum, families, genera, and species of bacteria was significantly varied between groups. This result indicates the effect of social networks on the composition of oral microbiota. This finding was in accordance with a previous study of the potential transmission of gut-microbiota between interacting networks (Raulo et al., 2021).

At the species level, group A showed a higher (P < 0.05) abundance of *Bacillus cereus* and *Staphylococcus epidermidis* compared to other groups, and these species are not commonly detected at the human oral cavity (Loberto et al., 2004), *Staphylococcus* epidermidis is frequently seen at the skin. The presence of such like an organism in the oral cavity may cause infection and could easily be resistant to antibiotics (Loberto et al., 2004).

The Acinetobacter lwoffii is normally found in the oropharynx, skin, and perineum of humans (Ku et al., 2000); in this study, it was significantly more in group A than in groups B and C.

Table 5
Frequencies (%) of the most common species among family members, which is generated by NCBI SRA Taxonomy Analysis Tool.

Participant ID	*Prevotella melaninogenica*	*Prevotella histicola*	*Prevotella nanceiensis*	*Streptococcus oricebi*	*Streptococcus sanguinis*	*Streptococcus pneumoniae*	*Veillonella atypica*	*Veillonella rogosae*	*Haemophilus parainfluenzae*	*Haemophilus pittmaniae*	*Porphyromonas pasteri*	*Granulicatella elegans*	*Nisseria subflava*
1H	12			10	9	4			8				
2H	28	29			7	2	13						
3H	14	3		2	2		7		26				
4H					2	24						10	
5H	7	–		–	15	5	4		14			5	7
1M*					8	1			44	7			–
2M	26	2			2	1	8		4				
3M	21	6	10		8	12	8		4				
4M	17				15	4	7		8				
5M**	4				31	9			2				
1S	19	5	12		4		5	12	19	3			
2S	25	4	9	5	4		9	9	10	5			
3S	13		3	9	10			9	18		11		
4S***	9		3		13			2	5		9	3	
5S****	7		6		9	1	2		11	4		7	
6S	14		2		8	18	2	7	10	1	11		1
2A	13				15	5			13		13		4
3A	8		3		14				20		12	5	
4A	9				2		4	5	32				
5A	12	2		17	13	2			2				

* Capnocytophaga leadbetteri = 11%, ** Streptococcus ovis = 10, *** Streptococcus phyrayngis, **** Streptococcus hongkongensis = 12, Streptococcus orisasini = 12.

Erwinia was also a characteristic bacterium of group B; this result agrees with a study conducted in Shanghai. They found Erwinia among the high-abundance species in toddler's mouths (Li et al., 2018). Group C showed a higher abundance of Actinobacillus parahaemolyticus and unclassified bacteria. We noticed that some core oral microbiota, like Prevotella, Streptococcus, Veillonella, Neisseria (Burcham et al., 2020), were significantly varied in our study groups. These variations could be attributed to the dietary intake, social networks, and lifestyle (Brito et al., 2019; Burcham et al., 2020) of different family groups participating in this study.

Prevotella nanceiensis, *Veillonella rogosae* and *Haemophilus pittmaniae* were characteristic of one family (S); this finding supports the hypothesis of possible passage of oral microbiota between family members (Ledder et al., 2018; Mukherjee et al., 2021). Additionally, among parents, different microbial species were more common; this could be due to intimate relationships between them, which could be a reason for bacterial transmission due to kissing (Kort et al., 2014).

The oral beneficial *Lactobacillus salivarius* bacteria was reported more commonly in some family members as shown in the heat map, this bacteria is used to improve the periodontal health, in a study conducted by Iwamoto and his colleagues, they found the *Lactobacillus salivarius* has the capacity to treat halitosis with beneficial effects on tooth bleeding (Iwamoto et al., 2010). Another beneficial bacteria (*Neisseria oralis*) was significantly higher in groups B and C, the presence of *Neisseria oralis* could imply that it is one of the bacteria that help keep the oral microbiota healthy (Lee et al., 2021).

A limitation of our study is the small sample size and difficulties in collecting other oral family health parameters.

5. Conclusions

In summary, this study highlights the precise and perceptible association of oral microbial between family members. Our findings documented the clustering of certain bacterial species in family groups, supporting the role of community in the development of oral microbiota. And support the presence of universally shared bacterial species among the human oral cavity; in addition, different microbial species were characteristics of some family members. These findings support the previous studies, which stated that the composition of the oral microbiome is influenced by environmental factors, lifestyle, and social networks, not by genetic factors (Jo et al., 2021; Mukherjee et al., 2021).

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CRediT authorship contribution statement

Hisham N. Altayb: Conceptualization, Methodology, Validation, Formal analysis, Investigation, Resources, Data curation, Writing – original draft, Writing – review & editing, Visualization, Funding acquisition. **Kamel Chaieb:** Methodology, Investigation. **Othman Baothman:** Validation, Writing – review & editing, Visualization. **Faisal A. Alzahrani:** Resources, Writing – review & editing. **Mazin A. Zamzami:** Validation, Writing – review & editing, Visualization. **Babiker Saad Almugadam:** Software, Formal analysis, Writing – original draft, Supervision.

Data availability

This study data were submitted to NCBI under the following Bioproject: PRJNA760299 and BioSample accessions in an additional file (Table S1).

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Appendix A. Supplementary material

Supplementary data to this article can be found online at https://doi.org/10.1016/j.sjbs.2022.103317.

References

Bartlett, A., Gullickson, R.G., Singh, R., Ro, S., Omaye, S.T., 2020. The link between oral and gut microbiota in inflammatory bowel disease and a synopsis of potential salivary biomarkers. Appl. Sci. 10 (18), 6421. https://doi.org/10.3390/app10186421.

Belkaid, Y., Hand, T., 2014. Role of the microbiota in immunity and inflammation. Cell 157 (1), 121–141.

Bik, E.M., Long, C.D., Armitage, G.C., Loomer, P., Emerson, J., Mongodin, E.F., Nelson, K.E., Gill, S.R., Fraser-Liggett, C.M., Relman, D.A., 2010. Bacterial diversity in the oral cavity of 10 healthy individuals. ISME J. 4 (8), 962–974.

Blaustein, R.A., Michelitsch, L.-M., Glawe, A.J., Lee, H., Huttelmaier, S., Hellgeth, N., Ben Maamar, S., Hartmann, E.M., 2021. Toothbrush microbiomes feature a meeting ground for human oral and environmental microbiota. Microbiome. 9 (1). https://doi.org/10.1186/s40168-020-00983-x10.21203/rs.3.rs-276097/v1.

Brito, I.L., Gurry, T., Zhao, S., Huang, K., Young, S.K., Shea, T.P., Naisilisili, W., Jenkins, A.P., Jupiter, S.D., Gevers, D., 2019. Transmission of human-associated microbiota along family and social networks. Nature Microbiol.

Burcham, Z.M., Garneau, N.L., Comstock, S.S., Tucker, R.M., Knight, R., Metcalf, J.L., 2020. Patterns of oral microbiota diversity in adults and children: A crowdsourced population study. Sci. Rep. 10 (1). https://doi.org/10.1038/s41598-020-59016-0.

Cho, T., Nagao, J.-I., Imayoshi, R., Tanaka, Y., 2014. Importance of diversity in the oral microbiota including candida species revealed by high-throughput technologies. Int. J. Dentistry 2014, 1–5.

Deo, P.N., Deshmukh, R., 2019. Oral microbiome: Unveiling the fundamentals. J. Oral Maxillofacial Pathol.: JOMFP.

Drell, T., Štšepetova, J., Simm, J., Rull, K., Aleksejeva, A., Antson, A., Tillmann, V., Metsis, M., Sepp, E., Salumets, A., Mändar, R., 2017. The influence of different maternal microbial communities on the development of infant gut and oral microbiota. Sci. Rep. 7 (1). https://doi.org/10.1038/s41598-017-09278-y.

Gomez, A., Nelson, K.E., 2017. The oral microbiome of children: Development, disease, and implications beyond oral health. Microb. Ecol. 73 (2), 492–503.

Griffen, A.L., Beall, C.J., Campbell, J.H., Firestone, N.D., Kumar, P.S., Yang, Z.K., Podar, M., Leys, E.J., 2012. Distinct and complex bacterial profiles in human periodontitis and health revealed by 16s pyrosequencing. ISME J. 6 (6), 1176–1185.

Hosgood, H.D., Cai, Q., Hua, X., Long, J., Shi, J., Wan, Y., Yang, Y., Abnet, C., Bassig, B.A., Hu, W., Ji, B.-T., Klugman, M., Xiang, Y., Gao, Y.-T., Wong, J.YY., Zheng, W., Rothman, N., Shu, X.-O., Lan, Q., 2021. Variation in oral microbiome is associated with future risk of lung cancer among never-smokers. Thorax 76 (3), 256–263.

Iwamoto, T., Suzuki, N., Tanabe, K., Takeshita, T., Hirofuji, T., 2010. Effects of probiotic lactobacillus salivarius wb21 on halitosis and oral health: An open-label pilot trial. Oral Surgery, Oral Med., Oral Pathol., Oral Radiol., Endodontol. 110 (2), 201–208.

Jo, R., Yama, K., Aita, Y., Tsutsumi, K., Ishihara, C., Maruyama, M., Takeda, K., Nishinaga, E., Shibasaki, K.-I., Morishima, S., 2021. Comparison of oral microbiome profiles in 18-month-old infants and their parents. Sci. Rep. 11 (1). https://doi.org/10.1038/s41598-020-78295-1.

Katz, K.S., Shutov, O., Lapoint, R., Kimelman, M., O'Sullivan, C., Brister, J.R., 2021. A fast, scalable, minhash-based k-mer tool to assess sequence read archive next generation sequence submissions. BioRxiv.

Kort, R., Caspers, M., van de Graaf, A., van Egmond, W., Keijser, B., Roeselers, G., 2014. Shaping the oral microbiota through intimate kissing. Microbiome. 2 (1). https://doi.org/10.1186/2049-2618-2-41.

Ku, S.C., Hsueh, P.R., Yang, P.C., Luh, K.T., 2000. Clinical and microbiological characteristics of bacteremia caused by acinetobacter lwoffii. Eur. J. Clin. Microbiol. Infect. Dis. 19 (7), 501–505.

Ledder, R.G., Kampoo, K., Teanpaisan, R., McBain, A.J., 2018. Oral microbiota in severe early childhood caries in thai children and their families: A pilot study. Front. Microbiol.

Lee, E., Park, S., Um, S., Kim, S., Lee, J., Jang, J., Jeong, H.-o., Shin, J., Kang, J., Lee, S., Jeong, T., 2021. Microbiome of saliva and plaque in children according to age and dental caries experience. Diagnostics. 11 (8), 1324. https://doi.org/10.3390/diagnostics11081324.

Lee, W.-H., Chen, H.-M., Yang, S.-F., Liang, C., Peng, C.-Y., Lin, F.-M., Tsai, L.-L., Wu, B.-C., Hsin, C.-H., Chuang, C.-Y., Yang, T., Yang, T.-L., Ho, S.-Y., Chen, W.-L., Ueng, K.-C., Huang, H.-D., Huang, C.-N., Jong, Y.-J., 2017. Bacterial alterations in salivary microbiota and their association in oral cancer. Sci. Rep. 7 (1). https://doi.org/10.1038/s41598-017-16418-x.

Li, F., Tao, D., Feng, X., Wong, M., Mei, C., Lu, H., 2018. Establishment and development of oral microflora in 12–24 month-old toddlers monitored by high-throughput sequencing. Front. Cellular Infect. Microbiol.

Li, Y., Ge, Y., Saxena, D., Caufield, P.W., 2007. Genetic profiling of the oral microbiota associated with severe early-childhood caries. J. Clin. 45 (1), 81–87.

Loberto, J.C.S., Martins, C.A.., Santos, S.S., Cortelli, J.R., Jorge, A.O.C., 2004. Staphylococcus spp. In the oral cavity and periodontal pockets of chronic periodontitis patients. Brazilian J. Microbiol. 35 (1-2). https://doi.org/10.1590/S1517-83822004000100010.

Marsh, P.D., Zaura, E., 2017. Dental biofilm: Ecological interactions in health and disease. J. Clin. Periodontol. 44, S12–S22.

Mukherjee, C., Moyer, C.O., Steinkamp, H.M., Hashmi, S.B., Beall, C.J., Guo, X., Ni, A., Leys, E.J., Griffen, A.L., 2021a. Acquisition of oral microbiota is driven by environment, not host genetics. Microbiome.

Mukherjee, C., Moyer, C.O., Steinkamp, H.M., Hashmi, S.B., Beall, C.J., Guo, X., Ni, A.i., Leys, E.J., Griffen, A.L., 2021b. Acquisition of oral microbiota is driven by environment, not host genetics. Microbiome 9 (1). https://doi.org/10.1186/s40168-020-00986-8.

Park, A.W., Yaacob, H.B., 1994. Pathogenic Microbes of the Oral Environment. J. Nihon Univ. School Dentistry 36 (1), 1–33.

Premaraj, T.S., Vella, R., Chung, J., Lin, Q., Panier, H., Underwood, K., Premaraj, S., Zhou, Y., 2020. Ethnic variation of oral microbiota in children. Sci. Rep. 10 (1). https://doi.org/10.1038/s41598-020-71422-y.

Qi, Y., Wu, H.-M., Yang, Z., Zhou, Y.-F., Jin, L., Yang, M.-F., Wang, F.-y., 2021. New insights into the role of oral microbiota dysbiosis in the pathogenesis of inflammatory bowel disease. Dig. Dis. Sci. 67 (1), 42–55.

Raulo, A., Allen, B.E., Troitsky, T., Husby, A., Firth, J.A., Coulson, T., Knowles, S.C., 2021. Social networks strongly predict the gut microbiota of wild mice. ISME J.

Schloss, P.D., Iverson, K.D., Petrosino, J.F., Schloss, S.J., 2014. The dynamics of a family's gut microbiota reveal variations on a theme. Microbiome. 2 (1).

Sedghi, L., DiMassa, V., Harrington, A., Lynch, S.V., Kapila, Y.L., 2021. The oral microbiome: Role of key organisms and complex networks in oral health and disease. Periodontol 2000.

Seerangaiyan, K., van Winkelhoff, A.J., Harmsen, H.J.M., Rossen, J.W.A., Winkel, E.G., 2017. The tongue microbiome in healthy subjects and patients with intra-oral halitosis. J. Breath Res. 11 (3), 036010.

Sulyanto, R.M., Thompson, Z.A., Beall, C.J., Leys, E.J., Griffen, A.L., 2019. The predominant oral microbiota is acquired early in an organized pattern. Sci. Rep. 9 (1).

Wade, W.G., 2013. Detection and culture of novel oral bacteria. Oral microbial ecology–current research and new perspectives. Caister Academic Press, Norfolk.

Weyrich, L.S., 2021. The evolutionary history of the human oral microbiota and its implications for modern health. Periodontol 2000. https://doi.org/10.1111/prd.12353.

Willis, J.R., Gabaldón, T., 2020. The human oral microbiome in health and disease: From sequences to ecosystems. Microorganisms.

Wingfield, B., Lapsley, C., McDowell, A., Miliotis, G., McLafferty, M., O'Neill, S.M., Coleman, S., McGinnity, T.M., Bjourson, A.J., Murray, E.K., 2021. Variations in the oral microbiome are associated with depression in young adults. Sci. Rep. 11 (1). https://doi.org/10.1038/s41598-021-94498-6.

Zhang, L., Liu, Y., Zheng, H.J., Zhang, C.P., 2020. The oral microbiota may have influence on oral cancer. Front. Cellular Infect. Microbiol.

Zhang, Y., Zhu, C., Cao, G., Zhan, J., Feng, X., Chen, X., 2021. Dynamic alterations of oral microbiota related to halitosis in preschool children. Front. Cellular Infect Microbiol.