Original article

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

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A B S T R A C T

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 pittmanae 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 sub-
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, Lactobacillus, 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 lies between an altitude of 21.54333 and longitudes of 39.172778 and covers an area of 1500 km². 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.

Table 1

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

Fig. 1. Shared and unique OTUs (378) across groups. Between the four groups A: Sudanese, B: Yemen, 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
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

![Figure 2](image_url)

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 OTUs with groups C and D, respectively. Moreover, group C showed 228 shared OTUs with group D (Fig. 1G). The highest numbers of unique OTUs 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
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 | Taxonomy level | Name Group |
|-----------------------------------------|----------------|------------|----------------|------------|
| Phylum Cyanobacteria                   |                | A          | Family Actinobacteria |                |
| Family Nocardiaceae, Oxalobacteraceae, and Staphylococaceae |                | D          | Family Acetobacteraceae, Enterobacteriaceae, Rikenellaceae, and Staphylococaceae |                |
| Rhodocyclus    |                | C          | Enterobacteriaceae, Mycoplasmataceae, Nocardiaceae, Oxalobacteraceae, Rikenellaceae, and Xanthomonadaceae |                |
| Erythrobacteraceae, Pseudomonadaceae, Rhodocyclaceae, and Sinobacteraceae |                | C          | Coriobacteriaceae, Microbacteriaceae, Mycoplasmataceae, Peptococaceae, and Staphylococaceae |                |
| Rs_045 and Staphylococaceae |                | A          | Enterobacteriaceae and Mycoplasmataceae |                |
| Erythrobacteraceae and Veillonellaceae |                | D          | Erythrobacteraceae and Veillonellaceae |                |

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                               |                | A than B, |                |
| Actinobacteria                         |                | B than C    |                |
| Nocardiaceae, Oxalobacteraceae,        |                | A than C    |                |
| Enterobacteriaceae, Rikenellaceae, and Staphylococaceae |                | A than C    |                |
| Enterobacteriaceae, Mycoplasmataceae, Nocardiaceae, Oxalobacteraceae, Rikenellaceae, and Xanthomonadaceae |                | A than C    |                |
| Erythrobacteraceae, Pseudomonadaceae, Rhodocyclaceae, and Sinobacteraceae |                | C than A    |                |
| Rs_045 and Staphylococaceae |                | A than D    |                |
| Coriobacteriaceae, Microbacteriaceae, Mycoplasmataceae, Peptococaceae, and Staphylococaceae |                | B than C    |                |
| Erythrobacteraceae and Veillonellaceae |                | C than B    |                |

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.

| 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, Nevska, Paludibacter, Pseudomonas, Ralibracter, Sphingomonas | C than A |
|                | Butyrivibrio and Staphylococcus | A than D |
|                | Klebsiella and Nevska | D than A |
|                | Alloscardovia, Atopobium, Erwinia, Johnsonella, Moryella, Mycoplasma, Peptococcus, Rothia, Staphylococcus | B than C |
|                | Actinobacillus, Burkholderia, Methyloversatilis, Ralibracter | C than B |
|                | Johnsonella, Lautropia, Moryella | B than D |
|                | Erwinia | D than B |
|                | Butyrivibrio, Erythromicrobium, Peptostreptococcus, Prevotella, Veillonella | C than D |
| Species        | Curvibacter, Erwinia, Klebsiella, Mycoplasma, Scardovia, Slackia | D than C |
|                | Acinetobacter lwoffii, Bacillus cereus, Escherichia coli, Staphylococcus epidermidis | A than B |
|                | Johnsonella ignava, Nevska_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 paraeaeolyticus, Neisseria oralis, Nevska ramosa, Sphingomonas yabuuchiae | C than A |
|                | Bacillus cereus, Staphylococcus epidermidis | A than D |
|                | Nevska ramosa, Sphingomonas yabuuchiae | D than A |
|                | Rothia mucilaginosa, Staphylococcus epidermidis | B than C |
|                | Actinobacillus paraeaeolyticus | 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.
studies link the development of the oral microbiota with the par-
the adult population as a whole (Sulyanto et al., 2019). Several
cavity with age, and more than 600 species are usually seen in
(Figs. S1-S20).

Johnsonella ignava in group C and
Prevotella melaninogenica, Prevotella histicola
than the other; reported to be more dominant among certain family members
the relative abundance of Actinobacillus parahaemolyticus
(P < 0.05). Analysis at the species level was also showed that
Rothia mucilaginosa
and
in groups B and C than A, respectively. Compared to group B,
B, C, or D (P < 0.05). Moreover,
Johnsonella ignava and Actinobacillus
unclassified bacteria was significantly greater in group C than B,
was significantly more in group A than in groups B and C.

Within family groups, different bacterial species were
reported to be more dominant among certain family members
than the other; Prevotella melaninogena, 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 pasteurii
was more commonplace in the parent of one family (2A, and
3A). Among one family of group C, the mother and her eldest
son were closer than another family member, and they were
commonly shared Prevotella melaninogena, 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 epi-
dermidis 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.
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 para-haemolyticus 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 microbiota, 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).

Funding

This project was funded by the Deanship of Scientific Research (DSR) at King Abdulaziz University, Jeddah, under grant no. (G: 615-130-1441). The authors, therefore, acknowledge with thanks DSR for their technical and financial support.

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 Baithman: 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.

Acknowledgements

In this section, you can acknowledge any support given which is not covered by the author contribution or funding sections. This may include administrative and technical support, or donations in kind (e.g., materials used for experiments).

Appendix A. Supplementary material

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

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