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Review Article

# Microbiota of Orofacial Cleft –A Comprehensive Review

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**Abstract**

Oral cavity microbiota act as an salient part of the human microbiota , which remains sterile throughout prenatal development, first hours following delivery diverse ecosystem starts to colonize ,which includes several hundred species and has an important function to protect against colonization of extrinsic bacteria which could affect systemic health. On the other hand, the most common oral diseases like caries, gingivitis and periodontitis are based on these microorganisms, and organized as biofilms. In orofacial cleft cases several thousand diverse species has been seen because due to irregular dentition and the oral cavity is always opening outside through the cleft. Sequencing to characterize bacterial flora in cleft cases samples collected at 3, 6, 12, 24 months and 7 years of age in 90 longitudinally followed children, 16S rRNA genes from sample DNA were amplified, cloned, and transformed into Escherichia coli. In 2,589 clones, 141 predominant species were detected, of which over 60% have not been cultivated. Thirteen new phylotypes were identified. Species common to all sites belonged to the genera *Gemella*, *Granulicatella*, *Streptococcus*, and *Veillonella*. in this review article literature discuss the role of microbial biofilms in orofacial cleft cases and the study utilize culture-independent molecular techniques to explain the bacterial diversity in the orofacial cleft oral cavity, and to determine the site and subject specificity of bacterial colonization.

**Keywords**: Orofacial Cleft ,Oral microbiota, *Simonsiella mitis, Simonsiella mitis* biovar, *Streptococcus infantis, Granulicatella elegans, G. hemolysans, Neisseria subflava*

## Introduction

Human oral cavity (mouth) is the area of a complex microbiome consisting of bacteria, protozoa, fungi archaea, and viruses. These bacterias are responsible for the infection of the periodontal (gum) and dental caries (tooth decay).[1] Periodontal diseases usually refer to common inflammatory disorders known as gingivitis and periodontitis, which are caused by a pathogenic microbiota in the subgingival biofilm, including *Porphyromonas gingivalis*, *Aggregatibacter actinomycetemcomitans*, *Tannerella forsythia* and *Treponema denticola* that trigger innate,Dental caries are caused by plaques,[2] which are a community of microorganisms in biofilm formation. Genetic and peripheral factors lead to variations in the oral microbiome. Usually, commensalism and coexistence between microorganisms and the host, In unusual situation the cariogenic microorganisms predominate, as a result make dental caries. The chance for developing tooth decay in individuals is dependent on factors such as immune system and oral microbiome which itself is affected by the environmental and genetic determinants. The oral microbs vary in person to person Utilizing advanced molecular biology techniques, new cariogenic microorganisms species have been discovered. The development and structural details of the neonatal microbiome have been almost elucidated, with a main focus on the microbial population inhabiting the lower intestinal tract and the oral cavity colonization following delivery, but the cleft palate cases is still quite different.[1] The oral microbiota represents an important part of the human microbiota, which includes several hundred diverse species. It is a normal part of the oral cavity and has an important function to protect against colonization of extrinsic bacteria which could affect systemic and mostly gastrointestinaltract health. While (medical) research focused on the planktonic phase of bacteria over the last 100 years, it is nowadays generally known, that oral microorganisms are organised as biofilms. On any non-shedding surfaces of the oral cavity dental plaque starts to form, which meets all criteria for a microbial biofilm and is subject to the so-called succession. When the sensitive ecosystem turns out of balance – either by overload or weak immune system – it becomes a challenge for local or systemic health.[3] It is necessary to first define the bacterial flora of the healthy oral cavity before we can determine the role of bacterial flora of the orofacial cleft The resident microbiota depends mainly on external factors, including gestational age, mode of delivery, type of feeding, the length of hospital stay following delivery, and general condition.[4,5,6]Bacterial composition patterns changed through time, starting with early colonizers including Streptococcus and Veillonella, other bacterial genera such as Neisseria settled after 1 or 2 years of age. Some of the study says Infants born by Caesarean section had initially indistinct bacterial content compared with vaginally delivered infants, Birth mode is known to influence the oral microbiota in infants at 3 months of age and infants born by vaginal delivery have a higher oral microbial diversity than CS infants in the first 6 months post-birth[7]

A higher abundance of health-associated streptococci and lactobacilli were detected in the oral cavity of vaginally delivered infants while infants delivered by CS acquired *Streptococcus mutans* nearly 12 months earlier than vaginally delivered infants[8] but this was recovered with age. Shorter breastfeeding habits and antibiotic treatment during the first 2 years of age were associated with a distinct bacterial composition at later age.

The saliva samples were taken from infants after birth at a mean age of 8.25 months.[9] 3 months [10] and at 1, 3 and 6 months [15]

without taking vaginal or skin samples from the mother, based on the mode of delivery. Studies of the impact of the feeding modality (breast or formula) on the oral microbiome have also demonstrated microbiota compositional changes .[11,12]

*Lactobacillus* species, such as *Lactobacillus grasseri*, were detected at higher abundance in breastfed infants, compared to formula-fed infants .and these oral lactobacilli have antimicrobial properties and probiotic qualities [13,14]. Some study indicates that the mode of delivery does not have any major influence on the infant oral microbiota Changes in diversity and composition were observed in the oral microbiota of the infant over time. These changes are more visible at 6 months and beyond, and again at 1 year of age, when both teeth begin to emerge, and weaning of introduced food begins.[15] The genus Streptococcus is one of the dominant bacterial groups found in human milk [16,17] and various species, including Streptococcus salivarius, are frequently found in the infant oral cavity. [18] The metabolic products derived from Streptococcus species from the dietary oligosaccharides in breast milk might pave the way for the establishment of other microorganisms in the oral cavity, an unhealthy oral microbiome can have important effects beyond the oral cavity, including elevated cardiovascular risk .[19] For instance, in vitro studies have demonstrated the ability of periodontal bacteria to increase the probability of thrombus formation, which could lead to ischaemic cardiovascular events.[20] Therefore, it is of interest to understand the colonization patterns of oral commensals during childhood and the potential benign effect of oral bacteria in preventing oral and systemic diseases, including microorganisms, which have been associated with health conditions [21,22]. A more detailed understanding of oral microbial communities' development in health and disease fundamental and the use of high-throughput sequencing techniques now allow exploring microbial composition and diversity in low volume oral samples to an unprecedented level of detail, [23] in comparison with culturing or early molecular methodologies. In this study, we aimed to address the temporal evolution and maturation of the oral and orofacial cleft microbial ecosystem during infancy, childhood.

In orofacial cleft cases the complex structure of the oral cavity, with its numerous recesses, the mucosal folds of the palate, and the invaginations of the cheeks and tongue, creates niches with different pH values, local oxygen concentrations,[24,25]

## Materials and Methods

Study Design, Ethics and Recruitment

First study is to explore the identification of microbial flora on basis of method of delivery The study design was to recruit a cohort of mother–infant dyads for longitudinal sampling (from birth to 1 year of age). The study cohort included pregnant mothers recruited antenatally, and their infants whom were followed and sampled from birth to 1 year of age.

All mothers were consented for the study within 1–2 months prior to their estimated date of delivery, and two groups of mother–infants dyads ($n$ = 185) were created based on their mode of delivery (full-term SVD infants and full-term CS delivered infants).in this study, although sampling began at birth, all neonates and infants are defined under the same label 'infant', irrespective to the age 28 days less is neonate and 29 days greater defined as infant. Inclusion criteria, applied to both SVD and CS delivered infants, were that all infants were born full term (>35 weeks' gestation) and medically healthy. Neonates did not receive antibiotic treatment at birth and infants were breastfed for a minimum of 4 weeks post-partum. Mothers who delivered neonates by CS were all given IV antibiotics prior to the surgery. Exclusion criteria included breastfeeding duration shorter than 4 weeks, Skin samples from 38

mothers of CS infants were collected, and 37 vaginal samples from SVD delivered infants. Some loss of samples from the mothers' skin or vaginal samples were not collected at birth due to circumstances, The saliva sample collected from the infant's oral cavity at each time point was labeled as 'Oral week 1, Oral week 4, etc.' in the "Results" section. The first saliva sample was collected from the newborn infants within 2 days of delivery, before the mother's left hospital. ($n$ = 77) (labelled as week 1). This was repeated again at 4 weeks ($n$ = 61), 8 weeks ($n$ = 60), 24 weeks (6 months) ($n$ = 64) and at 1 year ($n$ = 84) of age. All saliva samples were placed immediately on dry ice, transported to the laboratory, where they were frozen until further analysis, and stored at −80°C.Sample extraction and processing Extraction of DNA from all samples (vagina, skin and saliva/oral cavity) was carried out using the MO BIO PowerSoil DNA Isolation kit (Qiagen) along with the MO BIO PowerLyzer® 24 homogeniser with some initial optimisation for extraction from using a CatchAll™ .DNA was visualised on a 0.8% agarose gel and quantified using the Nanodrop 1000 (Thermo Scientific, Ireland). DNA was then stored at −80°C.

Secondly Microbiological smears of the normal Oral cavity mucosa and orofacial cleft malformation collected from neonates and elderly people, irrespective to the age and sex for this correlation study of both beneficial and harmful microbiota The inclusion criteria for newborns were as follows (1) complete Cleft lip and palate or Cleft soft palate , (2) gestational age over 37 weeks, (3) birth weight of 2,500–4,000 g, and (4) Apgar score of 9-10 at 1 min and of 10 at 5 min. (5) natal or neonatal teeth, (6) deciduous teeth at T2 [11]The exclusion criteria were (1) the coexistence of orofacial cleft with other developmental abnormalities, (2) antibiotic therapy, (3) respiratory tract infections, (4) tube feeding, (5) treatment with palatal plate, (6) past surgical repair of cleft lip and/or palate, and (7) failure to appear for the follow-up visit between the eighth and eighteenth week of life. The Inclusion criteria of elderly were (1) ranging in age from 23 to 55 (2) no clinical signs of oral mucosal disease, (3) did not suffer from severe halitosis, (4) The periodontia were healthy, (5) all periodontal pockets were less than 3 mm deep with no redness or inflammation of the gums. (6) did not have active white spot lesions or caries on the teeth.

The exclusion criteria were (1) antibiotic therapy for the last 6 months, (2) respiratory tract infections, (3) treatment with palatal plate, (4) past surgical repair of cleft lip and/or palate, Oral samples were directly suspended in 50 μl of 50 mM Tris buffer (pH 7.6), 1 mM EDTA, pH 8, and 0.5% Tween 20. Proteinase K (200 μg/ml; Roche Applied Science, Indianapolis, IN) was added to the mixture.

The samples were then heated at 55°C for 2 h. Proteinase K was inactivated by heating at 95°C for 5 min. Detection of species is dependent upon obtaining DNA that can be amplified.by using lysis technique, were able to detect many hard-to-lyse species, such as species of *Actinomyces* and *Streptococcus*. The 16S rRNA genes were amplified under standardized conditions using a universal primer set (forward primer, 5′-GAG AGT TTG ATY MTG GCT CAG-3′; reverse primer, 5′-GAA GGA GGT GWT CCA RCC GCA-3′) (27).

PCR was performed in thin-walled tubes with a GeneAmp PCR system 9700 (ABI, Foster City, CA). One microliter of the lysed sample was added to a reaction mixture (final volume, 50 μl) containing 20 pmol of each primer, 40 nmol of deoxynucleoside triphosphates, and 1 U of Platinum *Taq* polymerase (Invitrogen, San Diego, CA). In a hot-start protocol, the samples were preheated at 95°C for 4 min, followed by amplification under the following conditions: denaturation at 95°C for 45 s, annealing at 60°C for 45 s, and elongation at 72° for 1.5 min, with an

additional 15 s for each cycle. A total of 30 cycles were performed; this was followed by a final elongation step at 72°C for 15 min. The results of the PCR amplification were examined by electrophoresis in a 1% agarose gel. DNA was stained with ethidium bromide and visualized under short-wavelength UV light.

Cloning procedures.

Cloning of PCR-amplified DNA was performed with the TOPO TA cloning kit (Invitrogen) according to the instructions of the manufacturer. Transformation was done with competent *Escherichia coli* TOP10 cells provided by the manufacturer. The transformed cells were then plated onto Luria-Bertani agar plates supplemented with kanamycin (50 μg/ml), and the plates were incubated overnight at 37°C. Each colony was placed into 40 μl of 10 mM Tris. Correct sizes of the inserts were determined in a PCR with an M13 (−20) forward primer and an M13 reverse primer (Invitrogen). Prior to sequencing of the fragments, the PCR-amplified 16S rRNA fragments were purified and concentrated with Microcon 100 (Amicon, Bedford, MA), followed by use of the QIAquick PCR purification kit (QIAGEN, Valencia, CA).

16S rRNA gene amplification primers

Primers used for PCR amplification were the V4–V5 region primers 520F (AYTGGGYDTAAAGNG) and 926R (CCGTCAATTYYTTTRAGTTT) due to their high classification accuracy and consistent results.

Primers for Illumina sequencing contain the sequencing primer-binding sites, forward or reverse 16S rRNA gene-specific primer and a 10-nt in-line multiplexing identifier (MID).

Dual separate MIDs were attached to both ends of the PCR product. The V4–V5 amplicons for Illumina sequencing were generated using a two-step amplification procedure. The first step reaction mix contained 50 μl BIO-X-ACT™ Short Mix (BIOLINE), 10 μl of 2 nM forward and reverse primers, 50 ng genomic DNA and ddH2O to give a final volume of 100 μl. Cycling conditions were an initial 95°C, 5-min denaturation step; 30 cycles of 95°C for 15 s, 42°C for 15 s and 72°C for 30 s; and a final 10-min extension at 72°C. The products were purified using solid phase reversible immobilisation (SPRI) select beads (Beckman Coulter, IN) as per manufacturer's instructions, using a 0.9:1 volume ratio of beads to product. The purified PCR products were eluted in 40 μl of ddH2O. DNA quantity was assessed via Quant-iT™ PicoGreen® dsDNA Assay Kit (Invitrogen™). The samples were pooled in equimolar amounts (20 ng per sample) and then sequenced by Eurofins Genomics (Eurofins Genetic Services Ltd., I54 Business Park, Valiant way Wolverhampton WV9 5GB, UK) using Illumina MiSeq 2 × 300 bp paired end technology. Nextflex Rapid library preparation was carried out by the company to attach bridge adaptors necessary for clustering. Sequencing of 16S DNA was carried out on the V4/V5 region using a Miseq (300 bp paired-end reads). Sequence data were stored on a Linux server and backed up on external hard drives.

**Alpha- and beta-diversity analysis**

Alpha- and beta-diversity metrics were calculated in Qiime. To calculate diversity metrics, several additional steps were carried out (also in Qiime). The OTU table was rarefied (single_rarefaction.py) at 10,540 reads (the lowest read count in the data set). Representative OTU sequences were aligned using pyNAST (align_seqs.py) and filtered to remove columns that do not contribute to phylogenetic signal (filter_alignment.py). A phylogenetic tree was generated using FastTree (make_phylogeny.py). This tree is necessary for phylogenetic alpha- and beta-diversity metrics. The rarefied OTU table was used in the calculation of all diversity metrics.

The following alpha-diversity metrics were calculated: chao1, Shannon (Shannon's

index), Simpson (Simpson's index), observed species (OTU count) and phylogenetic (PD whole tree). The following beta-diversity metrics were calculated: weighted and unweighted UniFrac distances and Bray–Curtis dissimilarity.

**Statistics and data visualisation**

All statistics and data visualisation were carried out in R (v3.2.3 and v3.4.0) (Statistical and Computing, Vienna 2016). Alpha-diversity box plots were created using the package ggplot2. Unpaired analysis was also completed, with the Mann–Whitney *U* test being used to compare two groups and Kruskal–Wallis for three or more groups, both by paired and unpaired analysis. A Mann–Whitney *U* test was used to test whether SVD- and CS-born babies differed significantly for each time point. A Kruskal–Wallis test was performed on alpha-diversity metrics for each infant time point, followed by Mann–Whitney *U* pairwise comparisons corrected using the Benjamini–Hochberg method [52]. Dunn test was performed for the pairwise comparison of the alpha diversity for the mother samples with the infant time points. PCoA plots of beta-diversity metrics were created using the package ade4. Statistical differences in beta diversity were tested using the adonis function from the vegan package. Taxon abundance bar plots were created using the packages reshape2, ggplot2 and ggthemes. Taxon abundance was normalised to sample proportions for the bar plots. Kruskal–Wallis tests and Benjamini–Hochberg pairwise tests were used to test for statistical difference in particular taxa at different time points. Clustering of mother–infant pairs based on beta diversity was performed using the hclust function from base R, and the plot created using the rafalib package. The heatmaps to investigate clustering of mother–infant pairs and infant time points based on genera abundances were created using the metagenomeSeq package in R.

**Result and Discussion**

The diversity of the oral microbiota of the infant is influenced by birth mode at 1 week of age, but not beyond 1 week of age. To investigate the influence of birth mode on the oral microbiota composition as the infant increases in age, infant oral microbiota data were separated based on birth mode (SVD or CS) at the various time points (week 1, week 4, 6 months and 1 year). Alpha diversity was used to measure the overall diversity of the community present in the sample. Four indices were used (Fig: 1). The alpha diversity as represented by Shannon diversity index, of the infant oral microbiota at 1 week of age, was influenced by birth modality ($p$-value < 0.037), but at an older age, there was no influence of birth mode on the oral microbiota of the infant. Shannon diversity index takes the abundance of species into account, and in this case, this index indicates that the diversity of the infant oral microbiota at week 1 is lower in SVD infants, compared to CS infants. Therefore, the species abundance is richer in CS infant oral microbiota.[1]

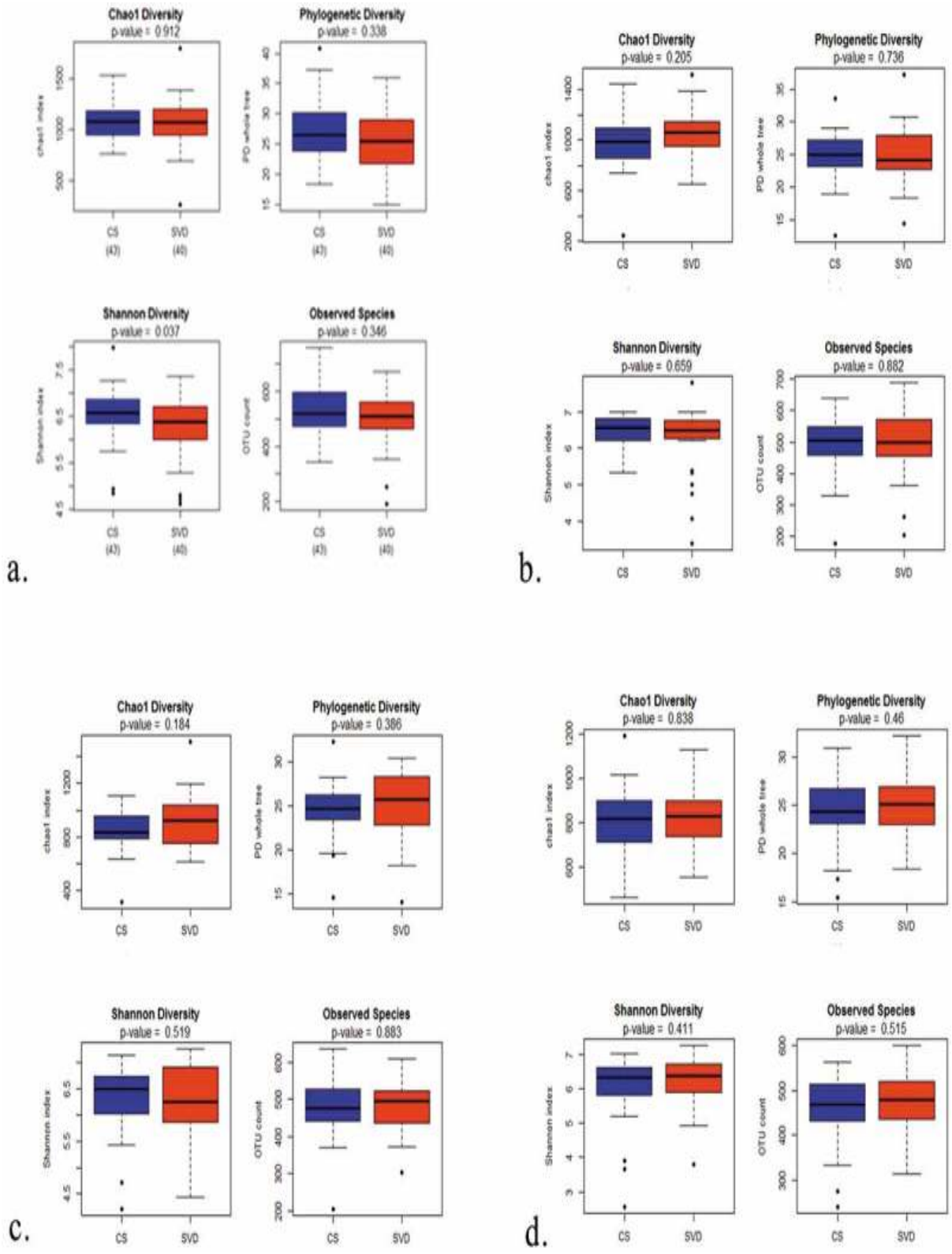


**Fig. 1:** Alpha -diversity measurement of the influence of mode of delivery Spontaneous vaginal delivery SVD Vs caesarean section [CS] on the infant oral microbiota at various time point [ week 1,week 2, week3 ,week 4 6 months and 1 year ]Boxplot of the cha01 diversity, Observed sepecies, phylogenetic diversity and Shannon diversity in the two groups [SVD and CS ] AT [a] week I, [b] week 4, [c] 6 months and [d] 1 year .outliers are represented by black points[26]

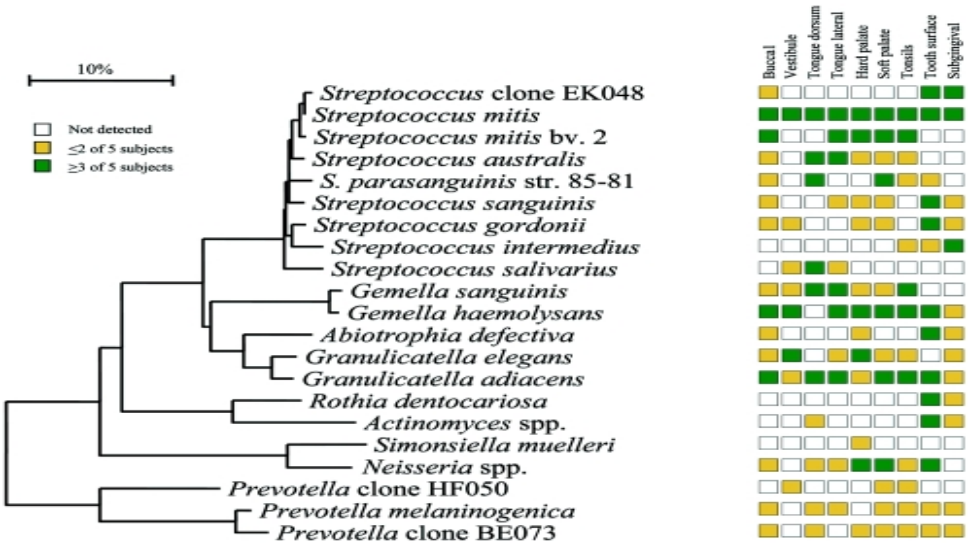


**Fig. 2:** Site specificity of predominant bacterial species in the oral cavity. In general, bacterial species or phylotypes were selected on the basis of their detection in multiple subjects for a given site. Distributions of bacterial species in oral sites among subjects are indicated by the columns of boxes to the right of the tree as follows: not detected in any subject (clear box), <15% of the total number of clones assayed (yellow box), ≥15% of the total number of clones assayed (green box). The 15% cutoff for low and high abundance was chosen arbitrarily. Marker bar represents a 10% difference in nucleotide sequences.

On the hard palate, the predominant bacterial species included S. mitis, S. mitis biovar 2, Streptococcus sp. clone FN051, Streptococcus infantis, Granulicatella elegans, G. hemolysans, and Neisseria subflava (Fig. 3). On the soft palate, S. mitis, other cultivable and not-yet-cultivable species of Streptococcus, G. adiacens and G. hemolysans were predominant (Fig. 4).

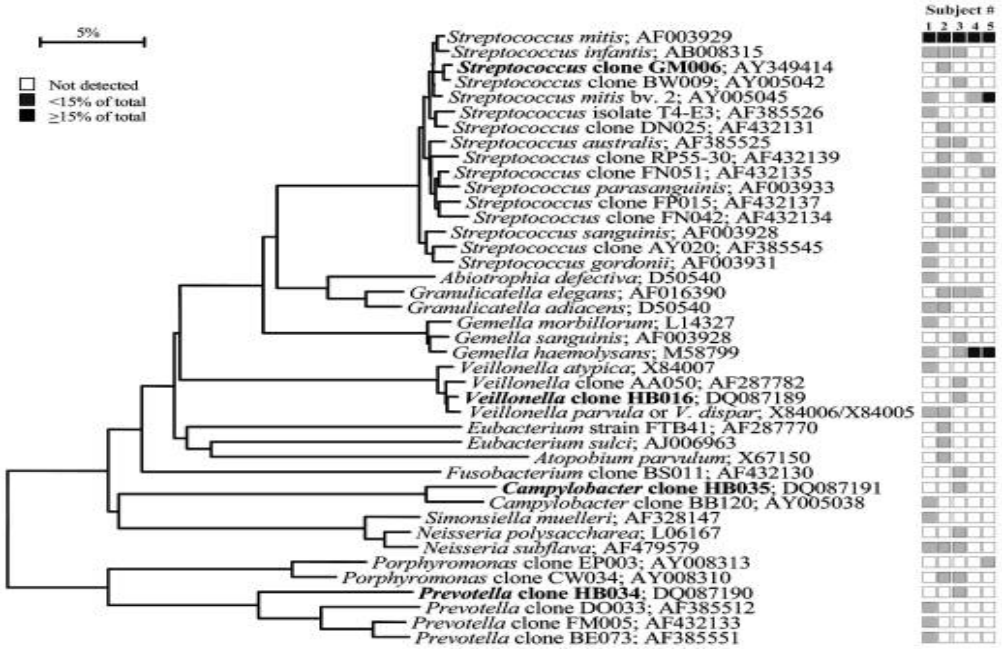


**Fig. 3:** Bacterial profile of the hard palate of healthy subjects .novel phylotypes identified in this study are indicated in bold Genbank accession numbers are provided. Marker bar represents a 5% difference in nucleotide sequences

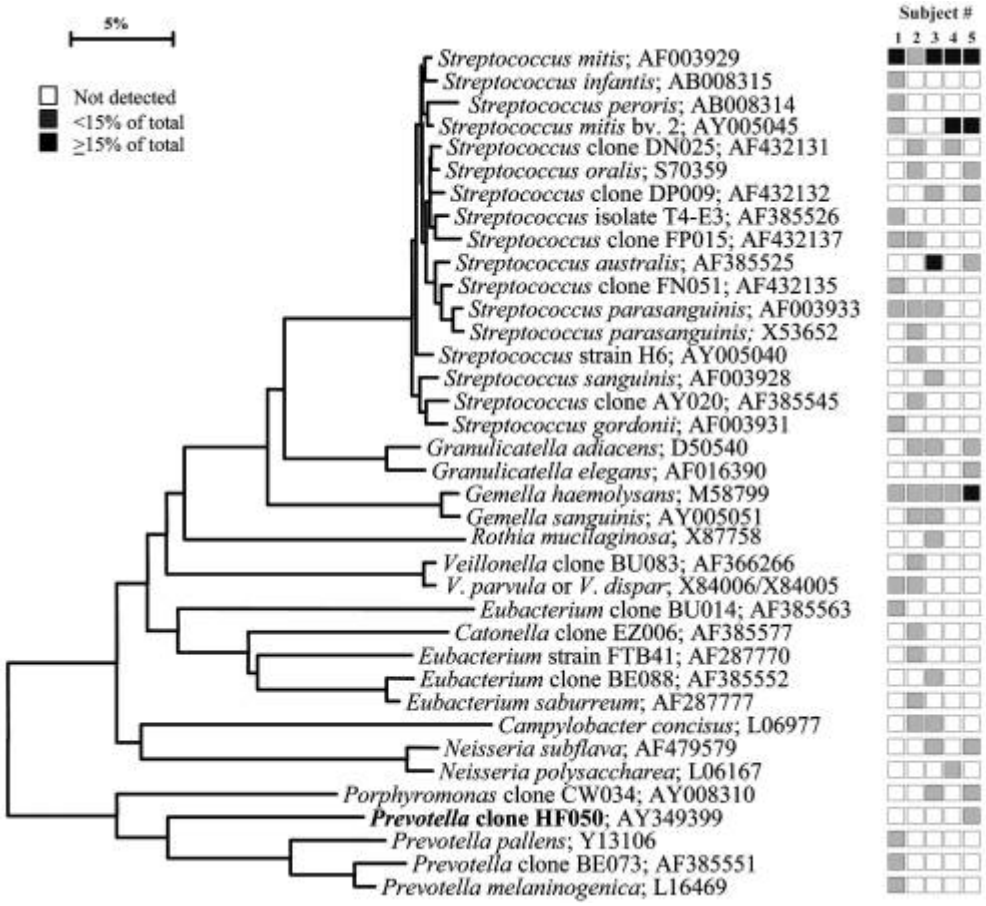


**Fig. 4:** Bacterial profile of the soft palate of healthy subjects .Novel phylotypes identified in this study are indicated in bold Genbank accession numbers are provided. Marker bar represents a 5% difference in nucleotide sequences

**Table 1: Statistical comparison of microorganism frequency [prevalence] colonization and growth intensity between cleft lip and palate group and cleft lip and soft palate group at T1**

| Microorganism | CLP group (n=30) | | | | *p* | CSP group (n=25) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (%) | Colonisation (%) | | GI | | (%) | Colonisation (%) | | GI |
| | | A1 | A2 | | | | B1 | B2 | |
| *Streptococcus mitis* | 63.3 | 73 | 100 | 3 | 0.458 | 60.0 | 53.3 | 93.3 | 3 |
| *Streptococcus oralis* | 6.6 | 100 | 100 | 3 | 0.665 | 4.0 | 100 | 0 | 3 |
| *Streptococcus pneumonia* | 3.3 | 100 | 100 | 3 | 0.590 | 4.0 | 100 | 100 | 3 |
| *Streptococcus sanguinis* | 20 | 100 | 50 | 3 | 0.486 | 28.0 | 85.7 | 57.1 | 3 |
| *Streptococcus salivarius* | 26.6 | 50 | 87.5 | 3 | 0.100 | 48.0 | 41.6 | 66.6 | 3 |
| *Streptococcus vestibularis* | 10 | 100 | 100 | 3 | 0.090 | 8.0 | 100 | 100 | 3 |
| *Streptococcus bovis biovar I* | 26.6 | 75 | 87.5 | 3 | 0.343 | 16.0 | 75 | 75 | 3 |
| *Streptococcus acidominimus* | 6.6 | 50 | 50 | 3 | 0.492 | 12.0 | 100 | 100 | 3 |
| *Streptococcus dysgalactiae* | 6.6 | 50 | 50 | 2 | 0.848 | 8.0 | 100 | 100 | 3 |
| *Streptococcus uberis* | 6.6 | 100 | 100 | 3 | 0.663 | 4.0 | 100 | 100 | 3 |
| *Streptococcus anginosus* | 6.6 | 80 | 100 | 2 | 0.188 | — | — | — | — |
| *Streptococcus intermedius* | 16.6 | 100 | 50 | 3 | 0.952 | 16.0 | 100 | 50 | 3 |
| *Streptococcus constellatus* | 6.6 | 100 | 50 | 3 | 0.188 | — | — | — | — |
| *Lactobacillus* spp. | 13.3 | 100 | 50 | 3 | 0.777 | 16.0 | 75 | 75 | 3 |
| *Gemella haemolysans* | 16.6 | 100 | 60 | 3 | 0.952 | 16.0 | 50 | 75 | 3 |
| *Gemella morbillorum* | 20 | 100 | 60 | 3 | 0.076 | 4.0 | 100 | 100 | 3 |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Enterococcus* spp. | 6.6 | 100 | 50 | 3 | 0.665 | 4.0 | 100 | 100 | 1 |
| *Staphylococcus aureus MSSA* | 40 | 83.3 | 83.3 | 3 | 0.020 | 12.0 | 100 | 75 | 2 |
| *Staphylococcus aureus MRSA* | 3.3 | 100 | 100 | 2 | 0.359 | — | — | — | — |
| *Staphylococcus xylosus* | 13.3 | 10 | 50 | 2 | 0.231 | 4.0 | 100 | 100 | 3 |
| *Staphylococcus epidermidis* | 33.3 | 20 | 23,3 | 2 | 0.833 | 36.0 | 88.8 | 88.8 | 2 |
| *Staphylococcus hominis* | 10 | 100 | 100 | 1 | 0.103 | — | — | — | — |
| *Staphylococcus haemolyticus* | 3.3 | 100 | 100 | 3 | 0.773 | 8.0 | 100 | 100 | 3 |
| *Staphylococcus lugdunensis* | 10 | 100 | 100 | 2 | 0.103 | 8.0 | 100 | 50 | 1 |
| *Lactococcus lactis* | 3.3 | 100 | 0 | 2 | 0.773 | 8.0 | 0 | 100 | 1 |
| *Neisseria* spp. | 16.6 | 100 | 80 | 2 | 0.099 | 36.0 | 88,8 | 100 | 2 |
| *Moraxella* spp. | 3.3 | 100 | 100 | 1 | 0.899 | 4.0 | 100 | 100 | 1 |
| *Acinetobacter lwoffii* | 6.6 | 100 | 100 | 1 | 0.665 | 4.0 | 0 | 100 | 1 |
| *Acinetobacter baumannii* | 3.3 | 100 | 100 | 1 | 0.899 | 4.0 | 100 | 100 | 3 |
| *Enterobacter cloacae* | 10 | 100 | 100 | 2 | 0.870 | 12.0 | 100 | 100 | 1 |
| *Enterobacter kobei* | 10 | 100 | 100 | 2 | 0.393 | 4.0 | 100 | 100 | 2 |
| *Enterobacter aerogenes* | 6.6 | 75 | 100 | 2 | 0.841 | 8.0 | 100 | 100 | 2 |
| *Enterobacter asburiae* | 3.3 | 100 | 100 | 2 | 0.889 | 4.0 | 100 | 100 | 2 |
| *Serratia liquefaciens* | 6.6 | 100 | 50 | 1 | 0.188 | — | — | — | — |
| *Serratia fonticola* | 10 | 100 | 33.3 | 2 | 0.465 | 4.0 | 100 | 100 | 1 |
| *Klebsiella pneumoniae* | 20 | 100 | 100 | 3 | 0.424 | 12.0 | 100 | 100 | 2 |
| *Klebsiella oxytoca* | 16.6 | 100 | 80 | 3 | 0.494 | 24.0 | 100 | 83,3 | 2 |
| *Citrobacter* spp. | 3,3 | 100 | 100 | 3 | 0.590 | 4.0 | 100 | 100 | 2 |
| *Escherichia coli* | 20 | 100 | 100 | 1 | 0.424 | 12.0 | 66.6 | 75 | 2 |

F, frequency, that is, percentage (%) of subjects from CLP or CSP group with a given microorganism; colonisation, percentage (%) of smears of a given bacterial species from the palate and/or from the tongue; A1, smears were obtained from palatal mucosa on the cleft margin in CLP subjects; A2, smears were obtained from the dorsum of the tongue in CLP subjects; B1, smears were obtained from the palatal mucosa in CSP subjects; B2, smears were obtained from the dorsum of the tongue in CSP subjects; GI, growth intensity; - square test; results printed in boldface have reached statistical significance ($p < 0.05$).

**Table 2: Statistical comparison of microorganism frequency [prevalence ] colonization and growth intensity between cleft lip and palate group and cleft lip and soft palate group at T2.**

| Microorganism | CLP group (n=30) | | | | *p* | CSP group (n=25) | | | |
|---|---|---|---|---|---|---|---|---|---|
| | (%) | Colonisation (%) | | GI | | (%) | Colonisation (%) | | GI |
| | | A1 | A2 | | | | B1 | B2 | |
| *Streptococcus mitis* | 100 | 60 | 100 | 3 | 0.002 | 56 | 64 | 100 | 3 |
| *Streptococcus oralis* | 10 | 100 | 100 | 3 | 0.103 | — | — | — | — |
| *Streptococcus pneumonia* | 13.3 | 100 | 100 | 3 | 0.777 | 16.0 | 100 | 100 | 3 |
| *Streptococcus sanguinis* | 50 | 100 | 46.6 | 3 | 0.458 | 40.0 | 100 | 60 | 3 |
| *Streptococcus salivarius* | 100 | 50 | 100 | 3 | 0.022 | 84.0 | 47.6 | 100 | 3 |
| *Streptococcus vestibularis* | 10.0 | 66.6 | 100 | 3 | 0.103 | — | — | — | — |
| *Streptococcus bovis biovar I* | 13.3 | 50 | 50 | 3 | 0.174 | 28.0 | 100 | 85,7 | 3 |
| *Streptococcus acidominimus* | 13,3 | 50 | 100 | 3 | 0.174 | 28.0 | 100 | 0.0 | 3 |
| *Streptococcus agalactiae* | 6.6 | 100 | 100 | 3 | 0.264 | 16.0 | 100 | 50 | 3 |
| *Streptococcus pyogenes* | 13.3 | 100 | 100 | 3 | 0.058 | — | — | — | — |
| *Streptococcus uberis* | 6.6 | 100 | 100 | 3 | 0.487 | 12.0 | 100 | 100 | 3 |
| *Streptococcus anginosus* | 13.3 | 50 | 100 | 2 | 0.058 | — | — | — | — |

| | | | | | | | | | |
|---|---|---|---|---|---|---|---|---|---|
| *Streptococcus intermedius* | 26.6 | 100 | 100 | 3 | 0.343 | 16.0 | 100 | 85.7 | 3 |
| *Lactobacillus* spp. | 13.3 | 75 | 100 | 3 | 0.058 | — | — | — | — |
| *Gemella haemolysans* | 26.6 | 62.5 | 100 | 2 | 0.177 | 12.0 | 100 | 100 | 3 |
| *Enterococcus* spp. | 13.3 | 75.0 | 100 | 3 | 0.885 | 12.0 | 100 | 66.6 | 3 |
| *Staphylococcus aureus MSSA* | 93.3 | 71.4 | 64.2 | 3 | <0.001 | 20.0 | 80 | 100 | 3 |
| *Staphylococcus epidermidis* | 83.3 | 92.0 | 100 | 2 | <0.001 | 28.0 | 71.4 | 57.1 | 3 |
| *Staphylococcus hominis* | 13.3 | 100 | 100 | 1 | 0.058 | — | — | — | — |
| *Lactococcus lactis* | 13.3 | 100 | 0.0 | 3 | 0.058 | — | — | — | — |
| *Neisseria* spp. | 53.3 | 56.2 | 93.7 | 3 | 0.695 | 48.0 | 83.3 | 66.6 | 3 |
| *Enterobacter cloacae* | 36.6 | 90.9 | 100 | 2 | 0.007 | — | — | — | — |
| *Enterobacter kobei* | 26.6 | 100 | 100 | 3 | 0.053 | — | — | — | — |
| *Enterobacter aerogenes* | 13.3 | 50 | 75 | 2 | 0.058 | — | — | — | — |
| *Hafnia alvei* | 3.3 | 100 | 100 | 2 | 0.359 | — | — | — | — |
| *Klebsiella pneumonia* | 53.3 | 87.5 | 100 | 3 | <0.001 | — | — | — | — |
| *Klebsiella oxytoca* | 76.6 | 43.4 | 56.5 | 3 | <0.001 | — | — | — | — |
| *Escherichia coli* | 36.6 | 100 | 100 | 2 | 0.311 | 24.0 | 100 | 83.3 | 2 |
| *Candida albicans* | 6.6 | 100 | 100 | 2 | 0.190 | — | — | — | — |

F, frequency, that is, percentage (%) of subjects from CLP group or CSP group with a given microorganism; colonisation, percentage (%) of smears of a given bacterial species from the palate and/or from the tongue; A1, smears were obtained from palatal mucosa on the cleft margin in CLP subjects; A2, smears were obtained from the dorsum of the tongue in CLP subjects; B1, smears were obtained from the palatal mucosa in CSP subjects; B2, smears were obtained from the dorsum of the tongue in CSP subjects; GI, growth intensity; -square test; results printed in boldface have reached statistical significance ($p < 0.05$).

Overall, cultivable and not-yet-cultivable species
of *Gemella*, *Granulicatella*, *Streptococcus,* and *Veillonella* were commonly detected in most sites. *S. mitis* was the most commonly found species in essentially all sites and subjects .On the other hand, *Neisseria* spp. were not found in subgingival plaque but were present in most other sites. *Simonsiella muelleri* colonized only the hard palate. Indeed, *S. muelleri* was initially isolated from the human hard palate[28]. On the hard palate, the predominant bacterial species included *S. mitis, S. mitis* biovar 2, *Streptococcus* sp. clone FN051, *Streptococcus infantis, Granulicatella elegans*, *G. hemolysans*, and *Neisseria subflava* (Tble 1). On the soft palate, *S. mitis*, other cultivable and not-yet-cultivable species of *Streptococcus*, *G. adiacens* and *G. hemolysans* were predominant (Table 2). *Prevotella* sp. clone HF050 was found in the maxillary anterior vestibule of one subject, dominating the bacterial flora as 44% of the clones. This clone was also found in lower proportions on the soft palate and tonsils of another subject M. catarrhalis has not been previously recognized as a pathogen in cleft palate repairs. This study demonstrates a higher fistula rate in procedures positive for M. catarrhalis. Other factors that may have contributed to the fistula formation include the severity of the initial cleft and technical factors. Further study is required before a definitive link can be established.[29]

**Result**

Conclusion study on how the orofacial cleft cases microbiome develops during early childhood and how external factors influence this ecological process . we establish the full bacterial diversity of the orofacial cleft oral cavity and then to determine variation and reproducibility using the microarrays. Consequently, it will

be relatively easy to compare the bacterial composition of a statistically significant number of samples to more precisely identify those species that are associated with health and oral disease .

**Conclusion**

This study indicates that the mode of delivery does not have any major influence on the infant oral microbiota. Changes in diversity and composition were observed in the oral microbiota of the infant over time. These changes are more visible at 6 months and beyond, and again at 1 year of age, there are more pathogenic microbes are seen in orofacial cleft cases than the microbiota present in the normal oral cavities. Our findings provide a closer insight into the oral microbiota development from orofacial cleft birth, and the influence of birth mode together with the documented changes in diversity and composition will aid us to get a better understanding of the long-term health impact within the oral cavity for the infant and provide a platform for additional studies to establish how orofacial cleft disturbances the health outcome of these infants.

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