Oral Microbial Community Composition in Young Children with Cystic Fibrosis.

Thesis

Presented in Partial Fulfillment of the Requirements for the Degree Master of Science in the Graduate School of The Ohio State University

By

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Abstract

Purpose: The purpose of this study was to compare oral microbial community composition in young children with cystic fibrosis (CF) to that of healthy controls, to determine if the oral cavity may play a role as an incubator of common pathogenic organisms responsible for pulmonary exacerbations in CF patients.

Methods: In this cross sectional pilot study, microbial community composition of salivary samples from 17 subjects with CF and 17 healthy controls 1 to 5 years of age was determined by 16S rRNA gene pyrosequencing. Caries experience (dft), gingival health, plaque levels and salivary pH were measured.

Results and conclusions: Significant differences in oral bacterial community composition were observed between CF and healthy young children for the two most abundant phyla, six genera and seven species. CF subjects had significantly less bacterial diversity and lower pH scores than controls. None of the major pathogens implicated by traditional culturing in exacerbations of CF were found in the oral cavity of young CF patients. However, many of the pulmonary bacterial genera that have been identified by recent studies using molecular techniques were found in the oral cavity. Therefore, even though oral cavity does not appear to be the primary source of the major cultivated bacterial pathogens responsible for CF exacerbations, it harbors several bacterial genera that co-inhabit CF lungs and may play a role in CF pathogenesis.
Dedication

This document is dedicated to my family.
Acknowledgments

I would like to thank the members of my thesis committee for their invaluable help and guidance on this research project. Without their continuous mentorship and support this project would not be possible. I would also like to thank Cliff Beall, Zach Thompson and Haella Holmes, for their collaboration and assistance with sample preparation, bioinformatics/statistics analyses, and valuable scientific discussions.
Vita

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Fields of Study

Major Field: Dentistry
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Introduction

The incidence of cystic fibrosis (CF) is 1 in 3000 newborn white Americans and it most commonly occurs in populations of northern European descent where it is the most common lethal genetic disorder [1]. Currently, there are more than 30,000 individuals with CF living in the US [2].

According to the US Cystic Fibrosis Foundation, the life expectancy for patients with CF has increased from 31 years to 37 years over the past decade [1]. The survival rate for patients with CF has continuously been rising due to advances in our understanding of the pathophysiology of the disease and constant improvements in treatment regimens. CF is a complex, multisystem disease that affects upper and lower airways, pancreas, bowel, sweat glands and reproductive tracts [3]. The main factor that leads to morbidity and mortality associated with CF is obstruction of exocrine glands with thick mucosal secretions. In the lungs, this obstruction leads to glandular hyperplasia, proliferation of pathogens within airway secretions, blockage of airway with neutrophil dominated mucopurulent debris, peribronchiolar inflammation and scar tissue formation [4].

CF is an autosomal recessive disorder. The defective gene involved in CF is located on the long arm of chromosome 7. This gene encodes the cystic fibrosis transmembrane conductance regulator (CFTR) protein which is a cAMP-regulated chloride channel located on the apical portion of mucosal epithelial cells of various organs [2]. There are
more than 1,500 different mutations of CFTR gene that can lead to CF. However, the
most common mutation of CFTR gene is the deletion of phenylalanine at codon 508
(named deltaF508), and this particular mutation is present in 70% of CF patients [3].
CFTR protein has many different regulatory functions, but it mainly acts as an ion
channel that regulates liquid volume on epithelial surfaces by secretion of chloride and
inhibition of sodium transport through epithelial sodium channels.

The most commonly accepted hypothesis regarding how dysfunctional CFTR protein
leads to airway disease is called the low-volume hypothesis. This hypothesis is based on
defective CFTR’s inability to inhibit the activity of the epithelial sodium channels.
According to this hypothesis, the loss of inhibition of sodium transport leads to excessive 
sodium and water absorption at the epithelial surface. This increased sodium and water absorption, coupled with reduced chlorine secretion by defected CFTR protein, ultimately
causes dehydration of mucous epithelium, reduced mucociliary clearance and formation
of hypoxic plaques that harbor various pathogenic organisms [1]. This chronic
colonization of the lungs by antibiotic-resistant microorganisms can ultimately lead to
progressive lung function decline and is the leading cause of morbidity and mortality in
CF patients [5]. A better understanding of airway infection in CF patients could provide
opportunities for improved treatment strategies with the goal of maintaining optimal lung
health and, therefore, improving the quality of life for these patients.

CF is characterized by alternating cycles of relatively stable lung function and periods of
pulmonary exacerbations that usually require antibiotic treatment. There is no clear or
uniformly accepted definition of a pulmonary exacerbation of CF. However, pulmonary
experts agree that the important features that are present during pulmonary exacerbations are increased cough, change in the color or quantity of the sputum, weight loss or decrease in appetite, and respiratory rate and auscultatory changes detected during respiratory exam [1].

The microbiological composition of the CF lung is very complex, and it changes based on patient’s age, antibiotic treatment and the stage of the disease. Common microorganisms in the CF airway detected by traditional culture-based methods include *Staphylococcus aureus, Haemophilus influenzae, Pseudomonas aeruginosa*, *Burkholderia cepacia, Streptococcus milleri* and *Stenotrophomonas maltophilia* [6,7].

Modern molecular methods have allowed the identification of other bacterial species in CF airways, such as *Prevotella oris, Fusobacterium gonidioformans, Bacteroides fragilis*, *Leptotrichia*-like sp., *A. defectiva, C. murliniae, Lautropia mirabilis*, and *Sarcina ventriculi* [8]. It is important to note that some of these newly identified organisms (e.g., *P. oris, Leptotrichia*-like sp., etc.) are considered to be oral-associated microbes [8], which implies a possible relationship between oral and pulmonary microflora. Recent studies on CF airway microbiota during pulmonary exacerbations used modern molecular techniques and identified a long list of bacterial genera, but the most prevalent genera were *Pseudomonas, Streptococcus, Prevotella, Achromobacter, Staphylococcus, Haemophilus, Fusobacterium, Veillonella, Ralstonia, Rothia, Abiotrophia, Gemella* and *Stenotrophomonas* [9,10].

The oral cavity can harbor respiratory pathogens that can cause airway infections, if aspirated. The relationship between oral health, oral microflora and respiratory diseases
such as bacterial pneumonia has been previously identified by various studies [11,12]. If a similar relationship exists between poor oral health, pathogenic oral microbiota and pulmonary exacerbations of CF, meticulous oral hygiene and maintenance of optimal oral health could decrease the morbidities associated with CF.

There have been numerous studies done in the past that looked at oral hygiene, caries risk, periodontal health, and cariogenic microbiota in children and adolescents with CF. When it comes to caries risk, various results were reported by different studies, but the majority of clinical studies found that children with CF are at a lower risk of dental decay than healthy children [13]. Different authors gave various explanations for a lower caries rate in CF children. Some authors attributed this to long term antibiotic therapy [14,15], others to altered salivary properties such as increased salivary pH and buffering capacity [16], and yet others to pancreatic enzymes that were previously linked to reduced dental decay in rats [17], and inhibited formation of dental calculus in animals and humans [18,19].

Some previous studies examined periodontal health in CF patients and concluded that CF patients have better periodontal health as manifested by significantly less gingival inflammation and significantly fewer bleeding sites as compared to controls [20-23]. It was not very obvious why CF patients had better gingival health, but among reasons proposed by several researchers were: long term antibiotics [23], possible difference in qualitative composition of dental plaque [23], and better oral hygiene habits [24]. When it comes to dental calculus accumulation, contradicting results were found. It is plausible for patients with CF to have different prevalence of calculus than healthy controls due to
alterations in the amounts of calcium and phosphate in their saliva. No clear relationship has been observed, however, as some studies have found that patients with CF have higher amounts of calculus in their mouth [24-26], while other studies have found contradicting evidence by reporting that either no difference in calculus accumulation exists between CF patients and healthy controls [14,21] or that CF patients had less dental calculus than healthy controls [22].

Several studies attempted to quantitatively compare dental plaque in CF children and adolescents and healthy controls, but no conclusive difference was found. A few studies found a tendency towards less dental plaque in CF patients [14,22] and one study found that CF homozygotes had significantly less plaque than healthy controls [23]. In contrast to those findings, one study found no difference in dental plaque among CF homozygotes, heterozygotes or healthy controls [21], and another study found a tendency towards more dental plaque in CF homozygotes than CF heterozygotes or healthy controls [20].

None of the studies done in the past did a comprehensive qualitative analysis of dental plaque or saliva of CF patients as compared to healthy controls. A few studies looked at two cariogenic microorganisms: *S. mutans* and lactobacilli and how their levels relate to dental decay prevalence in CF patients. Nezon and Liljemark found no difference in levels of either *S. mutans* or lactobacilli between CF and healthy controls and, therefore, they presumed that decreased caries experience in CF patients is not related to altered cariogenic microflora [27]. A more recent study by Aps et al., also looked at *S. mutans* and lactobacilli counts and correlated it with caries experience. Although they found no
difference in lactobacilli counts among CF homozygotes, heterozygotes or healthy controls, they found a significantly lower S. mutans count in CF homozygotes than in other two groups. However, the significantly lower S. mutans count did not translate into a corresponding decrease in caries experience for CF homozygotes as no significant differences were found in DMF among the three groups involved in that study [28]. Understanding the microbiologic basis of dental caries in CF patients entails more than looking exclusively at just two common cariogenic organisms. Both dental decay and pulmonary infections associated with CF are polymicrobial in nature. A recent study that used 16S rRNA pyrosequencing and traditional bacterial culturing to evaluate microbial communities present in CF patient sputum samples found that the majority of the identified genera represented common inhabitants of the human oral and nasal cavities, including Capnocytophaga, Fusobacterium, Neisseria, Porphyromonas, Prevotella and Veillonella [7]. However, this finding does not necessarily allow us to definitively establish the link between oral organisms and the inhabitants of CF airway due to the fact that sputum samples might have simply been contaminated by saliva during sputum collection. No studies done in the past attempted to analyze total oral bacteria in CF patients and compare it to the common pathogens associated with morbidities and mortalities of CF. Therefore, the major goal of this open-ended pilot study was to perform a comprehensive qualitative saliva analysis using 16S rDNA gene amplification and pyrosequencing in order to study the total bacterial community present in saliva of children with CF and compare it to that of healthy controls. First of all, this comparison would allow us to better understand the reasons for
decreased caries rate in CF children. But more importantly, if close similarities between oral bacteria in CF patients and common CF pathogens that typically inhabit CF airway are found, it would suggest that the oral cavity represents a major reservoir of the pathogens that are responsible for pulmonary exacerbations of CF.
Materials and Methods

Demographics

Subject group
Salivary samples were collected and oral exams were performed on seventeen 1-5 year old children who are regular patients at the CF clinic at Nationwide Children’s Hospital. All CF children had the same F508del/F508del mutation. All patients were of Caucasian descent due to the fact that CF is most prevalent in this population.

Control group
Salivary samples were collected from healthy seventeen 1-5 year old who are regular patients at the dental clinic and at the primary care clinic at Nationwide Children’s Hospital. Controls were all healthy, ASA I children with no systemic diseases and who are not taking any medications and who had not had any infectious illnesses within the last month. The control group was exactly matched to the CF group based on age, race, and dft status in order to eliminate confounding variables.

Clinical Methods
A verbal questionnaire was administered by asking parents/legal guardians questions pertaining to their children’s oral health, diet and oral habits.
Salivary samples were collected intraorally from buccal and sublingual mucosa by
saturating a dry, sterile, flocked swab (Copan Diagnostics Inc., Murrieta, CA) for 15-20 seconds. The saturated swabs were placed in individual tubes containing 0.2 ml of DNA stabilizing solution (Buffer ATL, QIAGEN, Germantown, MD) and placed into the freezer for storage until time of laboratory analysis. The same investigator (KB) collected all salivary samples.

Salivary pH was measured by rubbing the Q-tip against buccal and sublingual mucosa to promote salivation and absorb saliva, and then rolling the saturated tip over a pH strip (Macherey-Nagel pH-Fix 5.1-7.2).

A routine oral exam was performed in a knee-to-knee position by the same investigator (KB) and oral health information was obtained by looking at 3 criteria: 1) oral hygiene measured by the level of plaque, 2) past or present caries experience measured by dft (decayed and filled teeth), and 3) periodontal health measured by gingivitis. Because of the young age of participants most of which were pre-cooperate for a dental exam, a fast and easy recording technique was chosen to assess plaque and gingivitis. The visible absence of plaque was scored as 0, localized plaque was scored as 1, and generalized plaque was scored as 2. Similarly, the visible absence of gingivitis was scored as 0, localized gingivitis was scored as 1 and generalized gingivitis was scored as 2.

**Ethical Considerations**

This study passed the Institutional Review Board of Nationwide Children’s Hospital/The Ohio State University. The parents/legal guardians of all subject and control patients signed informed consent to participate in the study prior to data collection.
Laboratory methods

Comprehensive bacterial community analysis was performed by 16S rDNA 454 pyrosequencing. Isolation of bacterial DNA was done by using the QIAGEN QIAamp DNA Mini Kit according to the manufacturer’s Tissue Protocol with the addition of a bead-beating step. Bacterial 16S rRNA genes were amplified with the HMP primers (Broad Institute Version) focusing on the amplification of the V1 to V3 area of the 16S rRNA gene [29]. Amplified samples were subsequently purified twice by using Ampure Beads (Beckman Coulter, Brea, CA). Purified samples were quantitated by using a real time PCR library quantitation kit (Kapa Biosystems, Woburn, MA), and pooled before emulsion PCR. Pyrosequencing was done at the Plant-Microbe Genomics Facility (PMGF) at The Ohio State University in Columbus, Ohio. Roche 454 GS FLX system (Branford, CT) was used for pyrosequencing. Manufacturer’s protocol instructions were followed during pyrosequencing with the exception of reducing the copies per bead to 0.25. Amplicon Filtering Module was further used to eliminate sequences of insufficient length or quality.

Bioinformatics methods

Mothur [30] was used to further filter the sequences, to remove the primers and to identify the samples from which the sequences came. BLAST search [31] was then used to identify and taxonomically assign the sequences by comparing them to CORE database [32]. Sequences were required to be over 98% similar over 400 nucleotides to species in the database for identification. 91.6% of the sequence reads in this experiment aligned to
the CORE database. Previous experience has shown that reads that do not align are frequently chimeric sequences that are produced during the amplification reaction. Ordination analysis and biodiversity calculations were done with the vegan [33] library in R [34]. UniFrac calculations were done with the phyloseq package [35]. Welch two sample t-tests were done with the t.test function in R and Wilcoxon tests were done with the wilcox_test function of the coin package of R [36].
Results

In the CF group, the gender composition consisted of 59% females. Fifteen out of seventeen examined subjects had dft of 0, and two subjects had dft of 1. The mean dft was 0.12. The mean age was 2.59 years. In the control group, the gender composition consisted of 41% females, and those patients were exactly matched to the CF group in terms of race, gender and dft status (Table 1).

We limited our pool to children with the same F508del/F508del mutation. We decided to include only the children with the same genetic mutation because F508del is the most common mutation in CF and because previous studies have found that CF patients with different genetic mutations showed differences in microbial diversity in their airways [37].

The antibiotic history of the CF group included multiple exposures for all children. All had been treated with several different antibiotics, many of them broad spectrum (Table 2). The mean number of different antibiotics was 7.4 (range 4-13). As expected, increasing age was correlated with greater numbers of antibiotic exposures (p = 0.004). Also, seven of seventeen CF patients received antibiotics in the month prior to oral sampling. No significant relationship between microbial community composition in CF
and age, total number of antibiotics, or antibiotics in the last month was observed using the envfit function of the vegan package.

The mean sequence length was 515 base pairs. Levels of each phylum, genus, and species of bacteria identified by previously described laboratory and bioinformatics methods were calculated as a percent of total bacteria derived from each sample. Means of each phylum, genus and species were determined. Overall, 150 species, 48 genera and 9 phyla were identified in this study. An analysis using non-metric multidimensional scaling (NMDS) ordination based on Bray-Curtis Dissimilarity was performed on the entire bacterial community derived from 17 CF and 17 healthy control samples (Figure 1). This analysis positions the samples in two dimensions, and the distance between any two points on the graph represents the dissimilarity in bacterial composition between the two samples. From this graph, it can be clearly seen that CF patients and controls form two distinct clusters, which are significantly different (p=0.002 by permanova). The clustering indicates that CF and controls have distinctly different microbial community composition.

Welch two sample t-test was used to look at differences in species richness and species diversity between the two groups. While species richness was not found to be different between CF and controls, species diversity was found to be statistically significant (p=0.0375) indicating that CF patients’ bacterial community is less diverse than that of healthy controls (Figure 2). Statistically significant difference in bacterial community at the level of each phylum, genus and species was determined by using Wilcoxon test in R and setting the limit of significance at $\alpha = 0.05$. We found difference in bacterial
composition between CF and controls at the level of two phyla, six genera and seven species (Table 3).

In order to account for possible contributors for the evident differences in bacterial community composition between CF patients and healthy controls, we used JMP program (JMP, Version 9.0, SAS Institute Inc., Cary, NC) to analyze CF status by different dependent variables such as gingival health, plaque level and pH. The analyses of gingival health and plaque level by CF yielded no significant results, but the oneway analysis of pH by CF status found a statistically significant difference ($p = 0.0408$) in pH between CF patients and healthy controls (Figure 6). The graph indicates that CF patients had lower pH as compared to healthy controls.
Discussion

CF children examined in this study had a low caries rate with a mean dft of 0.12 (Table 1). Most CF children (88%) had no past or present decay and only a small fraction of them (12%) had a low dft scores of 1. This finding correlates with results from previous similar studies that found that CF children had low caries risk as compared to healthy controls [13]. As dental decay is a complex and polymicrobial infection, this low caries rate of CF children may be partially due to the difference in bacterial community composition that we saw between CF children and healthy controls.

This study focused on a very young age group of children and human oral microbial communities develop as children grow. But within the age group we studied, age related differences in microbial composition were not observed. In the future, if we expand the age group to include older children, we may see slightly different results due to the fact that bacterial community composition of the oral cavity is a dynamic and evolving entity that undergoes changes based on age.

Our results have shown that there were statistically significant differences in total oral microbial community composition between CF subjects and healthy controls, and that those differences were found at the level of two phyla, six genera and seven species. We have also found that CF patients had lower oral pH and lower oral bacterial diversity as
compared to healthy controls. Analyzing those differences at the level of phylum, CF patients had less *Bacteroidetes* but more *Firmicutes* (Figure 3). It is interesting to note that a recent study that analyzed CF airway microbiota found a similar result with *Bacteroidetes* phylum being less abundant (p=0.04) in the sputum of CF patients as compared to healthy controls [38]. At the level of genus CF patients had less *Bergeyella, Granulicatella* and *Neisseria*, but more *Abiotrophia, Actinomyces* and *Lautropia* (Figure 4). Finally, at the level of species CF patients had more *Streptococcus mitis, Abiotrophia defectiva, Actinomyces viscosus naeslundii oris* and *Lautropia mirabilis*, but they had less *Streptococcus cristatus, Bergeyella JF233961*, and *Granulicatella elegans* (Figure 5).

There may be several reasons to why we saw such differences in microbial community composition between CF and healthy children.

First of all, the pathophysiology and the complex multisystem nature of the disease may be altering the oral environment and driving the equilibrium towards certain microbial species. For instance, the finding in our study that CF patients had lower oral pH than that of healthy controls may be favoring acidophilic bacteria and discouraging acid sensitive bacteria from the oral cavity. It is worth mentioning, however, that one previous study that compared salivary pH and buffering capacity between CF individuals and healthy controls found a different result: young patients with CF had significantly higher salivary pH and buffering capacity than healthy controls [16]. However, the study had several limitations. One limitation was that siblings of CF subjects were used as controls. Some of those siblings may have been carriers of CF gene and, therefore, cannot be considered true controls. Another limitation of that study was that subjects and controls
were not matched based on caries activity. In fact, the caries experience in CF group was found to be considerably less than that of control group. The higher cariogenic activity of control group may have lowered oral pH in that group. In contrast to that study, more recent studies on airway pH in cystic fibrosis found that the epithelial lining fluid of CF patients is acidified and the pH of exhaled breath condensate is significantly lower in CF patients as compared to healthy controls [39,40].

In addition to pathophysiology of the disease, previous exposure to multiple antibiotics might have been a second factor that resulted in reduced bacterial diversity and altered the microbial community composition between the two groups. A recent longitudinal study on bacterial community dynamics in CF airways has found that antibiotic treatment is responsible for pronounced shifts in bacterial community composition of CF airways [41]. Similar shifts in bacterial community composition can be happening in the oral cavity after frequent antibiotic treatments and this may explain the differences between the two groups seen in our study. The heavy use of antibiotics in the CF group along with the small sample size did not allow analysis of the effect of individual antibiotic classes on bacterial composition and diversity in the oral cavity. In order to increase power of statistical analysis, similar future studies should increase the sample size. In addition, longitudinal study design should be done in the future. Previous studies have shown that bacterial population is a dynamic ecological system and it depends on various factors such as severity of disease, previous antibiotic treatment and clinical state of the patient at the time of sampling [41]. It is possible that antibiotic treatment administered during CF exacerbations to target pulmonary bacteria also had an effect on oral bacteria and
reduced oral bacterial diversity. If longitudinal design is used in the future, it will allow to sample and compare oral microbiota at different clinical states in order to make meaningful conclusions about effect of antibiotics on microbial composition of the oral cavity.

An important finding in this study was that among different oral bacterial species found in CF patients there were no major opportunistic pathogens traditionally identified by culture methods and thought to be responsible for morbidity and mortality of CF. Those pathogens include *Staphylococcus aureus*, *Haemophilus influenzae*, *Pseudomonas aeruginosa*, *Burkholderia cepacia*, *Streptococcus milleri* and *Stenotrophomonas maltophilia* [6,7], and none of them were identified in oral cavities of either CF patients or healthy controls in this study. In addition, none of the species that had previously been cultured from sputum samples were detected in our subsequent oral sampling. This suggests that in children, oral cavity may not be the principal source or an incubator of major pathogens conventionally thought to be responsible for chronic bacterial infections and pulmonary exacerbations of CF. However, we did see an overlap between several oral bacterial genera identified in this study and pulmonary genera that had been identified in CF airways by recent studies which used modern molecular techniques for bacterial identification. This overlap between oral and pulmonary microbiota was found at the level of several different genera: *Lautropia*, *Gemella*, *Prevotella*, *Leptotrichia*, *Abiotrophia*, *Rothia*, *Bergeyella*, *Fusobacterium* and *Veillonella*. It is interesting to note that new studies that use modern molecular techniques keep identifying new bacterial organisms from CF airway that may represent emergent pathogens implicated in
pulmonary exacerbations. For instance, the study by Dekhil et al. found that *Lautropia mirabilis* was present in substantial numbers in CF sputum samples [42]. Our study demonstrated not only that Lautropia genera was present in the oral cavity, but also that *Lautropia* genera and, in particular, *Lautropia mirabilis* species levels were significantly increased in CF patients as compared to controls (Figures 4 and 5). Also, a recent study that looked at changes in CF airway microbiota during pulmonary exacerbations found *Gemella* to be the most discriminative genus between baseline and exacerbations samples, thus implicating the role of *Gemella* as possible biomarker for pulmonary exacerbations in CF [9]. In order to make definitive conclusions about the role of oral bacteria in pulmonary exacerbations of CF, we need to expand our thinking beyond just a handful of microorganisms traditionally identified by culture based methods and thought to be the only culprits of CF morbidity and mortality.
Table 1. Demographics and dental status of subjects with CF and controls

<table>
<thead>
<tr>
<th></th>
<th>Control</th>
<th>CF</th>
<th>Significance</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Mean Age (range 1-5 years)</strong></td>
<td>2.59</td>
<td>2.59</td>
<td>P=1.00</td>
</tr>
<tr>
<td><strong>Mean dft (95% CI)</strong></td>
<td>0.12</td>
<td>0.12</td>
<td>P=1.00</td>
</tr>
<tr>
<td><strong>Gender (% Females)</strong></td>
<td>41</td>
<td>59</td>
<td>P = 0.30</td>
</tr>
<tr>
<td><strong>Gingivitis assessment (%)</strong></td>
<td></td>
<td></td>
<td>P= 0.55</td>
</tr>
<tr>
<td>0 no visible gingivitis</td>
<td>0 - 88</td>
<td>0 - 76</td>
<td></td>
</tr>
<tr>
<td>1 localized gingivitis</td>
<td>1 - 6</td>
<td>1 - 18</td>
<td></td>
</tr>
<tr>
<td>2 generalized gingivitis</td>
<td>2 - 6</td>
<td>2 - 6</td>
<td></td>
</tr>
<tr>
<td><strong>Plaque level (%)</strong></td>
<td></td>
<td></td>
<td>P=0.13</td>
</tr>
<tr>
<td>0 no visible plaque</td>
<td>0 - 82</td>
<td>0 - 59</td>
<td></td>
</tr>
<tr>
<td>1 localized plaque</td>
<td>1 - 18</td>
<td>1 - 41</td>
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<tr>
<td>2 generalized plaque</td>
<td>2 - 0</td>
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<tr>
<td>Antibiotic class</td>
<td>% exposed</td>
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<tr>
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<td></td>
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<tr>
<td>Aminoglycosides</td>
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<td>Sulfamethoxazole-Trimethoprim</td>
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<td>Penicillins plus beta-lactamase inhibitors or carbapenems</td>
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<td>Minocycline</td>
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Table 2. Summary of lifetime antibiotic exposures for subjects with CF
Table 3. Oral bacterial taxa whose relative levels differed significantly between subjects with CF and Controls

<table>
<thead>
<tr>
<th>Phyla</th>
<th>Genera</th>
<th>Species</th>
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<td>Bacteroidetes</td>
<td>Abiotrophia</td>
<td>Abiotrophia defectiva</td>
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<td>Firmicutes</td>
<td>Actinomyces</td>
<td>Actinomyces viscosus naeslundii oris</td>
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<td></td>
<td>Granulicatella</td>
<td>Granulicatella elegans</td>
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<td></td>
<td>Lautropia</td>
<td>Lautropia mirabilis</td>
</tr>
<tr>
<td></td>
<td>Neisseria</td>
<td>Streptococcus cristatus</td>
</tr>
<tr>
<td></td>
<td></td>
<td>Streptococcus mitis group</td>
</tr>
</tbody>
</table>
Figure 1. Non-metric multidimensional scaling (NMDS) of Bray-Curtis dissimilarities between the samples. The Bray-Curtis dissimilarities of proportional species abundances were used to perform NMDS using the metaMDS function of vegan, and a permanova analysis (adonis function) was used to assess differences between the groups. Dispersion ellipses for the two sets of samples were determined with the ordiellipse function based on a 95% confidence limit and the standard deviation of the points score. All functions used are from the vegan package.
Figure 2. Oral bacterial species richness and diversity in subjects with and without CF. Values were computed as the mean per 100 random samples of 100 sequences. Means are shown as blue diamonds, and bars are 95% confidence intervals based on single sample two-sided t-tests.
Figure 3. Fractional abundance of bacterial phyla in subjects with and without CF. The two most abundant phyla, the Bacteroidetes (p=0.015) and the Firmicutes (0.044), were significantly different between the two groups. The “other “ category included TM7, GNO2, Spirochaetes and SR1.
Figure 4. Fractional abundance of bacteria genera that were significantly different in subjects with and without CF ($\alpha=0.05$). Means are shown as blue diamonds. Bars are 95% confidence intervals based on single sample two-sided t-tests.
Figure 5. Fractional abundance of bacteria species that were significantly different in subjects with and without CF ($\alpha=0.05$). Means are shown as blue diamonds. Bars are 95% confidence intervals based on single sample two-sided t-tests.
Figure 6. Salivary pH in subjects with and without CF. Subjects with CF had significantly lower mean salivary pH than those without CF. The line across the middle of each diamond represents the group mean, and the apices the 95% intervals based on a two-sided t-test. The gray horizontal line represents the overall mean.


34. R Core Team (2013) R: A Language and Environment for Statistical Computing. Vienna, Austria.


