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Oral Microbiome and its Effect on Respiratory Disease

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Title: Oral Microbiome and its Effect on Respiratory Disease

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Abstract: The oral microbiome is composed of several different types of bacteria that can affect how our body operates. There are over 700 different bacteria living in our mouths. These bacteria can be helpful and harmful to our bodies, but they are synergistic in a way. There are several factors such as age, diet, smoking, and oral care that affect bacterial makeup. When there is a significant difference, our immune system becomes weakened and respiratory disease set in. The immune system then works to return the microbiome back to homeostasis. When this fails, the diseases become chronic. Thus, the oral microbiome be used as an environmental effects guide when looking at respiratory diseases in the future as preventative care.

One Sentence Summary: Oral bacteria helps our immune system.

Introduction

Homo sapiens are a complicated organism that is made from complex multicellular eukaryotic cells and mass amounts of microbes. Pre 2016, the ideology of the human body consists of ten times the number of bacteria to human cells. Since then, the information was tested and updated (*1*). The actual ratio was found to be 1.3:1 still in favor of bacteria with an uncertainty of 25% (*1*). Even with this updated number of bacteria to human cells, our body must work with these bacteria. This is done through a symbiotic relationship between microbes and human cells (*2*). Our bodies contain several different microbiomes that encompass varying symbiotic relationships. One of the most important microbiomes is the oral. The buccal cavity hosts an environment suitable for the second-largest microbiome in the body. It contains over 700 species of bacteria that reside on both the hard surfaces such as teeth and soft tissues that make up the oral mucosa (*2*). The buccal cavity also hosts other microbes like viruses, fungi, protozoa, and archaea (*2*). Due to the large number of microbes in the buccal cavity, the health

and maintenance of the cavity is crucial. Changes in our daily lifestyle can affect the oral microbiome makeup. Some examples of these changes are decreased brushing time, increased carbohydrate intake, tobacco product use, and stress (*3*). When the environment is disrupted, the microbes start to cause our bodies problems. This is when dental caries, periodontal diseases, and respiratory diseases are most likely to happen. Respiratory diseases have been linked to species of unmaintained oral bacteria invading the respiratory microbiota. It has also been shown that due to the proximity of oral and respiratory microbiotas the effects on one change both (*4*). Under this context, the purpose of this literature review is to find how oral microbiota can protect people from respiratory diseases.

Oral Microbiome

Bacterial Makeup

The buccal cavity contains several factors important in incubating bacteria. One factor that contributes to growth is the temperature of the buccal cavity. The cavity has a varying temperature depending on the location of measurement. The mean temperature was found to be 34.6°C at the incisor site and 35.8°C at the premolar site (*5*). These temperatures are close to 37°C which is the normal internal human body temperature and the optimal growth temperature for bacteria. The further we stray from this number, the harder it is for many bacteria to grow. Another factor that can impact bacteria growth is acidity levels. The pH level of the oral cavity is determined by the acidity of saliva, which can be manipulated by diet, carbohydrate intake, and the number of bacteria present. The pH of saliva has been found to be in the range of 6.2 and 7.6 and an average being 6.7 (*6*). This range is dependent on the bacterial count found in the mouth due to lack of oral hygiene and the amount of carbohydrates consumed. Some bacteria in the mouth undergo fermentation of sugars and produce lactic acid in response. This will cause fluctuation in the pH level of the saliva as this lactic acid builds up in the mouth. Naturally,

saliva acts as a buffer for acidic foods and drinks as it is close to a neutral pH of 7. This neutral pH of course is also optimal for bacteria to grow. A third factor to consider is the antimicrobial components in saliva. While saliva is 99% water, it still contains the compounds hydrogen peroxide, lactoferrin, and lysozymes (*7*). Hydrogen peroxide kills bacteria by releasing oxygen and water. This kills any anaerobic bacteria, but also the free oxygen models steal electrons that are needed for the bacteria to maintain their membrane protentional. The stealing of electrons by oxygen happens through a process called oxidation in which electrons are lost from the bacterial membrane. Lactoferrin works by tightly binding iron preventing bacteria from up taking the substance (*8*). Without iron, bacteria are unable to perform basic metabolic processes due to their role in the electron transport chain and other regulatory processes (*8*). This is due to iron's ability to transfer electrons (*8*). Lysozymes are known to target and degrade peptidoglycans. This is a common structure found in bacterial cell walls. By degrading the cell walls of the bacteria, the bacterial cells begin to undergo apoptosis (*7*). These three combinations are the body's natural control over bacterial growth in the buccal cavity. While the buccal cavity is a near-perfect environment for bacteria, the body does produce natural defenses to limit overgrowth.

Thanks to the perfect growing conditions, the buccal cavity contains over 700 documented species of bacteria (*2*). Even with a large number of species, the oral microbiome is highly similar between people in terms of what bacteria are present. The differences come down to a variance of ratios due to the different bacterial requirements for both growth and attachment to the buccal cavity. There are nine total sites that can support bacteria (*9*). They are the buccal mucosa, hard palate, palatine tonsils, throat, tongue dorsum, keratinized gingiva, saliva, subgingival plaque, and supragingival plaque (*9*). This can be observed in Table 1. The data from table 1 comes from members participating in Human Microbiome Project, HMP (*9*). When

looking at table 1, a key takeaway is the persistence of *streptococcaceae streptococcus* and *pasteurellaceae unclassified* in the buccal cavity. They are both present and dominate at every site. These were determined to be the core members of the oral microbiome (*9*). Some other important oral bacteria are *lactobacillales* and *staphylococcaceae gemella*. They are not present at every site but play major roles in the health of the oral microbiome. *Streptococcaceae streptococcus*, *pasteurellaceae unclassified*, *lactobacillales*, and *staphylococcaceae gemella* are some key bacteria found in the buccal cavity (9).

A closer look at these core members of the buccal cavity shows some interesting takeaways. The first is the *Streptococcaceae streptococcus (10)*. The *Streptococcaceae* indicates the family that the genus *streptococcus* is a member of (*10*). This family is known for being gram-positive with *streptococcus* being no exception (*10*). *Streptococcus* can also be further broken down into *streptococcus mutans (1b)*. *Mutans* is a commonly known cause of dental caries (*1b*). This would make sense that it has a high presence in the sites around teeth in the buccal cavity. *Pasteurellaceae unclassified* is an interesting find as they could not classify the genus (*9*). *Pasteurellaceae* are primarily gram-negative bacteria and the research on the human oral microbiome is limited (*11*). *Lactobacillales* was also unclassified, but they are gram-positive bacteria that are rarely found in caries-free individuals (*11*). The unclassified tag is applied when the bacteria cannot be identified further due to it not being in the HMP database (*9*). They also are known for their ability to produce lactic acid from carbohydrates and survive in more acidic conditions (*10*). This further amplifies dental caries. *Staphylococcaceae gemelli* is also a gram-positive bacterium (*10*). It favors conditions of high CO_2 and is found in people with poor dental care (*11*).

Carbon Dependence

The bacterial makeup has several factors that affect the microbiota, but a key component is the age of the individual. When babies are born, both the oral and respiratory microbiomes begin to develop immediately (*12*). Their development occurs simultaneously as air must pass through both the oral and respiratory microbiome (*12*). This leads to potential crossovers of bacteria and can determine the future health of both microbiotas. This is crucial when looking at respiratory diseases such as asthma. Asthma is a long-term respiratory disease that causes inflammation of the airways in the lungs. It may also cause a buildup of bacteria making it harder to breathe. These symptoms are caused by a lack of diversity in the microbiotas of people who develop asthma (*13*). In the oral microbiome, there was a clear increase of *Gemella haemolysans* in the children who develop asthma (*13*). There was also a lack of *Lactobacillus gasseri* and *L. crispatus* (*13*). The inverse was the case for the healthy children with increased levels of *Lactobacillus gasseri* and *L. crispatus* (*13*). Healthy children also had decreased levels of *Gemella haemolysans* (*13*). These swaps of bacteria are causing an immunological change which can cause diseases such as asthma to set in (*13*). Possible immunological changes have previously been examined in terms of eczema with *L. reuteri* (*13*). *L. reuteri* increases levels of anti-inflammatory IL-10 and proliferation of T cells (*13*). This would require more research and could be an interesting opportunity in the future.

The oral microbiome will also change from the time of being a fetus to the time of delivery. As the fetus is developing, it is encapsulated in amniotic fluid. The role of this amniotic fluid is to enable an exchange of nutrients for the developing fetus to utilize while also providing cushioning. This amniotic fluid has roughly a 70% chance of containing bacteria (*14*). When

bacteria are present, three are shown to be shared between the placenta, gut, and oral microbiomes (*14*). They are *Prevotella, Streptococcus, and Veillonella* (*14*). While *Prevotella* is normally found in the buccal cavity, this gram-negative bacterium can cause some problems in the respiratory tract (*10*). It is responsible for aspiration pneumonia or chronic sinusitis (*14*). *Streptococcus* was previously discussed in bacterial makeup. *Veillonella* is a gram-negative bacterium, in contrast to most firmicutes (*10*). *Veillonella* also possesses the ability to ferment lactate into lactic acid (*10*). The most interesting feature is its ability to reduce nitrate which can neutralize bacteria (*10*). Due to the presence of this bacterium in the amniotic fluid, it becomes some of the first microbes to become a part of the fetus's microbiomes.

Following infancy, the oral microbiome continues to develop throughout our lives. This was examined at key points throughout our development of the buccal cavity. These points are called dentitions, or the development of our teeth (*15*). The first detention in life is primary, commonly referred to as baby teeth (*15*). This is then followed by a mixture of the detentions (*15*). The mixture of detention is the stage from the first adult tooth to the time we lose our last (*15*). Finally, we have our permanent detention (*15*). These are the full adult set of teeth (*15*). The timing of the transition between detentions is different for each person (*15*). These are also not the only points in our lives where we have an oral microbiome. We also have them as an infant before teeth, and our permanent detention is supposed to last the rest of our life after about 16 years old (*15*). These stages of life produce different ratios of bacteria (*15*). The difference in development can be seen in figure 1. Some key takeaways are the spikes in *Firmicutes* when teeth and gums are developing (*15*). Thus, they are most prevalent from neonates until permanent detention. *Proteobacteria* is also very prevalent at an older age (15). This leads to the conclusion that *proteobacteria* is more controlling than firmicutes when fighting for resources (*15*). The

most common *firmicutes* are *Streptococcus, Lactococcus, Enterococcus, Granulicatella, Abiotrophia, Dolosigranulm, Alloiococcus, Lactobacillus, Gemella,* and *Bacillus* (*16*). *Proteobacteria* can be broken down into *Alphaproteobacteria*, *Betaproteobacteria*, *Gammaproteobacteria*, *Deltaproteobacteria*, and *Epsilonproteobacteria* (*16*).

Connection of the Oral and Respiratory Microbiomes

The oral and respiratory microbiomes are closely related. This is due to several factors, a big one being the proximity as stated previously (*12*). This proximity is vital in this situation as all pathogens trying to make their way into the lungs must pass through the oral microbiome at some point. Whether you breathe through your mouth or your nose, the air travels through the oral microbiome on its way to the lower respiratory tract and lower respiratory microbiome. This has led to two theories. The first is that human microbiomes naturally evolve into 'islands' as proliferation takes place at differing times and locations (*17*). The idea is that each microbiome is independent of one another and contains clear distinctions. The second theory is based on topological continuity which states that the bacteria found in the upper, lower, and oral microbiomes would be indistinguishable from each other due to contiguity (*18*). Recent studies have shown more favor for the second theory. This can be seen as several studies have found overlap between the oral, upper respiratory, lower respiratory, and even gut microbiomes (*19-21*). Studies have also found that the lower respiratory microbiome of the lungs consists of a sparse microbiome (*22-23*). They also found that the number of bacteria did not change in number in people with severe chronic obstructive pulmonary disease (COPD) from smoking when compared to healthy nonsmoking people (*22-23*). The only thing that did change was the bacterial compositions and the persistence of the bacteria present (*22-23*). They found that the

bacteria found in COPD patients was the more persistent set due to the damage to the lungs' natural defenses which causes a circle (*22-23*). An increase in the number of bacteria present leads to increased infections. These infections trigger more persistent bacteria to proliferate and cause additional inflammation to the infected tissues (*22-23*). This cycle causes persistent diseases like COPD and helps explain why bacteria from COPD cause 50% of exacerbations (*22-23*). Exacerbations are commonly referred to as flare-ups by the public.

The respiratory microbiome development is in line with the development of the oral microbiome. As previously stated, both microbiomes begin to develop prebirth, but do not truly get exposed until moments after birth. This happens as soon as air travels into the baby's lungs for the first time (*24*). This can be noted in the high conservation of bacteria found in both the oral and lung microbiomes (*24*). In a recent study, this conservation was investigated as the HMP did not consider the lungs as part of the respiratory tract (*25*). When the conservation was considered, it was found that some individuals have identical oral and lung microbiomes (*25*). But this could be caused by recent respiratory infection, recent antibiotic usage, or them just being naturally more susceptible to the immigration process (25). Another important find was the high conservation of *Prevotella*, *Veillonella*, *Streptococcus*, *Fusobacterium*, and *Haemophilus* (*25*). These are prevalent in high concentrations in both the lung and oral microbiomes (*25*). Another study that focused on the changes in microbiomes between smokers and non-smokers had significant evidence that smoking disrupts both microbiomes (*25*). A third paper was researching the lung microbiome observed that the lung microbiome is consistent with the oral microbiome, but roughly three magnitudes lower (*25*). They also found there was an 85-95% neutral overlap not including dental plaque and gingiva which did not overlap well (*25*).

The bacterium of the lungs is consistent with that of the oral microbiome. When we become ill, the composition of bacteria in the lung microbiome changes from Bacteroidetes *phylum* to Gammaproteobacteria *(10)*. Some common Bacteroidetes are *Gammaproteobacteria, Francisella, Escherichia Coli, Enterobacte,* and *Pasteurella (10)*. The phylum usually consists of unicellular rods that can be divided into two separate groups (*10*). The most important being heterotrophic, which means they steal nutrients from their environment for energy (*26*). The bacteria important for this paper comes from this grouping. This type would not survive in the lungs due to a lack of light to produce energy. Heterotrophic bacteria thus would thrive with the amply access to carbon (*26*). An example of the carbon source utilized by the bacteria is succinate (*26*). Succinate is a media of the citric acid cycle and the transitioning of macrophages from oxidative phosphorylation to aerobic glycolysis (*26*). Thus, in times of respiratory illness, the carbon supply of succinate is drastically increased numbers of M1 macrophages utilizing aerobic glycolysis (*26*). This would then signal an increase in replication as environmental factors are crucial in replication (*27*).

Immunological Impacts

The microbiomes of the body play an integral role in maintaining our immune system. The immune system can be broken down into two types, the adaptive and innate immune systems (*28*). The oral microbiome activates both to aid our body as part of its symbiotic relationship (*28*). The innate immune system is the non-memory section of the immune system that consists mainly of the physical barriers that pathogens must overcome to cause infections or inflammation (*28*). Examples of the innate immune system consist of the skin, mucus membranes, cilia, and gland excretions (*28*). The microbiomes of the body are also included in the innate immune system (*28*). Our bodies are designed to limit exposure to harmful antigens.

This includes things like bacteria; however, our bodies have found through evolution that some bacteria are beneficial (*28*). Our bodies try to let these advantageous bacteria grow, to limit the potential growth of other bacteria that could potentially be harmful (*28*). With that said, our body still has natural immune defenses to keep all bacteria in check. These would be the phagocytosis cells like macrophages and neutrophils (*28*). They are alerted of harmful pathogens through toll-like receptors (TLR) which is a crucial part of maintaining homeostasis in our microbiomes (*28*). These TLR can be found in the epithelial tissues that make up the organs and mouth (*28*). Once the TLR recognizes a PAMP or DAMP, then the phagocytosis begins (*28*). In the buccal cavity, a major gland excretion that is part of the innate immunity is saliva. As previously stated, saliva has natural antimicrobial makeup like hydrogen peroxide, lactoferrin, and lysozymes that are a part of the innate immune system (*7*). This is used to maintain the levels of bacteria in our buccal cavity and to prevent overgrowth to a degree.

The oral microbiome also plays a major role in the adaptive immune system. Adaptive immunity is the memory section of the immune system (*28*). This is the immunity section that vaccines trigger to help immunize us (*28*). The adaptive immune system becomes active when the innate immune system fails. The innate immune system fails when antigens manage to infiltrate our body's physical barriers or when the microbiomes become unmanaged (*28*). This section of the immune system is a crucial part of the oral and respiratory microbiomes as they are common sites of infection. When issues arise and they do not remember certain bacteria, autoimmune diseases become prevalent (*28*). This is the aspect of autoimmune diseases that make them so persistent as our body naturally lets these bacteria grow to a limited action from the innate immune system (*28*). The adaptive immunity boosts the overall health of an individual (*28*). The bacteria that make up the microbiomes are constantly evolving to overcome the

combination of immune systems (*29*). This then causes our immune system to become stronger as these bacteria become more resistant. The disruption of the delicate homeostasis between the immune system and microbiomes is what allows the bacteria to overcome the defenses and humans to become sick with respiratory diseases (*30*). This disruption can be seen in figure 2.

Respiratory Diseases

Several respiratory diseases can be contributed to bacterial colonization. Some common pathogens are *S. pneumoniae, H. influenzae,* and *Moraxella catarrhalis* which contribute to a majority of upper respiratory and lower respiratory tract infections (*30*). It should be noted that the upper and lower respiratory microbiomes do differ slightly, but it has recently been thought to be one microbiome. *S. pneumoniae* is a member of the firmicutes. Some variants of it are what is responsible for pneumonia, bacteremia, meningitis, and otitis media in humans (*31*). This will be discussed more in the inflammation section of the paper. *H. influenzae* and *Moraxella catarrhalis* are both proteobacteria. Influenza is caused by a virus and not *H. influenzae*. *H. influenzae* is linked to pneumonia, otitis media, epiglottitis, and meningitis (*32*). It has been found that the *H. influenzae* dominates *S. pneumoniae* due to the immune system recognizing dead *H. influenzae* as harmless. This is caused by *S. pneumoniae* destroying *H. influenzae* when *in vitro* (*33*). In our bodies, these two bacteria are found in the oropharynx region of the buccal cavity (*31*). *Moraxella catarrhalis* has been found to be associated with COPD, sinus infections, bronchitis, and bronchopneumonia (*34*). The one disease that these all have in common is pneumonia. Pneumonia is famous for the pus or fluid buildup in the air sacs that causes a crackling sound when breathing. A type of pneumonia called nosocomial pneumonia has been found to be closer in relation to the oral bacteria of *Pseudomonas aeruginosa* and *Staphylococcus aureus* (*35*). These florae are commonly found in dental plaque, which holds

about 100 million bacteria in a cubic millimeter of dental plaque (*35*). Dental plaque levels can be decreased with regular oral maintenance. This can then decrease the chances of getting nosocomial pneumonia.

Inflammation of the upper and lower respiratory tract causes problems and bacteria is a known trigger. One of the most common inflammations of the alveolar sacs is commonly referred to as pneumonia (*12*). Pneumonia can be caused by several factors, but an important cause is bacteria. Pneumonia can be triggered by the infection of *streptococcus pneumoniae (12)*. *Streptococcus pneumoniae* is a gram-positive bacterium that can cause Pneumonia, but there are over one hundred different variants in which only a minority can cause infection (*10*). In a study that looked at two newborn babies, eleven of the fifty-seven strains collected in the first ten days of life showed positive inflammation relating to BEAS-2B cells *in vitro* (*12*). While *Staphylococcus aureus* was found to be the sole bacteria on day one of life (*12*). Of the eleven strains that triggered inflammatory markers, seven did it without the need of a cofactor (*12*). The inflammatory marker response of these seven strains can be seen in figure 3. These seven strains consist of *Pantoea, Enterococcus,* and *Streptococcus* (*12*). Figure 3 also shows the number of up and down-regulated genes.

When looking at respiratory diseases like lung cancer, there has also been a link between the oral microbiome and susceptibility to contracting cancer in never smokers. Never smokers are people who have never smoked in their lives. It has been found that people with a lower alpha-diversity had a significant increase in the likelihood of getting lung cancer (*36*). When they were compared to those who had a much higher alpha-diversity (*36*). Alpha-diversity refers to the measure of microbiome variability in a local population (*36*). Beta-diversity did not provide significant evidence when compared to the case-control (*36*). Beta-diversity refers to the measure

of microbiome variability between two or more communities (*36*). Thus, alpha diversity and its ability to measure presence or absences was used and compared the time to diagnosis and saw significant linkages (*36*). During which it was found that the taxa in Bacteroidetes, Spirochaetes, and Firmicutes phyla were also associated with the increased risk of lung cancer (*36*). Bacteroidetes are gram-negative bacteria that along with firmicutes are typically found in the lung microbiome (*10*). Firmicutes on the other hand are typically gram-positive bacteria (*10*). They are also known for their production of endospores (*10*). Spirochaetes are gram-negative bacterium that is known for their role in acute bronchitis (*10*). Lack of diversity amongst these phyla is one of several issues that may lead to environmentally caused lung cancer.

Conclusions

The oral microbiome is a diverse and ever-evolving part of human life. The microbiome consists of over 700 types of bacteria that have found a place to grow in various locations and conditions of the buccal cavity. The ratio of bacteria that grows is dependent on several environmental factors such as food intake, oral maintenance, stress, age, and smoking. These factors can cause changes in the pH, temperature, and nutrient supply that bacteria have access to in the buccal cavity. These factors, while naturally favorable for bacteria growth, create additional instability in microbiome growth causing certain bacteria to replicate and overrun the oral microbiome. When the oral microbiome is unstable, this is when periodontal diseases become onset and cause problems for our immune system. This is also true for the respiratory microbiome which begins developing at the same time as the oral and shares several bacteria. There are currently two theories for if the microbiomes should be treated like islands or if they interact closer to one microbiome. The latter is the currently preferred theory, due to the similarities and proximity of the two microbiomes.

The body's immune systems try to keep the microbiomes in highly infected areas under control. This occurs mainly through the innate immune systems in which the microbiomes are members. The buccal cavity has glands that produce saliva which has antimicrobial agents such as hydrogen peroxide, lactoferrin, and lysozymes (*7*). There are also the M1 and M2 macrophages which not only work to kill bacteria and sites of inflammation but also produces succinate which heterotrophic bacteria use as a carbon source to survive. However, when the innate immune system fails, the adaptive immune system can succeed. In areas of microbiome instability, the adaptive immune system is the safety plan. When activated it kills the bacteria causing the infection to return the microbiome to homeostasis. While this works great on inflammatory diseases caused by bacteria, the adaptive immune system can fail or be avoided. When this occurs, we get chronic diseases such as COPD. With that said, there has even been a strong correlation found that people who have never smoked and receive lung cancer have a specific oral microbiome composition. This correlation makes sense as both cancer rates and microbiome composition are heavily affected by the environment with that being the actual cause of lung cancer in these patients.

This leaves room for further research in this field as correlations can be used to find potential causations. Specifically, how we can monitor the oral microbiome composition changes in people to potentially do preventative care. There is also future research being done with the COVID-19 pandemic that is looking into the respiratory microbiome as another way to help limit infections and severity of disease (*37*).

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Supplementary Materials:

Oral		Oral (continued)	
Buccal mucosa	• Streptococcaceae Streptococcus (2)	*Tongue dorsum*	• Streptococcaceae Streptococcus (2, 6)
	Pasteurellaceae unclassified (16, 19)		Veillonellaceae Veillonella (4)
	Staphylococcaceae Gemella (11)		Prevotellaceae Prevotella (10)
Hard palate	• Streptococcaceae Streptococcus (2, 6)		Pasteurellaceae unclassified (16)
	Pasteurellaceae unclassified (16)		Actinomycetaceae Actinomyces (14)
	Veillonellaceae Veillonella (4)		Fusobacteriaceae Fusobacterium (9)
	Prevotellaceae Prevotella (10)		Lactobacillales unclassified (13)
	Lactobacillales unclassified (13)		Neisseriaceae Neisseria (8)
	Staphylococcaceae Gemella (11)		
Keratinized gingiva	• Streptococcaceae Streptococcus (2)		
	• Pasteurellaceae unclassified (19)		
Palatine Tonsils	Streptococcaceae Streptococcus (2, 6)		
	Veillonellaceae Veillonella (4)		
	Prevotellaceae Prevotella (10)		
	Fusobacteriaceae Fusobacterium (9)		
	Pasteurellaceae unclassified (16)		
Saliva	Prevotellaceae Prevotella (10)		
	Streptococcaceae Streptococcus (2, 6)		
	Veillonellaceae Veillonella (4)		
	Pasteurellaceae unclassified (16)		
	Fusobacteriaceae Fusobacterium (9)		
	Porphyromonadaceae Porphyromonas (7)		
	Neisseriaceae Neisseria (−)		
Subgingival plaque	Streptococcaceae Streptococcus (2)		
	Fusobacteriaceae Fusobacterium (9)		
	Flavobacteriaceae Capnocytophaga (−)		
	Prevotellaceae Prevotella (−)		
	Corynebacteriaceae Corynebacterium (−)		
	Pasteurellaceae unclassified (−)		
Supragingival plaque	Streptococcaceae Streptococcus (2)		
	Flavobacteriaceae Capnocytophaga (−)		
	Corynebacteriaceae Corynebacterium (15)		
	Pasteurellaceae unclassified (−)		
	Neisseriaceae unclassified (21)		
	Fusobacteriaceae Fusobacterium (9)		
Throat	• Streptococcaceae Streptococcus (2, 6)		
	Veillonellaceae Veillonella (4)		
	Prevotellaceae Prevotella (10)		
	Pasteurellaceae unclassified (16)		
	Actinomycetaceae Actinomyces (−)		
	Fusobacteriaceae Fusobacterium (9)		
	Lachnospiraceae unclassified (−)		

Corresponding core OTUs underlying the taxonomy are labeled in parentheses.
A bullet (•) identifies core family and genera at >75% ubiquity and >10% abundance.
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Table 1. Shows the nine sites of bacterial growth in the mouth with the core genera of bacteria at each site (*9*). The table also shows the Operational Taxonomic Unit, OTU in parenthesis.

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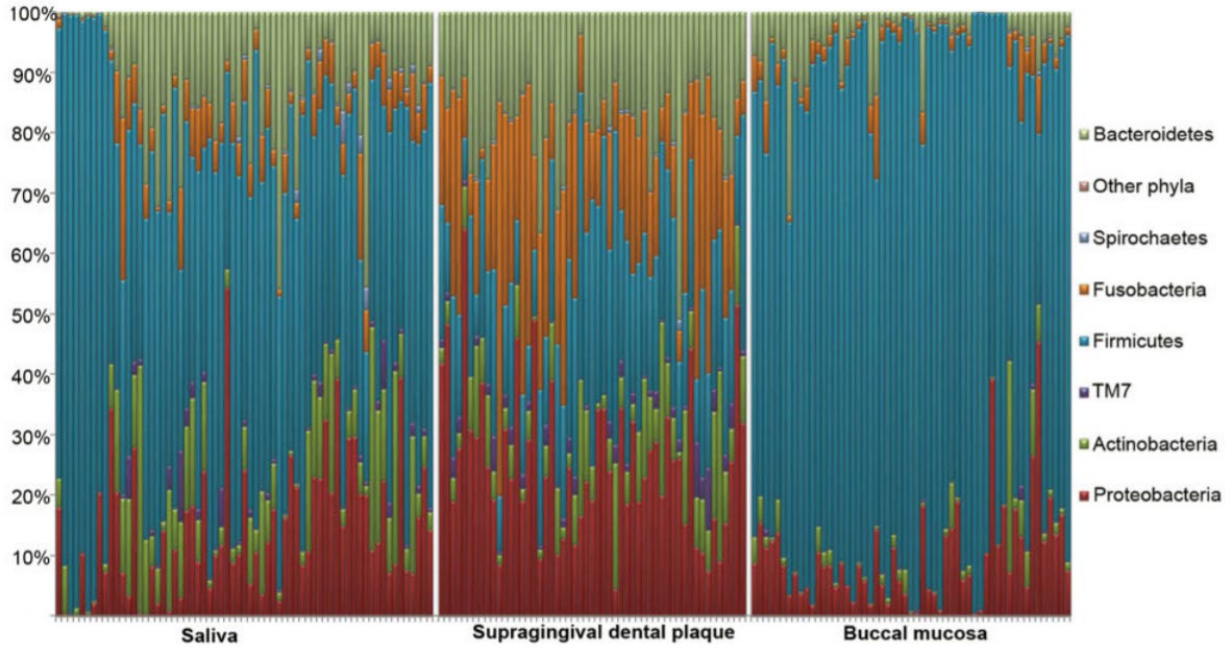
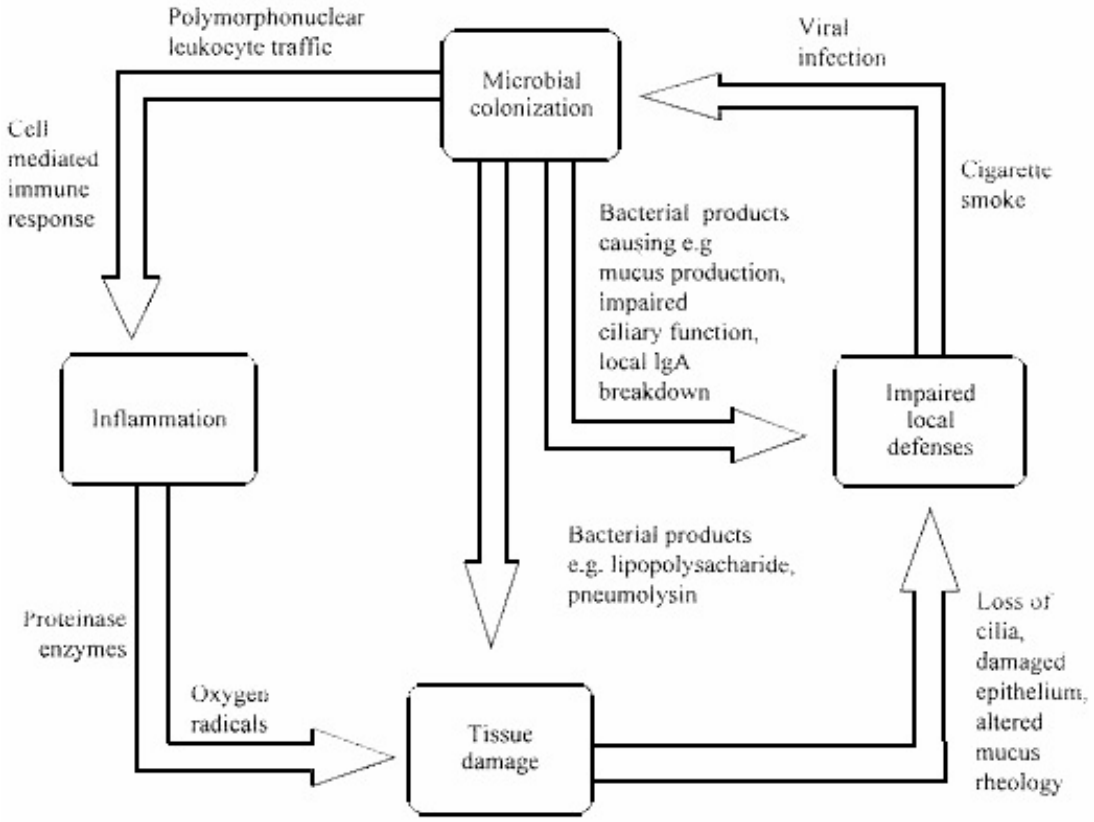


Figure 1. Shows the ratio of bacteria genera at different stages of life. Saliva is the one graph showing the 11 neonates. The order after that is primary, mixed, permanent, young adulthood, elderly. Each contain 11 subjects or 55 in total (*15*).



38. Figure 2: Shows the diagram of how the respiratory tract immune system can become impaired by the environmental factors and the outcomes of when it happens (*30*).

5

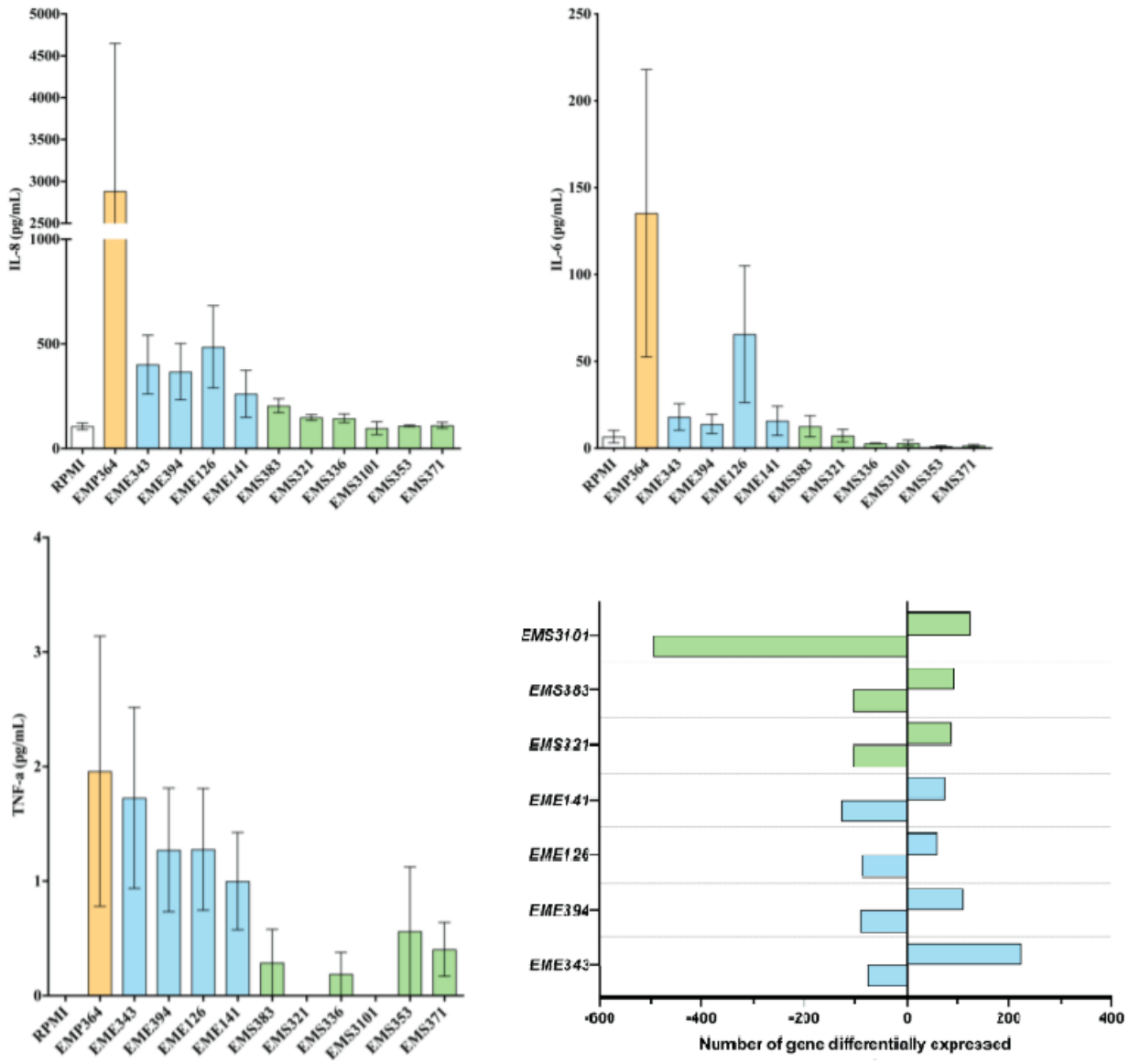


Figure 3: Inflammatory response of proinflammatory marker IL-8, IL-6, and TNF-a. While also show the number of genes that were up and down regulated. For this section only seven of the eleven were recovered (*12*).