REVIEW ARTICLE

Oral Microbial Shift: Factors affecting the Microbiome and Prevention of Oral Disease

[1]Namrata Dagli, [2]Rushabh Dagli, [3]Shrouq Darwish, [4]Kusai Baroudi

ABSTRACT

Background: Recently, oral microbiome has gained popularity among scientists. Microorganisms are no longer considered as disease-producing pathogens, rather they are now considered as partners of human in maintaining health. Since ancient times, changes in our lifestyle have affected our microbiome and the balance with their human host has been perturbed. The present review includes the description about factors affecting oral microbiome and establishing symbiosis with the human host so that they contribute in maintaining health rather than eliciting diseases.

Materials and methods: A comprehensive literature search was performed on databases such as Google Scholar, PubMed and Medline until April 2015. First, articles were selected on the basis of their titles and then abstracts were screened and unwanted articles were excluded. Articles obtained from all the databases were checked and duplicate articles were removed. Articles obtained from various databases: PubMed = 35, Google Scholar = 8. Out of these 43 articles, total 29 articles were finally selected for this review.

Results: The published literature suggests that the modern oral microbiome is less biodiverse, and possess more pathogenic bacterial species and lesser beneficial bacteria. The possible factors mainly responsible for this shift in microbiome were found to be change in diet, industrial revolution and indiscriminate use of antibiotics.

Conclusion: Various changes in lifestyles have affected oral microbiome adversely and perturb the symbiosis between the microbiome and their hosts. The present oral microbiome is found to be less diverse and more pathogenic. The present review may be helpful in understanding the relationship between the microbiome and their human hosts so that microbiome contributes in maintaining healthy state of the body.

Keywords: Ancient microbiome, Dietary shift, Oral microbiome.

INTRODUCTION

Recently, there is a change in concepts about microorganism. They are no longer considered only pathogens, rather they are now considered as partners of healthy human body. Humans are a kind of supraorganism, which is constituted by the human body and microorganisms.[1] The microbiome is found to affect normal physiologic function in human host such as digestion of food, synthesis of vitamins, immunity, drug metabolism, etc. There are several studies that suggest that even evolution of human race was not possible without their partners. The genome of these microorganisms is collectively termed as microbiome.[1] The term was coined by Lederberg and McCray.[2]

Past few decades have witnessed a significant change in human lifestyle and our closely associated microbiome. The results of analyses on gut microbiome show that ancient microbiota are different than the modern cosmopolitan human microbiomes and appear more related to rural microbiota.[3] Similar changes can be expected in oral microbiome too. Comparison of the present microbiome with that of ancient may enable

[1]Care Institute of Medical Science, Ahmedabad, Gujarat, India

[2]Department of Dental Public Health, Vyas Dental college and Hospital, Jodhpur, Rajasthan, India

[3]Department of Oral Medicine and Diagnostic Sciences Alfarabi Colleges, Riyadh, Saudi Arabia

[4]Department of Preventive Dental Sciences, Alfarabi Colleges Riyadh, Saudi Arabia

Corresponding Author: Namrata Dagli, Past Member, Ethical Committee of Clinical Research, Care Institute of Medical Science, Ahmedabad, India, e-mail: r.j.dagli@gmail.com

us to understand the shift of microorganisms due to various factors such as change in lifestyle, diet and use of antimicrobials.[4] This information can be helpful in the establishment of health-promoting oral microbiota.

MATERIALS AND METHODS

Comprehensive literature search was performed on databases such as Google Scholar, PubMed and Medline by using broad range of keywords such as oral microbiome, microbial shift, antient oral microbiota, diet and microbiome, micrbiota and mobile radiation, etc.

Articles were selected on the basis of their titles first and then abstracts of these selected articles were screened. Articles with the content not relevant for the present review were excluded. Articles obtained from all the databases were checked and duplicate articles were removed. The process of study identification and selection is shown in Flow Chart 1. After screening the articles found on literature search on the basis of their titles, total 43 articles were obtained. Out of these 43 articles, 35 were selected from PubMed database and eight were from Google Scholar.

After excluding four duplicate articles, abstract of the remaining 39 articles were screened. The content of 10 articles was not found relevant in abstract screening. Finally, 29 articles were selected for the review.

RESULTS

The published literature suggests that the modern oral microbiome is less biodiverse and possess more pathogenic bacterial species and lesser beneficial bacteria. The possible factors mainly responsible for this shift in microbiome were found to be change in diet, industrial revolution and indiscriminate use of antibiotics.

Human Oral Microbiome

Human cavity harbors several microorganisms, including bacteria, fungi, viruses, etc., which constitute the normal microbiome. It is very difficult to describe the normal flora because of its diversity. The microorganisms are listed in 'Human Oral Microbiome Database' (HOMD).[5]

Bacterial Taxa in Ancient Oral Cavity

The study of ancient microbiome is done under paleomicrobiology, a branch of paleontology. Dental calculus is gaining popularity among archeologist as a specimen to study ancient oral flora, as it preserves the DNA of oral microflora for years.

One archaeal and nine bacterial phyla were found to be dominant in ancient dental calculus. Firmicutes (most abundant) Actinobacteria, Proteobacteria, Bacteroidetes, TM7, Synergistetes, Chloroflexi, Fusobacteria, Spirochetes and Euryarchaeota were found, all of which are also dominant in the human oral microbiome today.[6,7] Other oral taxa such as *Aggregatibacter actinomycetemcomitans*, *Streptococcus mutans* and *Streptococcus mitis* were also identified.

According to Warinner et al,[7] periodontal pathogens such as *Tannerella forsythia, Porphyromonas gingivalis*

Flow Chart 1: Study identification and selection
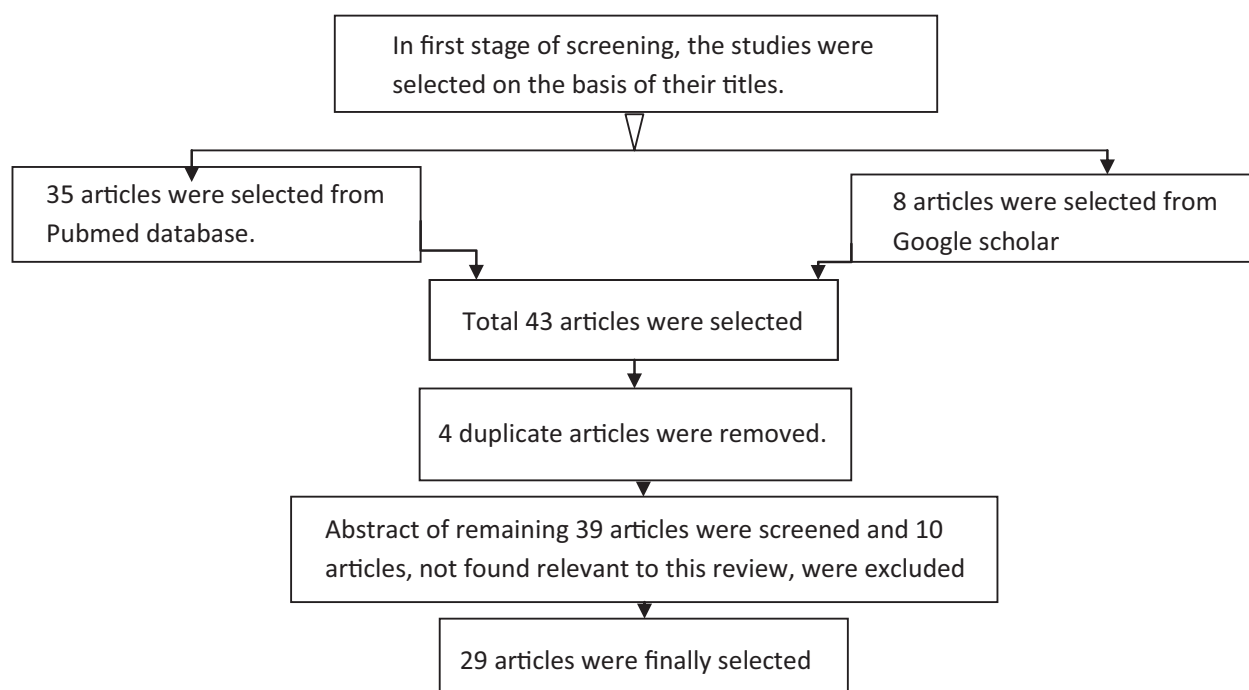
and *Treponema denticola* are particularly abundant and a significantly higher frequency has been noticed.

Additional pathogens include those implicated in acute dental infections (e.g., *Actinomyces odontolyticus*), caries (*S. mutans*) and endodontic biofilm microorganism (*Porphyromonas gingivalis, Streptococcus mitis, Streptococcus salivarius, Prevotella intermedia, Prevotella nigrescens, Streptococcus mutans* and *Actinomyces naeslundii*.)[8] and opportunistic upper and lower respiratory illness (e.g., *S. pneumonia, Streptococcus pyogenes*, and *Haemophilus influenzae*). Two obligate human taxa, *Neisseria meningitidis* and *Neisseria gonorrhoeae*, the causative agents of bacterial meningitis and gonorrhea, respectively, were also observed.[7]

Two additional oral taxa present at substantially higher frequency in at least one ancient dental calculus sample than the Human Microbiome Project healthy cohort: *Filifactor alocis* and *Olsenella uli*.

The archeological calculus was found to be dominated by Firmicutes, which is comparable to both the HOMD and modern oral samples. In addition, the ancient dental calculus samples contained all 15 phyla commonly found in the modern human oral cavity.[9] Actinobacteria are also comparable to the modern calculus sample.[9] Different time periods according to the major dietary shifts and major differentiating bacteria of these groups are listed in Table 1.[10]

Drug Resistance Mechanism in Ancient Microorganisms

Antibiotic resistance in pathogenic bacteria is generally considered as a modern phenomenon, but there are considerable evidences that suggest that genes related to drug resistance were found far before the beginning of the clinical use of antibiotics.

Bacteria, highly resistant to a wide range of antibiotics, are reported to be found in over 4 million years old samples. Enzyme-mediated mechanisms of resistance were also detected.[11] Metagenomic analyses of ancient DNA from 30,000 years old Beringian permafrost sediments identified highly diverse collection of genes encoding resistance to antibiotics such as β-lactam, tetracycline and glycopeptide. Furthermore, the vancomycin resistance element VanA showed similarity to its modern variants.[12] These studies suggest that antibiotic resistance is an ancient, natural phenomena.

SHIFT IN MICROBIOME SINCE ANCIENT TIMES

Major changes in carbohydrate intake in human history appear to have impacted the ecosystem of the mouth, opening up pathological niches for periodontal disease in the early neolithic and caries in the recent past. According to a recent paper by Adler et al,[10] related to analysis of the ancient microbial DNA in the calcified dental plaque from 34 early European skeletons, following changes in microbiome of hunter-gatherers (HGs) to modern humans can be observed:

- Less biodiverse: Modern oral microbiota are markedly less biodiverse than historic populations.[9] Higher phylogenetic diversity is associated with greater ecosystem resilience and, therefore, the current oral microbiome is likely to be less resilient to the changes in oral environment that may be either in the form of chemical imbalances or invasion by pathogenic microorganism.[10]
- Potentially cariogenic: Today's oral environment is dominated by potentially cariogenic bacteria.[10] The HG's dental calculus is found to have fewer bacterial species related to oral diseases. High frequency of *S. mutans* was seen only in the postindustrial samples.
- Incidence of periodontal diseases: Periodontal disease associated taxa such as *P. gingivalis, Tannerella* and *Treponema* were found similar to early agriculturists.[10]
- Less number of beneficial bacteria: In modern oral microbiome, bacteria associated with good health (e.g., *Ruminococcaceae*) are found in less frequency.[10]

Table 1: Different time periods according to the major dietary shifts and major differentiating bacteria of these groups[10]

Different time periods	Important features	Predominantly found bacterial taxa
Hunter gatherer microbial communities	Characterized by nonpathogenic flora	Clostridia, *Ruminococcaceae* (Nonpathogenic)
Farming groups from Neolithic and Medieval periods	Characterized by	*Clostridiales incertae sedis* (nonpathogenic)
	1. both pathogenic and nonpathogenic taxa	*Veillonellaceae* (Decay associated)
	2. periodontal disease associated taxa	periodontal disease-associated taxa, including *P. gingivalis, Tannerella* and *Treponema*
Modern oral environments	Predominated by decay-associated taxa	*Veillonellaceae Lachnospiraceae Actinomycetales*
	Only few taxa differentiate it from farming group of Medieval and Neolithic periods	
	The frequency of *S. mutans* is significantly higher in modern samples.	

Disappearance of these ancestral indigenous organisms, which are intimately involved in human physiology, might cause postmodern conditions such as obesity and asthma.[4]

Table 1 describes different time periods according to the major dietary shifts and major differentiating bacteria of these groups.[10]

Factors affecting Oral Microbiome

As reported by Adler et al,[10] two of the greatest dietary shifts in human evolution involved

- Carbohydrate-rich neolithic (farming) diets[13] (approximately 10,000 years back[14,15])
- Industrially processed flour and sugar (in ~1850).[10,16]

Except this dietary shift, other factors such as use of antibiotics, change in methods of oral hygiene, globalization and use of mobile phones are possible factors that affected the microbiome.

Diet

A study by Adler et al[10] indicates that the transition from HG to farming shifted the oral microbial community to a disease-associated configuration. This indicates the shift of oral microbiome due to change in diet.

Another study related to the consumption of dairy products demonstrated that the consumption of these products did not affect the quantity and composition of the oral microbiome.[17] Further research is required to understand which type of diet is associated with lesser oral diseases.

Industrial revolution

Oral microbiota remained quite constant between neolithic and medieval times. After this, cariogenic bacteria became dominant, apparently during the industrial revolution.[10] This shift may be attributed to increased consumption of refined carbohydrate. Less diverse oral microbiotic ecosystems than historic populations might contribute to chronic oral diseases in postindustrial lifestyles.[10]

Use of antibiotics

Antibiotic-treated subjects show unique dramatic shifts after antibiotic therapy. Some general changes can be observed such as an immediate decrease in actinobacteria count in throat and feces. Pre-treatment states are generally reestablished, but in some cases, it can take up to 4 years. It has been noted that antibiotic resistance can persist for years.[18] Substantial taxonomic changes in the microbiome have been observed in mice after administration of subtherapeutic doses of antibiotic by

Cho et al.[19] According to Blaser,[20] permanent changes in normal protective flora may lead to even more serious consequences than antibiotic resistance.

Mobile phone use

In past few decades, marked increase in mobile phone use has been seen. Their excessive use has been associated with various harmful effects on human body. To the best of our knowledge, no study has been done on the effect of mobile phone radiation on human oral microbiome, but there is possibility that it affects the microbiome indirectly, as it is associated with the increased salivary flow rate and increased volume of parotid gland, and decreased protein secretion.[9,21] However, another study demonstrates the decreased salivary flow, total protein, albumin and amylase activity in mobile phone users, which is indicative of oxidative stress.[22] All these studies indicate that excessive use of mobile phones can modify quantity and quality of saliva and, therefore, oral environment. In the absence of any such study that can correlate oral microbiome shift and mobile phone use, it is not possible to draw any conclusion.

Some other factors such as oral hygiene practices and increased globalization should be studied in this regard, as they have potential to influence our microbiome. Not enough information was found regarding their direct effect on oral microbiome till now.

The information obtained from the published literature clearly suggests that above-mentioned factors have adversely affected the oral microbiome and caused dysbiosis between microbiome and human host leading to an increase in incidence of oral diseases such as dental caries and periodontal diseases.

The Desirable Oral Microbiome

In order to achieve a microbiome that supports the host in maintaining health, it is required to identify the desirable and undesirable microorganism. The oral microbiota is natural and provides important benefits to the host, including immunological priming, downregulation of excessive pro-inflammatory responses, regulation of gastrointestinal and cardiovascular systems, and colonization by exogenous microbes.[14] Microbiota normally carry out following functions in the human host.

Confer immunity

The oral microflora forms biofilms on mucosal and dental surfaces of oral cavity . The normal resident oral microbiome plays an important role in preventing or inhibiting colonization by exogenous microbes and thus provides immunity.[13]

S. mutans has been found to produce the novel hybrid polyketide-nonribosomal peptide metabolite mutanobactin A, which can inhibit biofilm formation by *Candida albicans*, a pathogen responsible for oral fungal infections.[14]

Synthesis of vitamin

Food-related lactic acid bacteria (LAB) and commensals of the human gut such as bifidobacteria have ability to produce vitamins.[16] Certain strains of LAB are also found to produce vitamin B_{12}. Natural source of Vitamin B_{12} is available from meat, eggs and milk products.[23]

Aid in digestion

Microbiome helps in digestion of nutrients, particularly carbohydrates that are otherwise not digestible by the human host.[24] The human genome lacks most of the enzymes required for degrading the plant polysaccharides, but our microbiome has significantly enriched with genes for the metabolism of these glycans, amino acids, xenobiotics, methanogenesis and biosynthesis of vitamins, etc. Furthermore, they are the main substrates for microbial growth in the colon.[25]

Resident gluten-degrading microorganisms are also reported to be found in the oral cavity. These enzymes are found to be active in acidic pH and they have an advantage over enzymes associated with nonhuman sources that the changes produced might be more stable. Oral microbiome may bring a solution for gluten indigestion.[26]

Prevention of Oral Disease by reshaping the Microbiota

The increased understanding about the inter and intra-individual diversity of microbiome has changed our view and it suggests that merely removal of dental plaque is not sufficient for the control of gingival and periodontal diseases; the individual microbiome and its response to the treatment should also be considered. The treatment trends are changing and concepts of nonconventional treatments are now emerging. In this review, we are focusing on prevention of diseases by establishing a balance between the oral microbiome and their human host can support in maintaining healthy condition. The modification of gut microbiome has been proposed recently with the use of antibiotics, probiotics and prebiotics.[27] Desirable microbiome can be promoted by

• Reducing the number of pathogens by competitor microorganisms: *Streptococcus sanguinis* is considered a nonpathogenic and beneficial bacterium with regard to dental caries.[28] High levels of *S. mutans* in the oral cavity correlate with low levels of *S. sanguinis*. Growth of S. sanguinis should be promoted to control the growth of S. mutans.

• *Changing the oral environment that is unfavorable for the pathogenesis of disease* (*S. mutans* and acid-producing bacteria): Changing the pH of the oral environment can bring changes in microbial population too.[29] Previous researches have shown the variation in the ability of *Streptococcus* species to compete in acidic environments. *S. mutans* is most favored by an environment below pH 6.0, while '*S. mitior*' is relatively sensitive to low pH. At pH 7.0, *S. sanguinis* was dominant with *S. mutans* and '*S. mitior*' maintaining similar populations.[30]

Studies have indicated that when tested under the continuous limitation of glucose and sucrose, strains of *S. mutans* were found to have a lower affinity for glucose and sucrose than *S. sanguis* and *S. milleri*.[31]

Growth of desirable microorganism can be promoted by creating the oral environment more favorable for them. This can be achieved possibly by changes in diet and using mouthrinses that can aid in maintaining desirable pH.

• Use of probiotics: Probiotics are used to introduce missing microbial components with known beneficial functions for the human host. Prebiotics enhances the proliferation of beneficial microbes or probiotics, to maximize sustainable changes in the human microbiome.[27]

The study done by Hatakka et al[33] suggests that cheese-containing probiotic bacteria can be used to reduce the prevalence of oral *Candida* in the elderly.

Health-associated bacteria can be used as probiotics for prevention of oral diseases.[33] A study done on the individuals who had never suffered from dental caries have other dominant bacterial species rather than mutans streptococci shown to inhibit the growth of cariogenic bacteria, suggesting the use of these commensal bacterial strains as probiotics to promote oral health and prevent dental caries.[34]

• Increase in diversity: Modern oral microbiota are markedly less biodiverse and, therefore, less resilient to the changes in the oral environment.[10] Increasing the biodiversity with the use of probiotics can improve the resilience of microbiota for oral environment.

• Avoid unnecessary use of antibiotics: Although antibiotic resistance is not solely a result of antibiotic overuse, it is also a natural phenomenon, but indiscriminate use of antibiotic affects the delicate balance between normal flora and host. Beneficial bacteria are also eliminated depriving the host from their beneficial effects.

- Low concentrated of mouthwashes: Oral care products often contain antiseptics and antimicrobials. If they are used twice daily in recommended amount, they are effective for a longer time period at sublethal concentration and control oral microbiome by inhibiting unwanted, disease-producing bacterial traits without eliminating beneficial bacteria.[13]

- Low concentration of fluoride: Fluoride is found to prevent the enrichment of *S. mutans* in the culture by reducing the degree of fall in pH, although other oral bacteria including, *S. sanguis*, a beneficial commensal, were inhibited too. However, fluoride in subminimum inhibitory concentrations is also shown to exert antibacterial effect on dental plaque without causing pH-mediated imbalance of microbiome and suppress the selection of *S. mutans*.[35]

CONCLUSION

Published evidences suggest that various changes in lifestyles have affected the symbiosis between the microbiome and their hosts. As a result, the present oral microbiome has become more pathogenic. Although it is not possible to reverse the lifestyle changes, but there are other ways to promote the growth of the desirable microorganism. A shift in microbiome can significantly affect physiological processes such as the development of innate and adaptive immunity, resistance to infections and increase in digestive capability of host.

The present review may be helpful in understanding the balance between the microbiome and their human hosts and ecological reestablishment of the perturbed microbiome.

Factors are significantly affecting our environment and ecosystem like-diet, use of antiseptic mouthwashes and use of mobile phones. Further research is required for better understanding of the effect of these above-mentioned factors and some other factors such as increased globalization and change in oral hygiene practices. By understanding these factors, we can anticipate our future microbiome, and it will help us in planning to bring desirable changes. This ecological way of oral disease prevention may prove a major breakthrough in the field of preventive dentistry.

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