ORAL MICROBIAL PROFILE AND THEIR CONNECTION WITH SYSTEMIC DISEASES: A REVIEW

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Declaration

It is hereby declared that

1. The thesis submitted is our own original work while completing degree at Brac University.
2. The thesis does not contain material previously published or written by a third party, except where this is appropriately cited through full and accurate referencing.
3. The thesis does not contain material which has been accepted, or submitted, for any other degree or diploma at a university or other institution.
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Abstract/ Executive Summary

In countries like Australia, Spain, the UK, and the USA, the interrelation of systemic diseases with oral microbes, has grabbed the attention of the experts. Oral microbes are the causative agents of several systemic diseases, such as Cancers (Pancreatic Cancer, Colon Cancer, Liver Cancer), Diabetes, Cardiovascular diseases (Stroke, Heart failure, Myocardial infarction, Endothelial dysfunction), RA, and Obesity by their nature of formation of biofilm or the growing usage of antibiotics and its resistance. According to a report by WHO in 2022, they evaluated that around 3.5 billion people are affected by oral diseases, among them 3 out of 4 people live in middle-income countries, such as Bangladesh. This review discussed the composition of oral microbes and their habitats where they reside in a biofilm form, which enables them to interact with each other and facilitate them to interact with other systems of the body. Additionally, excessive usage of several drugs has caused the induction of resistance to several antibiotics by different oral microbes and these resistances are achieved by different approaches. Moreover, specific bacteria work in systemic and oral diseases, targeting those oral microbes, and different kinds of therapeutic approaches were selected. It was concluded with the correlating antibiotic usage in Bangladesh and other countries, along with their increased antibiotic resistance, which can be anticipated from the reviewed data that Bangladesh could resemble antibiotic resistance like other countries, and the excessive usage of antibiotics could lead to increased correspondence to systemic diseases.

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Sincerely,

Jannatul Mawa Namrata

Rifah Tabassum Purno

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List of Acronyms

GCF	Gingival Crevicular Fluid
DGGE	Denaturing Gradient Gel Electrophoresis
T-RFLP	Terminal Restriction Fragment Length Polymorphism
PCR	Polymerase Chain Reaction
EPS	Extracellular polymeric substance
ARGs	Antibiotic Resistance Genes
HGT	Horizontal Gene Transfer
PBP	Penicillin Binding Proteins
MRSA	Methicillin-resistant Streptococcus aureus
MFS	Major Facilitator Superfamily
RND	Resistance Nodulation-Division
SMR	Small Multidrug Resistance
ABC	ATP-Binding Cassette
MATE	Multidrug and Toxic Extrusion
Th17	T helper 17

Chapter 1

Introduction

The human oral cavity is a dynamic ecosystem which harbors numerous microorganisms that collectively form the oral microbiome. This diverse microbial community is composed of more than 700 species of bacteria, fungi, viruses and other microorganisms that plays a crucial role in maintaining oral health which also impacts the broader health of the human host. The first scientific recognition of oral microbiome was done by the father of microbiology, Antony van Leeuwenhoek, who observed his own dental plaque and reported observing various forms of microbes present in the plaque. But it took another 200 years to prove how the microbes in the dental plaque are related to diseases occuring in the dental cavity. (Yamashita & Takeshita, 2017) [1]

Oral cavity is the beginning part of the digestive tract which is followed by the esophagus, stomach, intestine and colon. The microbial composition is different in each of these parts depending on the environment of that organ. The oral cavity itself has several different microbial niches like saliva, supragingival, subgingival areas, and all of these areas are habitat of different microbial species based on the nutrient availability and suitable environment for their growth. In the oral cavity, the bacteria do not reside as their planktonic stage, rather they form biofilm with appropriate neighboring microorganisms to ensure their stability in the oral environment and also to create resistance against antimicrobial agents. The formation of biofilm does not occur randomly, but specific bacterial species form these biofilms according to their capability of co-aggregation and competence. Even in these biofilms, many bacterias have some symbiotic relationship with each

other as they are connected by metabolic communication that can facilitate both bacteria. As the formation of biofilm is done by aggregation of many bacterial species, it is hard to differentiate among all the existing species in any oral cavity. For differentiating among the oral microbes, culturing processes are not much viable due to prevalence of unculturable species. To study and analyze all the existing species and strains in an oral cavity, novel processes like 16S rRNA sequencing, DGGE, T-RFLP are used.

Recent studies are indicating that the oral microbes are not just static in the oral cavity, they have been migrating to other systems of the body like the digestive system and intestine. (Qin et al., 2014) [65] As they are transmitting to the other organs, they are getting linked up with many systemic diseases like pancreatic cancer, diabetes type II, pediatric Crohn's Disease, heart disease, and low weight, preterm birth. (Krishnan et al., 2016b) [62] Development of systemic diseases that are influenced by oral microbiome can also be caused by the rapid use of antibiotics in the daily life of people. For the treatment of any oral disease, the taken antibiotic can impact both oral and other microbiome in the body, which increases the danger of opportunistic pathogens somewhere else in the body (Taylor, 2021) [40].

This review paper will embark on a comprehensive exploration of the oral microbiota, its function, structure and characteristics in both oral cavity and other systems in the body, how they can be linked to other systemic diseases via increasing patterns of antibiotic resistance and possible therapeutic approaches to evade such conditions.

Chapter 2

Composition of oral microbes

Human oral cavity consists of numerous diverse microorganisms which is estimated to be at least 700 species from at least 12 phyla. (Arweiler & Netuschil, 2016) [2] All together these microorganisms are referred to as oral microbiomes. The term microbiome was formulated by Joshua Lederberg "to signify the ecological community of commensal, symbiotic, and pathogenic microorganisms that literally share our body space and have been all but ignored as determinants of health and disease" (Lederberg & McCray, 2001) [3] In this oral microbiome, the prevalent microbes are bacteria, fungi, virus, archaea and protozoa which are correlated with several oral diseases. The dysbiosis of the oral microbiome can cause oral infectious diseases like dental caries, periodontitis, endodontic infections, alveolar osteitis, tonsillitis and candidiasis. It has also been found that the oral microbes can also be linked to many chronic systemic diseases like cardiovascular diseases, preterm birth, diabetes, pneumonia, stroke and several kinds of cancer. (Duran-Pinedo & Frias-Lopez, 2015) [4]

Among the oral bacterias, the main phylums that are found are *Actinobacteria*, *Firmicutes*, *Chlamydia*, *Euryarchaeota*, *Fusobacteria*, *Tenericutes*, *Bacteroidetes*, *Spirochaetes*, and *Proteobacteria*. Other than these main phylums there are some other lesser-known phyla that persist in the oral cavity which are *Chloroflexi*, *Chlorobi* and *Synergistetes*. (Dewhirst et al., 2010) [5] Pathogenic bacterias that are found in the oral cavity are: *Enterococcus faecalis*,

Staphylococcus aureus, Streptococcus pyogenes, S. pneumoniae, Neisseria meningitidis, members of the family Enterobacteriaceae. Gram-positive aerobic *cocci, peptostreptococcus*, α-haemolytic *streptococci*, and gram-negative anaerobes are often isolated from oral infections. (Sweeney et al., 2004) [6] The availability of fungi in the oral cavity is way less than the bacteria, fungi account for only 0.004% of the overall oral microbiome. Here the fungi has been only detected in the specimen from the supragingival plaque, oral rinses and the hard palate. There are about 100 species of fungi in the oral cavity and the common genera among them are *Aspergillus, Aureobasidium, Candida, Cladosporium, Cryptococcus, Fusarium, Gibberella, Penicillium, Rhodotorula, Saccharomycetales,* and *Schizophyllum*. (Ghannoum et al., 2010) [7] The viruses that are existing in the oral microbiome are mainly eukaryotic viruses like *Anelloviridae, Herpesviridae,* and *Papillomaviridae* and phages. It has been found in one study that the oral viruses do not persist in the oral cavity for a short period of time, the virus in the saliva acts as a host for various pathogenic genes in the oral cavity. (Pride et al., 2011) [8] The diversity of oral archaea is much less than the oral bacteria and they are currently recognized as non-pathogenic. Even though archaeans have been detected in the subgingival biofilms and in the inflamed pulp tissue from the patients with periodontitis and peri-implantitis. The oral archaea were originally thought to be only methanogens but recent studies have detected the existence of non-methanogenic archaeans as well. (Aleksandrowicz et al., 2020) [9]

2.1 Ecological niches in the oral cavity:

The oral cavity has different types of tissue consisting of both hard and soft tissue types. These different kinds of tissue have unique properties that are colonized by microorganisms that are best

suited to that particular environment. Ecological niche is a term that describes the functions of microorganisms in their habitat. The different parts of the oral cavity are saliva, soft tissue surface of the tongue, hard tissue surface of teeth or supragingival area, palate, gingiva and buccal mucosa. (Zhang et al., 2018) [10]

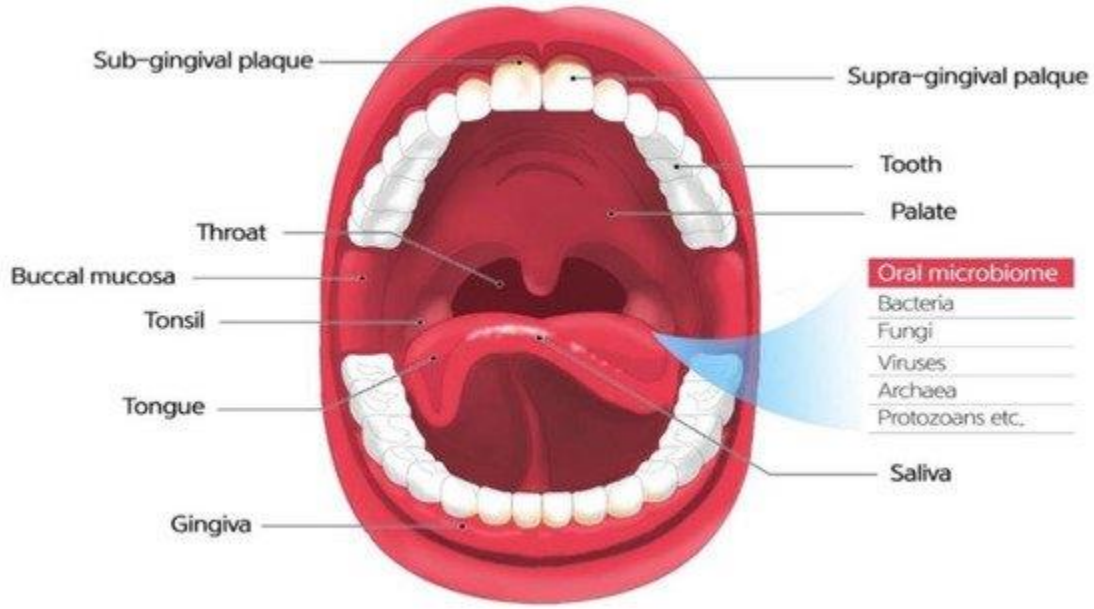

Figure 01: The different parts of the oral cavity ((Lee, 2021)) [11]

2.1.1 Saliva

Saliva includes all the microorganisms that come from other oral cavity parts but it does not represent the entire oral microbiome. The Firmicutes are more available in saliva but Actinobacteria and Fusobacteria are more available in the dental plaque. (Shi et al., 2018) [12] Saliva facilitates formation of a protective layer that works in microbial adhesion and colonization

process. Saliva plays an active role in immunological, chemical and physical defense of the oral cavity as it can influence both the colonization and clearance of microbes. It can contain defense proteins like lysozyme, salivary immunoglobulins, α-amylase, antimicrobial peptides, mucin and peroxidases. (Fábián et al., 2012) [13] It has been seen in different studies that the microbial composition of people from different countries varies. A study conducted with members of the Qatar has shown that the primary microbes in the saliva are the genera of *Neisseria, Gemella, Prevotella,Haemophilus, Porphyromonas, Streptococcus*, and *Veillonella* among them *Prevotella* is the most abundant. (Murugesan et al., 2020) [14] On the contrary, another study done on the people from Japan and China has shown that their microbial composition in saliva is different from the previous study where the most abundant genus is *Streptococcus* along with *Firmicutes, P. gingivalis, P. intermedia, T. forsythia* and *Fretibacterium*. It is predicted that these differences are caused by genetic, environmental and dietary factors. (Shaw et al., 2017) [15] As the variety of microbes changes in the saliva, this can be used to monitor the existence and prognosis of dental diseases like dental caries, periodontal diseases and even oral cancers.

2.1.2 Hard Tissue surface of teeth

As the tooth surface is the sole non-shedding surface of the oral cavity, it can facilitate the formation of dental plaque. The dental plaque has more microbial diversity and α-diversity compared to the tongue and saliva. (Ren et al., 2017) [17] The anatomy and physiology of the tooth surface area influence the oral microbial composition. There are 5 different areas of the crown surface of a tooth and they are: the proximal surface or contact point between teeth, the

occlusal or chewing surface, the buccal surface, the supragingival surface, and the lingual surface close to the tongue. Based on the location of these different parts, they have different bacterial composition. (Costalonga & Herzberg, 2014) [20] The hard tissue surface of the tissue is also known as the supragingival area which is the tooth surface that is coated with glycoproteins and proteins that are salivary components. The saliva is in constant contact with the hard surface of the teeth from where it supplies the nutrient for the microbes growing on the surface of teeth. The predominant species in the supragingival area are *Streptococcus* and *Actinomyces*. These bacterial species are able to form attachment between the adhesins on the bacterial cell surface and receptors that are found in the salivary coating and get attached to the saliva-coated tooth surface. These bacteria's are saccharolytic bacteria which can degrade carbohydrates that come from the food and transform it into organic acids like lactic, acetic, formic and succinic acids. This creates an anaerobic and acidic environment in the supragingival plaque which clears the way for colonization of the plaque by more aciduric and oxygen-labile bacteria like mutans *Streptococci*. This microbial shift and increase in aciduric bacteria can increase the cariogenic potential of the supragingival plaque (Takahashi, 2005) [16]

2.1.3 Soft Tissue surface of tongue

The oral mucosal epithelial is a shedding surface that sheds continuously. Compared to other mucosal tissues, the tongue has the greatest diversity of microorganisms. In the tongue coating, there are the existence of facultative and obligate anaerobes like *Streptococcus, Porphyromonas, Actinomyces, Prevotella,* and *Veillonella,* among them *Streptococcus* is the most common genus.

The tongue papillae provide an anaerobic environment which facilitates the existence of *Haemophilus, Leptotrichia,* and *Neisseria*. (Ren et al., 2017) [17] The oral mucosa contains both microbial immune barrier and physical barrier functions. So, the immunity of the oral mucosa also influences the composition of oral microbes. (X. Li et al., 2022) [21] Primarily the pattern recognition receptors are found on immune cells.

These receptors detect molecules associated with pathogens and activate a number of intracellular signaling pathways. Consecutively these pathways result in antimicrobial and pro-inflammatory reactions. (Şenel, 2021) [18] An example for this instance is that the oral immune cells can recognize the cell wall components of *Candida albicans* and it can produce antifungal reactions. For a healthy individual, the *Candida albicans* cannot induce high immune response and acts as a non-pathogenic yeast. But when it goes into the mycelial stage, it can activate IL17/Th17 pathway. Among the immunocompromised people, they might have an insufficiency of Th17 pathway. This deficiency can cause a fungal overgrowth and makes them more sensitive to oral candidiasis. (Gaffen & Moutsopoulos, 2020) [19]

2.1.4 Subgingival area

The subgingival site consists of an unstable epithelial surface and a stable tooth surface, these surfaces are washed with a continuous flow of gingival crevicular fluid. This fluid is a derivative of blood plasma which is rich in nitrogenous compounds like protein, peptide and amino acids. The subgingival site's environmental conditions, such as its neutral pH and anaerobic atmosphere, become more stable as a gingival crevice deepens. It facilitates the habitat of anaerobic,

asaccharolytic and proteolytic bacteria like *Eubacterium, Fusobacterium, Prevotella, Campylobacter,* and *Porphyromonas*. These bacteria get nutrition in the subgingival area from the gingival crevicular fluid and the desquamated epithelium. The proteolytic bacterias are able to degrade nitrogenous compounds by proteases that are either extracellularly secreted or cell membrane bound, and turn them into small amino acids and peptides. For example, *P. intermedia* is capable of degrading albumin and immunoglobulins by several proteases. The asaccharolytic bacterias have diverse metabolic pathways. *P. gingivalis* and *Prevotella intermedia* have many metabolic enzymes that can convert aspartic acid into succinic and acetic acids. Additionally, P. *gingivalis* can also convert glutamic acid and produce propionic and butyric acid by the propionic-butyric pathway. (Takahashi et al., 2000) [22] As the microbes from subgingival plaque require neutral pH and anaerobic conditions to thrive, bacteria like *P. gingivalis* are seen in the subgingival area and rarely in the supragingival area where the environment is acidic. (Takahashi, 2005b) [16]

The characteristics of supragingival area, soft tissue surface of tongue and subgingival area as different ecological niches are summarized in the following table.

	Supragingival area	Soft tissue surface of tongue	Subgingival area
Metabolic properties of microbial ecosystem	Saccharolytic	Facultative and obligate anaerobes	Asaccharolytic /proteolytic
Surface for microbial adhesion	Saliva-coated tooth	Saliva-coated epithelium	GCF coated tooth GCF coated epithelium
Nutrition	Saliva Carbohydrate	Saliva Desquamated epithelium	GCF Desquamated epithelium
Oxygen Concentration	High/low	High/low	Low
pH	Neutral/acidic	Neutral/acidic	Neutral

Table 01: Different characteristics of supragingival area, soft tissue surface on tongue and subgingival area

Chapter 3

Differentiating approaches of oral microbes

The microorganisms in the oral cavity that are the causative agents of several oral diseases usually exist in a biofilm formation, where the multispecies lifestyle is complex in nature. To analyze the bacterial species in any biofilm, traditional culture methods may be inadequate as most of the bacteria of an oral biofilm are not culturable. Among the oral microbiome, less than 1% of the microorganisms can be cultured in the laboratory. (Staley & Konopka, 1985) [23] As a result, alternative methods based on DNA analysis or molecular techniques have been developed for the assessment of dental biofilms.

By doing the DNA analysis, all the species present in the, both pathogenic and non-pathogenic, oral biofilm can be detected. Some mostly used molecular techniques for differentiating among the oral microbes are checkerboard DNA-DNA hybridization, 16S rRNA gene sequencing, Denaturing gradient gel electrophoresis (DGGE) and Terminal restriction fragment length polymorphism (T-RFLP).

3.1 Checkerboard DNA-DNA hybridization

The DNA-DNA hybridization is a DNA microarray technique considered as a gold standard of oral biofilm analysis which can process a large number of samples and simultaneously create profiles of multiple species. The whole process is done in a semi-quantitative manner. This technique is reliant on the process of binding DNA into a membrane, and that DNA has been isolated from the bacterial sample. After that, the hybridization with DNA probes that is specific to at least 40 separate bacterial species take place. (Nascimento et al., 2006) [24] The ability to do

simultaneous processing of a huge number of samples with multiple probes makes it very convenient. The microbes present on different oral surfaces, biofilm composition in periodontal diseases and prevalence of bacteria in specific oral communities can be detected using checkerboard DNA-DNA hybridization method. The original checkerboard hybridization methods use whole genomic DNA probes but it can be sometimes problematic due to the closely related sequences of some species. Like, in terms of identification of oral *Streptococci* that are very closely related phylogenetically . To evade such problems and differentiate between closely related species or subspecies, rRNA based DNA probes or oligonucleotides can be used. For using the oligonucleotide probes for analyzing, a PCR based, reverse capture, checkerboard hybridization protocol has been made. This modified procedure can detect and differentiate between the species of oral *Streptococci* directly from plaque samples. (Paster et al., 1998) [25] In this adapted approach to the conventional method, the 16S ribosomal RNA genes of 30 known bacterial species were amplified using PCR. Next, the PCR-amplified 16S rRNA genes are arranged on blots. Subsequently, a hybridization process is done with PCR-amplified 16S rRNA genes derived from unidentified plaque samples. These targets have primers that are labeled with universal probes and detected by chemifluorescence. The reverse capture hybridization technique enables simultaneous 1,350 hybridization reactions on a single membrane. (Hiyari & Bennett, 2011) [26]

3.2 16S rRNA gene sequencing

The 16S rRNA is used for the classification and identification of pure culture of bacteria and for estimating the bacterial diversity in various environmental samples. (Rajendhran & Gunasekaran, 2011) [28] 16S rRNA approach has been used to determine the phylogenetic identity of a bacterial

taxa and to determine in a mixed population if they are cultivable or non-cultivable. This 16S rRNA is present in almost every bacterial species and among them there are some differences in the sequences that helps discriminate between different species. This amplification process has also enabled the identification of unculturable species as well. (Janda & Abbott, 2007) [29] In this method, the identification of the species present in the oral biofilm is done by comparing the 16S rRNA sequence from the unknown sample with a database of species.

Firstly, from the biofilm sample the DNA is purified and from the genomic DNA, the 16S rRNA gene gets amplified by using gene-specific primers. This process can lead to amplification of the whole 16S rRNA gene or a smaller variable region of it. Following that, the PCR products are sequenced and the sequence gets compared against a database of known bacterial species. To determine the identity of the unknown species, there have been some threshold values that are considered while comparing the sample with the known database. For identification of a genus, a 97% similarity to a known database sequence is accepted. But to identify a species, 99% similarity to a known database sequence is accepted. It has been found that, by using the 16S rRNA analysis, over 300 bacterial species have been identified that were not initially identified by using culture-based methods. (Paster et al., 2001) [30] In a study of caries, the 16S rRNA sequencing and cloning has confirmed the presence of *S. mutans* and *Lactobacillus* spp as a primary member of pathogenic bacterias, and this result was also obtaining previously by culture and checkerboard methods. This analysis has also confirmed the presence of R. *dentocariosa* in the caries samples. (Munson et al., 2004) [31]

3.3 Denaturing gradient gel electrophoresis (DGGE)

The denaturing gradient gel electrophoresis or DGGE is a PCR and electrophoresis-based method used to analyze microbial communities. Here, the 16S rRNA and different marker genes are amplified by PCR and after amplification they are analyzed on a denaturing gel. The basis of separation is that a single-stranded DNA molecule migrates more slowly during electrophoresis than the equivalent double-stranded molecule because of enhanced interaction between the single-stranded molecule's unbonded nucleotides and the gel matrix. On the other hand, the double stranded molecules have hydrogen bonded nucleotides that pass through the gel easier. Here, a polyacrylamide gel is used that contains an increasing gradient of chemical denaturants like urea and formamide. When the double stranded DNA passes through the gradient in the gel, based on its GC content, the molecules begin to denature at a specific concentration of the denaturant. This can be visualized by electrophoresis where the separation is not done by size like typical gel electrophoresis but by the DNA sequence and its GC content. (Makovets, 2016) [32] Use of DGGE can aid in the detection of 50% of the sequence variants in DNA fragments up to 500bp. And this number can be increased to 100% by attaching a GC-rich sequence to one side of the DNA fragment. (Myers et al., 1985) In a DGGE gel, each band that is observed is represented by different bacterial populations within a community. So, the band patterns can be an identifier of the complexity and diversity of the biofilm sample This method has also been used in analysis of oral microbiome from dental biofilms of periodontitis and severe childhood caries. (Fischer & Lerman, 1983) [33]

3.4 Terminal restriction fragment length polymorphism (T-RFLP)

Terminal restriction fragment length polymorphism (T-RFLP) is a PCR-based method that can be used for the study of analyzing oral biofilms. This technique was initially used for analyzing bacterial diversity in the environmental samples but later it was utilized in the analysis of oral microbiome. (Liu et al., 1997) [34] T-RFLP has similarities to DGGE as it involves the amplification of specific gene markers like 16S rRNA, using PCR with gene-specific primers labeled with a fluorescent probe. The resulting amplified products go through the digestion process by using restriction endonucleases, following that the fragments are separated using capillary electrophoresis.

Next, the fragments carrying the fluorescent probes are detected and analyzed with the aid of specialized instruments and fragment analysis software. When gel electrophoresis is done with the samples, it produces distinctive banding patterns which indicates the complex microbial compositions in samples. This technology has proven successful in terms of identifying diverse microbial profiles in human saliva, identification of bacteria in infected root canals and also to identify any alterations in the microbial community in an oral cavity after it goes through any treatment. (Sakamoto et al., 2003) [35] Although the applications of T-RFLP are promising, it is still in its primitive stage. For an effective comparison, T-RFLP technique requires costly equipment, rigorous computational resources, and access to extensive genetic sequence databases. (Hiyari & Bennett, 2011b) [26]

A summarized version of the advantages and disadvantages of the molecular techniques of dental biofilm analysis is given below:

Molecular Method	Advantages	Disadvantages
Checkerboard DNA–DNA Hybridization	• Simultaneous profiling of multiple species • Simultaneous processing of large number of plaques	• Labor intensive • Traditional methods • limited to culturable species
16S rRNA gene sequencing	• High–throughput • Identifies unculturable species	• Low resolution at species level • No standardized threshold for distinguishing new species
DGGE	• Band pattern represent different bacterial population • Shows relative abundance of each species collected	• Difficulty maintaining reproducible results • Multiple species sequences may co–migrate
T-RFLP	• Fast detection of genetic diversity	• Novel software and database required • High computational power needed

Table 02: Summary of molecular techniques for dental biofilm analysis (Hiyari & Bennett, 2011b) [26]

Chapter 4

Formation of biofilm in oral cavity

Biofilms are described as an aligned aggregation of microorganisms that are connected to each other or attached to a surface and they are enclosed in some extracellular polymeric substances that are produced by themselves. (Hojo et al., 2009) [36] Biofilms can be found in nature on rocks on streams or in some industrial bioreactors. In animals, biofilms are also found in the host in otolaryngologic, vaginal or gastrointestinal tracts. There are more than 700 microbial species in the oral cavity but most of them are non-pathogenic and opportunistic microorganisms. The mouth constantly gets exposed to water-borne microorganisms, air-borne microorganisms and also microbes from food and droplets. The oral microbiome formation occurs right after birth and the *Streptococci* species is one of the first and dominant bacterial species that gets established in the oral microbiome. (Yumoto et al., 2019) [37] As more species get attached to the oral cavity, they aggregate together to form a biofilm structure to give them structural stability and be more resistant to various extraneous factors. The distribution of different species is not random in the biofilm formation, as they prefer specific sites with a beneficiary environment for them to grow and thrive. For the formation of biofilm, there are four stages in the process and they are: acquired pellicle formation, initial adhesion, maturation and dispersion. (Huang et al., 2011) [38]

4.1 Acquired pellicle formation

The attachment of acquired pellicle is the first stage of any oral biofilm formation. The tooth pellicle is produced from the salivary glycoproteins. As soon as the tooth is thoroughly cleaned, a thin film like tooth pellicle covers the tooth. (Figure 2).(Hojo et al., 2009) [36] The formation of

acquired pellicle is based on the Gibbs law of free enthalpy. It dictates that the attachment of glycoproteins to the surface of the tooth will cause the release of more energy. In the pellicle formation, the salivary glycoprotein is not the only active molecule, it interacts with the tooth surface along with other salivary components. These interactions cause various forces to form which can be categorized in three types:

- Long range force (50-100 nm between two interacting molecule): Includes van der Waals force, Coulomb interaction and dipole-dipole interaction
- Medium range force (10-50 nm between two interacting molecule): Includes hydrophobic interaction
- Short range force (less than 5 nm between two interacting molecule): Includes covalent bonds,ionic interactions , hydrogen bonds, electrostatic interactions, and Lewis acid-base interactions (Hannig & Hannig, 2009) [39]

These forces enable the proteins to be absorbed and make some conformational changes that prepares the newly formed pellicles for bacterial adhesion.

4.2 Initial adhesion

In the second step of biofilm formation, the bacteria get attached to the pellicle. By this adhesion the oral bacterial species interacts with the host molecules. The binding proteins in acquired pellicle are: α-amylase and proline-rich glycoproteins which are recognised by the planktonic bacteria. (Taylor, 2021) [40] The attachment in the initial adhesion stage is reversible so the initially attached bacteria can easily be removed from the pellicle. Electrostatic attraction and physical attachment cause the early attachments but in later stages the chemical forces become the

dominant factor. Many oral *Streptococci* has the ability to bind to *α*-amylase and proline-rich glycoproteins which gives them advantage in establishing the initial adhesion. (Kolenbrander et al., 2002) [41] Main early pioneer bacteria genera are: *Streptococcus* spp, *Haemophilus* Spp, *Actinomyces* spp, *Capnocytophaga* spp, *Neisseria* and *Veillonella* spp.(Dige et al, 2008) [43] *Streptococcus gordonii* is one of the early colonizers which can bind to acidic proline-rich proteins that are regarded as 25-30% of the total proteins in saliva. *Streptococcus sanguinis* is another early pioneer that can selectively attach and colonize the tooth pellicle. It is seen in the oral cavity after any tooth eruption occurs. (Rogers et al., 2001) [42] Soon after the pioneer bacteria attach to the pellicle, they secrete EPS and enable other bacteria to attach to the pellicle as well. Bacteria attach to the pellicle by their fimbriae and fibrils and this attachment is driven by the force of hydrophobic interactions, hydrogen bonds, van der Waals force and electrostatic interactions. (Hannig & Hannig, 2009) [39]

4.3 Maturation

As the early pioneer bacterias attach to the pellicle, they can provide specific binding sites. These binding sites can be provided directly or by binding to the salivary glycoproteins which facilitates subsequent binding of other bacteria and form the biofilm structure (Figure 2). The polysaccharide or protein receptors in the cell surface of pioneer bacterias are recognized by the late colonizers, consequently they start to attach to them. Among the late colonizers there are: *Treponema spp, Fusobacterium nucleatum, P. gingivalis Tannerella forsythensis, Aggregatibacter actinomycetemcomitans*. (Kreth et al., 2005) [44] During the maturation stage of biofilm formation, a proportional shift occurs. This shift causes a change in the microbial population from the initial to mature biofilm. Here, the amount of *Streptococci* and *Neisseria* decrease, and

Fusobacterium, *Actinomyces*, *Corynebacterium*, and *Veillonella* increase. When the biofilm forms its mature form, to provide the bacteria with necessary elements, they have porous layers and water channels. In the mature biofilm, the existing bacteria aggregate in a biofilm via cell-to-cell recognition process.

4.4 Dispersion

In Figure 2, it is shown that the bacteria leave biofilm by a single cell detachment or a group of cell detachment. The dispersion of biofilm includes erosion, sloughing and seeding. As the level of nutrition decreases at the original site of biofilm, the bacterias leave the site and try to find a new location with elevated nutritional content for growth. This search for nutrition divides the dispersion in 2 groups which are active and passive dispersion. The active dispersion is done by any particular bacteria but the passive dispersion occurs when other bacterial species compete with the particular bacteria for nutrition. (Kaplan, 2010) [45]

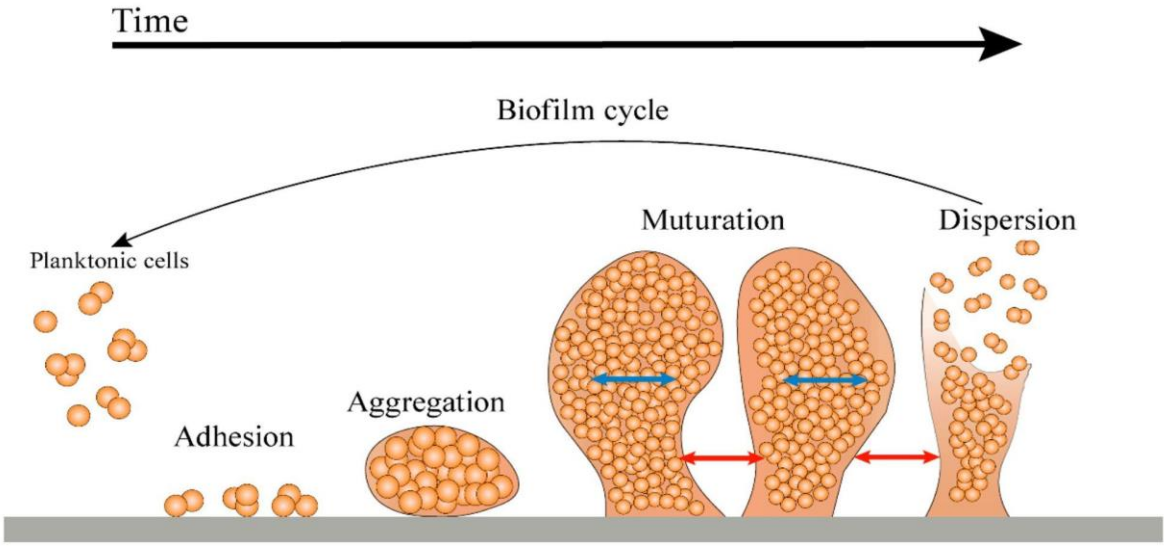

Figure 02: Diagram of steps of biofilm formation (Peng et al., 2022b) [46]

4.5 Components of Biofilm matrix

The matrix is the most dominant feature of any microbial biofilm as it also controls many characteristics of the bacterial cell when they are clustered together in a biofilm. The primary components that are the basis of most biofilms are proteins, carbohydrates, nucleic acids, cell wall polymers like lipid and peptidoglycan. (Flemming & Wingender, 2010) [47] This matrix facilitates binding of bacterial cells to the surface and also functions like an ion exchange resin that obstructs the flow of reactive or charged molecules through biofilm. The formation of the EPS matrix is dependent on the availability of substrate, creation and secretion of extracellular materials. One of the major matrix components that is related to dental caries is polysaccharides, specially the gulcans derived from the *S. mutans.* Some other soluble glucan and fructans are produced by some other species like *S. salivarius* and *S. gordonii*. (Bowen & Koo, 2011) [48] There has been a study conducted that showed the production of carbohydrate polymer by oral *Streptococci* which has an important function in development of dental caries. (Banas & Vickerman, 2003) [49] The carbohydrate contains almost 20% of the dry weight in a supragingival plaque and almost 2/3rd of this carbohydrate is water insoluble. In the mature biofilm matrix, there is also the presence of some extracellular DNA which is necessary for the stabilization of biofilm. It has been documented that many oral *Streptococci* depend on the extracellular DNA for maintaining the structural stability of the biofilm matrix. Additionally, it can also increase attachment to the tooth surface. For wild type matrix formation, *A. actinomycetemcomitans, Prevotella* spp, and *P. gingivalis* depend on the extracellular DNA. (Jakubovics & Burgess, 2015) [50] The matrix also works for positioning the cells in such a distance where the interaction between different species can be beneficial for both of them and the competition gets minimized. (Stacy et al., 2014) [51]

4.6 Biofilm vs. Planktonic Bacterial Cells

In the dental plaques, the bacterial cells act differently than their planktonic counterparts. Here the biofilm formation gives them both natural and acquired tolerance to various antimicrobial agents. Biofilms are heterogeneous in nature where they express different kinds of genes, metabolic activity and phenotypes compared to the planktonic stage. (Yumoto et al., 2019) [37] The difference between the external environment and the environment of the biofilm in plaque causes the phenotypic differences. When the bacteria reach its stationary stage, their growth rate decreases and there is less active replication occurring. So, the bacteria can channel their energy for growth to other functions like increasing resistance for antimicrobial activity. Also, the thick wall of dental plaque decreases the penetration of any antimicrobial agents, so their diffusion cannot reach the level where it can impact the longevity of the microbes. (Mosaddad et al., 2019) [52] The physical and metabolic changes in the microbes also contribute to the natural resistance of microbes in oral biofilms.

4.6.1 Co-aggregation among oral bacteria

Co-aggregation is the cell-to-cell reaction when the planktonic bacteria try to bind to the cell surface of early colonizers that are already present in the surface. This is the most important mechanism for colonization of oral microbes and formation of dental biofilm. At the bottom layer of the biofilm, the early colonizers bind by adhesins to the complementary pellicle receptors. After that, the secondary colonizers bind to the early colonizers and form a new surface to construct a bridge between the adjacent aggregated cells. *Poephyromonas gingivalis* is a secondary colonizer that binds to the early colonizers. When the process of bridging occurs between more than 3

species, it is important as it can connect species that are not natural co-aggregate partners. For example, *Fusobacterium nucleatum* can co-aggregate with other oral species like obligate anaerobes and *Streptococci*, which makes it one of the key components of dental biofilms bridge coordinators. (Kolenbrander et al., 2002) [41] This co-aggregation process doesn't only help in colonization of bacteria but also facilitates genetic exchange and metabolic communication considering that through this process, all bacteria can have an easy access to its neighbor bacterial metabolites.

4.6.2 Metabolic communication among oral bacteria

Oral bacteria get primary nutrients from the saliva, gingival crevicular fluids, food debris and foods that contain sugars (Figure 3) Along with it, there are metabolic communications among the oral bacteria where one microbe excrete some metabolite and it gets used as a nutrient by another microorganism. It can also happen when an organism breaks down a substrate by their enzymatic activity, it can be utilized as substrates by other organisms. For example, *S. oralis* hydrolyzes the host glycoproteins and utilizes monosaccharides that are important for the survival of both *S. oralis* and other oral bacteria. (Kolenbrander et al., 2002) [41] Similarly, some oral bacteria can produce short chain fatty acid that acts as an essential carbon source for other oral bacteria. It has been suggested by many studies that there is a symbiotic relationship between the *Streptococcus* and *Veillonella* species for the lactic acid production by *Streptococcus.* In both in vitro and in vivo growth of *Veillonella,* it is dependent on the lactate produced by other oral microbes. (Kumar et al., 2005) On the other hand, *Veillonella* can produce vitamin K_2 which is not synthesized in humans, is required for most of the *Prevotella* and *Porphyromonas* strains (Hojo et al., 2009)[36].

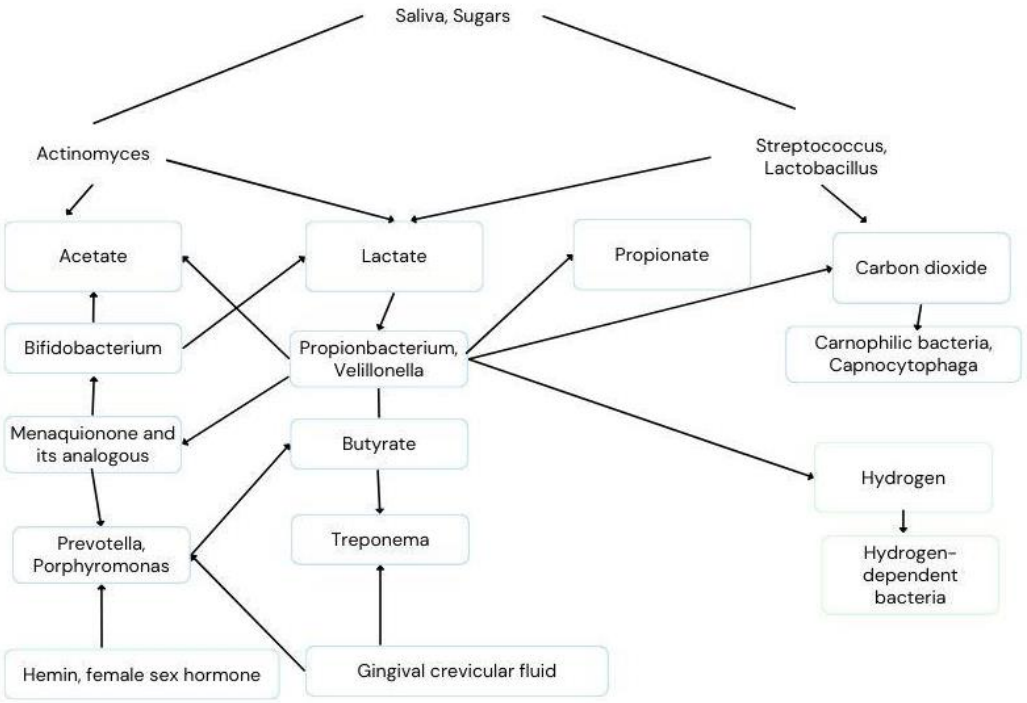

Figure 03: Metabolic relationship among oral bacteria in a dental biofilm (Generated using Canva)

4.7 Competition in biofilm

Biofilm being the habitat of a diverse colony of microbes, there are some competitive functions among the bacteria living within the biofilm. The competitive mechanisms that are thoroughly adopted by bacteria are synthesis of bacteriocin and quorum sensing.

4.7.1 Bacteriocin synthesis

Bacteriocin are proteinaceous bactericidal substances that are produced by bacteria so that it can suppress the growth of closely related bacterial species. The difference in bacteriocins from any antibiotic is that the bacteriocin produced in a bacteria can only act in its own strain or similar

strains. (Kuramitsu et al., 2007) [53] It has been seen in studies that among all oral bacterias, *Streptococci* has the strongest ability to produce bacteriocin. *S. mutans* can produce 2 types of bacteriocin which are lantibiotics and non-lantibiotics. Lantibiotics are an antibiotic peptide that contains mutacin I, II and III. On the other hand, non-lantibiotic contain mutacin IV and V. Compared to the non-lantibiotic, lantibiotic has wider spectrum of inhibition against gram positive bacteria. (Huang et al., 2011c) [38]

4.7.2 Quorum sensing

Quorum sensing is a signaling mechanism that is activated for responding to the cell density. This mechanism is present in both gram positive and gram-negative microorganisms. (Saini et al., 2011) [55] Here, the bacteria secrete autoinducers and it can regulate the interaction among different types of bacterial behavior. Through the quorum sensing, many responses can be controlled like adhesion to bacterial surface, synthesis of biosurfactants, extracellular matrix production, spore formation and virulence factor expression. As this system is very specific and accurate, it can accurately regulate different bacterial phenotypes. (Nadell et al., 2008) [56] There are 3 types of quorum sensing system prevalent in bacteria. In the first type, the gram-positive bacteria uses oligonucleotide as a signal molecule. In the second type, as their signal molecule, gram negative bacteria use acyl-homoserine lactone. Lastly, the third type is seen in both gram positive and negative bacteria where they use AI-2 as their signal molecule. (Karatan & Watnick, 2009) [57]

Chapter 5

Interaction of oral microbiota with another system

The oral microbiome is composed of a consortium of species that lives in a biofilm formation in the oral cavity. These bacteria can cause opportunistic infections when they get proper conditions for disease formation. (Krishnan et al., 2016) [58] The health of the oral cavity can work as an indicator for the health of the whole body. Some oral diseases can get linked up with systemic diseases of the other systems of the body. Oral microbiota can get spread in to the rest of the body by two major pathways which are:

- The biofilm bacteria can revert back to their planktonic stage and move freely to the other parts of the body
- Interaction between host immune system and the microorganism can impact the hosts sensitivity to any disease development (L et al., 2018) [59]

5.1 Presence of oral bacteria in the intestine

There are many microbial niches in the oral cavity which are divided into the supragingival surface, subgingival surface, soft buccal. Lingual and tongue mucosa. All of these areas have various kinds of microbiota and all of them have separate nutrient, pH and salivary levels. The oral bacterial microbiota is very stable compared to the microbial community in the intestine. (Halfvarson et al., 2017) [60]

Three ways have been described in how the oral microbiota can cross the distance and evade the organs of the digestive system, and they are:

- Direct invasion of oral microbiota through the esophagus and which will affect the digestive system and create an imbalance in intestinal microbiology. (Qin et al., 2014)
- *F. nucleatum* colonizes and enters into the colorectal tract by the blood cycling route. The pathogenic bacteria of periodontitis can enter the systemic circulation and pass on to the whole body by periodontal blood. (Abed et al., 2016) [66]
- Metabolites produced by oral microbes can enter the bloodstream and put the human body in an inflammatory state which causes the development of chronic disease in the digestive system. (Clemente et al., 2018) [67]

In a study, that analyzed the large fecal and salivary sample from various countries where it was seen that the intestine is colonized by high levels of oral species that account for almost 2% of classifiable microbial presence in the feces. Additionally, the profiling of microbial single nucleotides has demonstrated that oxygen tolerant species are highly present in oral bacteria, these oral bacteria have been translocated to the intestine of healthy individuals. These bacterias include *Fusobacterium spp., Streptococcus spp., Veillonella spp.* and *Haemophilus* spp (Schmidt et al., 2019) [61] In another study, it has been detected the presence of oral bacteria *Dialister invisus* at the DNA level at a stool sample. But transcriptome sequencing has proved that this bacteria might be transcriptionally inactive which indicates a non-viable population.(Schirmer et al., 2018) [62] On the contrary, viable oral bacteria have also been cultured from the content of the intestine which includes *Streptococcus salivarius, Veillonella parvula, Fusobacterium nucleatum* and *Campylobacter concisus* . (Van Den Bogert et al., 2013) [63] The existing resemblance in microbial composition of oral and fecal samples suggests that the presence of oral bacteria in the intestine is limited but not null and some viable species like *F. nucleatum* can translocate from the oral cavity to colonize the intestine. (Kirk et al., 2018) [64]

Chapter 6

Antibiotic resistance in Oral Microbiota:

Antibiotic resistance, which occurs due to the widespread availability of antibiotic resistance genes (ARGs) in oral microbiomes and the correlation between the presence of ARGs and the clinical use of antibiotics, is one of the significant threats to the global health system. According to the World Health Organization (WHO), antibiotic resistance significantly reduces motility rates attributed to numerous infectious illnesses and lifespan (World Health Organization: WHO, 2020) [167]. Antibiotic resistance could be associated with genetic changes or mutations, though resistance could be acquired through phenotypic resistance through specific growth in biofilms, persistence, or a stationary phase.

6.1 Approach to Antibiotic Resistance:

Antibiotic resistance phenotypes could be intrinsic where physiological traits of the species will exist or naturally, or acquire resistance which is achieved by two genetic mechanisms by which resistance is achieved such as horizontal gene transfer (HGT) or chromosomal mutation of the preexisting bacterial genome (Davies & Davies, 2010) [91].

6.1.1 Naturally/ intrinsic resistance:

Naturally resistant bacterial species are susceptible to antimicrobials, which could be due to the change in structure, which acts as the target molecule, or different metabolic processes vital for activating the antimicrobial (Reygaert, 2018) [143] . Additionally, bacteria with no cell wall are naturally resistant to antimicrobial activity against the cell, such as the β lactam antibiotic that

targets the cell wall. In contrast, *Mycoplasma* species consist of a cell wall. Usually, intrinsic resistance is linked to a lack of metabolic process that is also noticeable within oral microbes; for instance, *Actinomyces* species, *Streptococcus* species, and *Aggregatibacter*; these species lack the enzyme nitroreductase which is essential to convert metronidazole to its active metabolites; as a result of these not sensitive to the standard drug concentrations (Reygaert, 2018) [143].

6.1.2 Horizontal Gene Transfer:

Horizontal gene transfer is the transfer of a resistance gene into the plasmid and exchanged genetic material to build up resistance, and this is the most persistent way to circulate ARGs. The resistance is enhanced through microbial structures, for example, biofilm, where bacteria are placed perfectly to evolve their genomic material and exchange their genetic materials (Reygaert, 2018) [143]. Hence, bacteria within a biofilm are more resistant than planktonic counterparts. HGT occurs through three mechanisms: conjugation, transduction, and transformation.

At first, transformation is when the resistance gene carries exogenous DNA segments acquired from the bacteria. Bacteria alter their physiological state, which is known as competence, and during this, bacteria integrate exogenous DNA from the environment. So, this process is followed by naturally competent bacteria, for example, *Pneumococci*, *Haemophilus*, and several oral *Streptococci* (Chaguza et al., 2015) [84]. Moreover, transformation influences the progression of mosaic genes, such as developing the mosaic structure of a protein known as Penicillin Binding Proteins (PBP), which causes penicillin resistance in *Streptococci*. Secondly, transduction is a pretty similar process to transformation, but here, exogenous bacterial DNA is transferred from one bacterium to another bacterium through phage particles (Chaguza et al., 2015) [84]. Lastly,

conjugation was discovered by Edward Tatum and Joshua Lederberg in 1947 when they mixed two different strains of *E.Coli* and discovered a new type of recombinant bacteria, unlike the parental strains, which resulted in direct physical contact between two strains where the plasmid DNA was transferred from the donor to recipient bacterium (Raleigh & Low, 2013) [141]. Conjugation offers a wide range of bacteria the ability to transfer the resistance ability through plasmid. Though mobilized plasmids are not conjugative, they can be transferred to the recipient through a separate, self-transmissible plasmid in the donor so that they can play a role in the spread of ARGs (Suhartono & Savin, 2016) [154].

6.2 Four principal ways for bacteria to make antimicrobials ineffective:

Bacteria utilize several specialized defense mechanisms that are encoded by ARGs, such as target site modification (Zhang & Cheng, 2022) [171].

6.2.1 The alteration of the active binding site of the antibiotic:

Here, targeted molecules are structurally changed for the disruption of the antibiotic binding, such as the altering of the ribosomal target site in the DNA gyrase/topoisomerase gene, which is targeted by fluoroquinolones (Kapoor et al., 2017) [106]. Also, PBP is altered by mutation in chromosomal genes; this mechanism is widely used by Gram-positive cocci. Moreover, methicillin-resistant *Streptococcus aureus* (MRSA) produces substitute PBP, which reduces the effect of antibiotics, and by producing low-affinity PBPs, the resistance of β-lactam antibiotics could be increased. These mechanisms are widely used by *Streptococcus oralis, Streptococcus sanguis, and Streptococcus mitis* (Kapoor et al., 2017) [106].

6.2.2 Increases the activity of efflux pumps:

Efflux pump refers to the mechanism of pumping out the antibiotic from the cell, and bacteria efflux the antimicrobial agents from the cell. There are five leading families of efflux systems, which are the Major Facilitator Superfamily (MFS), Resistance Nodulation-Division (RND), Small Multidrug Resistance (SMR), ATP-Binding Cassette (ABC), and Multidrug and Toxic Extrusion (MATE) (Sharma et al., 2019) [150]

6.2.3 Restricting the entry of antibiotics into cells:

Numerous antibiotics utilize porin channels to enter gram-negative bacteria. As a defense mechanism, they lower the expression of porins, leading to impermeability and restricting the uptake of the antimicrobial, resulting in antibiotic resistance (Delcour, 2009) [93].

6.2.4 Enzymatic inactivation of antibiotics: The most common mechanism of resisting β-lactam antibiotics is inactivation through enzymatic degradation, such as β-lactamases ([Mechanism of Enzymatic Resistance to Beta-lactam Antibiotics], 1986) [128].

Several species achieved excellent resistance through utilizing one or more of the four principal and this resistance is widely applicable to some commonly used drugs; that are mentioned below:

Drugs	Species	Mechanism for resistance
Tetracycline	Gram positive, Gram negative	Enzymatic inactivation of antibiotic, Increasing the activity of efflux pump, Ribosomal protection protein. (Connell et al., 2003) [87] (Roberts, 1996) [144]
Erythromycin	Mostly Gram-positive	Enzymatic inactivation of antibiotic, alteration of the active binding site and increasing the activity of efflux pump. (Leclercq & Courvalin, 1991) (Leclercq & Courvalin, 1991a) [115-116]
Metronidazole	Only anaerobic and a few facultative anaerobes	Enzymatic inactivation of antibiotic (5-nitroimidazole reductase) (Carlier et al., 1997) [82]
Clindamycin	Aerobic and anaerobic Gram-positive, Anaerobic Gram-negative	Enzymatic inactivation of antibiotic, alteration of the

	(narrow-spectrum), *Haemophilus spp.* (Broad spectrum), anaerobic and a few facultative anaerobes, all Gram-positive	active binding site and increasing the activity of efflux pump (Leclercq & Courvalin, 1991) (Leclercq & Courvalin, 1991a) [115-116]
Ampicillin/Amoxicillin	Aerobic and anaerobic Gram-positive, Anaerobic Gram-negative (narrow-spectrum) and *Haemighilus spp.* (Broad spectrum)	Enzymatic inactivation of antibiotic (b-lactamase), alteration of the active binding site (Mosaic PBP) (Hakenbeck & Coyette, 1998) [101] (Livermore, 1995) [120]
Phenoxymethylpenicillin	Aerobic and anaerobic Gram-positive, Anaerobic Gram-negative (narrow-spectrum)	Enzymatic inactivation of antibiotic (b-lactamase), alteration of the active binding site (Mosaic PBP) (Hakenbeck & Coyette, 1998) [101] (Livermore, 1995) [120]

Table 03: Names of most used antibiotics, the species that are resistant and their mechanism to achieve resistance

Chapter 7

Diseases caused by oral bacteria:

Oral microbiota colonizes and produce metabolites in the mouth that directly affect a wide range of diseases; these microbiotas are mostly linked with several common oral diseases, for example, Caries, Oral cancer, Gingivitis, and Periodontitis. Recently, several studies have shown that oral microbiota is closely connected with systemic diseases, such as Cancers (Pancreatic Cancer, Colon Cancer, Liver Cancer), Diabetes, Cardiovascular diseases (Stroke, Heart failure, Myocardial infarction, Endothelial dysfunction), RA, and Obesity; the development of oral microbiota and progression of oral disease plays a role in the systemic disease. Moreover, the most common systemic infection is infectious endocarditis, which is caused by dental bacteria oral streptococci, which is a commonly reported causative agent.

7.1 Oral diseases:

7.1.1 Dental Caries:

In the oral cavity, dental caries is the most known chronic infectious disease that destroys the hard tissues of the teeth and is caused by several bacteria, such as *Lactobacillus spp., Prevetellaa spp., Dialister spp., Filifactor spp.,* and more that might be involved in the progression of dental caries (Lamont et al., 2018) [27,111]. During this disease, the acidity of the mouth increases, which causes a reduction of microbiota diversity in dental caries. However, the display of *S. acidophilus* was significantly shown in the salivary microbiota. When the host is not eating for a more extended period, these bacteria break down the glycoproteins into sugar and protein, and later, these are

metabolized and acidic, and small essential molecules neutralize each other (Lebeer et al., 2008) [114]. During dental caries, the acid-producing bacteria triumph in the metabolism process of sugar, and teeth will be oxidized more.

7.1.2 Oral cancer:

Oral cancer is a malignant tumor that occurs in the mouth, and this squamous cell carcinoma is known as mucosal variation. There are several varieties of oral cancer, for example, Gingival cancer, Tongue cancer, Oral cancer, Salivary gland cancer, Maxillary sinus cancer, Jaw cancer, and cancer in the facial mucosa. In the development of oral cancer, genetic factor, bacteria, and living habitats plays a significant role as oral microbiota and oral cancer is interlinked; several microorganisms were found on the surface of oral cancer and in cancer tissue, and their structure was different from normal mucosal microorganisms. When the oral squamous cell carcinoma increases, *S. mutants* and prednisone bacteria also increase (Watters, 2022) [165].

7.1.3 Gingivitis:

In healthy condition, the microbiota at the gingival sulcus is vast, but this area is regulated by gram-positive oral *Streptococci*; they develop the biofilm and starts gingival inflammation along with marginal swelling, pocket formation, and more. After a few weeks, the biofilm matures, and a complex microflora grows there, including gram-positive *cocci* and gram-negative *rods*, which comprise different sizes (Schulze et al., 2021) [146]. The development of the disease takes place due to unspecified biofilm formation, where all bacteria contribute to the progression of the disease.

7.1.4 Periodontitis:

In the gingival tissue, the inflation develops, allowing supragingival biofilm to merge with the gingival margin; with the maturation of biofilm, periodontal pockets develop into periodontitis. Periodontitis is usually termed as chronic or aggressive periodontitis with progression of the disease. Here, dental plaque bacteria, including plaque and subgingival plaque, spread on the surface of the tooth or periodontal pocket to develop the disease by destroying periodontal tissues. During this disease, the mouth is suitable for the microorganism to grow, and F. nucleatum in sailva is highly found in people with periodontitis (Bhuyan et al., 2022) [80]. After comparing the microbiota with ordinary people, the level of *Streptococcus pneumoniae, Clostridium, Carbachia, Porphyromonas, Helicobacter, Actinomycetes, Eugenia, Tannela, Hurdella,* and *Micromonas* in the patient of periodontitis are found in higher amount; at the same time these patients showed a low level of *Neisseria, Corynebacterium, and Carbonophili*. So, the change in microbiota in periodontitis patients could cause an alteration in functional gene structure and gene expressions (Okabe et al., 2021) [133]. A study by M. Lu et al. found that the Th17 in local inflammatory lesions increases significantly in periodontitis patients, which indicates that increased periodontitis is associated with Th-17 cells, which depend on oral microbiota. Therefore, oral microbiota could lead to an enhanced level of local Th17 cells, which promotes periodontitis. Due to this advanced and complex mechanism of periodontitis, it is interrelated with several systemic diseases as well (Huang et al., 2021) [103].

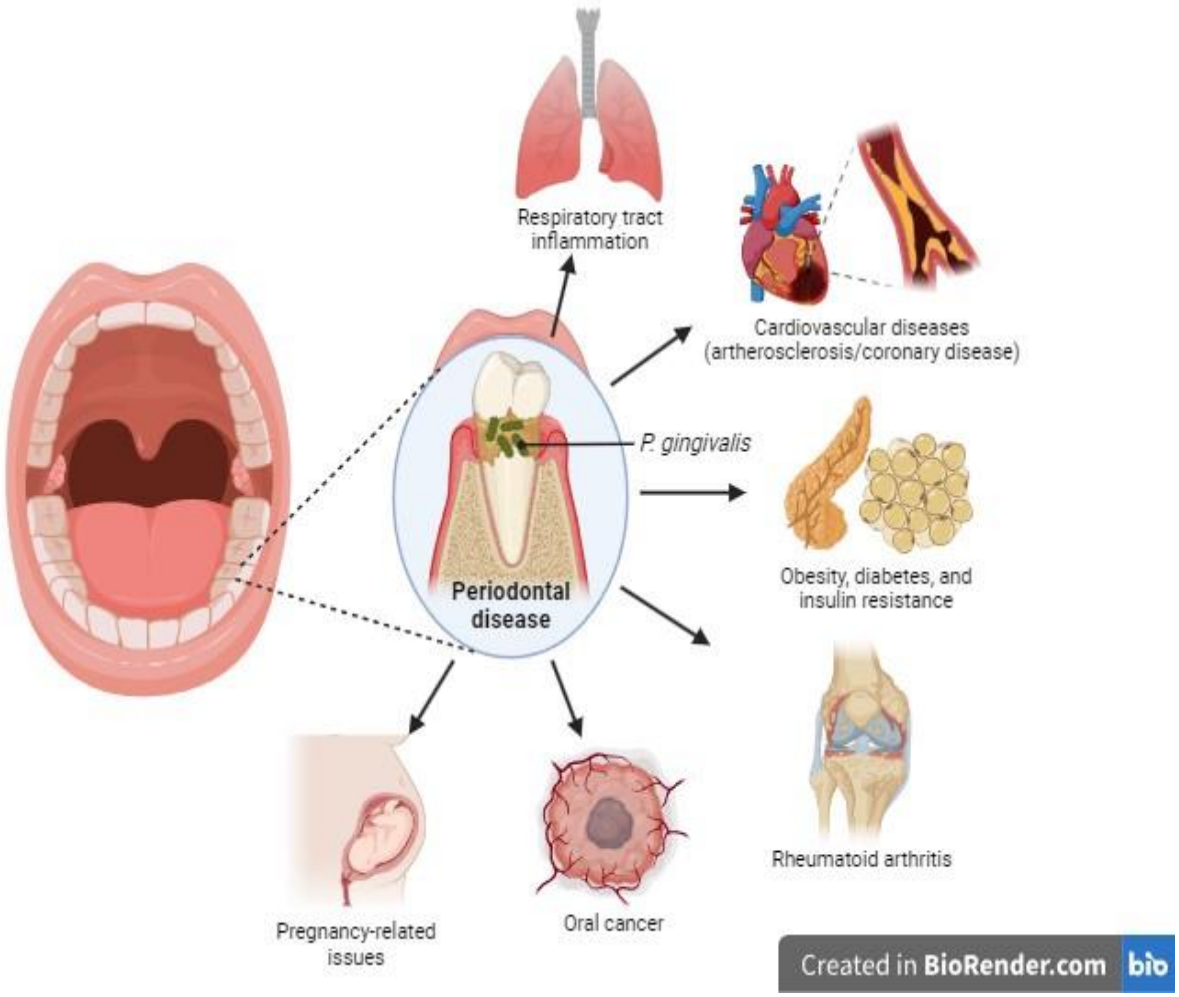

Figure 04: This figure represents the correlation between periodontal disease and other systemic diseases, such as oral cancer, RA, Obesity, Diabetes, CVDs, pregnancy, and respiratory tract inflammations; this figure is made by biorender.

7.2 Systemic diseases:

7.2.1 Cancers:

7.2.1.1 Pancreatic Cancer:

Pancreatic cancer is the fourth most common cancer with a high mortality rate. The major risk factor for this type of cancer is genetic factors, obesity, and smoking. However, the relation between pancreatic cancer and the imbalance of the microbiota in the oral exists. A study by How et al. showed that in the body of oral cavity patients, a higher rate of *P. gingivalis* and *Actinibacillus actinomycete* were found. In the same study, he also found an association between *P. gingivalis* and pancreatic cancer patients; By several analyses, he demonstrated that *P. gingivalis* is also found in higher amounts in the mouth of pancreatic patients, which promotes the progression of the tumor (Ma et al., 2023) [123].

Moreover, recent studies have shown that certain oral microorganisms contribute to cancer onset and progression by tuning on oncogenic signaling, boosting metabolic pathways, altering the proliferation of cancer cells, and stimulating chronic inflammation that suppresses tumor immunity (Chai et al., 2023) [85]. A study by Chai et al. showed that *P. gingivalis* survives and persists in host immune tissue by disrupting the host's immune responses by interacting with receptors, altering signaling pathways, such as activating the NF-B pathway, which increases the expression of cytokines and causing inflammation, promoting tumorigenesis (Chai et al., 2023) [85]. Also, *H. pylori* and pancreatic cancer have a positive correlation, found in high amounts in the oral microbiota of pancreatic cancer patients (Xu et al., 2022) [169]. As *H. pylori* was in the pancreatic tissue of the patients with pancreatic cancer and *H. pylori* was detected in 75% of pancreatic cancer patients. (Chai et al., 2023) [85]. Additionally, it infects the stomach and colonizes in an acidic

environment which is primarily responsible for causing gastric ulcers, pancreatic cancer is also related to gastric ulcers. They colonize the gastric mucosa by linking with antral gastritis, which leads to excessive acid secretion and increased susceptibility to pre-pyloric and duodenal ulcers; this colonization of *H. pylori* is a factor that leads to the development of pancreatic cancer, as they activated the molecular pathways related with pancreatic cancer initiation and maturation, therefore leading to pancreatic malignancy (Chai et al., 2023) [85]. However, a possible indirect mechanism is also used by *H. pylori* which are immune escape and inflammatory responses. Also, *H. pylor* can damage host DNA to trigger cellular carcinogenesis by secreting cytotoxin-associated proteins (Chai et al., 2023) [85].

7.2.1.2 Colon Cancer:

Colorectal cancer and gut microbiota are connected; colorectal cancer is also linked with *F. nucleatum* in the oral cavity, which could be transported to the other body parts by blood. During passing through blood, the immune system rejects them, leading to local inflammation and the promotion of tumor formation (Abed et al., 2020) [68]. In several studies, *F. nucleatum*, a gram-negative obligate anaerobic bacterium, was found in colorectal cancer patients' cancer tissue. Studies have found that *F. nucleatum* could develop in the mother’s oral cavity and affect fetal tissue by traveling from the mouth to another part, such as the uterus of the mother’s body, which could lead to fetal death; this indicates that *F. nucleatum* can travel from one place to other parts of the body (Sun et al., 2019) [155]. Additionally, Fly et al. showed that periodontal biofilm is consistent with the biofilm component of the colonic mucosa of colorectal cancer patients (Tomkovich et al., 2019) [162].

7.2.1.3 Liver Cancer:

In liver cancer, oral and gut microbiota imbalance plays an important role. A high difference in the oral microbiota of patients with liver cancer and ordinary people has been shown by M. Lu et al. he showed evidence of the composition of the oral microbiota in patients with liver cancer; for instance, *Clostridium, Oribacterium, Ciliate, Actinomycetes* and *Campylobacter* were seen high in number; in contrast, *Haemophilus, Streptococcus* and *Pseudomonas* showed low abundance. For diagnosis purposes, Clostridium and Oribacterium could be used (Lu et al., 2019) [121].

Moreover, the association between an imbalance of oral microbiota and liver cancer patients with cirrhosis is highly noticeable due to decreased oral symbiotic bacteria and an enhanced number of pathogenetic bacteria, such as *Enterococcus* and *Enterobacteriaceae* (Manzoor et al., 2020) [125]. In several studies, the gut microbiota of the patients with cirrhosis and healthy people were compared, and these patients' intestinal microbiota had a high number of oral-derived microorganisms, for example, *Streptococcus, Haemophilus, Lactobacillus, Clostridium, Weirong* and *Pasterurella genus* (Schwenger et al., 2019) [148]. As a result, the conclusion could be drawn that oral microorganisms could invade the gut microbiota of those patients.

7.2.2 Cardiovascular diseases:

7.2.2.1 Stroke:

Stroke is among the most common CVDs and mortality worldwide. Several factors could be identified as risk factors, such as hypertension, dyslipidemia, pre-existing heart diseases, and periodontitis, as potential causes of stroke. Pussinen et al. observed in a study that the elevated level of serum antibodies of *Aggregatibacter actinomycetemcomitans and Porphyromonas gingivalis* are interconnected with stroke. Moreover, patients with stroke showed peridotites at the dental site with *Porphyromonas gingivalis* and deep pockets. Also, *Porphyromonas gingivalis* is associated with ischemic stroke because of the correlation between the antibody present for *Porphyromonas gingivalis* and C-reactive protein level (Pussinen et al., 2011) [138].

7.2.2.2 Heart failure:

HF is one of the most commonly known CVDs, and it is interlinked with a common oral disease, periodontitis; patients with periodontal disease develop HF more. Also, between average people and HF patients, the patients have more severe periodontitis, linked with increased bone turnover markers. Moreover, several papers suggest that local and systemic factors such as cytokine and inflammatory mediators are also included in this mechanism, which needs to be researched vastly (Schulze-Späte et al., 2017) [147].

7.2.2.3 Myocardial infarction:

The risk factors of myocardial infarction and periodontal diseases are similar, such as diabetes, inflammation, and smoking. In 1980, Mattila et al. conducted a study where they found that dental health is worse in patients with myocardial infarction. Later, other studies also showed that chronic

dental infection and acute myocardial infarction are associated. Although several studies confirm the correlation between oral disease and myocardial infarction, the mechanism remains unknown (Mattila et al., 1989) [127].

7.2.2.4 Endothelial dysfunction:

The progress of atherosclerosis and other CVDs is mainly caused by Endothelial dysfunction, where the bioavailability of endogenous molecules, such as NO, is decreased along with inhibiting the attachment of leukocytes to endothelial cells. Periodontitis is also linked with this disease, and this positive correlation between salivary NO concentration and the patients with both endothelial dysfunction and periodontal disease was shown in a study by Moura et al., and she suggested that periodontal bacteria that can produce endotoxins and antigens play an essential role in the pathogenesis of this (Gimbrone & García-Cardeña, 2016) [100]. Additionally, periodontal bacteria up-regulate numerous adhesion and chemoattractant molecules on the surface of endothelial cells. For example, the endothelial monocyte chemoattractant protein-1 encounters robust expression, which is induced by *m* and it can also increase endothelial-1 expression and release, ET-1 acts as a vasoconstrictor factor and high expression of this molecule are linked with CVDs. Also, periodontal disease is incorporated with increased arterial stiffness and displays high atherosclerotic risk (Li et al., 2022) [117].

7.2.3 Diabetes:

Diabetes is a widely known and common chronic disease, precisely type 2 diabetes, extremely common where hyperglycemia or disrupted glucose metabolism are responsible. Many oral diseases, such as caries and periodontal disease, are related to type 2 diabetes, and these diseases can increase the complications of diabetes. Oral microbiota regulates the development stage of diabetes by affecting oral bone development, and type 2 diabetes patients had higher chances of losing teeth and increasing inflammation. Between type 2 diabetes patients and regular people, significant oral microbial differences were seen in terms of *TM7, Aggregatibacterm Neisseriam Mycobacterium, and Ekinella*; in diabetes patients, *Actinomyces, Selesnomonas, Streptococcus, Fusobacterium, Capnocytophaga,* and *Vellion* were increased (Xiao et al., 2017) [168].

7.2.4 Rheumatoid Arthritis (RA):

Rheumatoid Arthritis is a systemic autoimmune disease that is a chronic inflammation; this disease produces several enzymes that enhance self-antigenicity to start an autoimmune response, and the production of the enzymes is regulated by periodontitis. Moreover, both Periodontitis and RA use pathogenic mechanisms for inflammation and bone loss. RA initiates the inflammatory response in the periodontium and transforms into chronic systemic inflammation, resulting in the upregulation of inflammatory cytokines in oral tissues. Due to RA, the oral microbiota changes both quantitively and qualitatively, and it has an increased number of anaerobic species such as *Lactobacillus salivarius, Laptotrichia, Atopobium, Prevotella* and *Cryptobacterium curtum* (Çekici et al., 2013) [83]. In contrast, *Streptococcus* and *Corynebacterium* were found in reduced numbers.

7.2.5 Obesity:

The relationship between oral microbiota and obesity is quite unrevealed, but several studies anticipated the revelation between gut microbiota and obesity. However, the oral microbiota of the obese group is exceptionally different from ordinary people; the oral microbiota of these patients had an increased amount of *S. genus, S. mutans*, and *Plasmodium*, whereas the decreased amount of *Haemophilus, Corynebacterium, Carbonophilic phage* and *Staphylococcus*. In obese people, the adaptability of oral microbiota and biodegradability of exogenous compounds were low and showed immune disease characteristics (Benahmed et al., 2021) [79]. However, it has already revealed the correlation between microbial diversity and oral changes in obese people, but the mechanism is still unknown.

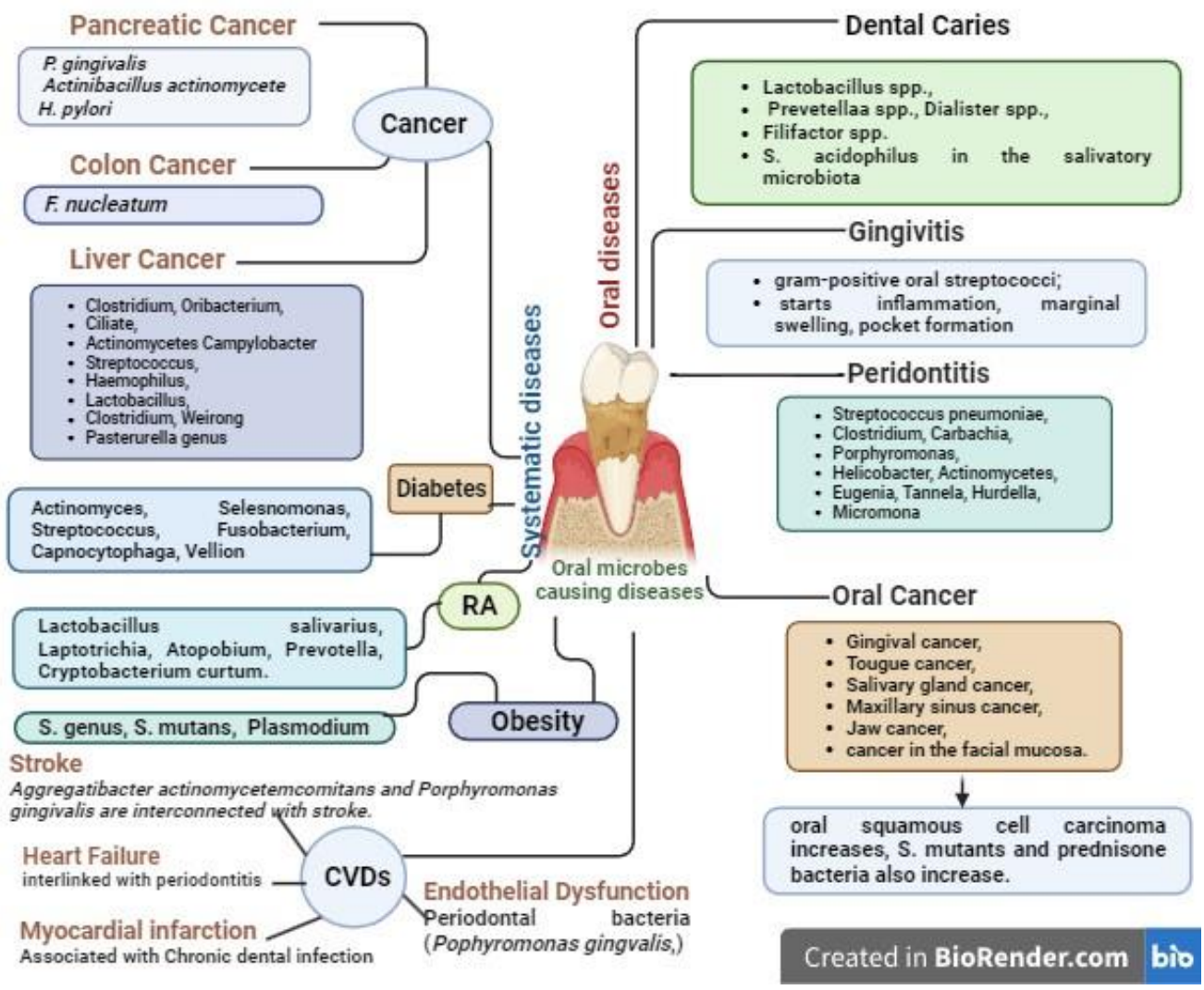

Figure 05: This is an overview of oral disease and systemic diseases; along with the oral microbes which are causing these diseases or they were found linked within these diseases; this figure is made by biorender.

Chapter 8

Prospective Therapeutic Approaches Targeting Oral Microbes:

8.1 Traditional treatments:

Conventional treatment options are primarily used for oral diseases rather than systemic diseases, and the options depend on the severity of the diseases. There are several methods for delivering treatments for healthy conditions, preventing bacterial biofilm growth, and maintaining hygiene; these traditional strategies include Local administration and Intraperitoneal treatment, which include individual strategies for treatment purposes.

8.1.1 Local Administration:

The local administration therapeutic approach includes preventing bacterial biofilm growth, as antimicrobials and antibiotics, powder, el, or oral administration are used (Preshaw et al., 2004) [137]. In the market, “Periostat therapy,” which includes a low dose of Doxycycline, reduces inflammation and boosts the host’s immunity. Moreover, as cation agents, Chlorhexidine and Cetylpyridinium chloride are used for anti-plaque activity for binding dental plaque, delta pellicle, and mucous membranes by disrupting bacterial growth through could calculus formation and staining (Eley, 1999) [95].

8.1.2 Intraperitoneal treatment:

Guided tissue regeneration is the advanced method used in Intraperidontal treatment, which involves putting a biocompatible gel or fabric that contains tooth enamel protein within the bone and tooth, which results in coping the mechanism of formation of the tooth and aiding in healthy bone regeneration (Barrington, 1981) [78]. These fabrics are hollow drug reserves made of polymers such as ethylene vinyl acetate, Polyglycolic acid, and polycaprolactone, which are utilized for sustained drug release by polymer diffusion. For specific periodontitis, tetracycline-loaded fiber and other materials are used (Demirel et al., 1999) [94].

In addition, polymer-based strips and packs such as Polyglycolic acid, Polyhydroxybutyric acid, chitosan, and ethyl cellulose are used to deliver antibiotics (Gheorghiță et al., 2021) [99]. Also, Mucoadhesive patches are used in this treatment for sustained drug release by diffusion, such as Amoxicillin trihydrate and Diclofenac Sodium. As specialized drug systems, denti-caps are used for Lidocaine Hydrochloride, hydrogels, and Amoxicillin trihydrate for delivering Tyramine (Manasadeepa et al., 2013) [124]. Moreover, In-situ gelling systems include thermoresponsive gets and PH-based responsive gelling systems for controlled drug release. Also, several factors regenerate cell growth, such as Gelatine microspheres that contain fibroblast growth factors that promote cell regeneration and bone formation and are used in treating periodontitis (Fernandes et al., 2018) [98].

8.2 Nanodrug delivery as a potential therapeutic strategy:

The failure of conventional drug delivery treatments and several drawbacks increased the demand for nanoparticle treatment. In the conventional method, there were systemic side effects, limited

accessibility of medicine reduced its effectiveness, higher doses were required for the medicine to work, which could increase the resistance and lead to the disruption of normal microflora of the body, shorter duration of action to achieve the desired effects, limited contact and it was time-consuming. A nano drug system was used as a solution, which has several advantages and withdraws all the limitations of the conventional method. For example, it has a higher retention time in the treatment that is usually inaccessible; as a result, it is highly effective with easy access to drugs (Van Winkelhoff et al., 2000) [163]. Also, it has versatile protein, which opens the door for customizable properties on the surface area, which increases drug release rate and flexibility and offers higher sensitivity and specificity for targeting selective bacteria in the oral cavity, which leads to saving the normal microflora and treating antibiotic-resistant microbes.

8.2.1 Use of nanoparticles:

Nanoparticles, specifically metal nanoparticles, have shown promising action in their antimicrobial activity; for example, the silver nanoparticle is the most well-known and influential metal that interacts with bacterial cells in several mechanisms. Firstly, the mechanism used by silver nanoparticles is disrupting bacterial DNA, which causes bacteria to lose their ability to replicate the enzymes needed for respiratory and producing ATP, which results in the generation of radical oxygen species because of the silver ions produced from the nanoparticle. Secondly, it interacts with bacterial membrane protein and destroys the structure and viability of the bacteria. Lastly, this technique destroys the bacterial cell membrane, increasing its permeability and inhibiting the bacteria’s growth (Kim et al., 2007) [109].

Moreover, Copper oxide can also act as an antimicrobial agent on various bacteria, including Methicillin-resistant *Staphylococcus aureus* and *E. coli*; meanwhile, Zinc oxide nanoparticles act against *Streptococcus obrinus*. Also, special silica nanoparticles work against *Candida albican* biofilm by depositing onto a polystyrene surface by polycationic binding (Ahamed et al., 2014) [71]. Additionally, Recaldent, which is also known as Casein Phophospeptide-Amorphous Calcium Phosphate Nano complex, increases the resistance to acidic conditions; another example is nanoparticles of quaternary Ammonium Poly (ethylene imine) that exhibit antimicrobial activity and restorative composite material at the concentration of 1% w/w and damages the bacterial cell wall of *Streptococcus mutans* to inhibit their growth (Emingil et al., 2004) [96].

8.2.2 Use of liposomes as nano drug delivery treatment:

Liposome is a potential treatment approach in periodontitis by enhancing the delivery and targeting antimicrobial agents by the presence of dental pellicle; the lipid bilayer structure of liposomes acts as a carrier for antimicrobial agents in the treatment due to the presence of dental pellicle, which is a protective layer formed by salivary protein on the enamel. This component acts as a barrier against acid attacks and protects against abrasion attrition, but it can also act as a base for the attachment stage of biofilm formation. Moreover, the salivatory protein in dental pellicles is amphiphilic phosphoproteins, which act as micelle structure and have a net negative charge; as a result, positively charged liposomes are highly absorbed into the enamel and mimic the structure of dental pellicle (Ash et al., 2014) [77].

However, liposomes form an aggregation of liposomes with salivary parts such as protein-rich proteins and divalent ions, which could reduce their effectiveness; this could be overcome by

coating liposomes with pectin, which has a net negative charge to compensate for the aggregation (Pistone et al., 2017) [136]. Liposome forms with retention on the dental enamel surface due to the affinity of pectin to the calcium present in the hydration layer of hydroxyapatite; this binding mechanism occurs because of the calcium bridge formation to promote selective targeting by adhesion of liposomes instead of uncoated negatively charged liposomes. Additionally, a new system involving liposomes that are loaded with water-insoluble Triclosan and water-soluble Chlorhexidine and this system targets *Streptococcus sanquis* biofilm. Triclosan and Chlorhexidine are influenced by Mucopolysaccharides, which leads to more effective delivery of drugs (Jones et al., 1997) [105].

8.2.3 Nanobubbles in the Therapeutic Approach:

Nanobubbles are another technique for reducing several conditions such as periodontitis, pockets depth, and eradicating bacteria by generating free radicals through cavitation; this is mainly nanoscopic gaseous cavities around 200 nm and made of small organic material in liquid solution and used a novel treatment for periodontitis. Nanobubbles are formed by collapsing microbubbles, which become larger by physical stimuli such as electrical discharge in an electrolytic solution (Yang et al., 2003) [170]. The stability of nanobubbles is attributed to the lower interfacial curvature and high contact angle, which ensure prolonged existence. The zeta potential changes in microbubbles and they shrink and transform more positively and reduce their size. As a result, it distributes anionic hydroxide ions over cationic hydrogen ions, which rescues diffusivity and stays stable in the electrolyte solution (Hayakumo et al., 2014) [102].

Moreover, nanobubbles generate free radicals during cavitation, where cavitation occurs due to string UV or high-pressure differentials in flowing liquids. Here, the pH of the solution influences the number of free radicals; acidic pH causes the production of more free radicals (Huth et al., 2011) [104]. Although Ozone nanobubbles form highly reactive hydroxyl radicals which reduce the growth of periodontal pathogens, such as *Porphyromonas gingivalis*, *Aggregatibactor actinomycetemcomitans*, and *Tanneralla forsythia*, these nanobubbles are also shown to be noncytotoxic to oral tissues which makes them suitable for therapeutic approach. Nanobubble eradicates *Streptococcus mutans bacilli*, which inhibits delta plaque accumulation and improves gingival health (Ohl et al., 2010) [132].

8.3 Novel and futuristic treatments:

8.3.1 Viral-mediated gene transfer as the latest treatment:

Viral-mediated gene transfer is the process that uses phages, which are proteins that encapsulate DNA and RNA genomes, and it is delivered to treat dental caries and periodontal disease by targeting the pathogenic bacteria while sparing healthy tissue (Kasimanickam et al., 2013) [107]. Here, phages provide a hydrolytic enzyme that breaks down the biofilm in dental caries by reducing bacterial growth. The phages used in this treatment are specific for pathogenic bacteria where non-targeting bacteria are unaffected; they go through fast mutation and propagation to inhibit bacterial growth, making them a suitable option compared to traditional antimicrobials. This phage therapy is highly effective with increased bacterial targets, so they can specifically target bacteria (Khalifa et al., 2016) [108]. However, it has a high risk of lysogen, which phages

incorporate into their genome into bacteria instead of killing them, which could result in potential pathogen strains; lysogens are more resistant to this therapy and more pathogenic (Abedon et al., 2011) [69]. Nevertheless, it can be a potential treatment option for periodontal disease; this can be used efficiently in root canal infections and multidrug-resistant infections (Vandenheuvel et al., 2015) [164]. It has more potential Use, but it requires more study.

8.3.2 For Disease Prevention the Use of Glucansucrase Inhibitor:

Glucansucrase is an enzyme that is secreted by *Streptococcus mutans* and is responsible for dental caries, where it converts sucrose into glucose to produce long-chain biofilm in dental caries (Raghavendra et al., 2009) [139]. Mainly, Glucansucrase has three mechanisms, namely hydrolysis sucrose, transfer of the glycosyl component, and successive glucosyl transfer into an acceptor molecule (Monchois, 1999) [130]. As this is responsible for the development of biofilm and dental caries, the inhibition of the progression of biofilm could be a potential therapeutic option, but it requires extensive research.

8.3.3 For eradication biofilm, the Use of photodynamic therapy:

Photodynamic therapy is the process of eliminating biofilm by sensitizing microorganisms to light where a light source and photosensitizer dye are needed, and as dye methylene blue, erythrosine, acridine orange, benzoporphyrin, and azulene are used (Raghavendra et al., 2009b) [140]. Photosensitizer dyes are selective as they penetrate the cell membrane, which depends on the solubility and lipophilicity; various types of bacteria have different types of cell wall structures, which highly influence the photosensitizer type. For example, hydrophilic dyes are used for gram-positive bacteria, and hydrophobic sensitizer dyes are used for gram-negative bacteria (Nagata et

al., 2012) [131]. Moreover, this process is specific, which allows them to target specific bacteria while sparing healthy tissues. This process is a promising approach because it has less risk of microbial resistance, and it activates the photosensitizer dye, which generates oxygen species that result in bacterial cell damage and biofilm destruction (Peng et al., 2016) [135].

8.3.4 Implementation of probiotics as therapeutic advancement:

Probiotics are living microorganisms, and in the presence of an adequate amount of dietary supplement, they can be helpful for the host (Chatterjee et al., 2011) [86]. It can be directly or indirectly used as a treatment, such as directly probiotic binds with the biofilm where they compete for resident bacteria and reduce their growth; for example, probiotic lactic acid bacteria in the oral cavities of healthy human inhibited the growth of the disease-causing bacteria, including *streptococcus mutans* and yeasts (Das et al., 2022) [90]. Indirectly, probiotics can improve immunity and non-immunological defense mechanisms, which could reduce the risk of disease (Ahola et al., 2002) [73]. Moreover, probiotics affect the inflammatory markers and decrease the levels of IL-1B, IL-6, IL-8, IL,10, and tumor necrosis factor-alpha in gingival crevicular fluid and blood. Several probiotics act as growth inhibitors for other bacteria, such as *Lactobacillus reuteri*, which secrete *reuteri* to inhibit bacterial growth; also, *Lactobacillus brevis* CD2 has inflammatory and antimicrobial effects (Emingil et al., 2004b) [97].

For dental caries treatment, the recombinant Streptococcus mutans strain opened a remarkable door for more effective treatment where non-naturally produced S. mutans produce recombinant nucleic acids, which reduce the number of pathogens that cause dental caries. Mainly, it prevents the colonization of the disease and displaces the indigenous strain of S. mutans, which permits the

hold to go through replacement therapy. Additionally, S. mutans has the selective advantage of producing bacteriocin in colonization; this bacteriocin removes a similar organism to the producer organism. Moreover, lactic acid is the leading cause of dental caries. As organisms metabolize, they produce lactate dehydrogenase (LDH), which causes tooth decay. As a result, LDH mutans streptococcus reduces the carcinogenicity of these microorganisms and is used for replacement therapy (Gupta and Marwah, 2010) [172].

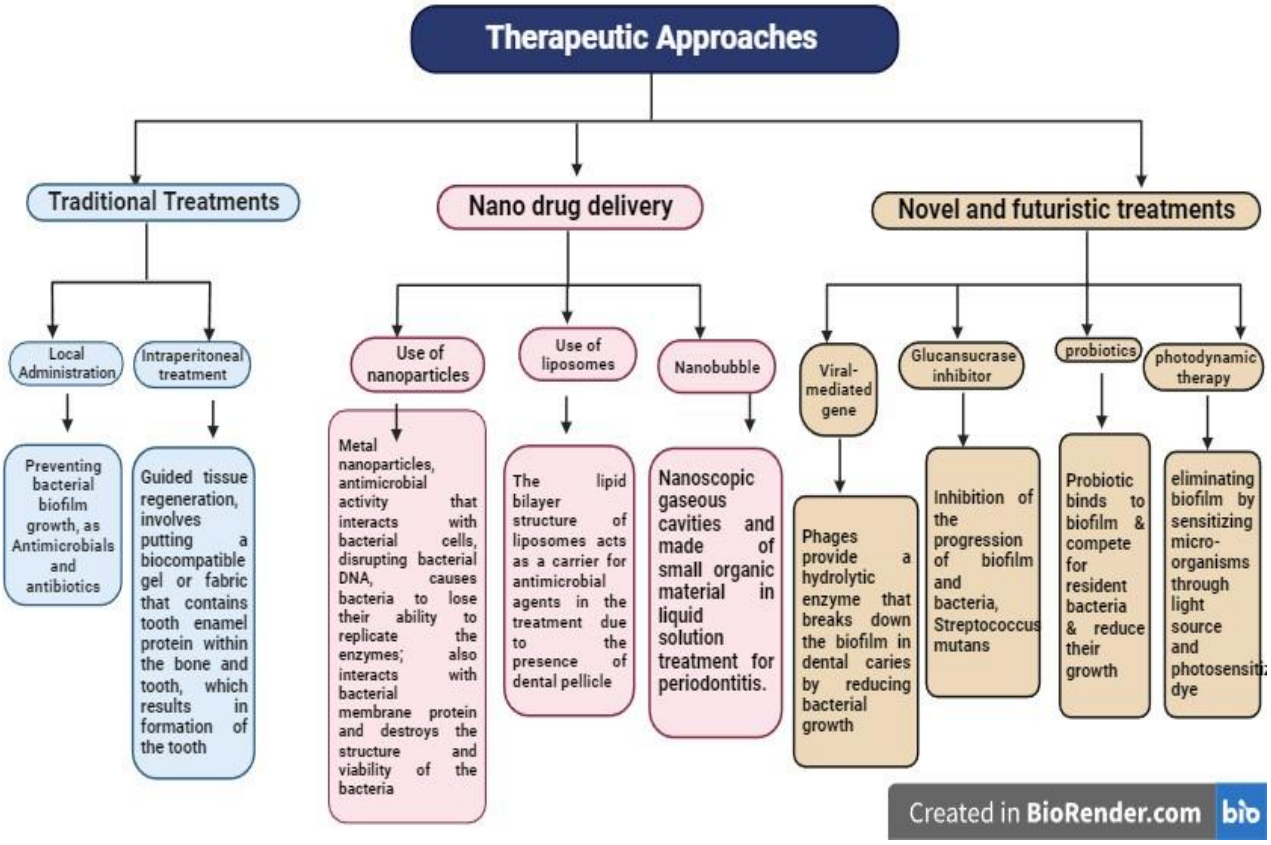

Figure 06: This figure showcases all the therapeutic choices are available and their mechanism to operate on the microbes; this figure is made by biorender.

Chapter 9

Antibiotic resistance around Dhaka city and other countries:

Antibiotic resistance is a challenge worldwide, and the consumption of dental-prescribed antibiotics increases the number of cases. Several surveys have revealed that dentists unnecessarily prescribed antibiotics are responsible for the increased resistance; a survey in urothelial revealed that 49% of dentists prescribe antibiotics with an odontogenic infection with cellulitis before doing any dental treatment; the similar survey also showed that 39% of dentists prescribe antibiotics for localized periapical abscess without any systemic involvement (Ahmadi et al., 2021) [72]. Moreover, another cross-sectional study in Wales showed that dentists tend to prescribe antibiotics without any systemic involvement, as evidence showed that 65.6% of the patients received antibiotics without any evidence of systemic involvement, and 70.6% of antibiotics were prescribed without dental treatment (Cope et al., 2015) [89]. Additionally, non-clinical factors are involved when the dentists' prescribing antibiotics are clinical time pressure, patients who do not want operative treatment, demands, and expectations for fast recovery; a study in the UK showed that dentists would prescribe antibiotics 33.3% of the time due to time pressure (Thompson et al., 2019) [161].

Also, in Australia, studies have revealed that dental practice does not adhere to the prescribing guideline for several antibiotics, such as Amoxicillin, which was prescribed by a dentist around 65% time for oncogenic interactions from 2013 to 2016; similarly, dentist-prescribed phenoxymethylpenicillin as the first line for odontogenic infections as it is efficient for 85% bacteria; as a result, clavulanic acid in increasing due to high use of this antibiotic; these are highly

influencing the bacteria resistance mechanism for the antibiotic (Teoh et al., 2018). A recent study in Australia showed that dentists are not following the current guidelines for prescribing antibiotics; for instance, around 11,000 dispensed prescriptions for the broad-spectrum antibiotic erythromycin were seen, which involved poor activity against *Fusobacterium* and *viridians streptococcus*, which are commonly seen for odontogenic infections (Teoh et al., 2019). Also, dentists prescribe trimethoprim with sulfamethoxazole, which has no entrance to practical dental diseases (Suda et al., 2019) [153].

Additionally, teethes are highly colonized by oral bacterium and 700 different bacterial species come from saliva; these microorganisms are highly involved in the development of diseases. To isolate these oral infections, dentists usually prescribe amoxicillin, penicillin, metronidazole, and more. These antibiotics are highly seen in antibiotic-resistant bacteria of the oral microflora as these are highly used by dentists around the world. Primarily, aminopenicillins are most commonly used by the dentists and it has shown great resistance *to Veillonella spp*. and *Prevotella denticola* which are usually isolated from the root canal; in a study, it was shown that 34 strains of facultative anaerobic bacteria collected from the root canal were susceptible to amoxicillin (Contaldo et al., 2023) [88]. However, anaerobic bacteria were considered as not susceptible to amoxicillin, as a result, Fosse et al also demonstrated that there is no concrete proof of amoxicillin being not sustainable to anaerobic bacteria (Brook et al., 2013) [81].

Moreover, penicillin is another most commonly used antimicrobial which is usually used against the most commonly found microorganism *Streptococcus* in periodontitis. *Streptococcus* produces

beta-lactamase from the subgingival plaque of patients with periodontitis; *Streptococcus* produces beta-lactamases but penicillin resistance could occur due to alteration to penicillin-binding proteins (Soares et al., 2012) [151]. In a sustainability test, it was found that 9 species of alpha-haemolytic *Streptococcus,* such as *Streptococcus mutans, Streptococcus aralis, Streptococcus salivarius* and *Streptococcus mitis* were not completely susceptible to penicillin, but *Streptococcus mutant* was susceptible to penicillin (Abranches et al., 2018) [70]. However, Potgieter et al also found out in blood culture S. mitis also shows resistance to penicillin, these were also resistant to aminoglycosides gentamicin, kanamycin, and tobramycin (Sweeney et al., 2004) [157]. Another study in Spain found out that *P. gingivalis* strain producing beta-lactamase showed penicillin resistance (Del Carmen Marca Andrés et al., 1998) [92]. Next, metronidazole is the combination of more than one antibiotic where resistance to this drug is shown to be associated with mobile genetic elements, aiding spread; the mechanism that could be used to develop resistance include mutation in the enzyme which causes the reduction of the drug to its active form, as a result, it decreases the entry antibiotic in the cell and mutation transporters are responsible for efflux of the drug (Weir, 2023) [166]. A study by Roche et al found that 8 out of some isolated bacteria including *Lactobacillus spp, Gemella morbillorum and Actinomyces israelii* were found resistant to metronidazole (Patait et al., 2015) [134].

Within these major resistant drugs, Cephalosporins are widely used, and *streptococcus* shows resistance to both penicillin and Cephalosporins because changes in penicillin-binding protein are associated with the transportation of the cefotaxime resistance determination. *Enterococcus spp., staphylococcus, Peptostreptococci* which is a bacterium of the genus *Porphromoas* and *Fusobacterium* also showed high-level resistance to cephalosporins (Livermore, 1987) [119].

Also, bacteria use different mechanisms to build resistance against tetracycline by synthesizing efflux protein and producing ribosomal protection protein, and using enzymatic modification of the antibiotic. Species that showed resistance to tetracyclines are alpha-hemolytic *Streptococci, S. mitis* (Speer et al., 1992) [152]. Besides, macrolides and related antibiotic such as erythromycin those codes for rRNA methylase which causes methylation of adenine residues in 23s rRNA, and this prevent the binding of macrolides to the 50s ribosomal subunit to building resistance (Svetlov et al., 2021) [156]. Bacteria could express macrolide resistance by drug inactivation through enzymes that code by mph and efflux of macrolide by an ATP binding transporter which is found in *S. aureus*. Particularly, for *S. aureus* along with *S. sanguis* dentists also prescribe chlorhexidine which is also resistant to them (Miklasińska-Majdanik, 2021) [129].

Around the world, including Dhaka dentist mostly uses amoxicillin, clindamycin, azithromycin, erythromycin, Cefuroxime, Doxycycline, penicillin, and more drugs are used for antibiotic therapy, before surgery or for faster recovery to treat several types of disease which are using the similar process to build antibiotic resistance by the similar oral bacterium; also, the study level in Dhaka is quite low about this issue, to confirm the correlation more studies should take place.

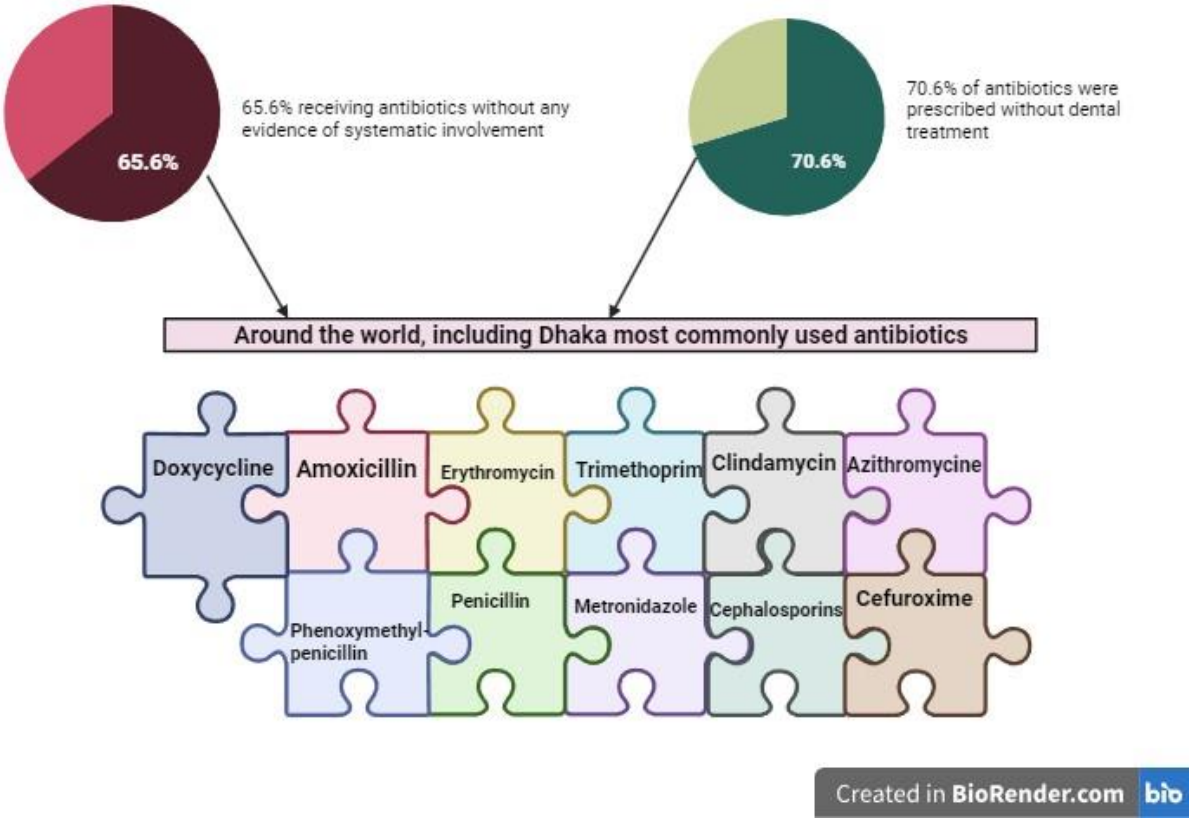

Figure 07: Worldwide most used antibiotics are shown which are mostly responsible for antibiotic resistance that causes several deadly diseases, also the percentage of unnecessary antibiotics by dentists. These common antibiotics are used in Dhaka as well, as a result, this resistance can be expected to be seen here; this figure is made by biorender.

Chapter 10

Conclusion:

The correlation between oral microbiota and various diseases has been evidently shown as a potential pathway for therapeutic approaches. While several systemic and oral diseases among the genders and kids have been known for their greater incidence and more advanced staged diagnosed cases; also, this has largely provided a lower survival rate and mortality rate. The study revealed a common and characteristic aspect of the progression of several diseases caused by oral microbiota. Targeting the common microorganisms and their structure of forming biofilm along with the mechanism of causing disease may also pave the way for new discovery of multiple treatments for significant oral diseases, such as dental caries, periodontitis, oral cancer, gingivitis; as well systemic diseases like diabetes, cardiovascular diseases, cancers, RA and obesity; knowing the mechanisms of these microorganism contribution and the providing treatment options could lead to a novel treatment option which may include the mechanism of the antibiotic resistance gene and how they are altering pathways and causing the disease could unveil latest aspect of treating these diseases.

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