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Chapter

Understanding Oral Diseases: Exploring Opportunities from Filipino Oral Microbiome Research

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Abstract

The human mouth houses the second most diverse microbial community in the body, with almost 700 species of bacteria colonizing the hard surfaces of teeth and the soft tissues of the oral mucosa. To compete in the relatively exposed oral cavity, resident microbes must avoid being replaced by newcomers. This selective constraint, coupled with pressure on the host to cultivate a beneficial microbiome, has rendered a commensal oral microbiota that displays colonization resistance, protecting the human host from invasive species, including pathogens. Current control of dental plaque-related diseases is non-specific and is centered on the removal of plaque by mechanical means. Several new methods based on the modulation of the microbiome that aim at maintaining and re-establishing a healthy oral ecosystem have been developed and has greatly expanded our knowledge of the composition and function of the oral microbiome in health and disease. Promoting a balanced microbiome is therefore important to effectively maintain or restore oral health. This review provides an updated body of knowledge on oral microbiome in health and disease and discusses the implications for modern-day oral healthcare. Filipino Oral Microbiome Research to develop a policy framework for microbiome-based management of dental diseases and opportunities will be discussed.

Keywords: oral microbiome, dysbiosis, modulation, systemic diseases, oral health policy

1. Summary

Resident microorganisms have co-evolved and co-existed in our body in a mostly harmonious symbiotic relationship. The mouth, for instance, houses the second most diverse microbial community in the body, with almost 700 species of bacteria colonizing the hard surfaces of teeth and the soft tissues of the oral mucosa. Synergy and interaction of variable oral microorganisms help human body against invasion of undesirable stimulation outside. To compete in the relatively exposed oral cavity, resident microbes must avoid being replaced by newcomers. This selective constraint, coupled with pressure on the host to cultivate a beneficial microbiome, has rendered a commensal oral microbiota that displays colonization

resistance, protecting the human host from invasive species, including pathogens. Perturbations of the oral microbiome through modern-day lifestyles can have detrimental consequences for our general and oral health. During dysbiosis, the equilibrium of the oral ecosystem is disrupted, allowing disease-promoting bacteria to manifest and cause conditions such as caries, gingivitis and periodontitis. In addition, rapid increases in carbohydrate consumption have disrupted the evolved homeostasis between the oral microbiota and dental health, as reflected by the high prevalence of dental caries. Development of novel modalities to prevent caries has been the subject of a breadth of research.

The current control of dental plaque-related diseases is nonspecific and is centered on the removal of plaque by mechanical means. The most prevalent oral diseases, dental caries and periodontal diseases, are microbiota-associated diseases. Due to this realization about the oral microbiome, several new methods based on the modulation of the microbiome that aim at maintaining and reestablishing a healthy oral ecosystem have been developed. Oral microbiomes play an important role in the human microbial community and human health. The use of recently developed molecular methods has greatly expanded our knowledge of the composition and function of the oral microbiome in health and disease. Studies in oral microbiomes and their interactions with microbiomes in variable body sites and variable health condition are critical in our cognition of our body and how to make effect on human health improvement. Through recent advances in technology, complexities of the oral microbiome have been slowly unraveled and new insights into its role during both health and disease are now made available. For practitioners and patients alike, promoting a balanced microbiome is therefore important to effectively maintain or restore oral health.

This chapter aims to give an update on our current knowledge of the oral microbiome in health and disease and to discuss implications for modern-day oral healthcare. More importantly, opportunities and future directions tapping Filipino Oral Microbiome Research will be explored toward developing a research-driven policy framework for microbiome-based management of dental diseases.

2. The oral microbiome: an overview

Humans have co-evolved with microorganisms [1], these commensals prevent colonization by exogenous microorganisms which translates their significant involvement in the normal development of the host defenses and gut mucosa, during the production of vitamin and energy as well as in the regulation of the cardiovascular system [2]. It was Antony van Leeuwenhoek who was first to identify oral microorganisms using his microscope, which is possibly the first account of oral microbiome [3], his “animalcules” [4].

The term “oral microbiome” has been coined to describe the microbial communities inhabiting the oral cavity behaving as a consortium involved in the modulation of various pathophysiological conditions of the host [5–7]. It is considered as the second most complex of the human body, after the gut-associated microbiome [8–10] with the average adult harboring about 50 to 100 billion bacteria represent about 200 predominant bacterial species [11]. Compared to other body sites, the oral microbiome is unique and readily accessible. However, there are only about 700 predominant taxa: 54% are cultivable and identified; 14% are cultivable but not yet identified, and the remaining 32% not even cultivated yet, the so-called “uncultivables” or phylotypes [5, 11–13]. These estimations are based on years of traditional identification of bacteria from cultural and phenotypic characterization

studies, but mostly from identification of bacteria from culture-independent molecular studies using 16S rRNA gene comparative analyses [5, 14–17].

Figure 1 describes the transition and changes related microbiome profile in the mouth from sterility to oral disease state such as dental caries or systemic disease state. The fetus in the womb is usually sterile [18, 19]. The baby comes in contact with the microflora of the uterus and vagina of the mother during delivery, and later with the microorganisms of the atmosphere at birth [20]. The oral cavity of the newborn is considered sterile even though there is a high probability of contracting contaminants. Since the mouth is exposed to various microorganisms from the first feeding onward, this starts the process of acquiring the resident oral microflora [19]. Typically, the earliest colonizers of the tooth surface are commensal streptococci, such as *Streptococcus mitis, Streptococcus sanguinis, Streptococcus gordonii,* and other closely related taxa [21]. They antagonize transient microorganisms by producing by-products such as alkali, bacteriocins, and hydrogen peroxides. High abundances of these commensal streptococci are favored in dental plaque particularly in the absence of a carbohydrate-rich diet. This dominance is strongly associated with good dental health.

Historically, humans recorded two shifts in diet preference as influenced by the development of agriculture and by the industrial revolution [22] which favored the increase of carbohydrate consumption, particularly sucrose. With frequent consumption of carbohydrates such as sucrose coupled with poor oral hygiene, bacterial production of a certain glucan matrix is enhanced which can lead to the development of dental plaque, where other carbohydrate-related fermentation can continue. Due to the described changes that would result, perturbation of homeostasis of the oral microbiome can now lead to the development of dental caries, which is considered as the most common chronic disease worldwide, affecting 60–90% of children and adults even in industrialized countries [23].

It was previously mentioned that about 400 to 500 oral taxa have been detected in the subgingival crevice alone [14, 16] with the remaining taxa distributed to other oral habitats such as the tongue, tooth surface, buccal mucosa, tonsils, soft and hard palate, and lip vestibule [14, 24–26]. Although there is compositional variation between sample sites taken from the oral cavity, a 'core' microbiome in health has been identified [24, 25, 27]. Studies have also demonstrated that oral

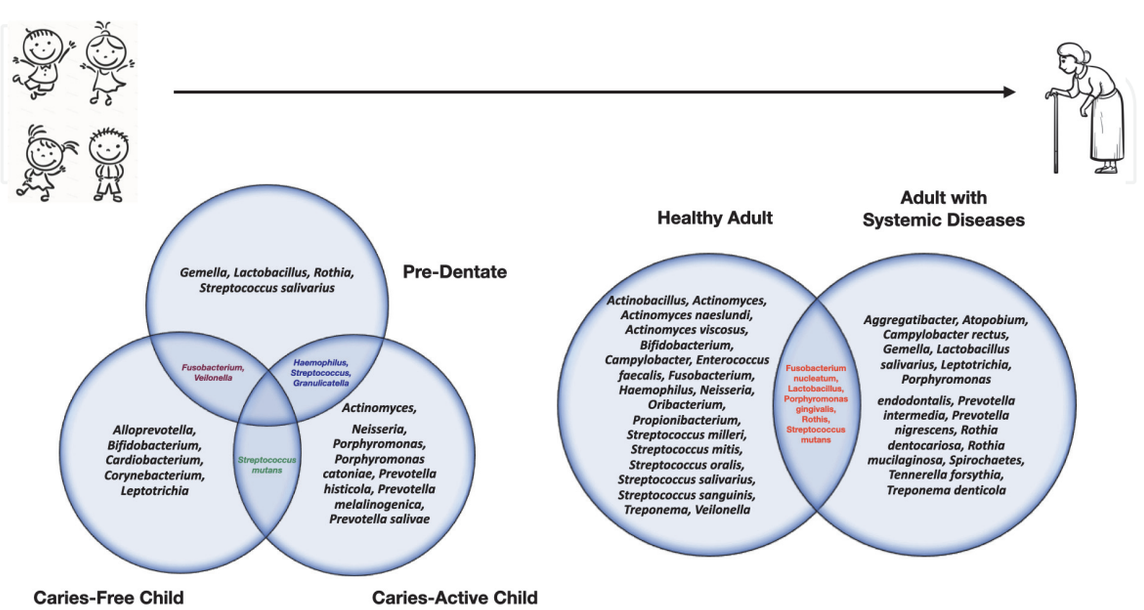

Figure 1.
Transition and changes related microbiome profile in the mouth from sterility to oral disease state such as dental caries or systemic disease state.

disease is not due to an isolated organism such as *Streptococcus mutans* causing caries, but is more polymicrobial in nature [5, 28, 29]. Studies have identified *Bifidobacterium, Veillonella, Granulicatetta, Scardovia, Fusobacterium, Prevotella* and *Actinomyces* as potential contributors to early childhood caries evidenced by their altered abundance in the caries microbiota [30].

Interestingly, there is a wide range of microorganisms that are said to inhabit the human oral cavity, including bacteria, fungi, viruses, archaea and protozoa which likewise contribute to influencing oral and systemic health [31]. Bacteria account for the main portion of oral microorganisms, and the major knowledge of the composition of oral bacteria comes from past culture-dependent methods, however, these data substantially underestimated the composition of the oral microbiome but development of culture-independent methods, particularly targeting 16S ribosomal RNA, offered expanded awareness of the great richness and diversity of the oral microbiome [31]. Fungi are present widely in the oral cavity not only as opportunistic pathogens of the elderly and immune-compromised but may also be present as members of the healthy oral microbiota [32]. Archaea constitutes only a minor part of the oral microbiome and is restricted to limited species [33, 34] observed in healthy subjects, but their prevalence and numbers are elevated in individuals with periodontitis [6]. Most viruses in the mouth are related to diseases including herpes simplex virus, human papilloma virus [35] and HIV infection indirectly related to observed oral manifestations, such as oral candidiasis, oral hairy leukoplakia, linear gingival erythema and necrotizing ulcerative periodontitis, and Kaposi's sarcoma [36].

The salivary or oral microbiome has been the target of interest for its diagnostic and prognostic value [37]. Since the commensal microbiome has an important role in the maintenance of oral and systemic health, altering its delicate balance may lead to the development of certain oral pathologies such as cavities endodontic disease, periodontal diseases, osteitis and tonsillitis [38–40] as well as various systemic diseases including cardiovascular disease [41], obesity [42–43], heart disease [44], diabetes [45], pediatric Crohn's Disease [46], pancreatic cancer [47], colon carcinoma [48], and even psychiatric disorders [49]. Studies during the last decade focused on the management and prevention of dental caries by modulating oral microbiome [37, 50]. However, it is yet to be established if there is a causal relationship between the oral microbiome and these systemic disorders. This battleground represents a significant opportunity for intervention and subsequent prevention of caries.

3. Methods employed in studying the oral microbiome: from "culturomics" to whole genome sequencing

Tables 1 and **2** show a comprehensive list of methods used in the study of oral microbiome from culture-dependent method to whole genome sequencing and OMICS. The relevant findings from the studies that utilized the various methods are also presented. Historically, as guided by Koch's postulates, cultivable bacterial taxa associated with various oral diseases were identified using culture-dependent methodologies such as microscopy, biochemical and other phenotypic tests, growth conditions, sugar utilization, and antibiotic sensitivity. However, this approach has presented various limitations when it comes to describing the actual diversity of the oral microbiome, or the so-called "great plate anomaly" [94, 95] and is unable to fully characterize complexity of bacterial communities such as those found in the oral cavity [60, 96].

Methods to study oral microbiome	Studies that have utilized the given methods	Important findings
Culture-Dependent Approach: "Culturomics"		
Heart Infusion Blood Agar (with menadione) Mitis Salivarius Medium (MS)	[51]	The cultivation on rich medium under anaerobic conditions, they investigated the overall composition of the microbiota of saliva, dental plaque, the gingival crevice, the cheeks, and the tongue dorsum They found organisms such as *Corynebacterium* spp., *Actinomyces* spp., and *Streptococcus sanguinis* colonizing teeth, treponemes in the gingival crevice, and *Streptococcus salivarius* on the tongue
MM10 Sucrose Medium MSB (MS + 20% sucrose, 0.2 U/ml bacitracin) Medium	[52]	Significant association between plaque levels of *S. mutans* and caries Saliva samples tended to have low levels of *S. mutans* and were equivocal in demonstrating a relationship between I and caries
MM10 Sucrose Medium MSB Medium LBS Medium	[53]	Clinical decay can occur in a few instances in the absence of detectable *S. mutans*, as was observed in the fissures high in lactobacilli
Trypticase Soy Broth (TSB) with 1% glucose	[54]	*Streptococcus mutans, Lactobacillus casei* and *Streprococcus faecalis* showed greater acid tolerance than strains of *Streptococcus sanguis*, *Streptococcus salivarius, Streptococcus mitis* and *Actinomyces viscosus* Species of plaque bacteria most closely associated with the initiation or progression of dental caries are more aciduric than non-cariogenic species
MSB medium GSTB medium (5% glucose, 5% sucrose, telurite, and bacitracin) TYCSB medium (tryptone, yeast extract, cysteine, sucrose, and bacitracin)	[55]	Early culture-based studies had shown that enamel caries was associated with increases in the numbers and proportions of mutans streptococci
Blood agar BM Medium	[56]	If such conditions of low pH are repeated on a regular basis, then the acidogenic/aciduric species are eventually able to increase their proportions and drive the plaque pH even lower, outcompeting the beneficial species
Tidd Hewitt Broth	[57]	There is heterogeneity in terms of expression of attributes among clinical strains belonging to a species, so that some strains of mutans streptococococci can be less acidogenic than isolates of other streptococcal species Emphasizes the need for detailed species and biovar identification of oral streptococci and for recognition of the significant physiological differences that occur within single species
BM Medium BMHGM Medium (BM supplemented with hog gastric mucin)	[58]	If such conditions of low pH are repeated on a regular basis, then the acidogenic/aciduric species are eventually able to increase their proportions and drive the plaque pH even lower, outcompeting the beneficial species

Methods to study oral microbiome	Studies that have utilized the given methods	Important findings
MTPY Medium TYCSB Medium	[59]	A total of 424 bifidobacteria were identified and these were *Bifidobacterium dentium*, *Parascardovia denticolens*, *Scardovia inopicata*, *Bifidobacterium longum, Scardovia* genomosp. C1 and *Bifidobacterium breve* Suggested that bifidobacteria may play a role in the progression of occlusal caries lesions in both children and adults Recent culture-based studies have correlated more diverse communities of bacteria with caries, including reporting on the association of *Actinomyces* and *Bifidobacterium* species with lesions, often with mutans streptococci comprising a relatively small percentage of the microbiota at diseased sites
BHI + HK Medium FAA Medium BUA medium	[5]	The human oral cavity contains a number of different habitats, including the teeth, gingival sulcus, tongue, cheeks, hard and soft palates, and tonsils, which are colonized by bacteria More than 700 prokaryotic taxa have been detected in the oral cavity, many of which cannot be isolated by common culture methods *Actinobacteria*, *Bacteroidetes*, *Chlamydiae*, *Chloroflexi*, *Euryarchaeota*, *Firmicutes*, *Fusobacteria*, *Proteobacteria*, *Spirochaetes*, SR1, *Synergistetes*, *Tenericutes*, and TM7
Cooked Meat Medium (CMM) CMM with mucin and serum Blood Agar	[60]	Cultivation of a *Synergistetes* strain representing a previously uncultivated strain from Subgingival plaque
Blood Agar MacConkey's Medium Chapman's Plate Growth Medium Chromagar-Candida	[61]	Samples deriving from oral cavity swabs, cultures, and *in vitro* tests showed the presence of various microorganisms belonging to different genera, species, and strains of bacteria, protozoans, and fungi in patient groups analyzed The pretreatment examination of oral cavity microbiota may be helpful in a preventive approach to the spread of infectious microorganisms, which may be etiological agents of human opportunistic infections and risk factors for treatment complications, particularly dangerous for older adults

Table 1.
Comprehensive list of methods used in the study of oral microbiome from culture-dependent methods to whole genome sequencing and OMICS. The relevant findings from the studies that utilized the methods are also presented.

Conventional cultivation of oral bacteria samples requires taking an appropriate sample, transferring the sample to an appropriate medium for transportation, and followed by correct storage following collection. Dispersion and plating the bacteria onto various culture media in the laboratory will then follow. The bacteria are then isolated and characterized by their colony morphologies (appearance) and biochemical testing. Species which do not grow are naturally "overlooked". One of the major challenges facing oral microbiology is the ability to culture the yet-to-be cultivated 32% of oral species. Furthermore, current knowledge existing about

Culture-Independent Approach: Pre-Next Generation Sequencing Era to Whole Genome Sequencing		
Studies that have utilized culture-independent methods	**Methods used**	**Important findings**
[14]	ABI Prism cycle sequencing kit	Highly common genera in various regions of cavity: *Gemella, Granulicatella, Streptococcus*, and *Veillonella*
[62]	16S ribosomal RNA genes polymerase chain reaction (PCR)	Tumor specimens: *Exiguobacterium oxidotolerans, Prevotella melaninogenica, Staphylococcus aureus, Veillonella parvula* Non-tumor specimens: *Moraxella osloensis, Prevotella veroralis, Actinomyces* **Oral squamous cell carcinoma*
[63]	Comparative 16S rRNA gene sequencing and QPCR	Dental Plaque: Microbial community is dominated by *Streptococci* (66%) followed by *Actinomyces* (6%)
[64]	16S ribosomal RNA genes polymerase chain reaction (PCR) Restriction fragment length polymorphism 16S ribosomal RNA gene sequencing	*L. salivarius* was more prevalent in children with moderate to high caries prevalence compared with children with low caries prevalence, while *L. fermentum* was the most predominant species in all study groups. Genetic heterogeneity of *Lactobacillus* species was found among the children and those with high caries prevalence tended to be colonized with more than one clonal type *L. salivarius* may be a putative caries pathogen among preschool Thai children
[27]	454 Pyrosequencing FLX system	Dental surfaces, cheek hard palate, tongue and saliva: *Firmicutes*: *Streptococcus, Veillonellaceae*, *Granulicatella* *Proteobacteria*: *Neisseria, Haemophilus* *Actinobacteria*: *Corynebacterium, Rothia, Actinomyces* *Bacteroidetes*: *Prevotella, Capnocytophaga, Porphyromonas*
[65]	Sanger Sequencing	Gingiva: 247 species-level phylotypes and nine bacterial phyla
[5]	ABI Prism cycle sequencing kit	Various Regions of the Oral Cavity: A total of 619 taxa in 13 phyla *Actinobacteria, Bacteroidetes, Chlamydiae, Chloroflexi, Euryarchaeota, Firmicutes, Fusobacteria* *Proteobacteria, Spirochaetes*, SR1, *Synergistetes, Tenericutes*, and TM7
[60]	Fluorescent *in situ* Hybridization (FISH) Quantitative PCR (Q-PCR)	*Synergistetes* W090/OTU 6.2 to be prevalent and 'abundant' in both periodontally healthy subjects and those with chronic periodontitis, which would suggest that this bacterium is a commensal, endogenous to the oral cavity
[66]	454 Pyrosequencing FLX system	Supragingival and subgingival plaques: Five major phyla are *Bacteroidetes,*

Culture-Independent Approach: Pre-Next Generation Sequencing Era to Whole Genome Sequencing		
Studies that have utilized culture-independent methods	**Methods used**	**Important findings**
		Actinobacteria, Firmicutes, Proteobacteria and *Fusobacteria*
[67]	Arbitrarily primed PCR (AP-PCR) Chromosomal DNA fingerprinting DGGE	Ascertained the role of lactobacilli in the caries process Found seven LB species in the oral cavity of the subjects: *L. vaginalis, L. oris, L. gasseri, L. salivarius, L. fermentum, L. rhamnosus* and *L. casei*.
[68]	16S rRNA-based microarray and PCR	Several species, including *S. wiggsiae* and *S. exigua,* are associated with the ecology of advanced caries Successful treatment is accompanied by a change in the microbiota, and that severe early childhood caries is diverse, with influences from selected bacteria or from diet
[69]	454 GLX Titanium pyrosequencing	Results show a given bacterial consortium associated with cariogenic and non-cariogenic conditions, in agreement with the existence of a healthy oral microbiome and giving support to the idea of dental caries being a polymicrobial disease
[70]	454 Pyrosequencing using GS- FLX sequencer	Supragingival dental plaque: Healthy one: *Bacilli* and *GammaProteobacteria* dominated with specific association of *Rothia* and *Aggregatibacter* Diseased one: *Clostridiales* and *Bacteroidetes* dominated
[71]	Next generation sequencing of 16S rRNA gene DNA deduction	Data included 9 phyla, 16 classes, 26 orders, 55 families, and 111 genera (OUT was defined within 3% genetic difference) Detected approximately 29% more types of microbes than those detected from the same sample without using DNA deduction DNA deduction technique will lead to a better understanding of the diversity of the human oral microbiota
[72]	DGGE and Sanger sequencing	Oral Tissue: Six major phyla: *Firmicutes, Bacteroidetes Proteobacteria, Fusobacteria, Actinobacteria* and uncultivated TM7 with 80 bacterial species/phylotypes
[73]	PCR-DGGE	First study that provides a baseline profile of the oral microbial diversity in caries-free and caries-active Filipino adults using culture- independent techniques The caries-free group exhibited a more diverse microflora compared with its caries-active
[74]	Next generation sequencing 16S rRNA whole genome (454 FLX Titanium)	Saliva microbiomes in human population were featured by a vast phylogenetic diversity yet a minimal organismal core Caries microbiomes were significantly

Culture-Independent Approach: Pre-Next Generation Sequencing Era to Whole Genome Sequencing		
Studies that have utilized culture-independent methods	**Methods used**	**Important findings**
		more variable in community structure whereas the healthy ones were relatively conserved Abundance changes of certain taxa such as overabundance of *Prevotella* Genus distinguished caries microbiota from healthy ones Caries-active and normal individuals carried different arrays of *Prevotella* species No 'caries-specific' operational taxonomic units (OTUs) were detected, yet 147 OTUs were 'caries associated', that is, differentially distributed yet present in both healthy and caries-active populations
[75]	RNA-Seq Paired-end sequencing (Illumina HiSeq)	The bacteria changing activity during biofilm formation and after meal ingestion were person-specific Some individuals showed extreme homeostasis with virtually no changes in the active bacterial population after food ingestion, suggesting the presence of a microbial community which could be associated to dental health
[76]	16S pyrotag sequencing	Subgingival Plaque: Smokers: elevated number of *S. mutans, Lactobacillus sali varius* and commensal poor anaerobes
[77]	Roche 454 FLX sy	Swab: Significant reduction in *Firmicutes and Actinobacteria* while *Fusobacteria* count proportionally elevated in all oral cancer patients
[78]	Arbitrary-primed PCR (AP-PCR) Multi-locus Sequence Analysis (MLSA)	Develop a streamlined method for identifying strains of *S. oralis* and *S. mitis* from plaque samples so that they could be analyzed in a separate study devoted to low pH streptococci and caries Novel primer sets offer a convenient means of presumptive identification that will have utility in many studies where large scale, in-depth genomic analyses are not practical
[79]	RNA-Seq Paired-end sequencing (Illumina HiSeq)	There were similar levels of *Actinomyces* gene expression in both sound and carious root biofilms These bacteria can be commensal in root surface sites but may be cariogenic due to survival mechanisms that allow them to exist in acid environments and to metabolize sugars, saving energy
[80]	PhyloChip microarrays	Oral buccal mucosa: IBS-overweight participants showed decreased richness in the phylum *Bacteroidetes*

Culture-Independent Approach: Pre-Next Generation Sequencing Era to Whole Genome Sequencing		
Studies that have utilized culture-independent methods	**Methods used**	**Important findings**
[81]	454 Pyrosequencing FLX system	Oral rinse samples: 13 phyla having 122 genera Core microbiome constituted 7 phyla with 55 genera
[82]	454-pyrosequencing	Subgingival plaque: Diabetics: *Fusobacterium, Parvimonas, Peptostreptococcus* *Gemella, Streptococcus, Leptotrichia, Filifactor, Veillonella, TM7, Terrahemophilus and* elevated levels of *Capnocytophaga Pseudomonas, Bergeyella, Sphingomonas, Corynebacterium* *Propionibacterium*, and *Neisseria* in hyperglycemic individuals
[83]	Deep shotgun sequencing	Saliva from three pairs of populations of hunter-gatherers and traditional farmers living in close proximity in the Philippines Comparing these microbiomes with publicly available data from individuals living on a Western diet revealed that abundance ratios of core species were significantly correlated with subsistence strategy, with hunter-gatherers and Westerners occupying either end of a gradient of *Neisseria* against *Haemophilus*, and traditional farmers falling in between Species found preferentially in hunter-gatherers included microbes often considered as oral pathogens, despite their hosts' apparent good oral health
[84]	Next-generation sequencing of V3-V4 region of 16S rRNA gene (Illumina MiSeq)	Salivary microbiome was characterized in a group of children stratified by the Simplified Oral Hygiene Index (OHI-S) Twenty taxonomic groups (Seventeen genera, two families and one class; *Streptococcus*, *Veillonella*, *Gemellaceae*, *Prevotella*, *Rothia*, *Porphyromonas*, *Granulicatella*, *Actinomyces*, TM-7-3, *Leptotrichia*, *Haemophilus*, *Selenomonas*, *Neisseria*, *Megasphaera*, *Capnocytophaga*, *Oribacterium*, *Abiotrophia*, *Lachnospiraceae*, *Peptostreptococcus*, and *Atopobium*) were found in all subjects and constituted 94.5–96.5% of the microbiome Of these twenty genera, the proportion of *Streptococcus* decreased while *Veillonella* increased with poor oral hygiene status, *Veillonella dispar* and *Veillonella parvula* tended to be elevated in the Poor oral hygiene group
[85]	Next-generation sequencing of V4 region of 16S rRNA gene (Illumina MiSeq)	A comprehensive analysis of the oral microbiome identified *Granulicatella* and *Neisseria* as bacteria enriched in subjects with MetS and *Peptococcus* as bacteria abundant in healthy controls

Culture-Independent Approach: Pre-Next Generation Sequencing Era to Whole Genome Sequencing		
Studies that have utilized culture-independent methods	**Methods used**	**Important findings**
		Results support that local oral microbiota can be associated with systemic disorders The microbial biomarkers identified would aid in determination of which individuals develop chronic diseases from their MetS and contribute to strategic disease management
[86]	Next-generation sequencing of V1-V2 region of 16S rRNA gene (Illumina MiSeq)	*Porphyromonas*, *Treponema*, *Tannerella*, *Filifactor*, and *Aggregatibacter* were more abundant in patients with periodontal disease, whereas *Streptococcus*, *Haemophilus*, *Capnocytophaga*, *Gemella*, *Campylobacter*, and *Granulicatella* were found at higher levels in healthy controls
[87]	Fluorescence in situ hybridization and Confocal Laser Scanning Microscopy	Establishes *S. oralis* as commensal keeper of homeostasis in the biofilm by antagonizing *S. mutans*, thus preventing a caries-favoring dysbiotic state
[88]	Next-generation sequencing of V3-V4 region of 16S rRNA gene (Illumina MiSeq)	Interproximal-associated microbiota was found to be similar to already described bacterial communities in other mouth niches. *Streptoccocus, Veillonella, Rothia, Actinomyces, Neisseria, Haemophilus* and *Fusobacterium* were the most abundant genera in this oral region
[30]	Next-generation sequencing of V4-V5 region of 16S rRNA gene (Illumina MiSeq)	Distinct differences between the caries microbiota and saliva microbiota were identified, with separation of both salivary groups (caries-active and caries-free) The major phyla of the caries active dentinal microbiota were Firmicutes (median abundance value 33.5%) and Bacteroidetes (23.2%), with *Neisseria* (10.3%) being the most abundant genus, followed by *Prevotella* (10%). The caries-active salivary microbiota was dominated by Proteobacteria (median abundance value 38.2%) and Bacteroidetes (27.8%) with the most abundant genus being *Neisseria* (16.3%), followed by *Porphyromonas* (9.5%). Caries microbiota samples were characterized by high relative abundance of *Streptococcus mutans*, *Prevotella* spp., *Bifidobacterium* and *Scardovia* spp.
[89]	Next-generation sequencing of V3-V4 region of 16S rRNA gene (Illumina MiSeq)	Provides thorough knowledge of the microbiological etiology of elderly individuals with caries and is expected to provide novel methods for its prevention and treatment
[90]	Next-generation sequencing of V3-V4 region of 16S rRNA gene (Illumina MiSeq)	Decrease in commensal saliva bacteria were observed in all the body sizes when compared to normal weight children. Notably, the relative abundance of bacteria

Culture-Independent Approach: Pre-Next Generation Sequencing Era to Whole Genome Sequencing		
Studies that have utilized culture-independent methods	**Methods used**	**Important findings**
		related to, *Veillonella*, *Prevotella*, *Selenomonas,* and *Streptococcus* was reduced in obese children Body size–specific saliva microbiota profiles open new avenues for studying the potential roles of microbiota in weight development and management
[91]	Fluorescence in situ hybridization and Confocal Laser Scanning Microscopy	Investigated the impact of various *Fusobacterium* species on *in vitro* biofilm formation and structure in three different oral biofilm models namely a supragingival, a supragingival "feeding", and a subgingival biofilm model Study showed variations in the growing capacities of different fusobacteria within biofilms, affecting the growth of surrounding species and potentially the biofilm architecture
[92]	Next-generation sequencing of V4 region of 16S rRNA gene (Illumina MiSeq)	Adult oral microbiomes were predominantly impacted by oral health habits, while youth microbiomes were impacted by biological sex and weight status The oral pathogen *Treponema* was detected more commonly in adults without recent dentist visits and in obese youth Oral microbiomes from participants of the same family were more similar to each other than to oral microbiomes from non-related individuals
[8]	Whole Genome Sequencing Real-time quantitative PCR microarray	Sampled oral micro-habitat included tongue dorsum, hard palate, buccal mucosa, keratinized gingiva, supragingival and subgingival plaque, and saliva with or without rinsing. Each sampled oral niche evidenced a different microbial community, including bacteria, fungi, and viruses Oral rinse microbiome was more representative of the whole site-specific microbiomes, compared with that of saliva Healthy oral microbiome resistome included highly prevalent genes conferring resistance to macrolide, lincosamides, streptogramin, and tetracycline.
[93]	Next-generation sequencing of V3-V4 region of 16S rRNA gene (Illumina MiSeq)	A comparison of oral bacteriome between two groups revealed the dominance of acidogenic and aciduric bacteria in diabetics which suggested the involvement of these eubacteria in oral dysbacteriosis in diabetes mellitus Phylum Firmicutes (p-value = 0.024 at 95% confidence interval) was significantly more abundant among diabetic patients than among the controls

Culture-Independent Approach: Pre-Next Generation Sequencing Era to Whole Genome Sequencing		
Studies that have utilized culture-independent methods	**Methods used**	**Important findings**
		Acidogenic bacteria *Prevotella* (p-value = 0.024) and *Leptotrichia* (p-value = 1.5×10^{-3}); and aciduric bacteria *Veillonella* (p-value = 0.013) were found to be in higher abundance in diabetic patients

Table 2.
Comprehensive list to describe methods in the study of oral microbiome from culture-dependent methods to whole genome sequencing and OMICS + studies that have utilized hem through the years).

pathogens linked to oral diseases are based on bacterial communities that have somehow survived transportation in a sample, grew easily or rapidly in the laboratory, based on a culture method employed in the laboratory [96, 97] which seems to limited and fundamentally incomplete.

Resultant knowledge of oral microbial ecology and dynamics have enabled development of novel culture techniques that have somehow helped the cultivation of some strains including the provision of distinct conditions such as an anaerobic (oxygen-free) environment, incubation in a variety of temperatures, use of chemically-defined media containing specific amounts of nutrients, and even the use of cytokine networks and microbial co-colonizers [98, 99]. But despite these advances, still many organisms remained uncultivable due to several assumptions [98, 100] such as (1) they exist in obligate metabolic associations with other organisms; (2) since oral bacteria do not live in isolation but in complex communities called biofilms, they depend on synergies and antagonisms for growth, along with mutual reliance for growth and survival; and (3) the lack of essential nutrients, growth factors and/or signaling molecules, overfeeding conditions, lack of cross-feeding partners, culture media toxicity and disruption of bacterial quorum-sensing and other signaling systems or other reasons that remained to be undiscovered until now.

Cultivation remains the foundation of oral microbiology in characterizing phenotypically, identification, including physiology and pathogenicity of particular species. The proportion therefore of the so-called 'overlooked' bacteria that are responsible for a number of oral diseases [99] must be studied using molecular signatures in order to include a huge number of uncultivable species found in healthy and diseased sites in the human mouth and to better understand the many aspects of microbial dynamics in the oral cavity [95, 98, 101]. The development and advances in molecular techniques, therefore, can help enhance and enable the study of complex host-associated bacterial communities such as those in the oral cavity.

Watson and Crick's discovery of the DNA structure helped clarify the mechanism of base pairing and explained how genetic information is stored and copied in living organisms. This knowledge then led to the power to investigate microbial communities or individual cells from detection to identification to diversity profiling using molecular-based technologies. Molecular techniques have enabled a more in-depth investigation and have resulted in different and more focused approaches compared to cultivation [102].

Early high-throughput analytical approaches in studying microbial communities utilized the fragment size separation differences of the DNA such as the denaturing gradient gel electrophoresis (DGGE) and the restriction fragment length polymorphism (RFLP) methods which based findings on [103, 104] following amplification

of target regions of interest through the polymerase chain reaction or PCR. These types of approach enabled analysis at the macro-level for microbial communities characterized by large shifts or variations in the population. DNA–DNA hybridization and DNA microarrays followed providing rapid assessment of specific bacterial associations in oral health and disease [105] with the use of hybridized DNA fragments and complementary probes arrayed on a glass slide for expression profiling [106].

The modern era of microbial genome analysis began in the early 2000s, with metagenomics and gene sequencing techniques [107]. The 16S rRNA gene is usually selected as the target gene because it comprises both variable and conserved regions, permitting the use of primers to conserved regions and more specific primers to amplify 16S rRNA genes from any source to discriminate between taxa [108, 109]. Bacterial taxa can be phylogenetically identified, whether they are cultivable or not-yet-cultivated, in a mixed population, e.g., plaque, by isolating DNA, amplification of universally conserved primers for 16S rRNA gene, a conserved gene of approximately 1500 bp, followed by cloning into *Escherichia coli*, and lastly Sanger sequencing [16] or the more modern next generation sequencing [110]. Among nine distinct hypervariable regions, V_1, V_2, V_3 and V_4 stretches are highly exploited sequences for studying microbial diversity due to their extreme variability; V_5 exhibits least variability [111] while $V_{4–6}$ region is considered as the most reliable stretch that represents the entire length of 16S rDNA for studying the majority of bacterial phyla [110]. Currently, oral microbiome-based next generation sequencing analysis chiefly relies on primers of either $V_{1–2}$ or $V_{3–4}$ regions. These bits and pieces of 16S rDNA regions are capable enough of providing the entire picture of bacterial phyla present in a niche; however, ambiguous data obtained from various targeted variable regions cannot be ignored, and it demands a massive full- length sequencing of 16S rDNA region for its validation. Interestingly, the use of V_1 region has been linked to differentiating *Streptococci*, a pioneer of the oral cavity, while region V_2 is associated to accurately identify various phylotypes of Gram-negative *Porphyromonas* and *Fusobacterium* [111]. Furthermore, amplifying $V_{1–3\ \text{works best for}}$ *Streptococcus, Fusobacterium, Prevotella, Porphyromonas*, and *Bacteroides*, but the use of $V_{4–6}$ region showed observed dominance of *Prevotella, Porphyromonas, Treponema, Enterococci* and *Campylobacter*-like oral inhabitants [112]. In the last decade, next generation sequencing methods have revolutionized the study of microbial diversity, and enabled large-scale sequencing projects to be completed in a few days or sometimes hours.

The use of universal bacterial primers for simultaneous analysis of a wide variety of bacteria in the oral microbiota is undeniably extensive. Some limitations related to its possible binding to the same conserved area of the 16S rRNA target population have been reported and cannot be ignored such as those linked to probable PCR competitive inhibition. In this scenario, the DNA of the predominant (major) bacterial species is much more likely to be amplified than DNA from bacteria that form a minority or small proportion of the overall mixed population which could lead (in theory) to the complete omission of the DNA of minority bacterial species from the analysis [113]. Amplification of the DNA species present in excess (competitor DNA) may result at the expense of the DNA species present in smaller amounts limiting the analysis to only the majority species present. To address this, Kuwamura and Kamiya [114] developed the "DNA deduction" approach where excess DNA of the majority bacterial species present in the target population is removed and the detection of minority bacterial members of the community is facilitated. This method was proved to be a useful technique to better understand the diversity and composition of the human oral microbiota since many researchers identify species based on 16S rRNA sequence information in studying the oral

microbiota [115, 116]. Since a large number of good-quality sequences is required in order to construct a precise DNA database for use in human microbiota analyses for next generation sequencing, DNA deduction technique may offer improvement of the human microbiota database.

Molecular techniques based on next-generation sequencing (NGS) of the 16 rRNA gene of the bacterial genome, allowed analysis of the complexity of the bacterial component of the oral microbiome and has represented the standard for studying the composition of microbial communities by allowing differentiation of bacteria by sequencing the variable regions of the gene coding for the 16S ribosomal RNA (rRNA) which greatly improved our knowledge of the bacterial component of the oral microbiome [13, 117]. However, it does not usually provide sufficient information to resolve communities at the sub-species level, nor it can detect eukaryotic microorganisms and/or viruses. Instead, the species-level resolution obtained by NGS is not adequate for transmission studies or for exploring subspecies variation in disease association, and the oral microbiome includes also important non-bacterial components, including eukaryotic microbes (fungi, protozoa) and viruses [8]. For instance, reports on normal microbiome have been almost exclusively restricted to the bacteriome, and there are limited published findings on the mycobiome–fungal microbiome and on other microorganisms. To simultaneously characterize the presence and amount of all the microbial components potentially present in the oral cavity, the Whole Genome Sequencing (WGS) was introduced very recently [118, 119]. In whole genome sequencing, the entire DNA (genome) of a single microbial culture or a complex microbial population can now be sequenced to great depth allowing us to generate reference genomes (de novo assembly) as a resource for future studies or identify the composition of microbial community respectively (mapping back to a reference genome).

Another important aspect that may not also be addressed in depth is the issue related to antimicrobial resistance or AMR, very limited reports are available on resistome of the healthy oral microbiome [120, 121]. Since AMR is a growing concern, it would be useful to have data on the prevalence and type of drug resistance of the microbes composing the healthy oral microbiome, which might be very easily acquired and transmitted through aerosol and contact. Recently, Caselli et al. [8] provided comprehensive and detailed picture of the healthy oral microbiome as determined by WGS analysis, including also the drug-resistance features of the bacterial component. These findings further strengthened laboratory capabilities and added more areas for oral microbiome research to enable evidence-based oral diseases management in practice.

4. Impacts of oral microbiome to oral health (dental caries) and non-oral health (systemic diseases)

Oral microbiome data have accumulated in recent years and somehow slowly influences how we see management of oral diseases. During the last decade, studies have focused on the management and prevention of certain and disorders such as dental caries by modulating oral microbiome [37, 50]. Interestingly, some member of the oral microbiota has also been tagged as possible effective biosensors or biomarkers of oral or systemic diseases [84, 122]. In addition, the salivary or oral microbiome has been the target of interest for its diagnostic and prognostic value [37]. **Table 3** comprehensively lists the studies that supported the impacts of oral microbiome studies to both Oral and Non-Oral or Systemic Health.

Dental diseases are now viewed as a consequence of a deleterious shift in the balance of the normally stable resident oral microbiome. It is known that frequent

Oral microbiota derived from microbiome data	Associated disease status	References
Fusobacterium nucleatum	Periodontal disease	[123]
Eubacterium minutum *Prevotella intermedia*	Peri-implantitis	[124]
Haemophilus	Oral leukoplakia (mucosal disease)	[125]
Fusobacteria *Leptotrichia spp* *Campylobacter concisus*		[126]
Prevotella spp. *Lactobacillus* spp. *Dialister* spp. *Filifactor* spp.	Pathogenesis and progression of dental caries	[127]
Veillonella *Porphyromonas* *Streptococcus mutans*	Severe early childhood caries	[128]
Streptococcus *Porphyromonas* *Actinomyces*		Ma et al., 2015
Streptococcus *Prevotella* *Neisseria* *Haemophilus* *Veillonella* *Gemella*	Inflammatory Bowel Disease	[129]
Klebsiella spp.		Atarashi et al., 2017
Acinetobacter calcoaceticus	Oral Squamous Cell Carcinoma	[130]
Atopobium rimae		[131]
Clavibacter michiganensis subsp. tessellarius		[132]
Bacillus mycoides		[72]
Capnocytophaga gingivalis		[133]
Citrobacter koseri		[134]
Curtobacterium flaccumfaciens		[7]
Delftia acidovorans		
Eikenella corrodens isolate		
Escherichia coli		
Enterococcus faecalis		
Lactobacillus gasseri		
Fusobacterium canifelinum, Fusobacterium naviforme,		
Fusobacterium nucleatum ssp. 1 *nucleatum*		
Gemella haemolysans, Gemella morbillorum		
Leptotrichia shahii		
Megasphaera micronuciformis		
Moraxella osloensis		
Ralstonia insidiosa, Ralstonia pickettii, Ralstonia solanacearum		
Rothia		
Novel Atopobium		
Olsenella uli		
Plantibacter flavus		
Propionibacterium acnes		
Parvimonas		
Peptostreptococcus micros, Peptostreptococcus stomatis		
Porphyromonas		
Prevotella melaninogenica		
Rhodococcus erythropolis		
Rothia mucilaginosa		

Oral microbiota derived from microbiome data	Associated disease status	References
Slackia *Streptococcus gordoniii, Streptococcus salivarius, Streptococcus sanguinis* *Tepidimonas aquatica* *Thermus scotoductus*		
Porphyromonas gingivalis *Aggregatibacter actinomycetemcomitans* *Leptotrichia*	Pancreatic Cancer	[135] [136] [39]
Streptococcus	Pancreatic Ductal Adenocarcinoma	[137]
Rothia *Peptostreptococcus* *Fusobacterium* *Leptotrichia*	Pancreatic Head Cancer	[138]
Porphyromonas gingivalis	Gingival Squamous Cell Carcinoma	[139]
Rothia mucilaginosa *Lactobacillus gasseri* *Lactobacillus johnsonii* *Lactobacillus gavinalis* *Streptococcus salivarius* *Streptococcus vestibularis* *Fusobacterium nucleatum*	Head and Neck Squamous Cell Carcinoma	[140]
Rothia mucilaginosa *Capnocytophaga gingivalis* *Prevotella melininogenica* *Gemella morbillorum* *Granulicatella adiacens* *Streptococcus gordonii* *Streptoccus parasanguinis* *Stretococcus salivarius* *Fusobacterium nucleatum*	Oral Potentially Malignant Disorder	[141]
Gemella morbillorum	Keratocytic Odontogenic Tumor	[142]
Streptococcus *Fusobacterium*	Colorectal Carcinoma	[143]
Porphyromonas gingivalis *Prevotella* *Streptococcus*	Esophageal Squamous Cell Carcinoma	[144, 145]
Streptococcus	Gastric Adenocarcinoma	[146]
Prevotella melaninogenica *Fusobacterium*	Oral Cancer	[77]
Rothia mucilaginosa *Streptococus*	Oral Mobile Tongue Carcinoma	[147]
Actinobacillus actinomycetemcomitans *Tannerella forsythia* *Porphyromonas gingivalis* *Fusobacterium nucleatum* *Prevotella intermedia*	Alzheimer's disease	[148, 149]
Aggregatibacter *Neisseria* *Gemella* *Eikenella*	Diabetes	[150]

Oral microbiota derived from microbiome data	Associated disease status	References
Selenomonas *Actinomyces* *Capnocytophaga* *Fusobacterium* *Veillonella* *Streptococcus*		
Bacteroides forsythus *Campylobacter rectus*	Adverse pregnancy outcomes	[151]
Fusobacterium nucleatum		[152]
Lactobacillus salivarius	Rheumatoid arthritis	[153]
Veillonella *Prevotella* *Megasphaera* *Campylobacter*	Human Immunodeficiency Virus infection	[154]
Chryseomonas *Veillonella* *Streptococcus*	Atherosclerosis	[155]

Table 3.
Comprehensive list of studies supporting the impacts of Oral microbiome data (healthy or dysbiosis) to Oral and non-Oral health.

carbohydrate consumption or reduced saliva flow can lead to caries, and excessive plaque accumulation increases the risk of periodontal diseases. In general, increase in fermentable carbohydrates in the oral cavity or saliva, which is usually the case in diabetes, establishes a favorable environment for the microbes involved in dental caries [13]. However, reports have also described that shifts in the proportion of plaque microflora may not be directly caused by the mere availability of certain fermentable carbohydrates but rather, brought about by pH-mediated mediated reactions generated from carbohydrate metabolism [56]. Hence, it can be suggested that modifying metabolism of certain carbohydrates may result in slowing down the enrichment of cariogenic species, preventing development of caries. The excess uptake of carbohydrates leads to acid production due to fermentation of carbohydrates by many oral cavity inhabitants which disturbs the buffering capacity of saliva and hence results in tooth decay or dental caries.

Changes in environmental conditions brought about by poor oral hygiene may favor certain members of the oral microbiota and consequently serve as biomarkers for certain oral disease condition. Saliva and diet were observed to alter *Lactobacillus* abundance in the pulpal layer [156], and that low levels of *Lactobacillus* were linked to the degree and duration of pain and length of caries destruction. Moreover, poor oral hygiene status seems to be linked to anaerobic conditions in the salivary milieu, this condition inhibits growth of *Streptococcus* and favors the growth of *Veillonella* [153, 157, 158]. Salivary and plaque microbiome data also revealed that *Veillonella* was frequently found in subjects with caries or periodontal subjects, whether children or adults, with poor oral health and having caries or periodontitis [84]. Based on these findings, the proportion of *Veillonella* in salivary microbiome, therefore, can serve as a biological indicator for poor oral hygiene and caries in children and adults.

Oral microbial dysbiosis is also linked to oral inflammation and may contribute to systemic conditions through bacteremia [159]. It contributes to variable systemic

diseases processing including gastrointestinal system diseases like inflammatory bowel disease, liver cirrhosis, pancreatic cancer, nervous system diseases like Alzheimer's disease, endocrine system diseases like diabetes, adverse pregnancy outcomes, obesity and polycystic ovary syndrome, immune system diseases like rheumatoid arthritis and HIV infection, and cardiovascular system diseases like atherosclerosis [160].

The dominant genera, *Streptococcus, Prevotella, Neisseria, Haemophilus, Veillonella* and *Gemella*, were found to largely contribute to dysbiosis observed in the salivary microbiota of IBD patients [129]. A growing interest regarding the variation of salivary microbiota between dental caries patients with comorbidities such as rheumatoid arthritis (RA) and atherosclerosis compared to healthy subjects. Alterations in the gut, dental or saliva microbiome distinguished individuals with RA from healthy controls, were correlated with clinical measures and could be used to stratify individuals on the basis of their response to therapy. It was observed that *Haemophilus* species seems to be depleted from saliva, dental plaque and fecal samples of RA patients but their numbers become normal upon standard RA treatment [153]. Interestingly, the levels of *Porphyromonas gingivalis*, *Treponema denticola, Tannerella forsythia* and *Prevotella intermedia* in the saliva, have been linked in lowering serum levels of high-density lipoproteins, which may be associated with an increased risk of atherosclerosis [122]. These findings also highlight the possibility of saliva-based screening as alternative to fecal samples in microbiologic studies of systemic diseases.

Microbes are believed to be released from the biofilm through the epithelium and spread systemically via the blood circulation, this is probably the reason why bacteria isolated from pancreatic tissues are believed to be possible members of the oral microbiome [161]. *Porphyromonas gingivalis* is an important bacterium that can be transferred from the mouth to gut in many diseases, including colon cancer, IBD, and diabetes [138]. This microorganism induces dysbiosis by impairing innate host defenses while promoting inflammatory responses in phagocytic cells. Since this microbe can disrupt the interaction between host microbiota and mucosa by modulating the innate immune system and signaling pathway enhancing high levels of inflammation in pancreatic cancer [162], it is believed to be a good candidate biosensor for early diagnosis of pancreatic cancer. Likewise, oral metabolome finds its application in determination of biomarkers in oral cancers [163]. An elevated rate of degradation of macromolecules was shown in metabolome profiling in periodontal disease. Shifts in metabolic profiles indicate survival of pathogens in given conditions.

Disruption of the oral microbiome leads to dysbiosis. Identifying the microbiome in health is the first step of human microbiome research, after which it is necessary to understand the role of the microbiome in the alteration of functional and metabolic pathways associated with diseased states [9]. Salivary microbiota composition and abundance were significantly associated with body size and dependent on gender; particularly notable was the decrease in the core bacteria in overweight and obese children [90]. Overweight and obese children are likely to stay obese into adulthood and develop diseases more frequently than normal weight children. Thus, the early identification of subjects at risk of developing obesity and the prevention of overweight and obesity is of great importance. As we increase our understanding of the interplay between the environment and the oral microbiome, it will become possible to identify new strategies to combat disease by actively promoting our natural microbiota and reducing the impact of the drivers of dysbiosis [164].

Future studies focusing on precision medicine and risk prediction for dental caries and even periodontitis may also be possible when changes in oral microbiota

profiles in the salivary milieu may be made available. Previous studies have supported this which targeted subtypes or strains of specific bacterial species such as *Porphyromonas gingivalis* or *Aggregatibacter actinomycemcomitans* [165] in periodontits or *Streptococcus mutans* in caries [166] in predicting common oral health risks. Characterizing oral biofilms by metabolic activity rather than by listing the predominant species may also be considered as a logical approach when defining plaque biofilms in health and disease.

5. Novel microbiome-based approaches to prevention and treatment of dental caries

Traditional intervention methods to addressing dental diseases include mechanical debridement and antibiotic use. Mechanical means are non-specific and may remove beneficial bacteria in the process, more importantly, the microbial richness and biodiversity are also significantly decreased after mechanical debridement [167] which is not beneficial to overall oral health. Antibiotics on the other hand are designed to target specific pathogenic bacteria in animals and humans [168]. It was reported that the number of amoxicillin-resistant oral bacteria was significantly higher in young children with amoxicillin use than that of children without [169]. In view of the role of oral microorganisms in the causation and pathogenesis of oral and systemic diseases, it is crucial to improve oral protection against pathogens and maintain the dynamic equilibrium of the oral microecology. Understanding the interactions between the microbial communities is a key to combating oral pathogens. Novel strategies have been developed, such as the use of probiotics and prebiotics to address limitations of traditional intervention methods.

Probiotics are well-known in health promotion, they are "live microorganisms when administered in adequate amounts, confer a health benefit on the host" [170]. The mechanism of action of probiotics in the mouth is presumed to be similar to those observed in other parts of the body [171] which is to act mainly through these paths: competition with potential pathogens for nutrients or adhesion sites, killing or inhibition of growth of pathogens through production of bacteriocins or other products, improvement of intestinal barrier integrity and upregulation of mucin production, modulation of cell proliferation and apoptosis, and stimulation and modulation of the mucosal immune system [171, 172]. Oral probiotics should have specific characteristics to perform effectively including the ability to stick to and colonize oral tissue including hard, non-shedding surfaces and become a part of the biofilm [171, 173]. Additionally, they should not ferment sugars; otherwise, they will decrease pH and develop caries [171]. Probiotic methods have been studied to treat caries mainly by interfering with the oral colonization of cariogenic pathogens. Some strains that were used include *Lactobacillus casei* [174], *L. rhamnosus* GG [175], *L. rhamnosus, Bifidobacterium* [176], *Lactobacillus reuteri* [177], *B. animalis* [178], and *L. paracasei* [179], all of which have been verified to be able to decrease the number of cariogenic bacteria and thus, prevent dental caries.

Prebiotics, on the otherhand are poorly digested oligosaccharides and have been demonstrated to be an aid to complement probiotics in the treatment of oral diseases [172] by stimulating the growth and activity of beneficial bacteria and simultaneously inhibiting the growth and activity of potentially detrimental bacteria [172]. Some examples are Lactose, Inulin, Fructo oligosacccharides, Galactooligosaccharides and Xylooligosaccharides [180]. However, studies on prebiotic utilization seems limited and should be further investigated. Based on these findings, probiotics and prebiotics could be alternatives to prevent and cure bacterial diseases because they can reestablish an ecological balance or regain the biodiversity of oral

microbiota in its early stages [181]. Prebiotics can drive beneficial changes in the oral microbiota and could increase resistance to dysbiosis and recovery of health. However, interaction between the oral microbiome and probiotics, as well as the exact mode of action of oral probiotics should be taken into serious consideration.

Other promising alternatives to control dental caries are the use of avirulent *S. mutans* produced by genetic engineering [182], which was designed to target glucosyltransferases and consequently inhibit biofilm formation [183].

The use of bacteriophage or phage is a virus that specifically targets and destroys disease-causing bacteria by invading bacterial cells [182], disrupting their metabolism and causing lysis such as phages against *Enterococcus faecalis* that showed reduction in bacterial viability in infected root canals [184]. Several targeted delivery systems have been designed and developed to treat oral diseases including nanoparticles [185–187].

Modulation of oral microbiota, therefore, in combination with novel drugs and delivery approaches serves as promising interventions and opportunities [188]. The application of ecological principles can help us understand how the tight interplay of the oral microbiota and the host dictates health or disease. We encourage advancing research directed toward developing ways or strategies to shift from traditional treatment to preventive and personalized dentistry.

6. Future directions for Philippine-based research and policy: oral microbiome and dental diseases in focus

The oral cavity is inhabited by hundreds of bacterial species that play vital roles in maintaining oral health or in shifting to a diseased state such as dental caries. The observed bacterial profile of the Filipinos compared with other populations may be brought about by the difference in their diet or oral hygiene practices. However, the possibility of this correlation has not been covered in a previous study on the Filipino oral microbiome [73]. Other factors influencing the shifts in the oral microbial diversity from a healthy to a diseased state may also be the focus of future studies.

The continued advancement of research and the resulting robust data on the oral microbiome is expected to lead to evidence-informed dentistry. The link of oral health diseases to systemic conditions have been established. Effective preventive measures, accurate diagnosis, sound treatment and the maintenance of good oral health will be expected outcomes when updated information on the oral microbiome are utilized optimally and may also lead to a decrease of the global burden of non-communicable diseases such as heart disease and diabetes mellitus. This strengthens the call for enhanced research on the Filipino oral microbiome and to shift the perspective that knowledge of the oral microbiome transcends health of the oral cavity but the general well-being of the individual and the populace.

Addressing the 87.4% dental caries and 48.3% periodontal disease prevalence [189] among Filipinos will not only require a responsive oral health care system but behavioral changes for Filipinos to prioritize oral health. The Lifecycle Approach has been laid out by the Department of Health in the delivery of the Basic Package of Oral Health Care [190]. Preventive Services must be done to provide specific protection from the occurrence of dental caries and other dental diseases, and this is initiated by the careful checking of the oral cavity.

Kits to test salivary pH levels are currently available. With its results analyzed with dietary patterns and oral hygiene habits, an individual's caries risk can be assessed. Minimum clinical intervention are done when appropriate preventive measures are adopted. Diagnostic tests to assess the oral microbiome were not specified in the DOH guidelines but utilization of these test kits, when available, can

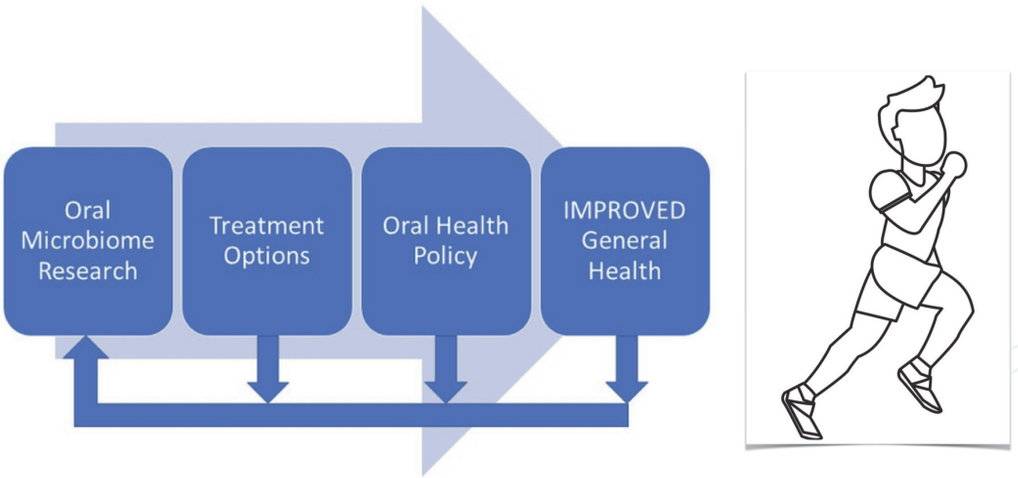

Figure 2.
Proposed framework directing possible future directions in Philippine-based research and policy focusing on Oral microbiome and dental caries.

aid in the development of individualized preventive programs. It should be noted that though the dental public health implications of diagnostic tests may be limited at the moment, its genomic value may be of promise. The high prevalence of oral diseases in the Philippines necessitates that unmet clinical needs be provided with proper preventive and promotive interventions. The development of a Filipino Oral Microbiome Database may support the advancement of effective oral health treatment options which could support responsive oral health policies in establishing oral health programs for Filipinos.

Figure 2 describes our proposed framework directing possible future directions in Philippine-based Research and Policy Focusing on Oral Microbiome and Dental Caries. It should be emphasized that monitoring and evaluation must be included in all stages. The process must be dynamic enough to allow for review. All stakeholders must be open to conducting new research when the desired outcomes are not being realized. With the enactment of the Universal Health Care (UHC) Act in 2018, the State is mandated to provide a comprehensive and integrated health care model to all Filipinos that include cost-effective promotive and preventive services. The translational data from oral microbiome researches can be referred for Health Technology Assessment to be reviewed for inclusion in the oral health care packages under the UHC.

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