Title: Culture-independent studies on bacterial dysbiosis in oral and oropharyngeal squamous cell carcinoma: A systematic review

Running Title: Bacterial dysbiosis in oral cancer

Divya Gopinath[1#],Rohit Kunnath Menon[2#],Moinak Banerjee[3], Richard *Su* Yuxiong[1], Michael George Botelho,[1] Newell W Johnson[1,4,5]

1. Faculty of Dentistry, University of Hong Kong, Hong Kong SAR, China.
2. International Medical University, Kuala Lumpur, Malaysia
3. Rajiv Gandhi Centre for Biotechnology, Trivandrum, India
4. Menzies Health Institute Queensland, Griffith University, Queensland, Australia
5. Dental Institute, King's College London.

# Equal contribution

*Corresponding Authors

Michael George Botelho (Associate professor) & Newell W Johnson (Honorary Professor)

Faculty of Dentistry

The University of Hong Kong

Prince Philip Dental Hospital

Street 34 Hospital Rd

Sai Ying Pun

HONG KONG

Tel 2859 0412

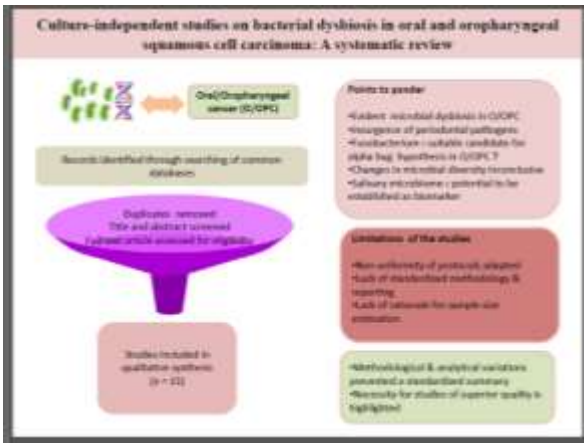
E-mail: botelho@hku.hk

n.johnson@griffith.edu.au

Total no of words: 4043 (excluding abstract and references)

Total no of words for abstract: 249

Graphical abstract



ABSTRACT

Imbalance within the resident bacterial community (dysbiosis), rather than the presence and activity of a single organism, has been proposed to be associated with, and to influence, the development and progression of various diseases; however, the existence and significance of dysbiosis in oral/oropharyngeal cancer is yet to be clearly established. A systematic search (conducted on 25/01/2018 and updated on 25/05/2018) was performed on three databases (Pubmed, Web of Science & Scopus) to identify studies employing culture-independent methods which investigated the bacterial community in oral/oropharyngeal cancer patients compared to control subjects. Of the 1546 texts screened, only fifteen publications met the pre-determined selection criteria. Data extracted from 731 cases and 809 controls overall, could not identify consistent enrichment of any particular taxon in oral/oropharyngeal cancers, although common taxa could be identified between studies. Six studies reported the enrichment of *Fusobacteria* in cancer at different taxonomic levels whereas three studies reported an increase in *Parvimonas*. Changes in microbial diversity remained inconclusive, with four studies showing a higher diversity in controls, three studies showing a higher diversity in tumors and three additional studies showing no difference between tumors and controls. Even though most studies identified

a component of dysbiosis in oral/oropharyngeal cancer, methodological and analytical variations prevented a standardized summary, which highlights the necessity for studies of superior quality and magnitude employing standardized methodology and reporting. Indeed an holistic metagenomic approach is likely to be more meaningful, as is understanding of the overall metabolome, rather than a mere enumeration of the organisms present.

Keywords: Oral cancer; Bacteria; Microbiome; Bacterial dysbiosis; Head and neck cancer; 16S rRNA sequencing

INTRODUCTION

Head and neck squamous cell carcinomas (HNSCC) are amongst the most common cancers in the world. Oral and Oropharyngeal cancers together constitute the major group of HNSCC and 51540 new cases are expected to be diagnosed and 10030 individuals are estimated to die in 2018 due to these cancers in US alone [1]. Numerous causative agents or factors have been described, notably tobacco-both smoked and smokeless, areca nut, alcohol, human papillomavirus (HPV), tooth-related factors/chronic mechanical irritation, dietary deficiencies and fungal infections, notably chronic candidiasis [2, 3]. However, the exact role of each factor remains uncertain despite the significant number of studies showing their association. HNSCC, like any other carcinoma, is a progressive chronic disease in which the healthy epithelium accumulates dysplastic changes and in some, but not all cases, finally transforms into invasive phenotypes. The events of carcinogenesis are complex and multifactorial and might not follow the same sequence in different individuals [3].

An increasing body of evidence has been accumulating in recent years which lead experts to suggest a possible role of microorganisms at different stages of carcinogenesis [4-6]. The connections between a consortium of bacteria and carcinogenesis have not been investigated thoroughly; perhaps because earlier research had identified individual pathogens such as *Helicobacter pylori*, linked to carcinogenesis [7]. Culture-dependent methods have examined the role of numerous individual or groups of bacteria and their association with cancers of oral

cavity [8]. However, it is now considered that 60-90% of the human microbiome is uncultivable which has prompted further research regarding the role of bacteria in carcinogenesis [9, 10]. Culture-independent techniques using molecular methods with recombinant DNA have endowed researchers with tools for recognizing the organisms that reside in microbial communities, reducing the potentially misleading results arising from the vagaries of cultivation. With the advent of next-generation sequencing, studies have focused on a more holistic approach for the characterization and identification of microbial communities and their possible involvement in various diseases, including different types of cancer [11-13]

Microbial dysbiosis can be defined as any change to the composition of resident commensal communities, relative to the community found in healthy individuals [14]. To date, very few studies have tried to address bacterial dysbiosis associated with oral/oropharyngeal cancers employing culture-independent approaches. Furthermore, clear evidence is still deficient on any association between this dysbiosis and oral /oropharyngeal cancers and whether the presence of specific bacterial taxa could be developed as biomarkers for diagnosis or risk assessment. Our aim in present article is to summarize current evidence on the association of bacterial dysbiosis and oral cancer by performing a systematic and comprehensive review of published studies which have utilized culture-independent techniques to study the characteristics of the whole oral microbial community in association with oral/oropharyngeal cancers. Whilst recognizing that a microbiome constitutes fungi, bacteria and viruses, and occasionally other organisms, the present review is restricted to the ‘bacteriome’. It is also important to differentiate the terms *microbiota*, which comprises all living microorganisms, and the *microbiome/bacteriome*, which includes all available genomes derived from dead or living microorganisms in its pure context.

METHODS

Literature search and data extraction methods for this review were specified and documented as a protocol and registered in PROSPERO (CRD42017051160). This systematic review follows the PRISMA (Preferred Reporting Items for Systematic Reviews and Meta-Analyses) criteria [15]. The strategies for the screening and inclusion were decided by authors 1 and 2 in advance. A systematic search of the published literature was conducted on 25/01/2018 and updated on

25/05/2018 in PubMed, Scopus and Web of science to identify articles on oral cavity microbiome and oral cancer-without date limitations using specific search terms. The search strategy included the following words: oral, oral cavity, saliva, oral tissue, microbiome, microbiota, microbial diversity, micro flora, microorganisms, oral cancer, oral squamous cell carcinoma and head & neck squamous cell carcinoma in combination. Boolean operators such as "and" "or" were used to stratify the search. After an initial screening of the titles and abstracts, two authors (DG and RKM) independently screened the full texts for eligibility. Moreover, manual searches of cross-references from relevant narrative reviews were also performed in order to exclude the possibility of omitting any important study. The search strategy was confined to English language comprising human studies.

Two authors independently assessed the risk of bias using an adapted Newcastle-Ottawa Scale for analytical non-randomized case-control studies similar to a recently published review on skin microbiome in atopic dermatitis [16].

Inclusion Criteria

Original research articles investigating the ‘bacteriome’ community through culture-independent methods in tissue, saliva or swabs in patients with oral and/or oropharyngeal cancers and controls were included.

Exclusion Criteria

Culture-based studies, conference abstracts, review articles, studies examining a cohort with oral or oropharyngeal cancer associated with other debilitating diseases and those studies without controls were excluded. Articles in languages other than English were excluded.

RESULTS

1546 articles were identified in the initial search after removal of duplicates. After reviewing the title and the abstract, 71 articles were included for full-text screening. Based on our selection criteria, 15 studies were selected and examined in detail (Figure 1).

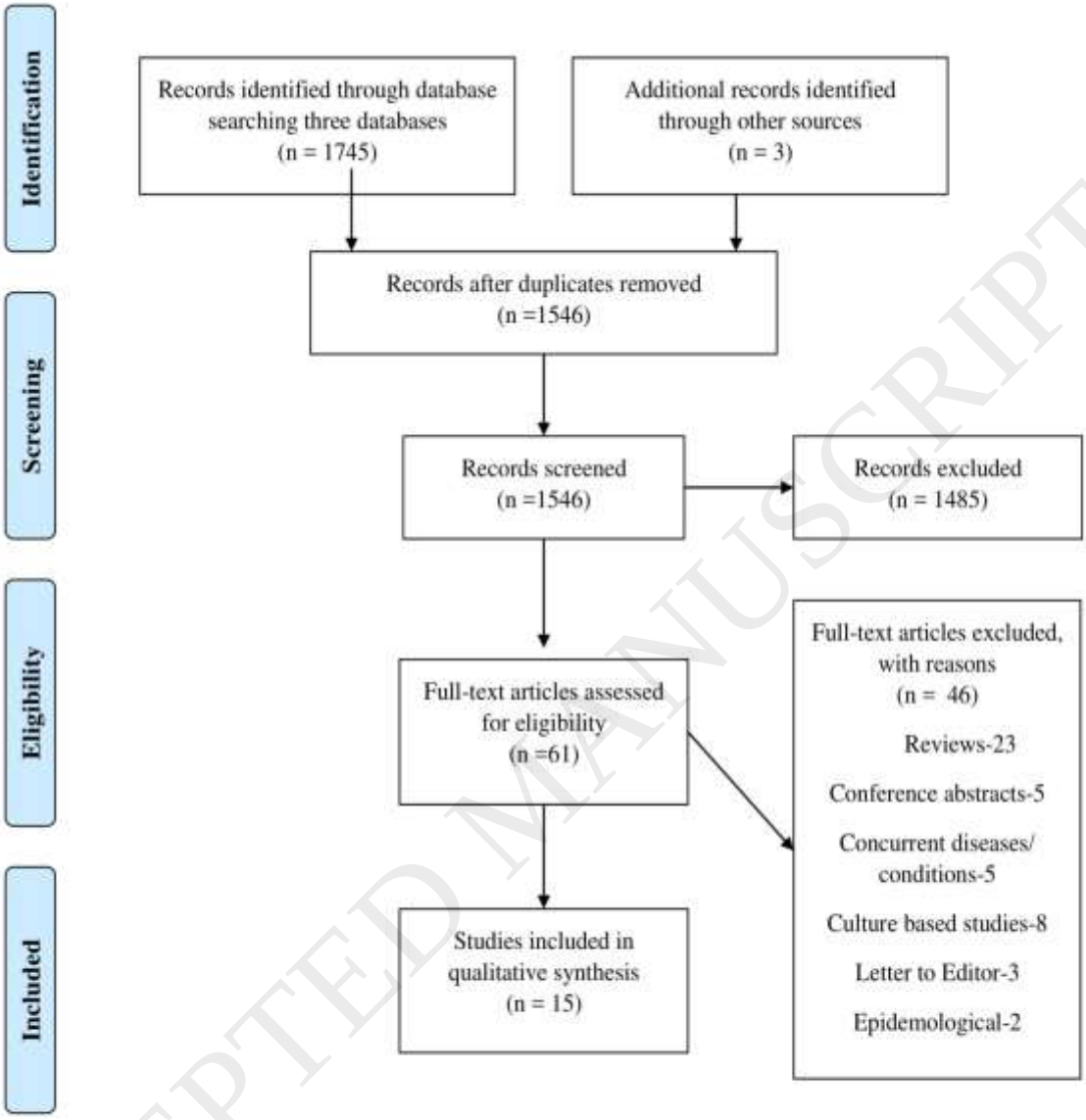


Figure 1: PRISMA flow diagram for systematic review depicting phases of identification of studies

Figure 1: PRISMA flow diagram for systematic review depicting phases of identification of studies

Sample characteristics & Methodology

Sample characteristics are described in detail in Table 1. There were a total of 731 cases and 809 controls represented in a total of fifteen publications. Various kinds of oral samples were used to study the 'bacteriome' including saliva [17-20], swabs from the surface of the tumor [21, 22], the tumor tissue [23-30] and oral rinses [31]. Samples from normal healthy individuals as well as patient's own mucosa were utilized as controls in the included studies. Those studies which used samples from normal healthy individuals as controls had varied matching criteria which included site [18, 27, 29], age [19, 27] and gender [27, 29]. Other studies used tissues from contra lateral mucosa of the same patient [21, 22, 28] or mucosa adjacent to cancer tissue from the patient [23-26, 30] as controls. The total sample size of the selected studies ranged from 5 to 363. However, none of the studies stated the rationale for their study population size: nonetheless, one of the studies claimed that their multivariate analysis remained well powered as the number of matched controls was twice that of cases [31].

We found significant heterogeneity in the methodology of studies (Table 2). Numerous studies have used archival frozen specimens collected for other varied purposes and thus strict exclusion criteria were not properly defined. Accepted protocols of microbial wall lysis such as lysozyme treatment or bead beating were not reported in the selected studies except for three [26,30,31]. DNA extractions were performed using different commercial DNA kits. The studies using 16S rRNA amplification approach targeted different hypervariabe regions; however, significant overlap was observed in constant regions of the 16S gene. One of the earlier studies used three sets of primers, which covered bacteria of three main phyla found in *Spirochaetes, Bacteroides* & *domain Bacteria* [22]. Amount of PCR cycles ranged from 25-35, where as one study did not provide any information regarding the PCR cycles [17]. Four studies utilized Topo TA cloning and Sanger sequencing for generating sequences with the number of colonies analyzed ranging from 30 to 95 per sample, whereas rest of the studies utilized 16S rRNA sequencing that allows deeper sequencing depths. One study utilized PathoChip^{TM} array (a type of microarray) coupled with next generation sequencing for identification of the putative pathogens [28].

Table 1: Summary of sample characteristics and 'bacteriome'

No	Author year	Sample	Age (Mean) Sex	Site	Study setting	Nature of control	Study population size		Other clinical factors studied	RESULTS	
							Cases	Controls		Diversity and Richness	Abundance of taxa *
1	Hooper 2007 (24)	Fresh Tissue	65.3 Both	Oral	UK	Normal mucosa from same subject	10	10	NA	NA	➢ *Clavibacter michiganesis subsp.tellarius, Fusobacterium naviforme, Ralstoniainsidosa* detected in >30% more of tumor ➢ *Granulicatella adiacens, Porphyromonas gingivalis, Sphingomona ssp pc5.28 and streptococcus mitis/oralis* detected in >30% more of non tumor tissue

2	Pushalkar 2011 (18)	Saliva	> 50 M	Oral	USA	Saliva of controls	3	2	NA	NA	➢ Most prevalent in OSCC- *Streptococcus*, *Gemella*, *Rothia*, *Peptostreptococcus*, *Lactobacillus*, *Porphyromonas* and *Lactobacillus* (genus) ➢ Most prevalent in controls- *Prevotella*, *Neisseria*, *Leptotrichia*, *Capnocytophaga*, *Actinobacillus*, *Oribacterium*, *SR1* and *TM7* (genus)
3	Bebek 2011 (23)	Fresh frozen and paraffin embedded tissue	62 M/F	HN-SCC	USA	Adjacent normal tissue	42	42	Age Sex Site Metastases HPV Tobacco Alcohol Node Methylation	NA	➢ *Enterobacteriace* (family) & *Ternericutes* (phyum) found to be significantly enriched in tumor tissue

4	Pushalkar 2012 (25)	Tissue	59 M/F	Oral	USA	Adjacent normal tissue	10	10	NA	Richness higher in tumor No differen-ce in diversity	➢ Taxa enriched in tumor - *Streptococcus sp*. oral taxon 058, *Peptostreptococcus stomatis, Streptococcus salivarius, Streptococcus gordonii, Gemella haemolysans, Gemella morbillorum, Johnsonella ignava* and *Streptococcus parasanguinis* ➢ Taxa enriched in non tumor- *Granulicatella adiacens*
5	Schmidt 2014 (21)	Swabs	62.4 M/F	Oral	USA	Contra lateral normal tissue	15	15	Smoking Nodal status	Diversity higher in tumor samples	➢ Tumor-Significant reduction in *Firmicutes* and *Actinobacteria* (phyla) ➢ Increase in *Fusobacteria*
6	Guerrero-Preston 2016 (17)	Saliva	NS	Oral /Oro pha-ryn-geal	USA	Saliva of healthy controls	17	25	HPV status Tumor site	Signific-antly higher diversity and richness in controls	➢ Tumor-Significant increase in *Streptococcus* and *lactobacillus* (genus) ➢ Non tumor-Significant increase in *Hemophilous, Neisseria, Leptotrichia, Aggregibacter, Lautropia* (genus)

7	Wang 2017 (26)	Archival Frozen Tissue	63 M/F	HNSCC	USA	Adjacent normal tissue	121	121	Clinical stage of cancer	No difference in diversity and richness	➢ Tumor-Significant reduction in *Actinobacteria*, Significant increase in *Parvimonas* (genus) up to parent family *Tissierellaceace*, ➢ Increase in *Fusobacteria*
8	Al-hebshi 2017 (27)	Archival Frozen Tissue	56.9 M/F	Oral	Yemen	Deep epithelial swabs from gender and age matched healthy controls	20	20	Nil	No difference in diversity and richness	➢ Tumor-*F.nucleatum subsp.polymorphum, Pseudomonas aeruginosa* and *Campylobacter sp. Oral taxon 44* (significantly abundant) tumors) ➢ Controls-*S. mitis, R. mucilaginosa and H. parainfluenzae*

9	Wolf 2017 (20)	Saliva	61.6 M/F	Oral /Oro pha-ryn-geal	Aust-ria	Saliva from healthy controls	11	11	Age, Alcohol Consum-ption, Tumor size, Lymph node status, Smoking, HPV status	Higher diversity in saliva of cancer patients**	➢ Highly abundant in SSC patients -Phylum *Actinomyces* (Actinobacteria), *Schwartzia* (Firmicutes), *Treponema* (Spirochaetes) and *Selenomonas* (Firmicutes). ➢ Highly abundant in Controls-Phylum Bacteroidetes (*Prevotella*), Proteobacteria (e.g. *Haemophilus* and *Neisseria*) and Firmicutes (e.g. *Streptococcus* and *Veilonella*)
10	Lee 2017 (19)	Saliva	53 M/F	Oral	Taiw-an	Saliva from healthy controls	127	125	Smoking, Chewing	Higher diversity in Saliva of normal controls	➢ *Bacteroides, Escherichia* and *Bulleidia* were present in over of 40% in cancer patients ➢ *Cloacobacillus*,*Gemmiger*, *Oscillospora* and *Roseburia* were 20 times abundant in cancer patients
11	Bornigen 2017 (31)	Oral rinse	NS M/F	Oral /Oro pha-ryn-geal	USA	Oral rinse from healthy controls	121	242	Smoking, alcohol, tooth status, HPV status, periodon-titis	NA	➢ Genus *Dialister* were relatively highly abundant in cases when compared to controls. ➢ Orders *Actinomycetales* and *Lactobacillales* were significantly underrepresented in oral cancer.

12	Zhao 2017 (22)	Swabs	62 M/F	Oral	China	Normal mucosa from same subject	80	80	Nil	Higher diversity in Cancer patients	➢ Spirochaetes, Fusobacteria and Bacteroidetes were significantly enriched in cases ➢ *Firmicutes* and *Actinobacteria* were significantly decreased in cases ➢ 17 taxa were enriched at genus level in cancers
13	Banerjee 2017 (28)	Formalin fixed paraffin embed-ded tissue	NS	Oral /Oro pha-ryn-geal	USA	Adjacent normal tissue as well as healthy control tissue	100	20 +20	Nil	NA	➢ Phylum *Proteobacteria* was more pronounced in cases ➢ Phylum *Bacteroides* were more pronounced in healthy controls ➢ 13 genera were specifically associated with tumor samples
14	Mukherjee 2017 (30)	Archival Frozen tissue	60.5 M/F	Oral	USA	Adjacent normal mucosa	39	39	Gender Race Age Smoking	Lower diversity & richness in cancer tissues	➢ Genera *Streptococcus, Actinomyces, Rothia, Corynebacterium, Enterococcus* and *Micrococcus* significantly increased in tumor group

15	Perera 2018 (29)	Deep Tissue	61 M	Oral	Sril-anka	Fibroepi-thelial polyp from age and gender matched controls	25	27	Nil	Lower diversity and richness in cancer tissues**	➢ Genera Capnocytophaga, Pseudomonas, and Atopobium were associated with tumor ➢ Lautropia, Staphylococcus, Propionibacterium, and Sphingomonas were the most significantly abundant in controls

*Described in terms used by authors in the respective articles as no standardized way of reporting was observed among the articles

** Not statistically significant #NA-Not available #NS- Not specified

Table 2: Summary of techniques of DNA extraction, amplification & sequencing

No	Author year	Sample	Method of DNA extraction	DNA amplification	Sequencing	Reference Data base
1	Hooper 2007(24)	Tissue	Pure gene DNA isolation kit	3 sets of primers targeting 16SrRNA genes of *Spirochaetes, Bacteriodes* & domain *Bacteria*	Topo TA cloning and sequencing of 30 clones samples	Gene bank
2	Pushalk-ar 2011(18)	Saliva	Incubation in Proteinase K & DNA purification kit (Epicenter)	V4-V5 region	454 parallel DNA sequencing	RDP ii
3	Bebek 2011(23)	Tissue	Tissue homogenized in trisodium citrate enzymatic lysis & DNA extraction by QIAmpDNA mini kit	V1-V4 region	Topo TA cloning and sequencing of 90 clones from each sample	RDP*
4	Pushalk-ar 2012(25)	Tissue	Incubation in Proteinase K &DNA purification kit(Epicenter)	V4-V5 region	Topo TA cloning and sequencing of 96-96 clones from each individual	HOMD**
5	Schmidt 2014(21)	Swabs	Incubation in Proteinase K & DNA easy kit (Qiagen)	V4 region	454 pyrosequencing	Green genes reference database
6	Guerrero Preston 2016(17)	Saliva	Saliva pellets were digested with SDS and Proteinase K & DNA extraction by phenol-	V3-V5 region	Roche/454 GS pyrosequencing	RDP

			chloroform method			
7	Wang 2017(26)	Tissue	Bead homogenization of tissue & enzymatic lysis & DNA extraction by QIAmpDNA mini kit(Qiagen)	V1-V4 region	Strataclone psc vector cloning and sequencing of 95 clones per sample	RDP
8	Al-hebshi 2017 (27)	Cases-Tissue /Controls-swab	DDK DNA isolation kit	V1-V3 region	MiSeq platform (Illumina, USA) (300 base pair chemistry)	HOMD
9	Wolf 2017(20)	Saliva	MagNA pure LC DNA Isolation Kit III	V4-V5 region	MiSeq platform (Illumina, USA)	Greengenes Database
10	Lee 2017(19)	Saliva	QIAamp DNA Blood Kit	V4 region	MiSeq platform (Illumina, USA)	SILVA database
11	Mukher-jee 2017(30)	Tissue	QiaAmp DNA Stool mini Kit	V4 region	Ion Torrent Thermofisher (USA)	UNITE 5.8s
12	Bornigen 2017(31)	Oral rinse	QIAsymphony kit	V4 region	MiSeq platform (Illumina, USA)	Greengenes Database
13	Zhao 2017 (22)	Swabs	QIAamp DNA Mini Kit	V4-V5 region	MiSeq platform (Illumina, USA)	RDP
14	Banerjee 2017(28)	Tissue	`All Prep DNA/RNA FFPE kit	NA	MiSeq platform (Illumina, USA)	RDP

15	Perera 2018 (29)	Tissue	Gentra Puregene Tissue kit	V1 - V3 region	MiSeq platform (Illumina, USA)	Species-level taxonomy assignment algorithm (23)

*RDP-Ribosomal Database Project** HOMD-Human Oral Microbiome Database

Risk of bias

The quality of the 'bacteriome' studies showed high variability (Table 3). None could be rated as very good. We qualified them as very good (total score=9; N=0), good (total score=7-8; N=8), fair (total score=5-6; N=5) and poor (<5; N=2). We could not identify any justification for sample size in the included studies. The main reason for downgrading the studies to fair was either lack of proper statistical analyses and lack of definable outcome measures.

Table 3: Risk of Bias Assessment by adjusted Newcastle-Ottawa Scale for Case Control studies

No	Studies	Selection (Definition and selection of cases and controls ;max=4*)	Comparability (cases and controls ;max=2*)	Outcome (outcomes measured as relative abundances of bacteria, Same method for cases and controls,statistical analysis ;max=3*)	Total (max=9)
1	Hooper 2007(24)	**	*	*	4
2	Pushalkar 2011(18)	**	*	*	4

3	Bebek 2011(23)	**	**	**	6
4	Pushalkar 2012(25)	***	**	*	6
5	Schmidt 2014(21)	***	**	**	8
6	Guerrero-Preston 2016(17)	***	*	***	7
7	Wang 2017(26)	**	**	***	7
8	Al-hebshi (2017)(27)	**	*	***	6
9	Wolf (2017)(20)	**	**	***	7
10	Lee (2017)(19)	***	**	***	8
11	Bornigen (2017)(31)	***	**	**	6
12	Zhao (2017)(22)	***	**	***	8
13	Banerjee (2017)(28)	**	**	**	6
14	Mukherjee (2017)(30)	***	**	***	8
15	Perera (2018)(29)	***	**	***	8

Microbial diversity & Relative abundance

Diversity and richness are two commonly used indices to report the diversity of the microbiome in human and environmental studies. Diversity can be defined as the number of phylotypes or operational taxonomic units found in a local habitat [31]. Interestingly, among the fifteen studies, only nine reported the change in bacterial diversity of the microbiome when compared to controls. Three studies did not observe any differences in microbial diversity in tumors compared to controls. However, higher diversity in controls over cases was reported by Preston et al. [17] and Lee at al. [19] in saliva and Perera et al. [29] and Mukherjee et al. [30] in tissues. Contrary to this, Schmidt et al. [21] and Zhao et al. [22] reported an increase in diversity in tumor swab specimens whereas Wolf et al. [20] reported an increase in diversity in tumor salivary samples respectively. Another study reported a relative shift from gram-negative to gram-positive organisms in oral cancer tissue samples [25].

Abundance is defined as the number of times each taxon is identified in the sample and often reported in terms of relative abundance, which helps in the comparison between the groups reported [33]. The studies varied in the reporting of abundance of taxa with a few concentrating on hierarchical differences from phyla all the way up to genera, whereas others only reported differences in genera.

The reported range of phyla varied from five to thirteen among the studies with most concentrating on the most abundant seven phyla in the oral cavity. *Firmicutes* is the most common phylum in the oral cavity [14] and all of the studies were in concordance regarding this finding except the study by Zhao et al which reported *Bacteroidetes* to be the most abundant [22]. However, the relative abundances of phylum *Firmicutes* varied between cases and controls among the studies. Three studies reported a reduction in *Firmicutes* in tumor tissue compared to controls [20,21,22] whereas three other studies identified an increase in phylum *Firmicutes* in the saliva [17, 18] and tissue [30] of cancer patients respectively when compared to controls. Reduction in abundances of *Actinobacteria* in the tumor samples was reported by three studies [21, 22, 26] and one among these studies observed a positive correlation between the reduction in the abundance of phyla *Actinobacteria* and the severity of the disease (increase in T stage) [21, 22, 26]. Two studies reported significant enrichment [22, 27] where as one study reported reduction [30] of *Fusobacteria* at phylum level in cancer samples compared to controls. Apart

from these three changes at the phylum level, significant enrichment in phylum *Tenericutes* was reported by Bebek et al. [23] and *Spirochaetes* and *Bacteroidetes* by Zhao et al. [22] in cancer samples.

Numerous changes were reported at the genus level (Table 1). A study conducted on a US population reported a significant reduction in the relative abundance of genus *Lactobacillus* and *Streptococcus* and enrichment of *Staphylococcus* and *Parvimonas* in the saliva of oral cancer patients when compared to controls [17]. Two other reports [25, 26] also identified an increase in *Parvimonas* in tumors relative to controls. Tumor samples also had loss of abundance of different taxa such as *Neisseria, Aggregatibacter, Haemophilus, and Leptotrichia* at genus level compared to controls as reported by Preston et al.[17]. Another study from USA reported the difference in abundance of 22 bacteria at genus level in tumor and non-tumor groups. Among them, *Streptococcus* was the most abundant genera to be significantly increased in tumor group compared to controls along with *Actinomyces, Corynebacteirum, Rothia, Enterocccus* and *Micrococcus* [30]. Bornigen et al. reported higher relative abundances of genus *Dialister* and depletion of *Scardovia* in oral rinse from oral cancer patients [31]. A study from Yemen reported strong association of genus *Fusobacterium, Campylobacter and Pseudomonas* with OSCC tissues, while *Streptococcus, Haemophillus* and *Rothia* were the most common genera in the controls [27]. Statistically significant differences in abundances of seven genera namely, *Lachnoanaerobaculum, Filifactor, Mycoplasma, Fretibacterium, Megasphaera, Tannerella* and *Peptostreptococcus* between the saliva of normal and oral cancer groups were reported in a Taiwanese population [19]. However, they reported a significant presence of *Escherichia coli* in normal controls and cancer tissue: this pathogen is not a common inhabitant of the oral cavity and is often considered as a contaminant in microbiome studies. Perera et al. reported the association of *Fusobacterium, Capnocytophaga, Pseudomonas,* and *Atopobium* with OSCC tissues from Sri Lankan males and *Lautropia, Staphylococcus, Propionibacterium,* and *Sphingomonas* with controls [29]. Wolf identified high abundance of genera, notably *Actinomyces* (Actinobacteria), *Schwartzia & Selenomonas* (Firmicutes), and *Treponema* (Spirochaetes) in SSC patients whereas *Prevotella (*Bacteroidetes), *Streptococcus* and *Veilonella* (Firmicutes) & *Haemophilus* and *Neisseria (*Proteobacteria) were more in controls in an European population [20]. Significantly higher abundances of *Campylobacter, Eikenella, Mycoplasma, Fusobacterium, Selenomonas, Treponema, Dialister, Centipeda,*

Lachnospiraceae_G_7, Alloprevotella, Peptostreptococcus, Filifactor, Peptococcus, Catonella, Parvimonas, and *Peptostreptococcaceae_XI_G_7, & Capnocytophaga* in cancer samples were reported in a Chinese population [22]. On the contrary, *Stomatobaculum, Lautropia, Veillonella, Megasphaera, Streptococcus, Scardovia, Rothia*, *Granulicatella,* and *Actinomyces* were more abundant in controls.

Apart from phylum and genus levels, two studies reported an increased abundance of families *Enterobacteriaceae* [23] and *Tissierellaceae* [26] in tumors compared to adjacent normal control tissue whereas another study reported a decrease in orders *Actinomycetales* and *Lactobacillales* in oral rinses of cancer patients relative to controls [31].

Association with clinical variables

Associations of microbiome with other variables commonly associated with HNSCC were also investigated by some of these studies (Table 1). Bornigen et al. observed a loss of bacterial diversity in oral rinse of smokers when compared to non smokers though not statistically significant [31]. However Schmidt et al could not find any overall changes in the microbiome that might be attributed to the use of tobacco owing to their limited sample size [21]. Bebek et al reported that microbiome subpopulations differentiated tobacco users from non-tobacco users with tobacco users showing a significant decrease in *Flavobacteria* (class) and *Tenericutes* (phylum) compared to the other group [23]. However, they could not find any association between specific bacterial sub-populations with alcohol use, nor with HPV status of the neoplasm. Wolf et al. observed that patients who were HPV+ exhibited a 'bacteriome' composition that resembled healthy controls [20] whereas Guerrero-Preston et al. reported a significant abundance of the genus *Gemellaceacae* and *Leuconostoc* in HPV+ HNSCC when compared to HPV− HNSCC patients [17].

DISCUSSION

Characterization of the microbiome as an entire community has broadened our views of microbial ecology, including community-level functions that integrate with the normal functioning of the human ecosystem. Results from the overall comparison of diversity and

richness in tumor versus normal tissue in oral/oropharyngeal cancers are conflicting. Similarity in overall taxonomical composition of the microbiota was found when paired samples from the same individual were compared. As suggested by Wang et al, similarity in the resident microbiome from paired samples in the same patient is unsurprising: oral fluids are common to both, and the situation may be due to the histological analogy of adjoining "normal" tissue to the tumor tissue: inappropriate due to the likelihood of ‘field cancerization' [26]. In contrast, the studies that have compared cases with normal healthy controls have identified a distinct pattern of the microbiome to be associated with cancer.

Our review demonstrates that the predominant phyla present in cases and controls are essentially the same. Nevertheless, there were notable discrepancies in relative abundances of three predominant phyla namely *Firmicutes, Actinobacteria and Fusobacteria*. Three of the published studies reported a significant decrease in the relative abundance of phylum *Actinobacteria* in tumors. It has been reported that *Actinomyces* spp. which is a genus under phylum *Actinobacteria,* exerts a shielding effect on the mucosa through the release of protease-inhibitors which are capable of inhibiting tumorigenesis [34]. It has also been postulated that *Actinomyces* spp. could have been outnumbered by oral commensal bacteria which grow faster in the relatively acidic tumor microenvironment, aided by hypoxia and glucose-deprivation [26, 32-35].

Taxa “*Fusobacterium*” was reported to be significantly more abundant in cancer samples: these bacteria have a special property to cohere with other species and therefore can play a critical role in helping other pathogens to colonize [36]. *F. nucleatum* is known to be associated with colorectal carcinoma (CRC) and has been shown to promote cellular proliferation and invasion in human CRC cell lines *in vitro* as well as to boost the evolution of OSCC and CRC in animal experiments [37-41]. However, this needs to be investigated in detailed prospective studies. Four studies reported the enrichment of *Parvimonas* at the genus level [17, 22, 25, 26]. *Parvimonas* are frequently seen to be enriched in colorectal cancer as well [39, 40]; however, the association has not been characterized in detail. The enrichment of these reported taxa in these studies is notable, despite numerous methodological variances between the studies, such as sample type, collection techniques and methods of sequencing.

We cannot discriminate whether the observed changes in the microbial community are cancer-associated, cancer-promoting or involved in cancer-pathogenesis. Two hypotheses have been proposed on the association between microbiome and cancers in the gut and extrapolation of these models to oral cancer can be convincing [40-45]. In the "alpha bug model", it is assumed that certain bacterial species might induce alterations in the mucosa that can support carcinogenesis such as epithelial cell hyper-proliferation and inflammation [40]. Moreover, these changes may also lead to the reduction of health-promoting bacteria. *Fusobacterium* can be considered as a plausible alpha bug in oral carcinogenesis, as it has been shown to initiate secondary colonization and induce cellular proliferation in carcinoma cells in vitro [27]. The "driver-passenger model" for colorectal cancer hypothesizes that the putative pathogenic microorganisms grow easily in the gut of those people prone to develop cancer, and exert their effect via a considerable reduction in the fraction of health-promoting microorganisms [44]. These pathogenic bacteria can be considered as "bacterial drivers", driving out the health-associated organisms, and inducing inflammation in the gut which can promote gene mutations within colonic epithelia which can lead to its proliferation. Inflammation increases intestinal permeability, modifying the microenvironment so as to allow substitution of the driver bacteria by opportunistic bacteria which, although considered as "bacterial passengers", may aid tumorigenesis as well [44-46]. The literature highlights that there is an insurgence of bacteria long regarded as periodontal pathogens in oral cancer samples, which can also act as bacterial "drivers". However, a "snapshot" of the community composition of tumor-associated microbiome captured in cross-sectional evaluations may highlight "passenger" bacteria which are present at that time and may mask the true drivers of carcinogenesis [46]. In other words, a temporal change in the oral microbiome might be involved in progression of cancer, and such perturbations may not be visible when samples are analyzed at a single time point.

Results from microbiome data will vary depending on the molecular methods adopted. Nine out of the fifteen studies included in this review have analyzed the microbiome after amplification of 16SrRNA genes. Amplification of 16S gene regions, which are significant for structural configuration of ribosome structure and function, using universal primers, can help to identify bacterial taxa [47]. Nevertheless, a significant limitation of this technique is that precision of the classification is dependent on the variable regions chosen to be amplified [48]. Moreover, some

bacterial species have multiple copies of 16S rRNA genes, which may lead to their artificial over-representation [48]. Some early studies used Topo TA cloning and Sanger sequencing, which is labor-intensive compared to the recent methods. The small quantity of clones (90-150) that are usually picked and sequenced in this way is unlikely to represent adequately the genetic makeup of the amplified material in a patient sample as compared to NGS (which accounts for 10000-50000 reads on average) [48]: conclusions thus drawn may bias by underestimation of important organisms [49, 50]. Newer whole metagenomic sequencing approaches, which allow investigating the entire gene content of a microbial population, have been utilized to study other types of cancer-associated microbiome [10, 51]: however, to our knowledge, there are no similar studies yet on oral cancer. An advantage of metagenomic sequencing over 16SrRNA-based sequencing is that we can understand the functional implications of the dysbiosis, which may be invaluable in identifying the role of the microbiome in carcinogenesis.

Microbiome studies in general including studies that have investigated the "bacteriome" of oral/oropharyngeal cancers face an ardent task of eliminating compositional and phylogenetic variations as a result of different variables like geographic location and factors that affect the lifestyle of an individual such as diet, habits and socioeconomic status. Bacteria commonly found in the intestines of swine and fowls have been reported to be present in oral cancer specimens from Taiwan. Constant exposure of the oral cavity to external environment and distinct dietary habits in Taiwan could have contributed to the presence of bacteria like *Cloacibacillus*, *Gemmiger*, *Oscillospira*, and *Roseburia* in samples procured from the oral cavity [52, 53]. Further, the published data on the oral and oropharyngeal cancer microbiome is, so far, limited to few geographic locations currently (Table 1), with sparse representation elsewhere in the globe, which highlights the need for more studies with a wider geographical coverage. A subject's recent use of an antibiotic is a common exclusion criterion in microbiome studies: antibiotics can influence the oral microbiome for as long as 3-36 weeks [54, 55] depending on the type of antibiotic and the personalized response of an individual to a particular antibiotic [57]. However, exclusion of patients with recent antibiotic use has not been considered in a number of studies.

Sample collection is a challenging yet important aspect of microbiome research which might critically influence the data generated [58]. Data generated from archival frozen specimens may

differ from DNA retrieved from fresh or paraffin-embedded tissue: all risk contamination in the clinic or laboratory.

Methodologic variations in the laboratory workflow can influence the microbiome composition. Chemical and mechanical methods for sample homogenization have been shown to break bacterial cell walls and consequently improve species representation [59]. It is noteworthy that only three of the included studies have reported the use of enzymatic lysis or bead beating [26, 30, 31] even though they are the recommended approaches of sample treatment for microbiome studies. One study included in the current review has reported the use of reagent controls to confirm the absence of contaminating bacteria which can influence data interpretation [26]. This is surprising, considering that the use of controls in order to identify contaminant taxa from reagents is highly recommended in microbial studies [60, 31, 62]. Biological variability prevails in all ecological communities and human microbiome can show evident inherent biological diversity. So it is imperative for the microbiome studies to be powered statistically in order to estimate the magnitude of microbial divergence between health and disease [57]. Nevertheless sample size estimation was not detailed in any of the studies included in the review. To summarize, inconsistencies and non-uniformity in data reporting and methodological approaches can be observed in oral microbiome studies.

A key undesirable outcome of all the aforementioned inconsistencies and variations in oral/oropharyngeal cancer microbiome studies is the consequent inability to perform a meta-analysis. Even though the raw data from some of the studies were publicly deposited and obtainable to everyone, a meta-analysis of datasets with clone libraries and next generation sequencing datasets could not be performed because of the differences in scale and magnitude of sequence counts and the laboratory procedures, which are fundamentally different [60]. Collective datasets generated with uniform methodology can bring us closer to deciphering microbial signatures specific to oral/oropharyngeal cancers. All these caveats highlight the emerging need for a standardized guidelines for analyzing and reporting microbiome data [63].

CONCLUSION

To our knowledge, this is the first systematic and comprehensive review that summarizes relevant epidemiological studies regarding microbial dysbiosis in association with oral/oropharyngeal cancer, and discusses discrepancies among them. Preliminary microbiome studies on oral cancer have demonstrated alteration in bacteriome which include pathogens of known significance. This could point towards the role of bacteriome associated inflammatory changes as a contributory event in oral cancer. Equally, bacterial changes could also be a reflection of the response to the changes in oral environment due to malignancy. Nevertheless identification of specific microbial changes is a positive stride towards developing salivary based microbial biomarkers for the clinical assessment of oral cancer progression. We highlight issues which are needed to be considered for future research. Despite accumulating evidence on the metagenomic characterization of the microbiome in several human cancers, few studies have so far used NGS in the study of cancers of oral cavity. Ethnicity, dietary patterns, behavioral risk factors, and timing of collection, have rarely been considered in any of these studies. Anatomical site and sub-site of the neoplasm, sample site within the tumor, physical size of samples, and sample handling, especially the need to avoid contamination with saliva, all need to be harmonized. Comparisons of healthy tissues within the same patient have value, but data from comparable anatomical sites from healthy, matched control patients are essential as well. Determination of adequate sample sizes in the statistical sense, standardized bioinformatic pipelines and analytic approaches are desirable to allow comparisons between studies. Ideally, metagenomic studies should also be designed to explore changes over time, in the total microbiome associated with oral potentially malignant disorders with high risk of malignant transformation. And finally an integrated approach with microbiomics, transcriptomics and proteomics/metabolomics will allow robust understanding of microbial role in carcinogenesis. These are demanding criteria, which will take many years to fulfill. We hope this review will encourage future collaborations towards meeting them.

Conflict of Interest
None declared

REFERENCES

1. Siegel RL,Miller KD,Jemal A.Cancer statistics,CA Cancer J Clin. 2018;68(1):7-30
2. Pollaers K, Kujan O, Johnson NW, Farah CS. Oral and oropharyngeal cancer in Oceania:Incidence, mortality, trends and gaps in public databases as presented to the Global Oral Cancer Forum. Translational Research in Oral Oncology. 2017;2:2057178X17726455.
3. Johnson NW. The Biology and Pathology of Squamous Cell Carcinomata in the Head and Neck.In:Warnakulasuriya S, Khan Z,editors.Squamous Cell Carcinoma: Molecular Therapeutic Targets, Springer; 2017 p. 1-35 ISBN 978-94-024-1083-9 DOI 10.1007/978-94-024-1084-6.
4. Althani AA, Marei HE, Hamdi WS, Nasrallah GK, El Zowalaty ME, Al Khodor S, et al. Human Microbiome and its Association With Health and Diseases. Journal of cellular physiology. 2016;231(8):1688-94.
5. Meurman JH, Uittamo J. Oral micro-organisms in the etiology of cancer. Acta odontologica Scandinavica. 2008;66(6):321-6.
6. Vogtmann E, Hua X, Zeller G, Sunagawa S, Voigt AY, Hercog R, et al. Colorectal Cancer and the Human Gut Microbiome: Reproducibility with Whole-Genome Shotgun Sequencing. PloS one. 2016;11(5):e0155362.
7. Wroblewski LE, Peek RM, Jr., Wilson KT. Helicobacter pylori and gastric cancer: factors that modulate disease risk. Clinical microbiology reviews. 2010;23(4):713-39.
8. Perera M, Al-Hebshi NN, Speicher DJ, Perera I, Johnson NW. Emerging role of bacteria in oral carcinogenesis: a review with special reference to perio-pathogenic bacteria. Journal of oral microbiology. 2016;8:32762.
9. D'Argenio V, Salvatore F. The role of the gut microbiome in the healthy adult status. Clinica chimica acta; international journal of clinical chemistry. 2015;451(Pt A):97-102.
10. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC, Yu WH, et al. The human oral microbiome. Journal of bacteriology. 2010;192(19):5002-17.

11. Khan S, Zakariah M, Rolfo C, Robrecht L, Palaniappan S.Prediction of mycoplasma hominis proteins targeting in mitochondria and cytoplasm of host cells and their implication in prostate cancer etiology.Oncotarget 2017;8(19):30830-43
12. Zakariah M, Khan S, Chaudhary AA, Rolfo C, Ben Ismail MM, Alotaibi YA. To Decipher the *Mycoplasma hominis* Proteins Targeting into the Endoplasmic Reticulum and Their Implications in Prostrate Cancer Etiology Using Next-Generation Sequencing Data.Molecules.2018;23(5):E994
13. Alshamsana A, Khan S, Imran A, Aljuffali IA, Alsaleh A. Predication of Chlamydia pneumonia protein localisation in host mitochondria an cytoplasm and possible involvements
14. Petersen C, Round JL. Defining dysbiosis and its influence on host immunity and disease. Cellular microbiology. 2014;16(7):1024-33.
15. Moher D, Liberati A, Tetzlaff J, Altman DG, The PRISMA Group.Preferred Reporting Items for Systematic Reviews and Meta-Analyses:The PRISMA Statement. PLoS Medicine.2009;6(7): e1000097.
16. Bjerre RD, Bandier J, Skov L, Engstrand L, Johansen JD. The role of the skin microbiome in atopic dermatitis: a systematic review. The British journal of dermatology. 2017;177(5):1272-8.
17. Guerrero-Preston R, Godoy-Vitorino F, Jedlicka A, Rodriguez-Hilario A, Gonzalez H, Bondy J, et al. 16S rRNA amplicon sequencing identifies microbiota associated with oral cancer, human papilloma virus infection and surgical treatment. Oncotarget. 2016;7(32):51320-34.
18. Pushalkar S, Mane SP, Ji X, Li Y, Evans C, Crasta OR, et al. Microbial diversity in saliva of oral squamous cell carcinoma. FEMS immunology and medical microbiology. 2011;61(3):269-77.
19. Lee WH, Chen HM, Yang SF, Liang C, Peng CY, Lin FM, et al. Bacterial alterations in salivary microbiota and their association in oral cancer. Scientific reports. 2017;7(1):16540.
20. Wolf A, Moissl-Eichinger C, Perras A, Koskinen K, Tomazic PV, Thurnher D. The salivary microbiome as an indicator of carcinogenesis in patients with oropharyngeal squamous cell carcinoma: A pilot study. Scientific reports. 2017;7(1):5867.

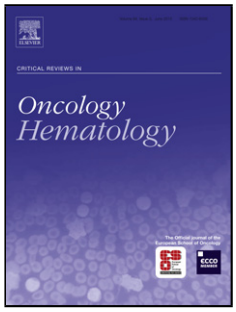
21. Schmidt BL, Kuczynski J, Bhattacharya A, Huey B, Corby PM, Queiroz EL, et al. Changes in abundance of oral microbiota associated with oral cancer. PloS one. 2014;9(6):e98741.
22. Zhao H, Chu M, Huang Z, Yang X, Ran S, Hu B, et al. Variations in oral microbiota associated with oral cancer. Scientific reports. 2017;7(1):11773.
23. Bebek G, Bennett KL, Funchain P, Campbell R, Seth R, Scharpf J, et al. Microbiomic subprofiles and MDR1 promoter methylation in head and neck squamous cell carcinoma. Human molecular genetics. 2012;21(7):1557-65.
24. Hooper SJ, Crean SJ, Fardy MJ, Lewis MA, Spratt DA, Wade WG, et al. A molecular analysis of the bacteria present within oral squamous cell carcinoma. Journal of medical microbiology. 2007;56(Pt 12):1651-9.
25. Pushalkar S, Ji X, Li Y, Estilo C, Yegnanarayana R, Singh B, et al. Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. BMC microbiology. 2012;12:144.
26. Wang H, Funchain P, Bebek G, Altemus J, Zhang H, Niazi F, et al. Microbiomic differences in tumor and paired-normal tissue in head and neck squamous cell carcinomas. Genome medicine. 2017;9(1):14.
27. Al-Hebshi NN, Nasher AT, Maryoud MY, Homeida HE, Chen T, Idris AM, et al. Inflammatory bacteriome featuring Fusobacterium nucleatum and Pseudomonas aeruginosa identified in association with oral squamous cell carcinoma. Scientific reports. 2017;7(1):1834.
28. Banerjee S, Tian T, Wei Z, Peck KN, Shih N, Chalian AA, et al. Microbial Signatures Associated with Oropharyngeal and Oral Squamous Cell Carcinomas. Scientific reports. 2017;7(1):4036.
29. Perera M, Al-Hebshi NN, Perera I, Ipe D, Ulett GC, Speicher DJ, et al. Inflammatory Bacteriome and Oral Squamous Cell Carcinoma. Journal of dental research. 2018;97(6):725-32.
30. Mukherjee PK, Wang H, Retuerto M, Zhang H, Burkey B, Ghannoum MA et al. Bacteriome and mycobiome associations in oral tongue cancer. Oncotarget 2017;8: 97273-97289

31. Bornigen D, Ren B, Pickard R, Li J, Ozer E, Hartmann EM, et al. Alterations in oral bacterial communities are associated with risk factors for oral and oropharyngeal cancer. Scientific reports. 2017;7(1):17686.
32. Schmidt TS, Matias Rodrigues JF, von Mering C. Ecological consistency of SSU rRNA-based operational taxonomic units at a global scale. PLoS computational biology. 2014;10(4):e1003594.
33. Li K, Bihan M, Yooseph S, Methe BA. Analyses of the microbial diversity across the human microbiome. PloS one. 2012;7(6):e32118.
34. Hozumi M, Ogawa M, Sugimura T, Takeuchi T, Umezawa H. Inhibition of tumorigenesis in mouse skin by leupeptin, a protease inhibitor from Actinomycetes. Cancer research. 1972;32(8):1725-8.
35. Schulze A, Harris AL. How cancer metabolism is tuned for proliferation and vulnerable to disruption. Nature. 2012;491(7424):364-73.
36. Bradshaw DJ, Marsh PD, Watson GK, Allison C. Role of Fusobacterium nucleatum and coaggregation in anaerobe survival in planktonic and biofilm oral microbial communities during aeration. Infection and immunity. 1998;66(10):4729-32.
37. Binder Gallimidi A, Fischman S, Revach B, Bulvik R, Maliutina A, Rubinstein AM, et al. Periodontal pathogens Porphyromonas gingivalis and Fusobacterium nucleatum promote tumor progression in an oral-specific chemical carcinogenesis model. Oncotarget. 2015;6(26):22613-23.
38. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. Fusobacterium nucleatum potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. Cell host & microbe. 2013;14(2):207-15.
39. Nakatsu G, Li X, Zhou H, Sheng J, Wong SH, Wu WK, et al. Gut mucosal microbiome across stages of colorectal carcinogenesis. Nature communications. 2015;6:8727.
40. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. Fusobacterium nucleatum promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. Cell host & microbe. 2013;14(2):195-206.
41. Chen W, Liu F, Ling Z, Tong X, Xiang C. Human intestinal lumen and mucosa-associated microbiota in patients with colorectal cancer. PloS one. 2012;7(6):e39743.

42. Sears CL, Pardoll DM. Perspective: alpha-bugs, their microbial partners, and the link to colon cancer. The Journal of infectious diseases. 2011;203(3):306-11.
43. So WK, Law BM, Law PT, Chan CW, Chair SY. Current Hypothesis for the Relationship between Dietary Rice Bran Intake, the Intestinal Microbiota and Colorectal Cancer Prevention. Nutrients. 2016;8(9).
44. Tjalsma H, Boleij A, Marchesi JR, Dutilh BE. A bacterial driver-passenger model for colorectal cancer: beyond the usual suspects. Nature reviews Microbiology. 2012;10(8):575-82.
45. Yang L, Chaudhary N, Baghdadi J, Pei Z. Microbiome in reflux disorders and esophageal adenocarcinoma. Cancer journal (Sudbury, Mass). 2014;20(3):207-10.
46. Wang J, Jia H. Metagenome-wide association studies: fine-mining the microbiome. Nature reviews Microbiology. 2016;14(8):508-22.
47. Isenbarger TA, Carr CE, Johnson SS, Finney M, Church GM, Gilbert W, et al. The most conserved genome segments for life detection on Earth and other planets. Origins of life and evolution of the biosphere : the journal of the International Society for the Study of the Origin of Life. 2008;38(6):517-33.
48. Liang B, Luo M, Scott-Herridge J, Semeniuk C, Mendoza M, Capina R, et al. A comparison of parallel pyrosequencing and sanger clone-based sequencing and its impact on the characterization of the genetic diversity of HIV-1. PloS one. 2011;6(10):e26745.
49. Palatinszky M, Nikolausz M, Svab D, Marialigeti K. Preferential ligation during TA-cloning of multitemplate PCR products--a factor causing bias in microbial community structure analysis. Journal of microbiological methods. 2011;85(2):131-6.
50. Schulze-Schweifing K, Banerjee A, Wade WG. Comparison of bacterial culture and 16S rRNA community profiling by clonal analysis and pyrosequencing for the characterization of the dentine caries-associated microbiome. Frontiers in cellular and infection microbiology. 2014;4:164.
51. Zhang C, Cleveland K, Schnoll-Sussman F, McClure B, Bigg M, Thakkar P, et al. Identification of low abundance microbiome in clinical samples using whole genome sequencing. Genome biology. 2015;16:265.
52. Ding J, Dai R, Yang L, He C, Xu K, Liu S, et al. Inheritance and Establishment of Gut Microbiota in Chickens. Frontiers in microbiology. 2017;8:1967.

53. Looft T, Levine UY, Stanton TB. Cloacibacillus porcorum sp. nov., a mucin-degrading bacterium from the swine intestinal tract and emended description of the genus Cloacibacillus. International journal of systematic and evolutionary microbiology. 2013;63(Pt 6):1960-6.
54. Lazarevic V, Manzano S, Gaia N, Girard M, Whiteson K, Hibbs J, et al. Effects of amoxicillin treatment on the salivary microbiota in children with acute otitis media. Clinical microbiology and infection : the official publication of the European Society of Clinical Microbiology and Infectious Diseases. 2013;19(8):E335-42
55. Abeles SR, Jones MB, Santiago-Rodriguez TM, Ly M, Klitgord N, Yooseph S, et al. Microbial diversity in individuals and their household contacts following typical antibiotic courses. Microbiome. 2016;4(1):39
56. Sinha R, Chen J, Amir A, Vogtmann E, Shi J, Inman KS, et al. Collecting Fecal Samples for Microbiome Analyses in Epidemiology Studies. Cancer epidemiology, biomarkers & prevention : a publication of the American Association for Cancer Research, cosponsored by the American Society of Preventive Oncology. 2016;25(2):407-16.
57. Kim D, Hofstaedter CE, Zhao C, Mattei L, Tanes C, Clarke E, et al. Optimizing methods and dodging pitfalls in microbiome research. Microbiome. 2017;5(1):52.
58. Dethlefsen L, Huse S, Sogin ML, Relman DA. The pervasive effects of an antibiotic on the human gut microbiota, as revealed by deep 16S rRNA sequencing. PLoS biology. 2008;6(11):e280.
59. Gill C, van de Wijgert JH, Blow F, Darby AC. Evaluation of Lysis Methods for the Extraction of Bacterial DNA for Analysis of the Vaginal Microbiota. PloS one. 2016;11(9):e0163148.
60. Adams RI, Bateman AC, Bik HM, Meadow JF. Microbiota of the indoor environment: a meta-analysis. Microbiome. 2015;3:49.
61. Adams RI, Miletto M, Lindow SE, Taylor JW, Bruns TD. Airborne bacterial communities in residences: similarities and differences with fungi. PloS one. 2014;9(3):e91283.
62. Salter SJ, Cox MJ, Turek EM, Calus ST, Cookson WO, Moffatt MF, et al. Reagent and laboratory contamination can critically impact sequence-based microbiome analyses. BMC biology. 2014;12:87.

63. Menon RK, Gopinath D. Eliminating bias and accelerating the clinical translation of oral microbiome research in oral oncology. Oral oncology. 2018;79:84-5.



Title: Culture-independent studies on bacterial dysbiosis in oral and oropharyngeal squamous cell carcinoma: A systematic review

Authors: Divya Gopinath, Rohit Kunnath Menon, Moinak Banerjee, Richard Su Yuxiong, Michael George Botelho, Newell W Johnson

PII: S1040-8428(18)30324-X
DOI: https://doi.org/10.1016/j.critrevonc.2019.04.018
Reference: ONCH 2738

To appear in: *Critical Reviews in Oncology/Hematology*

Received date: 10 July 2018
Revised date: 1 January 2019
Accepted date: 16 April 2019

Please cite this article as: Gopinath D, Menon RK, Banerjee M, Su Yuxiong R, Botelho MG, Johnson NW, Culture-independent studies on bacterial dysbiosis in oral and oropharyngeal squamous cell carcinoma: A systematic review, *Critical Reviews in Oncology / Hematology* (2019), https://doi.org/10.1016/j.critrevonc.2019.04.018