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REVIEW ARTICLE

Oral Microbiota Associated with Oral and Gastroenteric Cancer

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Abstract: When the normal microbiota-host interactions are altered, the commensal microbial community evolves to a dysbiotic status resulting in some species becoming pathogenic and acting synergistically in the development of local and systemic diseases, including cancer. Advances in genetics, immunology and microbiology during the last years have made it possible to gather information on the oral and gastrointestinal microbiome and its interaction with the host, which has led to a better understanding of the interrelationship between microbiota and cancer. There is growing evidence in support for the role of some species in the development, progression and responses to treatment of various types of cancer. Accordingly, the number of studies investigating the association between oral microbiota and oral and gastrointestinal cancers has increased significantly during the last years. Here, we review the literature documenting associations of oral microbiota with oral and gastroenteric cancers.

Keywords: Oral, Gastroenteric, Microbiota, Cancer, Pathogenic, *Helicobacter pylori*.

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1. INTRODUCTION

To date, *Helicobacter pylori* is the only bacterial species demonstrated to be a causative agent of cancer. It is involved in the etiology of gastric carcinomas and gastric lymphomas originating in mucosa-associated lymphoid tissue, and is the most important infectious cause of cancer in countries with a high human development index [1]. Yet, it has become apparent that some bacteria commonly found among the human oral and gastrointestinal microbiota may have the tumor-promoting capacity. Thus, an association with cancer has been shown for other bacteria, such as *Chlamydia trachomatis* with cervical squamous cell carcinomas [2, 3] and ovarian cancer [4] and *Fusobacterium nucleatum, Bacteroides fragilis, Streptococcus gallolyticus, Enterococcus faecalis, and Streptococcus bovis* with Colorectal carcinoma (CRC) [5, 6]. In support of this, epidemiological studies have established a clear relationship between some bacterial species that normally inhabit the oral cavity, such as *Streptococcus sp.*, *Prevotella melaninogenica*, *Porphyromonas gingivalis*, and *Capnocytophaga gingivalis* and oral squamous cell carcinoma (OSCC) as well as CRC and pancreatic cancers [7 - 11].

In the healthy subject, the oral cavity is colonized by complex bacterial, fungal and viral communities that coexist with the host in a balanced equilibrium [12]. When this balance is disrupted, some species promote a dysbiotic community and become opportunistically pathogenic, generating periodontal inflammation and, eventually, OSCC [13]. Outside the oral cavity, an association between *P. gingivalis* and pancreatic cancer was shown in a prospective study of 405 cases and 416 control subjects [14]. Individuals with high levels of antibodies against *P. gingivalis* (ATTC 53978) had a twofold higher risk of pancreatic cancer than individuals with lower levels of these antibodies. In other studies, *F. nucleatum* was one of the most abundant species within and around CRC tumors, and its levels correlated with the presence of lymph node metastases [8, 15, 16].

The fact that epidemiological studies show an association of oral bacteria with certain types of cancer does not require a causal relationship. Environmental and host factors can induce changes in the oral microbiota, which can cause damage in underlying tissues and even systemic spreading of bacteria. It is also conceivable that oral and gastrointestinal precancerous and cancer lesions can cause a dysbiosis that might support tumor growth. Nevertheless, a causal role of oral bacteria in the development of cancer has not been fully established yet and the precise mechanistic implication of specific microorganisms of the oral microbiota in the etiology of cancer remains to be demonstrated at the molecular level. Several mechanisms have been claimed to support a role of bacteria in cancer:

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Interference with signaling pathways and activation of transcription factors such as NF-κB, STAT3 and NFAT; suppression of apoptosis; release of metabolic carcinogens such as N-nitroso compounds and acetaldehyde; bacterial toxins that stimulate secretion of proinflammatory factors such as IL-18 and TNF-α; and immune-disruptive effects that chronic inflammation promoted by some bacteria may have on the tumor microenvironment [13].

In a mouse CRC model carrying a heterozygous mutation in the adenomatous polyposis coli gene ($APC^{Min/+}$) [17], it was shown that the Toll-like receptor (TLR)-adaptor MyD88 plays an essential role in tumor development suggesting that innate immune signaling pathways may be involved in the process of carcinogenesis. The inhibitory effect of MyD88 deficiency on tumor growth was due to a defective TLR signaling rather than an altered MyD88-dependent IL-1 and IL-18 signaling [18]. Moreover, it was found that altering the microbiota of $APC^{Min/+}$MSH-/- mice by germ-free rederivation (axenic mice) or antibiotic treatment prevents tumor development and tumor growth [19, 20]. It was shown that IL-23 produced by tumor-associated myeloid cells facilitates bacterial infiltration of the tumor, but not the adjacent tissue, and that infection, in turn, promotes tumor growth by a MyD88-dependent activation of STAT3 and NFAT accompanied by a tumor IL-17 response [21 - 23]. Altogether, these data suggest that bacteria provide tumor-stimulating signals, most likely through TLRs, which lead to the activation of transcription factors with anti-apoptotic and cell proliferation effects.

In the recent years, a significant number of studies have demonstrated changes in the composition of the microbiota in the digestive tract of cancer patients as compared with healthy subjects. The gram-negative, anaerobic bacteria *F. nucleatum* and *P. gingivalis* are the best-characterized regarding proinflammatory and possible oncogenic potential. Both bacteria enter human epithelial and endothelial cells and establish persistent intracellular infections, which can spread beyond the oral cavity [24]. In CRC, it has been demonstrated that *F. nucleatum* activates the β-catenin signaling pathway upon binding of the fusobacterial adhesion factor FadA to the endothelial cadherin (CDH5) leading to enhanced transcriptional activity of Wnt signaling genes, myc, cyclin D1 and NF-κB [25] and subsequently to the secretion of proinflammatory cytokines, such as IL-6, IL-8, IL-18 and TNF-α. Another fusobacterial protein, Fap2, binds to the inhibitory receptor TIGIT on human T and natural killer (NK) cells to block their cytotoxic activity over tumor cells [26, 27]. Additionally, *F. nucleatum* activates p38 resulting in enhanced secretion of the metalloproteinases MMP-9 and MMP-13, which are involved in tumor invasion and metastasis. Moreover it reduces the density of CD3 T cells in CRC tumors [27]. *P. gingivalis* can also induce inflammation and alter the normal immune status in the oral cavity. In infected epithelial and OSCC tumor cells, *P. gingivalis* can induce the expression of programmed death-ligand 1 (PD-L1), which upon binding to its receptor PD-1 on T cells inhibits T cell receptor (TCR)-mediated activation. This effect is mediated by the membrane fraction of *P. gingivalis*, rather than by other virulence factors such as lipopolysaccharide (LPS) [28]. One such factor, the fimbrial adhesion FimA, seems to promote epithelial cell proliferation by inducing cyclin-dependent kinase (CDK) activity and reducing the level of p53 [13]. Other bacteria with tumor-promoting capacity in the context of inflammation are the genotoxic colibactin-producing *E. coli* in colitis-associated carcinomas, and enterotoxin-producing *Bacteroides fragilis* and *Streptococcus spp*.

Metabolites and toxins produced by bacteria can have direct effects on tumor cells, as in the case of several bacterial toxins that have been associated with CRC [29]. For instance, anaerobic gut bacteria of the genus *Clostridium* are responsible for the 7a-dehydroxylation of primary bile acids resulting in the production of deoxycholic acid, which is considered a co-carcinogen that might be involved in colon and liver carcinogenesis [30, 31].

A contrasting aspect of the relationship of the microbiota with cancer is its capability to influence anti-tumor immune responses [32]. There is increasing evidence showing that dysbiosis induced by antibiotic medication correlates with increased frequency of some cancers. A large epidemiological study (125,441 patients and 490,510 matched controls) showed that the incidence of lung cancer increases upon repeated treatment with penicillin, cephalosporins or macrolides, and that prostate and bladder cancers increase in penicillin-treated patients [33]. Accordingly, treatment with metronidazole and ciprofloxacin of proto-neu transgenic mice enhances the growth of the mammary carcinomas that these mice develop [34]. A study comparing the growth of tumors (B16.SIY melanoma cells injected subcutaneously) and their infiltration by IFN-γ-producing cytotoxic T lymphocytes (CTLs) in mice harboring different microbiota showed that mice with higher tumor-specific CTL responses and slower tumor growth had a commensal microbiota with higher levels of *Bifidobacterium spp* [35]. Oral administration of *Bifidobacterium* improved anti-tumor immunity and when combined with anti-PD-L antibody therapy (checkpoint blockade), the tumor outgrowth was abolished. Another study in mice showed that disruption of the commensal microbiota interfered with the response of subcutaneous tumors to immunotherapy with CpG, a ligand of toll-like receptor 9 (TLR9), and oxaliplatin chemotherapy [36]. In addition, the presence of *Lactobacillus* species (*L. fermentum*) in the gut of these mice correlated with decreased response to tumor necrosis factor (TNF), while other bacterial species (*e.g.*, *Alistipes shahii*) favored hemorrhagic necrosis of tumors by TNF secreted by tumor-associated myeloid cells followed by CD8 T cell response [36]. A similar effect of the microbiota was found on tumor-bearing mice treated with non-myeloablative doses of cyclophosphamide [37]. In this case, the microbiota promoted an adaptive immune response against the tumors generating an increased frequency of a subset of T helper 17 (T_H17) cells and memory T_H1 cells that required the expression of MyD88. These responses were inhibited in mice treated with antibiotics. Further evidence in support of the role of the commensal microbiota in stimulating anti-tumor immunity come from studies on mice suggesting that bacterial metabolites such as butyrate have immunomodulatory effects [38]. Bacterial metabolites can have indirect effects on tumors by interfering with immunosurveillance, as it has been suggested for acetate, propionate and butyrate, which promote regulatory T cell (Treg) functions that prevent inflammation [39]. Although this may seem contradictory with an anti-tumor effect, some studies have shown that increased butyrogenesis

correlate with lower CRC risk [40]. Lastly, an interesting hypothesis to explain the interference of certain commensal bacteria with cancer progression is the possibility of cross-reactivity between bacterial and host tumor-associated antigens. Thus, under certain circumstances, commensal bacteria could prime T cells to recognize epitopes of self-antigens presented on the surface of tumor cells [32].

In contrast to the increasing number of reports on the oral bacterial microbiota, a limited number of studies have analyzed the fungal microbiota of the oral cavity using high throughput sequencing. A study using multitag pyrosequencing to identify the fungi in the oral cavity of 20 healthy subjects revealed a total of 101 species belonging to 74 culturable and 11 non-culturable genera, of which the most frequent were *Candida* species followed by *Saccharomycetales, Aspergillus, Fusarium and Cryptococcus* [41]. A more recent study on the fungal microbiome using for sequencing the fungal internal transcribed spacer (ITS) in oral wash samples of patients with periodontal disease compared with healthy subjects revealed 154 species and 81 genera across all samples [42]. The genera *Candida* and *Aspergillus* were the most abundant. The genus *Candida*, previously associated with periodontal disease in culture-base studies, showed a higher median relative abundance in patients with periodontal disease as compared to healthy subjects, although the difference was not significant. A study characterizing the oral fungi in HIV-infected patients revealed an inverse correlation between *Candida* and *Campylobacter*, while there was no correlation in healthy subjects [43]. This study also revealed that, in healthy subjects, an increase in the relative abundance of *Candida* was accompanied by a decrease in the genus *Picchia*, suggestive of an antagonistic correlation. In another study analyzing the bacteriome and mycobiome in tumor tissue of patients with squamous cell carcinoma of the tongue, the abundance of the fungal genus *Aspergillus* correlated negatively with some bacterial genera (*Actinomyces, Prevotella, Streptococcus*) [44]. An interesting case is that of *Malassezia* species, previously described as commensals and pathogens of skin and lungs [45], have been found to be abundant in saliva [46], and associated with pancreas ductal adenocarcinoma [47].

In summary, accumulating data on microbiome genomics, transcriptomics, proteomics and metabolomics is providing increasing evidence supporting different roles of commensal microbiota in cancer promotion, as well as its progression or regression, depending on its specific composition and on the infectivity and prevalence of the species that it contains. The oral microbiota is composed of more than 700 species or phylotypes and over 1000 different bacterial species [48]. Nowadays, the variable regions of the 16S rRNA of bacteria are usually sequenced to identify genera and species. The prokaryotic 16S rRNA is about 1500 bp and is made of conserved sequences intercalated with nine variable segments [49]. Nevertheless, there is significant subject-to-subject variation in the frequencies of the different bacterial species [50], which are determined by environmental, dietary and lifestyle factors [51] and conditioned by the health status, most importantly of the immune system [52, 53], the age and the anti-tumor therapy being applied [54, 55].

The advancement of genetics, immunology and microbiology during the last years has led to a better understanding of the relationship between microbiota and cancer. The number of studies investigating the association between oral microbiome and gastrointestinal cancers has increased significantly during the last years. Different types of cancers presented both in the upper and lower gastrointestinal tract have been the focus of these studies. In this review, we have explored the literature to provide an in-depth update of data documenting changes in the commensal oral microbiota of cancer patients, as well as healthy controls, which might allow establishing a correlation with oropharyngeal, esophageal, pancreatic, gastric, and colorectal cancers. Consistently, the phyla Actinobacteria, Bacteroidetes, Firmicutes, Fusobacteria and Proteobacteria are the most enriched in cancer and control groups of the reviewed studies.

2. OROPHARYNGEAL CANCERS

It has been well documented that the bacterial composition of the oral microbiota undergoes substantial changes in patients with oropharyngeal cancers as compared with healthy controls. The relative abundance of bacterial species, determined by 16S rRNA sequencing, is the most significant parameter in current studies.

Over the last years, a number of studies have shown that the relative abundance of selective species of bacteria increases in oropharyngeal cancers. Table **1** contains a summary of the literature reporting distinct oral microbiota profiles in association with oropharyngeal cancers. Most of these studies also described species of bacteria with higher representation in healthy controls as compared with oropharyngeal cancer patients, as shown in Table **2**. In 1998, Nagy *et al*. [56] compared cultured bacteria from OSCC tumor samples and healthy tissue samples of the same patients, finding an increased presence of *Porphyromonas, Prevotella, Streptococcus*, and *Fusobacterium* genera in OSCC tissue cultures. Accordingly, two other studies reported markedly increased abundance of *Fusobacterium* in OSCC [57] as well as in non-specified OC [58]. At the species level, *Fusobacterium nucleatum* has been frequently associated with tumor samples of OSCC [59, 60], OPMD [61], and HNSCC [62] patients. An elevated abundance of the *Streptococcus* genus in cancer samples has also been reported for OSCC [63], and OMTC [44]. Nevertheless, other studies have reported different results for OSCC [57] and non-specified OC [58] (Table **1** and Table **2**). Such discrepancies could be due to differences in the methods used or also to the different habits of the respective study populations. Moreover, an increased abundance of *Streptococcus gordonii* has been related to OSCC [64, 65], and OPMD [61]. In these studies, *Streptococcus parasanguinis* was also associated with OSCC [65] and OPMD [61]. Furthermore, two studies supported the association of *Streptococcus salivarius/vestibularis* with HNSCC [62] and OPMD tumorous samples [61]. However, in other studies on OSCC, the relative abundance of these species has been found increased in cancer samples [65], but also in healthy control samples [64]. In several studies on OSCC [59, 64 - 66] and a study on OPMD [61], *Streptococcus mitis* was predominantly associated with healthy tissue as compared with tumor tissues using metagenome sequencing. In contrast, in a previous study using DNA-DNA hybridization to analyze 40 common oral species of bacteria, Mager *et al*. [67] found *S. mitis* elevated in saliva of OSCC patients. The different methods used might account for such differences.

Table 1. Changes in oral bacteria in oropharyngeal cancer patients.

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control Sample	Reference
GSCC	Bacteroidetes	*Porphyromonas gingivalis*	Relative abundance increased in GSCC samples compared to the healthy epithelium	10 patients, and 5 controls	IHC	Biopsy paraffin-embedded blocks of GSCC	Non-neoplastic gingival tissue	Katz *et al*., 2011 [68]
HNSCC	Actinobacteria	*Rothia mucilaginosa*	Relative abundance increased in HNSCC samples compared to samples from healthy controls from JHU and HMP	17 HNSCC patients, and 25 healthy controls (JHU Cohort). Compared to 154 participants of the Human Microbiome Project (HMP)	16S rRNA next-generation sequencing	Saliva from HNSCC patients	Saliva from healthy controls	Guerrero-Preston *et al*., 2017 [62]
	Firmicutes	*Lactobacillus gasseri/johnsonii*	Relative abundance increased in samples from HNSCC patients treated with surgery and chemoradiation when compared to patients only treated with surgical removal of the tumour and to controls					
		Lactobacillus vaginalis						
		Streptococcus salivarius/ vestibularis	Relative abundance increased in HNSCC samples compared to samples from healthy controls from JHU and HMP					
	Fusobacteria	*Fusobacterium nucleatum*						
KCOT	Firmicutes	*Gemella morbillorum*	Relative abundance increased in KCOTs compared to RCs	6 KCOTs samples, and 10 RCs samples	Biochemical tests	Cyst fluid aspiration KCOTs	Cyst fluid aspiration RC	Scalas *et al*., 2013 [71]
OC	Bacteroidetes	*Prevotella melaninogenica*	Relative abundance significantly increased in cancer patient samples compared to healthy matching tissue	10 cancer patients, and 8 pre-cancer patients	16S rRNA pyrosequencing	Tumour sample from cancer and pre-cancer patients	Contralateral healthy tissue from the same patient	Schmidt *et al*., 2014 [58]
	Fusobacteria	*Fusobacterium*						

(Table 1) contd.....

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control Sample	Reference
OMTC	Actinobacteria	*Rothia mucilaginosa*	Relative abundance significantly increased in the tumour group compared to their matching non-tumour samples	37 patients	16S rRNA sequencing	Tumour sample	Matching healthy tissue from the same patient	Mukherjee *et al*., 2017 [44]
	Firmicutes	*Streptococcus*						
OPMD	Actinobacteria	*Rothia mucilaginosa*	Presence found in OPMD lesions but not presented in healthy tissue	7 patients	Metagenomic sequencing	Tumour sample	Healthy tissue from the same patient	Decsi *et al*., 2018 [61]
	Bacteroidetes	*Capnocytophaga gingivalis*						
		Capnocytophaga ochracea						
		Prevotella melaninogenica						
	Firmicutes	*Gemella morbillorum*						
		Granulicatella adiacens						
		Streptococcus gordonii						
		Streptococcus parasanguinis						
		Streptococcus salivarius						
	Fusobacteria	*Fusobacterium nucleatum*	Relative abundance increased in OPMD lesions compared to healthy tissue					
OSCC	Bacteroidetes	*Gemella haemolysins*	Relative abundance increased in OSCC lesions compared to healthy tissue	10 patients	16S rRNA sequencing	Tumour sample	Healthy tissue from the same patient	Pushalkar *et al*., 2012 [65]
		Gemella morbillorum						
		Peptostreptococcus stomatis						
		Streptococcus gordonii						
		Streptococcus parasanguinis						
		Streptococcus salivarius						
	Bacteroidetes	*Prevotella melaninogenica*	Presence found in tumour samples but not presented in healthy tissue	20 patients	16S rRNA sequencing	Tumour sample	Healthy tissue from the same patient	Hooper *et al*., 2006 [64]
	Firmicutes	*Gemella haemolysans*						
		Streptococcus gordonii						
	Fusobacteria	*Fusobacterium nucleatum*	Relative abundance increased in OSCC samples compared to healthy tissue	20 OSCC patients, and 20 matching controls	16S rRNA sequencing	Tumour sample	Anatomical matching sites from healthy controls	Al-Hebshi *et al*., 2017 [59]

(Table 1) contd.....

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control Sample	Reference
Bacteroidetes	*Porphyromonas gingivalis*	Relative abundance increased in OSCC samples compared to healthy tissue	6 patients	16S rRNA sequencing	Tumour sample and adjacent paracancerous tissue	Healthy tissue from the same patient (subgingival plaque)	Chang *et al*., 2019 [60]	
OSCC	Fusobacteria							*Fusobacterium nucleatum*
	Bacteroidetes	*Porphyromonas*	Presence increased in tumour samples compared to healthy tissue	21 patients	Cell culturing	Tumour sample	Contiguous healthy mucosa from the same patient	Nagy *et al*., 1998 [56]
		Prevotella						
	Firmicutes	*Streptococcus*						
	Fusobacteria	*Fusobacterium*						
	Actinobacteria	*Rothia*	Relative abundance increased in OSCC samples compared to healthy samples	3 patients, and 2 controls	16S rRNA pyrosequencing	Saliva sample from cases	Saliva sample from healthy control	Pushalkar *et al*., 2011 [63]
	Bacteroidetes	*Porphyromonas*						
	Firmicutes	*Gemella*						
		Lactobacillus						
		Peptostreptococcus						
		Streptococcus						
	Firmicutes	*Peptostreptococcus*	Relative abundance increased in the cancer patient group	125 cancer patients, 124 epithelial precursor lesion patients, and 127 healthy patients	16S rRNA sequencing	Saliva from OSCC patients	Saliva from controls	Lee *et al*., 2017 [69]
	Bacteroidetes	*Capnocytophaga gingivalis*	Increased counts in OSCC samples compared to healthy samples	45 OSCC patients, and 45 matching healthy controls	Checkerboard DNA-DNA hybridization	Saliva from OSCC patients	Saliva from healthy controls	Mager *et al*., 2005 [67]
		Prevotella melaninogenica						
	Firmicutes	*Streptococcus mitis*						
	Firmicutes	*Peptostreptococcus stomatis*	Relative abundance markedly increased in OSCC samples compared to healthy matching tissue	40 patients	Metagenomic sequencing	Swabs from oral lesions	Swabs from anatomically matching healthy sites	Zhao *et al*., 2017 [57]
	Fusobacteria	*Fusobacterium*						

Table 2. Oral bacteria associated with oropharyngeal healthy controls.

Cancer understudy	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control Sample	Reference
HNSCC	Fusobacterium	*Leptotrichia buccalis*	Relative abundance decreased in HNSCC samples compared to samples from healthy controls from JHU and HMP	17 HNSCC patients, and 25 healthy controls (JHU Cohort). 154 participants of the Human Microbiome Project (HMP)	16S rRNA next-generation sequencing	Saliva from HNSCC patients	Saliva from healthy controls	Guerrero-Preston *et al*., 2017 [62]

(Table 2) contd.....

Cancer understudy	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control Sample	Reference
OC	Actinobacteria	*Rothia*	Relative abundance significantly decreased in cancer patients compared to healthy matching tissue	10 cancer patients, and 8 pre-cancer patients	16S rRNA pyrosequencing	Tumour sample from cancer and pre-cancer patients	Contralateral healthy tissue from the same patients	Schmidt *et al*., 2014 [58]
	Firmicutes	*Streptococcus*	Relative abundance significantly decreased in cancer and pre-cancer patients compared to healthy matching tissue					
OPMD	Bacteroidetes	*Porphyromonas gingivalis*	Presence not found in OPMD lesions but presented in healthy tissue	7 patients	Metagenomic sequencing	Tumour sample	Healthy tissue from the same patient	Decsi *et al*., 2018 [61]
		Prevotella bergensis						
	Firmicutes	*Gemella haemolysans*	Relative abundance markedly decreased in tumorous samples compared to healthy tissue					
		Streptococcus mitis						
	Proteobacteria	*Neisseria meningitidis*	Presence not found in OPMD lesions but presented in healthy tissue					
		Neisseria subflava						
OSCC	Firmicutes	*Granulicatella adiacens*	Relative abundance decreased in OSCC lesions compared to healthy tissue	10 patients	16S rRNA sequencing	Tumour sample	Healthy tissue from the same patient	Pushalkar *et al*., 2012 [65]
		Streptococcus mitis						
	Actinobacteria	*Rothia mucilaginosa*	Presence predominantly associated with controls	20 OSCC patients, and 20 matching controls	16S rRNA sequencing	Tumour sample	Anatomical matching sites from healthy controls	Al-Hebshi *et al*., 2017 [59]
	Firmicutes	*Streptococcus mitis*						
	Actinobacteria	*Rothia mucilaginosa*	Relative abundance decreased in tumorous samples compared to healthy tissue	20 patients	16S rRNA sequencing	Tumour sample	Healthy tissue from the same patient	Hooper *et al*., 2006 [64]
	Bacteroidetes	*Prevotella veroralis*	Presence not found in OSCC lesions but presented in healthy tissue					
	Firmicutes	*Streptococcus mitis*	Relative abundance decreased in tumorous samples compared to healthy tissue					
		Streptococcus salivarius						

(Table 2) contd.....

Cancer understudy	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control Sample	Reference
Bacteroidetes	*Porphyromonas gingivalis*	Presence not found in OSCC samples but presented in healthy tissue	10 patients	16S rRNA sequencing	Tumour sample	Non-tumorous mucosal tissue specimen from the same patient	Hooper *et al*., 2007 [66]	
OSCC	Firmicutes	*Granulicatella adiacens*						Relative abundance decreased in tumorous samples compared to healthy tissue
		Streptococcus mitis/oralis						
	Bacteroidetes	*Capnocytophaga*	Relative abundance decreased in OSCC lesions compared to healthy tissue	3 patients, and 2 controls	16S rRNA pyrosequencing	Saliva sample from cases	Saliva sample from healthy control	Pushalkar *et al*., 2011 [63]
		Prevotella						
	Fusobacteria	*Leptotrichia*						
	Proteobacteria	*Neisseria*						
	Actinobacteria	*Rothia*	Relative abundance markedly decreased in OSCC samples compared to healthy control samples	40 patients	Metagenomic sequencing	Swabs from oral lesions	Swabs from anatomically matching healthy sites	Zhao *et al*., 2017 [57]
	Firmicutes	*Granulicatella*						
		Streptococcus						

The association of the *Prevotella* genus with cancer patients or healthy control groups has to be defined further at the species level. Thus, *Prevotella melaninogenica* has been unanimously associated with cancer samples of OSCC [64, 67], OPMD [61], and non-specified OC [58]. Contradictory to Nagy *et al*. [56], Pushalkar *et al*. [63] found the presence of *Prevotella* decreased in OSCC cancer lesions. Two studies support this finding at the species level, Decsi *et al*. found *Prevotella bergensis* only in the healthy control group of an OPMD study [61], while Hooper *et al*. [64] described *Prevotella veroralis* in the control group of an OSCC study. Concerning *Porphyromonas*, an association of this genus with cancer samples has also been reported for OSCC [63]. The increased relative abundance of the species *Porphyromonas gingivalis* has been reported in tumor samples of OSCC [60] and GSCC [68] patients, yet it could be cultured neither from OSCC by Hooper *et al*. [66] nor from OPMD cancerous samples by Decsi *et al*. [61].

A reportedly varying and controversial species of *Actinobacteria* is *Rothia mucilaginosa.* A study by Pushalkar *et al*. [63] described an increased abundance of *Rothia mucilaginosa* in the saliva of OSCC patients. Furthermore, Guerrero-Preston *et al*. found the relative abundance of *Rothia mucilaginosa* increased in the saliva of HNSCC patients [62], and it has also been found in OMTC [44] and OPMD [61] tumor tissues. Yet, another study reported a markedly increased abundance of the genus *Rothia* in healthy controls [57]. This is also supported by the described association of *Rothia mucilaginosa* with OSCC controls in two other studies [59, 64].

Chronic inflammation, which often accompanies the development of OSCC, has been attributed to an imbalance in the oral microbial community (dysbiosis). In addition, the tumor tissue provides a rich microenvironment that favors bacterial survival. Pushalkar *et al*. [63] analyzed saliva samples of OSCC and control subjects by pyrosequencing of 16S rRNA (V4-V5 region) to determine the total bacterial diversity and relative abundance of bacterial species in the samples. In this way, 8 phyla of bacteria were identified: *Firmicutes* (45% of classified sequences), *Bacteroidetes* (25%), *Actinobacteria* (14%); *Proteobacteria* (10%); *Fusobacteria* (5%); SR1 (0.6%); *Spirochaetes* (0.2%). Among 52 genera detected, the most prevalent in the OSCC samples were *Streptococcus, Gemella, Rothia, Peptostreptococcus, Porphyromonas* and *Lactobacillus*. In the control group, the most prevalent genera were *Prevotella, Neisseria, Leptotrichia, Capnocytophaga, Actinobacillus,* and *Oribacterium*. The increased relative abundance of *Peptostreptococcus* was found in saliva samples OSCC patients also by Lee *et al*. [69]. The species *Peptostreptococcus stomatis* has also been reported in such samples [57, 65].

The augmented relative abundance of the genus *Lactobacillus* in the saliva of OSCC patients [63] correlates with the increased abundance reported for *Lactobacillus gasseri/johnsonii* and *Lactobacillus vaginalis* in the saliva of HNSCC patients [62]. However, this association was only reported in patients treated with surgery and chemoradiation as compared to patients treated with just surgical removal of the tumor and to healthy controls. This suggests that chemoradiation might cause the increased relative abundance of these bacteria, which is in line with the known presence of a more complex oral microbiota in cancer patients treated with chemotherapy [70]. The relative abundance of the genus *Capnocytophaga* was found decreased in saliva samples of healthy controls in an OSCC study [63]. However, the species *Capnocytophaga gingivalis* and *Capnocytophaga ochracea*

were found increased in the saliva of OSCC and OPMD patients [61, 67]. The relative abundance of *Gemella* was reported increased in saliva samples of OSCC patients, in particular, *Gemella haemolysans* and *Gemella morbillorum* [64, 65] as well as in KCOT patients [71]. In contrast, Decsi *et al*. [61] reported an increased relative abundance of *Gemella morbillorum* but a decreased presence of *Gemella haemolysans* in OPMD patients. There is no clear explanation for this discrepancy.

Lastly, the relative abundance of some oral bacteria has been predominantly associated with healthy control samples in oropharyngeal cancer studies. For instance, the relative abundance of *Leptotrichia* was found increased in saliva samples of healthy controls [63] and the species *Leptotrichia buccalis* in saliva samples of healthy controls in an HNSCC study [62]. Similarly, an increased abundance of *Neisseria* has been reported in saliva samples of healthy controls, in particular, *Neisseria meningitidis* and *Neisseria subflava* [61]. Furthermore, the relative abundance of the genus *Granulicatella* has been reported markedly increased in swabs oral lesions versus healthy control tissue in an OSCC study [57]. Interestingly, the species *Granulicatella adiacens* was found to be more prevalent in the healthy controls of two other OSCC studies [65, 66]. Nonetheless, *Granulicatella adiacens* was not found increased in healthy control tissue in an OPMD study [61].

3. ESOPHAGEAL CANCERS

On average, 0.75-1.5 liters of saliva is generated per day by an adult person and about 0.5 liters by a child. Therefore, high numbers of oral-resident bacteria, fungi and viruses are ingested daily, which, directly or indirectly, may play a role in esophageal and gastroenteric pathologies. Two recent studies have shown the relationship between oral microbiome profiles and esophageal cancer. Their results are summarized in Table **3**. Chen *et al*. [72] found an increased abundance of *Prevotella, Streptococcus*, and *Porphyromonas* genera in saliva samples of ESCC patients. In addition, Peters *et al*. [73] reported the association between increased prevalence of the species *Porphyromonas gingivalis* and a higher risk of ESCC. These findings are in line with the results of oropharyngeal cancer studies regarding these bacteria.

4. PANCREATIC CANCER

Several recent studies on bacterial profiles in pancreatic cancer (PC) patients have shown dysbiosis in the oral cavity, duodenal mucosa and feces as compared with healthy controls. A summary of the reported associations of oral microbiome profiles and pancreatic cancers is shown in Table **4**, and the bacterial associations with healthy control individuals investigated in parallel are shown in Table **5**. In an earlier study, Farrell *et al*. [74] analyzed saliva samples of 10 resectable PC patients and 10 matched controls for the presence and abundance of bacterial species by array profiling (410 oligonucleotide probes) and real-time quantitative PCR. A total of 16 species/clusters showed significant differences between PC patients and healthy controls representing six genera: *Streptococcus*, *Prevotella*, *Campylobacter*, *Granulicatella*, *Atopobium* and *Neisseria*. In particular, the levels of *N. elongata* and *S. mitis* were significantly reduced and the levels of *G. adiacens* were increased in PC patients. The levels of *G. adiacens* and *S. mitis* were significantly different between PC and chronic pancreatitis and between PC and healthy individuals. Another study used high throughput sequencing to analyze the microbiome of saliva samples of a total of 108 patients [75], of which 8 were diagnosed with PC, 78 with other diseases, including cancer, and 22 were considered healthy. The results showed a higher proportion of *Leptotrichia* and a lower proportion of *Porphyromonas* and *Neisseria* in PC patients. Interestingly, the ratio of the bacterial genera *Leptotrichia* and *Porphyromonas* was significantly higher in PC patients as compared with the group of other diseases (including cancer) and the group of healthy subjects. Olson *et al*. [76] analyzed by 16S rRNA amplification and sequencing the oral microbiota in the saliva of about 50 newly diagnosed PDAC patients, 40 patients with intraductal papillary mucinous neoplasms and nearly 60 healthy controls. PDAC cases showed higher levels than controls of *Firmicutes* and related taxa (*Bacilli, Lactobacillales, Streptococcaceae, Streptococcus*). In turn, healthy controls showed higher levels of *Proteobacteria* and related taxa (*Gammaproteobacteria, Pasteurellales, Pasteurellaceae, Haemophilus; and Betaproteobacteria, Neisseriales, Neisseriaceae, Neisseria*). These differences were statistically significant.

Table 3. Oral bacteria associated with esophageal cancers.

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control sample	Reference
ESCC	Bacteroidetes	*Porphyromonas gingivalis*	Presence associated with a higher risk of ESCC	ESCC: 25 cases, and 25 controls EAC: 81 cases, and 79 controls	16S rRNA sequencing	Pre-diagnostic oral mouthwash from patients	Pre-diagnostic oral mouthwash from matching controls	Peters *et al*., 2017 [73]
	Bacteroidetes	*Porphyromonas*	Relative abundance increased in the ESCC group compared to non-ESCC groups	87 diagnosed ESCC, 63 patients with dysplasia, and 85 healthy controls	16S rRNA pyrosequencing	Saliva sample from cases	Saliva sample from controls	Chen *et al*., 2015 [72]
		Prevotella						
	Firmicutes	*Streptococcus*						

Table 4. Oral bacteria associated with pancreatic cancers

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control sample	Reference
PC	Bacteroidetes	*Porphyromonas gingivalis*	Relative abundance associated with a higher risk of PC	361 incident adenocarcinoma of the pancreas, and 371 matching controls	16S rRNA sequencing	Pre-diagnostic oral mouthwash samples from patients	Pre-diagnostic oral mouthwash samples from controls	Fan *et al*., 2018 [9]
	Fusobacteria	*Leptotrichia*	Increased relative abundance in PC samples compared to non-PC samples	8 pancreatic cancer patients, 22 healthy controls, and 78 diagnosed with other diseases (including other cancer types)	16S rRNA sequencing	Saliva samples from pancreatic cancer patients	Saliva samples from healthy patients and patients with other diseases	Torres *et al*., 2015 [75]
PDAC	Firmicutes	*Streptococcus*	Increased relative abundance in PDAC samples compared to healthy controls	40 newly diagnosed PDAC, and 58 healthy controls	16S rRNA sequencing	Saliva samples from cancer patients	Saliva samples from healthy controls	Olson *et al*., 2017 [76]
PHC	Actinobacteria	*Rothia*	Increased relative abundance in PHC samples compared to healthy controls	30 PHC patients, and 25 healthy controls	16S rRNA sequencing	Tongue coating sample from patients	Tongue coating sample from healthy controls	Lu *et al*., 2019 [77]
	Firmicutes	*Peptostreptococcus*						
	Fusobacteria	*Fusobacterium*						
		Leptotrichia						

Table 5. Oral bacteria associated with healthy controls in the pancreatic cancer studies.

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control sample	Reference
PC	Fusobacteria	*leptotrichia*	Higher relative abundance associated with a lower risk of PC	361 incident adenocarcinoma of the pancreas, and 371 matching controls	16S rRNA sequencing	Pre-diagnostic oral mouthwash samples from patients	Pre-diagnostic oral mouthwash samples from controls	Fan *et al*., 2018 [9]
	Porphyromonas	*gingivalis*	Higher relative abundance associated with a higher risk of PC					
	Aggregatibacter	actinomycetemcomitans						
	Bacteroidetes	*Porphyromonas*	Relative abundance decreased in PC samples compared to non-PC samples	8 pancreatic cancer patients, 22 healthy controls, and 78 diagnosed with other diseases (including other cancer types)	16S rRNA sequencing	Saliva samples from pancreatic cancer patients	Saliva samples from healthy patients and patients with other diseases	Torres *et al*., 2015 [75]
	Proteobacteria	*Neisseria*	Relative abundance decreased in PC samples compared to non-PC samples					
	Firmicutes	*Streptococcus mitis*	Relative abundance significantly decreased in PC samples compared to healthy control samples	10 patients, and 10 controls	qPCR	Saliva microflora from patients with pancreatic cancer	Saliva microflora from healthy controls	Farrell *et al*., 2012 [74]
	Proteobacteria	*Neisseria elongata*						

(Table 5) contd.....

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control sample	Reference
PDAC	Proteobacteria	*Neisseria*	Relative abundance significantly decreased in PDAC samples compared to healthy control samples	40 newly diagnosed PDAC, and 58 healthy controls	16S rRNA sequencing	Saliva samples from cancer patients	Saliva samples from healthy controls	Olson *et al*., 2017 [76]
PHC	Bacteroidetes	*Porphyromonas*	Relative abundance decreased in PHC samples compared to healthy controls	30 PHC patients, and 25 healthy controls	16S rRNA sequencing	Tongue coating sample from patients	Tongue coating sample from healthy controls	Lu *et al*., 2019 [77]

In a recent prospective microbiome study on the risk of oral dysbiosis for PC, Fan *et al*. [9] analyzed 361 PC patients and 371 matched healthy controls using oral wash samples and 16S rRNA sequencing. It was shown that the presence of the periodontal pathogens *Porphyromonas gingivalis* and *Aggregatibacter actinomycetemcomitans* and a decreased relative abundance of *F. leptotrichia* were associated with increased risk of PC. As mentioned above, *P. gingivalis* has also been associated with OSCC and ESCC. More recently, Lu *et al*. [77] analyzed the tongue coat microbiota of 30 pancreatic head carcinoma (PHC) patients and 25 healthy control subjects. In contrast to Fan *et al*., they found a higher relative abundance of *Fusobacteria* (*Fusobacterium* and *Leptotrichia*) in PHC patients. In addition, phyla *Actinobacteria* (*Rothia, Actinomyces, Corynebacterium*) *Clostridia* (*Peptostrep-tococcus*) and Epsilonproteobacteria (*Campylobacter*) were also found significant in number. In addition, they found that the relative abundance of opportunistic pathogens such as *Haemophilus* (Gammaproteobacteria) and *Bateroidetes* (*Porphyromonas* and *Paraprevotella*) was reduced in PHC patients as compared with healthy controls.

The oral microbiota is also composed of fungal species that have been associated with pancreas ductal adenocarcinoma [47]. *Malassezia* species known as commensals and pathogens of skin and lungs [45] have also been found as commensals in the saliva [46]. In their recent study, Aykut *et al*. [47] found that *Malassezia* species promote PDAC by driving the complement cascade through the activation of mannose-binding lectin (MBL).

5. GASTRIC ADENOCARCINOMA AND COLORECTAL CARCINOMA

Table **6** summarizes the most relevant taxa of oral bacteria associated with gastric adenocarcinoma (GAC) and colorectal carcinoma (CRC) and Table **7** displays the bacteria associated with matched healthy controls in the same studies. Several studies have reported specific changes in the relative abundance of fecal and colonic bacteria in CRC patients [5, 8, 78, 79]. However, only a few studies have reported to date significant differences in the bacterial profiles in the oral samples of CRC patients as compared with healthy controls [78, 80]. In their study, Kato *et al*. [80] found an increased presence of *Lactobacillus* and *Rothia* in oral rinse DNA samples of CRC patients. Surprisingly, no correlation was found in this study between oral *Fusobacterium* abundance and CRC. Oral rinse samples, however, are likely to contain more bacteria of saliva and the oral surfaces than from the dental plaques and periodontal pockets, which might explain the higher abundance of Firmicutes, as well as the absence of *Fusobacterium,* found in that study. In contrast, Flemer *et al*. [78, 81] analyzing oral swabs found that several oral taxa, such as *Prevotella* and *Streptococcus* were differentially abundant in the oral samples of CRC patients as compared with controls. Moreover, they detected an increased abundance of pathologic oral bacteria, including *Fusobacterium nucleatum*, in CRC tumor tissues.

Table 6. Oral bacteria associated with gastric and colorectal cancers

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control sample	Reference
GA	Firmicutes	*Streptococcus*	Relative abundance increased in GA patients compared to healthy controls	57 newly diagnosed GA, and 80 healthy controls	16S rRNA pyrosequencing	Tongue coating sample from patients	Tongue coating sample from healthy controls	Wu *et al*., 2018 [83]
CRC	Firmicutes	*Streptococcus*	Relative abundance increased in CRC patients compared to healthy controls	99 colorectal cancer patients, 32 colorectal polyps patients, and 103 healthy controls	16S rRNA sequencing	Saliva samples from cancer patients	Saliva and faecal samples from controls	Flemer *et al*., 2017 [78]
	Fusobacteria	*Fusobacterium*						

Table 7. Oral bacteria associated with healthy controls in the gastric and colorectal studies

Cancer	Phylum	Genus/species	Main findings	N	Technology used	Case sample	Control sample	Reference
GA	Bacteroidetes	*Porphyromonas*	Relative abundance decreased in GA samples compared to healthy control samples	57 newly diagnosed GA, and 80 healthy controls	16S rRNA pyrosequencing	Tongue coating sample from patients	Tongue coating sample from healthy controls	Wu *et al*., 2018 [83]
		Prevotella						
	Proteobacteria	*Neisseria*						
	Bacteroidetes	*Porphyromonas*	Relative abundance decreased in GA samples compared to healthy control samples	34 patients, and 17 healthy controls	16S rRNA sequencing	Tongue coating sample from patients	Tongue coating sample from healthy controls	Hu *et al*., 2015 [82]
	Fusobacteria	*Fusobacterium*						
	Proteobacteria	*Neisseria*						
CRC	Proteobacteria	*Neisseria*	Relative abundance decreased in CRC samples compared to healthy samples	99 colorectal cancer patients, 32 colorectal polyps patients, and 103 healthy controls	16S rRNA sequencing	Saliva samples from cancer patients	Saliva and faecal samples from controls	Flemer *et al*., 2017 [78]
	Fusobacteria	*F. nucleatum*	Detected also in control healthy subjects					

One of the first studies analyzing the microbiome profiles of the tongue coating in GAC patients showed a reduced microbiota diversity as compared with healthy subjects [82]. This study showed that the relative abundance of *Proteobacteria*, such as *Neisseria* and *Haemophilus*, as well as *Fusobacterium* and *Porphyromonas* was significantly reduced in GAC patients as compared with healthy individuals. In a more recent study, Wu *et al*. [83] analyzed the microbiome of the tongue coating of 57 newly diagnosed GAC patients and 80 healthy controls by pyrosequencing of 16 rRNA. They found that a higher relative abundance of *Firmicutes* and a reduced presence of *Bacteroidetes* was a characteristic of GAC patients as compared with healthy controls. The genus *Streptococcus* was found to be a common risk factor for GAC, while other gram-negative bacteria, such as *Porphyromonas*, *Prevotella*, *Prevotella7*, and *Neisseria*, correlated inversely with the risk of GAC in this study. These studies concluded that, although the results provide some evidence supporting that certain bacteria colonizing the tongue coating can be associated with GAC progression, while other bacteria may be related to a decreased risk, the nature of such associations is still unclear and further studies with larger cohorts and well-standardized methods will be required.

CONCLUSION

For the purpose of providing a simplified overview at a glance of the most significant reported oral microbiome associations with oral and gastroenteric cancers, the main findings of the studies addressed in this review have been summarized in Table **8**. As could be expected, oral neoplasms and, in particular, OSCC showed a higher number of different bacterial species significantly increased or decreased in the saliva of patients when compared with healthy donors. Nevertheless, a considerable number of oral bacteria, as well as fungi and viruses, are ingested with the 0.75-1.5 liters of saliva that is estimated to be generated daily by an adult. Indeed, some oral-resident bacteria seem to associate with gastroenteric tumors, most notably the *Streptococcus* and *Fusobacterium* genera (Table **8**).

Table 8. Summary of reported associations of oral bacterial species with gastroenteric cancers and healthy control groups (*).

Phylum	Genus/species	GSCC	HNSCC	KCOT	OC	OMTC	OPMD	OSCC	ESCC	PC/ PDAC	PHC	GAC	CRC
Actinobacteria	*Rothia*	-	-	-	[58]	-	-	[63] [57]	-	-	[77]	-	-
	R. mucilaginosa	-	[62]	-	-	[44]	[61]	[64] [59]	-	-	-	-	-
Bacteroidetes	*Capnocytophaga*	-	-	-	-	-	-	[63]	-	-	-	-	-
	C. gingivalis	-	-	-	-		[61]	[67]	-	-	-	-	-
	C. ochracea	-	-	-	-	-	[61]	-	-	-	-	-	-
	Porphyromonas	-	-	-	-	-	-	[56] [63]	[72]	[75]	[77]	[83] [82]	-
	P. gingivalis	[68]	-	-	-	-	[61]	[60] [66]	[73]	[9]	-	-	-
	Prevotella	-	-	-	-	-	-	[56] [63]	[72]	-	-	[83]	-
	P. bergensis	-	-	-	-	-	[61]	-	-	-	-	-	-
	P. melaninogenica	-	-	-	[58]	-	[61]	[64] [67]	-	-	-	-	-
	P. veroralis	-	-	-	-	-	-	[64]	-	-	-	-	-

(Table 8) contd.....

Phylum	Genus/species	GSCC	HNSCC	KCOT	OC	OMTC	OPMD	OSCC	ESCC	PC/ PDAC	PHC	GAC	CRC
Firmicutes	*Gemella*	-	-	-	-	-	-	[63]	-	-	-	-	-
	G. haemolysans	-	-	-	-	-	[61]	[65] [64]	-	-	-	-	-
	G. morbillorum	-	-	[71]	-	-	[61]	[65]	-	-	-	-	-
	Granulicatella	-	-	-	-	-	-	[57]	-	-	-	-	-
	G. adiacens	-	-	-	-	-	[61]	[65] [66]	-	[74]	-	-	-
	Lactobacillus	-	-	-	-	-	-	[63]	-	[76]	-	-	-
	L. gasseri/johnsonii	-	[62]	-	-	-	-	-	-	-	-	-	-
	L. vaginalis	-	[62]	-	-	-	-	-	-	-	-	-	-
	Peptostreptococcus	-	-	-	-	-	-	[63] [69]	-	-	[77]	-	-
	P. stomatis	-	-	-	-	-	-	[65] [57]	-	-	-	-	-
	Streptococcus	-	-	-	[58]	[44]	-	[56] [63] [57]	[72]	[76]	-	[83]	[78]
	S. gordonii	-	-	-	-	-	[61]	[65] [64]	-	-	-	-	-
	S. mitis	-	-	-	-	-	[61]	[67] [65] [64] [59] [66]	-	[74]	-	-	-
	S. parasanguinis	-	-	-	-	-	[61]	[65]	-	-	-	-	-
	S. salivarius	-	[62]	-	-	-	[61]	[65] [64]	-	-	-	-	-
Fusobacteria	*Fusobacterium*	-	-	-	[58]	-	-	[56] [57]	-	-	[77]	[82]	[78]
	F. nucleatum	-	[62]	-	-	-	[61]	[59] [60]	-	-	-	-	-
	Leptotrichia	-	-	-	-	-	-	[63]	-	[75] [9]	[77]	-	-
	L. buccalis	-	[62]	-	-	-	-	-	-	-	-	-	-
Proteobacteria	*Neisseria*	-	-	-	-	-	-	[63]	-	[75] [76]	-	[83] [82]	[78]
	N. elongata	-	-	-	-	-	-	-	-	[74]	-	-	-
	N. meningitidis	-	-	-	-	-	[61]	-	-	-	-	-	-
	N. subflava	-	-	-	-	-	[61]	-	-	-	-	-	-

(*) References in red: Bacteria associated with cancer patients. References in blue: Bacteria associated with matched healthy controls.

Abbreviations: GSCC, Gingival squamous cell carcinoma; HNSCC, Head and neck squamous cell carcinoma; KCOT, Keratocystic odontogenic tumor; OC, oral cancer; OMTC, Oral mobile tongue carcinoma; OPMD, Oral potentially malignant disorder; OSCC, Oral squamous cell carcinoma; ESCC, Esophageal squamous cell carcinoma; PC, Pancreatic Cancer; PDAC, Pancreatic ductal adenocarcinoma; PHC, Pancreatic head cancer; GAC, Gastric adenocarcinoma; CRC, colorectal carcinoma

An interesting outcome of these studies is the consistently increased presence of *Neisseria* genus and three different species in healthy control groups when compared with cancer patients. Similarly, *Granulicatella* is predominantly found associated with the samples of healthy control groups. Nonetheless, it is recognizable a lack of consensus among the different studies on which oral bacteria species or genera have been linked to different gastroenteric cancer types. Thus, for the genera *Rothia*, *Porphyromonas,* and *Leptotrichia,* there is no general consensus about their association with cancer. For instance, *Leptotrichia* is an opportunistic pathogen that causes some serious focal and distant infections, such as periodontitis, osteomyelitis and endocarditis; however, it triggers strong immune responses, which has been claimed to be a possible mechanism for a protective role against pancreatic carcinogenesis [9].

Some associations of various genera with either cancer patients or healthy subjects seem not to correlate with the findings at the species level. For example, while the genus *Capnocytophaga* was associated with matched healthy controls, the species *C. gingivalis* and *C. orchacea* were associated with OPMD and OSCC. This implies that different species associate inversely with patients and controls. Additionally, for the genus *Prevotella,* several studies have reported quite diverse associations for different species. Thus, *P. bergensis* and *P. veroralis* were found associated with healthy controls while *P. melaninogenica* has been repeatedly associated with samples from cancer patients. Lastly, the *Streptococcus* genus has been predominantly associated with samples from cancer patients, mainly the species *S. gordonii, S. parasanguinis*, and *S. salivarius,* while the species *S. mitis* has been predominantly associated with healthy controls. However, Olson *et al*. [76] could not replicate in their PDAC study, the findings of Farrell *et al*. [74] concerning the lower proportion of *S. mitis* in PC patients compared with controls. Overlooking some other contradictory results in different studies the genera *Gemella, Lactobacillus, Peptostreptoccocus*, and *Fusobacterium*, including some of their species, were predominantly found to be associated with cancer patients.

Notwithstanding, the conclusions of the different studies should be considered carefully, bearing in mind the enormous heterogeneity of the methodologies applied throughout the different studies. The primary limitation of the studies reviewed here is the small sample size, which can be due to the difficulty to find and recruit larger numbers of patients that match strict selection criteria (described below) and high costs associated with the analysis of the samples. Another limitation for comparing studies is the different types of samples used in each study. Among the studies addressed in this review, there was ample variation in the type of samples under study ranging from saliva, tongue coating, swabs, mouthwash, and biopsy samples to cyst fluid aspirations. The use of different sampling methods could have a considerable impact on the results obtained, since the different microenvironments provided by

the oral cavity can harbor separated microbial niches [84]. Additionally, a large number of factors, such as gender, age, oral hygiene, habits (smoking, alcohol used) diet or environment, have a marked impact on the oral microbiome status [85]. Hence, the patients, as well as the individuals selected as healthy controls, must be carefully chosen. Many recent studies included in this review did efforts to achieve this by recruiting patients and matching controls by including appropriate selection criteria. In some cases, such as the study by Olson *et al*. [76], the selection criteria were so strict that from 281 approached patients, 80% were considered ineligible for various reasons, most importantly because they had been previously treated with chemotherapy. The previous history of treatment with neo-adjuvant chemotherapy or chemoradiation influences substantially the microbiome profile [70]. Therefore, all the criteria followed for the selection of patients and control subjects should be well-documented and taken into account when performing the statistical analyses of results.

Another critical point is the use of different technologies for the analysis of the oral microbiome. Among the studies addressed in this review, there is variation ranging from the initial use of bacterial cultures, qPCR, IHC, biochemical test, checkerboard DNA-DNA hybridization, 16S rRNA sequencing, 16S rRNA pyrosequencing, 16S rRNA next-generation sequencing, and metagenomic sequencing. Such differences affect enormously the taxonomic resolution, allowing the classification of the microbiome at the genus level in some studies and the species level in others. Furthermore, the studies differed substantially in the way of analyzing the results, sometimes presented as a trend, others as significantly different results, or simply by explaining which species could or not be found in cancer or control groups.

The parameters discussed above may help explain the fact that some genera and species of bacteria have been found in different studies associated with cancer patients or with healthy donors. This is the case of OSCC, for which the results with the genera *Rothia*, *Streptococcus* and *Prevotella* and the species *P. gingivalis, S. mitis and S. salivarius* have been contradictory among different studies (Table **8**), although *S. mitis* has been generally associated with healthy subjects and only one study found this species associated with cancer.

The most recent studies gathered the sequences obtained from the 16S rRNA bacterial genes into operational taxonomic units (OTUs) assigning sequences with the similarity of 97% to the same OTU. Taxa with statistically significant overall differences are then pairwise compared and the results are used to construct a linear discriminant analysis (LDA) model to rank each taxon according to the size with which they differentiate between groups [76]. This seems the most appropriate method for analyzing the data resulting from the sequencing. Nevertheless, in some cases, the small number of samples included in the study did not demonstrate statistically significant differences. Finally, it is noteworthy to point out that even a significant correlation of a specific bacterial species with a type of cancer is not proof of causality, which requires further study at the molecular level.

Altogether, the studies summarized in this review provide a lot of relevant data on the oral microbiota associated with cancer as well as settle the basis for improving the design of future studies. In particular, a refinement in the selection criteria for the patient and healthy control recruitment, accurate analysis of the sequencing data and careful statistical analyses should improve the consistency of future studies and make them more comparable. Only this will make possible to develop reliable diagnostic and prognostic tests with predictive power and to design adjuvant therapeutic strategies based on attempts to fight dysbiosis and promote healthier bacterial balances that make the tumor microenvironment less favorable to tumor cell proliferation and more immunogenic. However, at present, the limited number of reliable studies and the low amounts of patients and healthy controls included in these studies do not define ideal types of microbiota that might help prevent or even treat cancer.

LIST OF ABBREVIATIONS

CRC	=	Colorectal Carcinoma
CTL	=	Cytotoxic T Lymphocyte
EAC	=	Esophageal Adenocarcinoma
ESCC	=	Esophageal Squamous Cell Carcinoma
GAC	=	Gastric Adenocarcinoma
GSCC	=	Gingival Squamous Cell Carcinoma
HNSCC	=	Head and Neck Squamous Cell Carcinoma
IHC	=	Immunohistochemistry
IL-18	=	Interleukin 18
KCOT	=	Keratocytic Odontogenic Tumor
NF-kB	=	Nuclear Factor Kappa B
NFAT	=	Nuclear Factor of Activated T Cells
MyD88	=	Myeloid Differentiation Primary Response Protein MyD88
OC	=	Oral Cancer
OMTC	=	Oral Mobile Tongue Carcinoma
OPMD	=	Oral Potentially Malignant Disorder
OSCC	=	Oral Squamous Cell Carcinoma
PC	=	Pancreatic Cancer
PDAC	=	Pancreatic Ductal Adenocarcinoma
PHC	=	Pancreatic Head Cancer
qPCR	=	Quantitative Polymerase Chain Reaction
PD-L1	=	Programmed Death-Ligand 1
RC	=	Radicular Cyst
STAT3	=	Signal Transducer And Activator Of Transcription 3
TIGIT	=	T Cell Immunoreceptor with Ig and ITIM Domains
TLR	=	Toll-Like Receptor
TNF-a	=	Tumor Necrosis Factor-alpha

CONSENT FOR PUBLICATION

Not applicable.

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CONFLICT OF INTEREST

The authors declare no conflict of interest, financial or otherwise.

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