



Does microbiome shift play a role in carcinogenesis? A systematic review

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Abstract: Recent scientific advances have presented substantial evidence that there is a multifaceted relationship between the microbiome and cancer. Humans are hosts to multifarious microbial communities, and these resident microbes contribute to both health and disease. Circulating toxic metabolites from these resident microbes may contribute to the development and progression of cancer. The aim of this systematic review was to evaluate microbiome and microbial shift contribution to the development and progression of cancer. This systematic review provides an analytical presentation of the evidence linking various parts of the microbiota to cancer. Searches were performed in databases such as PubMed, Google Scholar, Scopus, EBSCO, E-Journals and Science Direct from the time of their establishment until May 2018 with the following search terms: cancer or human microbe or cancer and human microbiome AND shift in microbes in cancer. The merged data were assessed according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) guidelines. Cochrane's Risk of Bias Tool was used to assess the bias. Initially, 2,691 articles were identified, out of which 60 full-text articles were screened and re-evaluated. Among them, 14 were excluded based on inclusion/exclusion criteria; eventually, 46 articles were included in the systematic review. The reports of 46 articles revealed that microbial shift involving *Candida* species, *Fusobacterium nucleatum*, *Porphyromonas gingivalis*, *Helicobacter pylori* and *Human papilloma virus (HPV)* 16 & 18 were most commonly involved in various human cancers. In particular, organisms, such as *Candida albicans*, *Fusobacterium nucleatum*, *Porphyromonas gingivalis* and *HPV-16* were found to be more prevalent in oral cancer. The present systematic review emphasizes that the role and diverse contributions of the microbiome in carcinogenesis will provide opportunities for the development of effective diagnostic and preventive methods.

Keywords: Carcinogenesis; human microbiome; mouth neoplasms; oral cancer; *Candida albicans*

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Introduction

The human microbiome is the collective genome of all bacteria, viruses, fungi and protozoa that survive in and on the body surfaces. There are nearly 30 trillion bacteria, and the number of microbial genes present in the human body is at least 100 times higher than the number of human genes. Based on factors such as environment, diet, lifestyle, antibiotic exposure and the immune system, the makeup of this microbial community varies between individuals. Any disturbance or alteration in these commensal organisms can have a negative impact on health and may lead to the development and progression of diseases such as cancer. The reason for this link is the immune system. The immune system not only controls microbial interaction with cancer therapies but also regulates the microbiome, which influences the development and progression of cancer. It is estimated that 20% of all fatal cancers in humans are caused by microorganisms (1,2).

Different groups of bacteria in or on the human body have been proposed to induce carcinogenesis either by interference with signaling pathways and the cell cycle or through induction of chronic inflammation. Cancer cells differ from normal cells in that the former display rapid and uncontrolled division, high metabolic rates and cellular morphology differences. The dysregulation of the cell cycle machinery is considered the hallmark of cancer. This dysregulation includes defects in cellular programs such as differentiation, proliferation, senescence and apoptosis. The term carcinogenesis is a multistep process that requires the accumulation of multiple genetic alterations that alters the functions of proto-oncogenes and tumor suppressor genes. All human cancers usually lead to increased cell proliferation, loss of cell adherence, local tissue infiltration and metastasis (3,4).

It is also believed that the microbiome causes mutagenesis by metabolic production of potentially carcinogenic substances such as acetaldehyde, nitrosamine and nitrosodiethylamine. These changes affect the symbiosis between the microbiome and the host. This systematic review aims to identify the roles and diverse contributions of the microbiome in carcinogenesis, which will provide opportunities for the development of effective diagnostic and preventive methods.

This systematic review is aimed at identifying specific components of the microbiome that play important roles in carcinogenesis and identifying microbial shifts associated with the transition from health to cancer. The investigative

techniques used to identify the microbiome will also be analyzed.

Methods

This systematic review was conducted according to the Preferred Reporting Items for Systematic Reviews and Meta-Analyses (PRISMA) checklist. The key question was the following: “Do the microbiome and microbial shift contribute to the development and progression of cancer?”

Study design

A systematic review of human studies was attempted to summarize the results of published studies that used the human microbiome as an etiological factor for cancer and evaluated its role in tumor development and progression, listing specific organisms involved in carcinogenesis.

Inclusion criteria

The articles included in the study were full-length, English-language articles that focused on microorganisms involved in the origins of various human cancers.

Exclusion criteria

The exclusion criteria for selecting the articles included the following:

- (I) Articles that did not examine the microbiome as an etiological factor for cancer.
- (II) Articles other than original research, such as reviews, editorial letters, books, personal opinions and abstracts.
- (III) Studies with insufficient data.

Data sources and search strategy

Databases such as PubMed, Google Scholar, Scopus, EBSCO, E-Journals and Science Direct were searched using keywords such as “Cancer or human microbiome or cancer and human microbiome” AND “shift in microbes in cancer”. In addition, PubMed searches were carried out for references cited in review articles dealing with the microbiome in cancer (Table 1). Articles that were published between May 2007 to May 2018 were included. The references of the selected articles were screened again for additional relevant studies that could have gone unnoticed during the electronic search.

Table 1 Methodology employed for the review

Statement of the objective	Method/methodology	Resources utilized	Key words used
To analyze and critically evaluate research articles that have used microbiome as etiological factor for cancer and to check the shift of human microbiome from normal to cancer	Collection of articles followed by critical evaluation of studies using human microbiome as an etiological factor for cancer and to assess the role of microbiome that played significant role in tumor development and progression, thus listing out specific microbiome that are involved in carcinogenesis	e-journals, Scopus, PubMed, EBSCO, Google scholar, Science direct	“Cancer or human microbiome or cancer and human microbiome” AND “shift in microbes in cancer”

Study selection

The study selection was carried out in two phases. Initially, the articles were evaluated overall. We listed the various microbes examined and how their populations changed from normal health to cancer. We also listed the specific microbes involved in each study. The second phase included evaluating the different techniques used and assessing the validation of the results mentioned in each article.

Data collection

The form used to collect overall data from individual articles included the following information: Authors, journal in which the article was published, year of publication, research focus, methodologies employed, results obtained, conclusion drawn by the authors, future scope of research in the given field.

Assessing risk of bias

The Cochrane Collaboration tool (5,6) was applied to assess the risk of bias for RCTs. Bias was evaluated as a judgment (high, low, or unclear) for individual elements from seven domains. Risk of bias was assessed for each included study from 6 aspects: (I) random sequence generation (selection bias), (II) allocation concealment (selection bias), (III) blinding of outcome assessment (detection bias), (IV) incomplete outcome data (attrition bias), (V) selective reporting (reporting bias), and (VII) other bias. Risk of bias was rated by two independent researchers (LM and DA). Disagreements were discussed and if they remained unresolved, the third author (RSR) was consulted.

Synthesis of results

The results of the individual studies were then summarized, and the various microbes involved in carcinogenesis were

listed. Data on the same microbes were grouped and analyzed. Individual points of interests were summarized across the selected studies.

Results

Search results

A search with the above mentioned keywords yielded a total of 2,691 results. However, these included conference presentations, letters to the editor, short communications, journal publications, books, reviews and case reports. A total of 108 potentially relevant articles were identified through screening of titles and abstracts; among those candidates, 60 full-text articles that fit the inclusion criteria were included. The selected articles were further screened by two researchers for their reliability. In case of any disagreement on the selection of a study, a third reviewer was consulted (*Figure 1*). A total of 46 articles were selected by the reviewers for the systematic review; these articles are listed in *Table S1*.

Study results

A total of 46 articles were selected based on the reviewers' decisions. The selected articles included original research articles in which the microbiome played an important role in carcinogenesis. Upon review of the selected articles, it was identified that oral, lung, gastric, pancreatic and colon cancers have been associated with altered microbial profiles. This shift is stimulated by changes in the microenvironment that interrupt the function of the normal microbiome and lead to alterations in the microbial composition that mediate carcinogenesis. Among the selected studies, microbes such as *Candida albicans* in oral cancer and *Fusobacterium nucleatum* (*F. nucleatum*) in colorectal cancer displayed shifts from normal health to cancer.

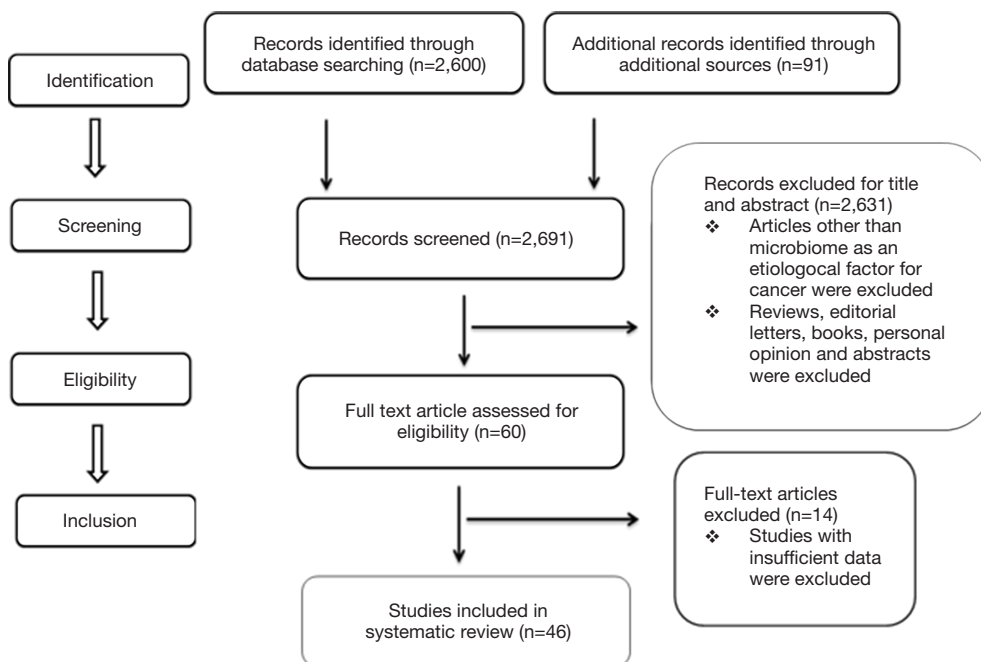


Figure 1 Study design as per PRISMA Guidelines.

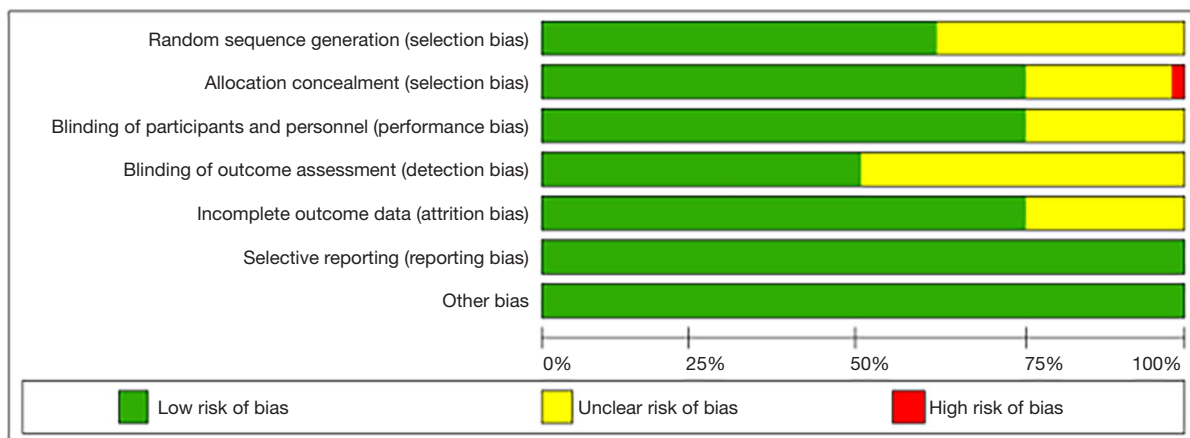


Figure 2 Representing the overall risk of bias for each domain.

Risk of bias of the included studies

The risk of bias in the RCTs is shown in Figure 2, and the overall risk of bias for each domain is shown in Figure 2. Only 2 studies (7,8) were of high risk of bias. The Cochrane Collaboration tool was applied to assess the risk of bias for RCTs. Bias was evaluated as a judgment (high, low, or unclear) for individual elements from seven domains.

The reviewed articles are listed in Table S1, which

describes the details of each study in terms of author, year, microbiome, sample, site, and methodology. A total of 22 microbes were considered as etiological factors for different cancers. The most commonly involved fungi was *Candida albicans* (9-21). The most commonly involved bacteria were *Porphyromonas gingivalis* (*P. gingivalis*) and *F. nucleatum* (7,22-24). Human papilloma virus (HPV) was strongly associated with various cancers (8,25-30).

Table 2 Microbiome and their association with cancer

Associated cancer	Microbiome involved	Literature report
Oral squamous cell carcinoma	<i>Candida albicans</i> , <i>Streptococcus alpha hemolyticus</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella</i> , <i>Leptotrichia</i> , <i>Streptococcus salivarius</i> , <i>Colliforms</i> , <i>Helicobacter pylori</i> , <i>Human papilloma virus - 16, 18</i> , <i>Epstein-Barr virus</i> , <i>Human herpes virus - 6</i> , mucosal microflora	Bandhary <i>et al.</i> 2018, Almstahl <i>et al.</i> 2018, Ainapur <i>et al.</i> 2017, Jain <i>et al.</i> 2016, Berkovits <i>et al.</i> 2016, de Sousa <i>et al.</i> 2016, Acharya <i>et al.</i> 2015, Alnuaimi <i>et al.</i> 2015, Jahanshahi and Shirani 2015, Ghosh <i>et al.</i> 2014; Bakki <i>et al.</i> 2014, Saravani <i>et al.</i> 2014, Metgud <i>et al.</i> 2014, Cankovic <i>et al.</i> 2013, Sonalika <i>et al.</i> 2012, Nola-Fuchs <i>et al.</i> 2012, Goot-Heah <i>et al.</i> 2012, Dayama <i>et al.</i> 2011, Saigal <i>et al.</i> 2011, Ahmed <i>et al.</i> 2010, Ang <i>et al.</i> 2009; Kurkivuori <i>et al.</i> 2007
Oropharyngeal squamous cell carcinoma	<i>Human papilloma virus - 16 & 18</i> , <i>Fusobacterium Nucleatum</i>	Wolf <i>et al.</i> 2017, Dang <i>et al.</i> 2015, Ang <i>et al.</i> 2010
Esophageal carcinoma	Salivary microbiome	Yamamura <i>et al.</i> 2016
Breast cancer	<i>Methylobacterium radiotolerans</i> , <i>Sphingomonas yanoikuyae</i> , <i>Human papilloma virus</i> , <i>Epstein-Barr virus</i>	Naushad <i>et al.</i> 2017, Xuan <i>et al.</i> 2014
Lung cancer	<i>Mycobacterium avium</i> complex	Lande <i>et al.</i> 2012
Gastric cancer	<i>Helicobacter pylori</i> , <i>Epstein-Barr virus</i> , oral bacteria, <i>Streptococcus mitis</i>	de Sousa <i>et al.</i> 2016, Hu <i>et al.</i> 2015, Kafle <i>et al.</i> 2014, Zaki <i>et al.</i> 2014
Pancreatic cancer	Salivary microbiota	Farrell <i>et al.</i> 2012
Colorectal cancer	Intestinal microbiome, <i>Cytomegalovirus</i> , <i>Fusobacterium nucleatum</i> , <i>Streptococcus bovis</i> , <i>Enterococcus Faecalis</i> , <i>Enterotoxigenic Bacteroides Fragilis</i> , <i>Escherichia coli</i> , <i>Streptococcus gallolyticus</i>	Andres-Franch <i>et al.</i> 2017, Zhou <i>et al.</i> 2016, Tsai <i>et al.</i> 2016, Tafvizi and Fard 2014; Mima <i>et al.</i> 2015; Weir <i>et al.</i> 2013
Squamous cell carcinoma of skin	<i>Staphylococcus aureus</i>	Kullander <i>et al.</i> 2009
Leukemia	<i>Herpes virus</i> , <i>Candida</i> species	Hasan and AL-Jubouri 2015; Steininger <i>et al.</i> 2009

Table 2 lists the microbiome and their association with cancer. In the selected studies, *Candida albicans*, *F. nucleatum*, *P. gingivalis* and HPV were the most common organisms involved in human cancers. *Helicobacter pylori* was reported to be linked to gastric cancer (31,32); *F. nucleatum* to colorectal cancer (22-24); *Methylobacterium radiotolerans*, *Sphingomonas yanoikuyae*, HPV, and *Epstein-Barr virus* to breast cancer (29,33); the *Mycobacterium avium* complex to lung cancer (34); and *Herpesvirus* and *Candida* species to leukemia.

Table 3 shows the cultural characteristics of the organisms associated with carcinogenesis and lists the microbes involved in microbial shift associated with carcinogenesis. Among the selected articles, most bacteria that were identified as involved in cancer were gram-positive facultative anaerobes.

Discussion

The microbiome of the human body consists of a broad variety of microorganisms that includes bacteria, virus, protozoa, and fungi. This microbial ecosystem shares the spaces in and on the body and creates an environment for commensal symbionts and pathobionts. The gastrointestinal tract has the largest microbial population in the human body, followed by the oral cavity. The advent of innovative molecular techniques has contributed to the isolation of approximately 700 microorganisms within the oral cavity. Recently, the conceptualization of microorganisms has changed. They have come to be considered only as pathogens rather than partners of the healthy human body (35-37).

Numerous studies have identified an interaction between the microbiome and cancer. The microbiome

Table 3 Cultural characteristics of the organisms associated with carcinogenesis

Microbiome	Kingdom	Gram reactivity	Environment	Culture
<i>Candida albicans</i>	Fungi	–	Aerobe or anaerobe	Sabouraud's dextrose agar (SDA), CHROM agar culture plate
<i>Streptococcus mitis</i>	Bacteria	Gram-positive cocci	Facultative anaerobe	Streptococcus mitis agar
<i>Streptococcus alpha-hemolyticus</i>	Bacteria	Gram-positive cocci	Facultative anaerobe	Blood agar, MacConkey agar, Tioglicolate surfaces
<i>Pseudomonas aeruginosa</i>	Bacteria	Gram-negative bacilli	Aerobe	Aerobic media
<i>Klebsiella</i>	Bacteria	Gram-negative bacilli	Facultative anaerobe	Aerobic media
<i>Leptotrichia</i>	Bacteria	Gram-negative bacilli	Anaerobe	Anaerobic media
<i>Streptococcus salivarius</i>	Bacteria	Gram-positive cocci	Facultative anaerobe	Brucella agar plates
<i>Streptococcus bovis</i>	Bacteria	Gram-positive cocci	Facultative anaerobe	Membrane bovis agar media
<i>Coliforms</i>	Bacteria	Gram-negative bacilli	Aerobic or facultative anaerobe	Violet red bile agar media, lauryl tryptase broth, brilliant green bile media
<i>Helicobacter pylori</i>	Bacteria	Gram-negative bacilli	Microaerophilic	Culture plate in microaerophilic jar, Brain heart infusion
<i>Staphylococcus aureus</i>	Bacteria	Gram-positive cocci	Facultative anaerobe	Mannitol salt agar media, tryptic soy agar
<i>Fusobacterium species</i>	Bacteria	Gram-negative bacilli	Anaerobe	Egg yolk agar media, brucella blood agar media, crystal violet erythromycin (CVE) agar media
<i>Enterococcus Faecalis</i>	Bacteria	Gram-positive bacilli	Facultative anaerobe	Pfizer selective enterococcus agar media
<i>Bacteroides Fragilis</i>	Bacteria	Gram-negative bacilli	Obligate anaerobe	Bacteroides Fragilis Selective media
<i>Escherichia coli</i>	Bacteria	Gram-negative bacilli	Facultative anaerobe	Luria broth, tryptic soy agar media
<i>Mycobacterium avium complex</i>	Bacteria	Gram-positive bacilli	Saprotrophic	Blood agar, mycobactin J-supplemented Herrold-egg yolk medium, Lowenstein-Jensen medium
<i>Methylo bacterium radiotolerans</i>	Bacteria	Gram-negative bacilli	Facultative methylotroph	Sheep blood agar, nutrient agar media
<i>Spingomonas yanoikuyae</i>	Bacteria	Gram-negative bacilli	Aerobe	Trypticase soy broth
<i>Human papilloma virus</i>	Virus	–	–	Tissue culture
<i>Epstein-Barr virus</i>	Virus	–	–	Chorioallantoic membrane of the chicken embryo
<i>Herpes virus</i>	Virus	–	–	Chick embryo culture

influences carcinogenesis using mechanisms that are independent of the immune system and inflammation. However, the most noticeable channel between the microbiome and cancer is through the immune system. It is believed that all these effects become more potent in combination and influence the initiation and progression of carcinogenesis. This systematic review was performed to identify the roles and diverse contributions of the microbiome in carcinogenesis, which will provide

opportunities for the development of effective diagnostic and preventive methods (35-37).

In the present systematic review, a total of 46 articles were selected by the reviewers, and the selected articles were original research articles in which the microbiome played an important role in carcinogenesis. Several microbes can cause chronic infections and produce toxins that disturb the cell cycle and alter cell growth. These chronic infections activate cell proliferation and DNA replication via activation

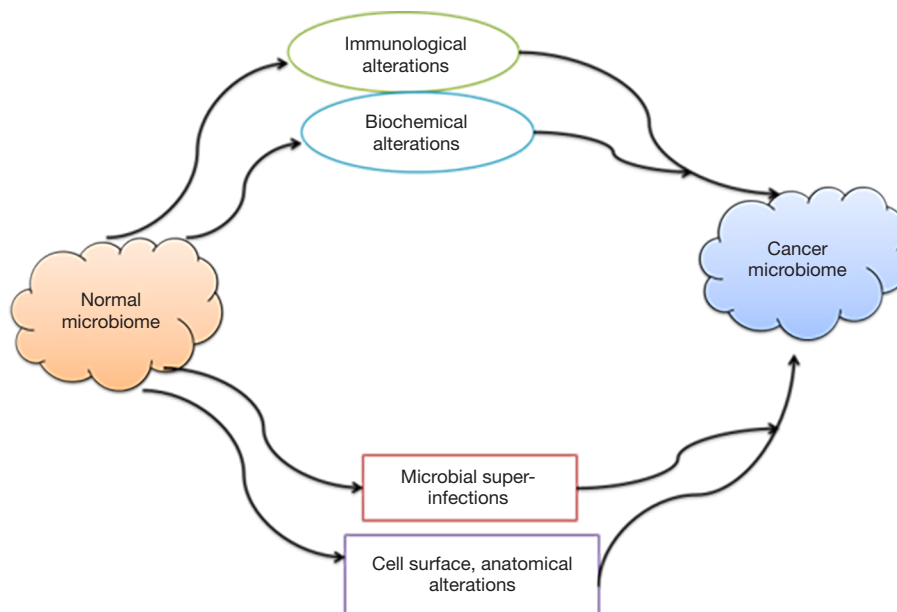


Figure 3 The possible mechanism for alteration of normal microbiome to cancer microbiome.

of cyclin D1 and the mitogen-activated kinase (MAPK) pathways, and they increase the activation of oncogenic transformation and the rate of tumor development through an increased rate of genetic mutation. Chronic infection results in intracellular accumulation of the pathogen, leading to suppression of apoptosis primarily by inactivating the retinoblastoma protein and modulating the expression of Bcl-2 family proteins. Thus, it allows the partially transformed cells to evade the self-destructive process and progress to a further level of transformation, ultimately becoming carcinogenic. The metabolism of potentially carcinogenic substances by the bacteria is another possible mechanism. The pre-existing microbes in oral cavity facilitate carcinogenesis by converting ethanol into its carcinogenic derivative, acetaldehyde. They also capable of inducing, mutagenesis, DNA damage and secondary hyperproliferation of the epithelium (38,39).

Cancer is a long-term process and is associated with changes in the body environment consisting of anatomical, physiological, biochemical, hormonal and immunological alterations (Figure 3). Hence, it is believed that changes during cancer development will also exhibit effects on the normal microbiome (40). On the other hand, the shift in the microbial community from eubiosis to dysbiosis is associated with host metabolic and cellular responses that modulate cancer risk. Various microbes play pivotal roles in preventing and protecting against cancer via modulation

of inflammatory processes due to close contact with host mucosa. In cancer, due to the increase in inflammation and oxidative stress, an imbalance occurs in the normal flora and drives the formation of specific metabolites—namely, nitric oxide synthetase (NOS2), reactive nitrogen species (RNS), and reactive oxygen species and an increase in cytokines such as interleukin-17 and tumor necrosis factor-alpha. As a result, various cellular responses occur, including a decrease in apoptosis, the promotion of cellular invasion and migration, an increase in cell proliferation, macrophage polarization toward the M1 phenotype, a decrease in DNA stability, and production of carcinogens. Overall, these processes result in a shift in commensal microflora to the pathogenic state (38).

In the present systematic review, it was identified that certain cancers including oral, lung, gastric, pancreatic and colon cancers have been associated with altered microbial profiles. This shift is stimulated by changes in the microenvironment that interrupt the function of the normal microbiome and lead to an altered microbial composition that mediates carcinogenesis. In the selected studies, microbes such as *Candida albicans* in oral cancer and *F. nucleatum* in colorectal cancer supported the idea of a shift in the microbiome from normal health to cancer (9-11).

According to this review, different types of cancer exhibit associations with different microbes. In the selected studies, *Candida albicans*, *F. nucleatum*, *P. gingivalis* and HPV were the

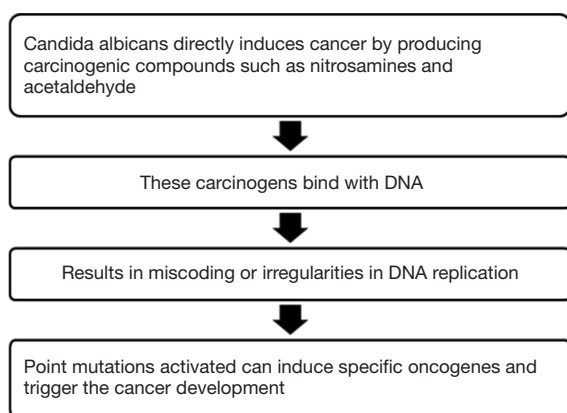


Figure 4 Steps in production of carcinogens and cancer development.

most common organisms involved in most human cancers. *Helicobacter pylori* was reported to be associated with gastric cancer (31,32); *F. nucleatum* (22-24) with colorectal cancer; *Methylobacterium radiotolerans*, *Sphingomonas yanoikuyae*, *HPV*, and *Epstein-Barr virus* with breast cancer (26,29,33); the *Mycobacterium avium* complex with lung cancer (34); and *Herpesvirus* and candidal species with leukemia (18,41).

In oral cancer, the most commonly isolated fungal species was *Candida albicans* (12-21), while the most commonly isolated bacterial species were *P. gingivalis* and *F. nucleatum* (22-24) and the most commonly isolated virus was *HPV* (8,25,27-30).

Common fungal species and cancer development

In the studies selected for this review, the most common fungal species in cancer was found to be *Candida albicans*. *Candida albicans* has the ability to colonize, penetrate and damage host tissues. These processes depend on an imbalance between host defenses and *Candida albicans* (Figure 4). The role of *Candida* in cancer development includes the following five elements:

- ❖ Colonization of the epithelium;
- ❖ Production of carcinogens;
- ❖ Ability to promote carcinogenesis in initiated epithelium;
- ❖ Ability to metabolize procarcinogens;
- ❖ Capability to alter the microenvironment and induce chronic inflammation.

Colonization of the epithelium

In the presence of specific defects in the immune system,

Candida albicans can colonize, penetrate and damage host tissue. There are two mechanisms involved:

- ❖ First is secretion of degradative enzymes such as aspartic proteases. This enzyme digests the epithelial cell surface and permit the candidal hyphae into or between epithelial cells.
- ❖ Second mechanism is the induction of epithelial cell endocytosis. Once the *Candida albicans* reach the keratinocytes, they stimulate the cells to form pseudopod-like structures that surround the fungi. Then, through the E-cadherin pathway, the cells can internalize the fungi (39).

Production of carcinogens

Ability to promote carcinogenesis in initiated epithelium

It is also proposed that candidal organisms initiate cell proliferation that can lead to clonal expansion of genetically altered cells (39).

Ability to metabolize procarcinogens

One important risk factor for the development of cancer is alcohol. In particular, the metabolic products of ethanol, including acetaldehyde, hydroxyl ethyl radicals, and ethoxy radicals, are active carcinogens. The conversion of alcohol to acetaldehyde is performed by the enzyme aldehyde dehydrogenase (ADH). Several studies have shown that bacteria and fungi can also be involved in this conversion process and can induce tumor development from various infections such as candidiasis.

Capability to modify the microenvironment and induce chronic inflammation

Currently, the relation among epithelial mesenchyme interaction, the role of chronic inflammatory cells and its mediators in cancer has been attaining attention. Different events may contribute to carcinogenesis and activate proteolytic enzymes that are able to degrade components of the epithelium and connective tissue. *Candida albicans* also secretes specific enzymes that have the capacity to degrade the basement membrane and matrix. They degrade laminin-332 and E-cadherin, which are present in the basement membrane and keratinocytes, respectively. The relation of *Candida albicans* to Toll-like receptors (TLR), NF- κ B, production of cytokines and cyclooxygenase-2 (COX2) implies that *Candida albicans* has the potential to alter the microenvironment and produce chronic inflammation that drives cancer development (42).

Common bacterial species and cancer development

In the studies selected for this review, the most common bacteria in cancer were *P. gingivalis* and *F. nucleatum*. *P. gingivalis*, a bacterium that has been isolated and found to be periopathogenic, plays a significant role in cancer by developing cellular invasion. The bacterial infection stimulates the ERK1/2-Ets1, p38/HSP27, and PAR2/NF-KB pathways to activate pro-matrix metalloproteinase (MMP)-9 expression. *P. gingivalis* also produces gingipains and cysteine proteinase leading to retainment of the PAR2 receptor as well as cleaving of MMP-9 proenzyme converting it into the mature active form. MMP-9 disintegrates the basement membrane and extracellular matrix, promoting tumor cell migration and invasion. This allows the tumor cells to reach the lymphatic system and blood vessels thereby causing metastasis. In this manner, *P. gingivalis* may add to the development and progression of cancer (43-51).

F. nucleatum is another periopathogenic bacterium associated with carcinogenesis. *F. nucleatum* can elevate cell proliferation and migration by targeting signaling molecules such as kinases involved in cell cycle control, causing cell proliferation and migration to increase. Furthermore, the bacterium secretes MMP-9 and MMP-13 (collagenase 3) by activating p38. It also plays a significant role in tumor invasion and metastasis. Recent studies have reported that *F. nucleatum*, with the help of a fusobacterial adhesin known as FadA that binds to E-cadherin on colon cancer cells, activates β -catenin signaling and results in colorectal cancer. This pathway leads to increased transcriptional activity of oncogenes, Wnt, and pro-inflammatory cytokines, as well as stimulation of cancer cell proliferation (Figure 5) (52,53).

It is well known that *P. gingivalis* and *F. nucleatum* play a pivotal role in periodontitis. Paradoxically, the role of these bacteria in the etiology and progression of oral cancer is less known. Chronic infection in the oral cavity aids carcinogenesis by various mechanisms that include atypical activation of infiltrating immune cells, induction of DNA damage through the formation of reactive nitrogen and oxygen species, and increased levels of immunocyte-derived bioactive molecules that promote tumor progression (52).

Common viruses and cancer

According to the present systematic review, the most common virus involved in cancer is HPV. HPVs are small, non-enveloped, icosahedral, epitheliotropic DNA tumor viruses that play important roles in carcinogenesis. The life

cycle of HPV begins at microlesions in the epithelium. This features a specialized differentiation program where viral DNA synthesis and expression are linked to capsid proteins. Virions directly infect the basal layer. The overlying stratum corneum and stratum granulosum synthesize capsids and release active virions. Once virion penetration occurs, three major mechanisms become active, including plasmid, vegetative, and productive replication. Because of these mechanisms, a change in keratinocytes from the basal layer to the surface of the epithelium occurs, providing a relevant microenvironment for productive cell replication. This also helps convert the keratinocyte into a permissive cell. Once the cells undergo epithelial differentiation, vegetative replication mechanisms occur. Finally, through viral episomal DNA rupture, the HPV genome is integrated into the genome of the host cell, and the preserved E6 and E7 segments undergo transcription (Figure 6). This results in a disturbance of cell control mechanisms and increased proliferation of infected cells, promoting cancer development and progression (53,54).

Among all cancers, 20% of oral cancers and 60% to 80% of oropharyngeal cancers are attributed to HPV infection. The International Agency for Research on Cancer (IARC) confirmed in 2012 that there was sufficient evidence to associate a subtype of HPV 16 with oral cancers. In the oral cavity, approximately 12 HPV types (2, 3, 6, 11, 13, 16, 18, 31, 33, 35, 52, and 57) have been associated with malignant lesions, and 24 HPV types (1, 2, 3, 4, 6, 7, 10, 11, 13, 16, 18, 30, 31, 32, 33, 35, 45, 52, 55, 57, 59, 69, 72, and 73) with benign lesions. HPV plays a pivotal role in the etiology of oral cancer because of its morphological association with squamous cell carcinoma (SCC) and its capability to immortalize oral keratinocytes and bring about transformation of epithelial cells. The most commonly identified type of genetic alteration in oral cancers was found to be loss of tumor suppressor proteins such as p53, antiproliferative proteins, and the product of the retinoblastoma gene (pRb). This is mainly due to genetic mutation or interaction with viral oncoproteins such as E6/E7 of HPV. Any interruption in the activity of these proteins permits the accumulation of genetic mutations, leading to a carcinogenic phenotype.

Commonly used investigative technique

The investigatory techniques used were culture, polymerase chain reaction (PCR), enzyme-linked immunosorbent assays, immunofluorescence assays, fluorescence staining and

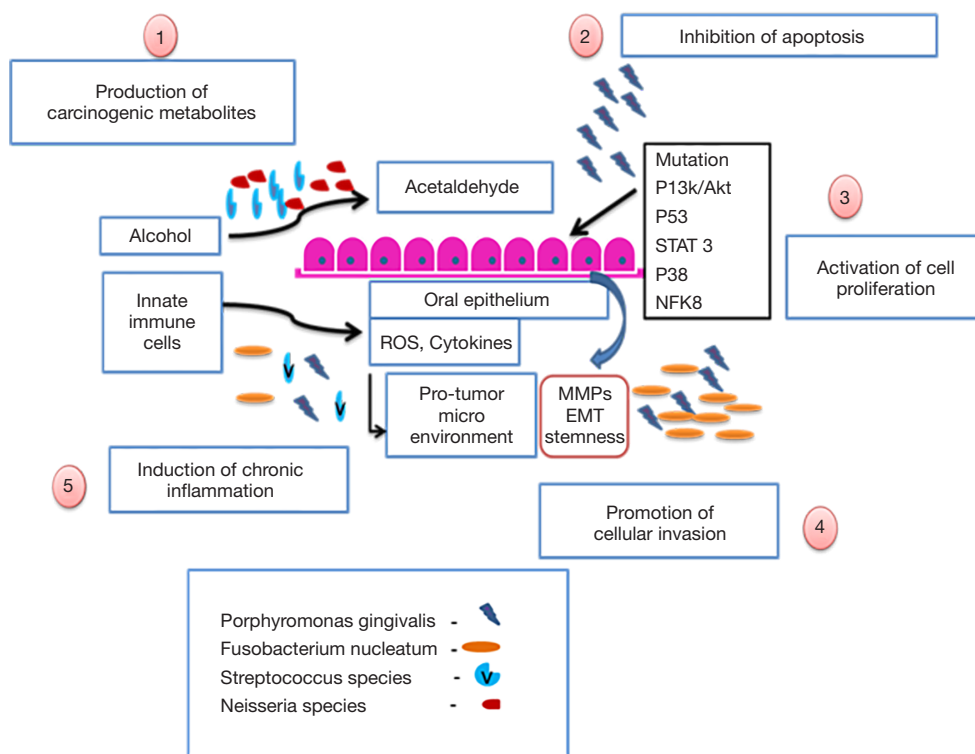


Figure 5 Possible mechanism of bacteria in carcinogenesis.

pyrosequencing. Among them, the most commonly used technique for *Candida* was culture, which was employed in the studies of Saini *et al.*, 2009; Saigal *et al.*, 2011; Bakki *et al.*, 2014; Tafvizi and Fard, 2014; Berkovits *et al.*, 2016; de Sousa *et al.*, 2016; Ainapur *et al.*, 2017. The advantage of using this method is that it is conventional, easy to use and cost effective. However, different culture media were used, with SDA being the most common. The growth of organisms in culture media was verified with help of confirmatory tests such as the sugar fermentation test, chlamyospore formation and the germ tube test (9,12-15,19,20,55).

For bacteria and viruses, the most commonly used investigative technique was PCR. The studies by Kang *et al.*, 2009; Kullander *et al.*, 2009; Ahmed and Eltoom, 2010; Dayama *et al.*, 2011; Farrell *et al.*, 2012; Goot-Heah *et al.*, 2012; Nola-Fuchs *et al.*, 2012; Faghihloo *et al.*, 2014; Saravani *et al.*, 2014; Tafvizi and Fard, 2014; Xuan *et al.*, 2014; Acharya *et al.*, 2015; Dang *et al.*, 2015; Mima *et al.*, 2015; Urbaniak *et al.*, 2016; Zhou *et al.*, 2016; Andres-Franch *et al.*, 2017; Naushad *et al.*, 2017; Bandhary *et al.*, 2018 used the technique to evaluate the microbiota associated with various cancers. The advantages of using this method are its high sensitivity, accuracy, reproducibility and specificity (26-31,33,56-63).

One of the limitations of this review is that it is limited

to English-language articles. Additionally, no animal studies were included in the systematic review. In view of recent advances that have brought novel, more targeted radiotherapy techniques and made new technology available to analyze the microbiome, the findings of older studies need to be interpreted cautiously.

There are several possible mechanisms—such as inflammation, metabolism and genotoxicity—by which the microbiome may regulate carcinogenesis, but there is no direct evidence for the relation between the microbiome and cancer. Research on the “oncobiome” continues to grow rapidly, but several key questions remain unanswered. On the other hand, it is becoming more apparent that the microbiome can also exert an anticancer effect through its products. Thus, researchers aim to identify and support microbes which fight cancer, thereby develop ways to eliminate those that promote development of cancer (61-63).

Conclusions

On the basis of the present review, many microbes have been found to play roles in carcinogenesis. In oral cancer, the commonly involved microbes were found to be *Candida albicans*, *P. gingivalis*, *F. nucleatum* and HPV. The present

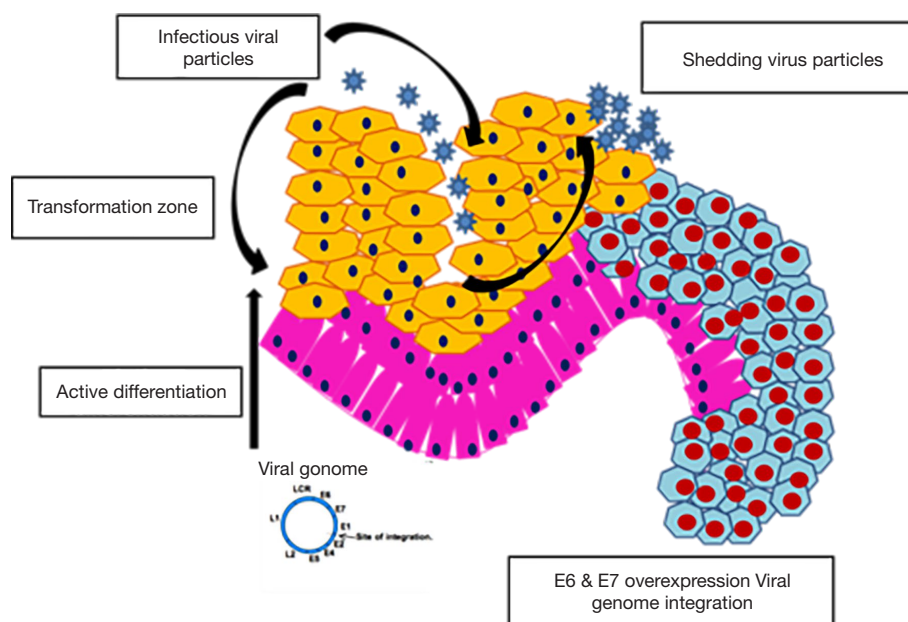


Figure 6 Mechanism of carcinogenesis induced by HPV.

systematic review emphasizes that the role and diverse contributions of the microbiome in carcinogenesis will provide opportunities for the development of effective diagnostic and preventive methods. The results have clarified the link between the microbiome and cancer, promoting the development of new methods to modulate a patient's resident microbial communities to improve their prognosis and treatment outcomes.

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Table S1 Summary of reviewed articles

Author & year	Microbiome	Type of cancer	Sample	Site	Methodology & validation	Conclusion
Bandhary <i>et al.</i> [2018]	Human papilloma virus (HPV) 16 & 18	Head & neck squamous cell carcinoma (SCC)	Tissue	Lesional site	Polymerase chain reaction (PCR)	There is no major role of HPV in carcinogenesis of head and neck SCC in coastal regions of south India
Almstahl <i>et al.</i> [2018]	Mucosal microflora	Head & neck cancer	Swab	Tongue & buccal mucosa	Culture method	There is increase in the mucosal pathogen, despite improvements in the treatment for cancer in the head and neck region
Ainapur <i>et al.</i> [2017]	<i>Candida</i> species	Oral squamous cell carcinoma (OSCC)	Oral swab	Lesional site	Culture method	An increase in candidal colonization in the oral cavity of OSCC patients undergoing radiotherapy was observed
Naushad <i>et al.</i> [2017]	HPV 16 & 18, Epstein-Barr virus (EBV) & mouse mammary tumor virus	Breast cancer	Tissue	Tissue blocks	PCR	The significant prevalence of viruses in breast cancer cases shows that they have a potential role in breast cancer development. The inactivation of tumor suppressor genes or activation of oncogenes by integration of HPV and MMTV viruses may lead to breast cancer development
Wolf <i>et al.</i> [2017]	Salivary microbiota	Oropharyngeal carcinoma	Swab	Saliva	16S rRNA gene sequencing method	Changes were found in the salivary microbiome of oral and oropharyngeal SCC patients and healthy controls. These changes may be promising biomarkers for SCC tumorigenesis, disease detection and the effectiveness of potential therapeutic interventions
Andres-Franch <i>et al.</i> [2017]	<i>Streptococcus gallolyticus</i>	Colorectal cancer	Tissues	Colonic mucosa	Quantitative real-time PCR & DNA methylation	Colorectal cancer patients showed low prevalence of <i>Streptococcus gallolyticus</i> infection
Jain <i>et al.</i> [2016]	<i>Candida</i> species	Oral cancer	Imprint	Tongue	Imprint culture technique, validated by the sugar fermentation test, chlamyospore formation and the germ tube test	Oral cancer patients undergoing radiotherapy & chemotherapy showed an increase in candidal colonization and an alteration in the growth pattern of <i>Candida</i> from the carrier state to the infective state
Zhou <i>et al.</i> [2016]	<i>Fusobacterium</i> species, <i>Enterococcus faecalis</i> , <i>Enterotoxigenic Bacteroides fragilis</i> , <i>Escherichia coli</i>	Colorectal cancer	Tissue	Normal and lesional sites	Quantitative real-time PCR	<i>Fusobacterium</i> species and <i>Escherichia coli</i> were significantly increased in tumor and adjacent tissues of colorectal cancer patients compared with the tissues of healthy individuals. These findings provide evidence supporting a possible association of <i>Fusobacterium</i> species and <i>Escherichia coli</i> with transformation of colorectal mucosa from the early adenomatous polyp stages to the colorectal cancer stage
Berkovits <i>et al.</i> [2016]	Oral yeast	OSCC	Oral swab	Lesional site	Culture method, matrix-associated laser desorption/ionization-time-of-flight mass spectrometry	Oral yeast carriage was significantly elevated in OSCC patients, supporting the notion that an altered microenvironment is associated with carcinogenesis
Tsai <i>et al.</i> [2016]	<i>Streptococcus bovis</i>	Colorectal adenocarcinoma	Blood	Colon	Gram staining method, colonoscopy	<i>S. bovis</i> bacteremia was associated with colorectal adenocarcinoma, especially in female patients
de Sousa <i>et al.</i> [2016]	<i>Candida</i> species	Orogastric cancer	Oral swab	Lesional site	Culture method, MALDI-TOF mass spectrometry	Increased virulence was observed in yeasts isolated from orogastric cancer patients
Yamamura <i>et al.</i> [2016]	<i>Fusobacterium nucleatum</i>	Esophageal carcinoma	Tissue	Tissue block	Quantitative real-time PCR	The presence of <i>Fusobacterium nucleatum</i> in esophageal carcinoma predicts shorter survival, suggesting that it might serve as a prognostic biomarker
Urbaniak <i>et al.</i> [2016]	Microbiome	Breast cancer	Tissue	Lesional site	16S rRNA sequencing & PCR amplification, validated by culture	The breast microbiome was observed to play important roles in both health and disease
Hu <i>et al.</i> [2015]	Oral bacteria	Gastric cancer	Tongue coating	Tongue	Tongue image analysis & gel electrophoresis	The microbiota of the tongue coating is an indicative tool for the observation and early diagnosis of gastric cancer
Dang <i>et al.</i> [2015]	HPV-16	Oropharyngeal squamous cell carcinoma (OPSCC)	Saliva	Whole mouth	Real-time PCR	HPV detection in oral rinse samples may be a useful screening tool to detect HPV-associated oral cancers
Sharma <i>et al.</i> [2015]	<i>Helicobacter pylori</i>	OSCC	Saliva	Whole mouth	Culture method	The patients with premalignant lesions and OSCC in the present study showed a high prevalence of <i>Helicobacter pylori</i>
Hasan and AL-Jubouri [2015]	<i>Candida</i> species	Leukemia	Oral swab	Lesional site	Culture method, validated by germ tube formation, chlamyospore production, the urease test, and the sugar fermentation test	Oral candidiasis is a discernible complication in leukemia patients, and this study showed that the most commonly isolated species was <i>C. guilliermondii</i> , which represented 31.66% of cases
Acharya <i>et al.</i> [2015]	EBV	OSCC	Oral swab	Buccal cavity	PCR, validated by nested PCR	The prevalence of EBV was much higher in the OSCC cases than in the controls. One observation suggested that EBV by itself is not a risk factors for OSCC but interacts with other risk factors such as tobacco smoking and alcohol
Alnuaimi <i>et al.</i> [2015]	<i>Candida</i> species	Oral cancer	Saliva	Whole mouth	Quantitative real-time PCR	Oral candida colonization was significantly higher in oral cancer patients than in healthy controls. Alcohol consumption and candidal carriage could be significant risk factors
Mima <i>et al.</i> [2015]	<i>Fusobacterium nucleatum</i>	Colorectal carcinoma	Tissue	Tissue blocks	Quantitative real-time PCR	<i>Fusobacterium nucleatum</i> DNA was associated with elevated colorectal-cancer-specific mortality
Jahanshahi and Shirani [2015]	<i>Candida</i> species	OSCC	Tissue	Tissue blocks	Fluorescence staining and periodic acid-Schiff staining	Fluorescence staining is more accurate than periodic acid-Schiff staining (PAS) in identifying <i>Candida</i> in OSCC
Faghihloo <i>et al.</i> [2014]	EBV	Gastric cancer	Tissue	Tissue blocks	Quantitative real-time PCR	A low prevalence of EBV-associated gastric cancer was observed in Iran
Ghosh <i>et al.</i> [2014]	Viable aerobic bacteria	OSCC	Tissue	Lesional site	Histological grading, culture method	Viable aerobic bacteria were more abundant in the deeper tissues of OSCC than closer to the surface
Tafvizi and Fard [2014]	Cytomegalovirus	Colorectal cancer	Tissues	Tissue blocks	Nested PCR	The findings in the study were statistically significant and showed that CMV could play an important role in creating malignancy and driving the progression of cancer through the process of oncomodulation
Zaki <i>et al.</i> [2014]	<i>Streptococcus mitis</i>	Oral and digestive cancer	Saliva	Whole mouth	Culture & Gram staining, validated by the sugar fermentation test and the catalase test	Increase in the number of <i>Streptococcus mitis</i> in saliva of oral and digestive cancer patients act as an early diagnostic marker
Bakki <i>et al.</i> [2014]	<i>Candida</i> species	Head & neck tumors	Saliva	Whole mouth	Culture, validated by carbohydrate assimilation and fermentation tests	A high prevalence of <i>Candida</i> was observed in the oral cavity of patients undergoing anticancer therapy
Saravani <i>et al.</i> [2014]	EBV & human herpes virus -6 (HHV-6)	OSCC	Tissue	Tissue blocks	Real-time PCR	HHV-6 and EBV are not directly involved in OSCC
Xuan <i>et al.</i> [2014]	Methylo bacteriumradiotolerans, spingomonas yanokuyae	Breast cancer	Tissue	Tissue blocks	16S pyro sequencing and quantitative real-time PCR	Bacterial load might be used in conjunction with current methods to monitor the progression of breast cancer as there is an inverse correlation between bacteria load and tumor staging
Kafle <i>et al.</i> [2014]	<i>Helicobacter pylori</i> (<i>H. pylori</i>)	Gastric cancer	Tissue & blood	Lesional site	Histological examination & Enzyme-linked immunosorbent assay (ELISA)	<i>H. pylori</i> along with hosts genetic and dietary factors play a major role in gastric carcinogenesis in patients infected with <i>H. pylori</i>
Metgud <i>et al.</i> [2014]	Aerobic and facultative anaerobic	OSCC	Saliva	Mucosa, whole mouth	Culture method	Higher degree of total number of microbial colony forming unit (CFUs)/mL was found in carcinoma site and saliva
Cankovic <i>et al.</i> [2013]	<i>Streptococcus alpha-haemoliticus</i>	OSCC	Saliva	Lesional site	Culture method	Presence of microbial flora on the irregular oral carcinoma surface contributes to chronic inflammation
Weir <i>et al.</i> [2013]	Intestinal microbiome	Colorectal cancer	Stool	Stools samples	Pyro sequencing analysis	There are "driver bacteria" with pro-carcinogenic features that contribute to tumor development and "passenger bacteria" that may outcompete drivers to flourish in the tumor environment as the cancer progress was observed
Sonalika <i>et al.</i> [2012]	Aerobes, anaerobes, coliforms, candida and gram negative, anaerobic bacilli	OSCC	Saliva	Whole mouth	Culture method	An appropriate antimicrobial protocol at the stage of diagnosis OSCC is mandatory
Nola-Fuchs <i>et al.</i> [2012]	HPV-16 & EBV	OSCC	Oral mucosal Swab, Venous blood	Oral mucosa, cubital fossa	QIA amp Mini Elute Virus Spin kit Digene HPV genotyping RH test & VIDAS EBV kit	Role of HPV-16 & EBV is less in OSCC patients
Lande <i>et al.</i> [2012]	<i>Mycobacterium avium</i> complex	Lung cancer	Sputum	Lesional site	Culture method	There was an association between the presence of mycobacterium Avium Complex in respiratory, cultures of lung cancer patients and SCC located in the periphery of the lung
Goot-Heah <i>et al.</i> [2012]	HPV-18	OSCC	Saliva	Whole mouth	Nested PCR & spectrophotometer technology	Low percentage of HPV-18 DNA was detected in OSCC, suggesting that HPV-18 may not play important role in development and progression of OSCC
Farrell <i>et al.</i> [2012]	Salivary microbiota	Pancreatic cancer	Saliva	Whole mouth	Human oral microbe identification by microarray, validation by Real-time quantitative PCR	An association between variations in salivary microbiota with pancreatic cancer and chronic pancreatitis patients was observed. It also provides evidence that salivary microbiota may act as a non-invasive biomarker of systemic diseases
Dayama <i>et al.</i> [2011]	<i>Helicobacter pylori</i>	OSCC	Tissue	Cancer site	Culture method & PCR, validation-oxidase and urease test	Increased risk of oral cancer is associated with <i>H. pylori</i> infection
Saigal <i>et al.</i> [2011]	<i>Candida albicans</i>	OSCC	Saliva	Whole mouth	Culture methods, validation-chlamyospore formation, corn meal broth + 5% milk, milk serum culture	The nitrosamine compounds produced by <i>Candida</i> species may be involved in which act directly or indirectly on oral mucosa
Ahmed and Eltoom [2010]	HPV-16 and HPV-18	OSCC	Tissue	Tissues blocks	PCR	Association between HPV-16 and HPV-18 infection and oral cancer was observed
Ang <i>et al.</i> [2010]	HPV-16	Oropharyngeal SCC	Tissue	Tissue blocks	In situ hybridization, validation-immunohistochemistry analysis	HPV -16 is a strong independent prognostic factor for survival among patient with oropharyngeal SCC
Steininger <i>et al.</i> [2009]	Herpes viruses	Chronic lymphocytic leukemia (CLL)	Blood	Cubital fossa	Semi-quantitative ELISA and qualitative immunofluorescence assay	Among all herpes virus cytomegalo virus (CMV)-seroprevalence was significantly higher in selected CLL cohorts than in age- and gender-matched healthy adult
Kullander <i>et al.</i> [2009]	<i>Staphylococcus aureus</i>	SCC	Tissue and swab	Lesional and normal area	Multiple displacement amplification and PCR	A strong association between staphylococcus aureus and SCC was found which was found to be greater than HPV and SCC
Kang <i>et al.</i> [2009]	Cariogenic bacteria, periodontopathic bacteria, <i>Candida</i> species	Oncological patients	Saliva	Whole mouth	PCR	<i>C.albicans</i> was significantly more prevalent in the oncological patients than in the healthy groups
Saini <i>et al.</i> [2009]	<i>Streptococcus viridians</i> , <i>Pseudomonas aeruginosa</i> , <i>Klebsiella</i> , <i>Candida albicans</i> & <i>Leptotrichia</i>	OSCC	Saliva	Lesional site	Culture & gram staining method	Hundred percent reduction in the normal microbial flora in oral cancer was observed
Kurkivuori <i>et al.</i> [2007]	Oral <i>Streptococci</i> group	Oral cancer	Strains	Bacterial and clinical	Culture method, Fluorescence analysis & gas chromatography	Oral streptococci play a pivotal role in fluctuation of salivary acetaldehyde levels after alcohol consumption and increases the risk of oral cancer development