REVIEW ARTICLE

# Oral Microbiota as a Diagnostic Biomarker of Digestive Cancer: A Systematic Review

SK Aziz Ikbal[1], Surendra Kumar Yadav[2], Roopanshi Mehrotra[3], Tasneem Fatima[4], Anjusha Sharda[5], Srashti Gupta[6]

## Abstract

**Aim:** This article aimed to review the association of oral microbiota with digestive cancer (DC).

**Background:** Oral microbiota is one of the most complex ecosystems in our body. The mouth, from which the digestive system starts, may be a source of an abundant taxonomic group of microbiotas that travel to the digestive system followed by growth, reproduction, and settlement, forming a complex microecological environment causing systemic and gastrointestinal (GI) disease.

**Review results:** A total of 14 articles were chosen for review. Most studies were case–control. Both positive and negative associations were seen between oral microbiome and DC.

**Conclusion:** Digestive cancer may be associated with distinctive oral microbial character.

**Clinical significance:** The present systematic review enlightens the risk of digestive carcinoma with oral microbiota that may act as a biomarker for early diagnosis of DC in a more comfortable, acceptable, and noninvasive way.

**Keywords:** Digestive cancer, Gastrointestinal cancer, Microbial dysbiosis, Oral microbiota, Systematic review.

*The Journal of Contemporary Dental Practice* (2023): 10.5005/jp-journals-10024-3598

## Introduction

Cancer is a leading cause of death worldwide. Gastrointestinal (GI) cancer refers to the malignant condition of the gastrointestinal tract (GIT) and other organs involved in digestion including the esophagus, stomach, biliary system, pancreas, small and large intestine, rectum, and anus. According to the World Health Organization (WHO) mortality database in 2018, a large number of new cases of digestive cancer (DC) (about 63%) is the leading cause of mortality over any other cancer-related death in Asia.[1] Among DC, esophageal, gastric, and liver cancers show more prevalence in Asia compared to colorectal and pancreatic cancers which are more prevalent in Europe and North America.[1]

There are well-established entities of interaction between genetic factors and developing cancer. Not only the age of a person, smoking, unhealthy diet, and inflammation are validated risk factors of cancer but also an association of *Helicobacter pylori* have been found with the development of cancer and defined as a class-I carcinogen. Several authors have identified common risk factors for DC among which well-recognized risks are environmental factors, high intake of fried food, alcohol consumption, old age, cigarette smoking, low intake of fruit or vitamin C, salted preserved food, and gut microbial infection.[2–6] Besides those, some authors admitted host interaction with environmental exposure as a risk factor.[7–9]

Some evidence suggested that not only the gut microbiota but also the dysbiosis of oral microbiota is correlative in conjunction with DC development.[10] The underlying mechanism of instituting between oral status and DC risk is not well understood. Although little, there is some evidence that establishes the oral microbiome as one of the modulating factors of developing cancer and other chronic diseases through the direct metabolism of chemical carcinogens and perpetuating systemic inflammatory mediators. The oral cavity is suitable for stable and harmonious colonization of bacteria unless disturbed by medication, disease, or significant changes in diet.[11]

[1–4,6]Career Post Graduate Institute of Dental Sciences & Hospital, Lucknow, Uttar Pradesh, India

[5]Sudha Rustagi College of Dental Sciences & Research, Faridabad, Haryana, India

**Corresponding Author:** Roopanshi Mehrotra, Career Post Graduate Institute of Dental Sciences & Hospital, Lucknow, Uttar Pradesh, India, Phone: +91 7275305475, e-mail: roopanshimehrotra12@gmail.com

**How to cite this article:** Ikbal SKA, Yadav SK, Mehrotra R, *et al*. Oral Microbiota as a Diagnostic Biomarker of Digestive Cancer: A Systematic Review. J Contemp Dent Pract 2023;24(11):902–911.

**Source of support:** Nil

**Conflict of interest:** None

Recent evidence shows that oral microbial dysbiosis plays crucial roles in human health such as host response, carcinogen metabolism, and nutrient digestion. The human microbiome is the second most frequently studied human microflora. Apart from *H. pylori*, many other microbiotas are associated with an increased risk of DC. Specific bacteria and/or their dysbiosis in the human microbiome can cause local mucosal inflammation and increased intestinal permeability. Coker OO et al. suggested some bacterial interactions across stages of gastric cancer (GC), indicating that GC is related to microbial composition shifted along with stages of gastric carcinoma that are superficial gastritis, atrophic gastritis, and intestinal metaplasia.[12]

Early detection and effective treatment improve cancer survival, yet this remains a great challenge. Most of the diagnostic methods of DC are invasive and they lack of symptoms until they proceed to the late stage, most times they remain undiagnosed.

Again, deoxyribonucleic acid (DNA) sequencing for digestive metastasis detection is limited in clinical use due to the need for fresh, high-quality specimens, tumor content, and tumor heterogeneity. Again, molecular markers such as mutant DNA or DNA methylomes are detectable until and unless the cancer has

advanced from an early stage. So, researchers have given stress to noninvasive ways of diagnosis to enhance diagnostic sensitivity or early detection of DC. Several authors hypothesized that there is a difference in the oral microbial ecosystem among DC patients and healthy people.[10,12–14] Again, saliva, dental plaque, and tongue coat are three relevant samples of oral microflora and are easily collected. Because of their ease of accessibility as compared to a sample from other body parts, they might become a biomarker for the detection of various systemic diseases, for example, diabetes, cardiovascular disease, weight loss, preterm birth, erectile dysfunction, and DC. In this perspective, recent evidence is suggestive of using the oral microbial composition as a noninvasive screening tool for the detection of GI carcinoma.[13–15]

To date, the oral microbial dysbiosis and development of DC remain uncertainty. It is far consequently imperative to unravel the possible influence of specific bacteria with digestive metaplasia. The present systematic review aims at the critical updated appraisal of existing literature to observe the association of oral microflora with digestive carcinoma and the differential quantification and possible influence of certain oral microbiomes on digestive inflammation and cancer.

## Materials and Methods

### Literature Research

This systematic review was performed according to the preferred reporting items for systematic reviews and meta-analysis (PRISMA) checklist.[16] The databases such as PubMed and Google Scholar were searched for eligible articles. A search was carried out by entering the following keywords and MeSH terms: Oral microbiota, oral microbiome, oral microbial dysbiosis, digestive cancer, digestive neoplasm, GI cancer, GI neoplasm, etc. Additionally, references regarding relevant topics were searched. The search was carried out for articles published between 2012 and 2022. Duplicates were removed and the abstracts and titles were checked for inclusion and exclusion based on the criteria provided below. The full texts were reviewed after meeting the inclusion and exclusion criteria as follows:

Inclusion criteria were (A) study relevant to the topic; (B) human studies; (C) original studies that adhered to samples collected from the oral cavity and (D) Descriptive (cross-sectional) and analytic studies (case–control and cohort).

The following criteria were excluded for this systematic review: (A) Animal study; (B) Irrelevant to the topic, (C) case reports and reviews; and (D) lack of effective statistical analysis.

For searching articles, the population, intervention, comparison(s), and outcome(s) (PICOs) schema was followed. A well-built question with the following criteria was used to facilitate searching the pertinent articles:

- Population: Adult patients with any type of GI cancer.
- Intervention: Oral microbial composition, and dysbiosis analyzed irrespective of methodology. No restriction was applied for different techniques, sample collection, preservation and analysis, and the type of microbiota that were analyzed.
- Comparison(s): Adult individual without cancer.
- Outcome(s): Association between oral microbiota composition both with abundance and quality of bacterial species.
- Study design: Descriptive (cross-sectional) and analytic studies (case–control and cohort) bounded to the magnitude of association between oral microbiota and DC.

### Data Extraction

Two of the authors (SAI and TF) independently screened titles and abstracts through the databases. This was followed by a full-text review of the relevant studies. For each eligible study, the following information was independently extracted and discussed with the third author (RM). The collected particulars were the author's name, country, year of publication, type of study, number of included patients with sample size, sample extraction, and detection method, and main result with the conclusion.

### Quality Assessment and Risk of Bias

The authors also performed a quality assessment with a risk of bias (ROB) evaluation of selected articles. The ROB was independently performed by two authors (SKY and AS) using the Newcastle–Ottawa scale (NOS)[17] (Table 1). The NOS contains eight items divided into the following three categories: selection, comparability, and outcome. One star was given for an appropriate assessment score ranging from 0–9 points and a high score indicating good methodological quality of the study based on the inclusion and exclusion criteria of the present study. Studies were thereafter classified into good, fair, and poor quality according to their star rating.

## Results

### Literature Search Result

The search strategy identified 1,399 potential articles, from PubMed and Google Scholar. After the removal of duplicates and irrelevant topics, 87 articles were screened on title and abstract. The full texts of 76 studies were further evaluated for eligibility. Based on inclusion and exclusion criteria, 14 articles were finally included in this review. The detailed selection process is presented in Flowchart 1. Five articles were further screened for group bar chart representation (Fig. 1) based on the provided odds ratio (OR).

### Studies Characteristics

The characteristics of the accepted articles depicted in Table 2 published between 2012 and 2022. Among 14 studies, 7 were from the USA, 6 were from China and one study was carried out in Italy. Most studies were case–control. Five studies were conducted on GC, five studies on pancreatic cancer (PC), two on esophageal cancer (EC), and one on colorectal cancer (CRC), and one on both CRC and chronic gastritis (CG). Table 3 represents the method of quantification of the oral microbiome with sample care provided in each article and Table 4 delineates the qualitative and quantitative results of various bacteria. The overall total number of DC or metaplasia patients who were considered was 1,171 who were compared with 1,277 controls. Four studies assessed tongue coat where saliva was taken as a sample in seven studies. After collecting the samples, they were stored at a temperature range from –20 to –80°C. Almost all of them used the method of 16S ribosomal ribonucleic acid (rRNA) sequencing whereas only one study has done both 16S rRNA and 18S rRNA sequencing.

### Oral Microbiota and Gastric Cancer

Five articles deal with the association of oral microbiota in healthy control and GC. The overall number of GC cases was 386 who were compared with 359 controls. WU J reported significantly higher level of *Prevotella*, *Neisseria*, and *Porphyromonas* in healthy control group but *Streptococcus* was higher in GC individuals.[18] Hu J et al.

**Table 1:** Newcastle–Ottawa scale (high score indicates lower ROB)

| *Category* | *Wu J et al.*[18] | *Hu J et al.*[19] | *Sun JH et al.*[20] | *Xu S et al.*[21] | *Contaldo M et al.*[22] | *Fan X et al.*[23] | *Farrell JJ et al.*[13] | *Chen X et al.*[14] | *Torres PJ et al.*[24] | *Kato I et al.*[25] | *Lu H et al.*[26] | *Salazar CR et al.*[27] | *Peters BA et al.*[10] | *Wei AL et al.*[28] |
|---|---|---|---|---|---|---|---|---|---|---|---|---|---|---|
| Selection | | | | | | | | | | | | | | |
| Is case definition adequate? | * | * | * | * | * | * | * | * | * | – | * | * | * | * |
| Case representativeness | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| Selection of control | – | * | * | * | * | * | * | * | * | – | – | – | – | – |
| Definition of control | * | – | * | – | * | – | – | – | * | * | – | * | – | – |
| Comparability | | | | | | | | | | | | | | |
| Comparability of cases and controls based on design or analysis (study controls for/study controls for any additional factor) | * | * | * | * | * | ** | * | * | * | * | ** | * | ** | * |
| Outcome | | | | | | | | | | | | | | |
| Ascertainment of exposure (secure record, Structured interview where blind to case/control status) | * | * | * | * | * | * | * | * | * | * | * | * | * | * |
| Same method of ascertainment of case and control | – | – | * | – | – | * | * | – | – | * | – | * | * | – |
| Nonresponse rate | * | * | – | – | * | – | – | – | * | – | – | – | – | * |
| Total score | 6 | 6 | 7 | 5 | 7 | 7 | 6 | 5 | 7 | 5 | 5 | 6 | 6 | 5 |

*Starred according to the assessment criteria of The Newcastle-Ottawa Scale (NOS)

reported significant difference in *Proteobacteria* and *Actinobacteria* seen between GC and control.[19]

### Oral Microbiota and Pancreatic Cancer

Five articles investigated the association of oral microbiota between control and pancreatic individuals. Overall total of 450 PC patients were assigned against 497 controls. Fan X et al. found higher levels of *Porphyromonas gingivalis* (Pg) and *Aggregatibacter actinomycetemcomitans* (Aa) in PC individuals.[23] Significant variation of *Neisseria elongata* and *Streptococcus mitis* between chronic pancreatitis and healthy control was reported by Farrell JJ et al.[13] Torres PJ reported a higher ratio of *Leptotrichia* to *Porphyromonas* in the saliva of PC patients.[24] *Streptococcus* and *Leptotrichina* found higher in PC group in another study.[28]

### Oral Microbiota and Colorectal Cancer

Contaldo M et al. collected saliva samples of CG healthy, and CRC individuals. reported statistically lower levels of *Fusobacterium nucleatum* and Pg in CRC group compared to healthy individuals.[22] However Kato I et al.[25] failed to support previous studies by denying the association of *Fusobacterium* and CRC.[25]

### Oral Microbiota and Esophageal Squamous Cell Carcinoma

Only two articles dealt with the association of oral microbiota in control and esophageal squamous cell carcinoma (ESCC) individuals. Chen X reported an overall decreased microbial diversity in ESCC compared to healthy control and dysplasia subjects.[14] Peters BA et al.[10] assessed microbial content in an oral wash of ESCC and esophageal adenocarcinoma (EAC) patients. The study identified an association between *Neisseria* and *Streptococcus pneumoniae* with a lower risk of EAC whereas Pg increases the risk of ESCC.[10]

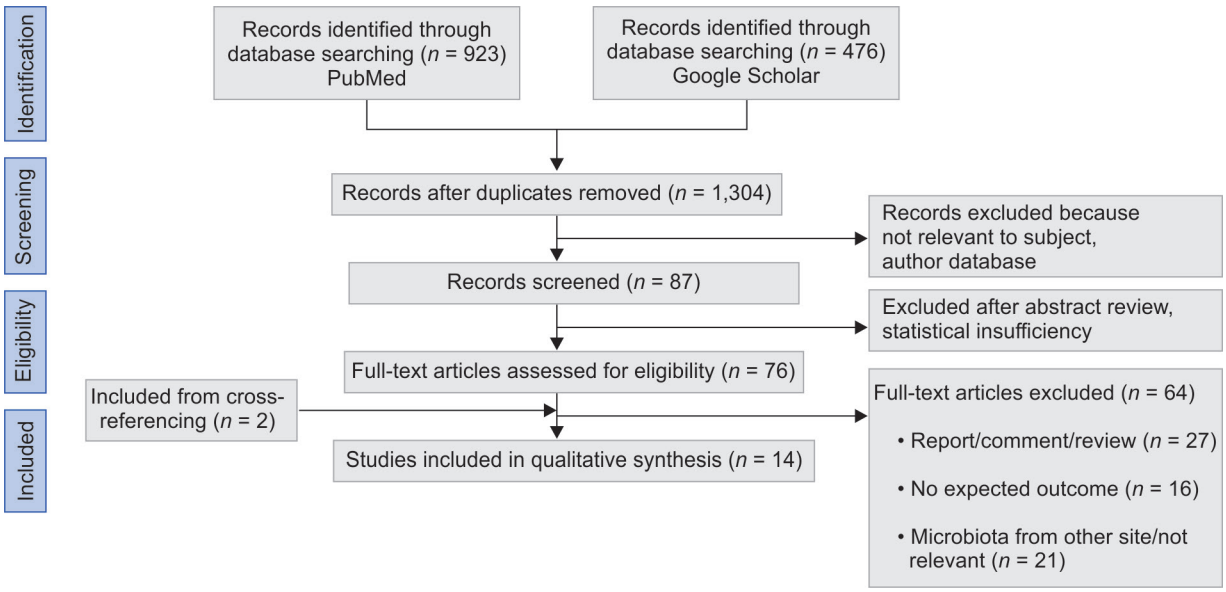
## Discussion

Digestive cancers that including cancers in the esophagus, stomach, liver, pancreas, colon, and rectum are increasing worldwide. Even though the diagnosis and treatment modalities of cancer are developing, the mortality rate is still disappointing. Despite relying on diagnostic procedures, the early stage DC patients often ignore endoscopy and biopsy for their invasiveness. Hence, easy, and successful diagnostic options such as biomarkers or any other noninvasive technique may be crucial later for early diagnosis and treatment of DC. Various authors reported the role of oral microbiota in DC development related to microbial composition, changes in the relative abundance (RA) of certain microorganisms along with the state of oral microbial dysbiosis.[10,29–31] The present systematic review enlightens the risk of digestive carcinoma (e.g., GC, PC, CRC and EC) with oral microbiota that may act as biomarker for early diagnosis of DC in more comfortable, acceptable and noninvasive way.

First. gastric cancer is the most common DC and one of the pertaining causes of cancer adhering global mortality.[32] Second, CG may proceed to GC by the following sequential stages: Chronic gastritis→atrophy→intestinal metaplasia→dysplasia. Therefore, this article also reviews the literature interlinking between oral microbial dysbiosis and CG.

The mouth, from which the digestive system starts, may be a source of an abundant taxonomic group of microbiotas that travel to the digestive system followed by growth, reproduction, and settlement, forming a complex microecological environment

**Flowchart 1:** The PRISMA flow diagram of article selection process



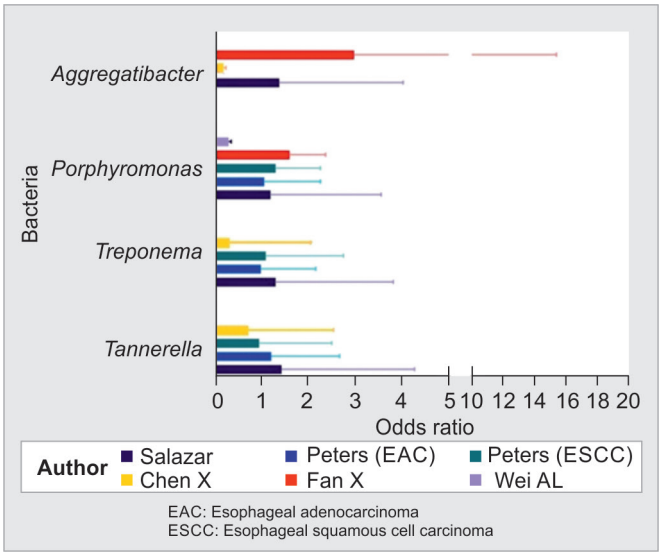




**Fig. 1:** Group bar chart representing OR of different bacteria in selected five studies

causing systemic and GI disease. The abundance or different typical count or pattern of oral microbiota could have a synergistic effect on onset or augmenting GC suggesting a possible role of oral microbiota in screening and risk assessment of cancer that could avail early detection and treatment initiation to combat this fatal cancer. Several authors showed strains of salivary bacteria, Pg, Aa, *Klebsiella* spp. could travel to the intestine and can cause gut floral dysbiosis followed by chronic inflammation and carcinoma.[33] The major phyla of oral bacteria include *Proteobacteria*, *Bacteroidetes*, *Firmicutes*, *Actinobacteria*, and *Fusobacteria*.[34]

Xu S et al. found significantly decreased *Neisseria* and *Rothia* in tongue coating in GC patients compared to healthy control whereas Sun JH et al. analyzed saliva and plaque and reported similar finding.[20,21] Cui J et al. analyzed 21 microbial species from tongue coating of gastritis and healthy individuals; 10 species were increased and were more diverse in gastritis patients whereas the levels of 11 species were less in gastritis patients compared to normal control.[35] Li X et al. suggested abundance of *campylobacter* in tongue-coating flora may be positively interlinked with the progression of gastric mucosa from normal to atrophic gastritis and GI metaplasia.[36]

Abundant *Peptostreptococcus* was reported by Coker OO et al. in the mucosa of GC patients.[12] Salazar conducted a study to detect the influence of oral pathogens on gastric precancerous lesion. Plaque and saliva samples were tested with reverse transcription-polymerase chain reaction (RT-PCR) for DNA levels of associated pathogens. They found a high but nonsignificant increase of Pg (OR = 1.12; 0.67–1.88) Aa (OR = 1.36, 0.87–2.12), and *Treponema denticola* (OR =1.34, 0.83–2.12) in patients with gastric precancerous lesions and hypothesized that periodontal pathogens are associated with increased incidence of gastric precancerous lesions.[27] Yamamura et al. and Hsieh et al. have given attention on *F. nucleatum*; they reported a significant increase of *F. nucleatum* DNA in EC, GC, and CRC.[37,38] Sun JH et al. analyzed bacterial distribution in saliva and plaque among different groups including GC patients.[20] They found an increased abundance of *Veillonella*, *Prevotella*, *Aggrigatibater*, and *Megasphaera* whereas *Leptotrichia*, *Rothia*, *Capnocytophaga*, *Campylobactor*, and *Tannerella* decreased in the GC group.[20] Several authors have reported the role of *Mycoplasma* that is not affected by many common antibiotics to be associated with various DC such as GC, CRC, and prostate cancer.[27,39,40]

Cordero and Varela–Calviño reported the influence of oral hygiene on intestinal inflammation interlinking them for their mutual involution in tumor development through signaling pathways.[41] They identified oral microbiota that travels to the gut and inspires the onset of CRC from chronic gastrointestinal (GI) inflammatory disease, for example, inflammatory bowel disease (IBD). Flemer et al. reported differently abundant oral taxa in CRC compared to healthy control and suggested heterogeneity of oral microbiota may be an alternative screening for the detection of CRC.[42]

The most common phyla that have been reported by several authors in oral samples of EC are *Firmicutes*, *Bacteroidetes*,

**Table 2:** General characteristics of selected studies

| *Reference* | *Country* | *Study type* | *Cancer type* | *Case (n)* | *Control (n)* |
|---|---|---|---|---|---|
| Wu J et al.[18] | China | Case–control | GC | 57 | 80 |
| Hu J et al.[19] | China | Case–control | GC | 74 | 72 |
| Sun JH et al.[20] | China | Case–control | GC | 37 | 13 |
| Xu S et al.[21] | China | Case–control | GC | 181 | 112 |
| Contaldo M et al.[22] | Italy | MECC | CG | 7 | 4 |
| | | | CRC | 4 | |
| Fan X et al.[23] | USA | Large nested Case–control | PC | 361 | 371 |
| Farrell JJ et al.[13] | USA | Case–control | PC | 10 | 10 |
| Chen X et al.[14] | USA | MECC | ESCC | 87 | 85 |
| | | | Dysplasia | 63 | |
| Torres PJ et al.[24] | USA | Case–control | PC | 8 | 22 |
| Kato I et al.[25] | USA | Population based case–control | CRC | 68 | 122 |
| Lu H et al.[26] | China | Case–control | PC | 30 | 25 |
| Salazar CR et al.[27] | USA | Case–control | GC | 37 | 82 |
| Peters BA et al.[10] | USA | Prospected study nested in two cohorts | EAC | 81 | 160 |
| | | | ESCC | 25 | 50 |
| Wei AL et al.[28] | China | Case–control | PC | 41 | 69 |

CG, chronic gastritis; CRC, colorectal cancer; GC, gastric cancer; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; MECC, multiple events case–control; PC, pancreatic cancer

**Table 3:** Sample care and methods of bacterial quantification of selected studies

| *Reference* | *Sample* | *Sample care* | *Method* | *Bacterial quantification* |
|---|---|---|---|---|
| Wu J et al.[18] | Tongue coat | Tongue scrap kept in tube with buffer then centrifuge and stored in –80°C | Pyrosequencing of 16S rRNA gene | RA |
| Hu J et al.[19] | Tongue coat | Tongue coating taken in tube with 1 mL of phosphate buffer solution then centrifuge at 5800 rpm for 5 minutes at –80°C | 16S rRNA sequencing | RA |
| Sun JH et al.[20] | Saliva and plaque | Plaque—taken in EDTA buffered tube then centrifuged<br>Saliva—taken with TE buffer | High throughput sequencing of 16S rRNA gene amplicon | Absolute amount |
| Xu S et al.[21] | Tongue coat | Tongue coat immersed in test tube with 10 mL phosphate buffered followed by centrifugation 2800*g* for 10 minutes and stored at –80°C | 16S rRNA and 18S rRNA genes sequencing | RA |
| Contaldo M et al.[22] | Saliva | Saliva sample frozen at –20°C | RT-PCR was done for genomic bacterial DNA extraction | RA |
| Fan X et al.[23] | Oral wash | Mouthwash stored at –80°C | 16S rRNA gene sequencing | RA |
| Farrell JJ et al.[13] | Saliva | Unstimulated saliva stored at –80°C | Bacterial DNA extracted. RT-PCR, q-PCR 16S rRNA sequencing | Absolute abundance |
| Chen X et al.[14] | Saliva | Saliva sample mixed to buffer and stored at –20°C | 16S rRNA was amplified and sequenced | RA |
| Torres PJ et al.[24] | Saliva | Stored at –80°C | DNA isolation, RT-PCR, and 16SrRNA sequencing | RA |
| Kato I et al.[25] | Mouthwash | – | 16S rRNA sequencing | RA |
| Lu H et al.[26] | Tongue coat | Tongue coat scrapped and kept in phosphate buffered saline followed by centrifugation and storage | 16S rRNA sequencing | Absolute amount |
| Salazar CR et al.[27] | Saliva and plaque | Both saliva and plaque samples were vortex mixed thoroughly for 30 seconds, then immediately placed into a container containing ice then transferred to laboratory within 1 hour for further processing | RT-PCR for DNA levels of pathogens | RA |
| Peters BA et al.[10] | Oral wash | Mouthwash and expectorate collected stored at –80°C | 16S rRNA gene sequencing | RA |
| Wei AL et al.[28] | Saliva | Fresh saliva placed on ice and stored in laboratory at –80°C | 16S rRNA gene sequencing | RA |

EDTA, ethylenediaminetetraacetic acid; RA, relative abundance; TE buffer, Tris-EDTA buffer

**Table 4:** Difference in abundance of oral bacteria among patients with digestive problems of the selected studies

| | | *Results* | | | | |
|---|---|---|---|---|---|---|
| *Reference* | *Cancer type* | *Microbiota* | *Higher in* | *p–value* | *Main finding* | *Authors conclusion* |
| Wu J et al.[18] | GC | *Prevotella* | HC | <0.001 | *Firmicutes* and *Streptococcus* increase and *Bacteroidetes*, *Neisseria*, *Porphyromonas*, and *Prevotella* decrease risk of GC | Tongue coating microbiome may help in early detection and prevention of GC |
| | | *Porphyromonas* | HC | 0.002 | | |
| | | *Neisseria* | HC | <0.001 | | |
| | | *Streptococcus* | GC | 0.004 | | |
| Hu J et al.[19] | GC | *Porphyromonas* | HC | 0.002 | Similar RA of *Firmicutes, Bacteroides, Fusobacteria,* seven transmembrane (TM7) and significant difference in *Proteobacteria* and *Actinobacteria* seen between GC and control | Tongue coating may be potential source of GC diagnosis |
| | | *Fusobacteria* | HC | 0.004 | | |
| | | *Neisseria* | HC | 0.008 | | |
| | | *Proteobacteria* | HC | <0.001 | | |
| | | *Actinobacteria* | GC | <0.001 | | |
| | | *Hemophilus* | HC | 0.007 | | |
| Sun JH et al.[20] | GC (Plaque) | *Prevotella* | – | 0.86 | 36 out of 37 patients with GC identified as high–risk population (sensitivity rate 97%) 1 among 13 control group showed high–risk population (*Helicobacter pylori* infection was detected) | Oral microbiome scoring may be potential for screening in suspected GC patients |
| | | *Rothia* | – | 0.67 | | |
| | | *Tannerella* | HC | 0.042 | | |
| | | *Aggregatibacter* | GC | 0.023 | | |
| | GC (Saliva) | *Prevotella* | GC | 0.042 | | |
| | | *Rothia* | HC | 0.0066 | | |
| | | *Aggregatibacter* | GC | 0.0049 | | |
| Xu S et al.[21] | GC | *Prevotella* | HC | <0.001 | The alteration of tongue coating microbiota had a possible linkage with the inflammation and metabolome and GC | Difference in tongue coating microbiome may be found in between GC and HC, may possibly link to inflammation and metabolome that could help tongue coating as biomarker in GC |
| | | *Porphyromonas* | HC | <0.001 | | |
| | | *Firmicutes* | GC | <0.05 | | |
| | | *Rothia* | HC | <0.001 | | |
| | | *Treponemma* | HC | <0.001 | | |
| | | *Peptostreptococcus* | HC | <0.001 | | |
| Contaldo et al.[22] | CG, CRC | *Porphyromonas* | HC | <0.05 | *F. nucleatum* was statistically lowest in CG and highest in control group followed by CRC group. Pg found lowest in CG group as compared to HC with a statistically significant difference | Total microflora sample may be used in future as noninvasive method of diagnosis of GI disease |
| | | *Fusobacteria* | HC | <0.05 | | |
| Fan X et al.[23] | PC | *Porphyromonas* | PC | 0.21 | Pg and Aa were associated with higher risk of pancreatic cancer | Oral microbiota play a role in the etiology of pancreatic cancer |
| | | *Aggregatibacter* | PC | 0.13 | | |
| Farrell JJ et al.[13] | PC | *N. elongata* | HC | <0.05 | *Neisseria elongata* and *S. mitis* showed significant variation between chronic pancreatitis and healthy control | Variation between salivary microbiota with pancreatic cancer, healthy control and chronic pancreatitis present that may be noninvasive biomarker |
| | | *S. mitis* | HC | <0.05 | | |
| | | *G. adiacens* | PC | <0.05 | | |
| Chen X et al.[14] | ESCC | *Lautropia* | HC | <0.01 | ESCC subjects had an overall decreased microbial diversity compared to control and dysplasia subjects | Correlation presents in between altered salivary microbiota and ESCC risk |
| | | *Bulledia* | HC | <0.01 | | |
| | | *Catonella* | HC | <0.01 | | |
| | | *Corynebacterium* | HC | <0.01 | | |
| | | *Moryella* | HC | 0.18 | | |
| | | *Peptococcus* | HC | <0.01 | | |
| | | *Cardiobacterium* | HC | <0.01 | | |

*(Contd...)*

**Table 4:** *(Contd...)*

| | | Results | | | | |
|---|---|---|---|---|---|---|
| *Reference* | *Cancer type* | *Microbiota* | *Higher in* | *p–value* | *Main finding* | *Authors conclusion* |
| Torres PJ et al.[24] | PC | *Leptotrichia* | PC | <0.01 | Significant higher ratio of *Leptotrichia* to *Porphyromonas* in saliva of PC patients | Bacterial profile in saliva may be useful biomarker for PC |
| | | *Porphyromonas* | PC | <0.01 | | |
| | | *Neisseria* | HC | 0.07 | | |
| | | *Aggregatibacter* | HC | 0.09 | | |
| Kato I et al.[25] | CRC | *Fusobacteria* | No association with disease or health | | No correlation between *Fusobacterium* and association of CRC | This study failed to support previous studies by denying the association of *Fusobacterium* and CRC |
| | | *Lactobacillus* | CRC | – | | |
| | | *Rothia* | CRC | – | | |
| | | *Neisseria* | HC | – | | |
| | | *Veillonella* | CRC | – | | |
| Lu H et al.[26] | PC | *Firmicutes* | PC | <0.05 | The microbiome diversity in tongue coat in pancreatic head cancer patient was significantly increased | Microbiota dysbiosis may be present in patient with PC |
| | | *Fusobacteria* | PC | <0.001 | | |
| | | *Actinobacteria* | PC | <0.001 | | |
| | | *Bacteroidetes* | HC | <0.001 | | |
| Salazar CR et al.[27] | GC (saliva) | Aa | – | 0.96 | Significant stronger relationship observed between bacterial burden score of periodontal disease-related microbe and gastric precancerous lesion | High level of colonization of periodontal pathogens are associated with an increased risk of gastric precancerous lesion |
| | | Pg | – | 0.45 | | |
| | | *T. denticola* | – | 0.90 | | |
| | GC (plaque) | Aa | GC | 0.96 | | |
| | | Pg | GC | 0.45 | | |
| | | *T. denticola* | GC | 0.90 | | |
| Peters BA et al.[10] | EAC | *T. forsythia* | EAC | 0.04 | *Neisseria*, *S. pneumoniae* were associated with lower EAC risk. Pg increases risk of ESCC | Early detection and prevention of EAC and ESCC are possible from oral microbial flora screening |
| | | Pg | – | 0.40 | | |
| | | *T.denticola* | – | 0.87 | | |
| | ESCC | Pg | ESCC | 0.09 | | |
| Wei AL et al.[28] | PC | *Streptococcus* | PC | 0.021 | *Streptococcus* and *Leptotrichina* associated with higher risk of PC where *Veillonella* and *Neisseria* have protective role | Saliva microbiome can distinguish PC patient from healthy control |
| | | *Leptotrichina* | PC | 0.016 | | |
| | | *Veillonella* | HC | 0.007 | | |
| | | *Neisseria* | HC | 0.041 | | |

CG, chronic gastritis; CRC, colorectal cancer; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; GC, gastric cancer; HC, healthy control; PC, pancreatic cancer

*Proteobacteria*, *Fusobacteria*, and *Actinobacteria*. Peters BA et al. observed an increased risk of EAC with an increase of *Tannerella forsythia* in oral microbiome where ESCC risk increased with Pg abundance.[10]

Fan X et al. claimed their study to be the first prospective evaluation of oral microbiome and PC and demonstrated a higher RA of Pg and Aa, and a decreased RA of *Fusobacteria* and its genus *Leptotrichia* are associated with risk of PC.[23] Decreased *N. elongata* and *S. mitis*, and increased *Granulicatella adiacens*, may elevate the incidence of PC.[13] This incidence supports the hypothesis that an elevation of *G. adiacens*, which often behaves like an opportunistic pathogen, may suppress the growth of *S. mitis*. Wei AL et al. claimed that *streptococcus* and *Leptotrichina* are associated with PC whereas *Veillonella* and *Neisseria* have a protective role in decreasing PC risk.[28]

Understanding the exact transmission pathway of the oral microbiome and how they commence DC may help in the early detection and control of cancer. Bacteria are the most common abundant and complex taxonomic group of oral microbes which is the reason for frequent studies conducted on them. Also, Pg *A. actinomicetemcomitans*, *Prevotella intermedia*, and *F. nucleatum* produce volatile sulfur compound (VSC) including hydrogen sulfide, methyl mercaptan that is linked to the onset of cancer.[43–45] The other way of developing DC may be due to the inhibition of T-cell proliferation by *F. nucleatum* that protect tumor cells.[37] Alcohol- and smoking-related carcinogens may be activated by oral microbiota because of high salivary aldehyde among drinkers and smokers.[46,47] Furthermore, *S. mitis*, *Streptococcus oralis*, *Streptococcus salivarius* produce acetaldehyde, the carcinogenic material by metabolizing alcohol.[48] Some microbes such as *Peptostreptococcus* produce various acids that can consolidate suitable hypoxic and acidic cellular environment enhancing GI metastasis.[49–51]

Zhang et al. suggested several ways that oral microbiota may induce carcinogenesis.[52] The first is related to chronic inflammation that accelerates the invasion process and metastasis of cells. First, anaerobic species especially *Porphyromonas*, *Prevotella*, and *Fusobacterium* stimulate the production of inflammatory mediators, for example, cytokines, tumor necrosis

factor-alpha (TNF-α) could develop fibroblast, epithelial and endothelial cells, and components of the extracellular matrix (ECM) and consequent increase of cell proliferation, growth, for example, and oncogenesis.[53,54] Second, Pg can activate host response through Toll-like receptor 2 (TLR2) and Toll-like receptor (TLR4) which can prevent apoptosis and increase the production of antiapoptotic factors followed by tumor growth. Third, bacterial generation such as *S. oralis*, *Lactobacillus*, and *Bifidobacterium* produce reactive oxygen species (ROS) and reactive nitrogen species (RNS), increasing epithelial mesenchymal transition (EMT) promoting mutagenesis.[55]

The most common fungi found in the oral cavity is *Candida*. *Candida albicans* (Ca) occasionally found in healthy individual's mouth can also be infectious after being dysregulated under various circumstances such as iatrogenic (trauma, prolonged steroid, antibiotics, significant dietary habit change) and physiological (e.g., pregnancy, elderly age, and puberty).[22,56–59] *Candida glabrata* can cause carcinogenesis by converting ethanol and glucose into aldehyde beyond normal level.[60] However, Contaldo M et al. reported no statistical difference in Ca levels between CG, CRC, and healthy individuals.[22]

Most of bacteria discussed in the reviewed studies were *Aggregatibacter*, *Porphyromonas*, *Treponema*, and *Tannerella*; establishing as a promising indicator for DC. However, not all studies found statistically significant results for association between those bacteria and DC. Overall, the reviewed studies suggest a multibacteria model in development and precipitating DC rather than a single oral bacterium that has limited ability to detect DC. There is a correlation between the right or left shift of oral microbiota and DC. These results suggested that the detection of samples derived from the oral cavity may be helpful in screening GIT cancer. This review article seems to be important not only for a better understanding of cancer growth regulation but also for clinical practice. However, the exact role of certain microbiomes in DC remains to be evaluated.

All the evidences suggested oral bacteria is linked to risk of DC regardless of type. However, the role of some other potential factors such as smoking, and alcohol abuse, drastically influence oral microbiota were adjusted in some studies.[10,23] Moreover other factors which may influence oral microbial patterns such as age, gender, diabetes, socioeconomic status were disregarded. Besides this factor, the role of oral hygiene, periodontal status, dietary pattern, and oral disease could influence the oral microbiome which should be taken into account in future studies.

The present systematic review has some limitations related to the interpretability and generalization of the results because of heterogeneity among studies. First, there is heterogeneity in sample selection and methodology (processing, storage temperature, storage medium, DNA extraction method, and statistical analysis). Second, different regions of study, lifestyles of people, culture, and taxonomical assignment may influence oral microbiota increasing probability of bias and heterogeneity.[61–63] Third, the protocol of assignment of participants to groups was not the same as most studies carried out definitive diagnosis only for case group, and the controls were included without endoscopies or histological study and there is no evidence of external validity proven till now. Lastly, the criteria of analysis and comparison of OR using the data available may be debatable because of different diagnostic criteria followed by authors.

## Conclusion

Despite the limited evidence available and different authors claiming different findings; this review article suggests oral microbial dysbiosis to be the noninvasive method for screening the probability of DC development. It is not possible to rule out the role of specific microbiota in DC to date.

There is large heterogeneity in sample collection and preservation; the method of performing tests limited the analyses of this review. Therefore, it is strongly recommended that homogeneity in aforesaid steps among studies would ascertain conclusion on a large basis with more comparable form. Future studies are needed for better knowledge of the dissemination pattern of oral microbiota from mouth to GIT and the possible role of specific oral microbiota on GI cancer.

## Orcid

*SK Aziz Ikbal* https://orcid.org/0000-0002-8014-2417
*Roopanshi Mehrotra* https://orcid.org/0000-0001-5356-7826

## References

1. Arnold M, Abnet CC, Neale RE, et al. Global burden of 5 major types of gastrointestinal cancer. Gastroenterology 2020;159:335–349. DOI: 10.1053/j.gastro.2020.02.068.
2. Wang Q, Chen Y, Wang X, et al. Consumption of fruit, but not vegetables, may reduce risk of gastric cancer: Results from a meta-analysis of cohort studies. Eur J Cancer 2014;50(8):1498–1509. DOI: 10.1016/j.ejca.2014.02.009.
3. Schwingshackl L, Schwedhelm C, Galbete C, et al. Adherence to mediterranean diet and risk of cancer: An updated systematic review and meta-analysis. Nutrients 2017;9(10):1063. DOI: 10.3390/nu9101063.
4. Karimi P, Islami F, Anandasabapathy S, et al. Gastric cancer: Descriptive epidemiology, risk factors, screening, and prevention. Cancer Epidemiol Biomark Prev 2014;23(5):700–713. DOI: 10.1158/1055-9965.EPI-13-1057.
5. Ishioka K, Masaoka H, Ito H, et al. Association between ALDH2 and ADH1B polymorphisms, alcohol drinking and gastric cancer: A replication and mediation analysis. Gastric Cancer 2018;21(6):936–945. DOI: 10.1007/s10120-018-0823-0.
6. Li WY, Han Y, Xu HM, et al. Smoking status and subsequent gastric cancer risk in men compared with women: A meta-analysis of prospective observational studies. BMC Cancer 2019;19(1):377. DOI: 10.1186/s12885-019-5601-9.
7. Castano-Rodriguez N, Kaakoush NO, Pardo AL, et al. Genetic polymorphisms in the Toll-like receptor signalling pathway in Helicobacter pylori infection and related gastric cancer. Hum Immunol 2014;75(8):808–815. DOI: 10.1016/j.humimm.2014.06.001.
8. Ghoshal U, Tripathi S, Kumar S, et al. Genetic polymorphism of cytochrome P450 (CYP) 1A1, CYP1A2, and CYP2E1 genes modulate susceptibility to gastric cancer in patients with Helicobacter pylori infection. Gastric Cancer 2014;17(2):226–234. DOI: 10.1007/s10120-013-0269-3.
9. Yang S, Park Y, Lee J, et al. Effects of soy product intake and interleukin genetic polymorphisms on early gastric cancer risk in Korea: A case–control study. Cancer research and treatment 2017;49(9):1044–1056. DOI: 10.4143/crt.2016.515.
10. Peters BA, Wu J, Pei Z, et al. Oral microbiome composition reflects prospective risk for esophageal cancers. Cancer Res 2017;77(23):6777–6787. DOI: 10.1158/0008-5472.CAN-17-1296.
11. Johansson I, Witkowska E, Kaveh B, et al. The microbiome in populations with a low and high prevalence of caries. J Dent Res 2016;95(1):80–86:2016. DOI: 10.1177/0022034515609554.

12. Coker OO, Dai Z, Nie Y, et al. Mucosal microbiome dysbiosis in gastric carcinogenesis. Gut 2018;67:1024–1032. DOI: 10.1136/gutjnl-2017-314281.
13. Farrell JJ, Zhang L, Zhou H, et al. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. Gut 2018;61(4):582–588. DOI: 10.1136/gutjnl-2011-300784.
14. Chen X, Winckler B, Lu M, et al. oral microbiota and risk for esophageal squamous cell carcinoma in a high-risk area of China. PLoS One 2015;10(12):e0143603. DOI: 10.1371/journal.pone.0143603.
15. Nagai H, Kim YH. Cancer prevention from the perspective of global cancer burden patterns. J Thorac Dis 2017;9(3):448–451. DOI: 10.21037/jtd.2017.02.75.
16. Stewart LA, Clarke M, Rovers M, et al. Preferred reporting items for a systematic review and meta-analysis of individual participant data: The PRISMA-IPD statement. JAMA 2015;313(16):1657–1665. DOI: 10.1001/jama.2015.3656.
17. Stang A. Critical evaluation of the Newcastle–Ottawa scale for the assessment of the quality of nonrandomized studies in meta-analyses. Eur J Epidemiol 2010;25(9):603–605. DOI: 10.1007/s10654-010-9491-z.
18. Wu J, Xu S, Xiang C, et al. Tongue coating microbiota community and risk effect on gastric cancer. J Cancer 2018;17:9(21):4039–4048. DOI: 10.7150/jca.25280.
19. Hu J, Han S, Chen Y, et al. Variations of tongue coating microbiota in patients with gastric cancer. Biomed Res Int 2015;2015:173729. DOI: 10.1155/2015/173729.
20. Sun JH, Li XL, Yin J, et al. A screening method for gastric cancer by oral microbiome detection. Oncol Rep 2018;39(5):2217–2224. DOI: 10.3892/or.2018.6286.
21. Xu S, Xiang C, Wu J, et al. Tongue coating bacteria as a potential stable biomarker for gastric cancer independent of lifestyle. Dig Dis Sci 2021;66(9):2964–2980. DOI: 10.1007/s10620-020-06637-0.
22. Contaldo M, Fusco A, Stiuso P, et al. Oral microbiota and salivary levels of oral pathogens in gastrointestinal diseases: Current knowledge and exploratory study. Microorganisms 2021;9(5):1064. DOI: 10.3390/microorganisms9051064.
23. Fan X, Alekseyenko AV, Wu J, et al. Human oral microbiome and prospective risk for pancreatic cancer: A population based nested case–control study. Gut 2018;67(1):120–127. DOI: 10.1136/gutjnl-2016-312580.
24. Torres PJ, Fletcher EM, Gibbons S, et al. Characterization of the salivary microbiome in patients with pancreatic cancer. Peer J 2015;3:e1373. DOI: 10.7717/peerj.1373.
25. Kato I, Vasquez AA, Moyerbrailean G, et al. Oral microbiome and history of smoking and colorectal cancer. J. Epidemiol Res 2015;2(2):92–101. DOI: 10.5430/jer.v2n2p92.
26. Lu H, Ren ZG, Li A, et al. Deep sequencing reveals microbiota dysbiosis of tongue coat in patients with liver carcinoma. Sci Rep 2016;6:33142. DOI: 10.1038/srep33142.
27. Salazar CR, Sun J, Li Y, et al. Association between selected oral pathogens and gastric precancerous lesions. PLoS One 2013;8(1):e51604. DOI: 10.1371/journal.pone.0051604.
28. Wei AL, Li M, Li GQ, et al. Oral microbiome and pancreatic cancer. World J Gastroenterol 2020;26(48):7679–7692. DOI: 10.3748/wjg.v26.i48.7679.
29. Cani PD. Human gut microbiome: Hopes, threats and promises. Gut 2018;67(9):1716–1725. DOI: 10.1136/gutjnl-2018-316723.
30. Huttenhower C, Gevers D, Knight R, et al. Structure, function and diversity of the healthy human microbiome. Nature 2012;486(7402):207–214. DOI: 10.1038/nature11234.
31. Lauka L, Reitano E, Carra MC, et al. Role of the intestinal microbiome in colorectal cancer surgery outcomes. World J Surg Oncol 2019:17(1):204. DOI: 10.1186/s12957-019-1754-x.
32. Ferlay J, Shin HR, Bray F, et al. Estimates of worldwide burden of cancer in 2008: GLOBOCAN 2008. Int J Cancer 2010;127(12): 2893–2917. DOI: 10.1002/ijc.25516.
33. McCoy AN, Araújo–Pérez F, Azcarate–Peril MA, et al. Fusobacterium Is Associated with Colorectal Adenomas. PLoS One 2013;8(1):e53653. DOI: 10.1371/journal.pone.0053653.
34. Sampaio–Maia B, Caldas IM, Pereira ML, et al. The oral microbiome in health and its implication in oral and systemic diseases. Adv Appl Microbiol 2016;97:171–210. DOI: 10.1016/bs.aambs.2016.08.002.
35. Cui J, Cui H, Yang M, et al. Tongue coating microbiome as a potential biomarker for gastritis including precancerous cascade. Protein Cell 2019;10(7):496–509. DOI: 10.1007/s13238-018-0596-6.
36. Li X, Wong GL, To K, et al. Bacterial microbiota profiling in gastritis without Helicobacter pylori infection or non-steroidal anti-inflammatory drug use. PLoS One 2009;4(11):e7985. DOI: 10.1371/journal.pone.0007985.
37. Yamamura K, Baba Y, Miyake K, et al. Fusobacterium nucleatum in gastroenterological cancer: Evaluation of measurement methods using quantitative polymerase chain reaction and a literature review. Oncol Lett 2017;14(6):6373–6378. DOI: 10.3892/ol.2017.7001.
38. Hsieh YY, Tung SY, Pan HY, et al. Increased abundance of Clostridium and Fusobacterium in gastric microbiota of patients with gastric cancer in Taiwan. Sci Rep 2018;8(1):158. DOI: 10.1038/s41598-017-18596-0.
39. Schmidt TS, Hayward MR, Coelho LP, et al. Extensive transmission of microbes along the gastrointestinal tract. Elife 2019;8:e42693. DOI: 10.7554/eLife.42693.
40. Mai X, Genco RJ, LaMonte MJ, et al. Periodontal pathogens and risk of incident cancer in postmenopausal females: The buffalo osteoperio study. J Periondontol 2016;87(3):257–267. DOI: 10.1902/jop.2015.150433.
41. Cordero OJ, Varela–Calviño R. Oral hygiene might prevent cancer. Heliyon 2018;4(10):e00879. DOI: 10.1016/j.heliyon.2018.e00879.
42. Flemer B, Warren RD, Barrett MP, et al. The oral microbiota in colorectal cancer is distinctive and predictive. Gut 2018;67(8):1454–1463. DOI: 10.1136/gutjnl-2017-314814.
43. Attene–Ramos MS, Wagner ED, Plewa MJ, et al. Evidence that hydrogen sulfide is a genotoxic agent. Mol Cancer Res 2006;4(1):9–14. DOI: 10.1158/1541-7786.MCR-05-0126.
44. Hellmich MR, Szabo C. Hydrogen sulfide and cancer. Handb Exp Pharmacol 2015;230:233–241. DOI: 10.1007/978-3-319-18144-8_12.
45. Singh SB, Lin HC. Hydrogen sulfide in physiology and diseases of the digestive tract. Microorganisms 2015;3(4):866–889. DOI: 10.3390/microorganisms3040866.
46. Kiyabu MT, Shibata D, Arnheim N, et al. Detection of human papillomavirus in formalin-fixed, invasive squamous carcinomas using the polymerase chain reaction. Am J Surg Pathol 1989;13(3):221–224. DOI: 10.1097/00000478-198903000-00007.
47. Rota MT, Poggi P. Reduction of oral acetaldehyde levels using a controlled-release chlorhexidine chip as a prevention strategy against upper digestive tract cancer. Med Hypotheses 2003;60(6):856–858. DOI: 10.1016/s0306-9877(03)00063-x.
48. Pavlova SI, Jin L, Gasparovich SR, et al. Multiple alcohol dehydrogenases but no functional acetaldehyde dehydrogenase causing excessive acetaldehyde production from ethanol by oral streptococci. Microbiology 2013;159(Pt 7):1437–1446. DOI: 10.1099/mic.0.066258-0.
49. Downes J, Wade WG. Peptostreptococcus stomatis sp. isolated from the human oral cavity. Int J Syst Evol Microb 2006;56(Pt 4):751–754. DOI: 10.1099/ijs.0.64041-0.
50. Lunt SJ, Chaudary N, Hill RP. The tumor microenvironment and metastatic disease. Clin Exp Metastasis 2009;26(1):19–34. DOI: 10.1007/s10585-008-9182-2.
51. Mazzio E, Smith B, Soliman K. Evaluation of endogenous acidic metabolic products associated with carbohydrate metabolism in tumor cells. Cell Biol Toxicol 2010;26(3):177–188. DOI: 10.1007/s10565-009-9138-6.
52. Zhang Y, Wang X, Li H, et al. Human oral microbiota and its modulation for oral health. Biomed Pharmacother 2018;99:883–893. DOI: 10.1016/j.biopha.2018.01.146.

53. Meng C, Bai C, Brown TD, et al. Microbiota and gastrointestinal cancer. Genomics Proteomics Bioinformatics 2018;16(1):33–49. DOI: 10.1016/j.gpb.2017.06.002.
54. Gholizadeh P, Eslami H, Kafil HS. Carcinogenesis mechanisms of Fusobacterium nucleatum. Biomed Pharmacother 2017;89:918–925. DOI: 10.1016/j.biopha.2017.02.102.
55. Vinasco K, Mitchell HM, Kaakoush NO, et al. Microbial carcinogenesis: Lactic acid bacteria in gastric cancer. Biochim Biophys Acta Rev Cancer 2019;1872(2):188309. DOI: 10.1016/j.bbcan.2019.07.004.
56. Petruzzi M, Della Vella F, et al. Dorsal tongue porphyrin autofluorescence and Candida saprophytism: A prospective observational study. PLoS One 2019;14(9):e0223072. DOI: 10.1371/journal.pone.0223072.
57. Milillo L, Lo Muzio L, Carlino P, et al. Candida-related denture stomatitis: A pilot study of the efficacy of an amorolfine antifungal varnish. Int J Prosthodont 2005;18(1):55–59. PMID: 15754893.
58. Sardaro N, Della Vella F, Incalza MA, et al. Oxidative stress and oral mucosal diseases: An overview. In Vivo 2019;33(2):289–296. DOI: 10.21873/invivo.11474.
59. Paoletti I, Fusco A, Grimaldi E, et al. Assessment of host defence mechanisms induced by Candida species. Int J Immunopathol Pharmacol 2013;26(3):663–672. DOI: 10.1177/039463201302600309.
60. Srinivasprasad V, Dineshshankar J, Sathiyajeeva J, et al. Liaison between micro organisms and oral cancer. J Pharm Bioallied Sci 2015;7(Suppl. 2):354–S360. DOI: 10.4103/0975-7406.163451.
61. Goodrich JK, Di Rienzi SC, Poole AC, et al. Conducting a microbiome study. Cell 2014;158(2):250–262. DOI: 10.1016/j.cell.2014.06.037.
62. Luo T, Srinivasan U, Ramadugu K, et al. Effects of specimen collection methodologies and storage conditions on the short-term stability of oral microbiome taxonomy. Appl Environ Microbiol 2016;82(18):5519–5529. DOI: 10.1128/AEM.01132-16.
63. Teng F, Nair SS, Zhu PF, et al. Impact of DNA extraction method and targeted 16S-rRNA hypervariable region on oral microbiota profiling. Sci Rep 2018;8(1):16321. DOI: 10.1038/s41598-018-34294-x.