

THE MICROBIOME IN OESOPHAGEAL DISEASE – A YEAR IN REVIEW

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Abstract: There is an increasing appreciation of the role that the host microbiome plays in the progression of oesophageal diseases. To date, potential relationships between the microbiota and the development of gastro-oesophageal reflux disease, Barrett's oesophagus, oesophageal adenocarcinoma, oesophageal squamous cell carcinoma, eosinophilic oesophagitis, and achalasia have been reported. Studies have not been limited to the local oesophageal microbiota but have also examined the oral and lower gut microbiotas. Comprehensive literature searches were performed on these topics for articles published from April, 2019 to March 2020 to provide an update on associations between the microbiota and oesophageal disease. The identified studies generally comprised cross-sectional profiling of differences in the microbiome between cases and controls. One robust study examined a mouse model of Barrett's oesophagus expressing human interleukin (IL)-1B and found that high fat diet accelerated the development of dysplasia partly through modifying the gut microbiota and this was independent of obesity. A substantial number of studies associated viral infections with oesophagitis; however, surprisingly, to date no virome studies have been attempted. Research areas currently lacking include holistic analyses of different microbiotas in the context of oesophageal disease, metabolomic studies, as well as studies profiling the virome and mycobiome. The oral, oesophageal and gut microbiota appear to play some role in the development of oesophageal disease; however, causation is currently absent in most published studies.

Keywords: Esophagus, Esophageal, Microbiota, Esophagitis, Adenocarcinoma.

INTRODUCTION

It has been proposed that several oesophageal diseases have some level of microbial contribution to their aetiology^{1,2}. These diseases include gastro-oesophageal reflux disease (GORD), Barrett's oesophagus (BO), oesophageal adenocarcinoma (OAC), oesophageal squamous cell carcinoma (OSCC), eosinophilic oesophagitis (EO), and achalasia^{1,2}. The purpose of this review was to provide an update on the literature in this research area that was published between April, 2019 and March, 2020. Thus, a search for articles published in this time period with the parameters [(Esophageal OR Esophagus OR Oesophageal OR Oesophagus) AND (Microbiota OR Microbiome)] was performed, yielding 56 articles. Twenty articles remained upon elimination of reviews and non-relevant articles. This search was adjusted to include the keywords virome or mycobiome and this resulted in no articles. The above searches were then complemented with less strict keywords replacing microbiome/microbiota with virus or viral (n=120 articles), bacterial, bacterium or bacterial (n=196 articles), as well as fungus, fungi or fungal (n=140 articles). Following filtration, 22 putatively relevant articles remained, resulting in a total of 42 articles potentially associated with this topic. Here, the evidence presented in these articles for a role for the microbiome in oesophageal disease is reviewed.

A ROLE FOR THE OESOPHAGEAL MICROBIOTA

Studies examining the oesophageal microbiota (n=11) were in the context of assessing clinical sampling as well as GORD, BO, OAC, OSCC and functional dyspepsia.

Kashiwagi et al³ recruited 17 healthy individuals and collected five mucosal samples from the upper gastrointestinal tract, one of which was from the middle of the oesophagus. The authors found that mucosal brushings were sufficient to obtain enough material to profile the microbiota without any adverse effects³. In support, Liu et al⁴ compared microbiota profiles from mucosal swabs and biopsies in 67 patients with OSCC, and found that both sampling methods result in similar microbial profiles. Okereke et al⁵ enrolled 17 patients with BO in an effort to compare the microbiota within mucosal biopsies from the oesophagus with the microbiota from swabs of the uvula and endoscope, and found no correlation across the samples, concluding that mucosal sampling is required for optimal profiling.

One study analysed the differences in oesophageal samples (mid-oesophagus) from control subjects classified as healthy and patients with functional dyspepsia, and reported a higher level of *Streptococcus* in samples from patients with functional dyspepsia⁶.

Two studies^{7,8} assessed the oesophageal microbiota in patients with GORD as compared to individuals with normal oesophagi. Yu et al⁷ collected mucosal biopsies two centimeters above the gastro-oesophageal junction and found no substantial differences in microbial profiles across the two groups, except for a decrease in relative abundance of *Prevotella*, *Helicobacter*, and *Moraxella* in patients with GORD. Kayar Dogan et al⁸ employed qPCR to determine bacterial load and the levels of specific bacterial species in 20 subjects with normal esophagi and 30 patients with GORD. The authors found no differences in bacterial load across the groups but showed higher levels of Gram-negative species in GORD.

Two studies investigated the oesophageal microbiota in patients with BO. Snider et al⁹ collected oesophageal brushings from 45 patients comprising controls (n=16), BO without dysplasia (n=14), low-grade dysplasia (n=6), high-grade dysplasia (n=5), and OAC (n=4). The authors profiled the microbiota using 16S rRNA amplicon sequencing and observed differences in beta-diversity upon transition from low-grade dysplasia to high-grade dysplasia, resulting from a decrease in *Veillonella* and increases in *Enterobacteriaceae* and *Akkermansia muciniphila*⁹. Patients on proton pump inhibitors were found to have higher levels of *Streptococcus*⁹. Okereke et al¹⁰, used normal oesophageal mucosal samples from within the same patients to examine differences in microbial profiles in BO tissue and identified higher relative abundance of *Haemophilus* in BO tissue. However, a meta-analysis including 46 articles published prior to September, 2018 and comprising 119,273 subjects concluded that the microbiome was not associated with an increased risk of BO ($p>0.05$, OR: 1.27, 95% CI: 0.66-2.43)¹¹.

The remaining studies profiled the oesophageal microbiota in the context of OSCC. Shao et al¹² analysed the microbiota in paired tumour and non-tumour samples from 67 patients and identified a higher relative abundance of *Fusobacterium* and less *Streptococcus* in tumour tissue. In support, Yamamura et al¹³ also found an important role for *Fusobacterium* in OSCC. The authors assayed tumour tissues from 551 patients and identified *Fusobacterium nucleatum* load to be associated with recurrence-free survival¹³. *F. nucleatum* levels within the tumour was also correlated with poor response to chemotherapy¹³.

Overall, the recent literature does not provide strong evidence for substantial shifts in the composition of the oesophageal microbiota with disease; however, differential abundance of specific bacterial species with pro-inflammatory capacity is common in subjects with disease.

A ROLE FOR THE ORAL MICROBIOTA

The oral microbiota has been implicated as a reservoir of detrimental microbial species in a range of conditions, in particular, in conditions where the resident microbiota loses stability. Four case-control studies examined the oral microbiota through sampling saliva samples, and this was in the context of EO and oesophageal cancers¹⁴⁻¹⁷.

Hiremath et al¹⁴ profiled the salivary microbiota of 26 paediatric subjects with EO and 19 age- and ethnicity-matched controls. Children with EO were found to have lower bacterial

richness and higher relative abundance of *Streptococcus* as compared to controls¹⁴. In EO patients, *Haemophilus* was observed to correlate with disease activity as assessed by the EO Histology Scoring System¹⁴.

The remaining studies assessed the salivary microbiota and its association with oesophageal cancers. Tanda et al¹⁶ examined if professional oral care had an influence on levels of acetaldehyde, a potential carcinogen, in 21 esophageal cancer patients and 20 age-matched controls and if this correlated with microbial load. While no changes in acetaldehyde levels were found after oral care in controls, a significant decrease in levels were seen in patients both after oral care and after surgery, and this was related to decreased in microbial acetaldehyde production from glucose¹⁶. In support of microbial metabolic differences between controls and patients with cancer, Kageyama et al¹⁵ assessed the salivary microbiota in 12 patients with oesophageal cancer and 112 age- and sex-matched controls, and found a higher bacterial richness in patients with esophageal cancer, as well as higher levels of *Corynebacterium*, *Porphyromonas gingivalis*, and *F. nucleatum*. In contrast, Wang et al¹⁷ examined saliva samples from 20 patients with OSCC and 21 controls and showed lower bacterial richness, higher *Actinomyces* and *Atopobium*, and lower *Fusobacterium* and *Porphyromonas* in OSCC. The differences across the studies may be related to the histological type of cancer.

While there is evidence of differences in the oral microbiota in oesophageal disease, the findings lack consistency. Studies should match profiling oral microbial communities with assessing microbial metabolic output to properly delineate these taxonomic inconsistencies.

A ROLE FOR THE INTESTINAL MICROBIOTA

A comprehensive study by Münch et al¹⁸ assessed the cross-talk between high fat diet, the intestinal (faecal) microbiota, and the oesophageal microenvironment in two mouse models that develop BO (humanised IL-1B and humanised IL-1B and IL-8) as well as a clinical cohort of BO and OAC. The authors found that mice expressing humanised IL-1B fed a high fat diet progressed to oesophageal dysplasia faster than mice on a control diet, and this was associated with a shift in the faecal microbiota and increased ratio of oesophageal neutrophils to natural killer cells¹⁸. Faster tumour progression was confirmed through faecal transfer from mice on high fat diet to mice on control diet, and decreased oesophageal dysplasia was shown in mice raised in germ-free conditions¹⁸, providing robust evidence that the gut microbiota influences the oesophageal microenvironment to modulate the risk of progression from metaplasia to dysplasia. In support of a strong systemic effect on the OAC cascade, Cook et al¹⁹ showed that increased circulating soluble tumour necrosis factor receptor 2 (sTNFR2) was associated with high risk of OAC after assessing inflammation levels in 296 cases of OAC and 296 matched controls. Notably, 33% of the effect of waist circumference on OAC risk could be attributed to sTNFR2 levels¹⁹.

There is some indication that the gut microbiota can influence outcome of chemotherapy in oesophageal cancer²⁰ as well as recovery following intervention²¹. Using a model of BALB/c nude mice injected with OSCC cells (Eca109), Zhou et al²⁰ showed that cepharanthine hydrochloride could modulate the efficacy of cisplatin, putatively through modulating the gut microbiota and activating innate immune pathways and apoptosis. Motoori et al²¹ provided 55 patients with oesophageal cancer with synbiotics prior to esophagectomy to assess the capacity of the gut microbiota to protect against post-operative infectious complications through the production of short chain fatty acids (SCFAs). The authors observed higher levels of lactic acid and lower production of the SCFAs acetic acid, propionic acid, and butyric acid in the ten patients that had infectious complications, with the ratio of SCFAs to lactic acid found to be a risk factor for infection following intervention²¹. Intriguingly, the use of antibiotics has been found to be associated with worse outcomes in OSCC, including lower progression-free survival and overall survival²².

Based on these findings, there is an urgent requirement for additional studies addressing the systemic effects of the intestinal microbiota on the local oesophageal microenvironment. The animal models employed and developed by Münch et al¹⁸, as well as the putative role of IL-1RA and IL-1A cancer cell survival, highlight the relevance of IL-1 signaling, as well as inflammasome activation given previous reports on the importance of IL-18 in oesophageal disease^{23,24}. Holistic assessment of the oral and oesophageal microbiotas in these animal models would be valuable (Figure 1).

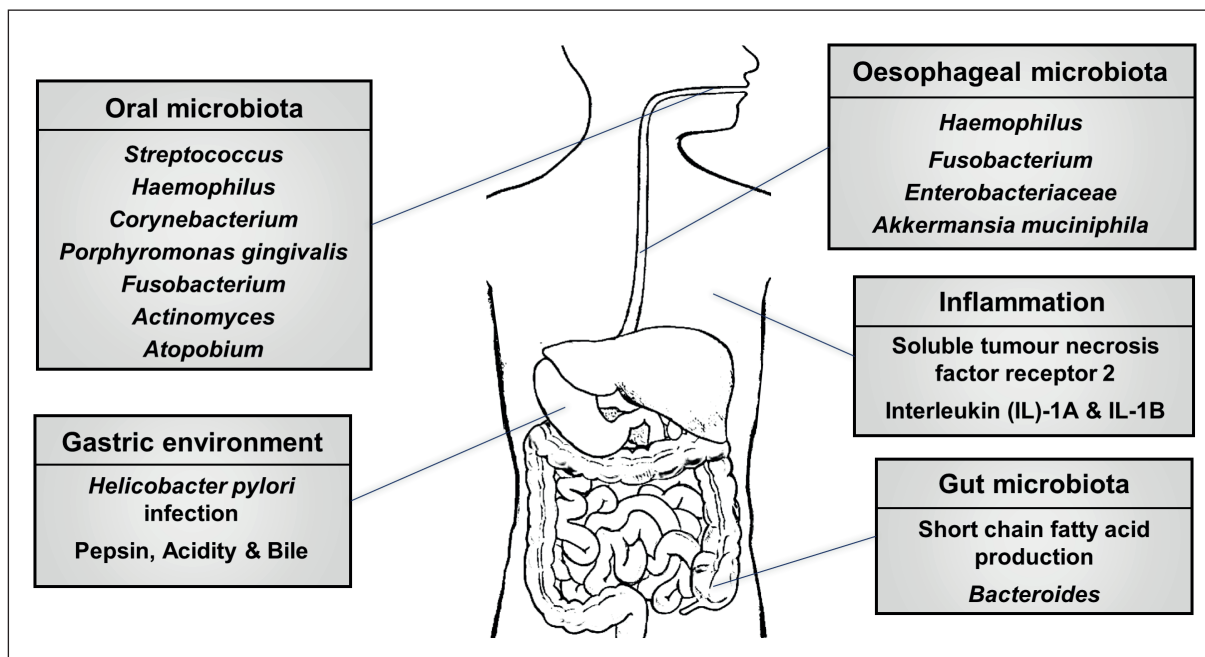


Figure 1. Microbes and associated factors that influence the oesophageal microenvironment. The oral microbiota can exert effects on levels of systemic inflammation and likely acts as a reservoir of pro-inflammatory species that populate the oesophagus when homeostasis is altered. The oesophageal microbiota can impact the local microenvironment through effects on host gene expression and inflammation, as well as production of metabolites. The gastric environment, in particular infection with *Helicobacter pylori*, production of pepsin, and levels of acid and bile in refluxate, can affect the progression of oesophageal disease. The gut microbiota plays an important role in host homeostasis. Its influence on systemic inflammation and host adiposity likely exerts a significant effect on the oesophagus. Only findings from the reviewed time period were included.

STUDIES IMPLICATING SPECIFIC MICROBIAL SPECIES

The literature searches also identified a large number of studies that assessed the carriage of specific viruses in oesophageal disease. These included studies on the possible role of herpes simplex virus in EO²⁵, achalasia²⁶, and oesophagitis²⁷⁻²⁹, as well as cytomegalovirus in oesophagitis³⁰. Infection with human immunodeficiency virus was also reported as a risk factor for OSCC but not OAC, with subjects with low CD4 counts being at most risk³¹.

The most studied virus was human papillomavirus (HPV). Tasneem et al³² detected HPV in ~3% of 121 patients with OSCC while Stelow et al³³ presented four cases of high-grade squamous intraepithelial lesions secondary to HPV. In contrast, Cappelleso et al³⁴ did not find an association between HPV and nine cases of verrucous carcinoma, a rare type of OSCC. Both Boon et al³⁵ and Xia et al³⁶ studied the impact of HPV on oesophageal cancer cells, and suggested that HPV can induce oncogenic pathways in these cells.

HPV has also been studied in the context of BO and OAC. Parameshwaran et al³⁷ employed droplet PCR to detect the E7 gene of HPV 16 and 18 in patients with Barrett's dysplasia (n=48) or OAC (n=41) and in controls (n=49). HPV was detected in 8.3% of Barrett's dysplasia and 14.6% OAC plasma samples as compared to 2% of controls, with detection in plasma correlating with oesophageal tissue positivity, and potentially, with disease severity³⁷. Similarly, Rajendra et al³⁸ found that antibodies against HPV were not frequent ($\leq 10.2\%$) in patients with reflux, BO, and Barrett's dysplasia/OAC, but that antibodies to HPV 16 E7 and HPV 18 E1 were significantly more prevalent in HPV-positive dysplasia and OAC than in controls. Notably, in another study by Rajendra et al³⁹, the authors reported that HPV positivity in combination with associated biomarkers was a significant predictor of survival benefit in Barrett's high-grade dysplasia and OAC, suggesting that HPV-associated cases may be less aggressive.

Given the strong evidence supporting several viruses as agents of oesophagitis, it is surprising that no oesophageal virome studies have been conducted to date. In addition to viruses, *Candida* species are a common cause of oesophagitis in immunocompromised and diabetic individuals. Given their presence and capacity to induce disease in this environment⁴⁰, studies should also assess the oesophageal mycobiome.

FACTORS ASSOCIATED WITH THE GASTROINTESTINAL MICROBIOTA

Helicobacter pylori infection has been implicated in the protection against of oesophageal diseases, in particular BO and OAC²⁴, and is known to influence the composition of the gastrointestinal microbiota. In a recent study, Doorackers et al⁴¹ showed that eradication of *H. pylori* does not increase the risk of developing BO or OAC in a cohort of 81,919 subjects, suggesting that the protective effects of *H. pylori* are more likely linked to immune training since childhood. In support of an association between *H. pylori* and oesophageal disease, a recent meta-analysis by Shah et al⁴² provided evidence for a protective role for *H. pylori* infection in EO.

Additional factors of relevance include use of proton pump inhibitors (PPIs), as they can increase the risk of developing oesophageal cancer⁴³, and obesity which appears to aggravate the immune responses in EO⁴⁴. It would be important to establish the contribution of the gut microbiota to these effects, given that both PPIs and obesity have been strongly linked to gut microbiota diversity.

CONCLUSIONS

There is accumulating evidence that gastrointestinal microbiotas are associated with several oesophageal diseases, through their differential effects on local and systemic inflammation; however, there is a paucity of data establishing causality. Future studies should focus on longitudinal clinical cohorts, holistic analysis of the oral, oesophageal and intestinal microbiotas in the context of oesophageal disease, as well as animal models that establish defined microbial communities in the oesophagus.

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Conflict of Interest

The author declares that no financial or potential competing interests exist.

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