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Review

Exploring Esophageal Microbiomes in Esophageal Diseases: A Systematic Review

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Studies that investigated esophageal microbiomes are limited when compared to those on intestinal microbiomes. Nevertheless, several studies have investigated the relationship between esophageal microbiomes and various esophageal diseases, owing to the advancement of next-generation sequencing techniques. *Streptococcus* is the most common bacterial taxon in a normal esophagus. Additionally, *Haemophilus*, *Neisseria*, *Prevotella*, and *Veillonella* are also found. However, gram-negative bacteria, including *Prevotella*, are more abundant in a diseased esophagus, such as in gastroesophageal reflux disease and Barrett's esophagus. This systematic review aims to summarize current evidences on esophageal microbiomes in various esophageal diseases.
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Key Words
Barrett esophagus; Eosinophilic esophagitis; Esophageal neoplasms; Gastroesophageal reflux; Microbiota

Introduction

While intestinal microbiomes have been relatively well studied, upper gastrointestinal tract microbiomes have not been thoroughly evaluated. Especially, studies on esophageal microbiomes are relatively limited. Traditionally, the esophagus is regarded as devoid of a significant bacterial population.[1,2] In addition, microbial flora in a normal esophagus has been considered transient and translocated from the oropharynx.[3] In 1998, Gagliardi et al[3] revealed that *Streptococcus viridans* is the most commonly found microorganism in esophageal cultures, which is also isolated from oropharyngeal cultures.

However, next-generation sequencing techniques such as 16S ribosomal RNA (rRNA) gene sequencing have been increasingly used to open a new horizon for microbial research nowadays.[4] The technique allowed recognition of uncultured bacteria, facilitating easy identification of differences in microbial composition between a normal and diseased esophagus.[5] Currently, the esophagus has been found to contain a diverse microbiome.[6,7] Additionally, several studies evaluated the microbial composition of a normal esophagus as well as various esophageal diseases such as gastroesophageal reflux disease (GERD), Barrett's esophagus, esophageal cancer, and eosinophilic esophagitis (EoE).[2] Here, we performed a systematic review on the variation in microbial composition according to the esophageal diseases.

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Methods

Search Strategy

We searched for all relevant studies published between January 1980 and February 2020 that examined the human esophageal microbiome using the MEDLINE, EMBASE, and Cochrane Library databases. The following search string was used: ([esophagus] OR [oesophagus] OR [esophageal] OR [oesophageal]) AND ([microbiome] OR [microbiota] OR [microbial] OR [microflora] OR [biota] OR [bacterial flora] OR [bacterial biofilm]). Appendix 1 shows the detailed search strategies in each database.

Inclusion/Exclusion Criteria

The inclusion criteria were as follows: (1) healthy individuals or patients with esophageal diseases including GERD, esophageal cancer, EoE, and achalasia, and (2) composition or any other findings about the esophageal microbiome. Non-original studies, non-human studies, abstract-only publications, and studies published in languages other than English were excluded.

Study Selection

First, we reviewed the titles and abstracts of the research papers found during our keyword search. Duplicates from multiple search engines were removed. Next, irrelevant studies were excluded by title and abstract review according to our inclusion and exclusion criteria. We screened the full text of all remaining studies. Two investigators (C.H.P. and S.K.L.) independently evaluated the studies for eligibility. Any disagreements were resolved through discussion and consensus.

Data Extraction

Data were extracted using a data extraction form that had been developed in advance. Two investigators (C.H.P. and S.K.L.) independently extracted the following information: first author, year of publication, country, study period, population, publication language, and study outcomes.

Results

Study Selection

Figure 1 shows the study flow diagram for our systematic review. Our literature search identified 682 studies. After examining the titles and abstracts, we discarded 200 duplicate articles, which were retrieved through multiple search engines. Another 444 irrelevant articles were excluded on the basis of their titles and abstracts. After reviewing the full text of the 38 remaining articles, we further excluded 5 articles that did not report the relevant outcomes. Additionally, 1 non-original article and 2 articles in which full-texts were unavailable were excluded. Finally, 30 studies were included in the systematic review[3,5,6,8-34] The main findings about esophageal microbiome of these studies are summarized in Table.

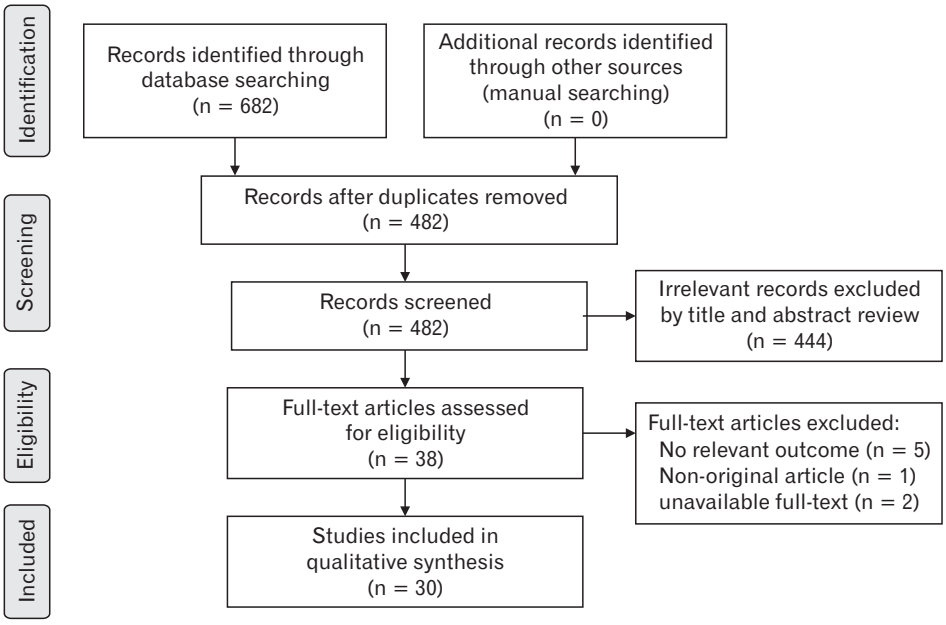

Figure 1. The study flow diagram.

Table. Summary of Studies on Esophageal Microbiome

Study	Country	Study period	Population	Analysis	Main findings about esophageal microbiome
1982, Finlay et al[8]	UK	N/A	12 patients with esophageal cancer	Culture	*Streptococcus*, Coagulase-negative *Staphylococcus*, and *Lactobacillus* were prevalent in patients with esophageal cancer.
1983, Mannell et al[9]	South Africa	N/A	50 individuals without esophageal disease, 51 patients with esophageal cancer	Culture	*Streptococcus viridans*, *Haemophilus influenzae*, and *Klebsiella pneumoniae* were abundant in both individuals with normal esophagus and patients with esophageal cancer.
1998, Gagliardi et al[3]	Brazil	N/A	30 patients with dyspepsia	Culture	*Streptococcus viridans* and group D *Streptococcus* were prevalent in patients with dyspepsia.
2004, Pei et al[6]	USA	N/A	4 normal individuals	16S rRNA gene sequencing	*Streptococcus*, *Prevotella*, and *Veillonella* were most prevalent in the esophageal microbiome.
2005, Pei et al[10]	USA	N/A	9 normal individuals, 12 patients with GERD, 3 patients with BE	16S rRNA gene sequencing	Bacteroidetes, Proteobacteria, and Firmicutes were abundant in the esophageal microbiome.
2007, Macfarlane et al[11]	UK	N/A	7 individuals with gastrointestinal symptoms requiring endoscopic examination, 7 patients with BE	Culture, 16S rRNA gene sequencing	*Campylobacter* was abundant in patients with BE, while it was not identified in patients with control.
2007, Zilberstein et al[12]	Brazil	N/A	10 normal individuals	Culture	*Streptococcus* (40.0%), *Staphylococcus* (20.0%), *Corynebacterium* (10.0%), *Lactobacillus* (10.0%), and *Peptococcus* (10.0%) were identified in the esophagus.
2009, Yang et al[13]	USA	N/A	12 normal individuals, 12 patients with esophagitis, 10 patients with BE	16S rRNA gene sequencing	Type I microbiome, dominated by the *Streptococcus*, correlated with normal esophagus, while type II microbiome contained a greater proportion of gram-negative anaerobes/microaerophiles correlated with esophagitis.
2012, Fillon et al[14]	USA	N/A	15 pediatric individuals who scheduled upper endoscopy	16S rRNA gene sequencing	*Streptococcus*, *Prevotella*, and *Veillonella* were most prevalent in the esophageal microbiome.
2013, Blackett et al[15]	Scotland	N/A	39 patients with iron deficiency anemia, 37 patients with GERD, 45 patients with BE	Culture	*Campylobacter* was prevalent in patients with GERD or BE compared to the control (patients with iron deficiency anemia).
2013, Liu et al[16]	Japan	2008-2009	6 normal individuals, 6 patients with reflux esophagitis, 6 patients with BE	16S rRNA gene sequencing	Proteobacteria was most prevalent in normal individuals (49.0%) and patients with reflux esophagitis (43.0%), while Firmicutes was most prevalent in patients with BE.
2013, Norder Grusell et al[5]	Sweden	2006-2009	40 individuals without gastrointestinal disease	Culture	*Streptococccus Viridans* was most prevalent in the esophagus.
2014, Amir et al[17]	Israel	N/A	15 individuals with normal esophageal mucosa, 13 patients with esophagitis, 6 patients with BE	16S rRNA gene sequencing	Enterobacteriaceae was associated with an abnormal esophagus. Proton pump inhibitor treatment changes the composition of esophageal microbiome.
2014, Yu et al[18]	China	2002	192 subjects without esophageal squamous dysplasia, 142 patients with esophageal squamous dysplasia	Human Oral Microbe Identification Microarray	Lower microbial richness was associated with the presence of esophageal squamous dysplasia.
2015, Gall et al[19]	USA	1983-2008	12 patients with BE	16S rRNA gene sequencing	*Streptococcus* to *Prevotella* species ratio was associated with progression of Barrett's esophagus.

Table. Continued

Study	Country	Study period	Population	Analysis	Main findings about esophageal microbiome
2015, Harris et al[20]	USA	N/A	25 normal individuals, 8 patients with GERD, 37 patients with EoE	16S rRNA gene sequencing	*Haemophilus* was significantly increased in untreated EoE subjects as compared with normal subjects. *Streptococcus* was decreased in GERD subjects on proton pump inhibitor as compared with normal subjects.
2015, Benitez et al[21]	USA	N/A	35 non-EoE pediatric individuals, 33 pediatric patients with EoE	16S rRNA gene sequencing	Proteobacteria including *Neisseria* and *Corynebacterium* was enriched in patients with EoE, while Firmicutes was predominant in non-EoE pediatric individuals.
2016, Yamamura et al[22]	Japan	2005-2013	325 patients with esophageal cancer	Polymerase chain reaction	*Fusobacterium nucleatum* in esophageal cancer tissues was associated with shorter survival.
2017, Elliott et al[23]	UK	N/A	20 normal individuals, 24 patients with non-dysplastic BE, 23 patients with dysplastic BE, 19 patients with BAC	16S rRNA gene sequencing	*Lactobacillus fermentum* was enriched in esophageal adenocarcinoma. Microbial diversity in patients with high-grade dysplasia decreased in comparison to control.
2017, Peters et al[24]	USA	N/A	210 normal individuals, 81 patients with EAC, 25 patients with ESCC	16S rRNA gene sequencing	Decreased abundance of *Neisseria* and *Streptococcus pneumoniae* was associated with lower risk of EAC. *Porphyromonas gingivalis* tended to be associated with higher risk of ESCC.
2018, Deshpande et al[25]	Australia	N/A	106 patients with gastrointestinal symptoms	16S rRNA gene sequencing	The interaction between *Streptococcus mitis/oralis/pneumoniae* and *Prevotella* spp. was found to be a co-exclusion interaction. The ratio of *Streptococcus* to *Prevotella* is an important defining characteristic across esophageal community types.
2018, Dong et al[26]	China	2015	27 normal individuals	16S rRNA gene sequencing	*Streptococcus* was more prevalent in the esophagus than in the oral cavity.
2018, Nobel et al[27]	USA	N/A	47 ambulatory patients scheduled to undergo endoscopy	16S rRNA gene sequencing	Increasing fiber intake was significantly associated with increasing relative abundance of Firmicutes.
2019, Liu et al[28]	China	2015-2016	67 patients with ESCC	16S rRNA gene sequencing	Mucosal swab specimens and biopsies yielded similar microbial profiles in patients with ESCC.
2019, Okereke et al[29]	USA	N/A	12 patients with BE	16S rRNA gene sequencing	*Streptococcus* were widespread throughout the esophagus (from proximal to distal esophagus).
2019, Okereke et al[30]	USA	N/A	17 patients with BE	16S rRNA gene sequencing	*Streptococcus* and *Alloiococcus* were more prevalent in the esophagus compared to the uvular.
2019, Shao et al[31]	China	2015	67 patients with ESCC	16S rRNA gene sequencing	The abundance of *Fusobacterium* was increased, while that of *Streptococcus* was decreased in the tumor tissues compared to non-tumor tissues in patients with ESCC.
2019, Snider et al[32]	USA	N/A	16 normal individuals, 14 patients with non-dysplastic BE, 10 patients with dysplastic BE, 4 patients with BAC	16S rRNA gene sequencing	Patients with high-grade dysplasia or adenocarcinoma increased Enterobacteriaceae and *Akkermansia muciniphila* and reduced *Veillonella*. Patients taking proton pump inhibitors increased *Streptococcus* and decreased Gram-negative bacteria.
2019, Yamamura et al[33]	Japan	2001-2016	551 patients with ESCC	Polymerase chain reaction	High burden of F. nucleatum was associated with poor recurrence-free survival in patients with ESCC.
2019, Yu et al[34]	China	2017	17 normal individuals, 32 patients with reflux esophagitis	16S rRNA gene sequencing	The richness and diversity of esophageal microbiome tended to be decreased in patients with reflux esophagitis.

N/A, not available; rRNA, ribosomal RNA; GERD, gastroesophageal reflux disease; BE, Barrett's esophagus; BAC, Barrett's adenocarcinoma; ESCC, esophageal squamous cell carcinoma; EoE, eosinophilic esophagitis.

Microbiome in a Normal Esophagus

The first study on microbiomes in a normal esophagus, based on bacterial cultures, was conducted by Mannell et al[9] in 1983. In their study, *S. viridans*, *Haemophilus influenzae*, *Neisseria catarrhalis*, *Streptococcus* group B, *Streptococcus faecalis*, and *Klebsiella pneumonia* were commonly isolated in aspirates from the normal esophagus. They also demonstrated that the esophagus is unsterile. The following studies also revealed that various bacteria can be found in a normal esophagus. In 1998, Gagliardi et al[3] tried to culture aspirate samples from 30 patients with nonspecific dyspepsia. Among them, *S. viridans* was most commonly found and isolated from 9 samples (30.0%). Group D *Streptococcus*, *Enterococcus*, *Staphylococcus aureus*, and *Klebsiella* were also isolated (20.0%, 10.0%, 6.6%, and 6.6%, respectively). In that study, *S. viridans* as well as *Neisseria*, non-group D *Streptococcus* were identified (45.5%, 27.3%, and 18.2%, respectively) in the oropharynx. Although the sample size was limited, the isolated bacteria in the esophagus were similar to those in the oropharynx, but not identical. Recently, Norder Grusell et al[5] investigated the bacteria found in both upper and lower esophagus through esophageal biopsy and brush. In their study, the most common cultured bacteria were *S. viridans*, followed by *Fusobacterium*, *Neisseria*, *Haemophilus*, and *Prevotella*, regardless of their location in the esophagus.

Since the early 2000s, esophageal microbiomes have been evaluated using culture-independent methods. Pei et al[6] examined esophageal biopsy samples obtained from 4 individuals. They performed a broad-range 16S rRNA gene polymerase chain reaction (PCR) analysis and obtained 900 PCR cloned products representing 833 unique sequences belonging to 41 genera. A majority of clones belonged to 13 of 41 genera, which were shared by all 4 individuals.[6] Specifically, *Streptococcus* (39.0%), *Prevotella* (17.0%), and *Veillonella* (14.0%) were most prevalent.[6] In 2012, Fillon et al[14] evaluated the esophageal microbiome in 15 individuals to investigate the performance of an esophageal string test (Enterotest) as compared to biopsy in the collected esophageal mucosal samples. They investigated the bacterial composition using the 16S rRNA gene sequencing technique. and they showed that the most prevalent bacterial taxa were *Streptococcus*, *Prevotella*, and *Veillonella*, which were similar with samples obtained through biopsy and those obtained through the esophageal string test.

In summary, the most common bacterial taxa in a normal esophagus include *Streptococcus*, *Haemophilus*, *Neisseria*, *Prevotella*, and *Veillonella*. However, the bacterial composition may differ depending on various factors, even in a normal esophagus. Age is the best-known factor associated with the esophageal microbiome,[25] which was positively correlated with *Streptococcus*, but negatively correlated with *Prevotella* in the Deshpande et al study[25] that investigated the bacterial community in the esophageal microbiome of 106 individuals. It is not yet clear why age affects the composition of esophageal microbiomes. However, the influence of age on the composition of gastric microbiomes has been also known.[35] Chronic gastric inflammation and decreased intragastric acidity by aging may change the microbial composition of the stomach. Given that gastric contents can affect the esophageal mucosa, change of gastric microbiome caused by aging may result in change of esophageal microbiomes.

Additionally, proton pump inhibitors (PPIs) may also affect esophageal microbiomes. Amir et al[17] showed a significant change of esophageal microbiomes after 8 weeks of PPI treatment (unweighted UniFrac analysis of similarities $R = 0.17$, $P < 0.05$). Decreased acid reflux by PPI administration may affect the esophageal microbiomes. Diet can also influence the esophageal microbiomes. In a previous study, dietary fiber intake was associated with increased number of *Firmicutes* and decreased number of gram-negative bacteria.[27] Conversely, low fiber intake was associated with a high number of gram-negative bacteria, including *Prevotella*, *Neisseria*, and *Eikenella*. It has been known that low fiber diet can lead to weight gain,[36] while high fiber diet may increase the production of short-chain fatty acid in the colon and improve systematic insulin sensitivity.[37] These changes may be related to the impact of dietary fiber on the esophageal microbiome.

The impact of low fiber intake is similar to that of reflux esophagitis or Barrett's esophagus on the esophageal microbiome composition, which will be described in the next section.

Reflux Diseases and Esophageal Microbiomes

In addition to demographic factors and medications, various diseases affect the esophageal microbial composition. In a study on gastric microbiomes, bacterial taxa other than *Helicobacter pylori* were hardly identified in patients infected with *H. pylori*.[38] Highly abundant *H. pylori* itself may be one of the causes; however, the acidic environment of the stomach is another cause for the decrease in number of other bacteria. In patients with severe atrophy and intestinal metaplasia, which decreased the intragastric acidity, various bacteria other than *H. pylori* are found.[39] Therefore, the esophageal microbial composition can easily be considered to change in patients with GERD and Barrett's esophagus.

In 2009, Yang et al[13] suggested that the esophageal microbiome could be classified into 2 groups: type I microbiome dominated by

Gram-positive taxa of *Firmicutes* phylum in normal individuals, and type II microbiome dominated by gram-negative taxa in patients with GERD and Barrett's esophagus. They concluded that inflammation and intestinal metaplasia are related with esophageal microbiome alteration. The main bacterial taxa in type I microbiome was *Streptococcus*, whereas type II microbiomes included *Veillonella*, *Prevotella*, *Haemophilus*, *Neisseria*, *Rothia*, *Granulicatella*, *Campylobacter*, *Porphyromonas*, *Fusobacterium*, and *Actinomyces*. As previously indicated, *Haemophilus*, *Neisseria*, *Prevotella*, and *Veillonella* are also commonly identified in the normal esophagus. In other words, the type II microbiomes are not exclusively found in a normal esophagus. They have a high probability to be found in an acid-exposed esophagus. Deshpande et al[25] classified bacterial taxa into several clusters. Among various bacterial taxa, *Streptococcus* and *Prevotella* were the representative bacterial taxa of clusters they belonged to.[25] Moreover, they revealed that the interaction between *Streptococcus* and *Prevotella* was consistently found in a co-exclusion interaction. These findings are consistent with results in the Yang et al study.[13] Another study suggested that the *Streptococcus*-to-*Prevotella* ratio was also a risk factor for the development of Barrett's esophagus.[19]

The difference in esophageal microbiome among the reflux disease status was also shown in the Liu et al study,[16] conducted using 16S rRNA gene sequencing. *Streptococcus* was the most common bacterial taxa in all the following 3 groups: normal esophagus, reflux esophagitis, and Barrett's esophagus. However, the proportion of *Streptococcus* was slightly higher in the normal group than in the reflux esophagitis or Barrett's esophagus groups. *Pasteurella*, *Haemophilus*, *Fusobacterium*, *Prevotella*, and *Neisseria* were more abundant in the reflux esophagitis group than in the normal group.

In another study by Blackett et al[15] conducted using a cultural analysis with PCR for specific bacterial taxa, the abundance of *Campylobacter* was increased in patients with GERD or Barrett's esophagus. Additionally, a significant increase in IL-18 expression was shown in esophagus colonized by *Campylobacter* among patients with GERD or Barrett's esophagus. IL-18 is known as an IFN-γ-inducing factor and plays a primary role in both innate and adaptive immunity.[40] Although the causal relationship has not been fully evaluated, an interplay between the esophageal microbiome and inflammatory markers is possible.

Based on results of these previous studies, the schematic diagram on differences in esophageal microbiome composition was observed according to the disease status in Figure 2.

Esophageal Cancer and Esophageal Microbiome

In contrast to changes toward increasing various bacterial taxa in GERD and Barrett's esophagus, microbial diversity decreased in esophageal adenocarcinoma (EAC) when compared with the control, which enriched acid-tolerant bacteria such as *Lactobacillus fermentum*.[23] EAC development may change peritumoral microenvironment including acidity. The production of lactic acid may also further acidify the intraesophageal environment. Additionally, noxious products from these bacteria, including hydrogen peroxide, may directly inhibit the growth of other bacteria and enable *Lactobacillus* to dominate in the lower esophagus.[23] A study by Snider et al[32] also showed that microbial diversity decreased in patients with EAC. The proportion of *Firmicutes* phylum (including *Streptococcus*) increased in the low-grade dysplasia, as compared to high-

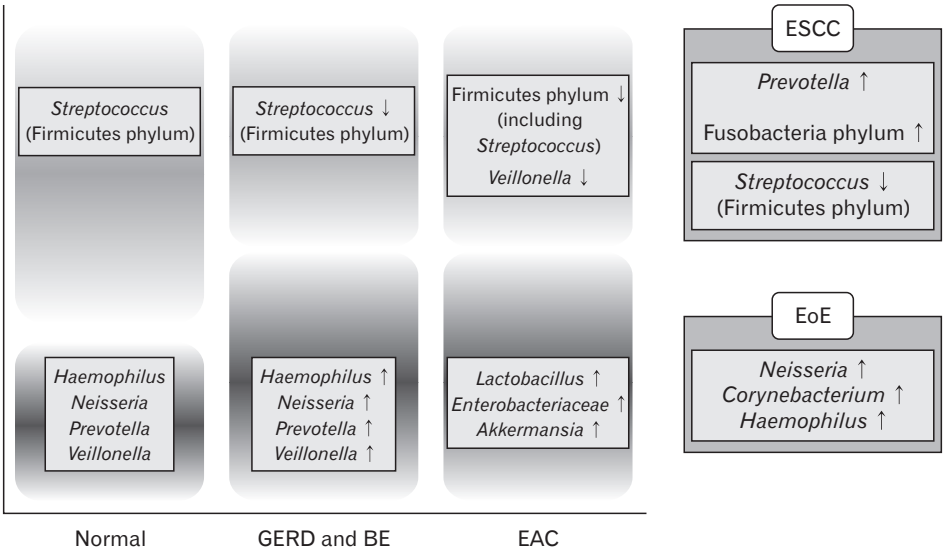

Figure 2. Schematic diagram of differences in esophageal microbiome composition according to esophageal diseases. GERD, gastroesophageal reflux disease; BE, Barrett's esophagus; EAC, esophageal adenocarcinoma; ESCC, esophageal squamous cell carcinoma; EoE, eosinophilic esophagitis.

grade dysplasia or adenocarcinoma. In this study, the proportion of Enterobacteriaceae and *Akkermansia* increased and *Veillonella* decreased in patients with EAC.

Until recently, characteristics of the esophageal microbiome in patients with esophageal squamous cell carcinoma (ESCC) have not been well known. However, in a recent case-control study including 25 patients with ESCC and 50 matched controls, *Prevotella*, especially *Prevotella nanceiensis*, was abundant in patients with ESCC.[24] Interestingly, *Porphyromonas gingivalis*, a periodontal pathogen, tended to increase in patients with ESCC. In a study on the oral microbiome in patients with ESCC, *Porphyromonas* was abundant in patients with ESCC as compared to those with dysplasia as well as the normal controls.[41] An association of *Fusobacterium nucleatum*, one of the periodontal bacteria, with the risk of colorectal cancer has been proven.[42] Another study by Shao et al[31] evaluated the difference in the esophageal microbiome between patients with ESCC and those with gastric cardia adenocarcinoma (GCA). Patients with ESCC showed a high proportion of Fusobacteria phylum (ESCC: 3.9% and GCA: 1.9%). Additionally, the microbiome in esophageal cancer tissue may be used for prediction of patient's prognosis. In the previous studies, intratumoral *F. nucleatum* was associated with poor recurrence-free survival as well as cancer-specific survival in patients with esophageal cancer.[22,33]

Eosinophilic Esophagitis and Esophageal Microbiome

EoE is a chronic immune/antigen-mediated disorder caused by T helper 2-mediated immune response triggered by food or environmental allergens.[43,44] As an increase in incidence and prevalence of EoE, interest in the esophageal microbiome in patients with EoE has been increasing.[43] In patients with EoE, *Neisseria* and *Corynebacterium* were enriched as compared to those with non-EoE.[21] In another study by Harris et al,[20] the bacterial load was increased regardless of the treatment status or degree of mucosal eosinophilia in patients with EoE as compared to healthy individuals. *Haemophilus* was significantly abundant in patients with untreated EoE.[20]

Achalasia and Esophageal Microbiome

Achalasia is a motility disorder presented as dysphagia, regurgitation of undigested food, weight loss, and chest pain.[45] It is caused by the inability to lower the esophageal sphincter to facilitate relaxation in the setting of absent peristalsis.[46] The relationship between achalasia and esophageal microbiome has not been evaluated. Although several case reports showed the association between *Mycobacterium goodii* pulmonary infection and achalasia and secondary achalasia due to human immunodeficiency viral infection,[47,48] evidence that support the association between achalasia and microbial composition in the esophagus of patients with achalasia were limited.

Conclusion

Owing to the advancement of next-generation sequencing techniques, associations between the esophageal microbiomes and various diseases have been widely investigated. Nowadays, the esophagus is found to be unsterile, and many bacterial taxa exist depending on the disease status. However, whether the esophageal microbiome induces esophageal diseases remains unknown. Most changes in esophageal microbiome composition may likely be a secondary change due to acid reflux, aggravation of inflammation, and other predisposing factors such as alcohol and smoking. To determine the causal relationship between esophageal microbiome and diseases, well-designed experiments using germ-free animal models are warranted. Nevertheless, understanding the esophageal microbiome in various diseases may have a clinical implication because oral microbiomes are usually correlated with esophageal microbiomes. We will be able to predict various esophageal diseases via oral samples that can be easily obtained compared to esophageal samples. Further researches will be conducted on oral and esophageal microbiomes in various esophageal diseases.

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