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Emerging Insights into the Esophageal Microbiome

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Abstract

Purpose of Review—Analysis of the esophageal microbiome remains a relatively new field of research, and most studies to date have focused on characterizing the esophageal microbiome in states of health and disease. Microbiome alterations have been implicated in the pathogenesis of inflammatory and neoplastic conditions in the colon and elsewhere in the gastrointestinal tract. The epidemiology of various esophageal conditions including Barrett’s esophagus (BE), esophageal adenocarcinoma (EAC), esophageal squamous cell carcinoma (ESCC) and eosinophilic esophagitis (EoE) point to the microbiome as a potential co-factor in disease pathogenesis, and the possibility exists that these microbiome alterations could contribute directly to the inflammatory environments necessary for the carcinogenesis or atopy involved in these conditions.

Recent Findings—The native esophageal microbiome is similar in composition to the oral microbiome, with a high relative abundance of the phylum Firmicutes and the genus *Streptococcus*. Limited studies to date suggest that there are certain microbiome alterations associated with esophageal diseases. Additionally, it may be possible to indirectly assess the esophageal microbiome via non-endoscopic means. This raises the possibility that non-invasive microbiome analysis could be used for disease screening and monitoring.

Summary—Further understanding of the role of the esophageal microbiome in disease pathogenesis, as well as methods for microbiome alteration may help elucidate future targets for disease modifying therapies, or minimally invasive screening tools in patients at high risk for development of various esophageal conditions.

Keywords

Microbiome; esophageal cancer; Barrett’s esophagus; eosinophilic esophagitis

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Compliance with Ethics Guidelines

Human and Animal Rights and Informed Consent

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Conflict of Interest

Michael May and Julian A. Abrams declare that they have no conflict of interest.

Introduction

The esophagus previously was felt to be largely absent of bacteria. However, with the advent of culture-independent methods such as 16S rRNA gene sequencing, it was found the esophagus contains a diverse microbiome [1]. However, relatively little is known with regard to the esophageal microbiome and its role in disease pathogenesis. This is due in part to the difficulty in sampling the esophageal microbiome; specifically, upper endoscopy is required for targeted specimen collection. This is in contrast to the mouth and colon, for example, where samples can be collected from large numbers of patients non-invasively.

The incidence of certain esophageal conditions such as Barrett's esophagus (BE), esophageal adenocarcinoma (EAC), and eosinophilic esophagitis (EoE) has risen dramatically over the past several decades. The reasons underlying these trends are incompletely understood. Coincident with the rise of these conditions has been a profound shift in the upper gastrointestinal microbiome since the mid-20th century, around the same time that widespread antibiotic use began. During this period, the prevalence of *Helicobacter pylori* infection of the stomach began to decrease, which at least raises the possibility that profound, population-level upper GI microbiome shifts resulted in an increased predisposition to the development of a variety of esophageal diseases. (Figure 1) In this paper we aim to summarize findings to date on microbiome alterations in certain esophageal diseases, discuss potential clinical implications, and lay out future research directions.

Techniques for Sampling and Analyzing the Esophageal Microbiome

Traditionally, sampling of the esophagus has involved endoscopy with brushings or biopsies. The invasiveness and expense of this procedure has limited sample sizes of esophageal microbiome studies and has greatly hampered the ability to assess longitudinal changes. Gall et al. compared endoscopic mucosal brushings to biopsies and found that brushings gave superior yield of bacterial DNA and increased the ratio of bacterial DNA to host DNA, which is significant in light of the low bacterial concentration in the esophagus [2]. We generally collect samples using endoscopic brushes, passing the brush back and forth ten times in each of four quadrants. Within individual patients in whom two brushings were taken from the same site, we have found that taxon-level relative abundances are very strongly correlated (unpublished data), and a second brushing from a particular site may not add markedly to findings from a single, thorough brush sampling.

There are non-endoscopic techniques and devices that have been proposed for esophageal microbiome sampling. Fillon et al. found that analyzing the microbiome from the proximal segment of string from the Enterotest™ (which the authors coined the “Esophageal String Test”) provided very similar microbiome profiles compared to matched endoscopic biopsies [3]. The Cytosponge is a tethered capsule designed for cell sampling in the esophagus and has been studied as a non-endoscopic method to diagnose Barrett's esophagus [4]. The capsule dissolves upon reaching the stomach, a spherical mesh of 3 cm diameter is exposed, and the mesh is then withdrawn through the mouth. As a tool for studying the esophageal microbiome, the Cytosponge yields higher quantities of microbial DNA than endoscopic brushes or biopsies [5]. Inflatable balloons have also been used to sample the upper

gastrointestinal microbiome [6]. However, none of these techniques exclusively sample the esophageal microbiome. The oral cavity has a much higher concentration of bacteria compared to the esophagus, and thus contamination with oral flora likely overwhelms the ability to assess microbiome alterations specific to the esophagus.

Culture independent analysis of the esophageal microbiome is largely restricted to 16S rRNA gene sequencing. Since the bacterial concentration of the esophagus is low compared to other sites such as the colon or mouth, the host:bacteria DNA ratio for esophageal samples is very high. As such, more detailed profiling of the esophageal microbiome using methods such as whole genome sequencing is challenging. While post-sequencing methods have been attempted to allow for detailed microbiome profiling of samples with low bacterial abundance[7, 8], these are not widely employed at the present time.

Native Esophageal Microbiome

The esophageal microbiome is broadly similar to the oropharyngeal microbiome but with key taxonomic differences [1, 9, 10]. The first studies on the esophageal microbiota, dating back to the early 1980s and based on culture, demonstrated that the esophagus was not a sterile site and did not simply contain a transient microbial population introduced from the oral cavity [9, 11, 12].

Pei et al. performed a detailed characterization of the esophageal microbiome in healthy individuals using culture-independent methods [1]. 16S rRNA gene sequencing was performed on esophageal endoscopy samples from four patients without esophageal disease. Ninety-five species-level operational taxonomic units were identified, belonging to six phyla: Firmicutes (70%), Bacteroidetes (20%), Actinobacteria (4%), Proteobacteria (2%), Fusobacteria (2%), and TM7 (1%). The most common genera of bacteria were *Streptococcus*, *Prevotella*, and *Veillonella*. While the normal esophageal microbiome had many commonalities with the oral microbiome, key differences, such as the absence of *Spirochaetes*, suggested a unique esophageal microbiome that was not solely the product of oropharyngeal colonization. Subsequent studies have confirmed the presence of the six phyla above and overall predominance of Gram-positive bacteria with *Streptococcus* as a consistently highly abundant genus [3, 13–15].

Factors that Influence the Esophageal Microbiome

Proton Pump Inhibitors (PPIs)

PPIs are theorized to alter the esophageal and gastric microbiome both through increasing pH of gastric secretions and also possibly by directly targeting the bacterial proton pumps of certain bacteria that contain P-type ATPase enzymes, such as *Streptococcus pneumoniae* and *H. pylori*. Amir et al. analyzed gastric refluxate and esophageal biopsies of eight patients with GERD, before and after eight weeks of PPI treatment (lansoprazole 30mg twice daily) [14]. The authors found that the microbial communities of esophageal biopsy samples were significantly altered after PPI treatment, with decreased *Comamonadaceae* and increased *Clostridiaceae* and *Micrococcaceae*. The family *Methylobacteriaceae*, which were increased in gastric aspirates among BE/esophagitis patients before PPIs, were markedly depleted after

PPI therapy. The authors speculated that PPI therapy may result in decreased availability of potentially toxic one-carbon compounds, a preferred carbon and energy source for *Methylobacteriaceae*. Sanduleanu et al. cultured gastric aspirates from 109 patients taking PPIs and 75 patients with untreated GERD and found that the prevalence of oropharyngeal bacteria, including *Neisseria*, *Streptococcus* and *Corynebacterium*, was significantly increased in patients on PPIs when compared with untreated patients [16]. These studies suggest that PPIs can have marked impacts on the upper GI microbiome, although the disease modifying effects of the microbiome changes is unknown.

Diet

Dietary factors are associated with altered risk of BE, EAC and ESCC, and dietary antigens are directly involved in EoE-related inflammation. It is possible that some effects of diet on esophageal disease risk and manifestations are mediated by changes to the local microbiome. Diet has profound effects on the colonic microbiome. High-fiber diets are generally associated with decreased colonic and systemic inflammation, whereas high-fat diets have the opposite effects [17–21]. A recent study by Mehta et al. found that “prudent” diets high in fiber were associated with decreased risks of colon cancer, but this protective effect only applied to *Fusobacterium nucleatum*-positive tumors [22]. These findings suggest that the effects of diet on colon cancer risk are mediated at least in part by alterations to the microbiome. However, there is little data on the effects of diet on the esophageal microbiome.

In a prospective study of 33 children with EoE, implementation of a six-food elimination diet on patients with active EoE did not have a marked impact on the esophageal microbiome [13]. However, the reintroduction of allergenic foods led to an increase in the prevalence of *Granulicatella* and *Campylobacter*[13]. Our group recently conducted a study of 47 patients who completed a 4-week dietary recall food frequency questionnaire validated for fat and fiber intake, and subsequently underwent endoscopy with esophageal brushings [23]. We found that increasing fiber intake positively correlated with increased relative abundance of Firmicutes and decreased relative abundance of Proteobacteria and Gram-negative bacteria as a whole. Interestingly, there were relatively few microbiome alterations based on dietary fat intake. However, longitudinal studies are necessary to better determine how diet alters the esophageal microbiome.

Barrett’s Esophagus (BE) and Esophageal Adenocarcinoma (EAC)

EAC is the predominant type of esophageal cancer in North America and Europe. Five-year survival from time of diagnosis is approximately 17%, as most tumors are discovered at later stages. BE is the precursor lesion that can be identified through endoscopy and followed for early detection of dysplasia and EAC. The incidence of EAC has been rising steadily and rapidly since at least the mid-1970s [24]. Between 1975 and 2009 the average annual percentage increase in incidence was 6.1% in men and 5.9% in women [25]. Data from the Connecticut Tumor Registry indicate that the age-adjusted incidence of EAC was very low (~0.4 cases per 100,000 person-years) and relatively stable until the late 1960s, and has had an overall approximately ten-fold increase since that time [26].

This pattern suggests that changes in exposures in the mid-20th century most likely led to an increase in rates of BE with a subsequent rise in the incidence of EAC. However, the dramatic increase in incidence of EAC is beyond what would be expected just based on increased rates of GERD, obesity and smoking[27]. GERD prevalence began to rise in the 1970s, obesity in the United States began to rise markedly starting in the 1980s, and smoking rates have dropped in recent decades [28–32].

Widespread use of antibiotics began in the 1940s, which potentially led to dramatic, population-level shifts in the upper gastrointestinal microbiome. *Helicobacter pylori* is inversely associated with EAC risk[33]. *H. pylori* infection rates began to fall in the mid-20th century, with a 26% decrease in seropositivity prevalence per decade between 1969 and 1989 [34]. The American and Western European diet also saw significant changes in the mid-20th century, with the widespread production and consumption of processed foods and sugar-sweetened beverages [35]. The combination of the rise in antibiotic use, decreased *H. pylori* prevalence and significant dietary changes in the mid-20th century, along with the inverse relationship between *H. pylori* infection and the development of EAC, all point to a role of the upper gastrointestinal microbiome in the pathogenesis of EAC. (Figure 1)

The Barrett's Esophagus- and Reflux-Associated Microbiome

Studies to date suggest that BE and reflux esophagitis are associated with a modestly distinct esophageal microbiome compared to patients without esophageal disease. This microbiome appears to be enriched with Gram-negative bacteria and a broader range of bacteria overall. Yang et al. compared the microbiomes of distal esophageal biopsies in 12 patients with normal esophagus, 12 with esophagitis and 10 with BE [36]. Using clustering analyses they classified the microbiome into two types, arbitrarily named Type I and Type II. Type I was dominated by *Streptococcus*, and Type II contained decreased relative abundance of *Streptococcus* and a greater proportion of Gram-negative anaerobes and microaerophiles. Almost all (13/14) of the patients with a Type II microbiome had reflux esophagitis or BE. MacFarlane et al. cultured and sequenced esophageal biopsy and aspirate samples from 7 patients with BE and 7 patients with normal esophagus [37]. High levels of *Campylobacter*, a Gram-negative genus that has been linked to enteritis, periodontal infections, and tumor formation in animals, was found in 4 of 7 patients with BE but in none of the control subjects. Also, BE patients were colonized with a wider variety of both Gram-negative and Gram-positive bacteria.

Amir et al. performed 16S rRNA gene sequencing on squamous tissue and gastric aspirates from 15 normal patients and 16 patients with reflux esophagitis (11) or BE (5) [14]. There was relatively little difference in the squamous tissue microbiome between the groups. However, the gastric aspirates in patients with BE or reflux esophagitis had an increased and very high relative abundance *Enterobacteriaceae*, particularly of the genus *Escherichia*. Certain strains of *E. coli* promote colon cancer in mice in the presence of colonic inflammation, raising the possibility that this bacterium could play a role in the development of BE [38].

The Microbiome in Esophageal Adenocarcinoma

The microbiome associated with EAC remains poorly described. Blackett et al. used 16S rRNA sequencing of prespecified species chosen based on culture isolation, comparing controls with patients with GERD, BE and EAC [39]. This group found that in many ways the microbiome of EAC was more similar to the normal esophageal microbiome than to BE. The control and EAC samples had increased relative abundance of *Bifidobacteria*, *Bacteroides*, *Fusobacteria*, *Veillonella*, *Staphylococcus* and *Lactobacilli* and decreased relative abundance of *Campylobacter* when compared with BE samples. Elliott et al. compared the microbiome of tissue from patients with EAC to BE and normal controls [5]. The authors reported a decrease in microbial diversity in EAC samples and increased relative abundance of *Lactobacillus fermentum*. Roughly half of the tumors were dominated by a single species within the order Lactobacillales.

Zaidi et al. studied the microbiome in a surgical rat model of EAC as well as tissue from patients with and without EAC [40]. *E. coli* was abundant in BE and EAC tissue but not in BE-associated dysplasia. Tumor tissue from the rat model also demonstrated significant upregulation of various toll-like receptors (TLRs), which likely play a key role in recognition of and response to bacteria. Based on these findings, the authors proposed a potential model in which TLR activation by microbes could contribute to development of EAC.

However, findings from these studies should be interpreted with caution. Tumors have a markedly altered topography, which may favor colonization and growth of certain bacteria. As such, it is unclear whether microbiome alterations in EAC point to potential causal mechanism for specific taxa or merely colonization due to an altered macro- and micro-environment.

Potential Mechanisms for Microbiome-Mediated EAC Risk

Mechanistic studies on the role of specific bacteria on the development of EAC are lacking. It has been posited that Gram-negative bacteria could contribute to chronic inflammation through a number of mechanisms. Lipopolysaccharides (LPS), expressed on the surface of Gram-negative bacteria, can bind to TLRs and other cell surface receptors, inducing the pro-inflammatory NF- κ B pathway and promoting neoplastic progression [41]. Another possible mechanism for Gram-negative bacteria to promote esophageal neoplasia is through reduction of dietary nitrates to nitrites, which can then be converted into carcinogenic N-nitroso compounds by the acidic environment of the distal esophagus in patients with acid reflux [42, 43].

F. nucleatum is an anaerobic Gram-negative bacterium that is highly abundant in the oral cavity, plays a role in periodontal disease, and has been associated with the development of colon cancer in humans [44–46]. *F. nucleatum* can activate the beta-catenin pathway through interaction between E-cadherin and *F. nucleatum*-produced FadA [47]. The presence of *F. nucleatum* has also been associated with the CpG island methylator phenotype in premalignant colorectal lesions and colorectal carcinoma, opening up the possibility that this

species may also mediate increased risk of carcinogenesis through epigenetic changes. [48]. However, a role of *F. nucleatum* in the development of EAC has not been described.

Esophageal Squamous Cell Carcinoma (ESCC)

ESCC accounts for roughly 90% of cases of esophageal cancer worldwide and remains the most common type of esophageal cancer in Asia, Africa, and South America and among African-Americans. In very high incidence areas such as Iran, Central Asia, and China, dietary factors such as poor nutritional status, low intake of fruits and vegetables, consumption of food and beverages at high temperatures, and consumption of carcinogenic food products are thought to account for a significant portion of cases[49, 50]. In the United States and Western Europe, rates of ESCC have been falling, with EAC now accounting for the majority of esophageal cancer cases[51].

The Microbiome in Esophageal Squamous Cell Carcinoma

Studies have demonstrated associations between diet and oral hygiene on risk for development of ESCC, suggesting a possible role of the esophageal and closely related oral microbiomes in the pathogenesis of ESCC. One study of 325 resected esophageal cancer specimens, of which 92% were ESCC, found increases in the quantity of *F. nucleatum* in esophageal cancer specimens compared to normal esophageal mucosa [52]. Further, the presence of *F. nucleatum* DNA in tumors was associated with shorter survival. These findings strongly suggest that specific bacteria such as *F. nucleatum* may play a role in ESCC development and may impact tumor behavior.

Yu et al. evaluated the upper GI microbiome collectively using a balloon device that sampled the stomach, esophagus and oral cavity in a Chinese population of 142 patients with esophageal squamous dysplasia and 191 controls and found that decreased alpha diversity was inversely associated with the presence of squamous dysplasia [6]. In an Iranian study of 19 patients with ESCC, 18 with squamous dysplasia, and 37 age- and sex-matched healthy controls, a greater abundance of Clostridiales and Erysipelotrichales was found in cancer and dysplasia tissue compared to normal esophagus from controls [53]. However, there were no significant differences in alpha diversity between cases and controls in this study. Collectively, these studies suggest that microbiome alterations occur in ESCC, although further studies are clearly warranted to assess causation.

Eosinophilic Esophagitis (EoE)

EoE is a chronic, immune/antigen-mediated, esophageal disease characterized clinically by symptoms related to esophageal dysfunction and histologically by eosinophil-predominant inflammation [54]. Although the pathogenesis is not fully understood, food or environmental allergens trigger a Th2-mediated response resulting in eosinophilic infiltration of the esophageal mucosa [55]. Esophageal tissue samples from affected patients are characterized by increased numbers of eosinophils, T cells and mast cells[56]. These findings, as well as the significant association between EoE and atopic conditions suggest an allergen-mediated pathogenesis of EoE [57, 58]. Elimination of specific dietary antigens can reduce or eliminate symptoms of EoE.

The first reported cases of probable EoE are from the 1960s, although it was not recognized as a unique clinical entity until about 1990 [59, 60]. Population based studies have demonstrated a progressively increasing incidence of EoE during the 1990s and 2000s [61]. One population based study that assessed the incidence of EoE in Olmsted County, Minnesota from 1976 to 2005 found significant increases in incidence from 1991 to 2005 [62]. EoE has a predilection for the more developed regions of the globe, with prevalence within these regions ranging from 4 to 400 per 100,000, and the highest prevalence reported by centers in Northern Europe and Australia [63]. One large population-based study in the United States estimated a prevalence of EoE of 57.7 per 100,000 persons and was highest in men 35–39 years old [64].

There are multiple hypotheses for the cause of the increased incidence of EoE. Allergic diseases in general have increased in developed countries in recent decades, possibly a result of a more sterile environment which results in exposure of the developing immune system to a smaller variety of antigens and a smaller range of antigens to which the body is tolerant [63]. In fact, a majority of patients with EoE have a history of other atopic conditions [57, 58].

Another theory is that changes in the esophageal microbiome may account for some of the increasing incidence of EoE. The historical trends in EoE incidence mirror those of EAC (Figure 1). Over the past half century there has been a major increase in antibiotic use and a steady decline in *H. pylori* infection rates, and as with EAC, *H. pylori* infection is associated with a decreased risk of EoE [65, 66].

The Microbiome of EoE

There is limited data on esophageal microbiome alterations associated with EoE. A prospective study was performed in 33 children with EoE, 18 of whom had active EoE and 15 of whom had inactive inflammation after dietary elimination treatment, and 35 healthy controls, all of whom underwent upper endoscopy with esophageal biopsies. [13] Patients with active EoE had increased relative abundance of *Neisseria* and *Corynebacterium* compared with controls, who had increased *Streptococcus* and *Atopobium*. This study did not find a significant effect of a six-food elimination diet on the esophageal microbiome. However, the reintroduction of certain foods led to an increase in the prevalence of *Granulicatella* and *Campylobacter*.

A study by Harris et al. used esophageal string testing to analyze esophageal samples from 70 children including 11 with untreated EoE and 26 with EoE in remission after treatment, 8 GERD controls and 25 healthy controls [15]. There was an increase in *Haemophilus* in the patients with EoE compared with the healthy controls. Also, there was evidence of increased bacterial load, but not diversity, in all subjects with EoE regardless of the degree of eosinophilia, compared with the healthy controls.

These studies hint at a distinct esophageal microbiome in EoE, although it is unclear whether any alterations play a role in the pathogenesis of EoE.

Potential Clinical Applications

The Oral Microbiome as a Screening Tool

The oral microbiome is broadly similar to the esophageal microbiome and very easy to sample. The oral microbiome has been studied as biomarker for various gastrointestinal malignancies, including ESCC, gastric cancer, pancreatic cancer, and colorectal cancer [67–69]. In a study of saliva samples from 87 incident and histopathologically diagnosed ESCC subjects, 63 subjects with squamous dysplasia, and 85 healthy controls, saliva in patients with ESCC had decreased microbial diversity and decreased relative abundance of several genera including *Lautropia*, *Bulleidia*, *Catonella*, *Corynebacterium*, *Moryella*, *Peptococcus* and *Cardiobacterium*[67]. These findings open up the possibility of using saliva to screen for ESCC in select populations.

Our unpublished data suggests that BE is associated with a unique oral microbiome including increased relative abundance of Firmicutes and decreased Proteobacteria. We created a three-taxon model that was able to distinguish patients with BE from controls based on their oral microbiome with 96.9% sensitivity and 88.2% specificity [70]. If specific taxa can be identified and validated for BE and/or EAC screening, then one could envision a rapid and inexpensive PCR-based test carried out in the primary care setting.

Can the Esophageal Microbiome be Modified to Alter Disease Risk or Outcomes?

If specific bacteria are involved in the development of esophageal diseases, then therapies aimed at modifying the esophageal microbiome could potentially lower risk or improve outcomes in those with disease. In a surgical rat model for EAC, rats given penicillin G and streptomycin had a non-significant reduction in EAC development [71]. There is relatively little research on the effects of probiotics on the esophageal microbiome. In a murine model of EoE, supplementation with *Lactococcus lactis* NCC 2287 significantly decreased esophageal and bronchoalveolar eosinophilia [72]. The use of systemic antibiotics may have negative effects elsewhere in the body, and topical therapies may prove more organ-selective and effective.

Given the close relationship between the oral and esophageal microbiome, another potential approach is to modify the oral microbiome. Our group is conducting a proof-of-concept trial to assess the effects of chlorhexidine mouth rinse on the esophageal microbiome (NCT02513784).

Future Directions for Research

Most research on the esophageal microbiome has involved characterizing the microbiome in disease states. Although these studies point to alterations in the microbiome associated with different diseases, data on causality are lacking. Prospective studies tracking longitudinal changes in the microbiome during disease progression and studies on the effects of various interventions on the esophageal microbiome are also needed. Such studies could both identify potential targets for risk modification and explore the clinical utility of markers for disease risk. If these changes in the microbiome are found to cause increased risk of esophageal disease, an important area of research will be to identify specific microbes that

carry responsibility for conferring increased or decreased risk of diseases. Identification of antibiotics and probiotics which have an effect specific to these species, and finding best methods for local application may then be an important area of study.

Conclusions

The microbiome has been identified as a key mediator of inflammatory and neoplastic conditions of the gastrointestinal tract and the colon in particular. The epidemiology of various esophageal conditions such as Barrett's esophagus and eosinophilic esophagitis point to the microbiome as potentially relevant players, although our understanding of the role of bacteria in the pathogenesis of these diseases remains limited. Future studies will hopefully elucidate the roles of specific bacteria in the esophagus and identify novel therapeutic approaches to modify disease risk and improve outcomes.

References

1. Pei Z, Bini EJ, Yang L, Zhou M, Francois F, Blaser MJ. Bacterial biota in the human distal esophagus. *Proc Natl Acad Sci U S A*. 2004; 101(12):4250–5. [PubMed: 15016918]
2. Gall A, Fero J, McCoy C, Claywell BC, Sanchez CA, Blount PL, et al. Bacterial Composition of the Human Upper Gastrointestinal Tract Microbiome Is Dynamic and Associated with Genomic Instability in a Barrett's Esophagus Cohort. *PLoS One*. 2015; 10(6):e0129055. • Multiple sites sampled in the upper GI tract of BE patients, demonstrating similar microbiome along the upper GI tract within individuals. [PubMed: 26076489]
3. Fillon SA, Harris JK, Wagner BD, Kelly CJ, Stevens MJ, Moore W, et al. Novel device to sample the esophageal microbiome--the esophageal string test. *PLoS One*. 2012; 7(9):e42938. [PubMed: 22957025]
4. Ross-Innes CS, DeBiram-Beecham I, O'Donovan M, Walker E, Varghese S, Lao-Sirieix P, et al. Evaluation of a minimally invasive cell sampling device coupled with assessment of trefoil factor 3 expression for diagnosing Barrett's esophagus: a multi-center case-control study. *PLoS Med*. 2015; 12(1):e1001780. [PubMed: 25634542]
5. Elliott DRF, Walker AW, O'Donovan M, Parkhill J, Fitzgerald RC. A non-endoscopic device to sample the oesophageal microbiota: a case-control study. *Lancet Gastroenterol Hepatol*. 2017; 2(1): 32–42. [PubMed: 28404012]
6. Yu G, Gail MH, Shi J, Klepac-Ceraj V, Paster BJ, Dye BA, et al. Association between upper digestive tract microbiota and cancer-predisposing states in the esophagus and stomach. *Cancer Epidemiol Biomarkers Prev*. 2014; 23(5):735–41. [PubMed: 24700175]
7. Zhang C, Cleveland K, Schnoll-Sussman F, McClure B, Bigg M, Thakkar P, et al. Identification of low abundance microbiome in clinical samples using whole genome sequencing. *Genome Biol*. 2015; 16:265. [PubMed: 26614063]
8. Robinson KM, Crabtree J, Mattick JS, Anderson KE, Dunning Hotopp JC. Distinguishing potential bacteria-tumor associations from contamination in a secondary data analysis of public cancer genome sequence data. *Microbiome*. 2017; 5(1):9. [PubMed: 28118849]
9. Mannell A, Plant M, Frolich J. The microflora of the oesophagus. *Ann R Coll Surg Engl*. 1983; 65(3):152–4. [PubMed: 6859774]
10. Snider EJ, Freedberg DE, Abrams JA. Potential Role of the Microbiome in Barrett's Esophagus and Esophageal Adenocarcinoma. *Dig Dis Sci*. 2016; 61(8):2217–25. [PubMed: 27068172]
11. Finlay IG, Wright PA, Menzies T, McArdle CS. Microbial flora in carcinoma of oesophagus. *Thorax*. 1982; 37(3):181–4. [PubMed: 7101222]
12. Lau WF, Wong J, Lam KH, Ong GB. Oesophageal microbial flora in carcinoma of the oesophagus. *Aust N Z J Surg*. 1981; 51(1):52–5. [PubMed: 7013751]
13. Benitez AJ, Hoffmann C, Muir AB, Dods KK, Spergel JM, Bushman FD, et al. Inflammation-associated microbiota in pediatric eosinophilic esophagitis. *Microbiome*. 2015; 3:23. ••

Prospective study in EoE demonstrating the impact of elimination diet and reintroduction on the esophageal microbiome. [PubMed: 26034601]

14. Amir I, Konikoff FM, Oppenheim M, Gophna U, Half EE. Gastric microbiota is altered in oesophagitis and Barrett's oesophagus and further modified by proton pump inhibitors. *Environ Microbiol.* 2014; 16(9):2905–14. • Demonstrates within-individual changes to the esophageal and gastric aspirate microbiome caused by PPI therapy. [PubMed: 24112768]
15. Harris JK, Fang R, Wagner BD, Choe HN, Kelly CJ, Schroeder S, et al. Esophageal microbiome in eosinophilic esophagitis. *PLoS One.* 2015; 10(5):e0128346. [PubMed: 26020633]
16. Sanduleanu S, Jonkers D, De Bruine A, Hameeteman W, Stockbrugger RW. Non-Helicobacter pylori bacterial flora during acid-suppressive therapy: differential findings in gastric juice and gastric mucosa. *Aliment Pharmacol Ther.* 2001; 15(3):379–88. [PubMed: 11207513]
17. Albenberg LG, Wu GD. Diet and the intestinal microbiome: associations, functions, and implications for health and disease. *Gastroenterology.* 2014; 146(6):1564–72. [PubMed: 24503132]
18. David LA, Maurice CF, Carmody RN, Gootenberg DB, Button JE, Wolfe BE, et al. Diet rapidly and reproducibly alters the human gut microbiome. *Nature.* 2014; 505(7484):559–63. [PubMed: 24336217]
19. Schulz MD, Atay C, Heringer J, Romrig FK, Schwitalla S, Aydin B, et al. High-fat-diet-mediated dysbiosis promotes intestinal carcinogenesis independently of obesity. *Nature.* 2014; 514(7523): 508–12. • Demonstrates that diet-induced microbiome alterations are capable of promoting intestinal cancer in mice. [PubMed: 25174708]
20. Kaakoush NO, Lecomte V, Maloney CA, Morris MJ. Cross-talk among metabolic parameters, esophageal microbiota, and host gene expression following chronic exposure to an obesogenic diet. *Sci Rep.* 2017; 7:45753. [PubMed: 28362001]
21. O'Keefe SJ, Li JV, Lahti L, Ou J, Carbonero F, Mohammed K, et al. Fat, fibre and cancer risk in African Americans and rural Africans. *Nat Commun.* 2015; 6:6342. [PubMed: 25919227]
22. Mehta RS, Nishihara R, Cao Y, Song M, Mima K, Qian ZR, et al. Association of Dietary Patterns With Risk of Colorectal Cancer Subtypes Classified by *Fusobacterium nucleatum* in Tumor Tissue. *JAMA Oncol.* 2017; 3(7):921–927. • Demonstrates that the effects of diet on colon cancer risk are mediated in part by the microbiome. [PubMed: 28125762]
23. Nobel Y, Snider EJ, Compres G, Freedberg DE, Toussaint N, Abrams JA. Dietary Fiber Intake is Associated with a Significantly Altered Human Esophageal Microbiome. *Gastroenterology.* 2017; 152(5):S632.
24. Brown LM, Devesa SS, Chow WH. Incidence of adenocarcinoma of the esophagus among white Americans by sex, stage, and age. *J Natl Cancer Inst.* 2008; 100(16):1184–7. [PubMed: 18695138]
25. Hur C, Miller M, Yin Kong C, Dowling E, Nattinger K, Dunn M, et al. Trends in Esophageal Adenocarcinoma Incidence and Mortality. *Cancer.* 2013; 6(119):1149–1158.
26. Abrams JA, Sharaiha RZ, Gonsalves L, Lightdale CJ, Neugut AI. Dating the rise of esophageal adenocarcinoma: analysis of Connecticut Tumor Registry data, 1940–2007. *Cancer Epidemiol Biomarkers Prev.* 2011; 20(1):183–6. [PubMed: 21127287]
27. Engel LS, Chow WH, Vaughan TL, Gammon MD, Risch HA, Stanford JL, et al. Population attributable risks of esophageal and gastric cancers. *J Natl Cancer Inst.* 2003; 95(18):1404–13. [PubMed: 13130116]
28. Kong CY, Nattinger KJ, Hayeck TJ, Omer ZB, Wang YC, Spechler SJ, et al. The impact of obesity on the rise in esophageal adenocarcinoma incidence: estimates from a disease simulation model. *Cancer Epidemiol Biomarkers Prev.* 2011; 20(11):2450–6. [PubMed: 21930957]
29. Flegal KM, Carroll MD, Kuczmarski RJ, Johnson CL. Overweight and obesity in the United States: prevalence and trends, 1960–1994. *Int J Obes Relat Metab Disord.* 1998; 22(1):39–47. [PubMed: 9481598]
30. Giovino GA. Epidemiology of tobacco use in the United States. *Oncogene.* 2002; 21(48):7326–40. [PubMed: 12379876]
31. el-Serag HB, Sonnenberg A. Opposing time trends of peptic ulcer and reflux disease. *Gut.* 1998; 43(3):327–33. [PubMed: 9863476]

32. El-Serag HB. Time trends of gastroesophageal reflux disease: a systematic review. *Clin Gastroenterol Hepatol*. 2007; 5(1):17–26. [PubMed: 17142109]
33. Islami F, Kamangar F. *Helicobacter pylori* and esophageal cancer risk: a meta-analysis. *Cancer Prev Res (Phila)*. 2008; 1(5):329–38. [PubMed: 19138977]
34. Banatvala N, Mayo K, Megraud F, Jennings R, Deeks JJ, Feldman RA. The cohort effect and *Helicobacter pylori*. *J Infect Dis*. 1993; 168(1):219–21. [PubMed: 8515114]
35. Popkin BM, Adair LS, Ng SW. Global nutrition transition and the pandemic of obesity in developing countries. *Nutr Rev*. 2012; 70(1):3–21. [PubMed: 22221213]
36. Yang L, Lu X, Nossa CW, Francois F, Peek RM, Pei Z. Inflammation and intestinal metaplasia of the distal esophagus are associated with alterations in the microbiome. *Gastroenterology*. 2009; 137(2):588–97. [PubMed: 19394334]
37. Macfarlane S, Furrie E, Macfarlane GT, Dillon JF. Microbial colonization of the upper gastrointestinal tract in patients with Barrett's esophagus. *Clin Infect Dis*. 2007; 45(1):29–38. [PubMed: 17554697]
38. Arthur JC, Gharaibeh RZ, Muhlbauer M, Perez-Chanona E, Uronis JM, McCafferty J, et al. Microbial genomic analysis reveals the essential role of inflammation in bacteria-induced colorectal cancer. *Nat Commun*. 2014; 5:4724. [PubMed: 25182170]
39. Blackett KL, Siddhi SS, Cleary S, Steed H, Miller MH, Macfarlane S, et al. Oesophageal bacterial biofilm changes in gastro-oesophageal reflux disease, Barrett's and oesophageal carcinoma: association or causality? *Aliment Pharmacol Ther*. 2013; 37(11):1084–92. [PubMed: 23600758]
40. Zaidi AH, Kelly LA, Kreft RE, Barlek M, Omstead AN, Matsui D, et al. Associations of microbiota and toll-like receptor signaling pathway in esophageal adenocarcinoma. *BMC Cancer*. 2016; 16:52. [PubMed: 26841926]
41. Yang L, Francois F, Pei Z. Molecular pathways: pathogenesis and clinical implications of microbiome alteration in esophagitis and Barrett esophagus. *Clin Cancer Res*. 2012; 18(8):2138–44. [PubMed: 22344232]
42. Potter L, Angove H, Richardson D, Cole J. Nitrate reduction in the periplasm of gram-negative bacteria. *Adv Microb Physiol*. 2001; 45:51–112. [PubMed: 11450112]
43. Suzuki H, Iijima K, Scobie G, Fyfe V, McColl KE. Nitrate and nitrosative chemistry within Barrett's oesophagus during acid reflux. *Gut*. 2005; 54(11):1527–35. [PubMed: 16227357]
44. Kostic AD, Chun E, Robertson L, Glickman JN, Gallini CA, Michaud M, et al. *Fusobacterium nucleatum* potentiates intestinal tumorigenesis and modulates the tumor-immune microenvironment. *Cell Host Microbe*. 2013; 14(2):207–15. [PubMed: 23954159]
45. Kostic AD, Gevers D, Pedamallu CS, Michaud M, Duke F, Earl AM, et al. Genomic analysis identifies association of *Fusobacterium* with colorectal carcinoma. *Genome Res*. 2012; 22(2):292–8. [PubMed: 22009990]
46. Kapatral V, Anderson I, Ivanova N, Reznik G, Los T, Lykidis A, et al. Genome sequence and analysis of the oral bacterium *Fusobacterium nucleatum* strain ATCC 25586. *J Bacteriol*. 2002; 184(7):2005–18. [PubMed: 11889109]
47. Rubinstein MR, Wang X, Liu W, Hao Y, Cai G, Han YW. *Fusobacterium nucleatum* promotes colorectal carcinogenesis by modulating E-cadherin/beta-catenin signaling via its FadA adhesin. *Cell Host Microbe*. 2013; 14(2):195–206. [PubMed: 23954158]
48. Ito M, Kanno S, Noshio K, Sukawa Y, Mitsuhashi K, Kurihara H, et al. Association of *Fusobacterium nucleatum* with clinical and molecular features in colorectal serrated pathway. *Int J Cancer*. 2015; 137(6):1258–68. [PubMed: 25703934]
49. Islami F, Pourshams A, Nasrollahzadeh D, Kamangar F, Fahimi S, Shakeri R, et al. Tea drinking habits and oesophageal cancer in a high risk area in northern Iran: population based case-control study. *BMJ*. 2009; 338:b929. [PubMed: 19325180]
50. Cook-Mozaffari PJ, Azordegan F, Day NE, Ressicaud A, Sabai C, Aramesh B. Oesophageal cancer studies in the Caspian Littoral of Iran: results of a case-control study. *Br J Cancer*. 1979; 39(3):293–309. [PubMed: 465299]
51. Cook MB, Chow WH, Devesa SS. Oesophageal cancer incidence in the United States by race, sex, and histologic type, 1977–2005. *Br J Cancer*. 2009; 101(5):855–9. [PubMed: 19672254]

52. Yamamura K, Baba Y, Nakagawa S, Mima K, Miyake K, Nakamura K, et al. Human Microbiome *Fusobacterium Nucleatum* in Esophageal Cancer Tissue Is Associated with Prognosis. *Clin Cancer Res.* 2016; 22(22):5574–5581. •• Demonstrates that *F. nucleatum*, which may have a causative role in colon cancer, is associated with ESCC outcomes. [PubMed: 27769987]
53. Nasrollahzadeh D, Malekzadeh R, Ploner A, Shakeri R, Sotoudeh M, Fahimi S, et al. Variations of gastric corpus microbiota are associated with early esophageal squamous cell carcinoma and squamous dysplasia. *Sci Rep.* 2015; 5:8820. [PubMed: 25743945]
54. Liacouras CA, Furuta GT, Hirano I, Atkins D, Attwood SE, Bonis PA, et al. Eosinophilic esophagitis: updated consensus recommendations for children and adults. *J Allergy Clin Immunol.* 2011; 128(1):3–20. e6. quiz 21–2. [PubMed: 21477849]
55. Sgouros SN, Bergele C, Mantides A. Eosinophilic esophagitis in adults: a systematic review. *Eur J Gastroenterol Hepatol.* 2006; 18(2):211–7. [PubMed: 16394804]
56. Straumann A, Bauer M, Fischer B, Blaser K, Simon HU. Idiopathic eosinophilic esophagitis is associated with a T(H)2-type allergic inflammatory response. *J Allergy Clin Immunol.* 2001; 108(6):954–61. [PubMed: 11742273]
57. Roy-Ghanta S, Larosa DF, Katzka DA. Atopic characteristics of adult patients with eosinophilic esophagitis. *Clin Gastroenterol Hepatol.* 2008; 6(5):531–5. [PubMed: 18304887]
58. Orenstein SR, Shalaby TM, Di Lorenzo C, Putnam PE, Sigurdsson L, Mousa H, et al. The spectrum of pediatric eosinophilic esophagitis beyond infancy: a clinical series of 30 children. *Am J Gastroenterol.* 2000; 95(6):1422–30. [PubMed: 10894574]
59. Kelley ML Jr, Frazer JP. Symptomatic mid-esophageal webs. *JAMA.* 1966; 197(2):143–6. [PubMed: 5952499]
60. Attwood SE, Furuta GT. Eosinophilic esophagitis: historical perspective on an evolving disease. *Gastroenterol Clin North Am.* 2014; 43(2):185–99. [PubMed: 24813509]
61. Hruz P, Straumann A, Bussmann C, Heer P, Simon HU, Zwahlen M, et al. Escalating incidence of eosinophilic esophagitis: a 20-year prospective, population-based study in Olten County, Switzerland. *J Allergy Clin Immunol.* 2011; 128(6):1349–1350. e5. [PubMed: 22019091]
62. Prasad GA, Alexander JA, Schleck CD, Zinsmeister AR, Smyrk TC, Elias RM, et al. Epidemiology of eosinophilic esophagitis over three decades in Olmsted County, Minnesota. *Clin Gastroenterol Hepatol.* 2009; 7(10):1055–61. [PubMed: 19577011]
63. Dellon ES. Epidemiology of eosinophilic esophagitis. *Gastroenterol Clin North Am.* 2014; 43(2): 201–18. [PubMed: 24813510]
64. Dellon ES, Jensen ET, Martin CF, Shaheen NJ, Kappelman MD. Prevalence of eosinophilic esophagitis in the United States. *Clin Gastroenterol Hepatol.* 2014; 12(4):589–96. e1. [PubMed: 24035773]
65. Dellon ES, Peery AF, Shaheen NJ, Morgan DR, Hurrell JM, Lash RH, et al. Inverse association of esophageal eosinophilia with *Helicobacter pylori* based on analysis of a US pathology database. *Gastroenterology.* 2011; 141(5):1586–92. [PubMed: 21762663]
66. Ronkainen J, Talley NJ, Aro P, Storskrubb T, Johansson SE, Lind T, et al. Prevalence of oesophageal eosinophils and eosinophilic oesophagitis in adults: the population-based Kalixanda study. *Gut.* 2007; 56(5):615–20. [PubMed: 17135307]
67. Chen X, Winckler B, Lu M, Cheng H, Yuan Z, Yang Y, et al. Oral Microbiota and Risk for Esophageal Squamous Cell Carcinoma in a High-Risk Area of China. *PLoS One.* 2015; 10(12):e0143603. • Suggests that specific alterations to the oral microbiome may be a useful marker to screen for ESCC. [PubMed: 26641451]
68. Farrell JJ, Zhang L, Zhou H, Chia D, Elashoff D, Akin D, et al. Variations of oral microbiota are associated with pancreatic diseases including pancreatic cancer. *Gut.* 2012; 61(4):582–8. [PubMed: 21994333]
69. Ahn J, Chen CY, Hayes RB. Oral microbiome and oral and gastrointestinal cancer risk. *Cancer Causes Control.* 2012; 23(3):399–404. [PubMed: 22271008]
70. Snider EJ, Nobel YR, Compres G, Freedberg DE, Toussaint NC, Abrams JA. Shifts in the microbiome associated with Barrett's esophagus and progression to dysplasia and adenocarcinoma. *Gastroenterology (DDW Abstract).* 2017; 152(5):S1011–S1012.

71. Sawada A, Fujiwara Y, Nagami Y, Tanaka F, Yamagami H, Tanigawa T, et al. Alteration of Esophageal Microbiome by Antibiotic Treatment Does Not Affect Incidence of Rat Esophageal Adenocarcinoma. *Dig Dis Sci*. 2016; 61(11):3161–3168. [PubMed: 27461059]
72. Holvoet S, Doucet-Ladeveze R, Perrot M, Barretto C, Nutton S, Blanchard C. Beneficial effect of *Lactococcus lactis* NCC 2287 in a murine model of eosinophilic esophagitis. *Allergy*. 2016; 71(12):1753–1761. [PubMed: 27259693]

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Figure 1. Temporal correlation between population-level alterations to the upper GI microbiome and emergence of various esophageal diseases in western countries

H. pylori infections began to decline and antibiotic use began to expand starting in the mid-20th century. This was followed in time by marked rises in the incidence of esophageal adenocarcinoma and eosinophilic esophagitis.