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Review

The Esophageal Microbiota in Esophageal Health and Disease

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Abstract: The esophagus, traditionally viewed as a sterile conduit, is now recognized as a dynamic habitat for diverse microbial communities. The emerging evidence suggests that the esophageal microbiota plays an important role in maintaining esophageal health and contributing to disease. The aim of this systematic review was to synthesize the current knowledge on the esophageal microbiota composition, its variation between healthy individuals and those with esophageal diseases, and the potential mechanisms through which these microorganisms influence esophageal pathology. A systematic literature search was conducted using multiple databases, including PubMed, Scopus, and Web of Science, to identify relevant studies published up to July 2024. The inclusion criteria encompassed original research articles that used molecular techniques to characterize the esophageal microbiota in human subjects, comparing healthy individuals with patients affected by esophageal conditions such as gastroesophageal reflux disease (GERD), Barrett's esophagus, eosinophilic esophagitis, and esophageal cancer. The primary outcomes were the composition and diversity of the esophageal microbiota, and the secondary outcomes included the correlations between microbial profiles and disease states. The esophageal microbiota of healthy individuals was dominated by Gram-positive bacteria, particularly *Streptococcus*. Conversely, the esophageal microbiota is considerably altered in disease states, with decreased microbial diversity and specific microbial signatures associated with these conditions, which may serve as biomarkers for disease progression and as targets for therapeutic intervention. However, the heterogeneous study designs, populations, and analytical methods underscore the need for standardized approaches in future research. Understanding the esophageal microbiota's role in health and disease could guide microbiota-based diagnostics and treatments, offering novel avenues for managing esophageal conditions.

Keywords: esophageal microbiota; health; esophageal diseases; esophageal cancer

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Citation: Bonazzi, E.; Lorenzon, G.; Maniero, D.; De Barba, C.; Bertin, L.; Barberio, B.; Salvador, R.; Valmasoni, M.; Zingone, F.; Ghisa, M.; et al. The Esophageal Microbiota in Esophageal Health and Disease. *Gastroenterol. Insights* **2024**, *15*, 998–1013. https://doi.org/10.3390/gastroent15040069

Academic Editor: Julio Plaza-Díaz

Received: 20 September 2024
Revised: 21 October 2024
Accepted: 18 November 2024
Published: 20 November 2024

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1. Introduction

The human body maintains symbiotic relationships with a complex and diverse array of microbial communities across various nonsterile body sites, which is collectively known as the human microbiota. The microbiome, instead, refers to the entire collection of genetic material (genomes) of the microorganisms within a particular environment. It includes the microorganisms as well as their genes, gene products, metabolites, etc. The abovementioned sites include the skin and various mucus membranes, such as those in the mouth, as well as the upper respiratory, lower genitourinary, and gastrointestinal tracts.

The composition of the human microbiota widely varies among individuals and with time, depending on several factors, such as genetics, food habits, and the environment [1]. Although the human esophagus had long been considered a relatively sterile environment, it is now recognized as a dynamic habitat for a diverse community of microorganisms known as the esophageal microbiota. In particular, owing to the advent of novel culture-independent techniques, such as 16S rRNA gene sequencing, researchers have finally been able to accurately characterize the esophageal microbiota both in healthy subjects and in those with various esophageal diseases [2,3]. The esophageal microbiota differs from the oral microflora, despite their close proximity, harboring its own, unique species [4]. The microbiota is thought to contribute to the maintenance of esophageal health via modulating several metabolic and immunologic pathways and playing a crucial role in the defense against microbial pathogens [5,6]. A balanced microbial community composition within the esophagus is essential for maintaining mucosal integrity, modulating immune responses, and potentially influencing the development of various esophageal diseases [7]. Interest is increasing in exploring the connection between the esophageal microbiome and the development of esophageal disorders, such as Barrett's esophagus or esophageal cancer, as changes in the human microbiome can strongly influence disease susceptibility [8]. This review focuses on the changes in the esophageal microbiota and/or microbiome that are associated with both the healthy and unhealthy esophagus, aiming to enhance the understanding of its differences under these conditions and different esophageal diseases, namely, achalasia, gastroesophageal reflux disease (GERD), Barrett's esophagus, eosinophilic esophagitis (EoE), and esophageal cancer. Clarifying the relationship between esophageal microflora and esophageal disorders will potentially offer new strategies for preventing or treating esophageal diseases.

2. Methods

2.1. Literature Search Strategy

A comprehensive literature search was conducted to identify relevant studies describing the esophageal microbiota in both healthy individuals and various in those with different esophageal diseases. The search spanned multiple. Searches were performed of electronic databases, including PubMed, MEDLINE, and Google Scholar, using targeted keywords and Boolean operators for optimized retrieval of relevant articles: examples of the keywords used: "esophageal microbiota", "esophageal disorders", "esophagitis", "gastrointestinal-reflux disease", "Barrett's esophagus", and "esophageal cancer". The search was restricted to articles published in English, primarily from the past decade. However, a few key studies older than ten years were included, as they provide essential insights on demonstrating the period of interest in the topic. Additionally, the references from selected articles were manually reviewed to uncover any further relevant studies.

2.2. Inclusion and Exclusion Criteria

Articles were included if they reported findings related to the esophageal microbiota in the context of esophageal diseases, with a primary focus on clinical studies. Exclusion was mostly considered. The exclusion criteria included non-English publications, reviews, commentaries, and studies not directly relevant to the scope of this review.

2.3. Data Extraction and Analysis

The data from the selected articles were extracted. The key details were study characteristics such as participant demographics (where applicable), microbiome analysis methods, outcome measures, and primary findings related to esophageal health and disease. A narrative synthesis was conducted, grouping studies according to diseases. The data were narratively synthesized, with studies grouped based on their thematic relevance to this review's objectives. The key findings were summarized, and emerging patterns were highlighted. Any discrepancies or conflicting results were noted and analyzed within the context of this review.

2.4. Quality Assessment

Given that formal quality assessment tools were not employed due to the narrative nature of this review, the quality assessment was not included. However, we considered the credibility and reliability of the selected studies during data synthesis and interpretation.

3. Results

3.1. The Esophageal Microbiota of the Healthy Esophagus

The esophageal mucosa hosts a resident microbiota that exhibits substantial qualitative and quantitative differences compared to the microbiota residing in the large intestine, with populations ranging from 10 cells per g/L of the sampled material in the esophagus to 10^{12} cells per g/mL of the sampled material in the large intestine [9]. Few studies have focused on the esophageal microbiota composition of healthy individuals alone [2,3,10,11]; however, more recent studies have investigated the esophageal microbiota associated with different esophageal diseases and included healthy patients as controls for comparison, thus allowing the characterization of the esophageal microbiota [12–14]. In 2004, Pei et al. were the first to determine the composition of the esophageal microbiota through 16S rRNA gene sequencing, thus demonstrating its complexity [2]. They revealed the presence of six predominant phyla: Firmicutes (mainly *Streptococcus*, *Veillonella*, *Megaspheaera*, *Granulicatella*, *Gemella*, *Clostridium*, and *Bulleidia*), Bacteroidetes (mainly *Prevotella* and *Bacteroides*), Fusobacteria, Actinobacteria (mainly *Rotia* and *Actinomyces*), and Saccharibacteria. The phyla of the Firmicutes were dominant, with *Streptococcus mitis* being the most abundant species. Further studies were conducted to more accurately characterize the esophageal microflora owing to the continuous progress of molecular technology (Table 1) [8,15]. These microbial communities are thought to play crucial roles in maintaining the integrity and function of the esophageal epithelium, contributing to the overall homeostasis of the esophageal environment. However, the delicate balance of the esophageal microbiome can be disrupted in various disease states, including GERD, Barret's esophagus, EoE, and esophageal cancer, which are characterized by alterations in the microbial ecosystem, which may contribute to the pathogenesis and development of these disorders [16,17].

Table 1. Main findings regarding esophageal microbiota in the healthy esophagus (last 20 years).

Study (Year)	Population	Sample Type; Method of Analysis	Main Findings
[2] Pei et al. (2004)	4 healthy individuals, with healthy esophagus	Distal esophagus	Members of 6 phyla were represented: Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria, and Sacchari bacteria
[8] Yang et al. (2009)	12 healthy individuals; 12 esophagitis patients; 10 BE patients	Distal esophagus	Esophageal microflora consisted of Gram-positive bacteria (dominated by *Streptococcus*) which mainly characterize the healthy esophagus
[15] Liu et al. (2013)	6 healthy individuals; 6 RE patients; 6 BE patients	Distal esophagus	There were 4 principal phyla associated with the healthy esophagus: Proteobacteria, Firmicutes, Bacteroidetes (8%), and Actinomycetes.

3.2. Esophageal Microbiota in Achalasia, Gastroesophageal Reflux Disease, and Barret's Esophagus

Achalasia is an esophageal motility disorder characterized by impaired relaxation of the lower esophageal sphincter during swallowing in the absence of peristalsis [18]. Achalasia is an immune-mediated inflammatory disease linked to the human herpes simplex virus type 1 (HSV-1). It involves immune responses associated with HSV-1, specifically Th-1-related responses, as well as Th2 and Th17-mediated immune pathways [19]. Therefore, it has been reported that the development of achalasia is mediated by different genetic

and environmental factors, among which is also the esophageal microbiota. It has been shown that microbiome alterations play a role in the pathogenesis of different inflammatory conditions in the gastrointestinal tract. Thus, it has been hypothesized that modification in the esophageal microbiota may be associated with altered immune responses recorded in achalasia patients. Achalasia is an esophageal motility disorder characterized by the impaired relaxation of the lower esophageal sphincter during swallowing in the absence of peristalsis [18]. Achalasia is an immune-mediated inflammatory disease that is associated with human herpes simplex virus type 1 (HSV-1), HSV-1-associated, as well as Th1-, Th2-, and Th17-related immune responses [19]. The development of achalasia is mediated by different genetic and environmental factors, among which is the esophageal microbiota. Microbiome alterations play a role in the onset and development of inflammatory conditions in the gastrointestinal tract; thus, changes in the esophageal microbiota may be associated with the immune response observed in patients with achalasia.

Ikeda et al. reported the esophageal microbiota alterations that occur in patients with achalasia [20]. The proportion of *Streptococcus* was altered in the majority of achalasia patients, with *Streptococcus* being implicated in the maintenance of a balanced esophageal environment [17]. Both *Actinomyces* and *Dialister* were associated with an increased Th17-related immune response, underlining that members of the esophageal microbiota can contribute to the development and/or exacerbation of achalasia. Geng et al. investigated the lower esophageal mucosal microbiota of patients with achalasia and controls in [21]. They also revealed esophageal microbiota alterations in patients with achalasia, specifically decreased microbiota diversity, which was associated with a predominance of Gram-negative bacteria compared with that in healthy controls. Moreover, the intestinal microbiota of patients with achalasia was associated with increased lipopolysaccharide levels, a microbial component known for triggering intestinal inflammation [22]. Together, these results highlight the implications of the esophageal microbiota in the onset and development of achalasia. Further studies are needed to more deeply understand the exact mechanisms involved.

Gastroesophageal reflux disease is a condition in which the stomach contents repeatedly flow back up into the esophagus, irritating its lining. This disease affects both adults and pediatric patients, with a worldwide incidence that has been increasing substantially in recent years [23–27]. GERD syndromes encompass typical reflux symptoms, characterized by regurgitation and heartburn, and are sometimes accompanied by non-cardiac chest pain, dysphagia, and dyspeptic symptoms. In some cases, patients can also experience extra-esophageal manifestations, including laryngeal hoarseness, coughing, dysphonia, asthma, and throat clearing [28–32]. In particular, most of the patients with GERD can be classified as patients with non-erosive reflux disease (NERD) or with erosive esophagitis. NERD has been commonly defined in the case of typical GERD symptoms in the absence of esophageal mucosal injury during upper endoscopy and presents in approximately 70% of patients with GERD [33–36]. The pathophysiology of GERD is multifactorial, involving, for example, delayed gastric emptying, esophageal motility abnormalities, reflux hypersensitivity, abnormal esophageal clearance, and failure of the anti-reflux barrier constituted by the esophagogastric junction and crural diaphragm [37–41]. In addition, mucosal damage can be affected by refluxate characteristics and esophageal clearance mechanisms [23,42,43]. Finally, due to its close proximity to esophageal mucosa and epithelial cells, the esophageal microbiota can be potentially involved in the onset and development of the disease. Indeed, several studies (Table 2) already reported differences in esophageal microbiota composition of healthy individuals compared to GERD patients, thus highlighting the potential role in disease pathogenesis.

Zhou et al. investigated the pathogenetic role of microbial dysbiosis in GERD, evaluating the characteristics of esophageal microbiota and the underlying host mucosa proteome [44]. A total of 70 patients were enrolled in the study and classified according to one of five phenotypes based on symptomatic and histopathologic features: (1) control subjects, (2) non-erosive reflux disease (NERD, (3) reflux esophagitis (RE), (4) Barrett's

esophagus, and (5) esophageal adenocarcinoma (EAC). Their results showed that the overall microbiota composition was altered in those with NERD and EAC compared with that of the controls and was associated with a reduction in the levels of proteins associated with external stimuli responses. In particular, the Chao1 richness estimator was significantly lower in microbial richness in the esophageal microbiota of patients with NERD than in controls. From the results of multivariate analysis, the authors identified 41 differential Operational Taxonomic Units (OTUs) within nine phyla: the esophageal microbiota of control subjects had higher levels of Gram-positive Firmicutes and Actinobacteria than that of the other groups, whereas the NERD microbiota composition was characterized by high levels of Proteobacteria and Bacteroidetes, with decreases in the levels of Fusobacteria and Actinobacteria [44]. Another study from Park et al. assessed the response of patients with NERD to proton pump inhibitor therapy, as well as the associated changes in their esophageal microbiota, together with the presence of biological markers in the esophageal biomarkers [13]. *Streptococcus* was found to be the most prevalent bacterial taxon in the esophageal microbiome of NERD patients. Moreover, the authors also observed that *Prevotella*, *Haemophilus*, *Veillonella*, *Neisseria*, and *Granulicatella* are common taxa in the esophagus of those with NERD. These results agree with the findings of Yang et al. [8].

Overall, these results emphasize the potential involvement of the esophageal microbiota in the onset and development of gastroesophageal-reflux-related diseases. However, further studies are needed to identify the mechanisms underpinning its role in disease pathogenesis and to understand whether these microbiota changes are a cause or consequence of these conditions

Barrett's esophagus (BE) is defined as the replacement of any portion of the esophageal normal distal squamous epithelium by metaplastic columnar epithelium, which is clearly visible endoscopically ($\geq$1 cm) above the gastroesophageal junction [45]. In particular, BE is a complication of GERD, with which it shares its symptoms. It has been estimated that in symptomatic patients with chronic GERD, the prevalence of BE is as high as 15% [46]. Since patients presenting BE carry a 30–40 times higher risk for developing esophagus adenocarcinoma than the general population, in recent years, the research on this particular condition has been growing [47]. In particular, several studies (Table 2) have already shown the relationship between the microbiome and BE, generally reporting that the esophageal microbiome associated with BE is altered compared to that of healthy individuals [48–50].

Okereke et al. aimed to further characterize the esophageal microbiota associated with BE [12]. The novelty of this study is that the authors investigated the esophageal microbiota at different locations along the esophagus, collecting multiple biopsies from different sites. A total of seventy-four patients were included in the study, and they were assigned either to a BE group (34 patients) or a without-GERD BE group (40 patients) based on the results of clinical evaluation. After collection of the biopsies, target organisms were identified using next-generation sequencing of the 16S rRNA gene to identify a specific microbiota pattern that can be used to predict which patients are at higher risk of developing esophageal adenocarcinoma. First, a microbiota community structure was found to be associated with the presence of BE, as already observed in previous studies on this topic [44,49]. Secondly, it was previously reported that, as the severity of BE increased, the likelihood of the detection of multiple organisms decreased as the BE severity increased, with a specific localization to the distal esophagus [12]. The evaluation of the esophageal mucosal microbiota and the identification of the presence/absence of a high-risk microbiota community could be a potential method to more effectively stratify the risk of esophageal cancer among patients with BE. Future studies should consolidate these first findings and further characterize (likely at the species level) the BE-associated microbial esophageal population to enable the development of microbiota-based treatment plans for these patients.

3.3. The Esophageal Microbiota in Eosinophilic Esophagitis

Eosinophilic esophagitis (EoE) is a chronic inflammatory condition of the esophagus driven by an immune-mediated response, which is unrelated to IgE and triggered by dietary

antigens. Clinically, EoE is characterized by symptoms of esophageal dysfunction, while histologically, it is marked by an inflammation dominated by eosinophils and confined to the esophagus [51]. In recent years, the incidence and prevalence of EoE have risen significantly, currently standing at 7.7 person-years and 42.2 cases per 100,000 adults, respectively, with no definitive cause identified [52,53]. Emerging evidence suggests that host and environmental factors may largely contribute to the increase in disease rates. Among these factors, it has been hypothesized that the esophageal microbiota could be involved in EoE pathogenesis [54–56]. It is already known that the gut microbiota plays crucial roles in metabolic function, influencing the development of the immune system [57]. Similarly, early microbiota studies in EoE (Table 2) have already demonstrated differences in the esophageal microbial community in EoE patients versus non-EoE controls [14,58].

We recently investigated the differences in the salivary, esophageal, and gastric microbiota between patients with active EoE and nonactive EoE controls [55]. We revealed that the esophageal microbiota could be used to differentiate between patients with and without EoE with a CE of 8%. Some members of the esophageal microbiota, namely, the *Actinobacillus*, *Bergeyella*, *Porphyromona*, and *Alloprevotella* genera, were associated with patients with active EoE who were characterized by biological samples with an eos/HPF > 15 [55]. Another interesting study by Benitez et al. investigated the esophageal microbiota of 68 children, 33 of whom were affected by eosinophilic esophagitis and 35 who were non-EoE controls [58]. The proportions of the bacterial communities in the esophageal microbiota significantly differed between those with and without EoE. In the EoE cohort, the esophageal microbiota was dominated by Proteobacteria, including *Neisseria* and *Corynebacterium*, whereas Firmicutes was the most abundant phyla in the esophagus of the non-EoE controls. Additionally, changes in the dietary intervention, in particular the reintroduction of highly allergenic foods, led to the enrichment of *Granulicatella* and *Campylobcater* genera in the esophagus of children with EoE [58]. In a later study, Laserna-Mendieta et al. assessed the changes in the esophageal microbiome through 16S rRNA sequencing using esophageal biopsies of adult patients with active EoE at baseline. After achieving remission with a proton pump inhibitor, the patients were swallowed topical corticosteroids or consumed food-elimination diets. The biopsies from those without EoE were also included in the study as controls [14]. The results of the analysis of the beta-diversity highlighted a clustering of the esophageal microbiota composition of patients with EoE that was different from that of the non-EoE controls at baseline. In particular, three genera, previously unassociated with the disease, were identified: *Filifactor*, *Parvimonas,* and *Porphyromonas*. In addition to changes in the esophageal microbiota due to the presence/absence of the disease, the authors reported microbial differences among patients with EoE who had been treated with different therapies. In detail, the post-treatment samples from the patients treated with a PPI and a food-elimination diet had a similar esophageal microbiota composition, whereas those administered swallowed topical corticosteroids had a microbial composition that was closer to that of the controls. These findings indicated that the esophageal microbiota of patients with EoE is different than that of people without EoE, suggesting that alterations in its composition depend on the therapy administered. In particular, swallowed topical corticosteroids made the esophageal microbiota composition of the patients with EoE similar to that of the non-EoE controls and less similar to that of people with EoE treated with proton pump inhibitors or a food-exclusion diet. However, not all studies have reported significant differences in the esophageal microbiota composition between those with and without EoE. For example, Johnson et al. found no significant differences in the esophageal microbiome between 24 adult patients with EoE and 25 adults without EoE [59]. Given the recent increases in the incidence and prevalence of EoE, additional studies are warranted to further understand the role played by the esophageal microbiota in EoE pathophysiology and investigate novel microbiota-based targeted therapies for EoE patients. Although some studies reported differences in people with EoE, not all studies agreed on this result. For example, a study conducted by Johnson and colleagues reported no differences in the esophageal microbiota of newly diagnosed EoE patients and non-eosinophilic esophagitis

patients. In particular, no significant differences were present both at the phyla and at the genus level [59]. In both groups, the most abundant phyla was Firmicutes, followed by Bacteroidetes, however further studies examining gut microbiota composition at the species level are needed to better elucidate the role of the esophageal microbiome in the pathogenesis of the disease.

Table 2. Main findings about esophageal microbiota in achalasia, Gastroesophagealin gastroesophageal reflux (GERD), Barrett's esophagus (BE), and eosinophilic esophagitis (EoE) (latest 5 last 10 years).

Study (Year)	Population	Sample Type; Method of Analysis	Main Findings
[13] Park et al. (2020)	18 NERD patients	NA *	Firmicutes, Proteobacteria, and Bacteroidetes dominated at the phylum level. At the genus level, the more common were *Streptococcus*, *Haemophilus*, *Prevotella*, *Veillonella*, *Neisseria*, and *Granulicatella*
[44] Zhou et al. (2020)	16 healthy patients; 11 NERD patients; 20 RE patients; 17 BE patients;	Proximal and distal esophagus	NERD patients had a shift from Fusobacteria and Actinobacteria to Proteobacteria and Bacteroidetes; RE and BE patients had a shift from Firmicutes to Fusobacteria and Proteobacteria
[12] Okereke et al. (2021)	34 BE patients; 40 GERD patients	Proximal and distal esophagus	Microbial diversity decreased with the increase in the length of Barrett's column
[55] Facchin S. et al. (2022)	Saliva: 29 people with EoE patients and 20 non-EoE controls Biopsies: 25 people EoE patients and 5 non-EoE controls	N/A *	In saliva samples, 23 ASVs were positively associated with EoE and 27 ASVs with controls, making it possible to discriminate between EoE, enabling differentiation between patients with and non-without EoE.; Analysis of observed esophageal microbiota samples showed a clear microbial pattern able to discriminate between patients with active and inactive EoE.
[21] Geng et al. (2023)	32 subjects with achalasia and 27 healthy individuals	Lower esophagus	Abundant taxa in achalasia patients: *Aquabacterium*, *Novosphingobium*; *Lactobacillus*, *Faecalibacterium*, *Acidovorax*, and *Ruminococcus*
[20] Ikeda et al. (2024)	16 subjects with achalasia and 11 control individuals	N/A *	*Actinobacteria* and *Bacteroides* were significantly higher in achalasia patients at the phylum level. At the genus level, *Streptococcus*, *Helicobacter pylori*, and *Xanthomonas* were significantly lower in achalasia patients while *Dialister* and *Actinomyces* were higher.
[60] Solfisburg et al. (2024)	125 non-BE patients; 20 non-dysplatic BE and 78 EAC patients	Oral swab	Increased relative abundance of *Streptococcus* in EAC patients

* NA represents not available.

3.4. Esophageal Microbiota in Esophageal Cancer

Esophageal cancer is classified into two main subtypes based on the type of cells involved: esophageal squamous cell carcinoma (ESCC) and Esophageal Adenocarcinoma (EAC). Moreover, other rare subtypes exist, such as small cell carcinoma, sarcomatoid carcinoma, and Lymphoepithwlioma-like carcinoma [61,62]. The increased incidence and mortality rates of esophageal cancer have pushed researchers to pay more and more attention to this disease in recent years. A major challenge remains in the early diagnosis and treatment of the disease, with many esophageal cancers that cannot be diagnosed until they present with symptoms such as odynophagia and dysphagia [61]. The etiology and pathophysiology of esophageal cancer are multifactorial and population dependent; thus,

the complex mechanisms leading to the insurgence of the disease are not completely understood. Some of the factors involved in the onset and development of the disease include genetics, smoking, consumption of alcoholic beverages, diet, and the microbiome [61,63]. With the deepening of research on human microbes and the evidence of their involvement in the onset and development of other malignant tumors [64,65], the esophageal microbiota has been investigated, in particular, its role in esophageal diseases and esophageal cancer etiology. Esophageal cancer, also known as esophageal squamous cell carcinoma (ESCC) and adenocarcinoma, is the sixth leading cause of death owing to cancer globally [61,62].

3.4.1. Esophageal Microbiota in ESCC

A recent study from Yang and colleagues investigating esophageal microbiota in 29 patients, 18 with diagnosed ESCC and 11 individuals with physiological normal esophagus (controls), revealed that microbiota composition in tumor tissues of ESCC was significantly different from that of controls [66]. In particular, ESCC microbiota were distinguished by an increased abundance of *Bacteroidetes*, *Fusobacteria*, and *Spirochaetes*, associated with a reduced microbial diversity. In more detail, the microbiota profiles of the two groups could be discriminated by the abundance of *Fusobacterium* spp. and *Klebsiella* spp. When these taxa were then employed to calculate the microbial dysbiosis index, it was reported that the dysbiosis microbiota had a good capacity to discriminate between ESCC and physiological normal esophagus. However, no comparisons were performed in patients with other esophageal diseases (i.e., Barrett's esophagus, GERD, or ADC) in order to corroborate the diagnostic value of this analysis. Nevertheless, these results suggest specific microbes may be associated with ESCC and potentially implicated in driving/mitigating ESCC carcinogenesis. Moreover, it has been speculated that these bacteria could play a role as cancer therapy's target. Another study by Zhang and colleagues recently investigated changes in the microbial community during cancer development with the aim of identifying latent pathogenic bacteria contributing to ESCC progression [67]. Firstly, this study reported a slight reduction in the esophageal microbiota diversity in tumor tissues compared to non-tumor tissues, although it was not significant. Interestingly, Linear discriminant analysis reported that four principal phyla and 28 genera were contributing to changes in the esophageal microbiota of ESCC patients. As an example, the probiotic Lactobacillus was enriched in non-tumor tissues, while the general pathogenic Fusobacterium was 4.35-fold higher in tumor tissues, which appears consistent with previous results [68,69]. Moreover, for tumor tissue samples, some genera were enriched in association with specific cancer stages, highlighting the importance of the specific cancer environment for the presence/growth of these bacteria. In particular, the genera *Treponema* and *Brevibacillus* were higher in the N1 and N2 stages, respectively, while *Acinetobacter* was enriched in the T3 stage [67]. Finally, in order to analyze and predict esophageal microbiota function, PICRUSt 1.1.4 software was used. The analysis carried out by this sophisticated software revealed that some pathways were significantly increased in the esophageal microbiota associated with tumor tissues (pathways related to base excision repair), while other pathways related to nitrotoluene degradation were increased in non-tumor tissues.

3.4.2. Esophageal Microbiota in EAC

Contrary to ESCC, esophageal adenocarcinoma typically occurs in the lower third of the esophagus, and its incidence rate in the US has increased to 7.2 per 100,000 people in recent years [70,71]. Many studies have already reported esophageal microbiota alteration in patients with EAC. Among them, Lopetuso and colleagues showed a reduction in *Streptococcus* and an increase in *Prevotella* in EAC patients compared to control individuals [72,73]. In another study, Zhou and colleagues showed that, compared with normal esophageal microbiota, EAC-associated microbiotas were enriched with *Proteobacteria* and *Firmicutes* [44]. Furthermore, *Candida albicans* and *Candida glabrata* were found to be abundant in more than half of the human EAC samples, suggesting that the fungal microbiota may play a pathogenetic role in the esophagus [72]. Yang et al. investigated the esophageal microbiota

in 29 patients, 18 with diagnosed ESCC and 11 with a physiologically normal esophagus (controls), finding that the microbiota composition in the tumor tissues of those with ESCC was significantly different from that of the controls [66]. In particular, ESCC microbiota was distinguished by higher abundances of *Bacteroidetes*, *Fusobacteria*, and *Spirochaetes*, which were associated with a reduced microbial diversity. In detail, the microbiota profiles of the two groups could be discriminated using the abundances of *Fusobacterium* spp. and *Klebsiella* spp. The dysbiosis of the microbiota was used to accurately discriminate between ESCC and physiologically normal esophagi, and these taxa were then employed to calculate the microbial dysbiosis index. However, no comparisons were performed with patients with other esophageal diseases (i.e., Barrett's esophagus, GERD, or ADC) to corroborate the diagnostic value of this analysis. Nevertheless, these results suggest that specific microbes are associated with ESCC and implicated in driving/mitigating ESCC carcinogenesis. Moreover, these bacteria may be a target for cancer therapy. Zhang et al. investigated the changes that occur in the microbial community during cancer development to identify latent pathogenic bacteria contributing to ESCC progression [67]. The authors reported a slight, nonsignificant reduction in the esophageal microbiota diversity in tumor tissues compared with that in nontumor tissues. The results of a linear discriminant analysis showed that four principal phyla and 28 genera contributed to the changes in the esophageal microbiota of the people with ESCC. As an example, the probiotic Lactobacillus was enriched in nontumor tissues, whereas the level of the general pathogenic Fusobacterium was 4.35-fold higher in tumor tissues, which appears consistent with previous results [68,69]. Moreover, some genera were enriched in association with specific cancer stages for tumor tissue samples, highlighting the importance of the specific cancer environment for the presence and growth of these bacteria. The genera *Treponema* and *Brevibacillus* were enriched in the N1 and N2 stages, respectively, whereas *Acinetobacter* was enriched in the T3 stage [67]. Finally, in order to analyze and predict esophageal microbiota function. The analysis conducted with this sophisticated software revealed that some pathways were significantly enriched for the esophageal microbiota associated with tumor tissues (pathways related to base excision repair); other pathways related to nitrotoluene degradation were enriched in nontumor tissues. Contrary to ESCC, esophageal adenocarcinoma typically occurs in the lower third of the esophagus, and its incidence rate in the USA has recently increased to 7.2 per 100,000 people [70,71]. Esophageal microbiota alterations have been reported in patients with EAC. Lopetuso et al. found lower levels of *Streptococcus* and higher levels of *Prevotella* in patients with EAC than in control individuals [73]. Zhou et al. found that, compared with normal esophageal microbiota, EAC-associated microbiotas were enriched with *Proteobacteria* and *Firmicutes*, such as *Staphylococcus aureus*, *Streptococcus infantis*, and *Lactobacillus salivarius* [44]. Additionally, high prevalences of *Candida albicans* and *Candida glabrata* were found in more than half of the human EAC samples, which suggested the presence of a fungal microbiota in the esophagus [72]. Recent studies (latest 5 years) on EAC are grouped in Table 3.

Together, these studies highlight the potential involvement of the esophageal microbiota in esophageal cancer onset and development. In particular, an important factor to take into account when analyzing the esophageal microbiota in relation to cancer development is the location of the disease, as well as the sampling size. Esophageal cancer can occur in different regions of the esophagus, and the microbiota composition can vary significantly between these areas. Sampling from the precise location of the tumor or inflamed tissue is crucial, as microbiota may differ in proximity to cancerous lesions, influencing both inflammation and disease progression. Additionally, variations in sampling techniques, such as endoscopic brushing or biopsy, can impact the accuracy and consistency of microbiota analysis. Additional studies, including multicenter trials and larger population samples, are warranted to further understand the interactions between the esophageal microbiota and the host's esophageal environment and to investigate the specific mechanisms involved in esophageal cancer pathophysiology. Figure 1 represents the esophageal microbiota changes associated with different esophageal diseases.

Table 3. Main findings on the esophageal/oral microbiota in esophageal cancer (latest 5 years).

Study (Year)	Population	Sample Type; Method of Analysis	Main Findings
[74] Jiang et al. (2021)	32 ESCC patients; 15 ES patients; 21 healthy individuals	NA *	At genus level, *Faecalibacterium*, *Curvibacter*, *Bacteroides*, and *Blautia* levels were lower in patients with ESCC
[75] Kovaleva et al. (2021)	48 ESCC patients	NA *	Two different groups were distinguished: one characterized by a high abundance of Gram-positive bacteria, while in the second one there was a lower abundance of Gram-positive bacteria
[76] Li et al. (2021)	41 ESCC patients	NA *	Most abundant phyla were *Actinobacteria*, *Firmicutes*, *Bacteroidetes*, *Fusobacteria* *Proteobacteria*, and *Spirochaetes*, which dominated the bacterial flora; the presence of *F. nucleatum* was strongly correlated with tumor clinical stage
[66] Yang et al. (2021)	18 ESCC patients; 11 healthy individuals	NA *	ESCC patients: decreased microbial diversity and lower presence of Bacteroidetes, Spirochaetes, and Fusobacterium
[77] Hao et al. (2022)	27 healthy subjects, 37 GERD, 32 BE and 25 EAC	Esophageal biopsy	Streptococcus was depleted in EAC compared to healthy control while Atopobiu, Actinomyces, Veillonella, etc., were increased. Tumor tissues *Brevibacillus* and *Treponema* were more common in N1 and N2 stages, respectively; *Acinetobacter* was more common in T3
[78] Shen et al. (2022)	19 ESCC patients	Tumor tissues and adjacent nontumor tissues	Tumor tissue: at genus level, bacterium with highest proportions in tumors was *Streptococcus* and was present in higher abundance; adjacent nontumor tissues: bacterium with highest proportions was *Labrys*.
[79] Zaramella et al. (2023)	58 patients with no dysplatic BE; 8 patients with low-grade dyspltatic BE; 8 patients with high-gradedysplasia; and 7 patients with EAC	Distal esophageal biopsy	The main genera in EAC were Prevotella and Streptococcus, although their amount was lower compared to the BE group. EAC microbiota was also characterized by an increase of Fusobacterium
[80] Jiang et al. (2023)	53 healthy individuals and 56 ESCC patients	Oral swab	Microbial richness and diversity were higher in oral microbiota of ESCC patients. At phylum level, Fusobacteria was increased in the ESCC group; Neisseriaceae were increased as family and Leptorichia was increased at genus level in the ESCC group.
[60] Solfisburg et al. (2024)	125 non-BE patients; 20 non-dysplatic BE and 78 EAC patients	Oral swab	Increased relative abundance of *Streptococcus* in EAC patients

* NA represents not available.

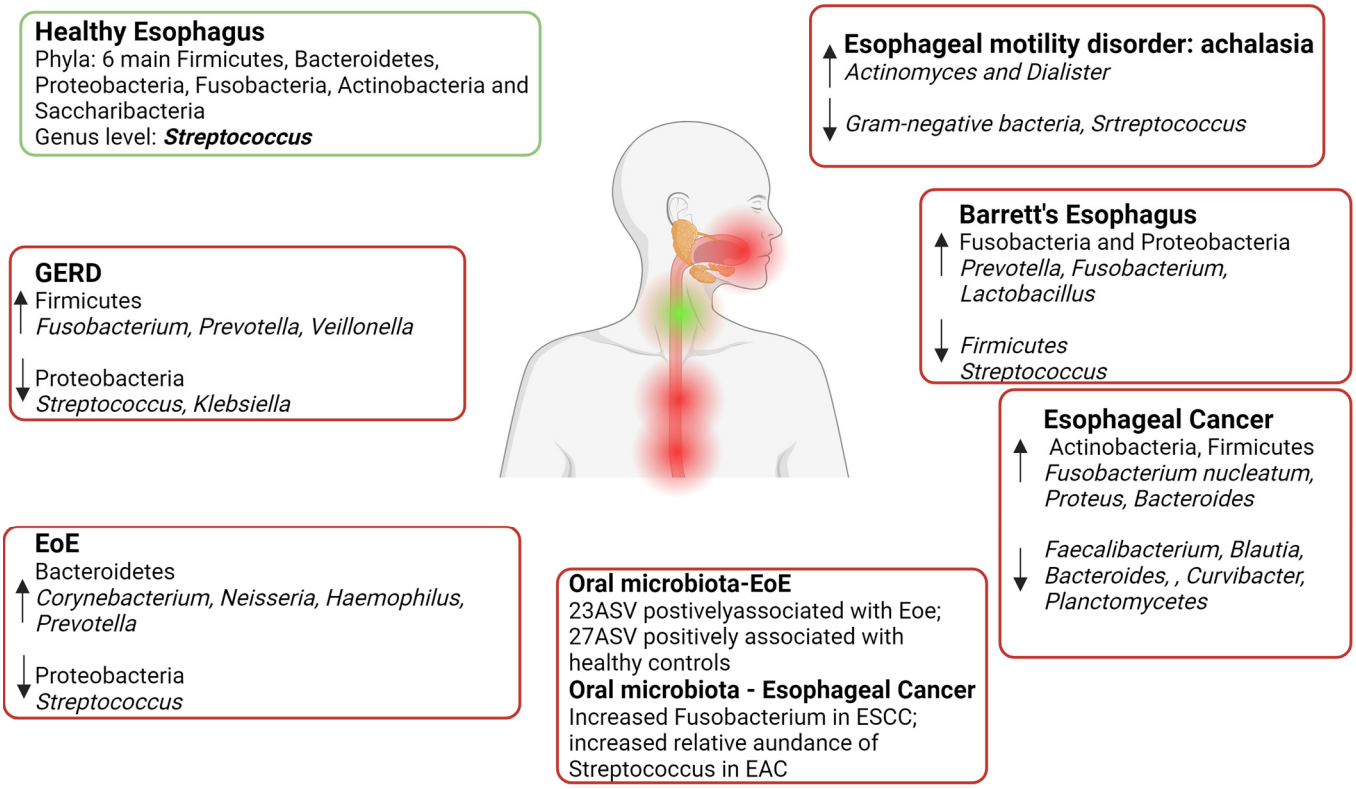

Figure 1. Changes in the esophageal microbiota in healthy individuals and in various esophageal diseases.

4. Conclusions

Due to the increased incidence and prevalence of esophageal diseases, in parallel with the increasing knowledge on the role of gut microbiota in the pathogenesis and management of several gastrointestinal and extraintestinal conditions [77–81], there are implications for the onset and development of these diseases [82–86]. Current studies report that there is a resident microbiota in the human esophagus, and its composition varies among individuals and, more importantly, between healthy individuals and people suffering from a specific esophageal disease, such as GERD, Barrett's esophagus, or EoE. In particular, a normal esophagus is mainly dominated by Gram-positive bacteria, including Firmicutes, Bacteroidetes, Proteobacteria, Fusobacteria, Actinobacteria, and Saccharibacteria, with *Streptococcus* being the most prevalent genus among the Firmicutes. Patients with esophagitis, including GERD, Barrett's esophagus, and EoE, are characterized by a microbiota enriched with Gram-negative bacteria, which are associated with decreased microbial diversity. These data suggest a mutual and reciprocal relationship between the esophageal microbiota and the esophageal environment, where the composition and function of the microbiota are shaped and influenced by the esophageal microenvironment and where the mucosal esophageal microenvironment is constantly remodeled by the resident and/or pathogenic microflora. Despite the increasing number of studies on esophageal microbiota in health and disease, some limitations, including differences in sampling and determination methods and inclusion and exclusion criteria, have led to heterogeneous results among the studies. The development of genomics and multiomics techniques, including metatranscriptomics, proteomics, and metabolomics, will help researchers to further understand the complex interactions in the esophageal environment and the role of the esophageal microbiome in esophageal diseases, potentially identifying molecular markers for predicting conditions such as Barrett's esophagus or esophageal cancer. These studies will help with developing more accurate screening and diagnostic tools to contribute to the prevention and monitoring of various esophageal diseases.

Author Contributions: Conceptualization, E.B. and E.V.S.; methodology, E.B. and E.V.S.; writing—original draft preparation, E.B.; writing—review and editing, R.S., M.V., M.G., E.B., L.B., G.L., C.D.B., B.B., F.Z., D.M. and E.V.S.; supervision, E.V.S. All authors have read and agreed to the published version of the manuscript.

Funding: This study received no external funding.

Conflicts of Interest: Edoardo Vincenzo Savarino has served as speaker for Abbvie, Agave, AG-Pharma, Alfasigma, Aurora Pharma, CaDiGroup, Celltrion, Dr Falk, EG Stada Group, Fenix Pharma, Fresenius Kabi, Galapagos, Janssen, JB Pharmaceuticals, Innovamedica/Adacyte, Malesci, MayolyBiohealth, Omega Pharma, Pfizer, Reckitt Benckiser, Sandoz, SILA, Sofar, Takeda, Tillots, and Unifarco; he has served as a consultant for Abbvie, Agave, Alfasigma, Biogen, Bristol-Myers Squibb, Celltrion, DiademaFarmaceutici, Dr Falk, Fenix Pharma, Fresenius Kabi, Janssen, JB Pharmaceuticals, Merck & Co, Nestlè, Reckitt Benckiser, Regeneron, Sanofi, SILA, Sofar, Synformulas GmbH, Takeda, and Unifarco; and he received research support from Pfizer, Reckitt Benckiser, SILA, Sofar, Unifarco, Zeta Farmaceutici. Sonia Facchin has served as a consultant for SILA, Unifarco, and Zeta Farmaceutici and has served as a speaker for Unifarco and SILA. Fabiana Zingone has served as a speaker for EG Stada Group, Fresenius Kabi, Janssen, Pfizer, Takeda, Unifarco, Malesci, and Kedrion and has served as a consultant for Galapagos. Brigida Barberio has served as a speaker for Abbvie, Agave, Alfasigma, AGpharma, Janssen, MSD, Procise, Sofar, Takeda, and Unifarco. The other authors declare no conflicts of interest.

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