Insights Into the Oral Microbiome and Barrett's Esophagus Early Detection: A Narrative Review

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Barrett's esophagus (BE) prevalence has increased steadily over the past several decades and continues to be the only known precursor of esophageal adenocarcinoma. The exact cause of BE is still unknown. Most evidence has linked BE to gastroesophageal reflux disease, which injures squamous esophageal mucosa and can result in the development of columnar epithelium with intestinal metaplasia. However, this relationship is inconsistent—not all patients with severe gastroesophageal reflux disease develop BE. There is increasing evidence that the host microbiome spanning the oral and esophageal environments differs in patients with and without BE. Several studies have documented the oral and esophageal microbiome's composition for BE with inconsistent findings. The scarcity and inconsistency of the literature and the dynamic phenomena of microbiota all warrant further studies to validate the findings and dissect the effects of oral microbiota, which are considered a viable proxy to represent esophageal microbiota by many researchers. This review aims to summarize the variability of the oral and esophageal microbiome in BE by using the example of *Streptococcus* to discuss the limitations of the current studies and suggest future directions. Further characterization of the sensitivity and specificity of the oral microbiome as a potential risk prediction or prevention marker of BE is critical, which will help develop noninvasive early detection methods for BE, esophageal adenocarcinoma, and other esophageal diseases.

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INTRODUCTION

Barrett's esophagus (BE) is a common gastrointestinal disease affecting 1.3–6.6 million US adults with a prominent male: female ratio of 2–3:1 (1). The disease is a premalignant condition characterized by the replacement of healthy squamous mucosa with specialized intestinal metaplasia (2). With a reported 30–125-fold relative risk of the lethal esophageal adenocarcinoma (EAC) (3), the incidence of BE has increased dramatically in the past 40–50 years, despite controlling for increased endoscopy rates (4–6). Patient quality of life suffers significantly because of BE-associated symptoms, which include regurgitation, difficulty swallowing, heartburn, chest pain, and justifiable fear of progression to cancer (7). Although the risk factors such as male sex, being non-Hispanic White, symptomatic gastroesophageal reflux disease (GERD), smoking, central obesity, physical activity, and alcohol intake for BE have been extensively investigated (8), the exact cause of BE is still unknown. In addition, among the patients with GERD symptoms, the prevalence of BE ranged from 1.5% to 19.8% depending on the study design and reference population (1). Screening of BE based on these risk factors, although noninvasive, are thus not sufficient.

Despite the personal and public health burden, the upper endoscopy is so far the only available screening tool for BE. Although a gold standard for accuracy, this procedure is invasive and expensive. Moreover, specialized training and operator expertise are necessary to maximize accuracy and yield. The value of effective novel screening lies in its ability to detect disease at an early stage, which can improve outcomes. In this circumstance, the earlier detection of BE as a precancerous lesion could reduce the risk of progression to EAC because identification would prompt intervention to prevent it.

The oral microbiome may be associated with BE. If this is true, then saliva testing of the oral microbiome could have a promising potential of prevention and prediction of BE occurrence and associated outcomes. BE patient' oral microbiota are significantly altered (9), which may shape the esophageal microbiota through distal migration, resulting in a constant microenvironment from mouth to esophageal sphincter (10,11). An oral sample-based screening tool to identify the signature microbial changes and their metabolomics effects in patients with BE is a promising early detection method, which may aid in reducing the increasing incidence trend of EAC.

This article reviews relevant research on the oral and esophageal microbiome in BE. The review highlights inconsistencies and potential confounders, and discusses remedial action in

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promoting microbiome profiling as an initial step to guide further microbiome studies, with a goal of developing an effective prevention, prediction, and early detection method.

METHODS OF LITERATURE SEARCH AND STUDY SELECTION FOR THIS NARRATIVE REVIEW

A systematic literature search for studies on oral microbiome or esophageal microbiome including information on the bacteria level identified from 16S gene sequencing and BE risk published from January 1966 to December 2020 was conducted in PubMed. The keywords used included "microbiome," "oral microbiome," "esophageal microbiome," "Barrett's esophagus," and "Barrett." In addition, manual searching of the references cited in review articles was performed. The full text was reviewed if studies reported the association between microbiome containing information on *Streptococcus* and BE risk in humans.

To be included in our narrative review, a study had to be conducted in human and report quantitative estimates of oral or esophageal microbiome containing information on the bacteria level. Studies including information on bacteria level by culture method were not included. The results were extracted by 2 independent reviewers. We use *Streptococcus* as an example for this narrative review. Specifically, we extracted information on the *Streptococcus* level, the most prevalent bacterial taxon in normal esophagus. We excluded studies that did not report *Streptococcus* level information. Information on the source of sample, sample size, study location, study findings on *Streptococcus*, collection and analysis, 16S sequencing primers, and reference were extracted (Table 2).

MICROBIOME IN HEALTHY ORAL CAVITY AND ESOPHAGUS

The human oral cavity is one of the most complex anatomic sites in the body and is colonized by trillions of diverse microorganisms (12). The dominant genera in healthy oral cavities include *Gemella, Veillonella, Neisseria, Fusobacteria, Streptococcus, Prevotella,* and *Actinomyces* (13). In 2005, a study conducted by Pei et al. (14) suggested that the esophageal microbiome resembles the oral microbiome and possesses 6 major phyla: *Firmicutes, Bacteroides, Actinobacteria, Proteobacteria, Fusobacteria,* and *TM7*. In particular, the *Firmicute* genus *Streptococcus* dominates the esophageal microbiota (14). Studies by Deshpande et al. (15) confirmed *Firmicute* and *Streptococcus* as the most abundant phylum and genus, respectively, using upper endoscopy of normal controls. Furthermore, Dong et al. (16) found that *Streptococcus, Neisseria, Prevotella, Actinobacillus*, and *Veillonella* genera were most abundant in both normal oral cavity and esophagus compared with other genera, whereas *Streptococcus* was much more prevalent in the esophagus and *Neisseria* dominated the oral cavity.

COMPARISON OF THE ORAL MICROBIOME DETECTED BY SEQUENCING OF 16S rRNA GENES BETWEEN BE AND CONTROLS THROUGH CASE-CONTROL STUDY DESIGNS

Disruption of microbial balance, dysbiosis, can initiate and promote the development of inflammation and carcinogenesis in various disease states. Yet, there is limited literature of dysbiosis in the oral microbiome of a patient with BE. Only one report compared 32 cases of BE (88% men) and 17 controls (58% men) using 16S rRNA sequencing (9). This study found a panel of taxa—including *Lautropia* and *Streptococcus* and an unspecified genus of the order *Bacteroidales*—could discriminate patients with BE with relatively high accuracy (96.9% sensitivity and 88.2% specificity) (9). The study also revealed a significant increase in *Enterobacteriaceae* among patients with BE with high-grade dysplasia and cancer, suggesting its significant association with progression from BE to EAC.

COMPARISON OF ESOPHAGEAL MICROBIOME BY SEQUENCING OF 16S rRNA GENES BETWEEN BE AND CONTROLS THROUGH CASE-CONTROL STUDY DESIGNS

The current literature on studying the microbiome and BE microbiota is mostly limited to tissue sampling of the esophageal microbiome (17). Notable changes in microbial populations between BE and controls are illustrated in Table 1. Table 2 lists available studies that compare relative changes in *Streptococcus* in BE as examples. The table highlights the inconsistent outcomes by comparing and contrasting the various methodologies and end points in the current literature.

The study by Macfarlane et al. (18) found that the total mean value of *Streptococcus* is higher among BE patients' biopsy and aspirate samples compared with that from controls. However, their study design only provided the absolute mean value without statistical support. Most reviewed studies found no significant difference in *Streptococcus* abundance between BE and controls. Worthy to note, these studies lack the sample sizes necessary for generalizable conclusions. A few studies have shown that *Streptococcus* levels decreased in patients with BE compared with controls. According to Yang et al., (19) in patients with esophagitis or BE, the abundance of *Streptococcus* species is reduced in esophageal tissue, whereas Gram-negative (type II, discussed later) anaerobes/microaerophiles constitute greater proportions.

Liu et al. (20) found *Streptococcus* as the most common bacterial taxa in normal esophagus, reflux esophagitis, and BE tissue

Table 1. Summary of current literature on changes in the esophageal and oral microbiome among patients with Barrett's esophagus vs controls

Microbiome location	Esophageal	Oral
Increased	Neisseria Prevotella Veillonella Haemophilus	Streptococcus[a] Veillonella Enterobacteriaceae[a]
Decreased	Streptococcus[a]	Neisseria Lautropia Corynebacterium[b]

Rows describe taxa decreased and increased in patients with Barrett's esophagus vs controls. Columns compare the samples taken from the esophagus to oral sample. Reference for esophageal microbiome is Park and Lee (17), reference for oral microbiome is Snider et al. (9).
[a]Firmicutes.
[b]Proteobacteria.

Table 2. Summary of literature of reporting microbiome composition including microbial *Streptococcus* level in association with Barrett's Esophagus through 16S gene sequencing

	Oral sample	Esophageal sample	Sample size study location	Findings on streptococcus	Collection and analysis	Primers/references	Comments
Increased *Streptococcus*	Snider et al., 2018 (9)	X	Control n = 17 BE n = 32 Columbia University Medical Center, New York, NY	Saliva from BE subjects had significantly decreased *Lautropia* ($P = 0.002$) and increased *Streptococcus* ($P = 0.009$) compared with the controls.	Saliva was collected fasting with drool technique and oral swabs; the esophagus sample was collected with brushing BE tissue or gastric cardia in controls.	16s rRNA gene V4 region, Greengenes database, Semiquantitative PCR. Primer(s): 515F and 806R. Strep: Str1, Str2	Relative abundance of *Lautropia*, *Streptococcus*, and a genus in the order *Bacteroidales* distinguished BE from the controls AUROC 0.94 (95% CI: 0.85–1.00). The optimal cutoff identified patients with BE with 96.9% sensitivity and 88.2% specificity.
	X	Macfarlane et al., 2007 (18)	BE n = 7 Controls n = 7 Gastroenterology Outpatients clinic at Ninewells Hospital (Dundee, United Kingdom)	*Streptococcus constellatus*, *Streptococcus crispatus*, *Streptococcus gordonii*, *Streptococcus mitis*, *Streptococcus salivarius*, *Streptococcus sobrinus* Total *Streptococcus* mean value is higher among BE vs controls	Aspirate and esophagus biopsy (middle or lower third) Cultured under aerobic, anaerobic, and microaerophilic conditions.	16s rRNA oligonucleotide probe, hybridization by FISH Strep: Str 0493	Campylobacter was abundant in patients with BE and not in controls.
No significant difference	X	Pei et al., 2005 (14)	BE n = 3 Normal n = 9 Department of Veterans Affair Medical Center, Nashville, TN	Streptococcal species 16S rDNA sequences detected in 1 normal esophagus patient and 0 patients with BE. *Streptococcus salivarius* detected in 1 normal esophagus patient and 1 patient with BE.	Esophageal biopsies obtained 2 cm above the squamocolumnar junction or in the case of Barrett's esophagus, 2 cm above the gastroesophageal junction.	Broad range 16S rDNA PCR Strep: M58839.1 GenBank at 98.1% Primer(s): fPB7I and rPB10I	
	X	Blackett et al., 2013 (33)	Controls n = 39 BE n = 45 EAC n = 30 Gut Group, Biomedical Research Institute, University of Dundee, Dundee, UK	*Streptococcus* contributes approximately 12% of total microbiota in both control and BE	Biopsies 5 cm above the esophagogastric junction or at the upper limit at the site of pathology.	Cultured, the MIDI system or 16s rRNA sequencing. Once key organisms and population changes were identified, molecular assays were designed to investigate biofilm composition using real-time PCR. Primer sets were designed or further optimized to target the small 16S rRNA gene subunit of a select range of pharyngoesophageal bacteria and cytokines, for use in real-time PCR. The PCR product for	An assay for the genus *Streptococcus* could not be designed. Previous study shows streptococci comprising between 12% and 78% of the total community

Table 2. (*continued*)

	Oral sample	Esophageal sample	Sample size study location	Findings on streptococcus	Collection and analysis	Primers/references	Comments
						each primer set was purified and ligated into a vector using the pGEM-T Easy Vector System I.	
	X	Amir et al., 2014 (34)	Controls n = 15 BE n = 6 Department of Gastroenterology and Hepatology at Meir Medical Center, Kfar Saba, Israel	No change of Streptococci in the esophageal biopsy samples from patients with BE	Esophageal mucosa, biopsies from normal-appearing mucosa above the tissue with esophagitis or BE.	Primer(s): 939F and 1492R V6 and V7 regions Ribosomal Database Project classifier and aligned with PyNAST pyrosequencing. Sequences from metagenomics.anl.gov	
	X	Elliot et al., 2017 (35)	Controls n = 20 Nondysplastic BE n = 24 Dysplastic BE n = 23 BEST2 study at 5 UK hospitals and the OCCAMS study at 6 UK hospitals	No significant difference in the relative abundance of Family of Streptococcaceae comparing BE with controls.	Fresh frozen tissue, fresh frozen endoscopic brushings, and a Cytosponge device	V1-V2 regions European Nucleotide archive # ERP005191. Primer(s): 331F and 797R.	
	X	Zaidi et al., 2016 (26)	Tumor adjacent normal epithelium n = 3 BE n = 13 EAC n = 5 Discovery pilot studies, undetermined location(s), and names. Authors at West Penn Allegheny Health System in Pittsburgh, PA.	*Streptococcus pneumonia* detected in high abundance in BE (50%–70%) in comparison to tumor adjacent normal epithelium	Esophageal and gastric samples, unspecified analysis technique	*Streptococcus pneumonia* targeted using "GTG ATG CAA GTG CAC CTT" PCR-ESI-MS-TOF technology with validation by FISH	
	X	Snider et al., 2019 (30)	Controls n = 16 NDBE n = 14 LGBE n = 6 HGBE n = 5 Columbia University Medical Center, New York, NY	No significant difference in the relative abundance of *Streptococcus* comparing BE with non-BE controls (35.7% vs 26.9%, $P = 0.18$) No significant overall alteration in the relative abundance of *Streptococcus* across levels of BE-related neoplasia (analysis of variance $P = 0.51$).	Brushing the squamous esophagus, BE tissue (BE cases) or gastric cardia, within 1 cm of the squamocolumnar junction (controls).	V4 hypervariable ribosomal RNA region Primer(s): 515F and 806R NCBI Sequence Read Archive and Greengenes.	Compared with controls not taking PPIs, patients taking PPIs had (A) reduced relative abundance of Gram- bacteria ($P = 0.05$) and (B) increased the relative abundance of *Streptococcus* ($P = 0.03$)
Decreased *3*	X	Yang et, al, 2009 (19)	Controls n = 12 BE n = 10 Division of Gastroenterology, Department of Medicine, Veterans Affairs New York Harbor Healthcare System, NY	Mean relative abundance of *Streptococcus* in the normal esophagus group (75.9%, n = 11) was significantly higher than that in the BE (54.1%, n = 10) groups	Distal esophageal tissue from endoscopy biopsy	Primer(s): 8F and 1510R SLOTU via RDP II.	The type I microbiome dominated by the genus *Streptococcus* and concentrated in the phenotypically normal esophagus. The type II

Table 2. (*continued*)

Oral sample	Esophageal sample	Sample size study location	Findings on streptococcus	Collection and analysis	Primers/references	Comments
						microbiome had greater proportion of Gram-negative anaerobes/microaerophiles and primarily correlated with esophagitis (OR: 15.4) and BE (OR: 16.5).
X	Liu et al., 2013 (20)	Controls n = 6 Reflux esophagitis n = 6 BE n = 6 Nagoya University Hospital, Japan	BE: *Streptococcus* (11%), Normal: *Streptococcus* (21%) Esophagitis *Streptococcus* (20%),	Distal esophagus at 1 cm above the gastroesophageal junction under endoscopic examination. Histological and DNA extraction analyses	Primer(s): 27F and 1492R Reverse searched in BLAST from GenBank with >97% match as baseline for homologous	
X	Gall et al., 2015 (21)	BE n = 12 Subset of Seattle Barrett's Esophagus Research Program (SBERP).	Esophageal microbiome consisting of the phyla Firmicutes, Actinobacteria, Bacteroidetes, Proteobacteria, and Fusobacteria. *Streptococcus* to Prevotella species ratio corresponds to phylogenetic distance sample clustering and correlates with BE progression No substantial difference in phylogenetic diversity between squamous and Barrett's mucosa microbiome within subjects.	Biopsy and then brush samples collected from squamous esophagus, Barrett's esophagus, stomach corpus, and stomach antrum	Broad range 16S PCR and 454 pyrosequencing for OTUs	Relative abundance of species varied between patients and intrasubject variability across biopsy sites less than intersubject comparison at each site. Stomach antrum microbiome more closely resembled the BE microbiome than the contiguous corpus.
X	Lopetuso, 2020 (36)	BE n = 10 EAC n = 6 Controls n = 10 September 2016 to January 2018, the Fondazione Policlinico A. Gemelli in Rome	BE: decreased *Streptococcus*, increased of Prevotella, Actinobacillus, Veillonella, and Leptotrichia.	Healthy controls (CTRL): 2 biopsies from the normal esophageal mucosa. BE: 2 biopsies from the esophageal metaplastic lesion (BEM) and 2 from the normal esophageal mucosa (BEU) EAC: 2 biopsies from the neoplastic lesion.	V3-V4 hypervariable, per Illumina. BLAST-aligned all reads belonging to genera to available reference sequences in NIH-NCBI database	Firmicutes to Bacteroidetes ratio reduction seen as progressive reduction of *Streptococcus* relative abundance and corresponding increase of Prevotella in BEM, even more marked and significant in EAC.

AUROC, area under the receiver operating characteristic; BE, Barrett's esophagus; CI, confidence interval; EAC, esophageal adenocarcinoma; FISH, Fluorescence In Situ Hybridization; GERD, gastroesophageal reflux disease; PCR, polymerase chain reaction; PPI, proton pump inhibitors.

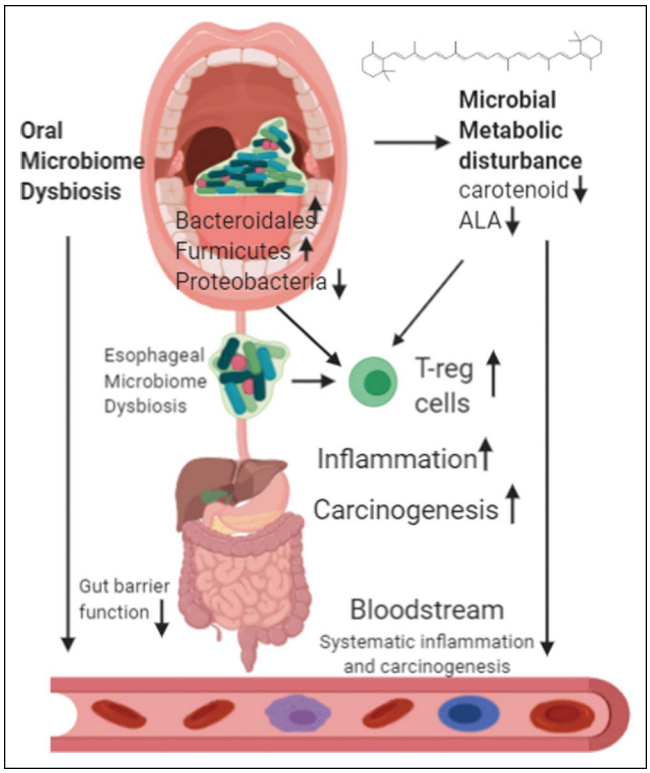

Figure 1. Key roles for oral microbiome/metabolites in the initiation and progression of Barrett's esophagus. The figure was created with BioRender.com.

samples. However, the proportion of *Streptococcus* to other genera was slightly higher in the normal group than in the reflux esophagitis or BE groups. Among a subset of participants from the Seattle BE Research Cohort, Gall et al. (21) reported that *Streptococcus* and *Prevotella* are dominant species in esophageal samples among patients with BE, with no substantial intra-individual difference between squamous and BE mucosal microbiota.

In summary, our literature review of the past 2 decades of BE microbiota research revealed a conflicting conclusion regarding whether and how the oral and/or esophageal microbiome are altered when comparing the samples from patients with BE and healthy controls. Case in point, the abundance of *Streptococcus* in BE was increased, decreased, and unchanged in 1, 4 and 6 studies, respectively (Table 1).

MECHANISMS OF MICROBIAL DYSBIOSIS IN ASSOCIATION WITH BE OR EAC

Despite the inconsistent and sometimes conflicting findings reported in studies evaluating oral/esophageal microbiome in BE, there are strong biological rationales supporting further research to supplement the literature. The dysbiosis of microbiota could induce metabolomics disturbance, inflammation, oxidant/antioxidant imbalance, and carcinogenesis (Figure 1). Mechanistic insights regarding the functional roles of specific bacteria in the development of BE or EAC are few. Yet, the contributions of microorganisms or their altered abundancies are evident. This is further supported by 16S metagenomics studies (22). Snider et al. suggested that patients with BE and reflux esophagitis harbor a distinct esophageal microbiome, characterized by a reduced level of Gram-positive *Streptococcus* in reflux-related conditions. The authors posited that the changes could be from gastric and bile acid reflux, changes in the distal esophagus microenvironment, or reflux from intestinal and gastric species (23).

Before the review by Snider, Yang et al. (19) hypothesized that the 2 types of gut microbiomes mediate health and disease. Type I is found more often in healthy individuals, predominantly of Gram-positive bacteria, and mostly of the *Streptococcal* species. Type II is represented by the dominance of Gram-negative, anaerobic/microanaerobic species, with the most dramatic increase in *Veillonella, Prevotella, Haemophilus, Neisseria, Campylobacter, Porphyromonas*, and *Fusobacterium*, most of which are also associated with periodontal disease conditions. The 2 types of organisms are projected as proxies for gastrointestinal health (at least in upper digestion) and may be useful in the clinical assessment, diagnostics, and management (12). *Streptococcus* is the most dominant genus in the esophageal microbiome, and its relative abundance is significantly higher in the type I microbiome (78.8%) compared with type II microbiome (30%) (12). The niche vacated by *Streptococcus* in type II microbiota is accompanied by an increase in relative abundance of mostly Gram-negative bacteria. The causes, or succession order, of this transition is yet to be determined. Relevant to our discussion, the type I microbiome dominates in the phenotypically normal esophagus, whereas the type II microbiome is representative in esophagitis and BE (19).

Yang et al. (19) predicted a stepwise increase in Gram-negative bacteria containing higher proportion of anaerobes/microaerophiles (type II microbiome) in esophagus microbiome as the disorder in the esophagus progresses from esophagitis to BE and potentially to EAC. The bacterial products may directly or indirectly stimulate pathogen pattern recognition receptors (i.e., Toll-like receptors) in the epithelial or inflammatory cells, with downstream expression of proinflammatory cytokines. This can potentially generate persistent innate immune responses in the esophagus (12). Gram-negative periodontal pathogens, type II microbiota, are known corollaries to oral inflammation and may potentially contribute to chronic inflammation in the esophagus, based on their anatomical continuity (12). The resulting population change in the esophageal microbiome could contribute to chronic inflammation through the proinflammatory NF-κβ pathway induced by the lipopolysaccharides from Gram-negative type II microbes. Lipopolysaccharides from Gram-negative bacteria bind to Toll-like receptor-4 and other cell surface receptors, which induces the NF-κβ pathway and promotes neoplastic progression. In addition, the dominance of esophageal Gram-negative bacteria can reduce dietary nitrates to nitrites, promoting carcinogenesis when converted into carcinogenic N-nitroso compounds by the acidic environment of the distal esophagus in patients with acid reflux (24).

REASONS FOR THE CONTRADICTORY FINDINGS ON MICROBIAL SPECIES LEVEL IN ORAL AND ESOPHAGEAL MICROBIOME

Current research on the oral and esophageal microbiome analysis in the context of BE's clinical implementation is relatively new

and represents an emerging study direction. The invasive nature of the collection of esophageal samples has so far limited study sample sizes in most cases. Furthermore, the validity of non-invasive sampling of oral microbiome has not been vigorously investigated, per current literature. Several reasons may explain the difference in microbial species such as *Streptococcus* between oral and esophagus microbiomes.

First, an inconsistent laboratory microbiome analysis. Different laboratories use different methods to extract DNA to perform polymerase chain reaction with different 16s rRNA gene fragments and to carryout differing bioinformatics analyses. Studies must agree on the level of taxonomic resolution—genus, species, etc.—and the source of verification, down to the DNA code and the database used. Establishing a standard methodology in microbiome studies is critical for reproducibility and validity.

Second, there are notable differences in the collected samples. There is heterogeneous spatial organization of microbial community in the gut because bacteria are not uniformly distributed (25). Therefore, the proportion of *Streptococcus* levels may alter dramatically in the esophagus compared with the oral cavity. This is a critical issue to iron out before implementing oral-based testing to screen for esophageal changes and dysbiosis. Dong et al. (16) reported that *Neisseria* was preferable in the oral cavity and *Streptococcus* in the esophagus, acting as an exclusive ratio where the sum of both remained constant, but the relative amounts changed according to sampling sites. According to Zaidi et al. (26), the oral microbiome harbors a larger and more diverse array of bacteria than its neighboring esophagus, potentially resulting in masking specific alterations to the esophagus because of contamination from the oral microbiota. Perhaps, much of the microbial taxa may adapt to the esophagus, and the reverse may not be true (27). These findings could be explained by a selective passage of taxa through the digestive system or preferential retention in the esophagus. Okereke et al. (28) collected biopsy samples from (i) proximal esophagus, (ii) midesophagus, (iii) distal esophagus, and (iv) BE with additional swabs from the uvula and the endoscope among patients with BE . They found *Streptococcus,* in addition to *Anaerococcus* and *Alloicoccus,* had the highest relative proportion in the esophagus, but such dominance diminishes in the uvula and on the endoscope. In addition, the study found more Gram-positive bacteria in the proximal esophagus (29). This suggests that there is an anatomical gradient of microbiota change in the esophagus in various disease states. Hence, sampling location can significantly affect the results. The overall pattern of microbial dysbiosis leads to disease progression, rather than just one specific organism's change that drives the disease progression. Thus, a complex microbial index may be necessary to derive useful microbial biomarkers, above tracking a single genus.

Third, the study groups selected for comparison were not uniformly defined. Some study protocols used healthy, non-GI patient participants as controls, whereas others opted for endoscopy participants who had normal esophagus biopsy results. Still, others chose adjacent healthy tissues from the same study subjects, and even then, the samples differed in margin sizes between metaplasia and defined normal tissues. Thus, it is difficulty to extract meaningful data informing differences between health and dysbiosis. It is desirable to establish a universally accepted standard to select study groups.

Fourth, recent or current treatments confound study outcomes. Proton pump inhibitors (PPI) are commonly prescribed for peptic gastrointestinal maladies. Administration of PPI increases the pH in gastric secretions by directly targeting proton pumps. Snider et al. (30) reported that patients taking PPIs had increased *Streptococcus* in the esophagus and overall decrease in Gram-negative bacteria. In a previous study, the same group published a similar series controlling for PPIs use and found a positive association between microbiome changes and BE status independent of PPI use (9). In comparison, Freedburg et al. (24) found decreased Gram-negative bacteria and increased *Streptococcus* in subjects on PPIs when compared with controls. Treatment for peptic diseases likely confounds the results and limits the ability to achieve homogeneity among study groups.

Fifth, the oral or esophageal microbial profiles may in part be determined by host genetics, age, other comorbidities (22), geographical locations, race/ethnicity, and dietary patterns (31). Sex seems to have less impact (22,32). For example, *Streptococcus* and *Prevotella* are the dominant bacteria in the upper gastrointestinal tract, and their ratio may be associated with central obesity and hiatal hernia length, the 2 known risk factors for BE (21). The studies with small sample sizes lacked sufficient statistical power to control for these impact factors. Geographical differences in microbiome because of local or traditional dietary patterns should be taken into consideration. Table 1 notes the differing locations of every study. Although it is important to not reflexively establish an ethnocentric healthy esophageal or oral microbiome, the variation between different race/ethnicities must also be accounted for when pooling and analyzing case-control studies.

FUTURE DIRECTIONS

The understanding of oral microbiome and its association with BE presents enormous future research and translational opportunities, given that existing studies used a wide variety of sample sourcing equipment and locations within the esophagus, reflecting the issue of inconsistence. Questions to be explored include the following: (i) Is dysbiosis a cause or a consequence of BE? For example, if there is acid regurgitation into oropharynx, could this alter a microbiome? (ii) Does dysbiosis contribute to the development of BE in patients with GERD? (iii) Does dysbiosis contribute to the progression of BE to malignancy? (iv) Can we manipulate the oral microbiome and prevent the progression of BE? (v) Could the dysbiosis of microbiota be a red flag that highlights ongoing carcinogenic and inflammatory processes, or does it act as a direct cause or a direct cause of these biological processes? To answer all of these questions, future studies should focus on individualized risk predictions incorporating most suitable and reproducible study designs, representativeness of the study population, anatomic location of the sampling, sequencing methods, and analytic methods for the signature microbiota. The current literature in our review on esophageal microbiome and BE had significant small samples sizes ranging from $n = 3$ to $n = 45$ for patients with BE in these studies (Table 2), which may not have adequate power to reach meaningful conclusions. Owing to the nature of oral samples' easy access, it is a priority to conduct oral sample-based population study with larger sample size in understanding microbiome's link to BE disease. Ultimately, population-based robust testing and research on oral microbiome

will enable the development of precise noninvasive prevention, prediction, and early detection methods tailored to individuals by their demographics, disease state, and treatment history.

CONCLUSIONS

Current research on the oral and esophageal microbiome analysis in the context of BE's clinical implementation represents an emerging and exciting direction for future studies. The invasive nature of the collection of esophageal samples has limited study sample sizes. Yet, the validity of noninvasive sampling of oral microbiome has not been thoroughly validated, as evident in the heterogeneity of the literature. Through reviewing studies performed in the past 20 years, we observed inconsistent results across studies. Particularly, the associations of microbiome changes, including *Streptococcus*—the most prevalent bacterial genus in both the oral cavity and esophagus-and BE. We identified some of the limitations of the current literature including small sample size, diverse study populations and geographic locations, inconsistent laboratory isolation and quantification methods, varying resolution of microbiome identification, and various esophageal sampling protocols. The avenue of saliva testing for esophageal disease and dysbiosis warrants further investigation and refinement. It is unlikely that BE can be predicted or identified by the distribution of a single bacterial genus; the development of risk prediction models that can be adaptable to clinical workflow need to be based on comprehensive understanding of microbe-host interactions that contribute to risk. Future studies must include (i) consistent laboratory microbiome analyses, (ii) robust sample sizes, (iii) uniform taxa surveillance methods and standard collection methods that are validated to be reproducible, (iv) carefully defined study patients including well-defined controls, patients with esophagitis, and patients with BE, (v) thorough collection of confounding factors including PPI use, obesity, race, environmental factors, dental hygiene factors for oral microbiome etc., and (vi) close collaboration between basic science and clinical research teams to facilitate timely translation of scientific discoveries to the development of effective diagnostic and prevention strategies.

CONFLICTS OF INTEREST

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