Microb Health Dis 2024; 6: e1111

WHAT IS TRUE AND WHAT IS NEW IN ESOPHAGEAL MICROBIOME IN 2024?

M. Jauvain[1,2], E. Bessède[1,2]

[1]Bordeaux Institute of Oncology, BRIC U1312, INSERM, University of Bordeaux, Bordeaux, France
[2]National Reference Centre for Campylobacters & Helicobacters, University Hospital of Bordeaux, Bordeaux, France

Corresponding Author: E. Bessède, MD; email: emilie.bessede@u-bordeaux.fr

Abstract – Research on the esophageal microbiome is a relatively new field. While the microbiota in the lower tract of the intestine, namely the small and large bowel, has been the core of scientific interest over the last 20 years, the resident microbial flora of the esophagus and its potential role in the pathogenesis of esophageal diseases have been neglected. The esophageal microbiota refers to the dynamic community of microorganisms that inhabit the esophagus, influenced by several factors, including diet, age, and diseases, and it is proposed to play a role in several esophageal disorders, including eosinophilic esophagitis (EoE), gastroesophageal reflux disease (GERD), esophageal carcinomas and carcinogenesis, and achalasia.
This review will outline data from studies published in the past year, mainly focusing on esophageal cancer and motility disorders, characterizing the esophageal microbiome in both health and disease states.

Keywords: Esophageal carcinoma, Microbiota, Microbiome.

INTRODUCTION

The study of the esophageal microbiome has become increasingly important in recent years due to its potential role in several esophageal diseases. It appears that the esophagus harbors its own microbial community, which seems to influence the development and progression of some diseases like esophageal cancer, eosinophilic esophagitis, gastroesophageal reflux disease, and achalasia. Understanding these microbial dynamics could provide valuable insights into the mechanisms driving and managing disease and can lead to novel diagnostic and therapeutic strategies. This review focuses on recent studies since 2023 on characterizing the esophageal microbiome and its implications.

METHODS

This review was drafted by searching articles on PubMed: articles corresponding to esophageal microbiome topic were selected, read, and summarized in order to retain the more important information.

Esophageal Cancer & Microbiota

In the context of esophageal cancer, a study examined the dysbiosis in the upper aerodigestive microbiota in patients with esophageal squamous cell carcinoma (ESCC) and oral cav-

ity squamous cell carcinoma (OSCC)[1]. The authors investigated the microbial communities in esophageal and oral samples from patients with ESCC and OSCC using V3-V4 16SrRNA sequencing. Compared to patients with no SCC, 32 and 45 distinctive bacterial genera were identified in ESCC and OSCC patients, respectively, with 20 genera common to both cancers. This suggests a convergent niche adaptation of upper aerodigestive SCC-associated bacteria that may play important roles in the pathogenesis of these malignancies. *Fusobacterium*, *Selenomonas*, *Peptoanaerobacter*, and *Peptostreptococcus* were enriched, while *Streptococcus* and *Granulicatella* were depleted in both cancer types. *Fusobacterium nucleatum* was notably abundant, and higher levels of *Selenomonas danae* and *Treponema maroon* were correlated with smoking. Predicted functional analysis revealed several depleted (notably lipoic acid and pyruvate metabolism) and enriched (notably RNA polymerase and nucleotide excision repair) pathways common to both cancers. These results suggest a convergent dysbiosis in the upper aerodigestive microbiota associated with both ESCC and OSCC.

Another study[2] explored the esophageal microbiome in patients with non-dysplastic Barrett's esophagus (BE), low- and high-grade dysplastic BE, and esophageal adenocarcinoma (EAC) to identify parameters characterizing cancer progression and to develop a score suitable for clinical practice to stratify cancer risk. 16SrRNA gene sequencing was applied on esophageal biopsies, and the microbial composition was evaluated at each different taxonomic level along the disease progression. Comparisons between non-dysplastic and dysplastic/cancer patients identified six significant microbial features. These features were used to create the Resident Esophageal Microbial Dysbiosis Test, a multiparametric score predicting the risk of progression toward EAC. The test's diagnostic accuracy and ability to identify dysplastic and cancer patients were validated. Additionally, the study examined the relationship between microbial parameters and patients' diet/lifestyle habits, as EAC is linked to obesity. This research highlights the potential of microbiome-based models as biomarkers for esophageal adenocarcinoma risk.

Moreover, concerning the metabolome, a study[3] investigated the role of cranberry proanthocyanidins (C-PAC) in preventing EAC by targeting the gut microbiome-esophageal metabolome axis in a rat reflux-induced EAC model. Sprague-Dawley rats, with or without reflux, received either water or C-PAC for 25 or 40 weeks. C-PAC demonstrated prebiotic effects, reversing reflux-induced dysbiosis and bile acid metabolism, significantly inhibiting EAC progression through TLR/NF-κB/TP53 signaling pathways. C-PAC attenuated pathogenic bacteria (*Streptococcus parasanguinis*, *Escherichia coli*, and *Proteus mirabilis*) and reversed inflammatory and immune-related genes and proteins linked to human EAC progression. This study suggests C-PAC as a dietary supplement that could potentially prevent EAC progression, either alone or as an adjunct to existing therapies, by reducing dysbiosis and inflammation.

It was demonstrated that in the microbiome, *Fusobacterium nucleatum* is associated with unfavorable clinical outcomes and inferior chemotherapeutic responses in esophageal cancer[4]. DNA methylation is crucial in cancer development, and LINE-1 (Long Interspersed Nucleotide Element 1) hypomethylation was associated with a poor prognosis in esophageal cancer. Since gut microbiota influences host cell DNA methylation, Baba et al[5] hypothesized that *F. nucleatum* affects LINE-1 methylation. Quantitative PCR was used to quantify *F. nucleatum* DNA and pyrosequencing assay for determined LINE-1 methylation in 306 esophageal tumor samples from patients. *F. nucleatum* was present in 21.2% of tumors, and LINE-1 methylation ranged from 26.9 to 91.8 (median = 64.8). *F. nucleatum* DNA was related to LINE-1 hypomethylation ($p < 0.0001$). The impact of *F. nucleatum* on clinical outcomes was not modified by LINE-1 hypomethylation (p for interaction = 0.34). Thus, *F. nucleatum* influences genome-wide methylation, which may be one of the mechanisms by which *F. nucleatum* contributes to esophageal cancer progression.

Finally, neoadjuvant chemoimmunotherapy (NACI) shows promise for treating resectable ESCC. Wu et al[6] explored how intratumoral microbiota affects the patient response to NACI. Variations in intratumoral microbiota β-diversity predicted NACI efficiency, with *Streptococcus* enrichment correlating with increased GrzB+ and CD8+ T-cell infiltration in tumors. *Streptococcus* abundance predicted longer disease-free survival. Single-cell RNA sequencing revealed that responders had more CD8+ effector memory T cells and fewer CD4+ regulatory T cells. Mice treated with fecal transplants or intestinal colonization with *Streptococcus* from responders showed increased tumor-infiltrating CD8+ T cells and better responses to anti-PD-1 treat-

ment. The study suggests that intratumoral *Streptococcus* could predict NACI response and highlights the potential clinical utility of intratumoral microbiota in cancer immunotherapy.

Achalasia & Esophageal Microbiota

Several studies focusing on achalasia and esophagus microbiota were published this past year. Esophageal achalasia is a rare motility disorder characterized by the selective degeneration of inhibitory neurons in the esophageal myenteric plexus.

Achalasia often presents with chronic food stasis and fermentation in the esophageal lumen, which may lead to alterations of the esophageal microbiome, with associated mucosal inflammation and dysplastic changes. A pilot study[7] aimed to characterize the esophageal microbiome in achalasia and its changes before and after peroral endoscopic myotomy. This prospective case-control study included 31 achalasia patients and 15 controls. Endoscopic brushing for esophageal microbiome collection was performed in all subjects, with additional follow-up endoscopy and brushing 3 months after PerOral Endoscopic Myotomy (POEM) in achalasia patients. A distinct esophageal microbial community structure was observed in achalasia patients with increased Firmicutes and decreased Proteobacteria. There was an enrichment of *Lactobacillus* in achalasia patients, and the amount of *Lactobacillus* was associated with the severity of achalasia. Finally, the altered esophageal microenvironment in achalasia leads to dysbiosis with a high abundance of *Lactobacillus*. After POEM, increased *Neisseria* and decreased *Lactobacillus* were observed.

Another study[8] aimed to characterize the composition of the esophageal microbiota in achalasia and explore the potential mechanisms involved in its pathogenesis. Here, the authors found that in esophageal achalasia patients, a lower diversity and a predominance of Gram-negative bacteria were observed compared to healthy patients. In addition, the authors used mice models to study the association between esophageal microbiota and achalasia by inducing esophageal dysbiosis in mice *via* chronic exposure to ampicillin sodium in their drinking water. The microbiome modifications were the same in the antibiotic-treated mice and in patients suffering from achalasia, i.e., increased abundance of Gram-negative bacteria, decrease of the relative abundance of *Rhodobacter*, and enriched Lipopolysaccharide biosynthesis. Thus, the authors conclude that patients with achalasia exhibit esophageal dysbiosis, which may induce aberrant esophageal motility.

A last study focused on achalasia and esophageal microbiota[9]. The authors aimed to determine the pathogenesis of achalasia by studying alterations in esophageal smooth muscle contraction and the associated inflammatory response and to evaluate the role of esophageal microbiota in achalasia development. They found that the hypophosphorylation of the myosin light chain in achalasia was associated with a downregulation of the myosin phosphatase-inhibitor protein CPI-17. Concerning the microbiota, an increase in the alpha-diversity index of the esophageal microbiota and the proportion of several microbes, including Actinomyces and Dialister, was observed and positively correlated with Th-17-related cytokines. Thus, the esophageal microbiota could be associated with the development and exacerbation of achalasia, simultaneously with an increased Th-17-related immune response.

Other Pathologies

Eosinophilic esophagitis (EoE) is a chronic inflammatory disease characterized by a T-Helper type 2 inflammatory response, with food antigens as the main causative factor, resulting in eosinophil accumulation within the esophageal mucosa.

Furuta et al[9] aimed to determine the mucosal microbiota associated with eosinophilic esophagitis and eosinophilic gastritis (EoG) in a geographically diverse cohort of patients compared to controls. This prospective study enrolled individuals with eosinophilic gastrointestinal disease, including pediatric and adult tertiary care centers, and collected clinical data, mucosal biopsies, and stool samples. One hundred thirty-nine mucosal biopsies were evaluated, corresponding to 93 EoE, 17 EoG, and 29 control specimens. Dominant community members (*Streptococcus* for EoE, *Prevotella* for EoG) were different in the mucosal biopsies but not

stool samples of individuals with Eosinophilic Gastrointestinal Diseases (EGIDs) compared to controls; taxa associated with EGIDs were highly variable across individuals. Further studies are needed to determine if therapeutic interventions contribute to the observed community differences.

Another study[10] aimed to investigate the effect of proton pump inhibitors (PPI) on esophageal mucosal transcriptome and active microbiota in children with normal esophagi. The authors used metatranscriptomics to capture the host transcriptional and active microbial profiles of 17 esophageal biopsy samples of children treated or not by PPIs. Compared with PPI-, the PPI+ children showed a more abundant presence of *Prevotella spp.* and *Streptococcus spp.*, associated with an upregulation of 27 genes, including the *MUC* genes. No differences in cell composition between the 2 groups were observed. Finally, this study described that in children with normal esophagus, PPI exposure can be associated with upregulation of esophageal mucosal homeostasis and epithelial cell function genes in a cell-independent manner. Further studies are mandatory to validate these descriptions.

In addition to these different original articles, two reviews were published last year[11,12]. A very interesting review focused on the role of the microbiota in esophageal cancer and reported all of the studies on this topic[11]. It is certain that esophagus cancer is associated with changes in the normal esophagus microbiota, but further work and multidisciplinary research efforts will be fundamental to better characterize these changes. The other review considered microbiota in reflux esophagitis and peptic ulcer disease and made an inventory of the studies performed in these fields[12]. It concludes that both RE and PUD are clearly linked to abnormal microbiota composition and diversity and that specific therapies that support, restore, or repair the microbiome should be investigated as complementary therapies to existing approaches.

CONCLUSIONS

The recent study highlights the significant role of microbiota in the pathogenesis and progression of various esophageal conditions, particularly ESCC, BE, and EAC. Dysbiosis, marked by specific bacterial genera, such as Fusobacterium, Selenomonas, and Streptococcus, appears to be a key factor in both ESCC and OSCC. Furthermore, microbiome-based tests have been developed to predict cancer progression, suggesting the use of microbiota as a biomarker for clinical application. In EAC, prebiotic use, like cranberry proanthocyanidins (C-PAC), have shown potential in reversing dysbiosis and reducing inflammation. Fusobacterium nucleatum has been linked to poor clinical outcomes in esophageal cancer, probably due to its impact on DNA methylation. Concerning intratumoral microbiota, Streptococcus spp. seems to predict response to chemoimmunotherapy, suggesting that the intratumoral bacteria could be useful for more personalized treatments. Concerning achalasia, studies have revealed microbial imbalances linked with treatments like POEM that alter the microbiome. PPIs also influence esophageal microbiota. Finally, in EoE, microbial profiles differ between affected patients and healthy controls, with the genera Streptococcus and Prevotella playing key roles. These findings underscore the importance of microbiota in esophageal health, opening new approaches for diagnostic and therapeutic strategies in esophageal diseases.

Conflict of Interest

The authors declare they have no conflict of interest.

ORCID ID

MJ: 0000-0002-2488-6892
EB: 0000-0002-0686-1763

AI Statement

The authors did not use any AI tool for the drafting of the manuscript.

REFERENCES

1. Zhu H, Yip HC, Cheung MK, Chan HC, Ng C, Lau EHL, Yeung ZWC, Wong EWY, Leung L, Qu X, Wang D, Cai L, Chan PKS, Chan JYK, Chen Z. Convergent dysbiosis of upper aerodigestive microbiota between patients with esophageal and oral cavity squamous cell carcinoma. Int J Cancer 2023; 152: 1903-1915.
2. Zaramella A, Arcidiacono D, Nucci D, Fabris F, Benna C, Pucciarelli S, Fassan M, Fantin A, De Re V, Cannizzaro R, Realdon S. Resident Esophageal Microbiota Dysbiosis Correlates with Cancer Risk in Barrett's Esophagus Patients and Is Linked to Low Adherence to WCRF/AICR Lifestyle Recommendations. Nutrients 2023; 15: 2885.
3. Weh KM, Howard CL, Zhang Y, Tripp BA, Clarke JL, Howell AB, Rubenstein JH, Abrams JA, Westerhoff M, Kresty LA. Prebiotic proanthocyanidins inhibit bile reflux-induced esophageal adenocarcinoma through reshaping the gut microbiome and esophageal metabolome. JCI Insight 2024; 9: e168112.
4. Yamamura K, Baba Y, Nakagawa S, Mima K, Miyake K, Nakamura K, Sawayama H, Kinoshita K, Ishimoto T, Iwatsuki M, Yasuo S, Yoichi Y, Naoya Y, Masayuki W, Hideo B. Human Microbiome Fusobacterium Nucleatum in Esophageal Cancer Tissue Is Associated with Prognosis. Clin Cancer Res 2016; 22: 5574-5581.
5. Baba Y, Hara Y, Toihata T, Kosumi K, Iwatsuki M, Iwagami S, Miyamoto Y, Yoshida N, Komohara Y, Baba H. Relationship between gut microbiome Fusobacterium nucleatum and LINE-1 methylation level in esophageal cancer. Esophagus 2023; 20: 704-712.
6. Wu H, Leng X, Liu Q, Mao T, Jiang T, Liu Y, Li F, Cao C, Fan J, Chen L, Yaki C, Quan Y, Shun L, Renchuan L, Lanlin H, Mingxin L, Yejian W, Zhaoshen L, Jun P, Qiyu L, Hang Z, Jun Y, Ke X, Mei L, Xinhao P, Haitao L, Gang L, Yongtao H, Xia Z, Zhi-Xiong JX, Junyo L, Guihua W, Chuan X. Intratumoral Microbiota Composition Regulates Chemoimmunotherapy Response in Esophageal Squamous Cell Carcinoma. Cancer Res 2023; 83: 3131–3144.
7. Yeh CC, Chen CC, Chen CC, Han ML, Wu JF, Wang HP, Wu MS, Tseng PH. Characteristics of the esophageal microbiome in patients with achalasia and its changes before and after peroral endoscopic myotomy: A pilot study. J Gastroenterol Hepatol 2023; 38: 1307-1315.
8. Geng ZH, Zhu Y, Chen WF, Fu PY, Xu JQ, Wang TY, Yao L, Liu ZQ, Li XQ, Zhang ZC, Wang Y, Ma LY, Lin SL, He MJ, Zhao C, Li QL, Zhou PH. The role of type II esophageal microbiota in achalasia: Activation of macrophages and degeneration of myenteric neurons. Microbiol Res 2023; 276: 127470.
9. Furuta GT, Fillon SA, Williamson KM, Robertson CE, Stevens MJ, Aceves SS, Arva NC, Chehade M, Collins MH, Davis CM, Dellon ES, Falk GW, Gonsalves N, Gupta SK, Hirano I, Khoury P, Leung J, Martin LJ, Menard-Katcher P, Mukkada VA, Peterson K, Spergel JM, Wechsler JB, Yang G-Y, Rothenberg ME, Harris JK. Mucosal Microbiota Associated With Eosinophilic Esophagitis and Eosinophilic Gastritis. J Pediatr Gastroenterol Nutr 2023; 76: 347-354.
10. Rajagopala SV, Shilts MH, Correa H, Das SR, Choksi YA, Jacobse J, Goettel JA, Hiremath G. Proton Pump Inhibitors Modulate Gene Expression Profile in Esophageal Mucosa and Microbiome. J Pediatr Pharmacol Ther 2023; 28: 504-508.
11. Moreira C, Figueiredo C, Ferreira RM. The Role of the Microbiota in Esophageal Cancer. Cancers (Basel) 2023; 15: 2576.
12. Rescigno M. Microbiota in Reflux Esophagitis and Peptic Ulcer Disease. Surg Infect (Larchmt) 2023; 24: 245-249.