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Systematic Review of the Bacterial Microbiome in Patients with Esophageal Cancer

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Keywords: Gastrointestinal microbiome; Barrett's esophagus; Esophageal adenocarcinoma; Esophageal squamous dysplasia; Esophageal squamous cell carcinoma.

Abbreviations: ESCC: Esophageal Squamous Cell Carcinoma; EAC: Esophageal Adenocarcinoma; EC: Esophageal Cancer; BE: Barrett's esophagus; ESD: Esophageal squamous dysplasia; GERD: Gastro esophageal Reflux Disease; HP: Helicobacter pylori

Abstract

Purpose: Esophageal Squamous Cell Carcinoma (ESCC) and Adenocarcinoma (EAC) are common and deadly diseases. The gut microbiome has been implicated in cancer development, yet its role in esophageal cancer (EC) pathogenesis remains unclear.

Methods: We performed a systematic review to summarize the literature on the microbiome and EC. Three databases were queried for studies performing microbial analysis in Barrett's Esophagus, EAC and ESCC.

Results: Thirty-six, out of 1589 articles identified, were synthesized after inclusion. Data-driven, 16s rRNA amplicon sequencing showed Firmicutes were the most abundant phyla in Barrett's esophagus (BE) and EAC, compared to both Firmicutes and Bacteriodetes in Esophageal Squamous Dysplasia (ESD) and ESCC. Associations between BE/EAC with *Campylobacter, Lactobacillus, Tannerella forsythia*, and *Escherichia coli* were found, whereas ESD/ESCC was associated with *Streptococcus anginosus, Porphyromonas gingivalis*, and *Fusobacterium nucleatum*. There was substantial heterogeneity in microbial analysis methods among reports and insufficient data to perform meta-analysis.

Conclusions: EC subtypes are associated with unique microbial compositions. However, standardized methodology for foregut microbiome research and further elucidation of the pathobiology of these microbial alterations is required to determine the clinical significance of these observations in EC.

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Introduction

Esophageal Cancer (EC) is a commonly diagnosed and deadly cancer that amounts to 1 in 20 cancer deaths worldwide [1]. The two primary EC subtypes are Squamous Cell Carcinoma (ESCC) and Adenocarcinoma (EAC) and both have distinct epidemiologic patterns. ESCC accounts for 90% of EC worldwide, while the incidence of EAC is greater that ESCC in Western countries [1]. Unique risk factors between ESCC and EAC may explain these observations. Smoking tobacco and alcohol consumption are associated with ESCC and it's believed these cancers develop from chronic squamous epithelial inflammation degenerating to a dysplasia-carcinoma sequence [2]. Barrett's Esophagus (BE) is the precursor to EAC and represents metaplasia of the distal esophageal squamous epithelium to specialized columnar epithelium, as a result of chronic injury from gastroesophageal reflux [3]. The primary risk factors for EAC include BE, chronic Gastroesophageal Reflux Disease (GERD), male sex, age over 50, and obesity [3]. Despite significant scientific advancement in the medical management of EC, the underlying mechanisms of EC subtypes are not fully understood. Microorganisms play a critical part in various gastrointestinal cancers [4]. Etiologic associations have been shown with Hepatitis B and C virus in hepatocellular carcinoma, Human papilloma virus in anal cancer, liver flukes (*Opisthorchis viverrini and Clonorchis sinensis*) in cholangiocarcinoma, and *Helicobacter pylori* (HP) in gastric cancer [4]. The divergence of the normal gastrointestinal microbiome, or dysbiosis, and its implications on cancer has been more recent, with advancements in genomic DNA sequencing. Commensal bacteria can modulate inflammation and immune system tone and, in turn, these interactions may confer protective-factors against, or risk-factors for, the development of gastrointestinal cancer [5]. Microbial population shifts from normal, dysbiosis, in the esophageal microbiome have been reported in a variety of foregut disease [6]. Periodontal pathogens, including *Streptococcus anginosus, Porphyromonas gingivalis*, and *Fusobacterium nucleatum*, are of particular interest and have been linked to multiple cancer types, including esophageal cancer [7-10]. However, whether dysbiosis represents a pathogen or a bystander effect in esophageal disease is unclear and reflects a limited understanding of mechanistic pathways for carcinogenesis [6]. As interest and methods for studying the gut microbiome has expanded, we performed a systematic review with the aim to better understand the association between EC and the microbiome.

Methods

Literature Search

Ovid MEDLINE, Embase and Scopus was searched by a trained librarian from January 1979 through March 2019 for studies evaluating the microbiome in BE, ESD, and EC. Our systematic review protocol was registered on PROSPERO (Reference number: CRD42020150027). The Rayyan webtool was utilized for the title and abstract review and our full search strategy can be found in supplemental file 1 [11]. A total of 1589 abstracts were screened and reviewed.

Inclusion and exclusion

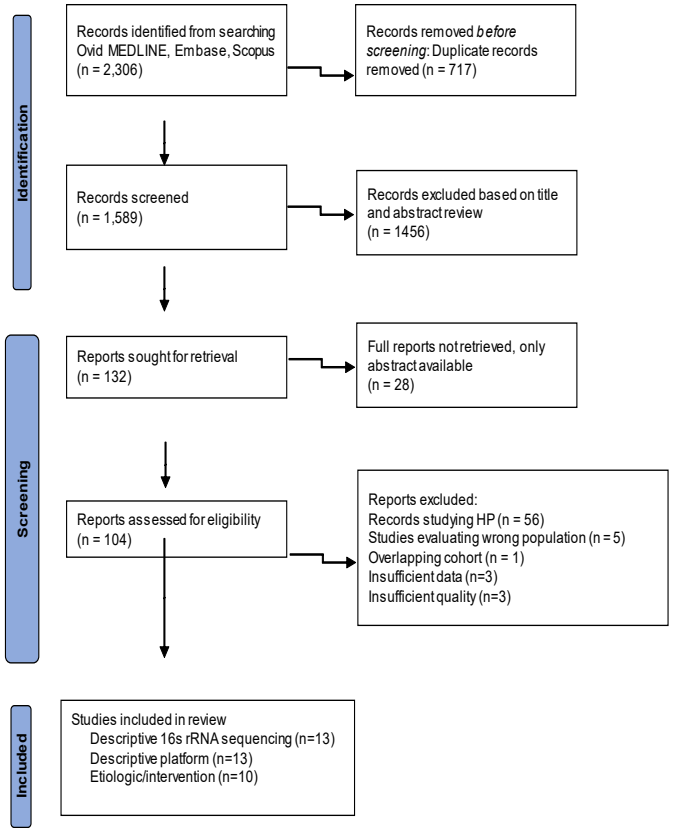
Titles and abstracts were independently screened by two reviewers (SJH and JRG). We included studies that evaluated the foregut microbiome as etiological, diagnostic, therapeutic, or prognostic factors in patients with BE, ESD, EAC, and ESCC. Pre-clinical work using human tissues, disease specific cell lines, or animal models were also included in effort to understand the underlying mechanisms of microbial-mediated pathogenesis. Records evaluating HP only and esophageal cancer were reviewed, but excluded from our synthesis given that these reports have been previously been studied in a systematic review and meta-analysis platform. Lastly, cohorts of less than 5 patients were also excluded. Disagreements between reviewers were resolved by consensus-based discussion between authors SJH, JRG, KKW.

Quality assessment and Data extraction

A meta-analysis was planned utilizing data from the systematic review. The Joanna Briggs Institute critical appraisal checklists for analytical cross-sectional studies and case-control studies were used for quality assessment. Consensus was reached by discussion in terms of including or excluding poor quality reports.

Results

A total of 1589 articles were identified after removing duplicates and 1529 were excluded based on title and abstract screening, leaving 60 articles for full-text review. Another 24 articles were excluded after full-text review, leaving 36 studies meeting inclusion criteria for synthesis **(Figure 1)** [12].



Source from: (Page, McKenzie et al. 2021)
HP, *Helicobacter Pylori*

Figure 1: PRISMA Systematic Review Flow Diagram.

Summary of Microbiome Studies Reviewed

Thirteen studies used 16s rRNA amplicon sequencing for microbiome analysis of saliva, esophageal tissue/fluid, or gastric tissue/fluid samples **(Table 1)**. Studies evaluating gastric samples were included if they were analyzing microbial associations with EC. Most studies utilizing tissue specimens were small (n = <20) [13-20], though two studies on ESCC included 20-50 cases [21,22]. The largest studies were performed using oral washes

in EAC and ESCC [23], saliva samples in ESCC and ESD [24], and Cytosponge and saliva samples in patients with BE [14,25]. A wide variety of bacterial taxa were reported to have high abundancy and prevalence in reviewed studies, as well as various methods of specimen acquisition and analytic platforms. The most studied disease state was BE (n=9), followed by GERD (n=4) and ESCC (n=4), EAC (n=2), and ESD and unspecified EC with 1 report each. Gram positive and negative organisms, as well as aerobic and anaerobic organisms, were represented in all groups studied. Firmicutes were the most abundant phylum in controls, GERD, BE and EAC. Microbial diversity measures included Operational Taxonomic Units (OTU), Shannon index, Shannon-Wiener index, Simpson index, Chao1 and Quadratic entropy [15,20-22,24,25]. ESCC, BE with high-grade dysplasia, and EAC were associated with decreased diversity compared to controls [14,24], whereas in other studies no differences in diversity were observed [15,21,22,25]. Due to the heterogeneity of qualitative data and acquisition methods, meta-analysis of these data could not be performed.

Table 1: Studies using 16s rRNA sequencing of microbiome as platform.

Author (Date) Country	Specimen Types	Method	Groups	N	Most abundant	Most prevalent	Enrichment in patient population
Narikiyo et. al. (2004)	EBx and Sal	16S rRNA sequencing	EC	20	Tumor Tissue: *Treponema denticola, Streptococcus mitis, Streptococcus anginosus.* Normal Tissue: *Streptococcus mitis, Treponema dencticola, Streptococcus anginosus*	NA	NA
Japan			Controls	20	*Streptococcus mitis, Streptococcus Sanguis, Streptococcus Parasanguis*	NA	NA
Pei et. al. (2005)	EBx	Broad range 16S rDNA PCR cloning resulting products	BE	3	*Prevotella pallens*	*Oral bacterium SH66** (50.5%), *Neisseria flavescens* (11.1%), *Prevotella pallens* (6%)	NA
USA			GERD	12	NA	Oral bacterium SH66* (12.5%), *Helicobacter pylori* (12.5%)	NA
			Controls	9	NA	Oral bacterium SH66* (33.3%), *Prevotella veroralis* (22.2%),	NA
Macfarlane et. al. (2007)	Ebx and EAsp	16S rRNA sequencing	BE	7	Various species of *Veilonella atypica*, *Campylobacter rectus/concisus*, *Megaspaera mucilaginosus*	*Campylobacter concisus* (biopsy) and *Streptococcus* (aspirate)	Campylobacter species uniquely present in BE
UK			Controls	7	Various species of *Streptococcus* and *Lactobacillus*	Various species of *Streptococcus* (biopsy) and *Lactobacillus* (aspirate)	
Yang et. al. (2009)	Ebx	16S rRNA sequencing	BE	10	*Streptococcus mitis, Streptococcus pseudopneumoniae, Streptococcus vestburalis*	NA	NA
USA			ERD	12	Streptococcus mitis, Streptococcus pseudopneumoniae, *Haemophilus paraphrophaemolyti*	NA	NA
			Controls	12	Streptococcus mitis, Streptococcus pseudopneumoniae, Haemophilus paraphrophaemolyti	NA	NA
Liu et. al. (2013)	Ebx	16S rRNA sequencing	BE	6	(Genus): *Veillonella, Prevotella, Streptococcus*	(Genus): *Prevotella*	NA
Japan			GERD	6	(Genus): *Streptococcus, Pasteurella*	(Genus): *Streptococcus, Fusobacterium*	NA
			Controls	6	(Genus): *Streptococcus, Klebsiella, Gemella*	(Genus): *Streptococcus*	NA
Amir et. al. (2014)	Ebx and GAsp	16S rRNA pyrosequencing	ERD+BE	19	*Proteobacteria and Firmicutes*	NA	*Enterobacteriacae* increased in gastric aspirate of ERD and BE
Israel			Controls	15	*Proteobacteria and Firmicutes*	NA	
			Subset: before and after PPI	8	*Proteobacteria and Firmicutes*	NA	

Chen et. al. (2015)	Sal	16S rRNA sequencing	ESCC	87	*Prevotella, Streptococcus, Porphyromonas*	No dominant genera	Decreased carriage of *Lautropia, Bulleidia, Catonella, Corynebacterium, Moryella, Peptococcus and cardiobacterium* in ESCC compared to non-ESCC
China			ESD	63	*Prevotella, Streptococcus, Veillonella*	No dominant genera	
			Controls	85	*Prevotella, Streptococcus, Veillonella*	No dominant genera	
Gall et. al. (2015)	Ebx, Ebr, Gbx	16S pyrosequencing	BE	15	*Streptococcus mitis, Streptococcus salivarius, Haemophilus parainfluenzae* (total number reads across all samples)	*Streptococcus mitis*	NA
Japan							
Nasrollahzadeh et. al. (2015)	Gbx	16S rRNA sequencing	ESCC + ESD	37	(Order): Clostridiales, Bacteroidales, Lactobacillales	NA	Clostridiales (Order) and Erysipelotrichales (Order) were more abundant in ESCC/ESD compared to healthy controls
Iran			ERD	17	(Order): Bacteroidales, Clostridiales, Lactobacillales	NA	
			Controls	37	(Order): Bacteroidales, Clostridiales, Lactobacillales	NA	
Peters et. al. (2017)	Sal	16S rRNA sequencing	EAC	81	(Order): Actinomycetales, (Class): Betaproteobacteria	(Order): Actinomycetales, *streptococcus pneumoniae*	In EAC, *Tannerella forsythia* is increased, while Neisseria and *Streptococcus pneumoniae* are decreased, relative to controls
USA			EAC control	160	(Order): Actinomycetales, (Class): Betaproteobacteria	(Order): Actinomycetales, *Streptococcus pneumoniae*	
			ESCC	25	(Order): Actinomycetales, (Class): Betaproteobacteria	(Order): Actinomycetales, *Streptococcus pneumoniae, Prevotella alloprevotella*	Porphyromonas gingivalis abundancy increased in ESCC relative to controls
			ESCC control	50	(Order): Actinomycetales, (Class): Betaproteobacteria	Actinomycetales (order), *Streptococcus pneumoniae, Neisseriaceae Neisseria*	
Elliott et. al. (2017)	Ebx, Ebr, Cytospge	16S rRNA sequencing	EAC	19	(Family): *Streptococcaceae, Lactobacillaceae, Prevotellaceae*	NA	*Lactobacillus fermentum* enriched in EAC, relative to BE and controls. There was reduced diversity in EAC
UK			HGD	23	(Family): *Streptococcaceae, Prevotellaceae, Pasteurellaceae*	NA	
			NDBE	24		NA	
			Controls	20	(Family): *Streptococcaceae, Prevotellaceae, Pasteurellaceae*	NA	
Snider et. al. (2018)	Sal	16S rRNA sequencing	BE	32	(Phylum): Bacteroidetes, Firmicutes, Proteobacteria	NA	Firmicutes increased and Proteobacteria decreased in BE
USA			Controls	17	(Phylum): Proteobacteria, Bacteroidetes, Firmicutes	NA	
Liu et. al. (2018)	Ebx	16S rRNA sequencing	ESCC	45	(Phylum): Firmicutes, Proteobacteria, Bacteroidetes	NA	*Streptococcus* and *Prevotella* abundance were associated with unfavorable survival
China							

*Unidentified/Non-cultured species

EC, Esophageal cancer; ESCC, esophageal squamous cell carcinoma; ESD, esophageal squamous dysplasia; EAC, esophageal adenocarcinoma; BE, Barrett's Esophagus; NDBE; Non-dysplastic Barrett's esophagus; HGD, Barrett's esophagus with High grade dysplasia; GERD, Gastroesophageal reflux disease; ERD, esophagitis; NA, not available; PCR, Polymerase chain reaction; rRNA, Ribosomal ribonucleic acid; Sal, saliva; Ebx, esophageal tissue biopsy; Ebr; esophageal brushing; EAsp, esophageal fluid aspirate; Gbx, gastric biopsy; Gasp, gastric fluid aspirate; Cytospge, cytospone

Table 2: Studies using other platforms to study the microbiome.

Author (Date) Country	Specimen Types	Method	Groups	N	Prevalence (%) and significant findings	Reported Risk factor(RF)/ protective factor(PF)
Muscroft et. al. (1981)	GAsp	Culture, morphology and biochemical reactivity	Gastroesophageal carcinoma (unspecified)	31	*Escherichia coli* 51% more frequently isolated vs. controls. *Clostridium spp.* 25.8% more frequently isolated vs. controls	RF: Clostridium spp., Escherichia coli
UK			Postoperative stomach, controls	57	*Escherichia coli 44%*more frequently isolated vs. intact stomach controls. *Clostridium spp.* 7%	NA
			Native stomach, controls	64	*Escherichia coli* 3.1% *Clostridium spp.* 1.6%	NA
Finlay et. al. (1982)	Ebx	Culture, morphology and biochemical reactivity	ESCC, EAC	12	Alpha and non-haemolytic streptococci comprised 49 % of the total aerobic growth. Coagulase negative staphylococci, lactobacilli, and corynebacterium species were frequent isolates	NA
UK						NA
Mannell et. al. (1983)	EAsp	Culture, morphology and biochemical reactivity	EC	NA	Isolation rate of all aerobic and anaerobic bacterial species were similar in both groups	NA
South Africa			Controls	NA		NA
Sasaki et. al. (1998)	Ebx, post-surgical specimens	PCR, primers for Streptococcus anginosus rDNA fragment	ESCC	15	*Streptococcus anginosus* 93% EC and 67% dysplastic esophagus, and 7% non-cancerous portions of the esophagus/stomach	RF: *Streptococcus anginosus*
Japan			Gastric cancer (unspecified)	43	*Streptococcus anginosus* 42%	NA
			Colorectal cancer (unspecified)	10	*Streptococcus anginosus* 10%	NA
			Extra-intestinal unspecified cancers (Lung, cervical, renal, bladder)	59	*Streptococcus anginosus* 0%	NA
Bohr et. al. (2003)	Ebx	PCR, primers for Helicobacteraceae	ESCC	9	*Helicobacteraceae* 56% within ESCC, 44% surrounding normal tissue. *Helicobacter Wolinella 33%*, *Helicbacter pylori 33%*	NA
South Africa						NA
Morita et. al. (2003)	Ebx and oral biopsy	PCR, primers for Streptococcus anginosus	ESCC	18	*Streptococcus anginosus* 44%. Quantitative DNA higher in ESCC compared to oral cancer	RF: *Streptococcus anginosus*
Japan			Normal tissue adjacent to ESCC	6	*Streptococcus anginosus* 17%	
			Oral SCC	28	*Streptococcus anginosus* 13%	
			Normal tongue tissue control	7	*Streptococcus anginosus* 0%	
Osias et. al. (2004)	Ebx	Gram stain, culture; Gram stain only on retrospective cohort;	BE/ERD/Controls (Retrospective cohort)	47	Bacterial scores higher in BE vs. non-BE and positively correlated with worsening dysplasia. Gram positive organisms were most abundant, but not further defined	RF: Higher bacteria counts were associated with BE and correlated positively with dysplasia
USA			BE (Prospective cohort)			
			GERD (Prospective cohort)	9		
Morita et. al. (2005)	Ebx and Sal	PCR, primers for S. anginosus, Streptococcus constellatus and Streptococcus intermedius (simultaneously)	ESCC	15/41	*Streptococcus anginosus* had similar levels in saliva of ESCC and healthy controls, but significantly higher levels noted in alcoholics	RF: *Streptococcus anginosus* in alcohol-related carcinogenesis
Japan			Benign disease	94		
			Controls	22		
Blackett et. al. (2013)	Ebx	Culture, primers bacterial DNA and cytokines	EAC	30	*Campylobacter concisus* 10%. *Helicobacter pylori* 77%, however, overall *Helicobacter pylori* gene copy counts less than other species	PF: *Helicobacter pylori*

UK			BE	45	*Campylobacter concisus* 42% *Helicobacter pylori* 58%	RF: *Campylobacter*
			GERD	37	*Campylobacter concisus* 51% *Helicobacter pylori* 54%	
			Controls	39	*Campylobacter concisus* 13% lower vs. GERD. BE *Helicobacter pylori* 74%	
Gao et. al. (2016)	Ebx	qPCR, primers for Porphyromonas gingivalis	ESCC	100	*Porphyromonas gingivalis* 61% in ESCCC and 12% in normal adjacent tissue. *Porphyromonas gingivalis* associated with severe disease (worse differentiation + lymph node spread)	RF: *Porphyromonas gingivalis*
China		IHC targeting whole bacteria and unique secreted protease gingipain Kgp	Controls	30	*Porphyromonas gingivalis* 0%	
Yamamura et. al. (2016)	Ebx	qPCR, primers for Fusobacterium nucleatum, microarray, and IHC for CCL20	EC (300 ESCC, 12 EAC, 13 other EC histology)	325	*Fusobacterium nucleatum* 23% and associated with EC severity and survival. Higher in tumor than matched adjacent normal tissue (investigated in subset n=60).	RF: *Fusobacterium nucleatum*
Japan			Controls	NA		
Yamamura et al (2017)	Ebx, post-surgical specimens	PCR, primers for Fusobacterium nucleatum	ESCC	20	*Fusobacterium nucleatum* 20% in ESCC and 5% in adjacent normal tissue	RF: *Fusobacterium nucleatum*
Japan			Gastric cancer (unspecified)	20	*Fusobacterium nucleatum* 10% in tumor, 0% in adjacent normal tissue	
			Pancreatic cancer (unspecified)	20	*Fusobacterium nucleatum* 0% in tumor tissue and adjacent normal tissue	
			Colorectal cancer (unspecified)	20	*Fusobacterium nucleatum* 45% in tumor tissue and 40% in adjacent normal tissue	
			Liver cancer (unspecified)	20	*Fusobacterium nucleatum* 0% in tumor tissue and adjacent normal tissue	
Yuan et al (2017)	Ebx, GBx	PCR, primers for Porphyromonas gingivalis, IHC anti bacterium	ESCC	50	*Porphyromonas gingivalis* 48% in ESCC, 23% in ESD, and non-cancerous tissue 3%	RF: *Porphyromonas gingivalis*
China			Gastric cardia adenocarcinoma	33	*Porphyromonas gingivalis* 18%	
			Gastric body adenocarcinoma	25	*Porphyromonas gingivalis* 4%	

EC: Esophageal Cancer; ESCC: Esophageal Squamous Cell Carcinoma; ESD: Esophageal Squamous Dysplasia; EAC: Esophageal Adenocarcinoma; BE: Barrett's Esophagus; NDBE; Non-Dysplastic Barrett's Esophagus; HGD: Barrett's Esophagus With High Grade Dysplasia; GERD: Gastroesophageal Reflux Disease; ERD: Esophagitis; NA: Not Available; PCR: Polymerase Chain Reaction; Sal: Saliva; Ebx: Esophageal Tissue Biopsy; Easp: Esophageal Fluid Aspirate; Gbx: Gastric Biopsy; Gasp: Gastric Fluid Aspirate; IHC: Immunohistochemistry

Thirteen studies used bacterial culture or specific PCR primers for bacterial DNA detection **(Table 2)** [26-28]. Most of these analyses were performed on tissues and a few studies used fluid aspirates [30,32,33]. The majority of reports evaluated carcinoma (n=11), of which ESCC were studied in 7, undefined EC subtype in 2, EAC in 1, and both EAC and ESCC in 1. The remaining 2 reports evaluated patients with BE and GERD. There was an expected, smaller variety of bacterial taxa reported amongst these studies as compared to those utilizing 16s rRNA sequencing. *Streptococcus anginosus, Porphyromonas gingivalis,* and *Fusobacterium nucleatum* were of specific interest for investigators and demonstrated higher relative prevalence in ESCC compared to controls, while positively correlating with disease severity and poor prognosis [29,31,32,35-38]. EAC had less *Campylobacter species* compared to GERD and BE, as well as less *HP* in GERD and BE compared to healthy controls [26]. Osias et al. 2004 demonstrated greater overall bacterial scores on gram stain in BE compared to GERD. Blackett et al. 2003 showed that healthy controls tended to have a greater overall bacterial flora, yet *Campylobacter concisus* was observed at significantly greater proportions in GERD and BE patients.

Studies Evaluating Mechanisms of Action

There were 10 studies evaluating the microbiome as an etiological factor or therapeutic intervention in BE and EC [39-48]. All of these studies were in vitro or *in vivo* and utilized either disease specific cell lines or animal models. These data were summarized in **supplemental file 2, Table 3**.

Systematic review of the bacterial microbiome in patients with Esophageal Cancer - SUPPLEMENTARY FILE 1

Ovid MEDLINE

Searched for Ovid MEDLINE(R) and Epub Ahead of Print, In-Process & Other Non-Indexed Citations and Daily between 1946 to March 20, 2019.

#	Searches	Results
1	exp Esophageal Neoplasms/mi [Microbiology]	160
2	Barrett Esophagus/mi [Microbiology]	106
3	exp esophageal neoplasms/ or barrett esophagus/	50829
4	3 and (microbiot* or microflora or microbiome).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	58
5	((esophag* or oesophag*) adj3 (cancer* or carcinoma* or adenocarcinoma* or eac or escc or barrett*)).mp. [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	49269
6	5 and (mi.fs. or microbiota.mp. or microbiome.mp. or bacteri*.mp.) [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	814
7	3 and (exp adenocarcinoma/ or exp carcinoma/)	24106
8	7 and (mi.fs. or microbiota.mp. or microbiome.mp. or microflora.mp. or exp bacteria/) [mp=title, abstract, original title, name of substance word, subject heading word, floating sub-heading word, keyword heading word, organism supplementary concept word, protocol supplementary concept word, rare disease supplementary concept word, unique identifier, synonyms]	269
9	1 or 2 or 4 or 6 or 8	945
10	limit 9 to english language	811

Embase

Searched from 1988 to 2019 Week 11.

#	Searches	Results
1	exp esophagus cancer/ or exp esophagus carcinoma/	58118
2	exp microbiome/	12592
3	microflora/ or exp bacterial flora/ or exp intestine flora/ or exp microbiome/ or exp mouth flora/	73133
4	exp bacterium/	1241975
5	1 and (2 or 3 or 4)	1066
6	((microbiota* or microbiome* or microflora*) and (esoophag* or oesophag* or barrett*)).mp.	182
7	1 and 6	75
8	5 or 7	1070
9	..l/ 8 lg=en	1007

Scopus

Search strategy:

#	Searches	Results
1	(TITLE-ABS- KEY ((esophag* OR oesophag*) W/3 (barrett* OR cancer* OR neoplas* OR carcinoma* OR adenocarcinom*)) AND TITLE-ABS-KEY ((microbiota OR microbiome OR microflora* OR flora)))	244

Table 3: Experimental studies on intervention or etiology of the microbiome.

Study	Model specifications	Intervention	Significance
Fein et al (2000)	GERD/BE/EAC model: Sprague-Dawley rats with esophagojejunostomy	1. Water (control group)	Triple antibiotics did not reduce Lactobacilus or Bacteriodes (both implicated in carcinogensis pathways). Therefore, these antibiotics may be of limited therapeutic value.
Germany		2. Acidified water pH 1.8	
		3. Water infused with triple antibiotics (Tobramycin, polymyxin, and vancomycin)	
Kauppila et al (2013)	BE-derived EAC cell line: OE33	1. *E.coli* DNA	Bacterial DNA ligand for TLR9, induces invasion, progression and metastasis. *E.coli* DNA was the most effective invasion ligand in the OE33 cell ine.
Finland		2. *H.pylori* DNA	
		3. Deoxyoligonucleotides	
Kohata et al (2015)	BE model: Wistar rats with esophagojejunostomy	1. control	Rebamipide decreases Clostridium and increases Lactobacillus relative to the control group, and reduced BE development.
Japan		2. rebamipide	

Namin et al (2015)	BE cell lines: CPA and CPD	1. *B.longum* and *Lactobacillus acidophilus* (Probiotic control)	Therapeutic probiotics B.longum and Lactobacillus acidophilus may reduce BE progression to EAC.
Iran		2. *C. concisus mediated infection, followed by "therapuetic" B.longum + Lactobacillus acidophilus*	
		3. "Prophylactic" *B.longum* and *Lactobacillus acidophilus administration, followed by C.concisus-mediated infection*	
Namin et al (2015)	BE and EAC cell lines: FLO-1, CPA and CPD	1. *Campylobacter concisus*	*Campylobacter consisus* and *Streptococcus salivarius* co-culture results in significant changes to TNFa, P53, and IL-18 expression, postulated to be factors in developing BE and its progression to EAC.
Iran		2. *Streptococcus salivarius*	
Sawada et al (2016)	BE model: Wistar rats with esophagojejunostomy	1. control	Penicillin G and streptomycin decrease Lactobacillales and increase Clostridium, resulting in a trend towards reducing incident EAC (not statistically significant).
Japan		2. Penicillin G and streptomycin	
Zaidi et al (2016)	GERD/BE/EAC model: Sprague-Dawley rats with esophagojejunostomy	NA	E.coli possibly increases TLR signaling, promoting malignant progression of BE.
USA			
Mozaffari et al (2018)	BE and EAC cell lines: FLO-1, CPA and CPD	*1. Campylobacter Concisus co-culture*	*Campylobacter Concisus* induces CDX1 expression in BE cell lines, promoting malignant progresion of BE.
Iran			
Meng et al (2019)	ESCC cell lines: Eca109 and KYSE510	*Porphyromonas gingivalis*	*Porphyromonas gingivalis* promotes proliferation and motility of ESCC cells, by activating NF-KB signaling
China			
Zhou et al (2018)	Oral and ESCC model: induced by 4-NQO-treatment of C57BL/6NTac mice	1. Germ-free	Increased chemical toxicity in germ-free mice indicating the microbiome alters at least in part the host gene expression in the liver, important for drug metabolism.
China		2. Conventionally housed	

ESCC: Esophageal Squamous Cell Carcinoma; EAC: Esophageal Adenocarcinoma; BE: Barrett's Esophagus; GERD: Gastroesophageal Reflux Disease.

Discussion

In our review of the literature, the most represented phyla in the normal esophagus microbiome were Firmicutes, followed by Bacteroidetes, Proteobacteria, Actinobacteria and Spirochaetes. Composites from 16s rRNA amplicon sequencing data revealed lower microbial abundance scores for top reported phyla among esophageal disease versus controls. Individual studies also reported on a positive correlation between decreasing microbial diversity and disease state severity [6,14]. However, the microbial composition found was heterogeneous and lacked validation in independent cohorts, yet the results obtained were hypothesis generating.

EAC and ESCC exhibit unique microbiome alterations at lower taxonomic ranks and may reflect differences in pathogenesis. Our review of the literature revealed associations between BE and EAC with *Campylobacter, Lactobacillus, Tannerella forsythia*, and *Escherichia coli* organisms. Firmicutes are in the top most abundant phyla in EAC tissue samples, largely due to increased prevalence of the family *Lactobacillaceae* compared to BE and healthy controls [14]. Oral washes from patients with EAC did not reproduce results from EAC tissue samples, as *Streptococcus pneumoniae*, the only species from the family *Lactobacillaceae* that was identified, was decreased in EAC compared to controls [23]. *Lactobacillus* and *Campylobacter* are particularly interesting organisms because they are adaptive to acid environments, as is present in the esophagus with increased exposure to gastric acid reflux. Direct and indirect oncoprotective effects of *Lactobacillus* organisms have been described in other gastrointestinal cancers, resulting in enhanced therapy efficacy, reduction of chemotherapy-induced toxicity, and a lower risk of post-surgical complications [49]. Experimental data on BE cell lines and a BE rat model have shown that *Lactobacillus,* enriched by either probiotic therapy (*Bifidobacterium Longum* and *Lactobacillus acidophilus*) [45], has protective effects against BE and EAC progression [41]. The protective mechanisms of *Lactobacillus* species appear to be mediated by TLR-4 signaling and reduced expression of pro-inflammatory mediators of IL-18, TNFa and downstream B-catenin mediated oncogene transcription, while also promoting p53 tumor suppressor gene expression [45,50-53]. In contrast, reduction of *Lactobacillus* by antibiotic therapy, with coincident increase of *Clostridium*, did not affect BE and EAC incidence in a rat model, suggesting multiple organisms may be involved in EAC carcinogenesis [46]. *Campylobacter* species was also studied and noted to have greater abundance in BE compared to controls [17]. Another group confirmed *Campylobacter* to be enriched in GERD and BE in comparison to EAC and controls [26]. Moreover, *Campylobacter* may play a role in BE by a variety of molecular pathways, among which include activation of the NF- κB pathway, but also via direct toxin mediated DNA stress injury [17,26]. Experimental analyses confirmed that *Camplylobacter concisus* induces CDX1 expression (a homeobox protein important in development of intestinal metaplasia) in BE cell lines and also enhances expression of IL-18 and TNFa, as well as inducing p53 gene mutations, all of which promote dysplasia [44,45,54,55]. Lastly, *Escherichia coli* was enriched in patients with esophagitis and BE compared with healthy controls and in patients with gastroesophageal carcinoma compared with controls [33]. These observations were consistent with in vitro work demonstrating that *Escherichia coli* activates TLR signaling pathways, promoting carcinogenesis in BE, as well as invasion, progression, and metastasis of EAC [40,47,56].

ESCC is associated with a higher abundance of some Firmicutes *(*orders Clostridiales and Erysipelotrichales) and lower abundance of others (genus *Bulleidia*, *Catonella, Moryella,* and *Peptococcus)* [21,24]. Bacteroidetes (specifically *Porphyromonas gingivalis*) is seen abundantly in ESCC, while Proteobacteria (genus *Lautropia* and *Cardiobacterium)* and Actinobacteria (genus *Corynebacterium)* abundance is lower [23,24]. ESCC has stronger links to microbes commensal to the oral cavity, as compared to EAC, and this includes *Streptococcucus anginosus, Porphyromonas gingivalis,* and *Fusobacterium nucleatum*. There was a paucity of mechanistic data as it pertains to microbial carcinogenesis in ESCC and thus it remains even less clear whether these organisms are bystander to associated microenvironment alterations. Only one record was found in this review, and it was an in vitro study on Porphyromonas gingivalis which demonstrated promotion of ESCC cell proliferation and motility by activation of NF-κB signaling [42].

Our systematic review of the literature on the microbiome and EC reveals that this area is still evolving. While there is data linking EC with certain bacterial phyla, organisms, and molecular mechanisms that promote carcinogenesis, inconsistent study methodology has dampened conclusions that can be made. There is a high degree of heterogeneity amongst the studies evaluating the microbial profiles of EC, including the type of specimens (saliva, tissue samples, aspirates), location (oral, esophageal, gastric), acquisition methods (spit sample, cytosponge, endoscopic sampling, surgical resection) and a variety of analytic methods (16s rRNA sequencing, gram stain, culture, and microbial specific PCR primers). Additionally, outcomes are subject to small sample sizes and lack of positive (spiked in) and negative (i.e., water collected from endoscopy room and sterile water) controls. The observed geographic variability in microbiome is another important factor to consider with the reviewed literature, given subjects were comprised of populations from the USA, UK, South Africa, China, Japan, Israel, and Iran. It's been shown that cultural and environmental factors including diet, hygiene, antibiotics, and climate can influence the gastrointestinal microbiome. Therefore, regional differences in the microbiome are expected and could confound comparisons made between different subject populations [57]. These limitations ultimately precluded the application of more robust analysis from the data reviewed. It should be acknowledged, that the optimal and most representative method of sample acquisition for foregut microbiome analyses is yet to be determined. The use of a swallowed sponge on a string has shown promise in being non-invasive and providing a high bacterial abundance relative to tissue biopsies and endoscopic brushings, though these may be diluted by oral or stomach organisms [14]. Other methods, such as electronic nose devices, take advantage of detecting volatile organic compounds as a surrogate marker for microbiome alterations, however this is not specific for foregut microbiota as the entire aerodigestive tract could be represented in analyzed samples.

Future directions

Future directions for studying the microbiome in BE and EC will need to address the aforementioned limitations related to study methodology, as well as solidify the clinical impact of the microbiome as a cause, contributor, or consequence of disease. Well-designed studies of the normal foregut microbiota from healthy individuals are also needed. While meta-genomics and meta-transcriptomics can be powerful tools for microbial study, its application for gut microbiome study may be limited due to insufficient biomass provided by small biopsy specimens and heavy contamination with human DNA and RNA. Optimizing and standard operating protocols for specimen acquisition may mitigate these concerns. Additionally, utilizing longer read sequencing of 16S in 16s rRNA amplicon sequencing would allow better differentiation of microbial DNA and the identification of more species, resulting in a more complete representation of the microbiome. Longitudinal application of 16s rRNA amplicon sequencing on biospecimens over time could reveal specific taxonomic changes in commensal gut microbes and host tissues that precede or coincide with the development of EAC and ESCC. This approach would have the added benefit of permitting each subject to serve as their own control in order to minimize microbiota inter-individual differences. Moreover, identifying potential disease-implicated taxa and experimentally validating their carcinogenic effects by using matched samples for 16s rRNA amplicon sequencing and microbial cultivations for in vitro and in vivo models of EC, will overcome problems in extrapolation of experimental data on strains not indigenous to the patient. These problems result from related strains considered to be of the same taxon because of similarities at the genomic level, but with considerable differences at the functional level. Finally, analyzing matched pre- and post-treatment samples might provide insights in the effectiveness of therapy in terms of changes in microbial populations and associations between the microbiome and progression or regression of disease. The gut microbiome is very sensitive to changes in the milieu and host states, and often precede visible changes observed by histology or endoscopy.[58] Therefore, changes in the gut microbiome could be clinically useful as markers for advancing disease, response to therapy, or even screening and early detection.

In conclusion, a complex relationship exists between the foregut microbiome and EC. EAC and ESCC may exhibit diminished microbial diversity, compared to controls, and appear to have distinct microbial profiles with biologic plausibility for carcinogenesis. However, standardized methodology for foregut microbiome research is lacking and this needs to be addressed in order to validate these results. Optimizing and standardizing methodology for foregut microbiome research will be necessary in order to further elucidate the pathobiological and clinical implications of these microbial alterations in EC.

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