

Dysbiosis Patterns in Glottic and Laryngeal Cancers: A Systematic Review of Microbiome Alterations[☆]

*†‡§ Jérôme R. Lechien *Mons, ‡Brussels, Belgium, †Paris, and §Poitiers, France

Summary: Background. This systematic review summarized current evidence regarding the role of upper aerodigestive tract microbiomes (UAM) in laryngeal squamous cell carcinoma (LSCC) development, progression, clinical, and oncological outcomes.

Methods. Two investigators systematically search PubMed, Scopus, and Cochrane Library databases for studies investigating microbiome characteristics, mechanistic roles, and associations with clinical and oncological outcomes in LSCC according to the Preferred Reporting Items For A Systematic Review And Meta-analysis statements. The bias analysis was conducted with the methodological index for nonrandomized studies.

Results. Ten studies were included, accounting for 491 LSCC patients. LSCC tissues demonstrated lower bacterial diversity compared with controls. Taxonomic analyses suggested an overrepresentation of Bacteroidetes (Prevotella) and Fusobacteriota (Fusobacterium) in LSCC, while Firmicutes (Stomatobaculum longum, Abiotrophia, Gemella, and Streptococcus) and Actinobacteria (Actinomyces, Corynebacterium, and Rothia mucilaginosa) were predominant in control tissues. Firmicutes demonstrated the largest compositional variation across studies, with 30.9%-63.6% abundance in LSCC compared with 13.9%-32% in controls. Two studies explored microbiome signatures: one for LSCC diagnosis and another for prognosis. Substantial methodological heterogeneity was observed across studies regarding confounding factor analysis, UAM assessment protocols, and control tissue selection.

Conclusion. The current literature supports potential distinct UAM signatures between LSCC and non-cancerous tissues, with Bacteroidetes and Fusobacteriota enriched in LSCC tissues.

Although emerging evidence supporting the key role of UAM in the development of LSCC, substantial methodological heterogeneity across studies necessitates standardized protocols for future investigations.

Key Words: Laryngeal—Cancer—Carcinoma—Microbiome—Microbiota—Bacteria—Surgery—Larynx—Oncological—Outcome—Review.

INTRODUCTION

Head and neck squamous cell carcinoma is the 6th most common adult cancer worldwide, corresponding to 5.3% of all cancers.¹ Laryngeal squamous cell carcinoma (LSCC) is the second most prevalent head and neck squamous cell carcinoma, accounting for 211 000 new cases and 126 000 deaths per year worldwide.^{1,2} The incidence of LSCC has significantly decreased in the past three decades, primarily because of the decrease of the incidence of localized disease, but the mortality did not decrease similarly, which results in an increased case-fatality rate overall.³ Thus, there is a critical need to renew attention to research on a new biologic cause of LSCC, and to develop effective new approaches for

prevention.⁴ The development of culture-independent molecular techniques for environment DNA analysis has led to increased investigation of microbiome roles in respiratory and digestive diseases over recent decades.⁵ In head and neck squamous cell carcinoma, the upper aerodigestive tract microbiome (UAM) has been shown to be involved in the carcinogenesis and the tumor progression, affecting the tumor microenvironment by promoting inflammation and producing carcinogenic metabolites.^{5,6} The UAM also affects the immune environment, modulating the response to immunotherapy, chemotherapy, or radiotherapy, and the related overall survival.⁷

This systematic review aimed to summarize current evidence regarding the role of UAM in LSCC development, progression, clinical, and oncological outcomes.

MATERIALS AND METHODS

The systematic review was conducted according to the Preferred Reporting Items for a Systematic Review and Meta-analysis (PRISMA) checklist.⁸ The criteria for considering studies were based on the population, intervention, comparison, outcome, timing, and setting framework.⁹

Studies

The systematic review included studies published in English-language peer-reviewed journals from January 2000 to January 2025. Eligible studies included prospective

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From the *Department of Surgery, UMONS Research Institute for Health Sciences and Technology, University of Mons (UMons), Mons, Belgium; †Department of Otolaryngology and Head and Neck Surgery, Foch Hospital, School of Medicine, UFR Simone Veil, Université Versailles Saint-Quentin-en-Yvelines (Paris Saclay University), Paris, France; ‡Department of Otolaryngology and Head and Neck Surgery, CHU Saint-Pierre, Brussels, Belgium; and the §Department of Otolaryngology, Elsan Hospital of Poitiers, Poitiers, France.

Address correspondence and reprint requests to: Jérôme R. Lechien, Department of Surgery, UMONS Research Institute for Health Sciences and Technology, University of Mons (UMons), Mons, Belgium. E-mail: Jerome.Lechien@umons.ac.be

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and retrospective cohorts, cross-sectional analyses from cancer registries, and controlled trials investigating associations between UAM and LSCC. Case reports and experimental animal studies were excluded.

Participants and inclusion criteria

Studies were included if they considered patients with subglottic, glottic, or supraglottic LSCC or pharyngeal SCC with laryngeal involvement. Microbiome analyses of laryngeal, pharyngeal, oral (saliva), or tracheal specimens were considered eligible. There was no selection criteria based on the treatment modalities, microbiome characterization methods, or demographic factors. Studies examining head and neck squamous cell carcinoma without specific LSCC subgroup analyses were excluded.

Outcomes

Two investigators independently carried out data extraction with disagreements resolved through consensus. The primary outcomes included UAM compositional profiles (taxonomic classification: phylum, class, order, family, genus, and species) and their associations with LSCC. Secondary outcomes were study characteristics (design, evidence level), patient demographics (mean/median age, sex ratio), oncological findings (cTNM staging, anatomical subsite, and treatment modalities), and methodological aspects (specimen types, analytical techniques).

Intervention and comparison

There was no criterion for intervention. In case of investigation of the prognosis value of UAM, the type of intervention (surgery, chemo-/radiotherapy) had to be specified.

Timing and setting

There were no criteria for specific timing in the disease process.

Search strategy

The author and a librarian independently conducted the PubMed, Scopus, and Cochrane Library searches for relevant peer-reviewed publications related to UAM features in LSCC. The following keywords were used for the search strategy: Larynx; Laryngeal; Cancer; Squamous Cell Carcinoma; Oncological; Microbiome; Microbiota; Bacteria; and Outcomes. The studies reporting database abstracts, available full texts, or titles with the search terms were considered. The research findings have been reviewed for relevance and the reference lists of some articles (eg, reviews or meta-analyses) were examined for additional pertinent studies. The included studies were analyzed for the number of patients, study design, inclusion and exclusion criteria, quality of trial/evidence-based level (EBL),¹⁰ demographics, and outcomes. A critical attention was paid to the potential overlap between cohort studies. Ethics committee approval was not required.

Bias analysis

The bias analysis was conducted with the methodological index for nonrandomized studies (MINORS), which is a validated tool for assessing the quality of retrospective, prospective, uncontrolled, controlled, or randomized surgical studies.¹¹ MINORS includes items rated 0 if absent, 1 when reported but inadequate or partly adequate, and 2 when reported and adequate. The following items compose the MINORS: 1) aim of the study [clearly stated (2), unclear (1), or absent (0)]; 2) inclusion of consecutive (2), nonconsecutive (1), or undetermined (0) patients; 3) prospective data collection (prospective (2), retrospective analysis of prospective collected data (1), or absent (0)); 4) appropriateness of endpoints (adequate evaluation of UAM and oncological outcomes (2), adequate evaluation of one outcome (1), and no adequate outcome evaluation (0)); 5) adequate follow-up period (in case of prospective-predictive value studies); and 6) the 5% rate of lost to follow-up ((2) vs (0) if more than 5%). The item related to the study size prospective calculation was only considered for prospective studies and judged as good (2), mentioned as unnecessary or not provided (1), or absent (0). The ideal MINORS score was 16 for noncomparative studies and 24 for comparative studies.¹¹

RESULTS

Of the 29 identified studies, 10 studies met our inclusion criteria.^{12–21} Eight studies were prospective controlled (EBL: 3 C),^{12,13,15–20} and the others were uncontrolled prospective (EBL: 4)^{14,21} (Table 1 and Figure 1). Two studies demonstrated potential sample overlap; however, after careful evaluation, both were retained for analysis due to their distinct outcome measures and complementary findings.^{18,19} Analysis of cohorts revealed potential participant overlap between one large-cohort study¹⁵ and two smaller investigations.^{18,19} Moreover, 3 studies were excluded from the final analysis due to confirmed overlap of patient data with previously included publications.^{22–24}

Demographics, patients, and tumor stages

Excluding potential overlaps, the findings of 491 patients with LSCC were included. There were seven females and 370 males. Gender was not detailed in one study.¹⁷ The mean age of patients ranged from 57.1 to 68.8 years (Table 1). The tumor stage and anatomical location features were described in six studies.^{12,15,16,19–21} The UAM was mostly investigated in cT3 and cN0 glottic and supraglottic LSCC (Table 2). There was no study including patients with distant metastasis. The treatments were reported in two studies.^{12,21} Riva et al²¹ conducted correlation analysis between tracheal microbiome profiles and clinical outcomes, reporting no statistically significant associations. Control specimens were predominantly derived from vocal cord polyp tissue,^{13,15,16,18,19} while only two investigations used tissue samples from healthy subjects.^{12,17}

TABLE 1. Demographic, Clinical, and Microbiota Outcomes

References	Design	Demographics				Treatment Features (N, %)					Phyla Outcomes	Primary Results
		EBL	N	F/M	Age (y)	C/RT	Surgery	U/BND	Postop CRT	MM		
Dorobisz et al, 2024 ¹²	Prospective	3 C	44 LC	9/35	63.4	17 (39)/ 40 (91)	20 (45)	NP	14 (32)	La	FIR (<22.1%)	CT > LC
Yu et al, 2023 ¹³	Controlled		30 CT	11/19	62.6						ACB, ACT, and BAC (≥1.7%) FUS, PRB, and PSE BDT (≥24.7%) FUS, ACB, and PS	CT = LC LC > CT LC > CT
	Prospective Controlled	3 C	77 LC 76 VCP	6/71 10/66	60.4	-	-	-	-	Or		
Dhakal et al, 2022 ¹⁴	Cross-sectional	4	78 LHC	17/61	63.1	-	-	-	-	La	PSE, ACT, and FIR	+LC
Hsueh et al, 2022 ¹⁵	Prospective	3 C	110 LC	3/107	NP	-	-	-	-	La	FUS, PE, and ST	LC > CT
	Controlled		61 LC	2/59							FUS and LC recurrence (r)	LCr > LC
Dong et al, 2021 ¹⁶	Prospective	3 C	35 VCP									
	Controlled		19 LC	1/18	NP	-	-	-	-	La	Bacterial richness	NAT > LC
Hayes et al, 2018 ¹⁷	Prospective	3 C	21 VCP	6/15	NP							
	Controlled		58 LC	NP	63-71					Or	ACB, BPR, and BAC Microbiome β-diversity	CT > LC CT = LC
Gong et al, 2017 ¹⁸	Prospective	3 C	40 LC	6/62	57.1	-	-	-	-	La	Species richness and evenness BDT, FIR, and ACB	LC = VCP
	Controlled		28 VCP									
Gong et al, 2017 ¹⁹	Prospective	3 C	29 LC	2/29	NP	-	-	-	-	La	BDT, FUS	LC > CT
	Controlled		28 VCP	4/28	NP						FIR	CT > LC
Shin et al, 2017 ²⁰	Prospective	3 C	19 LHC	0/19	48-83	-	-	-	-	La	Bacterial diversity (α-β) FIR, ACB	CT > LC CT > LC
	Controlled											
Riva et al, 2015 ²¹	Unprospective	4	25 LC	1/24	68.8	0	25	NP	10	Tr	BDT, FUS, PRB, SPI, and TEN Association of clinical findings, age, tobacco, and allergies – MM	CT = LC P > 0.05

Abbreviations: ACB, actinobacteria; ACT, actinomycetota; BAC, bacillota; BDT, bacteroidetes; BPR, betaproteobacteria; CT, controls; FIR, firmicutes; FUS, fusobacteriota; GE, gemella; La, larynx; LC, laryngeal cancers; LHC, laryngeal-hypopharyngeal cancers; MM, microbiome material; NAT, normal adjacent tissue; PE, prevotella; PRB, proteobacteria; PS, pseudomonas; PSE, pseudomonadota; ST, streptococcus dysgalactiae; SPI, spirochaetes; TEN, tenericutes; VCP, vocal cord polyp; VP, Veillonella parvula.

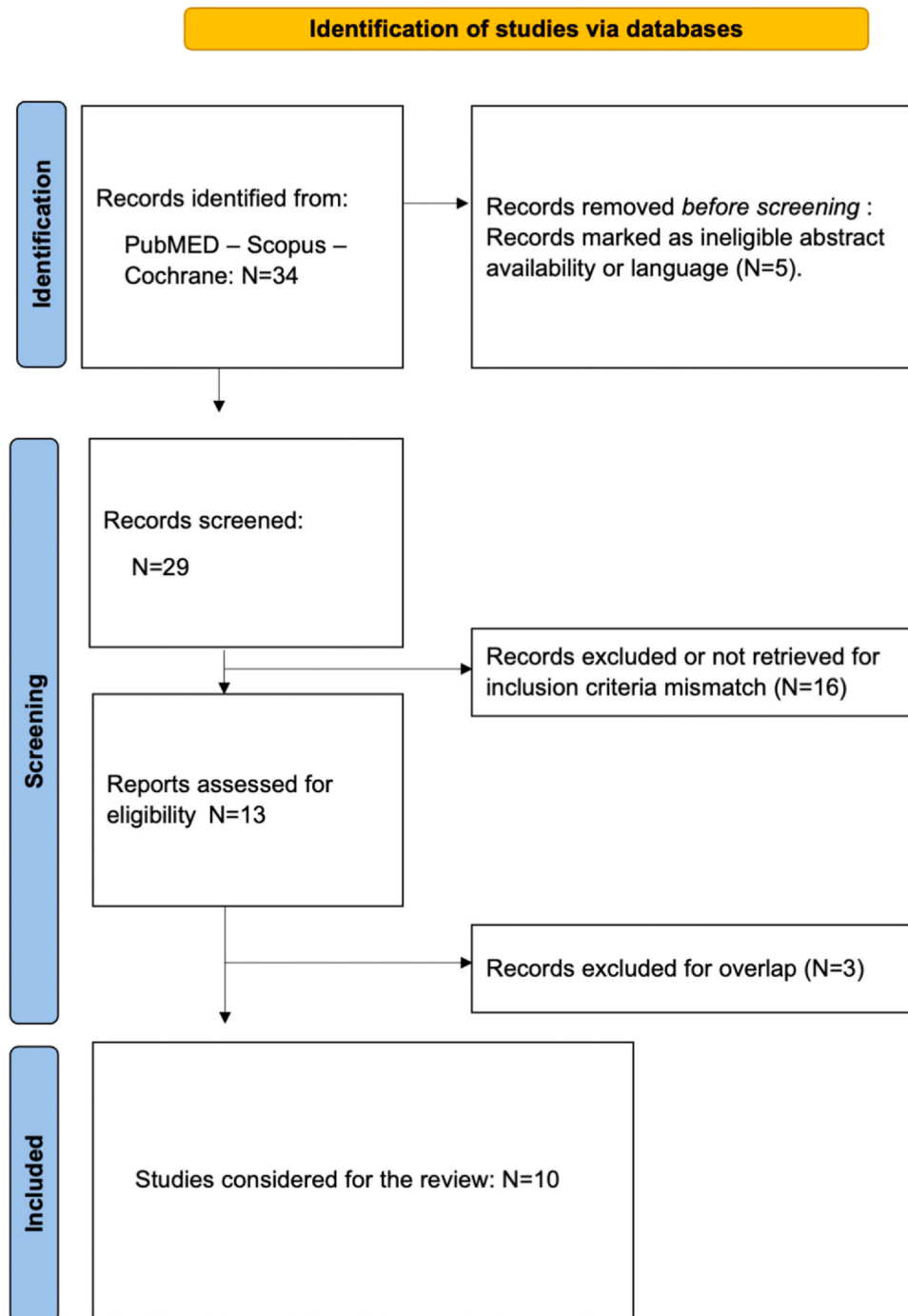


FIGURE 1. PRISMA flowchart.

Microbiome outcomes

The most important phyla outcomes are reported in Table 1. The differences in bacterial population between patients with LSCC and controls are shown in Table 3. Assessment of laryngeal microbial diversity revealed a statistically significant reduction in LSCC tissue samples compared with control specimens (eg, normal adjacent tissue, healthy donor tissue, and vocal cord polyp tissue samples) in two studies,^{16,20} while Hayes et al did not find significant differences in oral microbiome β -diversity between LSCC and control individuals.¹⁷

Bacteroidetes,^{12,19} Bacillota,¹² and Fusobacteriota^{16,18} phyla have been identified as predominant in LSCC compared with controls. Proteobacteria,¹⁷ Firmicutes,^{12,16,18,20} and Actinobacteria^{16,17,20} were predominant in control tissues compared with LSCC specimens (Table 3). Phylum-level taxonomic analysis identified Firmicutes as exhibiting the most substantial compositional disparity, with relative abundance ranges of 30.9%-63.6% in LSCC specimens versus 13.9%-32% in control tissues.^{12,16,18,20} Dorobisz et al determined a cutoff for diagnosing LSCC with bacterial population considering Firmicutes $\leq 22.1\%$, Bacillota

TABLE 2.
Oncological Outcomes

References	N	T Stage			N Stage							Anatomical Location			
		cT1-is	cT2	cT3	cT4	N0	N1	N2a	N2b	N2c	N3a	M+	Subglottic	Glottic	Supraglottic
Dorobisz et al ¹²	44	11	17	13	3	25	5	13	0	0	1	0	0	33	11
Gong et al ¹⁹	31	12		19		NP						0	0	20	11
Dong et al ¹⁶	19	NP	NP	NP	NP	NP	NP	NP	NP	NP	NP	0		19	0
Riva et al ²¹	25	0	3	16	6	16	4	5			0	0	1	17	7
Shin et al ²⁰	19	0	3	10	6	5	1	13			0		NP	NP	NP
Hsueh et al ¹⁵	110	12	40	43	15	79	9	21			1	0	0	71	39
	61	19		42		38	23					0	34		27
Total number	309	54	63	143	30	163	42	52	0	0	2	0	35	160	95

Abbreviations: NP, not provided.

≥1.7%, and Bacteroidetes ≥24.7% as a biomarker of LSCC.¹² Hsueh et al reported that Fusobacteriota (*Fusobacterium nucleatum*) impairs DNA mismatch repair and stability in patients with squamous cell carcinoma of the head and neck, and the abundance of *Fusobacterium nucleatum* was associated with a higher risk of LSCC recurrence.¹⁵

Among bacterial genera and fungi, the taxonomic profiling of at least two studies revealed an abundance of *Actinomyces*,^{18,20} *Candidatus Saccharimonas aalborgensis*,^{16,18} *Fusobacterium*,^{13,15} *Prevotella*,^{12,15} and *Stomatobaculum longum*^{12,18} in LSCC tissues compared with controls; while the following species were more abundant in controls: *Abiotrophia* defective,^{17,18} *Corynebacterium*,^{16,17} *Gemella*,^{12,17,18} *Rothia micilaginosa*,^{12,13,15,20} and *Streptococcus*.^{12,13,15,18,20} Supplementary taxonomic differences between LSCC and control specimens were reported in single studies; complete microbiota compositional data are summarized in Table 3.

Epidemiological analysis

The mean MINORS was 10.8 ± 3.8 , indicating substantial methodological limitations among included studies (Table 4). None of the studies considered the inclusion of consecutive patients with LSCC. The prospective data collection and unbiased endpoint assessment scores were heterogeneous across studies. Significant methodological limitations were identified for confounding factors: several studies lacked documentation of alcohol and tobacco exposure,^{14,20} while others, despite recording these variables, did not analyze their potential impact on microbiome composition.^{12,13,15–19,21} Additionally, antibiotic exposure was not adequately controlled for in one investigation.¹² The consideration of patients with and without a history of head and neck radiation was an additional potential confounding factor.²¹ The missing information related to the LSCC stage in some studies^{13,14,16–18} is an additional bias, limiting the finding interpretation. None of the studies reported study size calculation (Table 4). The selection of controls is an additional limitation of most studies with the consideration of vocal cord polyp^{13,15,16,18,19} or adjacent tumor tissue¹⁶ as controls. Finally, some studies reported

heterogeneity in the methods used for DNA extraction, amplification, quantification, and sequencing (Table 5).

DISCUSSION

The development and accessibility of metagenomic shotgun sequencing, which fragments environmental DNA, has enabled phylogenetic analysis and microbiota dynamics characterization through functional gene and pathway assessment in otolaryngological and head and neck surgical contexts.

The present systematic review identified specific phyla/bacteria significantly associated with the development/progression of LSCC. Precisely, the LSCC tissues demonstrated lower bacterial diversity compared with controls, and different population patterns. Phylum-level taxonomic analysis suggested an overrepresentation of Bacteroidetes (ie, *Prevotella*) and Fusobacteriota (ie, *Fusobacterium*) in LSCC, while Firmicutes (ie, *Stomatobaculum longum*, *Abiotrophia*, *Gemella*, and *Streptococcus*) and Actinobacteria (ie, *Actinomyces*, *Corynebacterium*, and *Rothia mucilaginosa*) were predominant in control tissues. Recent investigations of colorectal and head and neck squamous cell carcinoma tissues revealed a negative association between *Fusobacterium* abundance and DNA mismatch repair (MMR) pathway expression.^{15,25} MMR, a highly conserved cellular mechanism, identifies and corrects base-pair mismatches and insertion/deletion loops during DNA replication and recombination, suggesting a potential mechanism linking bacterial dysbiosis to carcinogenesis. In this review, Firmicutes demonstrated the largest compositional variation across studies, with 30.9%–63.6% abundance in LSCC compared with 13.9%–32% in controls.^{12,16,18,20} The low abundance of Firmicutes was similarly identified in oral, pharyngeal, and esophageal malignancies,^{26,27} supporting a potential transversal role of this phylum in the development of head and neck malignancies.

Current microbiome research primarily consists of prospective controlled and cross-sectional studies of patients with established cancer diagnoses and existing dysbiosis.

TABLE 3.
Species

Species	Dorobisz et al ¹² (%)			Gong et al ¹⁸ (%)			Dong et al ¹⁶ (%)			Hayes et al ¹⁷ (TC median)			Riva et al ²¹ (%)			Shin et al ²⁰ (+/++)			Hsueh et al ¹⁵			Yu et al ¹³ (%)		
	LC	CT		LC	VCP		LC	NAT	VCP	LC	CT		LC	NAT		LC	NAT		LC	NAT		LC	VCP	
<i>Phyla</i>																								
Bacteroidetes	33.2	10.0		21.2	10.1		27.6	31.8	27.5	-	-		+	+		+	+		-	-		-	-	
Bacteroidota	3.2	3.8		-	-		4.6	6.2	5.2	-	-		+	+		+	+		-	-		-	-	
Fusobacteriota	2.5	1.3		24.5	7.6		8.9	3.9	3.8	-	-		+	+		+	+		-	-		-	-	
Proteobacteria	15.4	19.4		-	-		28.5	27.5	24.1	19.5	81.3		+	+		+	+		-	-		-	-	
Firmicutes	13.9	35.9		32.0	63.6		19.0	21.8	30.9	-	-		+	+++		+	+++		-	-		-	-	
Bacillota	2.2	0.4		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Actinomycetota	7.1	9.6		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Pseudomonadota	0.7	1.5		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Actinobacteria	6.7	5.4		-	-		4.7	6.9	6.5	0.0	3.62		+	+++		+	+++		-	-		-	-	
Spirochaetes	-	-		-	-		-	-	-	-	-		+	+		+	+		-	-		-	-	
Other phylum	0.7	1.4		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
<i>Bacterial Genera and Fungi</i>																								
Abiotrophia defectiva	0.0	0.0		24.5	65.4		2.3	2.3	12.4	21.1	48.8		-	-		-	-		-	-		-	-	
Actinobacteria	1.9	1.5		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Actinomycetes	3.7	3.0		17.9	8.4		3.7	5.0	4.0	-	-		+++	+		+	+		-	-		5.5	0.3	
Aggregatibacter	0.0	0.1		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Atopobium	0.0	0.0		0.9	0.04		-	-	-	-	-		-	-		-	-		-	-		-	-	
Bifidobacteriaceae	0.0	0.0		3.4	1.9		-	-	-	-	-		-	-		-	-		-	-		-	-	
Campylobacter	1.8	1.1		1.0	2.6		-	-	-	-	-		+	+		+	+		-	-		-	-	
Candidatus Sacharimonas aalborgensis	0.0	0.0		25.8	6.9		7.8	3.1	3.0	-	-		-	-		-	-		-	-		-	-	
Capnocytophaga granulosa/gingivalis	0.3	0.8		-	-		-	-	-	-	-		+	+		+	+		-	-		-	-	
Cloacibacterium	0.0	0.0		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Clostridiales	0.5	0.0		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Corynebacterium	0.0	0.1		-	-		0.6	1.1	1.2	1.3	9.5		-	-		-	-		-	-		-	-	
Dialister	-	-		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Eikenella corrodens	0.0	0.0		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Flavobacterium	-	-		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Fusobacterium	1.3	0.8		-	-		-	-	-	-	-		-	-		-	-		-	-		0.3	0.0	
Gemella	0.4	1.7		1.6	0.3		-	-	-	9.4	20.8		-	-		+	+		14.0	< 14.0		8.8	2.2	
Granulicatella elegans	0.0	0.0		0.9	0.2		-	-	-	-	-		-	-		-	-		-	-		-	-	
Granulicatella	0.0	0.0		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Haemophilus	4.0	6.6		-	-		-	-	-	-	-		-	-		-	-		+++	+++		-	-	
Helicobacter	-	-		-	-		-	-	-	-	-		+++	+		+	+		-	-		-	-	
Kingella	0.0	0.0		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Klebsiella	-	-		-	-		-	-	-	-	-		-	-		-	-		-	-		0.2	0.0	
Lactobacillales	2.7	2.6		-	-		-	-	-	-	-		+	+		+	+		-	-		0.2	1.0	
Lautropia	0.0	0.0		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	
Leptotricha	0.7	0.2		-	-		-	-	-	-	-		-	-		-	-		-	-		-	-	

TABLE 3 (Continued)

	Dorobisz et al ¹² (%)			Gong et al ¹⁸ (%)			Dong et al ¹⁶ (%)			Hayes et al ¹⁷ (TC median)		Riva et al ²¹ (%)		Shin et al ²⁰ (+/++)		Hsueh et al ¹⁵		Yu et al ¹³ (%)	
	LC	CT		LC	VCP		LC	NAT	VCP	LC	CT	LC	CT	LC	NAT	LC	NAT	LC	VCP
Megasphaera	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mogibacterium	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Mycoplasma	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	0.2	0.0
Neisseria	2.0	4.4	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Parvimonas	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+++	-	-	-	-
Pasteurellaceae	0.2	0.5	-	-	-	-	-	-	-	-	-	-	-	+	+++	-	-	-	-
Peptoniphilus	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+++	-	-	-	-
Peptostreptococcus	-	-	-	0.03	0.004	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Porphyromonas	2.1	1.5	-	-	-	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Prevotella/alloprevotella	15.7	5.0	-	-	-	-	-	-	-	-	-	-	-	+	+	15.6	<15.6	-	-
Prevotella melaninogenica	13.2	5.5	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Pseudomonas	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	-	8.3	5.5
Ralstonia	-	-	-	-	-	-	-	-	-	-	-	-	-	+	+++	+	+	1.6	23.3
Rothia micilaginosa	3.2	9.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-	+++	0.3	1.5
Selenomonas	-	-	-	0.5	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Shaalii odontolytica	1.1	0.0	-	1.5	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Solobacterium	-	-	-	0.8	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Stomatobaculum longum	0.3	0.0	-	1.1	0.2	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Streptococcus	7.4	29.6	-	0.1	0.1	-	-	-	-	-	-	-	-	+	+++	10.9	<10.9	2.4	3.9
Tannerella	0.0	0.0	-	1.4	0.1	-	-	-	-	-	-	-	-	-	-	-	-	-	-
Treponema	0.0	0.0	-	0.3	0.1	-	-	-	-	-	-	-	-	+	+	-	-	-	-
Veillonella	0.0	0.0	-	4.3	0.4	-	-	-	-	-	-	-	-	+++	+	-	-	-	-

Abbreviations: CT, controls; LC, laryngeal carcinoma; NAT, normal adjacent tumor tissue; TC media, transformed count median; VCP, vocal cord polyps.

TABLE 4.
Bias Analysis

References	Clearly Stated		Inclusion of Consecutive Data		Prospective Endpoints Appropriate to Study		Unbiased Assessment		Follow-Up Adequate		<5% of Lost to		Study Size Prospective		Adequate Control		Contemporary Groups		Baseline Equivalence		Adequate Analyses		Total MINORS	
	Aim	Patients	Collection	to Study	Assessment	P	eriod	Follow-Up	Adequate	Calculation	Group	Groups	Equivalence	Analyses	Stat	Score								
Dorobisz et al, 2024 ¹²	2	0	2	2	2	-	-	-	-	0	1	1	1	2	-	13								
Dhakal et al, 2022 ¹⁴	2	0	0	2	1	-	-	-	-	0	-	-	-	-	-	5								
Gong et al, 2017 ¹⁸	2	0	2	2	2	-	-	-	-	0	0	0	-	2	2	10								
Gong et al, 2017 ¹⁹	2	0	2	2	2	-	-	-	-	0	0	0	-	2	2	10								
Dong et al, 2021 ¹⁶	2	0	2	2	1	-	-	-	-	0	0	0	-	2	2	9								
Hayes et al, 2018 ¹⁷	2	1	2	2	2	2	2	-	2	0	2	2	2	2	2	19								
Riva et al, 2015 ²¹	2	0	2	2	1	-	2	-	-	0	-	-	-	-	-	9								
Shin et al, 2017 ²⁰	2	0	1	2	1	-	-	-	-	0	0	0	-	2	2	8								
Hsueh et al, 2022 ¹⁵	2	0	1	2	2	2	2	-	-	0	0	0	-	2	2	11								
Yu et al, 2023 ¹³	2	0	1	2	2	2	2	-	-	0	1	1	1	2	2	14								

TABLE 5.
Microbiome Analysis Methods

Reference	DNA Extraction Kit/Method	PCR Amplification Region	Quantification Method	Sequencing Platform	Additional Technical Notes
Gong et al, 2017 ¹⁸	QIAamp DNA Mini Kit + enzymatic/bead-beating	V1-V3 (27F/534R)	Not specified	454 FLX Titanium	Negative controls used
Gong et al, 2017 ¹⁹	QIAamp DNA Mini Kit + enzymatic/bead-beating	V1-V2 (347F/803R)	Not specified	454 FLX Titanium	Used PowerSoil DNA Isolation Kit
Hayes et al, 2018 ¹⁷	PowerSoil DNA Isolation Kit	V3-V4	DESeq2	454 FLX Titanium	Used mouthwash samples
Yu et al, 2023 ¹³	QIAamp Fast DNA Stool Mini Kit	V3-V4	NEB Next® Ultra™ DNA Library Prep	Illumina NovaSeq	Paired-end reads
Shin et al, 2017 ²⁰	Qiagen RNeasy Mini Kit	V4 (51/806)	Not specified	Ion Torrent PGM	Used QC specimens
Riva et al, 2015 ²¹	Culture-based method	Not applicable	Not applicable	Not applicable	Multiple culture media
Dorobisz et al, 2024 ¹²	GeneMATRIX Swab-Extract DNA Kit	V3-V4	Qubit + DeNovix spectrophotometer	Illumina MiSeq	300-bp paired-end reads
Dhakal et al, 2022 ¹⁴	TCGA data reanalysis	Not applicable	Not applicable	Not applicable	Used exotic pipeline
Dong et al, 2021 ¹⁶	QIAGEN QIAamp DNA Mini Kit	V3-V4	Not specified	Illumina MiSeq	Used tissue samples
Hsueh et al, 2022 ¹⁵	QIAamp DNA FFPE Tissue Kit	V3-V4	Not specified	Illumina HiSeq 2500	Fluorescence-based PCR (MSI analysis)

Thus, the temporal relationship between microbial dysbiosis and LSCC development is still unresolved. The contributing factors of LSCC, including tobacco, alcohol (supraglottic carcinoma), and laryngopharyngeal reflux disease, may be currently considered as the primary etiological factors of dysbiosis. In oral squamous cell carcinoma, alcohol and poor oral health status combining to induce chronic inflammation have been associated with the development of dysbiosis and an increased acetaldehyde level, leading to a tumor-promoting environment.²⁸ In oropharyngeal and LSCC, the tobacco consumption had a significant influence on the global community structure, specifically at lower taxonomic levels.²⁹ In the same vein, preliminary evidence suggested that laryngopharyngeal reflux disease patients exhibit distinct laryngopharyngeal and oral microbiota profiles compared with healthy controls,^{30,31} while human papilloma virus (HPV)-microbiome interactions potentially modulate local immune responses through complex mechanisms.³² Despite increased evidence supporting their significance, the key UAM-influencing factors—including HPV status, laryngopharyngeal reflux disease, nutritional status, and alcohol and tobacco consumptions—were insufficiently considered in the analyzed studies, potentially confounding the observed differences between LSCC and control UAM profiles.

Despite the anatomical continuity of the upper aerodigestive tract mucosa, distinct bacterial diversity patterns and community compositions have been observed across different anatomical subsites. Thus, the heterogeneity in the microbiome samples (eg, saliva, tracheal, pharyngeal, and laryngeal secretions) is the primary limitation of the present review. The variability in UAM sequencing methods potentially biases study comparisons, consisting of another limitation of this review.

Importantly, the present review reports a male:female ratio of greater than 50:1, which does not represent the common ratio in LSCC that is 4-7 times greater in males compared with females. Because inflammatory and oncological processes can be influenced by gender,³³ this low representation of females can limit the generalizability of the microbiome findings.

Finally, laryngeal specimens of patients with vocal cord polyps^{13,15,16,18,19} or adjacent tumor tissue¹⁶ were considered as controls in many studies. Vocal cord polyps are benign lesions of the vocal folds that primarily develop in patients with underlying disorders compromising vocal fold mucosal integrity, such as laryngopharyngeal reflux disease.³⁴ Consequently, considering vocal cord polyp specimens as controls may introduce systematic bias in group comparisons, as their associated UAM cannot be represent the UAM of healthy vocal fold mucosa.

CONCLUSION

The current literature supports potential distinct UAM signatures between LSCC and noncancerous tissues, with Bacteroidetes and Fusobacteriota enriched in LSCC

tissues. To date, data investigating the prognostic value of UAM in carcinoma oncological outcomes are lacking. Although emerging evidence supporting the key role of UAM in the development of LSCC, substantial methodological heterogeneity across studies necessitates standardized protocols for future investigations.

CRedit Authorship Contribution Statement

Jerome R. Lechien: Patients were recruited from the Reflux Consultation of the author of the paper. Contributions: Design, acquisition of data, data analysis and interpretation, drafting, final approval, and accountability for the work; final approval of the version to be published; agreement to be accountable for all aspects of the work in ensuring that questions related to the accuracy or integrity of any part of the work are appropriately investigated and resolved.

Declaration of Competing Interest

The author has no conflicts of interest.

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