BMC Oral Health

SYSTEMATIC REVIEW

Open Access

Comparative analysis of bacterial abundance and diversity in tumour tissue of oral squamous cell carcinoma and non-tumour tissue: insights from a systematic review of 16S ribosomal RNA sequencing

Swagatika Mohapatra[1], Swagatika Panda[1*], Neeta Mohanty[1] and Bibhu Prasad Mishra[2]

Abstract

Background As per the recent research findings, there is a significant difference between the bacteriome of normal tissue (NT) and tumor tissues (TT) of oral squamous cell carcinoma (OSCC). Identifying this distinct bacteriome is crucial for understanding their potential contribution to oral carcinogenesis. This systematic review (SR) aims to identify exclusive and relative bacterial abundance and bacterial diversity in TT and NT.

Methodology The review was conducted following the PRISMA guidelines. PUBMED and SCOPUS databases were searched for studies in English published till 31st August 2024. The inclusion criteria focused on identifying bacteriome in NT versus TT at either species,/genus, and/or phylum level through 16 s ribosomal RNA sequencing. Quality assessment was performed using an eleven-parameter tool combining the Newcastle–Ottawa Scale and customized criteria.

Result Evaluating the selected 13 articles, we have identified the exclusive and relative abundance of bacteriome in TT and NT at phylum, genus, and species levels. Three phyla such as Chloroflexota, Deinococcus-Thermus, and Mycoplasmatota, are found exclusively in TT. Seven genus such as *Eubacterium, Campylobacter, Aeromonas, Oceanivigra, Rheinheimera, Weissella,* and *Catonella* are exclusively found in TT. Ten species such as *Micrococcus luteus, Prevotella melaninogenica, Exiguobacterium oxidotolerans, Fusobacterium naviforme, Staphylococcus aureus, Veillonella parvula, Parvimonas sp oral taxon 110, Eubacterium ll G1 infirmum, Eubacterium XI G3 Brachy, Weissella viridescens* are found in TT. Six genus such as *Capnocytophaga, Selenomonas, Leptothrix, Desulfovibrio, Desulfoplanes, Pelospora* are found exclusively in NT. Eleven species, such as *Streptococcus sp. Oral taxon 071,Selenomonas sputigena, Treponema pedis, Acholeplasmatales bacterium, Capnocytophaga haemolytica, Eubacterium sp., Syntrophomonadaceae genomosp.,Treponema putidum, Mitsuokella sp., Actinomyces sp. Oral taxon 848 str. F0332, p- 2534 - 18B5-gut-group* are found in NT. Seven common genera within which different species are identified in TT and NT, suggesting differences in bacterial behaviour and characteristics within the same genus. A total of 12 phyla, 35 genera, and 54 species were found to be relatively more abundant in TT compared to NT. Conversely, 7 phyla, 32 genera, and 45 species were relatively more abundant in NT than in TT. Considerable variations in diversity metrics were found between TT and NT.

*Correspondence:
Swagatika Panda
swagatikapanda@soa.ac.in
Full list of author information is available at the end of the article

Conclusion This systematic review is the first to identify a distinct bacteriome exclusive to OSCC tumour tissue compared to normal tissue using 16S ribosomal RNA sequencing. This pioneering work lays the foundation for future studies on the oral microbiome as a potential diagnostic or therapeutic target in oral cancer management. It emphasizes the importance of exploring species-level differences for a deeper understanding of their roles in OSCC.

Trial registration Not applicable.

Keywords Bacteria, 16 s ribosomal RNA sequencing, Bacterial abundance, Diversity, Oral squamous cell carcinoma

Background

Oral cancer ranks as the sixth most prevalent cancer globally, with 3,77,713 new cases and 177,757 deaths reported in 2020 [1–3]. This marks an increase in new cases compared to 2018, which saw 354,864 cases and 177,384 deaths [4]. More than 90% of oral cancers are oral squamous cell carcinoma (OSCC). Traditionally, OSCC has been most prevalent among older individuals, typically affecting those in their sixth to seventh decade of life. In recent years, this trend has shifted, with increasing incidence observed in younger age groups. The pathogenesis of OSCC is complex, and its multifactorial nature makes it challenging to fully comprehend. Tobacco use and/or alcohol consumption have long been recognized as significant clinical risk factors [5]. In recent years, there is a progressive increase in OSCC among patients without any habit of tobacco and/or alcohol [6]. Yet, there has been a notable rise in OSCC among patients without any history of tobacco or alcohol use. This diversion has led to exploring additional risk factors in oral carcinogenesis, with the microbiome emerging as a potential contributor. While the role of viruses and Candida [5, 7, 8] in oral carcinogenesis is well established, the involvement of bacteria in oral carcinogenesis remains a topic of ongoing debate. Drawing parallels to other cancers, bacterial species like *Chlamydia trachomatis*, *Streptococcus bovis*, and *Helicobacter pylori, Salmonella typhi* have been implicated in malignancies such as cervical carcinoma, colon cancer, gastric adenocarcinoma, and gall bladder cancer respectively [9–12]. The oral microbiome is one of the most diverse in the human body, containing over 700 bacterial species from various niches like the tongue, buccal mucosa, and saliva [13]. In 2007, the National Institutes of Health launched the Human Microbiome Project to investigate the microbiome's role in health and disease [14]. Since then, numerous studies have profiled the bacteriomes of oral cancer, uncovering patterns of bacterial dysbiosis, which may be linked to carcinogenesis [15]. Both pathogenic and commensal bacteria can induce genetic changes in epithelial cells, which may drive oral carcinogenesis [16]. Proposed mechanisms to promote bacteria-mediated oral carcinogenesis include chronic inflammation, disruption of cell cycles, and the production of carcinogenic substances like acetaldehyde [16].The conclusiveness of these studies requires a clear consensus on differences in the abundance and diversity of oral bacteriome between OSCC patients and healthy individuals, which is inadequately addressed. Although multiple studies have demonstrated that there exist differences in the bacterial abundance and diversity between OSCC patients and healthy patients, the results are inconclusive owing to the differences in sites and subsites of bacteria isolation, and techniques of bacterial identification. In fact, there are four systematic reviews [17–20]that have previously addressed this research question. However, these reviews have grouped all bacteriome sources such as saliva, tissue, tissue scrapings together, despite clear differences in the bacteriome across different oral subsites. Additionally, Gopinath et al. [18] demonstrated the bacteriome data from pharyngeal and oropharyngeal tumours, which may be different from the bacteriome of tumour tissue of OSCC. Although metagenomic sequencing and 16S ribosomal RNA sequencing are the most advanced bacterial identification technologies, previous reviews have evaluated the oral bacteriome irrespective of any specific technology [17–20]. Given these inadequacies, we focused our analysis on the bacteriome of tumour tissue in OSCC, comparing it exclusively to non-tumour tissue through 16S ribosomal RNA sequencing alone, we now can explore the bacteriomes associated with oral cancer more precisely. Therefore, we included studies that used exclusively 16S ribosomal RNA sequencing for bacteriome identification, which would ensure accuracy and comparability. By concentrating solely on the bacteria present in tumour tissue, and avoiding the potential confounding introduced by combining bacteriomes of all subsites of oral cavity, we aim to offer a better understanding of the differences in the bacterial abundance and bacterial diversity between OSCC tissues and non-tumour or healthy tissues. This approach will help provide better clarity on bacterial interactions specific to OSCC tissues.

Methodology

Protocol and guidelines

This systematic review was conducted in accordance with the Preferred Reporting Items for Systematic Reviews

and Meta-Analyses (PRISMA) [21] guidelines to ensure transparency and reproducibility. The review protocol was registered with the International Prospective Register of Systematic Reviews (PROSPERO) under registration number CRD42023458745.

Research question and PECO

The research question of this review was to evaluate whether there are any differences in bacterial abundance and diversity between OSCC tumour tissues (TT) with that in non-tumour adjacent healthy tissue of the same patient or healthy tissue from another patient (NT). This systematic review focuses on observational cross-sectional studies comparing the bacteriome in TT (Population) with that in NT (Comparison) through 16S ribosomal RNA sequencing (Exposure) to evaluate the differences in bacterial abundances and diversity (Outcome) in both groups. Outcomes were expressed in bacterial abundances and/or bacterial diversity. Bacterial abundances were expressed in metrics such as prevalent species and/or genus and/or phylum in both group of tissues. Bacterial diversity was expressed in indices of alpha and beta diversity such as Chao1, observed richness, Good's Coverage, Simpson, Shannon, Bray–Curtis and Jaccard index.

Search strategy

The search strategy was developed based on the PECO criteria. Searches were conducted across PUBMED and SCOPUS databases using both keywords and MeSH terms. Additionally, bibliographies of previous systematic reviews, and grey literature databases like OpenGrey, were reviewed to identify relevant studies. The search strategy was built around key concepts of the research question. The search was limited to English-language publications up to August 31, 2024.

PubMed Search Strategy: (("Oral Squamous Cell Carcinoma"OR"Oral Cancer")) AND ("Normal tissue"OR "Paracancerous"OR"Healthy"OR"Adjacent")) AND ("Microbiome"OR"Bacteriome"OR"Bacteria"OR"Microorganism"OR"Microbe")).

Scopus Search Strategy: TITLE-ABS-KEY ('oral AND cancer' OR 'oral AND squamous AND cell AND carcinoma') AND TITLE-ABS ('microbe' OR 'bacteria' OR 'microorganism') AND TITLE-ABS ('normal' OR 'healthy' OR 'precancerous').

Selection criteria

This review included observational human studies that compared the bacteriome in OSCC tumour tissues with non-tumour healthy oral tissues of same or different patients by 16S ribosomal RNA sequencing. Studies were excluded if those compared bacteriomes between tumour tissue and potentially malignant disorder tissues [22], or tumour tissues subjected to radiotherapy [23], or identified the bacteriome from oral rinse specimens, swabs or saliva specimens [24], or bacteriome derived from epithelial cells collected via brush biopsy [25]. Additionally, studies that identified bacteria solely through immunohistochemical analysis were also excluded [26]. One article [27] was excluded because the bacteriome was identified from a secondary database..

Quality assessment

We have evaluated the quality of each of the included study based upon three criteria of New-Castle Ottawa scale (NOS) for case control study [28] and 8 customized criteria tailored to the specific needs of bacteriome research. The customised criteria included well-defined research question, potential contaminants during sample collection, region of 16 s ribosome gene targeted, sequencing depth, clearly outlined bioinformatics software used to minimize sequencing errors, taxonomic database assignment accuracy, histopathologic evaluation of tumour tissue, and tissue site heterogeneity. The NOS criteria assessed three broad domains such as selection of TT and NT, comparability of TT and NT and outcome. The maximum score in NOS was 9 stars which was equivalent to 9 points. Additionally, with each customised criterion scored as 0 or 1, with each criterion scored as 1 (Yes) if adequately addressed and 0 (No) if not, for a maximum of 8 points, the total possible quality score was 17, and we classified studies as high quality if they scored at least 12.

Data extraction

Data from the selected studies were systematically recorded using Microsoft Excel (2019 version). Extracted data included Author and publication year, sample types which included OSCC tumour tissues (TT) as cases and non-tumour tissues (NT) as controls. The control tissue sources were either para-cancerous tissue or contralateral mucosa from the same individual, or healthy tissue from different individuals. Other parameters were sample size, age, gender ratio, tumour site, clinical stage, grading, and habit history (e.g., tobacco or alcohol use), DNA extraction kits, PCR regions targeted, 16S ribosomal RNA sequencing techniques, bioinformatics software, sequencing depth, reference databases for taxonomic identification, species/genus/phylum level relative and exclusive bacterial abundances in TT and NT, bacterial diversity expressed as alpha diversity indices (Chao1, observed richness, Good's Coverage, Simpson, Shannon) and beta diversity indices (Bray–Curtis, Jaccard index). While assigning the bacteria reported in each of the included article to TT or NT, we considered only those

with an abundance difference of at least 10% between the groups or a statically significant difference ($p < 0.05$). These were then designated as relatively abundant. Similarly, bacterial taxa reported as present exclusively in one group were noted as exclusively abundant. If taxonomy at the species level was mentioned in the articles, the complete lineage of the taxa was assigned and reported based on the NCBI database [29].

Data analysis

Qualitative assessment was conducted on the included articles to evaluate the bacterial abundances and diversity in TT and NT. Given the significant heterogeneity in reporting of metrics of alpha and beta diversity among the studies, a meta-analysis was deemed inappropriate.

Results

Study selection

A total of 223 articles were initially identified across PubMed and Scopus. After removing duplicates and irrelevant studies 118 articles remained. A thorough review of titles and abstracts resulted in the exclusion of 47 articles. 5 articles could not be retrieved. After conducting a comprehensive literature search, 66 articles were assessed for eligibility, and 47 were excluded due to various reasons. After conducting an in-depth literature analysis, 19 articles were screened for full text reading. We have excluded six articles [22–27] due to many factors such as comparison of TT with pre-cancerous tissue, bacteriome of oral swab and cytology specimen, immunohistochemical expression of bacterial proteins, tumour tissue irradiated with radiotherapy and bacteriome identified from a secondary data base. Thirteen articles [28–40] met the inclusion criteria and were included in this systematic review. A detailed PRISMA flowchart illustrating the study selection process is provided in Fig. 1.

Study characteristics

Demographic and clinical features

The study populations varied geographically, encompassing China [30–34], England [35], Sri Lanka [36], Saudi Arabia [37], New York [38], Spain [39], Philadelphia [40], India [41], and the USA [42]. Sample sizes differed across studies, with the number of tumour tissues (TT) and normal tissues (NT) per study ranging from 10 to 65. In total, the 13 studies provided data on 374 TT and 341 NT samples. The age of patients in both TT and NT groups was presented differently across all studies. Three studies [35, 39, 40] reported the age as a mean, one study [38] used the median, and the remaining studies [30–34, 36, 37, 41, 42] expressed it as mean ± standard deviation. It ranged from 49.3 ± 13.24 to 63.0 ± 9.6 in TT and 50.67 ± 6.81 to 63.0 ± 9.6 in NT.

Ten studies [30, 32–35, 37–40, 42] have reported male to female proportion according to which there was higher prevalence of oral cancer in males compared to females. Additionally, those 10 studies have matched the male to female proportion between TT and NT groups. Subsites of oral mucosa were mentioned only in 8 studies [30–32, 36–38, 40, 42]. 10 studies [30–34, 36–38, 40, 42] have reported the smoking, alcohol and snuff habit in the OSCC patients. Staging and grading of TT in OSCC were reported in six [32–34, 39, 40, 42] and four [32, 33, 36, 40] of the thirteen studies. Four studies [33, 39, 40, 42] have reported higher number of patients with OSCC at stages III and IV while the remaining two studies [32, 34] observed fewer patients in advanced stages of OSCC compared to early stages. These data are tabulated in Table 1.

Sample collection

Sample collection of TT and NT varied among the included studies. Tumour tissues were collected from the tumour sites, with only one study [35], separately collecting superficial and deep tumour tissue samples. NT were obtained from para-cancerous sites [30–35, 38, 40, 42] or the healthy mucosa of different patients [37, 39, 41]. Perera et al. [36] compared the bacteriome in OSCC with that of fibroepithelial polyps from the same anatomical sites in different individuals. The distance between TT and NT collected from the para-cancerous mucosa was recorded in several studies [30–32, 34–36, 38, 42] ranging from 2 to 5 cm. All studies, except one [38] reported the size, mass, volume of the biopsied tissues. Five studies documented tissue volumes ranging from 5 mm^3 to 10 mm^3 [30, 31, 34, 35, 41]. Three studies [36, 37, 42] reported tissue weights between 25 to 100 mg. Only two studies [32, 33] mentioned the width of tissue specimens.

Whole metagenomic shotgun sequencing

All of the included studies, except pushalkar et al. [38] have used various commercial DNA extraction kits while Pushalkar et al. [38] employed a modified DNA extraction method. DNA amplification has targeted single or multiple hypervariable regions of the bacterial 16S ribosomal genes. While one study focused exclusively on the V4 [42], the remaining studies targeted multiple regions, includingV1–V3 [36, 37], V3–V4 [30–34, 39, 41], and V4-V5 [38]. Most of the included studies [32–34, 36, 37, 39, 41] employed the Illumina MiSeq system for sequencing, with one study using the Illumina PE250 [20], and another employing the Illumina HiSeq 2500 platform (Novogene, Beijing, China) [31]. Additional sequencing techniques included the ABI PRISM 3100 Genetic Analyzer [35], and the ABI

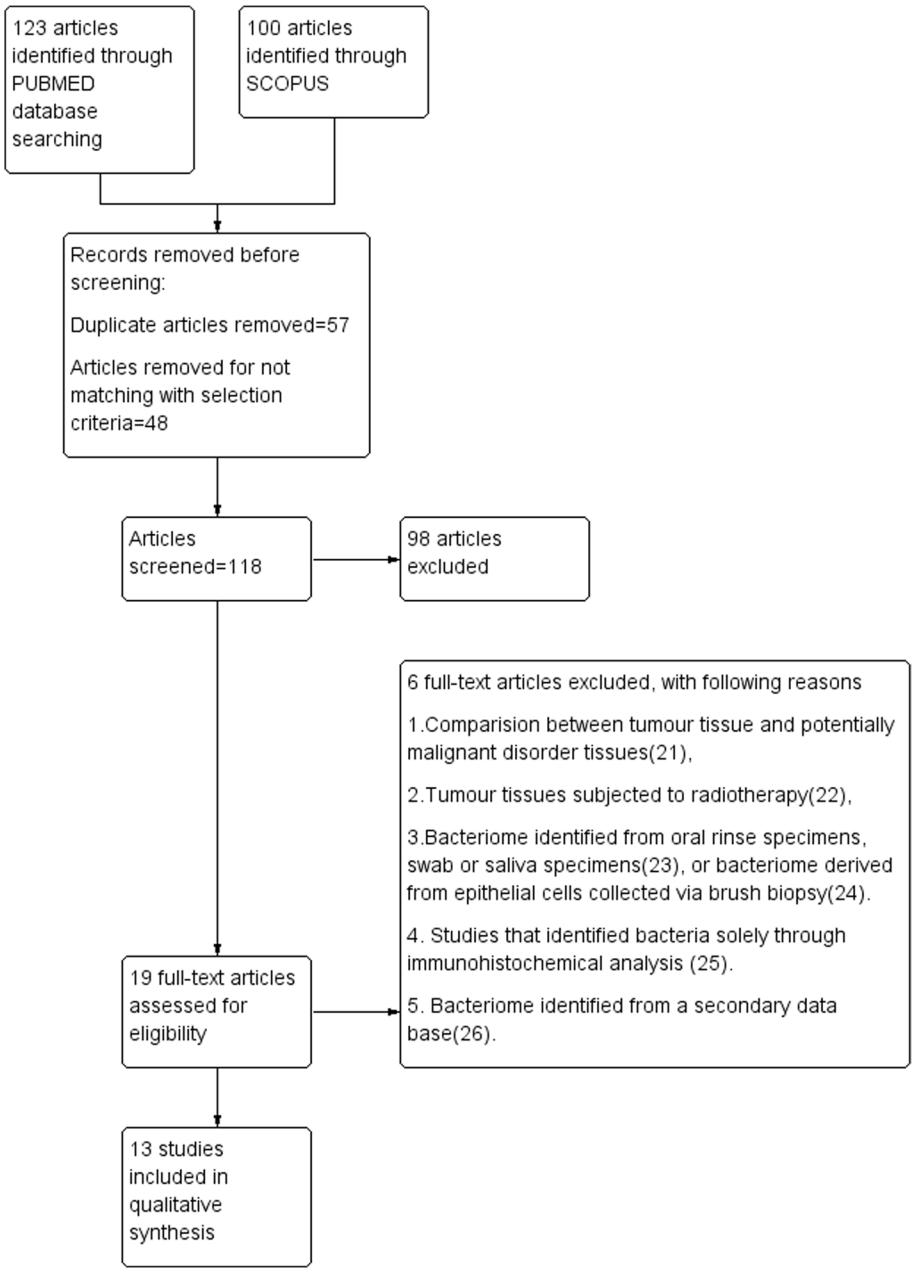

Fig. 1 PRISMA flow diagram

PRISM 3730xl [38] One study utilized NEB Next rRNA Depletion Kits E7850 & E7400 [40]. Notably, Mukherjee et al. [42] used QiiME sequencing platform. Several reference database were used for taxonomic classification which included GenBank [35],KEGG [30, 36],Silva [30, 34, 39, 40],RDP [31, 32, 38], HOMD [32, 37, 38], UNITE 5.8 s [42] for the taxonomic classifications. Chang et al. and Gopinath et al. [33, 41] did not specify a reference database, which may affect taxonomic comparability.

Bacterial abundance

The included studies reported bacteriome data across three taxonomic levels such as species, genus, and phylum. Nine articles [31–33, 35–39, 42] compared the bacteriome at the species level. There are only four articles [35, 37–39] which described exclusive abundance of TT and NT. A total of 10 and 11 exclusively abundant species were identified in TT and NT respectively. There were only 5 species such as *Atopobium parvulum, Parvimonas micra, Prevotella melaninogenica, Rothia mucilaginosa,*

Table 1 Clinical and Demographic characteristics of TT and NT

Author, Year (ref)	Sample size		Age (mean or median)		Male:Female		Habit (TT)	Staging (TT)	Grading (TT)	Size/weight/volume of tumour tissue		Distance between TT and NT
	TT	NT	TT	NT	TT	NT				TT	NT	
Hooper et al., 2006 [35]	20	12	66.9	66.9	4.01	4.01	NA	NA	NA	1 cm^3	1 cm^3	5 cm
Yuchao Li et al., 2020 [30]	10	15	61 + _9.49	60.87 + _7.04	5.05	7.08	Smoking, alcohol	NA	NA	1 cmX1 cm^2	1 cm^3	2 cm
Perera et al., 2018 [36]	25	27	61.00 ± 9.5	50.58 ± 13.5	NA	NA	Smoking, alcohol	NA	WDSCC = 11 MDSCC = 11	100 mg	100 mg	2 cm
Nie et al., 2022 [31]	65	65	57.9 + _9.6	58.1 + _9.7	NA	NA	Smoking, alcohol	NA	NA	5 mm^3	5 mm^3	2 cm
Al hebshi et al., 2017 [37]	20	20	53.6 + _10.4	52.3 + _8.9	1.01.00	1.01.00	Arabian snuff	NA	NA	25 mg	NA	NA
Yang et al., 2021 [32]	23	23	61.9 + _12.3	61.9 + _12.3	11.12	11.12	Smoking, alcohol	I-II 15 III-IV 8	WDSCC 6 MDSCC 14 PDSCC 3	> 3 mm	NA	2 cm
Pushalkar et al., 2012 [38]	10	10	59	59	53.47.00	53.47.00	smoking, alcohol	NA	NA	NA	NA	5 cm
Chang et al., 2019 [33]	61	30	57.4 + _10.4	55.4 + _10.2	39.22.00	18.12	Smoking, alcohol	I-II 28, III-IV 33	WDSCC = 34	4 µm	4 µm	NA
Herreros pomaros et al., 2023 [39]	10	11	70.31	70.31	48.52.00	48.52.00	NA	I-II 4, III-IV 6	NA	NA	NA	NA
Vinay Jain et al., 2023 [40]	20	20	61	63.5	15;5	15;5	Smoking, alcohol	I- 4, II- 5, III- 3, IV- 7	WDSCC- 3, MDSCC- 14, PDSCC- 1, NA- 2	NA	NA	NA
Divya gopinath et al., 2021 [41]	48	46	49.31 + _13.24	50.67 + _6.81	NA	NA	NA	NA	NA	0.5 cm^3	0.5 cm3	NA
Peng ye et al., 2021 [34]	23	23	63.0 ± 9.6	63.0 ± 9.6	18.05	18.05	Smoking, alcohol	T1-T2 = 13, T3-T4 = 10, N0 = 9, N1-N2 = 14, M0 = 23	NA	0.7 cmX0.7 cmX0.5 cm	0.7 cmX0.7 cmX0.5 cm	2 cm
Mukherjee et al., 2017 [42]	39	39	60.5 ± 13.2	60.5 ± 13.2	30.09.00	30.09.00	smoking, alcohol	I-II 7, III-IV 27, NA- 5	NA	30–50 mg	NA	2 cm

WDSCC Well Differentiated Oral Squamous Cell Carcinoma, *MDSCC* Moderately Differentiated Oral Squamous Cell Carcinoma, *PDSCC* Poorly Differentiated Oral Squamous Cell Carcinoma, *UN/ANA* Undifferentiated/Not Assessable, *NA* Data not available

Streptococcus salivarius, commonly found between TT and NT. 11 articles [30–34, 36–39, 41, 42] have compared the bacteriome at genus level. A total of seven and six genera were identified exclusively in TT and NT respectively. There were seven common genera such as *Pseudomonas, Eubacterium, Staphylococcus, Streptococcus, Veillonella, Prevotella,* and *Fusobacterium* in TT and NT. This systematic review identified several phyla associated with TT and NT, including *Proteobacteria, Actinobacteria, Firmicutes, Bacteroidetes, Spirochaetes, Fusobacteria, and Mycoplasmatota,* though only three phyla such as *Chloroflexii*, Deinococcus-Thermus, and Mycoplasmatota were identified exclusively in TT. Other than exclusive abundance, we have also observed the relative abundance of bacteria at species, genus and phylum level. A total of 54 species, 35 genera, and 12 phyla were found to be relatively more abundant in TT compared to NT. Conversely, 45 species, 32 genera, and 7 phyla were relatively more abundant in NT compared to TT. The recent nomenclature of bacterial phyla has updated the older names as follows: Bacteroidota has replaced *Bacteroidetes*, Bacillota has replaced *Firmicutes*, Actinomycetota has replaced *Actinobacteria*, Pseudomonadota has replaced *Proteobacteria*, Chloroflexota has replaced *Chloroflexi*, Fusobacteriota has replaced *Fusobacteria*, Spirochaetota has replaced *Spirochaetes*, and Mycoplasmatota has replaced *Tenericutes*. Bacterial abundance in TT and NT at three taxonomic levels such as phylum, genus and species were schematically depicted in supplementary Table 1 and Fig. 2. The bacteria which were found to be exclusively abundant in NT included a range of normal commensals typically found in the human body, particularly in the oral cavity or part of the normal oral microbiota in dental plaque.

Bacterial diversity

Apart from only three studies [33, 35, 40] rest all studies have evaluated the bacterial diversity either in the form of alpha or beta diversity. The studies included in this review used various indices to evaluate alpha diversity, including the Shannon, Simpson, and Chao indices, as well as observed richness and evenness indices. Yuchao Li et al. [30]observed that the Chao index, which estimates species richness, was significantly higher in TT. Al Habeshi et al. [37], Herreros-Pomares et al. [39], and Pushalkar et al. [38] found that the Chao index was higher in NT though Pushalkar et al.'s [38] finding not statistically significant. The Chao index ranged from 93.57 [38] to 284.18(166.95) [39] and 71.22 [38]to 637.94(306.85) [39] in TT and NT respectively. This wide variation in Chao index suggested potential differences in species richness patterns between TT and NT, possibly influenced by factors such as sample size and patient characteristics.

The Shannon index reflects both richness and evenness within a sample. Nie et al. [31], Peng Ye et al. [34], and Al Habeshi et al. [37] found that the Shannon index was higher in TT. In contrast, Gopinath et al. [41] and Perera et al. [36] found no significant difference in the Shannon index between TT and NT. Mukherjee et al. [42] reported a significant decrease in the Shannon index in TT. The range of Shannon index was found to range from 3.2 [38] to 4.033 ± 0.939 [37] and 3.37 [38] to 4.6 ± 1.04 [39] in TT and NT respectively. The broader range in NT compared to TT highlights greater variability in microbial diversity in NT. These conflicting findings suggest that microbial diversity patterns may be influenced by differences in patient characteristics, sample size, and sample collection methods across studies.

The Simpson index, another measure of diversity and evenness, showed varied results across studies. Nie et al. [31] and Peng Ye et al. [34] both reported higher Simpson indices in TT. On the other hand, Gopinath et al. [41]found no significant difference in the Simpson index between TT and NT, while Mukherjee et al. [42] observed a decrease in the index in TT. These discrepancies highlight the heterogeneous nature of microbial diversity patterns in TT, which may also be influenced by differences in sample size, sequencing methods, and patient characteristics across studies. On a similar note, ang et al. [32] and Pushalkar et al. [38] reported higher Simpson evenness index and evenness index in NT respectively, though this difference was not statistically significant. Higher ACE estimator of species richness in TT was only reported by Pushalkar et al. [38].

Beta diversity measures the difference in bacterial composition between TT and NT. This metric was reported only by five studies [30, 31, 34, 37, 39]. Al hebshi [37] and Peng et al. [34] expressed beta diversity as Jaccard indices and UniFrac index respectively and found differences between TT and NT but did not mention the differences. Yuchao Li et al. [30] expressed it in Bray–curtis indices and did not find any difference between, and Fujiao Nie [31] et al. expressed it in UniFrac index. Pomares et al. [39] too demonstrated the differences between the two groups by concluding that bacteriome of TT is more homogenous as compared to NT.

Quality assessment

Overall, the quality assessment showed that the majority of included studies scored at least 12/17, with seven studies [31, 34–36, 38, 41, 42]achieving 14 or higher, and one study [30], 2020 achieving a perfect score of 17/17. These findings suggested that most studies included in this review exhibit a high level of methodological rigor and reporting quality, enhancing the reliability of the overall results. Limitations, particularly regarding

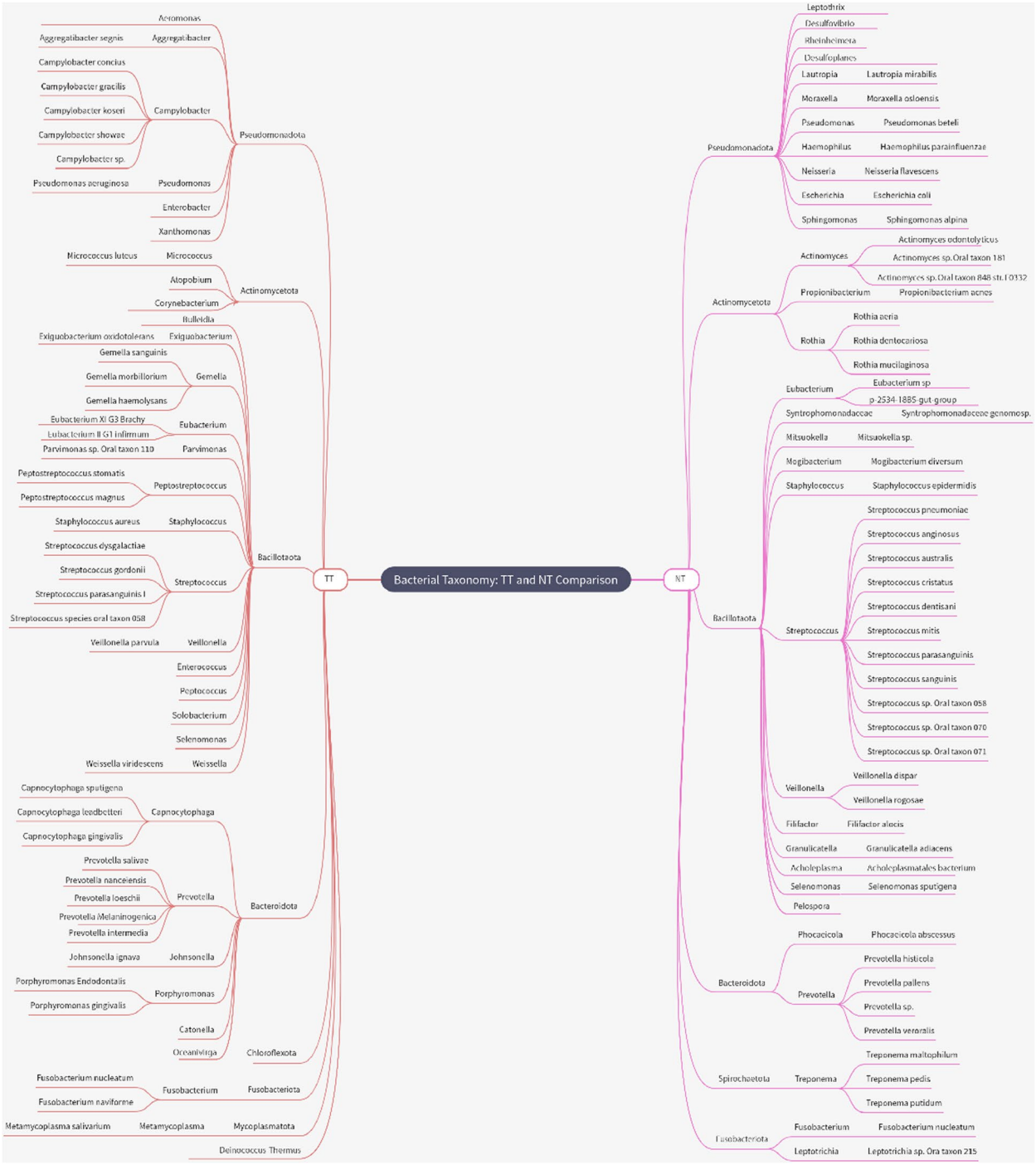

Fig. 2 Abundance of bacteria in TT and NT at phylum, genus, and species level

histopathologic (HP) evaluation of NT and tissue site heterogeneity in NT, no mention on sequencing depth were noted in a few studies [32, 33, 37, 39, 40] indicating areas for improvement in future research. Quality assessment scores of all articles were found above 12 as reported in Table 2.

Discussion

For the first time ever, we compared the bacteriome abundance and diversity of OSCC tissues (TT) and compared it with healthy/para-cancerous tissues (NT) to identify the exclusive bacterial abundance at species, genus and phylum level. We included 13 studies that compared

Table 2 Quality assessment of the articles by a combined NOS and customized criteria

Article identification	NOS criteria			Customized criteria								
Author, Year	Selection	Comparability	Outcome	Research question	Sample contamination	Region of 16 S ribosome	Sequencing depth	Bioinformatics	Taxonomic database assignment	HP evaluation	Tissue site heterogenity	Total score
Hooper et al., 2006 [35]	4	2	3	1	1	0	1	0	1	0	1	14
Yuchao Li et al., 2020 [30]	4	2	3	1	1	1	1	1	1	1	1	17
Perera et al., 2018 [36]	3	2	3	1	1	1	1	1	1	1	0	15
Fujiao et al., 2022 [31]	2	2	3	1	1	1	1	1	1	1	1	15
Al Hebshi et al., 2017 [37]	2	1	3	1	1	1	1	1	1	0	0	12
Yang et al., 2021 [32]	3	1	2	1	1	1	1	1	1	0	1	13
Pushalkar et al., 2012 [38]	3	2	1	1	1	1	1	1	1	1	1	14
Chang et al., 2019 [33]	4	2	1	0	1	1	0	1	1	0	1	12
Herraoes pomares et al., 2023 [39]	3	1	3	1	0	1	1	1	1	1	0	13
Vinay Jain et al., 2023 [40]	4	1	2	1	0	0	1	1	1	1	1	13
Divya Gopinath et al., 2021 [41]	3	2	3	1	1	1	0	1	1	1	0	14
Peng Ye et al., 2021 [34]	3	1	3	1	1	1	1	1	1	0	1	14
Mukherjee et al., 2017 [42]	3	1	3	1	1	1	1	1	1	0	1	14

bacterial abundances and diversity between TT and NT through 16S ribosomal RNA sequencing technique. The study populations were diverse, with broad geographical variation that may introduce variability due to population-specific differences in the bacteriome [43]. While the inclusion of diverse populations strengthens the generalizability of the result, it also emphasizes the need for further studies that control for this regional influence.

The abundance of the opportunistic pathogens such as *Escherichia coli, Pseudomonas beteli* and *Moraxella osloensis*, *Streptococcus pneumoniae, Staphylococcus epidermidis,* and *Sphingomonas alpina* in NT [31, 36, 38] can be ascribed to the dynamic ecosystem of the oral microbiome, the role of the host immune system, the subclinical nature of these bacteria, and antibiotic use. Those pathogens may coexist with normal commensals in a controlled state, and as long as this balance is maintained, they do not cause disease.

Upon reviewing the bacterial abundance in TT at genus level revealed the exclusive presence of seven genera such as *Eubacterium, Campylobacter, Catonella, Aeromonas, Oceanivirga, Rheinheimera, Weissella and* relative abundance of 35 genera, both of those are depicted in Fig. 2.

Seven bacterial genera such as *Pseudomonas, Eubacterium, Staphylococcus, Streptococcus, Veillonella, Prevotella, Fusobacterium* were found both in TT and NT. Ten genera, including, *Atopobium, Bulleidia, Eubacterium, Peptococcus, Solobacterium, Johnsonella,Weissella, Oceanivirga, Aeromonas, Metamycoplasma* were identified for the first time in this SR and were not reported in previous SRs [17–20]. Notably, *Gemella*, and *Catonella* were observed by only Shen et al. [20] while *Vellionella* was reported in all prior SRs except for Gopinath et al. [18]. Interestingly, *Neisseria* which was found in all previous SRs [17–20] was not identified in the present analysis. Similarly, the genera *Lactobacillus*, and *Haemophillus* were reported by three SRs Gopinath et al. [18] and Abdus et al. [19], Mun et al. [17] but were absent in our findings. Additionally, *Rothia*, and *Leptotrichia* were observed in two SRs [18] [19] but were not found in this SR. *Alloprevotella,* observed by both Mun et al. [17] and Shen et al. [20] but not found in the present review. Moreover, Mun et al. [17] and Shen et al. [20] reported the abundance of *Granulicatella* and *Proteobacteria* respectively which were neither reported by other two SRs [18, 19] nor in the present SR. *Dialister, Scardovia, Lachnoanaerobaculum, Fretibacterium, Megasphaera, Tannerella*, were exclusively observed by Gopinath et al. [18] and not found in any other SRs [17, 19, 20] nor in the present SR. A possible explanation for the identification of these additional bacteria in SR by Gopinath et al. [18] could be the inclusion of oropharyngeal cancer samples along with oral cancer specimen. The differences in bacterial abundance between the present SR and previously conducted SR may also be attributed to the inclusion of different sample types such as saliva, tissue scrapings, and tissue specimens in earlier studies. In contrast, this review focused exclusively on tissue specific bacterial abundance.

Qualitative synthesis on bacterial abundance at species level revealed presence of ten bacteria such as *Micrococcus luteus, Prevotella melaninogenica, Exiguobacterium oxidotolerans, Fusobacterium naviforme, Staphylococcus aureus, Veillonella parvula*, *Parvimonas sp. Oral taxon 110, Eubacterium II G1 infirmum, Eubacterium XI G3 Brachy, Weissella viridescens* exclusively in TT.. There are twenty species which were never reported in any of the previously conducted related SRs [17–20] were *Aggregatibacter segnis, Campylobacter concius, Campylobacter showae, Pseudomonas aeruginosa, Gemella morbillorium, Gemella haemolysans, Peptostreptococcus stomatis, Streptococcus dysgalactiae, Streptococcus gordonii, Streptococcus parasanguinis I, Streptococcus species oral taxon 058, Capnocytophaga gingivalis, Prevotella intermedia, F. nucleatum, Eubacterium II G1 infirmum, Eubacterium XI G3 Brachy, Exiguobacterium oxidotolerans, Weissella viridescens, Johnsonella ignava, Porphyromonas Endodontalis.* We may infer that, the presence of this group of bacteria may be due to the tissue specific nature of the sample from OSCC patients contrary to the previous SRs where nature of the sample from OSCC patients included tissue, swab, saliva, and cytological smear. *Prevotella tannerae,* was reported by Mun et al. [17] and Abdus et al. [19] but not found in the present SR. Gopinath et al. [18] and Abdus et al. [19] observed *Parvimonas micra* which we did not find in the TT in the present SR. *Streptococcus salivarius*, was observed only by Gopinath et al. [18] whereas *Streptococcus mitis, Rothia mucilaginosa, and Peptostreptococcus anaerobius* were exclusively observed by Abdus et al. [19]. Mun et al. [17] found the exclusive abundance of *Lactobacillus gasseri, Lactobacillus vaginalis,* and *Streptococcus mutans* in TT. *Capnocytophaga sputigena, Catonella morbi,* and *Dialister invisus* were seen exclusively in SR conducted by Shen et al. [20]. Taxonomic resolution at species level is a critical requirement to evaluate the bacterial abundance in TT as observed in the present SR. Interestingly we observed several species of the genus *Prevotella* such as *Prevotella histicola, Prevotella pallens, and Prevotella veroralis* were found in NT, whereas *Prevotella intermedia, Prevotella loeschii, Prevotella nanceiensis, and Prevotella salivae* were prevalent in TT. *Prevotella melaninogenica* in TT was found in the present SR, well supported by findings of previous SRs [17–20]. Li et al. [44] reported the abundance of *P gingivalis, Fusobacterium nucleatum* in OSCC in their bibliometric analysis. Behaviour of the bacteria

can vary significantly as per the characteristics of the species within the same genus.

While comparing the bacterial abundance between NT and TT at the phylum level, we observed no significant differences between the groups, except for the phylum *Chloroflexota, Deinococcus-Thermus, Mycoplasmatota* which were exclusively found in TT. These phyla were not reported in any previous systematic reviews, and its presence in this study could be due to differences in sequencing techniques, increased sensitivity of detection methods, or the specific tumour tissue microenvironment sampled in this review. Li et al. [27] collected the data of bacteriome from a secondary database, The Cancer Microbiome Atlas (TCMA) and identified the increased relative abundance of phylum as Fusobacteriota and Spirochaetota and genus *fusobacteria* and decreased relative abundance of phylum Bacillota and Actinomycetota in OSCC as compared to normal tissue. The relative abundance of phyla and genera in TT as compared to NT was not possible in the present SR owing to the inconsistency in reporting among the included studies. Proportion of phyla and genera was not reported in many studies. Li et al. [27] also established the association of key oral microbiomes with clinical characteristics of OSCC, which we were unable to report because of the inconsistent reporting of the clinical characteristics of TT among the included studies.

Bacteria may contribute to carcinogenesis through multiple mechanisms, including the production of aldehydes that cause DNA damage and mutations, as well as by promoting dysbiosis and perturbing the microbiota [45, 46]. For instance, cytolethal distending toxins (CDT) produced by *Aggregatibacter actinomycetemcomitans* act as virulence factors. Inside the cytoplasm, CdtB's phosphatase activity disrupts PIP3, leading to activation of PI3 K, a KRAS effector, which may result in KRAS mutations and drive carcinogenesis. Additionally, CdtB induces double-strand breaks (DSB), activating ATM kinase, which halts the G1/S and G2/M phases of the cell cycle. promoting carcinogenesis through cell senescence. Errors in homologous recombination (HR) and non-homologous end-joining (NHEJ) repair mechanisms, coupled with the activation of RhoA and p38 MAPK signalling for cell survival, further exacerbate carcinogenesis [47, 48]. Our findings support prior studies suggesting that *Capnocytophaga gingivalis* is abundant in OSCC with potential roles in inducing epithelial-mesenchymal transition (EMT), and enhancing invasiveness [47, 48]. Similarly, several genera such as *Prevotella, Fusobacterium, and Actinomyces* have been proposed as diagnostic biomarkers for OSCC [49]. Specific associations have been reported for *Porphyromonas gingivalis, Fusobacterium nucleatum*, and various *Prevotella* species with initiation and progression of OSCC [25, 50, 51]. The virulence factors of *P. gingivalis*, such as fimbriae and lipopolysaccharides, stimulate pro-inflammatory cytokines and activate the JAK2/STAT3 anti-apoptotic signalling cascade, contributing to carcinogenesis [52–54]. Additionally, *Porphyromonas gingivalis* has been shown to mediate G1 phase arrest, further facilitating carcinogenesis [55]. Our results also suggest potential additive effects of *P. intermedia* and *Fusobacterium nucleatum* in OSCC [25] akin to their synergistic roles in colorectal cancer [56]. *Fusobacterium nucleatum* promotes carcinogenesis through its FadA adhesin, facilitating attachment to and invasion of oral epithelial cells, which activates the β-catenin pathway. This activation increases the expression of LEF/TCF, NF-κB, and cytokines such as IL- 6, IL- 8, and IL-18, creating a pro-inflammatory microenvironment conducive to OSCC progression [57]. Contrary to previous reports associating *Streptococcus anginosus* with OSCC, our review did not find it abundant in TT. Instead, we observed higher abundance of *Streptococcus dysgalactiae, Streptococcus gordonii, Streptococcus parasanguinis, and Streptococcus species oral taxon 058* in TT. While the mechanisms of *Streptococcus anginosus* have been explored, the other *Streptococcus* species found in this review require further investigation [58]. It is essential to also consider the potential anti-tumour role of certain commensal bacteria. Notably, some bacteria may exhibit dual roles in carcinogenesis acting as both pro- and anti-tumour depending upon the microenvironmental dynamics. For instance, a recent transcriptomic study [59] in oral squamous cell carcinoma cell lines found that *Streptococcus* exhibited significant antitumor properties whereas *Neisseria aureus*, and *Haemophilus parainfluenzae* exhibited dual effect, and *Porphyromonas gingivalis* showed pro-tumour activity [60]. Exploring the pro- and anti-tumour effects of bacteria prevalent in TT could provide insights into novel strategies for cancer treatment.

Although oral bacteriome-based diagnostics and therapies hold promise for OSCC, the current literature is limited, and inconsistent reporting of clinicopathological characteristics in TT has hindered comprehensive analyses. Liu et al. [61] have associated oral bacteriome in OSCC with the depth of invasion (DOI) demonstrating a direct association with the abundances of *Porphyromonas endodontalis, Gemella morbillorum* and *Gemella haemolysans* and inverse association with the abundances of *Prevotella melaninogenica, Haemophilus parainfluenzae* and *Neisseria flavescens*. *Salmonella typhimuricum, Eschereria coli,* and *Bifidobacterium* have demonstrated anti-tumour effects in targeting therapies in oral cancer [62–65]. Our findings underscore the need for future research to elucidate the role of bacteria in OSCC, particularly their association with clinicopathological factors, and to explore their potential in developing treatment and prognostic strategies.

The findings on bacterial diversity in TT and NT are variable, with some studies indicating greater ACE estimator of species richness higher in TT as the study conducted by Pushalkar et al. [38] while Al-hebshi et al. [37] suggested higher Observed richness in NT. The results for alpha diversity, particularly the Shannon and Simpson indices, also vary across studies, possibly due to heterogeneity of NT, or the staging of OSCC of the given TT. Additionally, evenness indices tend to be higher in NT, indicating a more balanced microbial composition in NT. This variability underscores the complexity of the microbiome's role in oral carcinogenesis and highlights the need for more standardized approaches in studying bacterial diversity in OSCC. Several studies reported conflicting results regarding the Chao index, another measure of alpha diversity, which estimates species richness. These discrepancies in species richness suggest that bacterial diversity may not follow a consistent pattern between TT and NT, possibly influenced by the specific sample types. In terms of species evenness, which measures how uniformly individual organisms are distributed among species, both authors;Yang et al. [32] & Pushalkar et al. [38] reported that the evenness index was higher in NT than in TT. Whereas the ACE estimator of species richness, as reported by Pushalkar et al. [38], was higher in TT, adding another layer of complexity to the understanding of bacterial diversity in OSCC. Collectively, these findings suggest that bacterial diversity in OSCC is highly variable across studies, with no clear consensus on whether TT or NT consistently harbours greater microbial diversity. The observed differences in alpha diversity metrics are likely influenced by factors such as the limited number of studies, population differences, and tissue heterogeneity of NT as NT from the same patient tends to be more comparable than NT from another healthy individual because of the individual specific nature of the oral microbiome. While beta diversity provides insights into the differences in bacteriome of TT and NT, there are only five studies which inadequately reported the differences in beta diversity metrics between TT and NT. These factors highlight the gap in the literature regarding bacterial diversity comparisons between TT and NT and the need for more robust studies focusing on bacterial diversity.

As the outcome of the review is dependent upon the sequencing platforms, bioinformatics tool and Reference databases used in the included articles, each included study was critically evaluated by an eleven-parameter based quality assessment tool combining NOS and customised criteria. Majority of the included studies were of high quality which make this review finding reliable. High-throughput sequencing technologies and deeper sequencing data in most of the studies improved the reliability of the result. [66–69].

This systematic review encountered several limitations, including variability in sample sizes and differences in patient cohorts, which may affect the generalizability of findings. Variations in genetics, lifestyle, and environmental exposures contribute to differences in the oral microbiome. While studies comparing tumour and healthy tissues from the same patient aim to control for individual variability, intra-patient differences and spatial heterogeneity within the oral cavity can still impact results. Variations in biopsy methods, clinical stage reporting, and microbial profiling techniques, as well as potential publication bias, may also affect the findings. Additionally, the diversity of studied populations and confounding factors such as lifestyle and diet were not always reported. Further, the heterogeneity among studies could be attributed to the incomplete data on habits of smoking and alcohol use among both groups. Lastly, lack of comprehensive beta diversity analysis, with only a few studies reporting it, limits our ability to assess bacterial community shifts.

Conclusion

This review highlights and compares the exclusive as well as relative abundance of bacterial taxa in TT as compared to NT. While these findings provide valuable insights into the distinct microbial profiles, variability in sampling, sequencing technique, taxonomic resolution and population differences limits the generalisability of the present result. To obtain reliable and reproducible results, future research must address these limitations by adopting standardized sampling protocols, consistent sequencing methodologies, and precise species-level identification of bacteria. Such advancements are essential to elucidate the role of bacteria in etiopathogenesis of OSCC and to translate these findings into clinically applicable diagnostic and therapeutic strategies. Further investigation into the bacterial taxa identified in this systematic review holds the potential to advance our understanding of bacteria-mediated carcinogenesis in OSCC.

Abbreviations

OSCC	Oral Squamous Cell Carcinoma
PRISMA	Preferred Reporting Items for Systematic Reviews and Meta-Analyses
PROSPERO	International Prospective Register of Systematic Reviews
TT	Tumour Tissue
NT	Non tumour Tissue
NOS	New-Castle Ottawa scale

Supplementary Information

The online version contains supplementary material available at https://doi.org/10.1186/s12903-025-05941-3.

Supplementary Material 1: Table 1: Relative and exclusive abundance of bacteria in TT and NT at specie, genus and phylum level.

Acknowledgements
Not applicable.

Authors' contributions
Author 1. Dr Swagatika Mohapatra (SM). Role: Conceptualization, Methodology. Contributions: Developed the research question, designed the study, and performed data analysis. Author 2. Dr Swagatika Panda (SP). Role: Data Collection, Analysis, Writing and editing. Contributions: Assisted in data interpretation, Drafted the manuscript, revised sections for clarity, and ensured coherence in writing. Author 3: Dr Neeta Mohanty (NM). Role: Supervision, Editing. Contributions: Oversaw the review, Proof reading. Author 4: Dr Bibhu Prasad Mishra (BM). Role: Data Collection, Analysis. Contribution: provided guidance on methodology and data analysis. All authors read and approved the final manuscript.

Funding
Open access funding provided by Siksha 'O' Anusandhan (Deemed To Be University)

Data availability
Data is provided as supplementary information files.

Declarations

Ethics approval and consent to participate
Not applicable.

Consent for publication
Not applicable.

Competing interests
The authors declare no competing interests.

Author details
[1]Department of Oral Pathology and Microbiology, Institute of Dental Sciences, Siksha 'O' Anusandhan University, Bhubaneswar, Odisha, India. [2]Department of General Surgery, Maharaja Krushnachandra Gajapati Medical College and Hospital, Berhampur, Odisha, India.

Received: 19 October 2024 Accepted: 3 April 2025
Published online: 16 April 2025

References

1. Rivera C. Essentials of oral cancer. Int J Clin Exp Pathol. 2015;8(9):11884–94.
2. Sarode G, Maniyar N, Sarode SC, Jafer M, Patil S, Awan KH. Epidemiologic aspects of oral cancer. Dis-Mon DM. 2020;66(12):100988.
3. Vigneswaran N, Williams MD. Epidemiologic trends in head and neck cancer and aids in diagnosis. Oral Maxillofac Surg Clin N Am. 2014;26(2):123–41.
4. Bray F, Ferlay J, Soerjomataram I, Siegel RL, Torre LA, Jemal A. Global cancer statistics 2018: GLOBOCAN estimates of incidence and mortality worldwide for 36 cancers in 185 countries. CA Cancer J Clin. 2018;68(6):394–424.
5. Johnson N. Tobacco use and oral cancer: a global perspective. J Dent Educ. 2001;65(4):328–39.
6. Lalremtluangi R, Dangore-Khasbage S. Non-habit-related oral squamous cell carcinoma: a review. Cureus. 2024;16(2):e54594. https://doi.org/10.7759/cureus.54594.
7. Lissowska J, Pilarska A, Pilarski P, Samolczyk-Wanyura D, Piekarczyk J, Bardin-Mikołłajczak A, et al. Smoking, alcohol, diet, dentition and sexual practices in the epidemiology of oral cancer in Poland. Eur J Cancer Prev Off J Eur Cancer Prev Organ ECP. 2003;12(1):25–33.
8. Cawson RA. Leukoplakia and oral cancer. Proc R Soc Med. 1969;62(6):610–4.
9. Gold JS, Bayar S, Salem RR. Association of Streptococcus bovis bacteremia with colonic neoplasia and extracolonic malignancy. Arch Surg Chic Ill 1960. 2004;139(7):760–5.
10. Toyokawa T, Yokota K, Mizuno M, Fujinami Y, Takenaka R, Okada H, et al. Characterization of elongated Helicobacter pylori isolated from a patient with gastric-mucosa-associated lymphoid-tissue lymphoma. J Med Microbiol. 2004;53(Pt 3):207–12.
11. Shukla VK, Singh H, Pandey M, Upadhyay SK, Nath G. Carcinoma of the gallbladder–is it a sequel of typhoid? Dig Dis Sci. 2000;45(5):900–3.
12. Wallin KL, Wiklund F, Luostarinen T, Angström T, Anttila T, Bergman F, et al. A population-based prospective study of Chlamydia trachomatis infection and cervical carcinoma. Int J Cancer. 2002;101(4):371–4.
13. Minarovits J. Anaerobic bacterial communities associated with oral carcinoma: intratumoral, surface-biofilm and salivary microbiota. Anaerobe. 2021;68:102300.
14. Peterson J, Garges S, Giovanni M, McInnes P, Wang L, Schloss JA, et al. The NIH human microbiome project. Genome Res. 2009;19(12):2317–23.
15. Talapko J, Erić S, Meštrović T, Stipetić MM, Juzbašić M, Katalinić D, et al. The impact of oral microbiome dysbiosis on the aetiology, pathogenesis, and development of oral cancer. Cancers. 2024;16(17):2997.
16. Panda S, Gopinath D, Johnson NW. Bacteria-induced carcinopathogenesis in oral squamous cell carcinoma. In: Routray S, editor. Microbes and oral squamous cell carcinoma: a network spanning infection and inflammation. Singapore: Springer Nature; 2022. p. 107–24. Available from: https://doi.org/10.1007/978-981-19-0592-6_9. Cited 2024 Sep 24.
17. Su Mun L, Wye Lum S, Kong Yuiin Sze G, Hock Yoong C, Ching Yung K, Kah Lok L, et al. Association of microbiome with oral squamous cell carcinoma: a systematic review of the metagenomic studies. Int J Environ Res Public Health. 2021;18(14):7224.
18. Gopinath D, Menon RK, Banerjee M, Su Yuxiong R, Botelho MG, Johnson NW. Culture-independent studies on bacterial dysbiosis in oral and oropharyngeal squamous cell carcinoma: a systematic review. Crit Rev Oncol Hematol. 2019;139:31–40.
19. Salam A, Khan F. Periodontopathogens in oral cancer: a meta-analysis of bacterial taxa of the oral microbiome associated with risk factors of oral squamous cell carcinoma. medRxiv; 2022. p. 2022.03.11.22272244. Available from: https://www.medrxiv.org/content/10.1101/2022.03.11.22272244v1. Cited 2024 Sep 23.
20. Shen X, Zhang Y lun, Zhu J fei, Xu B hua. Oral dysbiosis in the onset and carcinogenesis of oral epithelial dysplasia: A systematic review. Arch Oral Biol. 2023;147:105630.
21. Page MJ, McKenzie JE, Bossuyt PM, Boutron I, Hoffmann TC, Mulrow CD, et al. The PRISMA 2020 statement: an updated guideline for reporting systematic reviews. BMJ. 2021;29(372):n71.
22. Hashimoto K, Shimizu D, Ueda S, Miyabe S, Oh-Iwa I, Nagao T, et al. Feasibility of oral microbiome profiles associated with oral squamous cell carcinoma. J Oral Microbiol. 2022;14(1):2105574. https://doi.org/10.1080/20002297.2022.2105574.
23. Anjali K, Arun AB, Bastian TS, Parthiban R, Selvamani M, Adarsh H. Oral microbial profile in oral cancer patients before and after radiation therapy in a cancer care center – a prospective study. J Oral Maxillofac Pathol JOMFP. 2020;24(1):117.
24. Yang J, He P, Zhou M, Li S, Zhang J, Tao X, et al. Variations in oral microbiome and its predictive functions between tumorous and healthy individuals. J Med Microbiol. 2022;71(8):001568.
25. Castañeda-Corzo GJ, Infante-Rodríguez LF, Villamil-Poveda JC, Bustillo J, Cid-Arregui A, García-Robayo DA. Association of Prevotella intermedia with oropharyngeal cancer: a patient-control study. Heliyon. 2023;9(3):e14293.
26. Listyarifah D, Nieminen MT, Mäkinen LK, Haglund C, Grenier D, Häyry V, et al. Treponema denticola chymotrypsin-like proteinase is present in early-stage mobile tongue squamous cell carcinoma and related to the clinicopathological features. J Oral Pathol Med Off Publ Int Assoc Oral Pathol Am Acad Oral Pathol. 2018;47(8):764–72.
27. Li Z, Fu R, Wen X, Wang Q, Huang X, Zhang L. The significant clinical correlation of the intratumor oral microbiome in oral squamous cell carcinoma based on tissue-derived sequencing. Front Physiol. 2022;13:1089539.
28. Luchini C, Stubbs B, Solmi M, Veronese N. Assessing the quality of studies in meta-analyses: advantages and limitations of the Newcastle Ottawa Scale. World J Meta-Anal. 2017;5(4):80–4.
29. Federhen S. The NCBI taxonomy database. Nucleic Acids Res. 2012;40(Database issue):D136-143.

30. Li Y, Tan X, Zhao X, Xu Z, Dai W, Duan W, et al. Composition and function of oral microbiota between gingival squamous cell carcinoma and periodontitis. Oral Oncol. 2020;107:104710.
31. Nie F, Wang L, Huang Y, Yang P, Gong P, Feng Q, et al. Characteristics of microbial distribution in different oral niches of oral squamous cell carcinoma. Front Cell Infect Microbiol. 2022;12:905653.
32. Yang K, Wang Y, Zhang S, Zhang D, Hu L, Zhao T, et al. Oral microbiota analysis of tissue pairs and saliva samples from patients with oral squamous cell carcinoma – a pilot study. Front Microbiol. 2021;12(12):719601.
33. Chang C, Geng F, Shi X, Li Y, Zhang X, Zhao X, et al. The prevalence rate of periodontal pathogens and its association with oral squamous cell carcinoma. Appl Microbiol Biotechnol. 2019;103(3):1393–404.
34. Ye P, Liu Y, Cai YJ, Yang H, Xu HT, Lu ZY. Microbial community alteration in tongue squamous cell carcinoma. Appl Microbiol Biotechnol. 2021;105(21–22):8457–67.
35. Hooper SJ, Crean SJ, Lewis MAO, Spratt DA, Wade WG, Wilson MJ. Viable bacteria present within oral squamous cell carcinoma tissue. J Clin Microbiol. 2006;44(5):1719–25.
36. Perera M, Al-Hebshi NN, Perera I, Ipe D, Ulett GC, Speicher DJ, et al. Inflammatory bacteriome and oral squamous cell carcinoma. J Dent Res. 2018;97(6):725–32.
37. Al-Hebshi NN, Nasher AT, Maryoud MY, Homeida HE, Chen T, Idris AM, et al. Inflammatory bacteriome featuring Fusobacterium nucleatum and Pseudomonas aeruginosa identified in association with oral squamous cell carcinoma. Sci Rep. 2017;7(1):1834.
38. Pushalkar S, Ji X, Li Y, Estilo C, Yegnanarayana R, Singh B, et al. Comparison of oral microbiota in tumor and non-tumor tissues of patients with oral squamous cell carcinoma. BMC Microbiol. 2012;20(12):144.
39. Herreros-Pomares A, Hervás D, Bagan-Debón L, Jantus-Lewintre E, Gimeno-Cardona C, Bagan J. On the oral microbiome of oral potentially malignant and malignant disorders: dysbiosis, loss of diversity, and pathogens enrichment. Int J Mol Sci. 2023;24(4):3466.
40. Jain V, Baraniya D, El-Hadedy DE, Chen T, Slifker M, Alakwaa F, et al. Integrative metatranscriptomic analysis reveals disease-specific microbiome–host interactions in oral squamous cell carcinoma. Cancer Res Commun. 2023;3(5):807–20.
41. Gopinath D, Menon RK, Wie CC, Banerjee M, Panda S, Mandal D, et al. Differences in the bacteriome of swab, saliva, and tissue biopsies in oral cancer. Sci Rep. 2021;11(1):1181.
42. Mukherjee PK, Wang H, Retuerto M, Zhang H, Burkey B, Ghannoum MA, et al. Bacteriome and mycobiome associations in oral tongue cancer. Oncotarget. 2017;8(57):97273–89.
43. Govender P, Ghai M. Population-specific differences in the human microbiome: factors defining the diversity. Gene. 2025;15(933):148923.
44. Li Z, Fu R, Huang X, Wen X, Zhang L. A decade of progress: bibliometric analysis of trends and hotspots in oral microbiome research (2013–2022). Front Cell Infect Microbiol. 2023;12(13):1195127.
45. Hampelska K, Jaworska MM, Babalska ZŁ, Karpiński TM. The role of oral microbiota in intra-oral halitosis. J Clin Med. 2020;9(8):2484.
46. Koliarakis I, Messaritakis I, Nikolouzakis TK, Hamilos G, Souglakos J, Tsiaoussis J. Oral bacteria and intestinal dysbiosis in colorectal cancer. Int J Mol Sci. 2019;20(17):4146.
47. Sun Z, Xiong C, Teh SW, Lim JCW, Kumar S, Thilakavathy K. Mechanisms of oral bacterial virulence factors in pancreatic cancer. Front Cell Infect Microbiol. 2019;4(9):412.
48. Zhu W, Shen W, Wang J, Xu Y, Zhai R, Zhang J, et al. Capnocytophaga gingivalis is a potential tumor promotor in oral cancer. Oral Dis. 2024;30(2):353–62.
49. Mager DL, Haffajee AD, Devlin PM, Norris CM, Posner MR, Goodson JM. The salivary microbiota as a diagnostic indicator of oral cancer: a descriptive, non-randomized study of cancer-free and oral squamous cell carcinoma subjects. J Transl Med. 2005;7(3):27.
50. Neuzillet C, Marchais M, Vacher S, Hilmi M, Schnitzler A, Meseure D, et al. Prognostic value of intratumoral Fusobacterium nucleatum and association with immune-related gene expression in oral squamous cell carcinoma patients. Sci Rep. 2021;11(1):7870.
51. Inaba H, Sugita H, Kuboniwa M, Iwai S, Hamada M, Noda T, et al. Porphyromonas gingivalis promotes invasion of oral squamous cell carcinoma through induction of proMMP9 and its activation. Cell Microbiol. 2014;16(1):131–45.
52. Chattopadhyay I, Verma M, Panda M. Role of oral microbiome signatures in diagnosis and prognosis of oral cancer. Technol Cancer Res Treat. 2019;1(18):1533033819867354.
53. Olsen I, Yilmaz Ö. Possible role of Porphyromonas gingivalis in orodigestive cancers. J Oral Microbiol. 2019;11(1):1563410.
54. Binder Gallimidi A, Fischman S, Revach B, Bulvik R, Maliutina A, Rubinstein AM, et al. Periodontal pathogens Porphyromonas gingivalis and Fusobacterium nucleatum promote tumor progression in an oral-specific chemical carcinogenesis model. Oncotarget. 2015;6(26):22613–23.
55. Cho TJ, Wee SW, Woo VH, Choi JI, Kim SJ, Shin HI, et al. Porphyromonas gingivalis-induced autophagy suppresses cell proliferation through G1 arrest in oral cancer cells. Arch Oral Biol. 2014;59(4):370–8.
56. Lo CH, Wu DC, Jao SW, Wu CC, Lin CY, Chuang CH, et al. Enrichment of Prevotella intermedia in human colorectal cancer and its additive effects with Fusobacterium nucleatum on the malignant transformation of colorectal adenomas. J Biomed Sci. 2022;27(29):88.
57. Stasiewicz M, Karpiński TM. The oral microbiota and its role in carcinogenesis. Semin Cancer Biol. 2022;1(86):633–42.
58. Mizumoto A, Ohashi S, Hirohashi K, Amanuma Y, Matsuda T, Muto M. Molecular mechanisms of acetaldehyde-mediated carcinogenesis in squamous epithelium. Int J Mol Sci. 2017;18(9):1943.
59. Lan Z, Liu WJ, Cui H, Zou KL, Chen H, Zhao YY, et al. The role of oral microbiota in cancer. Front Microbiol. 2023;14. Available from: https://www.frontiersin.org/journals/microbiology/articles/10.3389/fmicb.2023.1253025/full. Cited 2024 Nov 18.
60. Baraniya D, Chitrala KN, Al-Hebshi NN. Global transcriptional response of oral squamous cell carcinoma cell lines to health-associated oral bacteria - an in vitro study. J Oral Microbiol. 2022;14(1):2073866.
61. Liu Y, Li Z, Qi Y, Wen X, Zhang L. Metagenomic analysis reveals a changing microbiome associated with the depth of invasion of oral squamous cell carcinoma. Front Microbiol. 2022;13:795777.
62. Zhou S, Gravekamp C, Bermudes D, Liu K. Tumour-targeting bacteria engineered to fight cancer. Nat Rev Cancer. 2018;18(12):727–43.
63. Xiong S, Qi Z, Ni J, Zhong J, Cao L, Yang K. Attenuated Salmonella typhimurium-mediated tumour targeting imaging based on peptides. Biomater Sci. 2020;8(13):3712–9.
64. Li Q, Li Y, Wang Y, Xu L, Guo Y, Wang Y, et al. Oral administration of Bifidobacterium breve promotes antitumor efficacy via dendritic cells-derived interleukin 12. OncoImmunology. 2021;10(1):1868122.
65. Kouidhi S, Zidi O, Belkhiria Z, Rais H, Ayadi A, Ayed FB, et al. Gut microbiota, an emergent target to shape the efficiency of cancer therapy. Explor Target Anti-Tumor Ther. 2023;4(2):240–65.
66. Quast C, Pruesse E, Yilmaz P, Gerken J, Schweer T, Yarza P, et al. The SILVA ribosomal RNA gene database project: improved data processing and web-based tools. Nucleic Acids Res. 2013;41(Database issue):D590-6.
67. Cole JR, Wang Q, Fish JA, Chai B, McGarrell DM, Sun Y, et al. Ribosomal database project: data and tools for high throughput rRNA analysis. Nucleic Acids Res. 2014;42(Database issue):D633-642.
68. Benson DA, Cavanaugh M, Clark K, Karsch-Mizrachi I, Ostell J, Pruitt KD, et al. GenBank. Nucleic Acids Res. 2018;46(Database issue):D41-7.
69. Chen T, Yu WH, Izard J, Baranova OV, Lakshmanan A, Dewhirst FE. The human oral microbiome database: a web accessible resource for investigating oral microbe taxonomic and genomic information. Database J Biol Databases Curation. 2010;2010:baq013.

Publisher's Note