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Cultivable Oral Microbiota Associated with Oral Squamous Cell Carcinoma: A Paired Comparison of Tumor and Clinically Intact Mucosal Surfaces

Bence Zalánfi [1], Zsanett Kövér [1], Márió Gajdács [2,3,*] and Edit Urbán [4]

[1] Department of Dentistry, Oral and Maxillofacial Surgery, Medical School and Clinical Center, University of Pécs, Tüzér u. 1., 7623 Pécs, Hungary; zalanfi.bence@pte.hu (B.Z.); kover.zsanett@pte.hu (Z.K.)
[2] Department of Public Health, Albert Szent-Györgyi Medical School, University of Szeged, Dóm tér 10., 6720 Szeged, Hungary
[3] MTA-SZTE Lendület "Momentum" Anthropogenic Stress and Plant Resilience Research Group, Közép Fasor 52., 6726 Szeged, Hungary
[4] Department of Medical Microbiology, Medical School and Clinical Center, University of Pécs, Szigeti út 12, 7624 Pécs, Hungary; urban.edit@pte.hu
* Correspondence: gajdacs.mario@med-u-szeged.hu

Abstract

Background/Objectives: Oral squamous cell carcinoma (OSCC) is associated with complex alterations of the oral microbial environment, although the local microbiota colonizing tumor surfaces remains incompletely characterized. This study aimed to compare the cultivable microbiota of OSCC-affected and clinically intact oral mucosal surfaces within the same patients. **Methods**: A prospective, single-center study was conducted among patients with—histologically and microbiologically confirmed—HPV-negative OSCCs treated at the University of Pécs Clinical Centre, Hungary, between June 2024 and January 2026. Paired microbiological samples were collected from a standardized 1 cm^2 area of the tumor surface and a clinically tumor-free oral mucosal surface on the contralateral side in each patient. Conventional microbiological methods and MALDI-TOF MS were used for the culture and identification of microbial species. Species richness and the occurrence of individual taxa were compared between tumor and control surfaces; statistical analyses were performed using IBM SPSS 22.0 (threshold: $p < 0.05$). **Results:** Forty-one patients were included ($N = 41$; 31 males and 10 females; mean age, 59.9 ± 13.9 years). Overall, 33 aerobic-capnophilic and 65 anaerobic bacterial species, and 4 *Candida* species were identified. Anaerobic species richness was significantly greater on tumor surfaces than on clinically intact surfaces (6.89 ± 1.24 vs. 4.07 ± 1.65 species, $p = 0.026$), with 29 anaerobic species detected exclusively on tumor surfaces. *Fusobacterium* spp. and *Prevotella* spp. were significantly more frequently isolated from tumor surfaces ($p = 0.013$ and $p = 0.009$, respectively). *F. nucleatum* was detected on 58.5% of tumor surfaces vs. 19.5% of control surfaces. **Conclusions**: OSCC tumor surfaces exhibited pronounced cultivable anaerobic and fungal dysbiosis, compared with clinically intact mucosal surfaces from the same patients, whereas differences in aerobic-capnophilic bacterial richness were limited. The enrichment of fusobacteria, particularly *F. nucleatum*, and *Prevotella* spp. supports their potential relevance within the OSCC-associated microbial community. Larger longitudinal and molecular studies are warranted to clarify the biological and clinical importance of these microbial alterations.

Keywords: mouth neoplasms; microbiota; *Fusobacterium*; *Prevotella*; *Candida*; bacteria; anaerobic; dysbiosis

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1. Introduction

Lip and oral cavity cancers represent the 16th most common type of cancer worldwide (12th in men and 18th in women; the first three being lung, prostate and colorectal cancer among men, while breast, lung and colorectal among women); however, when grouped together, malignancies of the oral cavity and pharynx are the sixth most common type of cancer globally, with a 5-year period prevalence of 1,157,226 cases [1–3]. According to the data from the World Health Organization (WHO) Global Cancer Observatory (GCO) for 2024, over 452,205 incident cases of oropharyngeal cancer were recorded, causing over 194,108 deaths in the same period [3]. Based on data from the Global Burden of Disease (GBD) Study 2021, the global age-standardized prevalence (17.7/100,000 population) of lip and oral cavity cancers increased by 27.5% since 1990 [4]. The disease exhibits considerable disparities according to geography and demographic characteristics: both incidence and mortality rates are highest in Southeast and South Asia (i.e., India, Pakistan, Bangladesh and China, which are associated with certain socio-economic factors and traditions), with disease prevalence being 2–3 times higher in middle-to-elderly-aged men than in women across most ethnic groups [4–6].

The vast majority (~90.0%) of confirmed oral cancer cases are histologically classified as oral squamous cell carcinoma (OSCC), while the remaining cases are usually secondary manifestations of blood cancers, sarcoma or melanoma [7,8]. OSCCs may manifest in diverse forms—most commonly on the lip, the lateral part of the tongue or the floor of the mouth—usually including white patches (leukoplakia), red patches (erythroplakia), endophytic ulcerative lesions, or exophytic masses characterized by raised margins and tissue friability [9,10]. Warning signs of OSCCs often include persistent ulcers, enlarged and indurated cervical lymph nodes, unexplained discomfort, oral pain or numbness, loose teeth, and dysphagia; however, patients may experience symptoms only in the later stages of disease development, which delays timely detection of the illness [11]. Although early diagnosis of oral cancers is critical—as adherence to routine oral health screenings may improve overall survival rates by as much as 50.0%—timely detection of the disease remains unacceptably low (~30.0%) [12]; this may partly be due to the low attendance rates at routine dental check-ups (i.e., where the opportunistic oral health screening for and detection of suspicious lesions could occur), owing to dental care-related costs, socio-cultural influences and oral health literacy and dentophobia [13,14].

On the other hand, late-stage detection of oropharyngeal cancers severely limits available therapeutic alternatives and heavily impacts patient morbidity, mortality, and post-treatment quality of life (QoL) [15]. Furthermore, OSCC exhibits a high propensity for metastasis formation, most frequently to the regional cervical lymph nodes. Cervical metastasis—whether unilateral or bilateral—may further reduce survival rates by up to 50.0% [16]. If the disease is detected early, and prompt treatment is initiated, 1- and 5-year survival rates of oropharyngeal cancer may be as high as 85.0% and 65.0%, respectively; however, the prognosis is considerably worsened by the presence of extranodal extension and distant metastasis (most commonly to the lungs, kidneys, or brain), at which point, treatment often becomes palliative, and ~90.0% of patients succumb to the disease within 12 months [17,18].

The development of OSCCs is frequently preceded by oral potentially malignant disorders (OPMDs); the overall mean rate of malignant transformation of OPMDs is estimated at 7.9% [19]. Proliferative verrucous leukoplakia (PVL)—a rare and multifocal type of OPMD, most commonly associated with individuals not using tobacco or consuming alcohol (predominantly older women)—carries an exceptionally high malignant transformation rate ranging from 60.0% to 100.0% [20]. Homogenous and non-homogenous leukoplakias (including speckled and nodular variants) represent the most frequent clinical manifestations

of white mucosal lesions, requiring histopathological surveillance due to variable dysplastic progression. Erythroplakia may also be considered a severe warning sign; although rare (with an estimated prevalence between 0.02–0.2%), 85.0% of these lesions show dysplastic characteristics or carcinoma in situ at the time of biopsy, with malignant transformation occurring in ~45.0% of cases [21]. Other high-risk precursor conditions for oral cancers include oral submucous fibrosis (OSMF; often triggered by consumption of areca nuts, tobacco use, spicy foods and nutritional deficiencies, leading to excess collagen build-up) and oral lichen planus (OLP). OLP is a chronic, immune-mediated inflammatory disorder, characterized by presenting in diverse clinical forms (i.e., reticular, papular, plaque-like, erosive and ulcerative subtypes), requiring careful differentiation, long-term monitoring and personalized therapeutic approaches, owing to its potential for malignant transformation (particularly in the case of erosive-atrophic variants) [22–24].

The etiological factors associated with the development of oral cavity and pharyngeal cancers are deeply tied to established lifestyle (smoking, alcohol use, inappropriate oral hygiene, oncogenic microorganisms, dietary factors) and systemic (inherited genetic predisposition, presence of chronic underlying conditions, immunological factors) risks. Tobacco use—whether via cigarettes, cigars, or novel electronic or smokeless tobacco products—exposes the oral mucosa to potent tobacco-specific nitrosamines, including N'-nitrosonornicotine (NNN) and 4-(methylnitrosamino)-1-(3-pyridyl)-1-butanone (NNK), respectively [25,26]. These compounds metabolize into reactive intermediates that may form DNA adducts, leading to damage to the genetic material, initiation and subsequent tumor formation [25,26]. Alcohol consumption has a dual effect on OSCC development: on one hand, mammalian cells metabolize alcohol into acetaldehyde, a compound that directly causes DNA damage and interferes with DNA synthesis and repair (classified as a Group 1 carcinogen by the International Agency for Research on Cancer (IARC)) [26]. Furthermore, frequent alcohol consumption causes irritation and hyperemia, and acts as a solvent for organic carcinogens, increasing the permeability of the oral mucosa, facilitating the penetration of other carcinogens (e.g., from cigarette smoke) [27,28]. The synergistic effect of concurrent tobacco and alcohol use is staggering: depending on their intensity, the risk of developing head and neck cancers is 10–100 times higher in these individuals, compared to their non-smoking, abstinent counterparts [29,30]. Viral aetiologies, specifically high-risk Human Papilloma Virus (HPV) strains (including genotypes 16, 18, 31, 33, 35, 45, 52, 58, 64, 67, 69, 73; IARC Group 1) are responsible for up to 90.0% of HPV-mediated oral and genital malignancies, actively disrupting host cell cycle mechanisms through oncogene integration; the relevance of HPV-mediated oral cancers may be especially relevant in younger, non-smoking, female patients [31].

Several published studies have also highlighted the notable role of systemic conditions as important risk factors for facilitating oral cancer development. A recent meta-analysis by Gong et al.—corresponding to 6465 cases of oral cancer and 1407 cases of precancerous lesions—highlighted that Type 2 diabetes mellitus (T2DM) increases the likelihood of oral cancer by 15.0%, driven by hyperglycemia, insulin resistance, and compensatory hyperinsulinemia [32]. Elevated insulin levels boost concentrations of insulin-like growth factor-1 (IGF-1), stimulating cell proliferation and inhibiting apoptosis, which has been suggested as a possible underlying mechanism. Additionally, insulin resistance triggers the release of pro-inflammatory cytokines, such as tumor necrosis factor-alpha (TNF-α) and interleukin-6 (IL-6), leading to conditions favorable for malignant transformation [31]. While traditional risk factors for the development of OSCCs are well-documented, emerging research focuses on the intricate role of the oral microbiota in carcinogenesis. The oral cavity hosts the second most diverse microbial community in the human body—surpassed only by the gut—containing over 700 bacterial species, alongside various fungi and protozoa [33]. Dysbiosis

of this microbiota is linked not only to local pathophysiological processes, such as dental caries and periodontal disease, but may also be implicated in the development of other systemic conditions, such as cardiovascular disease, dementia, colorectal and pancreatic cancer, among others [34,35].

Current evidence suggests that the oral microbiota may contribute to tumor formation through three primary mechanisms: (i) the induction of chronic inflammation (which promotes sustained cell division and angiogenesis), (ii) the manipulation of cytoskeletal architecture and rearrangement with subsequent inhibition of host cell apoptosis, and (iii) the production of carcinogenic substances, leading to direct damage to cells or to the genetic material [36]. The main inflammatory pathways implicated are triggered by the lipopolysaccharides (LPS) of Gram-negative bacteria (often late colonizers of the dental biofilm and/or periodontopathogens), leading to the activation of periodontal and immune cells, resulting in elevated IL-1β, IL-6, and TNF-α production [37]. IL-1β acts aggressively by degrading E-cadherin (enhancing cell migration) and stimulating matrix metalloproteinase-9 (MMP-9), which breaks down the extracellular matrix, facilitating the invasion of tumor cells [37,38]. IL-6 further drives tumor growth by inhibiting apoptosis, causing elevated levels of oxidative stress (i.e., reactive oxygen species (ROS) and reactive nitrogen species (RNS)), and increasing the expression of intercellular adhesion molecules (ICAMs) [36]. Concurrently, TNF-α activates critical oncogenic signaling pathways, including Wnt and nuclear factor kappa-B (NF-κB), while drastically increasing the production of ROS and RNS, culminating in direct oxidative DNA damage in oral epithelial cells [37,39].

Specific bacterial strains are able to uniquely maneuver these pathways; *Porphyromonas gingivalis* (*P. gingivalis*), an aggressive periodontal pathogen, directly inhibits host cell apoptosis by activating the anti-apoptotic Jak1/Akt/Stat3 signaling pathway, suppressing p53 levels, and secreting nucleoside diphosphate kinase (NDK), which neutralizes ATP-induced cell death [37,40]. Furthermore, microbial metabolic by-products are actively responsible for cultivating a pro-tumorigenic microenvironment; for example, species such as *Solobacterium moorei* (*S. moorei*), *P. gingivalis*, and *Fusobacterium nucleatum* (*F. nucleatum*) are able to generate toxic volatile sulfur compounds (VSCs)—such as hydrogen sulfide (H_2S) and methyl mercaptan (CH_3SH)—which exhibit genotoxic effects, accelerating tumor spread [37,41,42]. The enrichment of anaerobic taxa—particularly those producing VSCs, such as *Fusobacterium* and *Prevotella* species—may hold notable clinical implications regarding the symptoms of the patients, as VSCs generated by these microbial communities frequently manifest clinically as persistent halitosis or oral malodor. While halitosis is commonly associated with non-cancerous conditions, such as periodontal disease or poor oral hygiene, persistent, localized, or unexplained oral malodor accompanying mucosal changes should be recognized as a potential clinical indicator of OSCCs [43]. Furthermore, other genera—including members of the *Lactobacillus* and *Streptococcus* species—ferment sugars into lactic acid, which results in local pH reduction, mirroring the acidic, hypoxic environments characteristic of advanced solid tumors, and indirectly improving the efficiency of metastasis formation [37].

Beyond bacterial species, numerous studies have demonstrated the dramatic increase in fungal infections—particularly those caused by *Candida* species—in patients with OSCC, forming complex, protective biofilm scaffolds alongside pathogenic bacteria in their oral cavity [44]. *C. albicans* drives a distinct immunological response by increasing IL-17A production via Th17 cells; in turn, due to the activation of the IL-17RA pathway, tumor cells release the chemokine CCL2, recruiting macrophages to the tumor microenvironment [45]. These recruited macrophages adopt a remarkably immunosuppressive phenotype, characterized by the increased expression of IL-10, arginase-1, PD-L1, and galectin-9, thereby shielding the tumor from host immunity [46]. *Candida* species also engage in interking-

dom metabolic and physical crosstalk: by interacting with oxygen and ROS, and utilizing adhesion proteins like Als1, Als3, and Hwp1, they cultivate a protective, low-oxygen microenvironment that remarkably enhances the survival, invasiveness, and overall virulence of strictly anaerobic bacteria, such as *P. gingivalis* [44–46]. A critical element of *C. albicans* virulence is candidalysin, a cytolytic toxin encoded by the *ECE1* gene, which may induce severe cellular damage, trigger inflammation, and promote angiogenesis in the oral microenvironment [47]. Furthermore, in terms of chemical carcinogenesis, *C. albicans* behaves similarly to human tissues—by metabolizing ethanol into its carcinogenic end-product acetaldehyde and actively producing nitrosamines from external sources [45].

Against this background, the present study aimed to provide a comprehensive qualitative and compositional characterization, focusing specifically on cultivable species richness and differential taxa recovery (including aerobic-capnophilic, anaerobic, and fungal species), associated with HPV-negative OSCCs versus clinically intact oral mucosal surfaces within the same patients, originating from the southwestern region of Hungary. We hypothesized that tumor surfaces would present a distinct qualitative microbial profile, marked by notably elevated cultivable anaerobic species richness and localized compositional shifts compared to paired control sites. Particular attention was given to differences in microbial species richness and the occurrence of potentially relevant taxa, which were associated with facilitating tumor development and progression. By using paired sampling of tumor-affected and clinically intact tumor-free sites, we sought to identify local microbial alterations associated with the OSCC microenvironment, while minimizing within-person variability. The findings may contribute to a better understanding of tumor-associated oral dysbiosis and provide a basis for future studies investigating its biological and clinical significance.

2. Materials and Methods

2.1. Study Design and Setting, Inclusion Criteria

The present prospective, single-center study was carried out among patients receiving treatment for oropharyngeal cancers at the Maxillofacial Clinic of the Department of Dentistry, Oral and Maxillofacial Surgery, University of Pécs (PTE) Clinical Centre, between 1 June 2024 and 31 January 2026. The Clinical Centre operates as a university teaching hospital—situated in the Southern Transdanubia region of Hungary—providing primary to tertiary healthcare services to the inhabitants of the region (~800,000 individuals).

The inclusion criteria for the study were: (*i*) individuals aged ≥18 years, (*ii*) the presence of a clinically and histologically confirmed, HPV-negative OSCC (*Carcinoma planocellulare*) in the oral cavity. Specifically, our cohort was strictly restricted to OSCCs originating from the mucosal surface of the oral cavity (such as the tongue, floor of the mouth, gingiva, palate, and lips), (*iii*) patients who were cooperative following the receipt of their OSCC diagnosis, have undergone cancer treatment, and were presenting on time at their scheduled follow-up visits, (*iv*) patients who provided informed consent to participate. The HPV status of the tumors was determined via histopathological methods; in addition, confirmatory tests by polymerase chain reaction (PCR) were performed at the Department of Medical Microbiology, PTE Clinical Centre. Patients with either oral tumors of other histological characteristics or HPV-positive cancers were not eligible. Cancers originating from major or minor salivary glands, which represent a distinct glandular histogenesis and etiological spectrum independent of mucosal dysbiosis, as well as tumors localized to the tonsils or posterior oropharyngeal structures, were explicitly excluded to maintain a homogeneous mucosal microenvironment. In addition, those patients who reported receiving antimicrobial treatment one month preceding the time of sampling were also excluded. The patients served as their own controls (self-controlled study); therefore, the involvement of an additional control group was not necessary.

2.2. Sampling Procedure, Controls, Data Collection and Follow-Up

During the study, the non-invasive microbiological sampling—in the areas of the oral cavity described earlier—was carried out from the surface of a tumor-affected and clinically tumor-free area, performed using a sterile flocked swab (resulting in better elution of the sample into the transport media) before initiation of any treatment modality following OSCC diagnosis (chemotherapy, radiotherapy and surgical interventions). The sterile swab was then placed in an anaerobic transport medium (COPAN, Brescia, Italy). The tonsils were not included in the region to be examined and sampled, according to the rationale described previously. Sampling was conducted on a 1 cm^2 tumor-affected area and a 1 cm^2 clinically tumor-free area; depending on the extent of the tumor spread, the sample from the opposite (contralateral) side of the lesion was collected. The area was marked with a 1 cm^2 3D-printed square sterile localization frame measuring 1 × 1 cm, and then the specific area was swabbed with a sterile cotton swab (Figure 1).

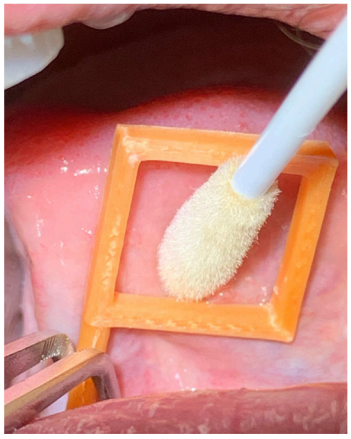

Figure 1. Sampling procedure using a 3D-printed square sterile localization frame, measuring 1 × 1 cm, and the sterile flocked swab.

Patients were instructed not to brush their teeth, rinse their mouths, eat, drink, chew gum, or smoke for 1 h prior to sampling; during this time, demographic and clinical data were collected, including the sex and age of the patients, localization of the tumor, Tumor-Nodes-Metastasis (TNM) classification, patient stratification according to the treatment approach, presence of relevant comorbidities (e.g., T2DM) and lifestyle-related risk factors (tobacco use, alcohol consumption), respectively. After receiving treatment, patients presented at the clinic every three months for stomato-oncological follow-up visits; patient status and outcomes associated with participants were recorded up to 24 months post-sampling.

2.3. Traditional Microbiological Culture Method

The samples obtained (swabs from the tumor-affected and clinically tumor-free area) were processed immediately after arrival at the Department of Medical Microbiology; the time elapsed between microbiological sampling and processing was <30 min in all instances. The samples were vortexed (using Stomacher 80, Labsystem, Budapest, Hungary) for 30 s at low speed in 1.0 mL of reduced BHI (Brain Heart Infusion, pH 7.2) broth (Oxoid, Basingstoke, UK). Following this, a dilution series was prepared from the stock solution, which was diluted to a 10^{-1} to 10^{-6} dilution range. From the stock solution itself and from each dilution, 100 µL was plated in parallel onto selective and non-selective agar media. Fastidious Anaerobe Agar (FAA), supplemented with 5% (v/v) bovine blood, haemine, and Vitamin K_1, was used for the isolation and enumeration of all cultivable anaerobic bacteria.

Columbia blood agar and cooked blood (chocolate) agar were used for the isolation and enumeration of aerobic bacterial flora [48]. For the selective cultivation of any aerobic Gram-negative species from the Enterobacterales order that might be present, Endo agar (bioMérieux, Marcy l'Etoile, France) was used; Sabouraud Dextrose agar (bioMérieux, Marcy l'Etoile, France) was used for the cultivation of fungal species. For the easier isolation of black-pigmented anaerobic Gram-negative bacteria (such as *Prevotella* spp., *Porphyromonas* spp.), they were isolated using Kanamycin Vancomycin Laked Blood (KVLB) agar (Oxoid, Basingstoke, UK), while Cadmium-Fluoride-Acriflavine-Tellurite (CFAT) agar (Oxoid, Basingstoke, UK), was used for the selective isolation of *Actinomyces*/*Schaalia* species, respectively [33].

The cultures were incubated for 5 days in an anaerobic chamber (Bactron Sheldon Man, Cornelius, OR, USA) under anaerobic conditions (90% N_2, 5% H_2, and 5% CO_2) and aerobically for 24 h at 37 °C; cultured bacteria and fungi were counted by determining the exact number of colonies. Strains with different colony morphology were identified at the species level, using matrix-assisted laser desorption–ionization time-of-flight mass spectrometry (MALDI-TOF MS; Bruker Daltonics, Bremen, Germany). Sample preparation procedures and the technical details of the MALDI measurements were described in detail previously [49]. During analyses, the microFlex LT Biotyper instrument Bruker Daltonics, Bremen, Germany) and the MALDI Biotyper Library 3.1 were used for spectra analysis. Based on consensus criteria, genus-level identification of bacterial isolates was deemed reliable if the $log(\text{score}) \geq 1.7$, while for reliable species-level identification, a $log(\text{score}) \geq 2.0$ was needed [49].

2.4. Statistical Analysis

During our analysis, continuous variables were expressed as a median and range (minimum–maximum), while categorical variables were expressed as frequencies and percentages (n, %). McNemar's test was used to determine differences between proportions, while paired-sample and independent-samples t-tests were used for the comparison of continuous variables. Statistical analyses were performed using SPSS Statistics version 22.0 (IBM Inc., Chicago, IL, USA). During analyses, p values < 0.05 were considered statistically significant. An *a priori* sample size determination for the study was not performed. Due to the previously detailed exclusion criteria, handling of missing data was not relevant during our analyses.

2.5. Ethical Considerations

The study was conducted in accordance with the Declaration of Helsinki (1975, last revised in 2024 [50]), and national and institutional ethical standards. Ethical approval for this study protocol was obtained from the Human Institutional and Regional Biomedical Research Ethics Committee, Medical School, University of Pécs (approval ID: 9503-2023, approval date: 31 March 2023; and approval ID: 9593-PTE 2024, approval date: 13 December 2024).

2.6. Informed Consent

Before participation in our study, OSCC patients were provided with detailed information regarding the study's purpose, procedures, potential risks, and benefits. Written informed consent was obtained from each participant, ensuring that they understood their right to participate voluntarily and their right to withdraw at any time without any disadvantageous consequences or influence on their treatment. Confidentiality and anonymity were maintained throughout the study. The participants did not receive any incentives (monetary or otherwise) to take part in the study.

3. Results

3.1. Sample Characteristics, Outcomes

Out of the 49 OSCC patients—meeting inclusion criteria—who were approached to take part in the study during the data collection period, a total of $N = 41$ patients were enrolled, while the remaining ($n = 8$) refused participation (i.e., did not consent) or did not accept any treatment/did not present again at the clinic following their cancer diagnosis. Of these patients, $n = 31$ (75.6%) were male, and $n = 10$ (24.4%) were female. The mean age of participants was 59.9 ± 13.9 years. The summary of sample characteristics—stratified by sex—is shown in Table 1.

Table 1. Characteristics of OSCC patients included in our study, stratified by sex ($N = 41$).

OSCC patients ($N = 41$)			
	Males ($n = 31$)	Females ($n = 10$)	Statistics
Age (years, mean ± SD)	57.8 ± 14.8	65.9 ± 6.2	$p = 0.041$
Anatomical localization of the tumor			
Labial ($n = 2$)	2 (6.5%)	0 (0.0%)	n.s. ($p = 0.11$)
Palatal ($n = 6$)	4 (13.0%)	2 (20.0%)	
Lingual ($n = 15$)	10 (32.3%)	5 (50.0%)	
Sublingual ($n = 12$)	12 (38.7%)	0 (0.0%)	
Gingival ($n = 6$)	3 (9.5%)	3 (30.0%)	
TNM classification			
T1 ($n = 12$)	7 (22.6%)	5 (50.0%)	n.s. ($p = 0.33$)
T2 ($n = 5$)	4 (12.9%)	1 (10.0%)	
T3 ($n = 11$)	10 (32.3%)	1 (10.0%)	
T4 ($n = 13$)	10 (32.3%)	3 (30.0%)	
N0 ($n = 26$)	14 (45.2%)	8 (80.0%)	n.s. ($p = 0.35$)
N1 ($n = 5$)	5 (16.1%)	0 (0.0%)	
N2 ($n = 9$)	7 (22.6%)	2 (20.0%)	
N3 ($n = 1$)	1 (3.2%)	0 (0.0%)	
M0 ($n = 41$)	31 (100.0%)	10 (100.0%)	n.s. ($p = 1.00$)
M1 ($n = 0$)	0 (0.0%)	0 (0.0%)	
Underlying disease, lifestyle factors			
Tobacco consumption (yes) ($n = 26$)	20 (64.5%)	6 (60.0%)	n.s. ($p = 0.90$)
Alcohol consumption (yes) ($n = 18$)	16 (51.6%)	2 (20.0%)	n.s. ($p = 0.16$)
T2DM (yes) ($n = 9$)	5 (16.1%)	4 (40.0%)	n.s. ($p = 0.25$)
Outcome at 24-months post-sampling			
Stable ($n = 16$)	10 (32.3%)	6 (60.0%)	$p = 0.027$
Recurrence ($n = 3$)	1 (3.2%)	2 (20.0%)	
Exitus ($n = 22$)	20 (64.5%)	2 (20.0%)	

SD: standard deviation; TNM: Tumor-Nodes-Metastasis; T2DM: Type 2 diabetes mellitus; n.s.: not significant.

Regarding the anatomical localization of the OSCC, the tongue was the most common site of the tumor, accounting for $n = 15$ cases (36.6%), followed by sublingual localization in $n = 12$ cases (29.3%); the gingiva and the palate were affected in 6 cases each (14.6%), while the lip was involved in $n = 2$ cases (4.9%), respectively. Based on the TNM classification, to ascertain the anatomical extent of the primary tumor (T category), the majority of patients presented with T4 tumors ($n = 13$, 31.7%), followed by T1 ($n = 12$, 29.3%), T3 ($n = 11$, 26.8%) and T2 ($n = 5$, 12.2%), respectively. Involvement of regional lymph nodes (i.e., N categorization) was assessed using two distinct clinical pathways: in the case of $n = 17$ (41.5%) patients, who received primary oncotherapy based on the decisions of the multidisciplinary tumor board (oncoteam), N categories were determined using diagnostic imaging, resulting in $n = 8$ (47.1%) N0, $n = 2$ (11.8%) N1, $n = 6$ (35.3%) N2, and $n = 1$ (5.9%) N3 cases, respectively. Conversely, $n = 24$ (59.5%) patients underwent surgical treatment, including selective neck dissection (SND), where postoperative histopathological evaluation was used for nodal staging: this surgical cohort yielded $n = 18$ (75.0%) N0 cases, and $n = 3$ cases each (12.5%) for the N1 and N2 categories, respectively. At the time of first detection, distant metastases (M category) were absent in all patients (M0: 100.0%).

During the analysis of the patients' anamnestic data, $n = 11$ (26.8%) had no underlying disease or risk factors present, $n = 9$ (21.9%) had T2DM, and $n = 18$ (43.9%) consumed alcohol, while $n = 26$ (63.4%) consumed tobacco, respectively; furthermore, in the case of $n = 13$ (31.7%), alcohol and tobacco consumption occurred concurrently, while in $n = 5$ (12.2%) patients, all tested risks (tobacco, alcohol and T2DM) were present. At 24 months post-sampling, $n = 22$ (53.7%) patients have died; of the $n = 19$ (46.3%) remaining patients, $n = 3$ (7.3%) currently receive treatment due to recurrence of the disease. Among the patients who died, $n = 13$ presented with stage T4, $n = 7$ with T3 and $n = 2$ with T2 at the initial detection of the OSCC.

3.2. Microbiological Culture of Samples from the Surface of OSCC and Clinically Intact Tissues

During our study, traditional bacterial culture was used from samples obtained from OSCC-affected and control surfaces to determine cultivable microbiota and cultivable species richness; a more global summary of these results is found in Table 2. Overall, 33 different aerobic-capnophilic species and 65 different anaerobic species were identified, in addition to 4 species of *Candida* spp. (Tables 3 and 4). In addition to cultivable species richness, bacterial density on OSCC surfaces was substantially higher (10^8–10^{10} CFU/mL), compared to that of clinically intact tissue surfaces (10^4–10^6 CFU/mL). Regarding the distribution of aerobic species, no notable differences were found between OSCC vs. clinically intact surfaces, with an average of 2–3 species (OSCC vs. clinically intact: 2.63 ± 0.48 vs. 2.39 ± 0.67; $p = 0.483$) identified on both surfaces. On the other hand, strains of three species (*Enterobacter ludwigii*, *Haemophilus influenzae* and *Morganella morganii*) were identified exclusively on the control surfaces, while strains of twelve species (*Abiotrophia defectiva*, *Corynebacterium* spp., *Enterobacter cloacae*, *Enterococcus avium*, *Granulicatella adiacens*, *Haemophilus parainfluenzae*, *Klebsiella aerogenes*, *K. oxytoca*, *K. variicola*, *Streptococcus anginosus*, *S. canis*, *S. constellatus*) were found exclusively on the tumorous surfaces (Table 3). However, some species were only found in a single patient (i.e., *Corynebacterium* spp., *E. ludwigii*, *G. adiacens*, *K. variicola*, *M. morganii* and *S. canis*), respectively. *S. mitis/oralis* were present in the highest proportions on both surfaces (OSCC vs. clinically intact: 80.5% vs. 82.9%). Regarding fungal isolates, no relevant differences were shown for the species distribution between surfaces; however, they were detected significantly more commonly on the tumorous side (18 [43.9%] vs. 7 [17.0%]; $p = 0.015$), with *Candida albicans* mainly driving this difference (Table 3).

Table 2. Summary of the results of traditional bacterial culture in samples obtained from OSCC-affected and control surfaces of oropharyngeal cancer patients.

	OSCC Side	Control Side
Overall number of distinct aerobic-capnophilic species detected	30	21
Average number of aerobic-capnophilic species/sample	2.63 ± 0.48	2.39 ± 0.67
Overall number of distinct anaerobic species detected	62	35
Average number of anaerobic species/sample	6.89 ± 1.24	4.07 ± 1.65
Number of fungal species detected	4	4
Bacterial density (CFU/mL)	10^8–10^{10}	10^4–10^6

OSCC: oral squamous cell carcinoma.

Table 3. Results of traditional bacterial culture in samples obtained from OSCC-affected and control surfaces of oropharyngeal cancer patients: aerobic-capnophilic species and fungi.

Microorganisms	OSCC Side (*n*, %)	Control Side (*n*, %)
Abiotrophia defectiva	**1 (2.4%)**	0 (0.0%)
Aggregatibacter segnis	1 (2.4%)	1 (2.4%)
Bacillus spp.	1 (2.4%)	1 (2.4%)
Citrobacter koseri	1 (2.4%)	1 (2.4%)
Corynebacterium spp.	**1 (2.4%)**	0 (0.0%)
Enterobacter cloacae	**3 (7.3%)**	0 (0.0%)
Enterobacter ludwigii	0 (0.0%)	**1 (2.4%)**
Enterococcus avium	**1 (2.4%)**	0 (0.0%)
Escherichia coli	2 (4.9%)	1 (2.4%)
Granulicatella adiacens	**1 (2.4%)**	0 (0.0%)
Haemophilus influenzae	0 (0.0%)	**2 (4.9%)**
Haemophilus parainfluenzae	**1 (2.4%)**	0 (0.0%)
Klebsiella aerogenes	**2 (4.9%)**	0 (0.0%)
Klebsiella oxytoca	**2 (4.9%)**	0 (0.0%)
Klebsiella pneumoniae	1 (2.4%)	1 (2.4%)
Klebsiella variicola	**1 (2.4%)**	0 (0.0%)
Morganella morganii	0 (0.0%)	**1 (2.4%)**
Neisseria flavescens	4 (9.8%)	3 (7.3%)
Nocardia spp.	1 (2.4%)	1 (2.4%)
Staphylococcus aureus	3 (7.3%)	2 (4.9%)
Streptococcus agalactiae	1 (2.4%)	2 (4.9%)
Streptococcus anginosus	**9 (21.9%)**	0 (0.0%)
Streptococcus canis	**1 (2.4%)**	0 (0.0%)
Streptococcus constellatus	**4 (9.8%)**	0 (0.0%)

Table 3. *Cont.*

Microorganisms	OSCC Side (*n*, %)	Control Side (*n*, %)
Streptococcus gordonii	1 (2.4%)	3 (7.3%)
Streptococcus infantis	1 (2.4%)	1 (2.4%)
Streptococcus intermedius	2 (4.9%)	1 (2.4%)
Streptococcus mitis/oralis	33 (80.5%)	34 (82.9%)
Streptococcus mutans	1 (2.4%)	1 (2.4%)
Streptococcus parasanguinis	9 (21.9%)	15 (36.6%)
Streptococcus salivarius	3 (7.3%)	7 (17.1%)
Streptococcus sanguinis	2 (4.9%)	1 (2.4%)
Pseudomonas aeruginosa	1 (2.4%)	1 (2.4%)
Candida albicans	14 (34.1%)	4 (9.8%)
Candida glabrata	3 (7.3%)	2 (4.9%)
Candida krusei	1 (2.4%)	1 (2.4%)
Candida lusitaniae	1 (2.4%)	1 (2.4%)

OSCC: oral squamous cell carcinoma; distinct species only present for either the OSCC or clinically intact surfaces are denoted in **boldface.**

Table 4. Results of traditional bacterial culture in samples obtained from OSCC-affected and control surfaces of oropharyngeal cancer patients: anaerobic species.

Microorganisms	OSCC Side (*n*, %)	Control Side (*n*, %)
Acidaminococcus intestinii	0 (0.0%)	**1 (2.4%)**
Actinobacillus porcinus	**1 (2.4%)**	0 (0.0%)
Actinomyces graevenitzii	**1 (2.4%)**	0 (0.0%)
Actinomyces naeslundii	**1 (2.4%)**	0 (0.0%)
Actinomyces oris	4 (9.8%)	1 (2.4%)
Alloprevotella tannerae	3 (7.3%)	1 (2.4%)
Alloscardovia omnicolens	2 (4.9%)	1 (2.4%)
Bifidobacterium dentium	2 (4.9%)	3 (7.3%)
Camplylobacter rectus	**1 (2.4%)**	0 (0.0%)
Camplylobacter showae	**1 (2.4%)**	0 (0.0%)
Capnocytophaga gingivalis	**1 (2.4%)**	0 (0.0%)
Capnocytophaga sputigena	3 (7.3%)	1 (2.4%)
Clostridium cadaveris	**1 (2.4%)**	0 (0.0%)
Clostridium perfringens	**1 (2.4%)**	0 (0.0%)
Clostridium tertium	**1 (2.4%)**	0 (0.0%)
Cutibacterium acnes	2 (4.9%)	3 (7.3%)
Cutibacterium avidum	2 (4.9%)	1 (2.4%)
Eggerthella cateniformis	**2 (4.9%)**	0 (0.0%)
Eggerthella lenta	2 (4.9%)	2 (4.9%)
Eikenella corrodens	2 (4.9%)	1 (2.4%)
Eubacterium brachy	**1 (2.4%)**	0 (0.0%)

Table 4. *Cont.*

Microorganisms	OSCC Side (*n*, %)	Control Side (*n*, %)
Finegoldia magna	2 (4.9%)	1 (2.4%)
Fusobacterium canifelinum	**2 (4.9%)**	0 (0.0%)
Fusobacterium varium	2 (4.9%)	1 (2.4%)
Fusobacterium necrophorum	**1 (2.4%)**	0 (0.0%)
Fusobacterium nucleatum	24 (58.5%)	8 (19.5%)
Fusobacterium periodonticum	4 (9.8%)	3 (7.3%)
Gemella hemolysans	3 (7.3%)	5 (12.2%)
Gemella morbillorum	2 (4.9%)	1 (2.4%)
Lachnoanaerobaculum orale	5 (12.2%)	4 (9.8%)
Lachnoanaerobaculum saburreum	**1 (2.4%)**	0 (0.0%)
Lactobacillus acidophilus	4 (9.8%)	3 (7.3%)
Lactobacillus fermentans	**1 (2.4%)**	0 (0.0%)
Leptotrichia buccalis	**3 (7.3%)**	0 (0.0%)
Parascardovia denticolens	**1 (2.4%)**	0 (0.0%)
Parvimonas micra	7 (17.1%)	4 (9.8%)
Pediococcus pentosaceus	**1 (2.4%)**	0 (0.0%)
Peptinophilus coxii	1 (2.4%)	1 (2.4%)
Peptostreptococcus anaerobius	**1 (2.4%)**	0 (0.0%)
Peptostreptococcus stomatitis	19 (46.3%)	13 (31.7%)
Porphymonas assacholytica	**1 (2.4%)**	0 (0.0%)
Porphyromonas somerae	3 (7.3%)	1 (2.4%)
Prevotella baroniae	**1 (2.4%)**	0 (0.0%)
Prevotella buccae	15 (36.6%)	5 (12.2%)
Prevotella denticola	2 (4.9%)	0 (0.0%)
Prevotella histicola	1 (2.4%)	1 (2.4%)
Prevotella heparinolytica	**1 (2.4%)**	0 (0.0%)
Prevotella intermedia	8 (19.5%)	1 (2.4%)
Prevotella melaninogenica	7 (17.1%)	2 (4.9%)
Prevotella oulorum	**1 (2.4%)**	0 (0.0%)
Prevotella oralis	1 (2.4%)	1 (2.4%)
Prevotella nigrescens	2 (4.9%)	1 (2.4%)
Prevotella timonensis	1 (2.4%)	1 (2.4%)
Rothia dentocariosa	**2 (4.9%)**	0 (0.0%)
Rothia mucilaginosa	6 (14.6%)	1 (2.4%)
Schaalia odontolytica	15 (36.6%)	8 (19.5%)
Selenomonas infelix	**1 (2.4%)**	0 (0.0%)
Selenomonas sputigena	**3 (7.3%)**	0 (0.0%)
Slackia exigua	**4 (9.8%)**	0 (0.0%)
Solobacterium moorei	2 (4.9%)	1 (2.4%)

Table 4. *Cont.*

Microorganisms	OSCC Side (*n*, %)	Control Side (*n*, %)
Tissierella praeacuta	**1 (2.4%)**	0 (0.0%)
Vagococcus lutrae	**1 (2.4%)**	0 (0.0%)
Veillonella dispar	11 (26.8%)	11 (26.8%)
Veillonella parvula	17 (41.5%)	15 (36.6%)
Veillonella rogosae	8 (19.5%)	5 (12.2%)

OSCC: oral squamous cell carcinoma; distinct species only present for either the OSCC or clinically intact surfaces are denoted in **boldface.**

Within the context of anaerobic species, tumorous surfaces were significantly more species-rich vs. the control surfaces (6.89 ± 1.24 vs. 4.07 ± 1.65; $p = 0.026$), representing nearly twice the cultivable species richness per 1 cm^2 surface sample. The strain of one species (*Acidaminococcus intestinii*) was detected exclusively on the control surface, while strains of 29 species were detected exclusively on the tumorous surfaces (i.e., *Actinobacillus porcinus*, *Actinomyces graevenitzii*, *A. naeslundii*, *Camplylobacter rectus*, *C. showae*, *Capnocytophaga gingivalis*, *Clostridium cadaveris*, *C. perfringens*, *C. tertium*, *Eggerthella cateniformis*, *Eubacterium brachy*, *Fusobacterium canifelinum*, *F. necrophorum*, *Lachnoanaerobaculum saburreum*, *Lactobacillus fermentans*, *Leptotrichia buccalis*, *Parascardovia denticolens*, *Pediococcus pentosaceus*, *Peptostreptococcus anaerobius*, *Porphymonas assacholytica*, *Prevotella baroniae*, *P. heparinolytica*, *P. oulorum*, *Rothia dentocariosa*, *Selenomonas infelix*, *Slackia exigua*, *S. sputigena*, *Tissierella praeacuta* and *Vagococcus lutrae*) (Table 4). However, some species were only found in a single patient (i.e., *A. porcinus*, *A. graevenitzii*, *A. naeslundii*, *C. rectus*, *C. showae*, *C. gingivalis*, *C. cadaveris*, *C. perfringens*, *C. tertium*, *E. brachy*, *F. necrophorum*, *L. saburreum*, *L. fermentans*, *P. anaerobius*, *P. assacholytica*, *P. baroniae*, *P. heparinolytica*, *P. oulorum*, *S. infelix*, *T. praeacuta*, *V. lutrae*), respectively. Statistically significant differences were noted in the presence of *Fusobacterium* spp. (33 vs. 12 isolates; $p = 0.013$) and *Prevotella* spp. (40 vs. 12; $p = 0.009$) between OSCC and control surfaces, respectively. In addition to the above-mentioned genera, *Veillonella* spp., *Peptostreptococcus* spp. and *Schaalia/Actinomyces* spp. were the most commonly detected.

4. Discussion

Oral and oropharyngeal cancers represent a substantial global public health burden, ranking among the most common malignancies worldwide and accounting for considerable cancer-related mortality [1–6]. Despite the availability of effective diagnostic and therapeutic approaches, delayed detection remains an important challenge for clinicians and healthcare systems as a whole, as patients may remain asymptomatic or present with non-specific symptoms until relatively advanced stages of disease. Consequently, late diagnosis is associated with increased morbidity and mortality and substantially poorer quality of life [11,15–18]. Furthermore, recent meta-analyses also point out that the receipt of a diagnosis of oral and oropharyngeal cancers is associated with some of the highest rates of suicidal ideation and suicide attempts in comparison to all cancers, compounding OSCC-associated disease burden [51,52]. In addition to established behavioural, environmental, viral, and host-related risk factors, increasing evidence suggests that alterations of the oral microbial environment may be relevant to the development and progression of OSCC [33–47,53]. Against this backdrop, the present self-controlled study investigated the cultivable aerobic-capnophilic, anaerobic, and fungal microbiota directly associated with HPV-negative OSCC tumor surfaces and compared it with that of clinically intact oral mucosal surfaces within the same patients, in a prospective, single-center design. While there were similar studies conducted in another major health centre in Hungary [54], no

similar research has previously been conducted in the Southern Transdanubia region, to the best of our knowledge. Our study therefore fills an important regional gap by providing the first comparative data on microorganisms associated with tumor surfaces in this specific OSCC patient population, which may serve as regional reference data and provide a basis for further investigations into the clinical significance of tumor-associated microbial alterations, the evaluation of targeted oral hygiene and infection-control procedures, and the design of larger multicenter studies.

Our main findings show that the microbial environment of tumor surfaces substantially differed from that of clinically intact mucosa, with the most pronounced differences involving the anaerobic component of the cultivable microbial flora. Whereas the richness of aerobic-capnophilic bacteria was broadly comparable between the two sampling sites, tumor surfaces harboured significantly greater anaerobic species richness, with numerous anaerobic species detected exclusively on tumor surfaces. Nonetheless, these results should be interpreted cautiously, particularly due to the fact that some of the species of interest were detected in only one patient. These results are consistent with studies linking OSCC not to the presence of a single pathogen but to the structural and functional reorganization—or dysbiosis—of the oral microbiome [33–47,53]. In particular, *Fusobacterium* spp. and *Prevotella* spp. were significantly more frequently recovered from tumor-associated samples than from clinically intact mucosa, with *F. nucleatum* being detected in 58.5% of tumor samples compared with 19.5% of control samples. Fungal recovery was also significantly more frequent on tumor surfaces, with *C. albicans* being the predominant fungal isolate. Therefore, our findings indicate that OSCC tumor surfaces are characterized by a distinct cultivable microbial environment, particularly involving enrichment of anaerobic bacteria and increased fungal recovery. Importantly, because samples were obtained from paired tumor and clinically intact sites within the same patients, these observations are less likely to be attributable solely to within-person differences (e.g., due to lifestyle habits, diet, oral hygiene practices, periodontal status, antimicrobial exposure) in the oral microbiota; this study design strengthens the interpretation that the observed differences are associated with the local tumor environment [55]. Nevertheless, the cross-sectional nature of the microbiological comparison does not allow us to determine whether these microbial alterations contribute to tumor development and progression, arise as a consequence of the tumor-associated microenvironment, or reflect a bidirectional interaction between the host, tumor, and resident microbiota. Following 24 months post-sampling, 53.7% of patients had succumbed to the disease. It must be noted that this high event rate reflects the advanced stage at initial presentation—characterized by a high proportion of T3 and T4 tumors—rather than administrative loss to follow-up. While regular stomato-oncological surveillance was maintained, this severe baseline prognosis considerably limits the feasibility of long-term longitudinal microbial analysis post-treatment, representing an important clinical constraint when evaluating microbial shifts in advanced OSCCs.

The greater anaerobic species richness observed on tumor surfaces is broadly consistent with previously published evidence demonstrating alterations in the oral or intratumoral microbiota across the development and progression of OSCC. Singh et al. demonstrated changes in intratumoral microbial composition from premalignant conditions through advanced stages of OSCC and reported associations with tumor immunological characteristics [56]. Zhang et al. similarly identified microbiological and functional differences according to OSCC stage and observed enrichment of *F. nucleatum* and other potentially pathogenic taxa in poorly differentiated tumors [57]. Using saliva samples, Mäkinen et al. also reported differences between patients with OSCC and healthy controls, including a higher proportion of potentially pathogenic bacteria [58]. Taken together, these findings suggest that the local environment associated with OSCC may favor the persistence

or enrichment of anaerobic microorganisms. The tumor surface may provide an altered ecological niche, characterized by factors such as reduced oxygen availability, tissue disruption, nutrient availability, and changes in local host immunity. Such conditions may facilitate colonization by anaerobic microorganisms and contribute to the maintenance of a distinct tumor-associated microbial community [33]. Importantly, our findings should not be interpreted as demonstrating that anaerobic bacteria are responsible for the development of OSCC (i.e., causal inference). Rather, they demonstrate that cultivable anaerobic species richness is greater at the tumor surface than at clinically intact mucosal sites in the same patients. This distinction is particularly important when interpreting culture-based observations in the context of the increasingly complex evidence base on oral dysbiosis and carcinogenesis.

One of the most notable findings was the higher prevalence of *Fusobacterium* spp. on tumor surfaces. *Fusobacterium* was detected on 26.8% of clinically intact surfaces compared with 68.3% of tumor surfaces. The difference was particularly pronounced for *F. nucleatum*, which was identified on 58.5% of tumor surfaces compared with 19.5% of clinically intact surfaces. This nearly threefold difference is consistent with previous clinical and experimental studies suggesting an association between *F. nucleatum* and OSCC [33–47,53]. Several earlier experimental studies provide plausible biological explanations for this association: for example, Li et al. reported that *F. nucleatum* may enhance OSCC cell proliferation through activation of the CDH1/β-catenin signaling pathway [59]; in addition, Nie et al. described a CXCL2-mediated interaction involving *F. nucleatum*, tumor cells, and macrophages that may contribute to tumor progression and an immunosuppressive microenvironment [60]. Similarly, Selvaraj et al. further demonstrated that *F. nucleatum* may promote invasive growth, inflammatory responses, and angiogenic processes in malignant oral keratinocytes, although these effects were dependent on bacterial strain and cell line [61]. The higher prevalence of *F. nucleatum* observed in our clinical samples is therefore biologically plausible in light of these experimental findings. However, the culture-based design of the present study cannot establish a causal relationship between the microorganism and tumor development or progression. Thus, the increased recovery of *F. nucleatum* may represent a factor contributing to the tumor microenvironment, a consequence of the ecological conditions created by the tumor, or a combination of both; to clarify the relationship, further studies are necessary.

Prevotella spp. were also detected substantially more frequently on tumor surfaces than on clinically intact surfaces, with prevalence rates of 61.0% and 29.3%, respectively. Namely, *P. buccae* was identified in 36.6% of tumor samples compared with 12.2% of clinically intact samples, while *P. intermedia* was detected in 19.5% and 2.4%, respectively. In contrast, *Veillonella* strains were relatively common at both sampling sites, with only modest differences between tumor and clinically intact surfaces. These results support the interpretation that OSCC-associated microbial alterations are likely to involve a polymicrobial community rather than a single pathogenic species. Interactions among anaerobic bacteria, including potential metabolic cooperation and effects on inflammatory and immune pathways, may collectively contribute to the characteristics of the tumor-associated microbial environment. Previous studies and reviews have proposed several mechanisms through which oral microorganisms may influence carcinogenesis and tumor progression, including chronic inflammation, immune modulation, epithelial–mesenchymal transition, invasion, and the effects on cancer stem cell-like properties [33–47,53].

In addition to bacterial differences, the present study identified a significant difference in fungal recovery between tumor and clinically intact surfaces. Fungal isolates were recovered more frequently from tumor surfaces than from clinically intact mucosa. *C. albicans* was the predominant fungal isolate, consistent with previous observations—including previous

similar results from Hungary [54]—that *Candida* species may be relevant components of the oral microbial environment associated with OSCC. The increased recovery of fungi from tumor surfaces may have several possible explanations. Local tissue damage, altered immune conditions, changes in oxygen availability, irregularities of the tumor surface, and interactions between bacteria and fungi may all influence fungal colonization. These findings therefore suggest that OSCC-associated microbial alterations should not be considered exclusively from a bacterial perspective. The biological relevance suggested for *Candida* spp. is also supported by existing experimental evidence [36,44–47,53,62,63]. In particular, *C. albicans* has been proposed to influence the local tumor microenvironment through inflammatory mechanisms and production of biologically active metabolites. Candidalysin, for example, may induce epithelial damage and inflammatory responses, while *Candida*-associated metabolism has been implicated in the generation of potentially carcinogenic compounds [37,44–47,53,62,63]. These findings were reinforced by the study of Vadovics et al. [64], where *C. albicans* resulted in enhanced pro-tumor signaling, inflammation and MMP activity, and processes associated with epithelial–mesenchymal transition in various *in vitro* and *in vivo* models. However, it must be noted, that the present study demonstrates increased fungal recovery rather than fungal infection, and the observed association should therefore be interpreted primarily as evidence of altered fungal colonization or recovery from tumor surfaces.

The findings of the present study may be interpreted within the broader concept of tumor-associated oral dysbiosis. The enrichment of anaerobic bacteria (such as *Fusobacterium* and *Prevotella* species) and increased recovery of fungi (predominantly *C. albicans*) on tumor surfaces may reflect an altered ecological niche generated by the tumor, which microorganisms may in turn influence via local inflammatory, metabolic, and immune processes. This creates the possibility of a bidirectional relationship between the tumor microenvironment and the resident microbiota, where structural and functional community reorganization promotes a pro-inflammatory, hypoxic niche favorable for tumor progression [33–47,53,62,63]. The molecular complexity of the relationship between tumors and the microbiota is illustrated by the comprehensive study of Cai et al. [65], where they simultaneously analyzed mucosal bacterial communities, the complete host transcriptome, and DNA CpG methylation in tumor and adjacent clinically intact tissues from 98 patients with HPV-negative OSCC, linking dysbiosis to genetic, transcriptional, and epigenetic alterations in host cells. Owing to its paired sampling design and specific inclusion criteria, this study is methodologically particularly comparable to our own tumor–control investigation.

The clinical significance of our findings should also be emphasized from the perspective of prevention and structured dental follow-up. In addition to reducing smoking and excessive alcohol consumption, regular dental examinations provide an opportunity for systematic assessment of the oral mucosa, recognition of potentially malignant lesions, and timely referral of suspicious lesions to specialist care. Following a cancer diagnosis, and preferably before the initiation of oncological treatment, a comprehensive dental assessment, management of odontogenic foci of infection, and individualized oral hygiene education are warranted, as emphasized in recent clinical frameworks focusing on comprehensive oral care after cancer therapy [66], as these measures may reduce the risk of opportunistic infections during treatment and late oral complications [67]. Based on the results of our study—i.e., the marked difference in the anaerobic component of the cultivable microbiota, suggesting the presence of a distinct microbial environment at the OSCC tumor surface—improvements in professional and home oral hygiene could theoretically reduce the elevated anaerobic and fungal burden observed on tumor surfaces [68]. However, interventional studies are needed to establish whether such improvements would also modify dysbiosis or the clinical outcome of the tumor. Taken together, the involvement of dentists and dental hygienists in

the work of the multidisciplinary oncological team should be regarded as a fundamental element of comprehensive OSCC care—not only to reduce treatment side effects but also to support prevention, early detection, and sustained QoL.

An important strength of the present study is its paired sampling design—in which each patient serves as their own control—in addition to the standardized sampling from 1 cm^2 areas of tumor and clinically intact mucosa, which enabled direct comparison within individuals and reduced the influence of inter-individual variation in oral microbial composition. The prospective design and use of conventional culture in combination with MALDI-TOF MS also enabled the identification of viable microorganisms recovered under the applied cultivation conditions. This is particularly relevant when considering the potential biological activity of viable bacterial and fungal isolates. Nonetheless, limitations of our study should be considered when interpreting its findings: firstly, the study was conducted at a single center and included a relatively small number of patients ($N = 41$, with no preceding sample size determination performed), which limits the generalizability and statistical power of the findings. Second, the study was cross-sectional with respect to microbiological sampling; therefore, temporal or causal relationships between microbial alterations and tumor development or progression cannot be established. The existing literature provides several potential mechanisms through which oral microorganisms may participate in carcinogenesis or tumor progression, including chronic inflammation, oxidative stress, modulation of host immune responses, microbial metabolites, epithelial signaling, invasion, and angiogenic processes; however, the present study cannot directly evaluate the relevance of these mechanisms. Third, culture-based methods do not capture the complete oral microbial community, and the observed species richness should therefore be interpreted as cultivable species richness rather than total microbial diversity. Furthermore, the limited discriminatory capacity of MALDI-TOF MS for closely related species (e.g., between oral streptococci, such as *S. mitis* and *S. oralis*) should be acknowledged [69]. Molecular sequencing approaches would be expected to detect microorganisms that were not recovered under the culture conditions used in the present study. Therefore, the present culture-based findings complement, rather than replace, the evidence generated by molecular and sequencing-based investigations. In addition, the clinically intact mucosal surfaces sampled in our study should not be regarded as equivalent to mucosa from healthy individuals without OSCC. Although this self-controlled, paired approach is advantageous for controlling inter-individual variation, the comparison cannot determine whether the clinically intact sites themselves differ from the oral microbiota of healthy individuals. Limitations listed above should be considered when formulating future studies, with the inclusion of culture-based, metagenomic, meta-transcriptomic, and functional methods.

Despite the above-listed methodological constraints, the broader clinical and public health relevance of this work must be emphasized: due to the considerable disease burden associated with OSCCs, there is an urgent and growing need for studies examining tumor-associated oral dysbiosis to heighten clinical awareness among dental and medical professionals, improve understanding of local microenvironmental factors, and advocate for the systematic integration of dental oncology into multidisciplinary treatment workflows. Our study contributes to this by providing foundational regional baseline data, paving the way for larger, multi-method investigations aimed at translating microbiological insights into enhanced supportive care and patient outcomes.

5. Conclusions

This prospective paired-sampling study demonstrates that OSCC tumor surfaces harbor a distinct cultivable microbial environment, compared with clinically intact oral mucosal surfaces within the same patients. The most pronounced differences involved the anaerobic

bacterial component, with significantly greater anaerobic species richness observed on tumor surfaces, whereas the richness of aerobic-capnophilic bacteria was broadly comparable between sampling sites. In particular, *Fusobacterium* spp. and *Prevotella* spp. were recovered more frequently from tumor-associated samples, with *F. nucleatum* showing a marked enrichment on tumor surfaces; furthermore, fungal recovery was also more frequent from tumor surfaces, with *C. albicans* representing the predominant fungal isolate. Our results support the notion that a distinct cultivable microbial environment (characterized by local oral dysbiosis) on OSCC surfaces may be relevant to future approaches involving oral monitoring, dental care, and supportive management of patients with oral malignancies. Nonetheless, causal associations remain to be established, as it is yet to be determined whether the presence of these microorganisms is a consequence of the tumor microenvironment, a contributor to tumor biology, or a manifestation of bidirectional host–tumor–microbiota interactions. Thus, longitudinal studies with larger and more diverse samples, involving multi-method (combining culture-based, metagenomic, meta-transcriptomic, and functional methods) analyses are needed to ascertain whether the identified microbial alterations can be used as diagnostic or prognostic biomarkers or as microbiome-based adjunctive therapeutic targets.

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Institutional Review Board Statement: The study was conducted in accordance with the Declaration of Helsinki (1975, last revised in 2024), and national and institutional ethical standards. Ethical approval for this study protocol was obtained from the Human Institutional and Regional Biomedical Research Ethics Committee, Medical School, University of Pécs (approval ID: 9503-2023, approval date: 31 March 2023; and approval ID: 9593-PTE 2024, approval date: 13 December 2024).

Informed Consent Statement: Before participation in our study, OSCC patients were provided with detailed information regarding the study's purpose, procedures, potential risks, and benefits. Written informed consent was obtained from each participant, ensuring that they understood their right to participate voluntarily and their right to withdraw at any time without any disadvantageous consequences or influence on their treatment. Confidentiality and anonymity were maintained throughout the study. The participants did not receive any incentives (monetary or otherwise) to take part in the study.

Data Availability Statement: The original contributions presented in this study are included in the article. Further inquiries can be directed to the corresponding author.

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References

1. World Cancer Researc Fund: Mouth and Oral Cancer Statistics. Available online: https://www.wcrf.org/preventing-cancer/cancer-statistics/mouth-and-oral-cancer-statistics/ (accessed on 12 September 2026).
2. Weatherspoon, J.D.; Chattopadhyay, A.; Boroudmand, S.; Garcia, A.I. Oral Cavity and Oropharyngeal Cancer Incidence Trends and Disparities in the United States: 2000–2010. *Cancer Epidemiol.* **2015**, *11*, 497–504. [CrossRef] [PubMed]
3. Ferlay, J.; Ervik, M.; Lam, F.; Laversanne, M.; Colombet, M.; Mery, L.; Piñeros, M.; Znaor, A.; Soerjomataram, I.; Bray, F. *Global Cancer Observatory: Cancer Today*; International Agency for Research on Cancer: Lyon, France. Available online: https://gco.iarc.who.int/today (accessed on 12 September 2026).
4. GBD 2021 Oral Disorders Collaborators. Trends in the global, regional, and national burden of oral conditions from 1990 to 2021: A systematic analysis for the Global Burden of Disease Study 2021. *Lancet* **2025**, *405*, 897–910. [CrossRef] [PubMed]
5. Filho, A.M.; Warnakulasuriya, S. Epidemiology of oral cancer in South and South-East Asia: Incidence and mortality. *Oral Dis.* **2024**, *30*, 4847–4854. [CrossRef] [PubMed]
6. Li, M.; Wang, C.; Wei, Y.; Qin, X.; Huang, W.; Zhou, B.; Huang, X. Global burden of lip and oral cavity cancer across adults aged ≥45 years from 1990 to 2021 and projections to 2050. *Tob. Induc. Dis.* **2025**, *23*, e192. [CrossRef] [PubMed]
7. Badewelan, M.; Muaddi, H.; Ahmed, A.; Lee, K.T.; Tran, S.D. Oral Squamous Cell Carcinoma and Concomitant Primary Tumors, What Do We Know? A Review of the Literature. *Curr. Oncol.* **2023**, *30*, 3721–3734. [CrossRef] [PubMed]
8. Rivera, C.; Venegas, B. Histological and molecular aspects of oral squamous cell carcinoma (Review). *Oncol. Lett.* **2014**, *8*, 7–11. [CrossRef] [PubMed]
9. Jayasinghe, R.D.; Siriwardena, B. Clinical Aspects of Oral Cancer and Potentially Malignant Disorders with Special Relevance to South Asia. *Ann. Maxillofac. Surg.* **2025**, *14*, 128–136. [CrossRef] [PubMed]
10. Kumari, P.; Debta, P.; Dixit, A. Oral Potentially Malignant Disorders: Etiology, Pathogenesis, and Transformation Into Oral Cancer. *Front. Pharmacol.* **2022**, *13*, e825266. [CrossRef] [PubMed]
11. Bradley, P.T.; Lee, Y.K.; Albutt, A.; Hardman, J.; Kellar, I.; Odo, C.; Randell, R.; Rousseau, N.; Tikka, T.; Patterson, J.M.; et al. Nomenclature of the symptoms of head and neck cancer: A systematic scoping review. *Front. Oncol.* **2024**, *14*, e1404860. [CrossRef] [PubMed]
12. González-Ruiz, I.; Ramos-García, P.; Ruiz-Ávila, I.; González-Moles, M.Á. Early Diagnosis of Oral Cancer: A Complex Polyhedral Problem with a Difficult Solution. *Cancers* **2023**, *15*, e3270. [CrossRef] [PubMed]
13. Khairuddin, A.N.M.; Bogale, B.; Kang, J.; Gallagher, J.E. Impact of dental visiting patterns on oral health: A systematic review of longitudinal studies. *BDJ Open* **2024**, *10*, e18. [CrossRef] [PubMed]
14. Garrido, C.; Sepúlveda, D.; Zúniga, R.; Cartes-Velásquez, R. Non-attendance at dental appointments: A brief review of causes and strategies for prevention. *Rev. Estomatol. Hered.* **2024**, *34*, e5331.
15. Rogers, S.N.; Chieng, C.Y.; Davies, A.; Aziz, A.; Lowe, D. Health-related quality of life and concerns in patients attending an oral and maxillofacial oncology review clinic: Late effects 5- and 10-year following cancer diagnosis. *Front. Oral Maxillofac. Med.* **2022**, *4*, e22. [CrossRef]
16. Bugshan, A.; Farooq, I. Oral squamous cell carcinoma: Metastasis, potentially associated malignant disorders, etiology and recent advancements in diagnosis. *F1000Research* **2020**, *9*, e229. [CrossRef] [PubMed]
17. Cancer Research UK: Survival for Mouth and Oropharyngeal Cancer. Available online: https://www.cancerresearchuk.org/about-cancer/mouth-cancer/survival (accessed on 12 September 2026).
18. Akhter, M.; Hossain, S.; Rahman, Q.B.; Molla, M.R. A study on histological grading of oral squamous cell carcinoma and its co-relationship with regional metastasis. *J. Oral Maxillofac. Pathol.* **2011**, *15*, 168–176. [CrossRef] [PubMed]
19. Liu, Y.; Wu, W.; Diao, J.; Yang, W.; Zheng, S.; Han, Y.; Yuan, C. Metabolic and microbial alterations in oral potentially malignant disorders versus oral squamous cell carcinoma. *Sci. Rep.* **2025**, *15*, e40637. [CrossRef] [PubMed]
20. Zhang, C.; Lan, Q.; Wei, P.; Gao, Y.; Zhang, J.; Hua, H. Clinical, histopathological characteristics and malignant transformation of proliferative verrucous leukoplakia with 36 patients: A retrospective longitudinal study. *BMC Oral Health* **2024**, *24*, e639. [CrossRef] [PubMed]
21. Villa, A.; Villa, C.; Abati, S. Oral cancer and oral erythroplakia: An update and implication for clinicians. *Aust. Dent. J.* **2011**, *56*, 253–256. [CrossRef] [PubMed]
22. Abati, S.; Bramati, C.; Bondi, S.; Lissoni, A.; Trimarchi, M. Oral Cancer and Precancer: A Narrative Review on the Relevance of Early Diagnosis. *Int. J. Environ. Res. Public Health* **2020**, *17*, e9160. [CrossRef] [PubMed]
23. Sowmya, S.; Sangavi, R. Prevalence of Oral Submucous Fibrosis With Other Oral Potentially Malignant Disorders: A Clinical Retrospective Study. *Cureus* **2023**, *15*, e49642. [CrossRef] [PubMed]

24. Dus-Ilnicka, I.; Malysa, A.; Mazur, M.; Stolarz-Paradowska, A. Different Approaches to Oral Lichen Planus Treatment: A Narrative Review. *Int. J. Mol. Sci.* **2026**, *27*, e914. [CrossRef] [PubMed]
25. Hecht, S.S.; Hatsukami, D.K. Smokeless tobacco and cigarette smoking: Chemical mechanisms and cancer prevention. *Nat. Rev. Cancer* **2022**, *22*, 143–155. [CrossRef] [PubMed]
26. Jiang, X.; Wu, J.; Wang, J.; Huang, R. Tobacco and oral squamous cell carcinoma: A review of carcinogenic pathways. *Tob. Induc. Dis.* **2019**, *17*, e29. [CrossRef] [PubMed]
27. Stornetta, A.; Guidolin, V.; Balbo, S. Alcohol-Derived Acetaldehyde Exposure in the Oral Cavity. *Cancers* **2018**, *10*, e20. [CrossRef] [PubMed]
28. Feng, L.; Wang, L. Effects of alcohol on the morphological and structural changes in oral mucosa. *Pak. J. Med. Sci.* **2013**, *29*, 1046–1049. [CrossRef] [PubMed]
29. Znaor, A.; Brennan, P.; Gajalakshmi, V.; Mathew, A.; Shanta, V.; Varghese, C.; Boffetta, P. Independent and combined effects of tobacco smoking, chewing and alcohol drinking on the risk of oral, pharyngeal and esophageal cancers in Indian men. *Int. J. Cancer.* **2003**, *105*, 681–686. [CrossRef] [PubMed]
30. Japan Esophageal Cohort Study Group. Alcohol consumption, smoking, and the implications of their cessations for field carcinogenesis in the esophagus: A 10-year prospective cohort study. *Lancet Reg. Health West Pac.* **2026**, *67*, e101798. [CrossRef] [PubMed]
31. Baba, S.K.; Alblooshi, S.S.E.; Yaqoob, R.; Behl, S.; Saleem, M.A.; Rakha, E.A.; Malik, F.; Singh, M.; Macha, M.A.; Akhtar, M.K.; et al. Human papilloma virus (HPV) mediated cancers: An insightful update. *J. Transl. Med.* **2025**, *23*, e483. [CrossRef] [PubMed]
32. Gong, Y.; Wei, B.; Yu, L.; Pan, W. Type 2 diabetes mellitus and risk of oral cancer and precancerous lesions: A meta-analysis of observational studies. *Oral Oncol.* **2015**, *51*, 332–340. [CrossRef] [PubMed]
33. Kövér, Z.; Nordskag, V.J.; Bán, Á.; Gajdács, M.; Urbán, E. The role of *Actinomyces* spp. and related organisms in cervicofacial infections: Pathomechanism, diagnosis and therapeutic aspects. *Anaerobe* **2023**, *82*, e102767. [CrossRef] [PubMed]
34. Shen, Y.; Fan, N.; Ma, S.X.; Cheng, X.; Yang, X.; Wang, G. Gut Microbiota Dysbiosis: Pathogenesis, Diseases, Prevention, and Therapy. *MedComm* **2025**, *6*, e70168. [CrossRef] [PubMed]
35. Li, E.; Hunag, J.; Yao, J.; Lei, M.; Wu, M. Oral microbiota dysbiosis and the oral-X axis in chronic diseases. *Genes Dis.* **2026**, 102274. [CrossRef]
36. Karpiński, T.M. Role of Oral Microbiota in Cancer Development. *Microorganisms* **2019**, *7*, e20. [CrossRef] [PubMed]
37. Bhuyan, R.; Bhuyan, S.K.; Mohanty, J.N.; Das, S.; Juliana, N.; Abu, I.F. Periodontitis and Its Inflammatory Changes Linked to Various Systemic Diseases: A Review of Its Underlying Mechanisms. *Biomedicines* **2022**, *10*, e2659. [CrossRef] [PubMed]
38. Cheng, C.Y.; Kuo, C.T.; Lin, C.C.; Hsieh, H.L.; Yang, C.M. IL-1β induces expression of matrix metalloproteinase-9 and cell migration via a c-Src-dependent, growth factor receptor transactivation in A549 cells. *Br. J. Pharmacol.* **2010**, *160*, 1595–1610. [CrossRef] [PubMed]
39. Liu, S.; Liu, J.; Wang, Y.; Deng, F.; Deng, Z. Oxidative Stress: Signaling Pathways, Biological Functions, and Disease. *MedComm* **2025**, *6*, e70268. [CrossRef] [PubMed]
40. Al-Taweel, F.B.; Douglas, C.W.I.; Whawell, S.A. The Periodontal Pathogen *Porphyromonas gingivalis* Preferentially Interacts with Oral Epithelial Cells in S Phase of the Cell Cycle. *Infect. Immun.* **2016**, *84*, 1966–1974. [CrossRef] [PubMed]
41. Lan, Z.; Liu, W.-J.; Cui, H.; Zou, K.-L.; Chen, H.; Zhao, Y.-Y.; Yu, G.-T. The role of oral microbiota in cancer. *Front. Microbiol.* **2023**, *14*, e1253025. [CrossRef] [PubMed]
42. Kushkevych, I.; Martínkova, K.; Mráková, L.; Giudici, F.; Baldi, S.; Novak, D.; Gajdács, M.; Vitézova, M.; Dordevic, D.; Amedei, A.; et al. Comparison of microbial communities and the profile of sulfate-reducing bacteria in patients with ulcerative colitis and their association with bowel diseases: A pilot study. *Microb. Cell* **2024**, *11*, 79–89. [CrossRef] [PubMed]
43. Barrak, I.; Stájer, A.; Gajdács, M.; Urbán, E. Small, but smelly: The importance of *Solobacterium moorei* in halitosis and other human infections. *Heliyon* **2020**, *6*, e05371. [CrossRef] [PubMed]
44. Semlali, A.; Al-Zharani, M.; Dahdah, M.; Chandad, F. *Candida albicans* in Oral Squamous Cell Carcinoma: From Microbial Dysbiosis to Tumor-Promoting Mechanisms and Translational Opportunities. *Int. J. Mol. Sci.* **2026**, *27*, e6118. [CrossRef] [PubMed]
45. Ayunintyas, N.F.; Mahdani, F.Y.; Pasaribu, T.A.S.; Chalim, M.; Ayna, V.K.P.; Santosh, A.B.R.; Santacroce, L.; Surboyo, M.D.C. Role of *Candida albicans* in Oral Carcinogenesis. *Pathophysiology* **2022**, *29*, 650–662. [CrossRef] [PubMed]
46. Park, K.; Veena, M.S.; Shin, D.S. Key Players of the Immunosuppressive Tumor Microenvironment and Emerging Therapeutic Strategies. *Front. Cell Dev. Biol.* **2022**, *10*, e830208. [CrossRef] [PubMed]
47. Lortal, L.; Lyon, C.M.; Sprague, J.L.; Sonnberger, J.; Paulin, O.K.A.; Wichramasinghe, D.N.; Richardson, J.P.; Hube, B.; Naglik, J.R. Candidalysin biology and activation of host cells. *mBio* **2025**, *16*, e00603–e00624. [CrossRef] [PubMed]
48. Kövér, Z.; Gajdács, M.; Polgár, B.; Szabó, D.; Urbán, E. The Microbiological Background of Medication-Related Osteonecrosis of the Jaw (MRONJ): Clinical Evidence Based on Traditional Culture and Molecular Biological Detection Methods. *Antibiotics* **2025**, *14*, e203. [CrossRef] [PubMed]

49. Opota, O.; Croxatto, A.; Prod'hom, G.; Greub, G. Blood culture-based diagnosis of bacteraemia: State of the art. *Clin. Microbiol. Infect.* **2015**, *21*, 313–322. [CrossRef] [PubMed]
50. Nashwan, A.J.; Joy, G.V. The 2024 Declaration of Helsinki Revision: Relevance to Nursing Research. *J. Adv. Nurs.* **2026**, *82*, 3986–3996. [CrossRef] [PubMed]
51. Bushi, G.; Khatib, M.N.; Singh, M.P.; Pattanayak, M.; Vishwakarma, T.; Ballal, S.; Bansal, P.; Gaidhane, A.M.; Tomar, B.S.; Ashraf, A.; et al. Prevalence of suicidal ideation, attempts and associated risk factors in oral cancer patients: A systematic review and meta-analysis. *BMC Oral Health* **2025**, *25*, e140. [CrossRef] [PubMed]
52. Nassar, S.I.; Durgham, R.A.; Nguyen, S.A.; Newman, J.G.; Kejner, A.E.; Albergotti, W.G. Risk Factors for Suicide Among Head and Neck Cancer Patients: A Systematic Review and Meta-Analysis. *Head Neck* **2025**, *47*, 2231–2247. [CrossRef] [PubMed]
53. Talapko, J.; Erić, S.; Meštrović, T.; Stipetić, M.M.; Juzbašić, M.; Katalinić, D.; Bekić, S.; Muršić, D.; Flam, J.; Belić, D.; et al. The Impact of Oral Microbiome Dysbiosis on the Aetiology, Pathogenesis, and Development of Oral Cancer. *Cancers* **2024**, *16*, e2997. [CrossRef] [PubMed]
54. Berkovits, C.; Tóth, A.; Szenzenstein, J.; Deák, T.; Urbán, E.; Gácser, A.; Nagy, K. Analysis of oral yeast microflora in patients with oral squamous cell carcinoma. *Springerplus* **2016**, *5*, e1257. [CrossRef] [PubMed]
55. Gran, S. Within-person studies—What, when and how? *Br. J. Dermatol.* **2019**, *180*, 445–446. [CrossRef] [PubMed]
56. Singh, R.P.; Kumari, N.; Gupta, S.; Jaiswal, R.; Mehrotra, D.; Singh, S.; Mukherjee, S.; Kumar, R. Intratumoral Microbiota Changes with Tumor Stage and Influences the Immune Signature of Oral Squamous Cell Carcinoma. *Microbiol. Spectr.* **2023**, *11*, e0459622. [CrossRef] [PubMed]
57. Zhang, M.; Zhang, H.; Hong, A.; Huang, J.; Yang, L.; Long, Y.; Yu, Z. Dynamic changes of dental plaque and saliva microbiota in OSCC progression. *Clin. Oral Investig.* **2025**, *29*, e314. [CrossRef] [PubMed]
58. Makinen, A.I.; Pappalardo, V.Y.; Bujis, M.J.; Brandt, B.W.; Makitie, A.A.; Meurman, J.H.; Zaura, E. Salivary microbiome profiles of oral cancer patients analyzed before and after treatment. *Microbiome* **2023**, *11*, e171. [CrossRef] [PubMed]
59. Li, Z.; Liu, Y.; Huang, X.; Qang, Q.; Fu, R.; Wen, X.; Liu, J.; Zhang, L. *F. Nucleatum* enhances oral squamous cell carcinoma proliferation via E-cadherin/β-Catenin pathway. *BMC Oral Health* **2024**, *24*, e518. [CrossRef] [PubMed]
60. Nie, F.; Zhang, J.; Tian, H.; Zhao, J.; Gong, P.; Wang, H.; Wang, S.; Yang, P.; Yang, C. The role of CXCL2-mediated crosstalk between tumor cells and macrophages in *Fusobacterium nucleatum*-promoted oral squamous cell carcinoma progression. *Cell Death Dis.* **2024**, *15*, e277. [CrossRef] [PubMed]
61. Selvaraj, A.; McManus, G.; Healy, C.M.; Moran, G.P. *Fusobacterium nucleatum* induces invasive growth and angiogenic responses in malignant oral keratinocytes that are cell line- and bacterial strain-specific. *Front. Cell. Infect. Microbiol.* **2024**, *14*, e1417946. [CrossRef] [PubMed]
62. Di Cosola, M.; Cazzolla, A.P.; Charitos, I.A.; Ballini, A.; Inchigolo, F.; Santacroce, L. *Candida albicans* and Oral Carcinogenesis. A Brief Review. *J. Fungi* **2021**, *7*, e476. [CrossRef] [PubMed]
63. Malavika, G.; Ravi, S.S.S.; Maheswary, D.; Leela, K.V.; Lathakumari, R.H.; Priya, K.S.L. Role of *Candida albicans* in chronic inflammation and the development of oral squamous cell carcinoma. *Cancer Pathog. Ther.* **2025**, *3*, 402–410. [CrossRef] [PubMed]
64. Vadovics, M.; Ho, J.; Igaz, N.; Alföldi, R.; Rakk, D.; Veres, É.; Szücs, B.; Horváth, M.; Tóth, R.; Szücs, A.; et al. *Candida albicans* Enhances the Progression of Oral Squamous Cell Carcinoma *In Vitro* and *In Vivo*. *mBio* **2022**, *13*, e0314421. [CrossRef] [PubMed]
65. Cai, L.; Zhu, H.; Mou, Q.; Wong, P.Y.; Lan, L.; Ng, C.W.K.; Lei, P.; Cheung, M.K.; Wang, D.; Wong, E.W.Y.; et al. Integrative analysis reveals associations between oral microbiota dysbiosis and host genetic and epigenetic aberrations in oral cavity squamous cell carcinoma. *npj Biofilms Microbiomes* **2024**, *10*, e39. [CrossRef] [PubMed]
66. Jawor, N.; Pogoda, K.; Ziętek, M.; Skośkiewicz-Malinowska, K. Current approaches to comprehensive oral care after cancer therapy. *Dent. Med. Probl.* **2026**, *63*, 1043–1057. [CrossRef] [PubMed]
67. Behkar, S.; Golchini, P.; Kocamaz, Ö.F.; Altundongan, S. Evaluation of dentists' awareness, knowledge, and clinical practices regarding early-stage oral cancer lesions in Türkiye: A cross-sectional study. *PLoS ONE* **2026**, *21*, e0341849. [CrossRef] [PubMed]
68. Gupta, N.; Pal, M.; Rawat, S.; Grewal, S.M.; Garg, H.; Chauhan, D.; Ahlawat, P.; Tandon, S.; Khurana, R.; Pahuja, A.K.; et al. Radiation-induced dental caries, prevention and treatment—A systematic review. *Natl. J. Maxillofac. Surg.* **2015**, *6*, 160–166. [CrossRef] [PubMed]
69. Ikryannikova, L.N.; Filimonova, A.V.; Malakhova, M.V.; Savinova, T.; Filimonova, O.; Ilina, E.N.; Dubovickaya, V.A.; Sidorenko, S.V.; Govorun, M.V. Discrimination between Streptococcus pneumoniae and Streptococcus mitis based on sorting of their MALDI mass spectra. *Clin. Microbiol. Infect.* **2013**, *19*, 1066–1071. [CrossRef] [PubMed]

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