

ISOLATION AND IDENTIFICATION OF ORAL MICROBIOTA IN HEAD AND NECK CANCER PATIENTS BEFORE AND AFTER RADIOTHERAPY

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ABSTRACT

Head and neck cancer (HNC) is one of the most common malignancies worldwide and is associated with significant oral complications following radiotherapy. Radiotherapy alters the oral environment by reducing salivary flow, changing oral pH, and disrupting the normal microbial ecosystem, thereby increasing the risk of dental caries and opportunistic infections. The present hospital-based analytical study evaluated changes in oral microflora and dental problems in head and neck cancer patients before and after radiotherapy. Twenty patients diagnosed with HNC were recruited after informed consent. Oral swabs were collected from the buccal mucosa, tongue, and dental plaque before and after radiotherapy. Samples were cultured on nutrient agar, and bacterial isolates were identified using Gram staining and biochemical tests. Dental status was assessed using the Decayed, Missing, and Filled Surfaces (DMFS) index. Healthy individuals were included for comparison. The predominant microorganisms isolated were *Streptococcus mitis*, *Streptococcus mutans*, *Ligilactobacillus salivarius*, *Staphylococcus aureus*, *Staphylococcus epidermidis*, *Micrococcus luteus*, *Actinomyces* spp., *Proteus vulgaris*, and *Bacillus subtilis*. Candida species were isolated from two patient samples. A reduction in bacterial colony counts after radiotherapy and an increase in DMFS scores in most patients indicated deterioration of oral health following treatment. These findings emphasize the importance of preventive dental care, maintenance of oral hygiene, and microbiological monitoring in patients undergoing radiotherapy.

KEYWORDS: Head and Neck Cancer, Oral Microflora, Radiotherapy, Dental Caries, Xerostomia, and Oral Health.

INTRODUCTION

Head and neck cancer (HNC) comprises a heterogeneous group of malignant tumors arising from the oral cavity, pharynx, larynx, nasal cavity, paranasal sinuses, and salivary glands.^[1] It is one of the leading causes of cancer-related morbidity and mortality worldwide, with a particularly high burden in developing countries such as India. The incidence of HNC has increased over recent decades due to widespread use of tobacco in both smoked and smokeless forms, excessive alcohol consumption, poor oral hygiene, and infection with human papillomavirus (HPV).^[2] Squamous cell carcinoma (SCC) constitutes approximately 90% of all head and neck malignancies and commonly affects the tongue, buccal mucosa, floor of the mouth, gingiva, and oropharynx. The treatment of HNC include surgery, radiotherapy and chemotherapy.^[3] Radiotherapy is an essential treatment modality for head and neck cancer, either as a primary treatment or in combination with surgery and chemotherapy. Ionizing radiation destroys malignant cells by causing irreversible DNA damage and

inhibition of cell division.^[4] Despite its effectiveness against tumors, radiotherapy also damages adjacent healthy tissues within the radiation field. The salivary glands, oral mucosa, periodontal tissues, teeth, muscles of mastication, and jaw bones are particularly susceptible to radiation injury. Consequently, patients often develop xerostomia, oral mucositis, dysphagia, taste disturbances, trismus, radiation caries, periodontal disease, and osteoradionecrosis. These complications significantly impair nutrition, speech, oral function, and quality of life.^[5]

The oral cavity is a complex ecological environment inhabited by more than 700 microbial species, including bacteria, fungi, viruses, and protozoa.^[6] Under healthy conditions, these microorganisms coexist in a balanced relationship with the host and contribute to oral health by preventing colonization by pathogens, supporting immune defense, and maintaining ecological stability.^[7] The composition of the oral microbiome is influenced by saliva, diet, oral hygiene, immune status, and

environmental conditions. Any disturbance in this balance, known as microbial dysbiosis, may result in dental caries, periodontal disease, candidiasis, and other oral infections.^[8]

Saliva plays a central role in maintaining oral homeostasis. It lubricates oral tissues, facilitates mastication and swallowing, buffers bacterial acids through bicarbonate ions, provides calcium and phosphate for enamel remineralization, and contains antimicrobial components such as lysozyme, lactoferrin, lactoperoxidase, and secretory immunoglobulin A (IgA).^[9] Radiotherapy-induced damage to the salivary glands causes a marked reduction in salivary flow and changes in saliva composition.^[10] These changes decrease the natural protective mechanisms of the oral cavity, creating an environment favourable for acidogenic bacteria and opportunistic fungi.^[11] Among the oral complications associated with radiotherapy, radiation caries is one of the most destructive. Unlike conventional dental caries, radiation caries progresses rapidly and frequently involve the cervical margins, incisal edges, and cusp tips of teeth. The disease develops due to the combined effects of salivary dysfunction, altered oral microflora, reduced buffering capacity, increased plaque accumulation, and changes in tooth structure. If untreated, radiation caries may lead to extensive destruction of dentition within a short period.^[12]

Radiotherapy also causes significant alterations in oral microflora. Studies have demonstrated an increase in cariogenic microorganisms such as *Streptococcus mutans* and *Lactobacillus* species, along with opportunistic fungal pathogens including *Candida* species. In contrast, several beneficial commensal bacteria decrease following radiotherapy. This microbial imbalance contributes not only to dental caries but also to oral mucosal infections, delayed healing, and reduced quality of life in cancer patients.^[13]

Considering these complications, early diagnosis and continuous monitoring of oral microflora are important components of comprehensive cancer care. Preventive dental evaluation before radiotherapy, maintenance of oral hygiene, fluoride application, antimicrobial mouth rinses, and regular dental follow-up can reduce oral complications and improve treatment outcomes. An interdisciplinary approach involving oncologists, dentists, microbiologists, and oral medicine specialists is therefore essential for the successful management of head and neck cancer patients receiving radiotherapy. The present study was undertaken to evaluate dental problems associated with oral microflora in head and neck cancer patients before and after radiotherapy. Oral microorganisms were isolated and identified using culture-based microbiological techniques, while dental status was assessed using the DMFS index. The findings are expected to contribute to improved understanding of radiation-induced microbial alterations and to support the

development of preventive strategies aimed at preserving oral health and enhancing the quality of life of patients undergoing cancer therapy.

MATERIALS AND METHODS

The present study was a hospital-based prospective analytical study conducted to evaluate dental problems associated with oral microflora in head and neck cancer patients undergoing radiotherapy. The study compared oral microbial changes and dental status before and after radiotherapy and also included healthy individuals as controls for comparison.

Patient recruitment and Duration of Study

The patient was selected and recruited from OPD of Dr. Vidhi Gupta, Department of Radiation Oncology, Jawaharlal Nehru Cancer Hospital and Research Centre (JNCHRC), Bhopal, Madhya Pradesh, India. The study time period includes 6 months. The patient selected with oral and written consent on the basis of inclusion and exclusion criteria given below and before sample collection ethical approval taken.

Ethical Approval

The study was conducted after obtaining approval from the Institutional Ethics Committee of Jawaharlal Nehru Cancer Hospital and Research Centre. Written informed consent was obtained from every participant before sample collection. Confidentiality of patient information was maintained throughout the study.

Inclusion Criteria

- Patients diagnosed with head and neck cancer
- Patients scheduled for radiotherapy
- Patients above 18 years of age
- Patients willing to participate in the study
- Patients who provided written informed consent

Exclusion Criteria

- Patients receiving antibiotic or antifungal therapy
- Patients with systemic diseases affecting oral flora
- Patients who had undergone previous radiotherapy
- Patients unwilling to participate
- Patients with severe systemic illness

Demographics and History of Patient

The patient demographics and history collected from Medical Record Department (MRD), JNCH&RC, Bhopal. The history of those patients collected which agreed to participate in the study.

Dental Assessment

Dental health was assessed using the Decayed, Missing and Filled Surfaces (DMFS) Index, a standardized method for evaluating dental caries experience. DMFS scores were recorded before and after radiotherapy to assess treatment-related changes in dental status. As per analysis total number of 28 teeth present in patient mouth were counted before and after radiotherapy.^[14] A patient

having increased tooth decay post- radiotherapy (RT) was placed in the DMFS [+] (increase) group.

Sample Collection

Oral swab samples were collected under aseptic conditions from the buccal mucosa, tongue, and dental plaque before initiation of radiotherapy and repeated sample taken after completion of radiotherapy. Sterile cotton swabs were immediately transported to the microbiology laboratory for culture and identification of microorganisms. Similarly, samples were also collected from healthy individuals for comparison of oral flora.

Buccal Smear

The oral samples were examined for human epithelial, cheek cells morphology and abnormality with micronucleus. The buccal smear prepared on clean slide, allowed to dry at room temperature and fixed in methanol. The slides were stained with giemsa for 10 minutes, washed, dried and examined under microscope 10 X and 20 X for nuclear abnormalities, micronucleus and cytomorphology.

Microbiological Analysis

Collected samples from HNC patients and healthy individuals were inoculated on Nutrient Agar plates and incubated for 24 hours, observed for colony morphology and characteristics. The bacteria identified by gram's staining and biochemical tests. The results are compared in samples of pre and post radiotherapy, as well as healthy individuals.

Biochemical tests

The bacterial colonies were identified by gram's staining and cultured in nutrient broth. The isolated bacteria were identified by biochemical tests. After gram's staining, bacterial identification was confirmed by biochemical tests. The biochemical tests include catalase, coagulase, triple sugar iron (TSI), hemolysis, citrate utilization, urease, methyl red and voges – proskauer test.

Catalase Test

This test helps to differentiate *Staphylococci* from *Streptococci*. The enzyme catalase, acts as a catalyst in the breakdown of hydrogen peroxide to oxygen and water. On a clean slide colonies were emulsified of test organism in distilled water taken from petri dish. To it 1-2 drops of hydrogen peroxide (H_2O_2) was added and covered with the plate. Formation of gas bubbles indicated positive reaction. The *Staphylococcus* species gives positive results whereas, *Streptococcus* gives negative result. Fresh culture was taken about 24-hour old for test and H_2O_2 solution kept in black bottle and shaken before use.

Coagulase Test

This test is used to differentiate *Staphylococcus aureus* from other *Staphylococci*. The enzyme, coagulase, causes plasma to clot by converting fibrinogen to fibrin. Coagulase is produced by *Staphylococcus aureus*. It is considered a pathogenicity test for *Staphylococcus*

aureus. The test is performed on slide by taking two drops of saline, to it colonies were emulsified in each drops to make thick suspensions. A loop was taken dipped in undiluted plasma and mixed with the bacterial suspensions in such a way that adhering traces of plasma goes into one of the bacterial suspensions. The immediate coarse clumping of the mixture (within 5 – 10 seconds) were examined. The clumping indicates positive coagulase test. No plasma was added to the other suspension. It serves as negative control to the test and to differentiate non – specific granular appearance from true coagulase clumping.

The Triple Sugar Iron (TSI) Test

The Triple Sugar Iron (TSI) test is a microbiological test named for its ability to test a microorganism's ability to ferment sugars and to produce hydrogen sulfide. Carbohydrate fermentation is indicated by the production of gas and a change in the color of the pH indicator from red to yellow. The TSI media was prepared 500 mL and poured in sterile test tube with cotton plug. The tubes were kept at tilting position to prepare slants. After solidification. The media used further for inoculation of bacteria and bacterial suspension was taken using sterile loop. The loop was sterilized by heating in flame to red hot. The TSI slants were inoculate by streaking on surface of agar slant as well as stabbing in center of medium. Cotton plugs were applied and tubes were kept in incubator at 37 °C in ambient air for 18 to 24 hours. The medium reaction was examined by color change given below.

- An alkaline/acid (red slant/yellow butt) reaction: It is indicative of dextrose fermentation only.
- An acid/acid (yellow slant/yellow butt) reaction: It indicates the fermentation of dextrose, lactose and/or sucrose.
- An alkaline/alkaline (red slant, red butt) reaction: Absence of carbohydrate fermentation results.
- Blackening of the medium: Occurs in the presence of H_2S
- Gas production: Bubbles or cracks in the agar indicate the production of gas formation of CO_2 and H_2S

The test is used primarily to differentiate members of the Enterobacteriaceae family from other gram-negative rods. It is also used in the differentiation among Enterobacteriaceae on the basis of their sugar fermentation patterns.

Hemolysis Test

Hemolysis test is one to identify bacteria which causes hemolysis of blood. Blood agar plates were used for the growth of pathogenic organisms that are capable of producing extracellular enzymes that cause hemolysis of the blood. Sheep blood is mostly used for Group A *Streptococci* as it provides the best results, but it fails to support the growth of *Haemophilus haemolyticus*. It is because sheep blood is deficient in pyridine nucleotides.

Four different types of hemolysis are observed on blood agar which can each be identified by a zone of hemolysis present around the growing colonies alpha by greenish – grey or brownish discoloration around colony due to partial lysis of red blood cells. Beta hemolysis is defined by a clear zone of hemolysis under and around the colonies when grown on blood agar. β -hemolytic bacteria include group A *Streptococci* like *S. pyogenes* and group B streptococcus like *S. agalactiae*, both of which are associated with severe infections in humans. Gamma hemolysis is also called non-hemolysis as no lysis of red blood cells occurs. As a result, no change of coloration or no zone of hemolysis is observed under or around the colonies. Species like *Neisseria meningitidis* are non-hemolytic or gamma-hemolytic. In the present study Sheep blood agar prepared plates were procured from Himedia were used for the study. The pure culture strains were inoculated onto plates and plates were incubated at 37 ° C for 24 hours. The plates were examined after 24 hours for hemolysis and bacterial identification.

Citrate Utilization test

This test is used for the identification of enterobacteria and one of the important test. Some bacteria can utilize citrate as a sole source of carbon and metabolise to acetoin and CO₂, as well as ammonium as its only source of nitrogen. The pure culture bacterial colonies inoculated on Simmon's Citrate agar (Himedia) by stab culture. A growth of blue colour in Simmon's agar, indicates positive result with citrate utilization. The *E. coli* gives negative result and *Klebsiella pneumonia* gives positive result.

Urease Test

The enzyme urease is possessed by some bacteria. The urease is able to decompose urea by hydrolysis to give ammonia and carbon dioxide. This reaction turns the medium alkaline which is shown by a change in colour of the indicator to red pink. Christensen's Urea basal medium prepared without glucose and urea, pH adjusted to 6.8 – 6.9. The medium sterilized at 121° C for 20 minutes, cooled to 50°C, glucose and urea added and sterilized beforehand by filtration. The medium prepared, solidified and inoculated entire surface of slope with bacterial colonies, incubated at 37 ° C in incubator. After overnight incubation plates observed for red – pink colour of urease positive test. The *Proteus vulgaris* gives positive result and *Escherichia coli* gives negative.

Methyl Red (MR) Test

It detects the production of sufficient acid during the fermentation of glucose and differentiate enterobacteria. Some bacteria with extended incubation times, are able to maintain the pH levels at below 4.5 demonstrated by a change in colour of methyl red indicator which is added at the end of the incubation period. Methyl Red is an azo dye pH indicator that is specifically designed to detect highly acidic environments. The bacterial colonies inoculated in a tube containing MR – VP broth,

incubated at 37 ° C for 48 hours up to 5 days to allow sufficient acid to be produced. A few drops of the Methyl Red indicator solution are added to the incubated broth. A red colour indicates a positive (acid) reaction. The positive control is *E. coli* and negative control is *Enterobacter aerogens*.

Voges – Proskauer Test

Some bacteria ferment carbohydrates with the production of acetoin (acetyl methyl carbinol). Under alkaline condition, the acetoin is oxidised to diacetyl which gives a pink colour with the reagent. The test is usually done in conjunction with the methyl red (MR) test. The production of acetoin usually results in inadequate acid concentration during fermentation to give an MR positive reaction. Therefore, an organism is either MR or VP positive.

The bacterial colonies inoculated in a tube containing MR – VP broth, incubated at 37°C for 48 hours. After 48 hours 1 mL 10% potassium hydroxide added, incubated for one hour at room temperature and examined for appearance of pink color for positive result. The *Escherichia coli* is positive and *Klebsiella pneumonia* is negative with no color.

Statistical analysis

All experiments were performed in triplicate and results are expressed as mean $\pm$ standard deviation (SD). The mean, standard deviation and percentage reduction values were calculated in MS excel.

RESULTS

A total of 20 patients diagnosed with head and neck cancer were included in the study as per inclusion and exclusion criteria and consent. Patients represented different age groups and both sexes. The majority of cases belonged to the middle-aged and elderly population, reflecting the higher prevalence of head and neck cancer in these age groups. Healthy individuals were included as controls for comparison of oral microflora. Ethical approval taken from Institutional Ethical Committee of Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. The ethical approval number 1130/JNCH/RES/08/04/2026, dated 08 April 2026.

Demographics and Clinical History Collection

The demographics and history of HNC patients were collected from Medical Record Department (MRD), Jawaharlal Nehru Cancer Hospital and Research Centre, Bhopal. Out of 20 patients 13 patients were of Squamous Cell carcinoma and most of them were of grade – 2. All of them except two were habitual of tobacco eating. Eight were having smoking habits smokers and 5 were alcoholic. The age limits were from 30 – 75 age group and most of them were middle aged in fifties. No previous history was found in most of the patients except diabetes in two and hypertension in one patient. The diet habits showed most of them are vegetarian. The patient

selected for the study includes pre and post radiotherapy. The radiotherapy patients were given betadine mouth wash thrice daily to resist infection.

Assessment of Tooth decay

The results were taken after a week to 10 days and the time period is very short to calculate DMFS score. The time period to calculate should be one to two month ideally to determine DMFS score. The other reason of better DMFS score in most of the patients is due to dental care also as most of the patients are prescribed

mouth wash by oncologist. Radiotherapy to the head and neck region causes xerostomia and salivary gland dysfunction which dramatically increases the risk of dental caries and its sequelae. Radiation therapy (RT) also affects the dental hard tissues increasing their susceptibility to demineralization following RT. Post radiation caries is a rapidly progressing and highly destructive type of dental caries. The patient does not develop plaque or major dental problems. The results were given in tabular form 1.

Table 1: Patient DMFS Score before and after Radiotherapy.

No. of patients	Decayed (D)		Missing (M)		Filled Surfaces (F)		Score (S)	
	Before	After	Before	After	Before	After	Before	After
1.	3	3	12	11	0	0	+	-
2.	6	6	6	6	0	0	+	+
3.	4	4	2	2	0	0	+	+
4.	5	5	7	4	0	0	+	-
5.	7	7	5	5	0	0	+	+
6.	3	3	6	6	0	0	+	+
7.	2	2	4	4	0	0	+	+
8.	4	4	4	4	0	0	+	+
9.	3	3	8	8	0	0	+	+
10.	1	1	2	2	0	0	+	+
11.	3	3	12	12	0	0	+	+
12.	4	2	5	5	0	0	+	-
13.	5	5	3	3	0	0	+	+
14.	3	3	6	6	0	0	+	+
15.	2	1	6	6	0	0	+	-
16.	4	4	4	4	0	0	+	+
17.	6	6	4	1	0	0	+	-
18.	4	4	1	1	0	0	+	+
19.	4	4	2	2	0	0	+	+
20.	2	2	1	1	0	0	+	+

Buccal Smear

Buccal smear prepared and analyzed in head and neck cancer patients for exfoliative cytology to monitor cytogenetic damage, side effects of radiotherapy and cellular atypia. For head and neck cancer patients, buccal smears (exfoliative cytology) are primarily used to monitor cytogenetic damage, radiation/chemotherapy side effects, and track cellular atypia. Buccal smear is prepared to examine human epithelial cells especially in tobacco consumption people. The HNC patients slide showed fragmented nuclei, binucleated nuclei and micronucleus (Figure 3). Out of 20 patient samples 5 samples showed micronuclei, especially in male buccal smear. The male patients prone to eat tobacco had shown micronuclei in their buccal smear. The post radiotherapy patient's results were same as pre radiotherapy samples taken before radiotherapy and not much difference seen. The results were compared with healthy individuals and no prominent changes in epithelial cells were observed as none of the healthy individual are tobacco user.

In another study done on head and neck cancer patients the genetic damage and environmental exposure in buccal cells were evaluated. The micronucleus (MN) and Bi-nucleated (BN) in exfoliated cells of buccal mucosa showed significant increase ($P < 0.001$ and < 0.05 , respectively) in the cases in relation to the control group after controlling risk factors like tobacco smoking and chewing, alcohol drinking, amalgam fillings, and occupational exposure of HNC. There was a significant difference found between smokers and nonsmokers in the frequency of BN among patient and control groups ($P = 0.046$ and 0.045 , resp.). Furthermore, a significant difference between smokers and nonsmokers was found in the MN frequency among HNC patients ($P = 0.030$). Among patient group, the MN frequency of participants occupationally exposed (6.99/1000 cells) was significantly higher than that of nonexposed (4.70/1000 cells) ($P = 0.014$). There was no significant difference between participants with filling material amalgam and those without filling material amalgam, in the frequency of anomalous cells in cancer patients and healthy controls.^[15]

A case control study conducted in Khartoum, Sudan. Papanicolaou stained oral mucosal cells were obtained from 100 cancer patients receiving radiotherapy and/or chemotherapy (ascertained as cases), 50 cancer patients not exposed to either therapy (control 1), and 50 apparently healthy individuals (control 2). The data was analyzed by using a computer SPSS program, to obtain the Chi-square test. Without prior knowledge of the subjects' group, oral epithelial atypia was detected in 7% of the cases. Inconclusive features of cytological atypia were observed in 13% of the cases. Atypia was not observed in both the control groups. Inflammatory infiltrate and viral cytopathic effects were identified in 32% and 8% of the cases respectively. Cytological atypia, viral infections, and inflammatory infiltrates were detected after exposure to radiotherapy and/or chemotherapy.^[16]

Results of growth on culture plates of HNC patients and healthy Individuals

The growth observed after 24 hrs of incubation. The colonies were counted, its morphology, color and texture noted given in table 7 & 8 for Pre samples, and 8 & 9 for Post and 10 & 11 for Healthy Individuals. The colonies morphology in most of the samples were of white and yellow. The number of colonies counted and it varies from approximately 500 to 20 colonies. The colonies were smooth, mucoid and butyrous. The growth of

bacteria reduced in post samples when compared with pre. The mouth of the sample contains commensal which reduces due to radiotherapy. Two of the samples showed growth of fungus along with bacterial growth (Figure 15 – 19). The growth after 24 hours similar like radiotherapy patients but type of colonies was found different than HNC patients (Figure 20 and 21). Following completion of radiotherapy, significant alterations in oral microflora were observed. A reduction in overall bacterial colony counts was noted, whereas opportunistic microorganisms became relatively more prominent. *Candida* species were isolated from two patients after radiotherapy. These findings indicate that radiation therapy altered the normal oral microbial ecology and favored microbial dysbiosis.

Biochemical tests

The bacterial strains identified by biochemical tests and results are given below in tabular form. Total 10 bacterial isolates were identified were gram positive and negative cocci, bacilli *Streptococcus mitis*, *Ligilactobacillus salivarius*, *Streptococcus mutans*, *Micrococcus luteus*, *Staphylococcus epidermidis*, *Staphylococcus aureus*, *Actinomyces*, *Proteus vulgaris* and *Bacillus subtilis*. As well as fungus isolated in two samples *Candida* species. The bacterial identification by biochemical tests are given in Table 7.

Table 2: Results of Biochemical tests.

Bacterial strains	Catalase	Coagulase	Citrate Utilization	Urease	MR/VP	TSI	Hemolysis
<i>Streptococcus mitis</i>	-	-	-	-	+/-	No gas, no acid, no H ₂ S (Negative)	Alpha hemolysis
<i>Ligilactobacillus salivarius</i>	-	-	-	-	-/-	Yellow slant and butt due to acid production	Gamma hemolysis
<i>Streptococcus mutans</i>	-	-	-	-	-/+	Yellow slant and butt due to acid production	Alpha hemolysis
<i>Micrococcus luteus</i>	+	-	-	+	-/-	Negative with no color change	Gamma hemolysis
<i>Staphylococcus epidermidis</i>	+	-	-	+	-/+	Negative with no color change	Gamma hemolysis
<i>Staphylococcus aureus</i>	+	+	+	+	+/+	Yellow slant and butt due to acid production	Beta hemolytic
<i>Actinomyces</i> species	-	-	+	-	-/-	H ₂ S production in TSI with blackening of media	Gamma hemolysis
<i>Proteus vulgaris</i>	+	-	-	+	+/-	H ₂ S production in TSI with blackening of media	Beta hemolytic
<i>Bacillus subtilis</i>	+	-	+	-	-/+	Yellow butt due to acid production	Gamma hemolysis
<i>Candida</i> species	+	-	-	-	-/-	-	-

Note: Positive (+) and Negative (-)

The bacteria *Streptococcus mitis* is mouth commensal cocci shaped and beneficial bacteria inhibit mouth and lives in mouth in symbiotic relationship and protect from pathogens. This bacteria form stable biofilms and loss of balance of this microbe can lead to disease. *Lactobacillus* is a natural opportunistic colonizer of the human oral cavity. Its elevated levels in saliva due to consumption of sugar rich diet. *Ligilactobacillus salivarius* commonly found in healthy individuals is gram positive bacilli in

chains. *Streptococcus mutans* is another coccus isolated from oral swabs is gram positive, facultative anaerobe. *Micrococcus luteus* gram positive, strict aerobe forms bright yellow, smooth colonies on nutrient agar. It is commensal of oral flora found in healthy individuals but can cause opportunistic infections in immunocompromised people and HNC patients are immunocompromised patients. *Staphylococcus epidermidis* gram positive, cocci shaped is a normal commensal of mouth. It is found in both healthy and

HNC patients. *Staphylococcus aureus* is gram positive cocci found in oral flora and causes opportunistic infections in immunocompromised patients. *Actinomyces* species found in oral flora of one of HNC patient. The bacteria are gram positive rod shaped, typically resides in gums and causes opportunistic infection in immunocompromised patients of HNC. It causes deep tissue abscess and systemic infections. *Proteus vulgaris* is gram negative bacteria isolated from HNC patients is an opportunistic bacterium. *Bacillus subtilis* gram positive isolated from oral swabs of HNC patients. The bacteria are opportunistic usually develop after radiotherapy treatment. For fungus *Candida* species also invade immunocompromised patients.

Oral microflora consists of gram positive and gram negative microflora. They are beneficial for mouth and do not cause any harm but in immunocompromised patients some flora can cause diseases and causes opportunistic infections.

A number of researchers had identified oral flora in healthy individuals which remain as a commensal and maintain symbiosis and attains pathogenic potential when there is an imbalance between host immunity and microorganisms. In one of the study oral flora isolate from age group of 20 and 24 of both genders and cultured on blood agar and MacConkey agar. The most common commensals reported was gram-positive cocci, and among them, Coagulase-negative *Staphylococcus* species (85%) are predominant, followed by *Staphylococcus aureus* (13.33%), and *Streptococcus* species (1.67%). The presence of colonies remains the same in all three samples obtained from the same healthy individuals. Loss of balance between commensals and pathogens can lead to dysbiosis, which results in disease.^[17] Cancer patients receiving chemotherapy and/or radiation therapy are prone to many predisposing factors like immunosuppression, imbalance in the oral flora, hypo-salivation and local tissue damage. Therefore, considered to be at higher risk for oral bacterial and fungal infection than the general population.

A previous study done on 110 patients divided in 2 groups 55 chemotherapy (CT) and 55 radiochemotherapy (RCT), as well as 50 healthy individuals. Saliva sample was collected and inoculated on Blood agar, MacConkey agar and Sabouraud's Dextrose Agar (SDA). The identification of bacterial and fungal isolates was done by standard microbiological methods and result was calculated according to cycles of Chemotherapy and Radio chemotherapy combined. The result showed 149 culture isolates from 110 patient in which gram negative bacilli (GNB) found in 63.6%, *Candida* spp. in 50%, *Staphylococcus aureus* in 8% and normal commensal of oral cavity in 13.6% patients in study group and this was higher than control group and this difference was statistically significant in relation to all isolates individually. Relatively more microorganism was isolated during RCT (56%) in compare to CT alone (44%), among GNB- *Pseudomonas* (27.7%,32.3%) and *Klebsiella* (25%,29.4%) were most frequently isolated during CT, RCT respectively. *Candida* spp. were more commonly isolated from patient on RCT (63.6%) than CT (36.3%) when compared to control group (20%) among which *C. tropicalis* was more prevalent species. Both GNB & *Candida* spp. were more commonly isolated in later chemotherapy cycles (CT4, CT5 CT6). The common bacteria reported gram negative bacilli & *Candida* spp. is directly related to number of chemotherapy cycles and combined cancer therapy.^[18]

The oral microbiome plays a key role in the development of head and neck cancer (HNC). Studies reported that certain bacteria increase cancer risk, while others may be protective. However, the overall diversity of bacteria represents the key to health, since the balanced microbiome supports healthy mucosa, while dysbiosis—an imbalance of microbes—can promote cancer by triggering inflammation and weakening the immune response. Researchers are exploring the microbiome as a biomarker for early detection and as a target for new treatments, including probiotics and prebiotics. Though promising, more research is needed to fully understand these links and improve cancer care.^[19]

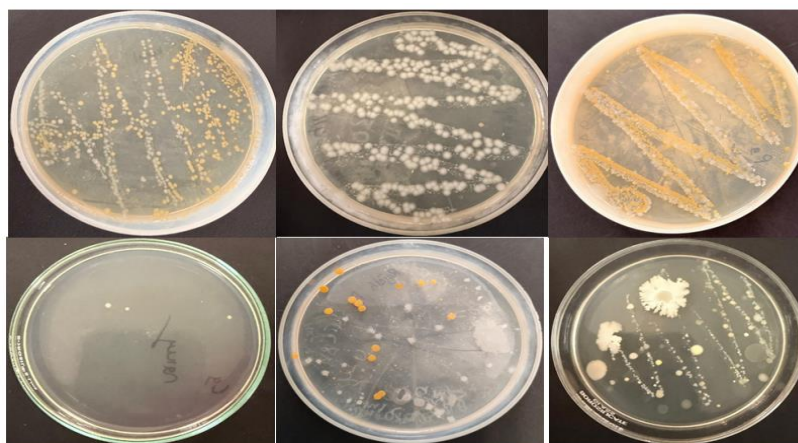


Figure 1: Bacterial colonies on culture plates inoculated with oral swabs of HNC Pre & Post radiotherapy samples.

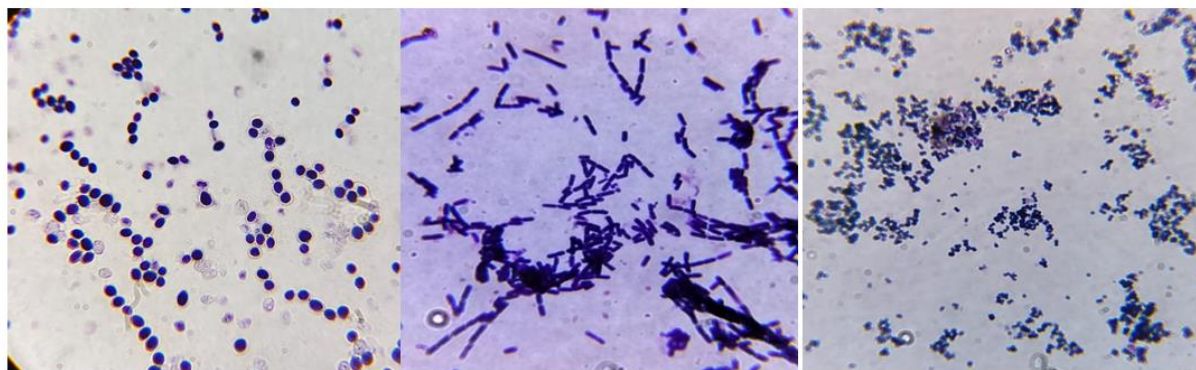


Figure 2: Bacterial colonies on culture plates inoculated with oral swabs of HNC Pre & Post radiotherapy samples.



Figure 3: Catalase and Coagulase.

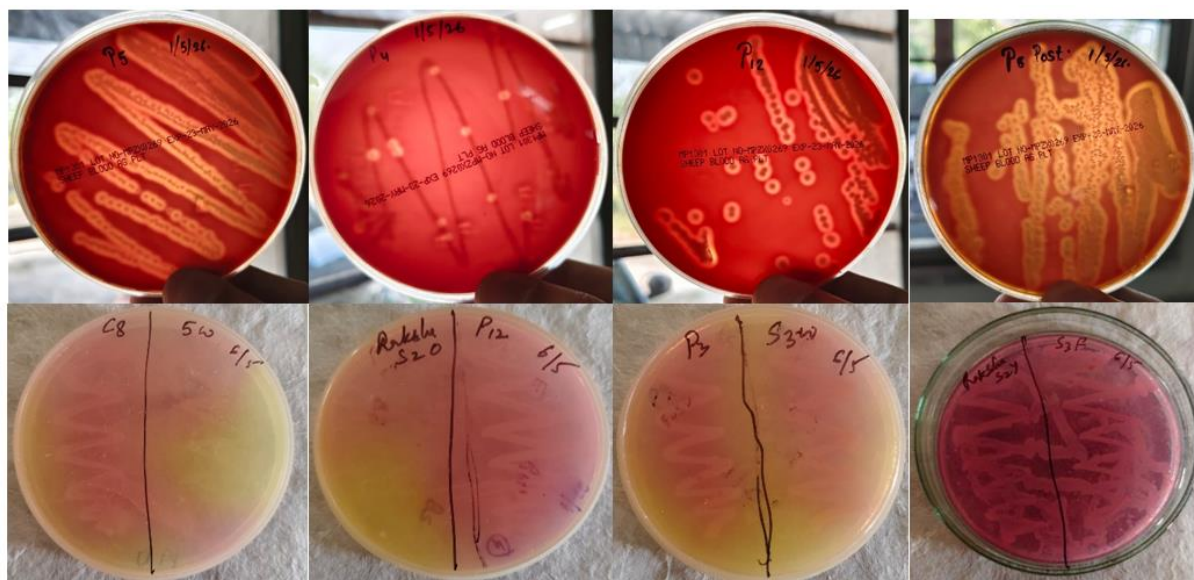


Figure 4: Blood and Urease Agar plates.

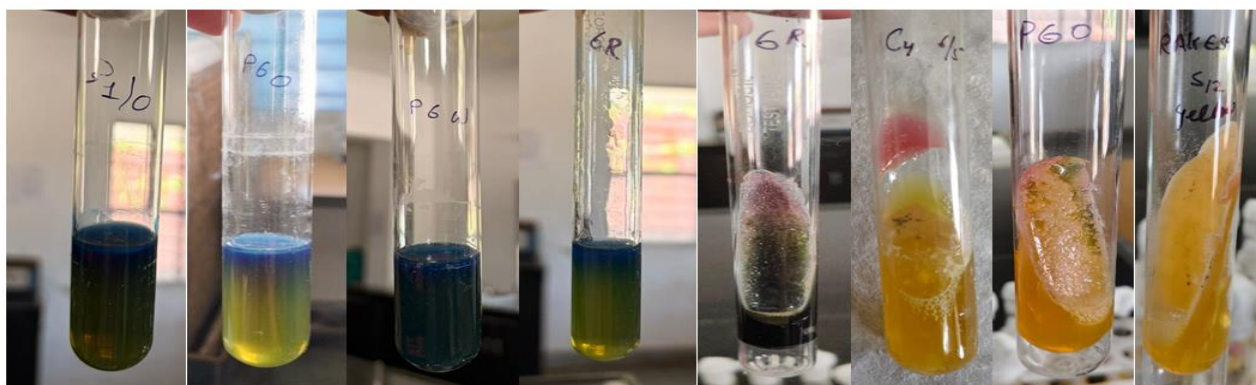


Figure 5: Simmons Citrate Agar and Triple Sugar Iron Test.

CONCLUSION

The present study demonstrated that radiotherapy significantly affects the oral ecosystem of head and neck cancer patients. Alterations in the oral microflora were observed following radiation therapy, with changes in the prevalence of normal bacterial flora and the emergence of opportunistic microorganisms such as *Candida* species in some patients. These microbial changes were accompanied by deterioration in dental health, as reflected by increased DMFS scores after treatment. Radiation-induced xerostomia, reduced salivary buffering capacity, mucosal damage, and changes in oral pH contribute to an environment favourable for microbial imbalance and dental caries. The findings emphasize that oral complications are among the most common and clinically significant adverse effects of radiotherapy. Further studies with larger sample sizes, longer follow-up periods, and molecular microbiological techniques are recommended to better understand radiation-induced changes in the oral microbiome and to develop more effective preventive strategies.

CONFLICTS OF INTEREST

The author declares no conflict of interest for this research.

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REFERENCES

1. Karunya AS, Kumara KS, Kumar A, Shenoy K. Head and neck cancer: a comprehensive review of epidemiology, global burden and advances in radiotherapy. *J Egypt Natl Canc Inst.* 2026; 38(1): 62.
2. Sun H, Yu M, An Z, Liang F, Sun B, Liu Y, Zhang S. Global burden of head and neck cancer: Epidemiological transitions, inequities, and projections to 2050. *Front Oncol.* 2025; 15: 1665019.
3. Badwelan M, Muaddi H, Ahmed A, Lee KT, Tran SD. Oral Squamous Cell Carcinoma and Concomitant Primary Tumors, What Do We Know? A Review of the Literature. *Curr Oncol.* 2023; 30(4): 3721-3734.
4. Alfouzan AF. Radiation therapy in head and neck cancer. *Saudi Med J.* 2021; 42(3): 247-254.
5. Sroussi HY, Epstein JB, Bensadoun RJ, Saunders DP, Lalla RV, Migliorati CA, Heavilin N, Zumsteg ZS. Common oral complications of head and neck cancer radiation therapy: mucositis, infections, saliva change, fibrosis, sensory dysfunctions, dental caries, periodontal disease, and osteoradionecrosis. *Cancer Med.* 2017; 6(12): 2918-2931.
6. Dewhirst FE, Chen T, Izard J, Paster BJ, Tanner AC R, Yu WH, Lakshmanan A, and Wade WG. The human oral microbiome. *Journal of Bacteriology.* 2010; 192(19): 5002-5017.
7. Zhao Z, Yuan Z, Li Y, Huang X. The Balance and Imbalance of Microbial Communities: Oral-Gut Microbiota and Colorectal Cancer. *Am J Clin Oncol.* 2025; 48(10): 501-508.
8. Rajasekaran JJ, Krishnamurthy HK, Bosco J, Jayaraman V, Krishna K, Wang T, Bei K. Oral Microbiome: A Review of Its Impact on Oral and Systemic Health. *Microorganisms.* 2024; 12(9): 1797.
9. Kumar, Brij. The composition, function and role of saliva in maintaining oral health: A review. 2018.
10. Jasmer KJ, Gilman KE, Muñoz Forti K, Weisman GA, Limesand KH. Radiation-Induced Salivary Gland Dysfunction: Mechanisms, Therapeutics and Future Directions. *J Clin Med.* 2020; 9(12): 4095.
11. Xu, H & Dongari-Bagtzoglou, A. Shaping the oral mycobiota: Interactions of opportunistic fungi with oral bacteria and the host. *Current opinion in microbiology.* 2015; 26: 65-70.
12. Gupta N, Pal M, Rawat S, Grewal MS, Garg H, Chauhan D, Ahlawat P, Tandon S, Khurana R, Pahuja AK, Mayank M, Devnani B. Radiation-induced dental caries, prevention and treatment - A systematic review. *Natl J Maxillofac Surg.* 2015; 6(2): 160-6.

13. Ayoubi S, Rahmanian M, Hakimiha N, Rezaei Tavirani M, Jahani-Sherafat S. An Overview of How HNCs Radiotherapy Affects Oral Microbiota: Shifts, Complications, and Management Strategies. *J Lasers Med Sci.* 2026; 17.
14. Bandyopadhyay D. From mouth-level to tooth-level DMFS: conceptualizing a theoretical framework. *J Dent Oral Craniofac Epidemiol.* 2013; 1(1): 3-8.
15. Khlifi R, Trabelsi-Ksibi F, Chakroun A, Rebai A, Hamza-Chaffai A. Cytogenetic abnormality in exfoliated cells of buccal mucosa in head and neck cancer patients in the Tunisian population: impact of different exposure sources. *Biomed Res Int.* 2013.
16. Ahmed HG, and Al Elemirri D. Assessment of oral cytological changes associated with exposure to chemotherapy and/or radiotherapy. *CytoJournal.* 2009; 6: 8.
17. Narayanarao BNG, Chandrasekaran D, Rakeeba F. Oral Commensals in Healthy Individuals: A Clinicocytological Study. *Cureus.* 2024; 16(7): e65317.
18. Soni P, Parihar RS and Soni LK. Opportunistic Microorganisms in Oral Cavity According to Treatment Status in Head and Neck Cancer Patients. *J Clin Diagn Res.* 2017; 11(9): DC14-DC17.
19. Golusinska-Kardach E, Yadav H, Jain S, Masternak MM, Golusiński W. The Oral Microbiome and Head and Neck Cancer: A Narrative Review. *Cancers (Basel).* 2025; 7(17): 2736.