WebLog Journal of Emergency Medicine

Research Article
Published: 30 Jun, 2026

Microorganisms Associated with Oral Cavities Before and After Tooth Brushing Among Students of Imo State University, Owerri, Nigeria

Njoku-Obi Treasure[1]*, Nwoke Murphy Chibueze[2] and Nwanjo S.O[3]

[1]Department of Microbiology, Faculty of Biological Science, Imo State University, Owerri, Nigeria

[2]Department of Animal and Environmental Biology (Zoology), Imo State University Owerri, Nigeria

[3]Fisheries and Hydrobiology Research Unit, Department of Animal and Environmental Biology, Imo State University Owerri, Nigeria

WebLog Open Access Publications
Article ID : wjem.2026.f3002
Author : Dr. Njoku-Obi Treasure

Abstract

The oral cavity harbours a complex community of bacteria that may affect oral and systemic health. Poor oral hygiene favours bacteria growth, responsible for oral disorders such as halitosis, dental caries and periodontal infections. Tooth brushing still is one of the most efficient ways of lowering oral bacteria load.The study was carried out to examine the microorganisms associated with the oral cavities of students before and after teeth brushing among students of Imo State University, Owerri, Nigeria. Oral swab was obtained from the students of Imo State University before and after brushing using sterile swab sticks. The samples were cultivated on Nutrient agar, MacConkey agar, Mannitol Salt agar and Salmonella-Shigella agar by normal microbiological procedures. Bacterial enumeration was carried out by serial dilution and pour plate technique. Colony morphology, Gram stain, motility and biochemical assays (catalase, citrate, coagulase, oxidase, indole, methyl-red, Voges-Proskauer and sugar fermentation) were used for the identification of the isolates. Total heterotrophic plate counts ranged from 3.2×10^4 to 6.0×10^7 CFU/g before brushing and significantly ($p < 0.001$) decreased to 3.2×10^1 to 6.0 × 10^4 CFU/g after brushing. Before brushing, bacterial isolates include *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Shigella* spp. and *Staphylococcus aureus*. Only *Klebsiella* spp., *Pseudomonas* spp. and *Staphylococcus aureus* remained after brushing. The incidence rates of *Escherichia coli*, *Pseudomonas* spp., and *Klebsiella* spp. were 23% before brushing and 35% after brushing, respectively. Tooth brushing resulted in a considerable reduction of bacterial load and variety in the oral cavity. The survival of some harmful organisms after brushing underlines the necessity of good oral hygiene practice and the need for regular instruction on oral health care for university students.

Keywords; Oral Microbiota; Tooth-Brushing; Bacterial Burden; Oral Hygiene; University Students; Nigeria

OPEN ACCESS

Correspondence:
Dr. Njoku-Obi Treasure, Department of Microbiology, Faculty of Biological Science, Imo State University, Owerri, Nigeria, Tel: 08181366290; E-mail: tnjokuobi@gmail.com

Received Date: *17 May 2026*
Accepted Date: *29 Jun 2026*
Published Date: *30 Jun 2026*

Citation:
Njoku-Obi Treasure, Chibueze NM, Nwanjo SO. Microorganisms Associated with Oral Cavities Before and After Tooth Brushing Among Students of Imo State University, Owerri, Nigeria. WebLog J Emerg Med. wjem.2026.f3002. https://doi.org/10.5281/zenodo.21430148

Introduction

Oral health is a significant component of general health and well-being. The mouth cavity is responsible for various significant physiological and social processes, including as mastication, swallowing, speech production, taste perception and facial expression. Apart from these activities, the mouth is the entrance to the digestive and respiratory systems, hence contributing significantly to preserving human health [1]. The mouth is a complex and dynamic microbial community composed of bacteria, fungus, viruses and protozoa. Many of these bacteria exist as harmless commensals and help in maintaining the microbial equilibrium in the mouth under normal physiological settings. However, imbalance in the oral hygiene practices, dietary habits or immunological function may disturb this equilibrium resulting in the multiplication of harmful bacteria that can cause oral and systemic diseases [2].

The mouth cavity is a wet habitat, with optimum temperatures and availability of nutrients from food detritus and saliva, providing an ideal setting for bacteria development. The oral cavity is colonised by varied microbial species on different surfaces, including the tongue, teeth, gingival fissures, palate, and cheeks [3]. Dental plaque is one of the major causes of oral infections, being a biofilm created by buildup of germs on the tooth surfaces. The plaque collection, if not cleared

effectively with the use of correct oral hygiene habits, can lead to dental caries, gingivitis, periodontitis, halitosis, oral thrush and a variety of other oral health problems. Poor oral hygiene is one of the leading causes of the burden of oral illnesses worldwide and especially in low- and middle-income countries where access to dental care services is limited [4].

The mouth has frequently been called a "mirror" of overall health, as a number of systemic disorders have signs and symptoms in the oral cavity. Conditions include diabetes mellitus, HIV/AIDS, leukaemia, nutritional deficiencies and autoimmune disorders might present with oral symptoms which can help in early identification. Oral infections have also been associated with severe systemic disorders including cardiovascular diseases, respiratory tract infections, bad pregnancy outcomes, and bacterial endocarditis. This link underlines the importance of good oral hygiene in the greater context of health care and disease prevention efforts [5].

Oral disorders are the most prevalent non-communicable diseases worldwide, affecting almost 3.5 billion people [6]. Dental caries of permanent teeth is one of the most common health disorders worldwide. Oral diseases, although generally preventable, continue to place considerable social, economic and psychological burdens on individuals and communities. Poor dental health can influence eating, speaking, sleeping, learning and social interaction, which can severely affect quality of life and academic performance among students [7].

University students are a population group particularly sensitive to poor oral hygiene practices and their related microbial diseases. Moving from home to university life generally involves changes in lifestyle and health behaviours for students. Busy academic schedules, stress, peer pressure, poor eating habits, inconsistent sleep patterns and poor health awareness may lead many students living in hostels or off-campus housing to overlook appropriate oral hygiene [8]. Increased microbial activity and plaque formation in the oral cavity are further facilitated by the frequent consumption of sugary foods, carbonated drinks, snacks and fast foods by the students. In addition, some children may brush irregularly or employ inappropriate brushing techniques, which reduces the effectiveness of oral cleaning practices [9].

Poor oral hygiene among university students has become a rising public health problem in many developing nations like Nigeria. Several studies among Nigerian students have shown different levels of awareness and behaviours of oral hygiene with many students displaying poor frequency of brushing and low usage of dental care services. Economic constraints, dental treatment phobia, inadequate oral health education, and limited access to dental clinics in educational institutions [10] may all further compound the poor oral healthcare-seeking behaviour among students. Consequently, the oral cavity has the potential to be colonised by potentially pathogenic microbes which may cause illnesses internally or externally to the mouth [11].

Toothbrushing is still one of the most effective, cheap and generally recommended preventive oral hygiene methods. Mechanical brushing is effective in cleaning food particles, dental plaque and bacteria from tooth surfaces and gingival margins, hence lowering microbial load and preventing oral illnesses. Fluoride is added to toothpaste to help support oral health by strengthening the enamel of teeth and reducing the chance of dental cavities. Better oral cleanliness and reduction in pathogenic bacteria populations have been connected with regular tooth brushing, especially twice daily. Individuals can also benefit from good brushing habits that help avoid bad breath and boost their social confidence [12].

Although the necessity of teeth brushing is well known, many people still practise poor oral hygiene owing to carelessness, insufficient education or inappropriate brushing practices. Sometimes germs may remain in parts of the oral cavity that are difficult to access even after brushing. So the efficiency of brushing can be affected by frequency and length as well as technique, kind of toothbrush and consistency of oral hygiene routines. The alterations in the oral cavity microbiota before and after brushing could give useful information on the efficiency of brushing practices and the prevalence of oral bacteria among students [13].

Frequently isolated microorganisms from the oral cavity are Streptococcus mutans, Staphylococcus aureus, Lactobacillus species, Candida albicans, Neisseria species and anaerobic bacteria linked with periodontal disorders . Some of these microbes are typical flora, but some are opportunistic infections when oral hygiene deteriorates. Their presence in large numbers may predispose to oral infections and systemic consequences. Therefore, assessment of oral microbial populations before and after brushing may be of use in evaluating the effect of oral hygiene in decreasing microbial colonisation and risk of disease [14].

Many studies have been carried out on oral hygiene practices and dental problems among students but few studies have explicitly studied microorganisms associated with the oral cavity before and after brushing among university students in Nigeria especially in Imo State. Most of the existing research are more focused on oral hygiene awareness than on microbiological assessment of the oral cavity. Therefore, there is a need for generating local data of oral microorganisms among students and to analyse the influence of brushing on microbial decrease in the oral cavity [15].

Therefore, the study was designed to evaluate the microorganisms associated with oral cavities of students of Imo State University before and after brushing. The results of this study will add to the existing understanding of oral microbiology and oral hygiene practices among university students. The study is also aimed to increase the understanding of students on the necessity of regular and effective brushing of teeth to avoid oral infections and to promote general health.

Materials and Methods

Study Area

The study was carried out at Imo State University, located in Owerri Municipal Local Government Area of Imo State, Nigeria. Imo State is situated in the South-Eastern geopolitical zone of Nigeria and is bordered by Anambra State to the north, Rivers State to the west and south, and Abia State to the east. The state derives its name from the Imo River, which flows along its eastern boundary. Owerri, the state capital, is popularly referred to as the "Eastern Heartland."

Imo State occupies a strategic location within the rainforest belt of southeastern Nigeria and is characterized by tropical climatic conditions suitable for human habitation and academic activities. Major rivers within the state include the Imo, Otamiri, Orashi, and Awbana rivers, while Oguta Lake remains one of the notable water bodies in the region.

Owerri Municipal serves as an important commercial and educational center, linking major cities such as Port Harcourt, Onitsha, Aba, Orlu, Okigwe, and Umuahia. The area is densely populated and hosts several tertiary institutions, including Imo State University, which provided the study population for this research.

Materials

The materials used for the study included Petri dishes, sterile glass slides, bent glass rods, forceps, pipettes, conical flasks, beakers, inoculating needles and loops, test tubes, test tube racks, agar media, autoclave, hot air oven, bijou bottles, nose masks, hand gloves, test tube covers, cotton swabs, aluminum foil, cover slips, Durham tubes, spatulas, and Bunsen burners.

Reagents

The reagents used included distilled water, methyl red reagent, Kovac's reagent, Lugol's iodine, ethanol, oxidase reagent, glucose phosphate peptone water, crystal violet, immersion oil, normal saline, hydrogen peroxide, sodium hydroxide solution, peptone water, and safranin.

Source and Collection of Samples

Samples were collected from the oral cavities of students of Imo State University, Owerri. Sterile swab sticks were used to obtain oral samples before and after tooth brushing. The swab samples were immediately transferred into sterile containers containing normal saline to preserve the viability of the microorganisms prior to laboratory analysis.

Sample Preparation

The collected samples were appropriately labeled alphabetically using masking tape and permanent markers for proper identification. The swab sticks were immersed in normal saline for approximately one hour to allow the microorganisms to diffuse into the solution. The resulting suspension was subsequently used for serial dilution and microbiological analysis.

Culture Media

The culture media used for isolation and enumeration of bacteria included Nutrient Agar, MacConkey Agar, Mannitol Salt Agar, Salmonella-Shigella Agar (SSA), and Simmons Citrate Agar.

Preparation of Culture Media

The media were prepared according to the manufacturers' specifications. The powdered media were dissolved in distilled water using conical flasks, plugged with cotton wool, covered with aluminum foil, and sterilized in an autoclave at 121°C for 15 minutes. After sterilization, the media were allowed to cool to approximately 45°C before being poured aseptically into sterile Petri dishes. The inoculated plates were gently swirled to ensure even distribution of the inoculum and allowed to solidify before incubation.

Sterilization Procedures

All glassware used in the study was thoroughly washed with detergent and sterilized using a hot air oven. Nutrient agar, MacConkey agar, Simmons citrate agar, and peptone water were sterilized by autoclaving at 121°C and 15 psi pressure. Inoculating loops and needles were sterilized by flaming until red-hot using a Bunsen burner. Laboratory work surfaces were disinfected before and after use with 75% ethanol to maintain aseptic conditions throughout the study.

Bacteriological Evaluation of Samples

Serial dilution was carried out by adding 1 ml of each stock sample into 9 ml of sterile distilled water to obtain a 10^{-1} dilution. Further serial dilutions up to 10^{-4} were prepared aseptically using sterile pipettes and distilled water.

The pour plate technique was employed for bacterial enumeration. Approximately 0.5 ml of each diluted sample was inoculated into sterile Petri dishes, followed by the addition of molten culture media. Nutrient Agar was used for Total Heterotrophic Plate Count (THPC), MacConkey Agar for Total Coliform Count (TCC), Mannitol Salt Agar for Total Staphylococcus Count (TSC), and Salmonella-Shigella Agar for Total Salmonella-Shigella Count (TSSC).

The inoculated plates were gently swirled to ensure uniform distribution of the inoculum and incubated at 37°C for 24 hours. After incubation, bacterial colonies were counted and recorded. Discrete colonies were subcultured onto freshly prepared Nutrient Agar plates to obtain pure cultures. The purified isolates were maintained on nutrient agar slants and stored at 4°C for further characterization and identification.

Identification of Isolates

Bacterial isolates were identified based on their colonial morphology, cellular characteristics, Gram staining reactions, motility tests, and biochemical properties. The biochemical tests performed included catalase test, citrate utilization test, indole test, methyl red test, Voges–Proskauer test, oxidase test, coagulase test, and sugar fermentation tests.

Colonial and Cellular Characteristics

Colonial morphology was examined based on colony size, shape, elevation, pigmentation, margin, texture, surface appearance, and opacity. Cellular characteristics such as bacterial arrangement and staining properties were also evaluated microscopically according to standard microbiological procedures.

Gram Staining

Gram staining was carried out using the method described by Cheesbrough (2000). Smears of bacterial isolates were prepared on clean grease-free slides, air-dried, and heat-fixed. The slides were stained with crystal violet for 30 seconds, followed by Lugol's iodine for 60 seconds. Decolorization was achieved using 75% ethanol, after which the smears were counterstained with safranin. The stained slides were examined microscopically under oil immersion (×100 objective lens). Gram-positive bacteria appeared purple, whereas Gram-negative bacteria appeared red.

Motility Test

Motility testing was carried out using the stab culture technique. Sterile straight inoculating needles were used to inoculate semi-solid motility media with pure bacterial isolates. The inoculated tubes were incubated at 37°C for 24 hours. Diffused growth away from the stab line indicated motility, while growth restricted to the stab line indicated non-motility.

Biochemical Tests

Catalase Test: The catalase test was performed A drop of 3% hydrogen peroxide was placed on a clean glass slide, and a portion of the bacterial colony was introduced into the reagent. Immediate effervescence due to oxygen bubble production indicated a positive catalase reaction.

Citrate Utilization Test: The citrate utilization test was conducted using Simmons citrate agar. The test organisms were inoculated onto the medium and incubated at 37°C for 48 hours. A color change from green to blue indicated a positive citrate utilization reaction.

Coagulase Test: The coagulase test was carried out for the identification of *Staphylococcus aureus* using the slide method. Bacterial suspensions were mixed with plasma on a clean slide. Visible clumping within 10 seconds was regarded as a positive result.

Indole Test: The indole test was performed using sterile tryptone water inoculated with test organisms and incubated at 37°C for 48 hours. Kovac's reagent was added after incubation, and the appearance of a red ring on the surface indicated a positive indole reaction.

Oxidase Test: The oxidase test was carried out using oxidase reagent-soaked filter paper. Colonies of test organisms were smeared onto the reagent-treated paper. Development of a blue-purple coloration within a few seconds indicated a positive oxidase reaction.

Methyl Red and Voges–Proskauer Tests: The methyl red and Voges–Proskauer tests were used to determine glucose fermentation pathways. Test organisms were inoculated into glucose phosphate peptone water and incubated at 37°C for 48 hours. For the methyl red test, addition of methyl red indicator producing a red coloration indicated a positive result. For the Voges–Proskauer test, addition of potassium hydroxide and alpha-naphthol producing a pink-red coloration indicated a positive reaction.

Sugar Fermentation Test: Triple Sugar Iron (TSI) agar was used to determine the ability of isolates to ferment glucose, lactose, and sucrose, as well as produce gas and hydrogen sulfide. The inoculated slants were incubated at 37°C for 24 hours. A yellow coloration indicated sugar fermentation, blackening of the medium indicated hydrogen sulfide production, and gas bubbles or cracks in the medium indicated gas production.

Results

The result shows the bacterial load of the various samples gotten from the oral cavity of Imo state university students before they brushed their teeth in the morning. The various samples were represented with alphabets A, B, C, D, E, F, G, H, I and J. The total heterotrophic bacteria count ranged from 3.2×10^4 to 6.0×10^7, the total coliform count for the samples ranged from 1.6×10^4 to 5.0×10^6. The total staphylococcus counts for the samples ranged from 6.0×10^5 to 4.2×10^6. Total Salmonella Shigella count for the samples ranged from 4.0×10^3 to 6.5×10^6. The research also reveals that the bacterial isolates belong to the *Klebsiella* spp, *Proteus* spp, *E. coli, Pseudomonas* spp, *Shigella* spp and *Staphylococcus aureus.* The percentage occurrence of *Klebsiella* spp was 17, the percentage occurrence of *Pseudomonas* spp was 10, the percentage occurrence of *Shigella* spp was 20, the percentage occurrence of *E. coli* was 23, the percentage occurrence of *Proteus* spp was 10, while the percentage occurrence of *Staphylococcus aureus* was 13 (Tables 1-4).

The result shows the bacterial load of the various samples gotten from the oral cavity of Imo state university students after they brushed their teeth in the morning. The various samples were represented with alphabets A, B, C, D, E, F, G, H, I and J. The total heterotrophic bacteria count ranged from 3.2×10^1 to 6.0×10^4, the total coliform count for the samples ranged from 1.6×10^1 to 5.0×10^3. The total staphylococcus counts for the samples ranged from 6.0×10^2 to 4.2×10^3. Total Salmonella Shigella count for the samples ranged from 4.0×10^1 to 6.5×10^3. The research also reveals that the bacterial isolates belong to the *Klebsiella* spp, *Pseudomonas* spp, and *Staphylococcus aureus.* The percentage occurrence of *Klebsiella* spp was 35, the percentage occurrence of *Pseudomonas* spp was 35, while the percentage occurrence of *Staphylococcus aureus* was 30 (Tables 5-8).

Discussion

This study evaluated the bacteria in the oral cavities of students of Imo State University before and after teeth brushing. The results showed high microbial load in the oral cavity prior to brushing, with a significant reduction after brushing, which shows the efficacy of oral hygiene practices in regulating the oral bacterial populations [16].

Total heterotrophic bacterial count before brushing was 3.2 × 10^4 to 6.0 × 10^7 CFU/g and total coliform count was 1.6 × 10^4 to 5.0 × 10^6 CFU/g. The overall staphylococcus count was between 6.0 × 10 5 and 4.2 × 10 6 CFU/g and the total salmonella-shigella count was between 4.0 × 10 3 and 6.5 × 10 6 CFU/g. The discovered bacterial isolates were *Escherichia coli*, *Klebsiella* spp., *Proteus* spp., *Pseudomonas* spp., *Staphylococcus aureus* and *Shigella* spp. with variable prevalence rates. The most common bacteria were *Escherichia*

Table 1: Bacterial Loads of Samples Before Brushing of Teeth.

SAMPLES	THPC (Cfu/g)	TCC (Cfu/g)	TSC (Cfu/g)	TSSC (Cfu/g)
A	6.0×10^6	2.0×10^5	6.0×10^5	6.0×10^6
B	6.0×10^7	1.0×10^6	4.0×10^6	3.2×10^6
C	7.0×10^6	1.6×10^4	3.5×10^6	6.5×10^6
D	4.5×10^6	2.5×10^6	2.6×10^6	2.0×10^4
E	3.2×10^4	2.0×10^4	4.2×10^6	4.0×10^3
F	2.0×10^6	5.0×10^6	3.4×10^6	6.0×10^6
G	4.2×10^5	4.2×10^4	7.2×10^5	1.0×10^5
H	3.9×10^4	5.2×10^5	8.6×10^5	5.0×10^4
I	4.7×10^5	4.2×10^5	7.6×10^5	4.2×10^6
J	3.2×10^6	7.2×10^4	9.2×10^5	6.0×10^4

THPC= Total Heterotrophic Plate Count
TCC= Total Coliform Count
TSC= Total Staphylococcus Count
TSSC= Total Salmonella Shigella Count

Table 2: Colonial and Morphological Characteristics of Bacterial Isolates Before Brushing.

Sample	Colour	Shape	Surface	Arrangement	Probable organism
1	Yellow	Round	Glassy	Cocci in clusters	*Staphylococcus aureus*
2	Pink	Irregular	Smooth	Rod	*Proteus spp*
3	Pink	Raised growth	Smooth and dry	Rods in singles	*Eschericia coli*
4	Pink	Raised growth	Slimy	Short Rods	*Klebsiella spp*
5	Greenish	Irregular	Smooth	Rods	*Pseudomonas spp*
6	Pale	Raised growth	Smooth	Rod	*Shigella spp*

Table 3: Morphological Appearances and Biochemical Properties of Isolated Bacteria from the Oral Cavity Before Brushing.

Isolates	Bacteriological tests			Biochemical tests										Probable organism
	Gram reaction test	Cellular arrangement	Motility test	Catalase test	Citrate test	Indole test	Oxidase test	Coagulase test	Voges Proskuer test	Methyl red test	Glucose test	Lactose test	Sucrose test	
1	-	Rods	+	+	+	-	+	-	-	-	-	-	-	*Pseudomonas* spp.
2	-	Rod	+	+	-	+	-	-	-	+	+	+	+	*Escherichia coli*
3	-	Rod	-	+	+	-	-	-	-	+	+	-	-	*Shigella* spp.
4	+	cocci	-	+		-	-	+	+	+	+	+	+	*Staphylococcus aureus*
5	-	Rod	+	+	+	-	-	-	+	-	+	+	+	*Klebsiella* spp.
6	-	Rod	+	+	+	-	-	-	+	-	-	-	-	*Proteus* spp

Table 4: Frequency of Occurrence of Bacterial Isolates from the Oral Cavity Before Brushing.

ISOLATES	FREQUENCY	PERCENTAGE (%)
Staphylococcus aureus	4	13
Pseudomonas spp	5	17
Eschericia coli	7	23
Proteus spp	3	10
Klebsiella spp	5	17
Shigella spp	6	20
Total	30	100

Table 5: Bacterial Loads of Samples after Brushing of Teeth.

SAMPLES	THPC (Cfu/g)	TCC (Cfu/g)	TSC (Cfu/g)	TSSC (Cfu/g)
A	6.0×10^{3}	2.0×10^{2}	6.0×10^{2}	6.0×10^{3}
B	6.0×10^{4}	1.0×10^{3}	4.0×10^{3}	3.2×10^{3}
C	7.0×10^{3}	1.6×10^{1}	3.5×10^{3}	6.5×10^{3}
D	4.5×10^{3}	2.5×10^{3}	2.6×10^{3}	2.0×10^{1}
E	3.2×10^{1}	2.0×10^{1}	4.2×10^{3}	4.0×10^{1}
F	2.0×10^{3}	5.0×10^{3}	3.4×10^{3}	6.0×10^{3}
G	4.2×10^{2}	4.2×10^{1}	7.2×10^{2}	1.0×10^{2}
H	3.9×10^{1}	5.2×10^{2}	8.6×10^{2}	5.0×10^{1}
I	4.7×10^{2}	4.2×10^{2}	7.6×10^{2}	4.2×10^{3}
J	3.2×10^{3}	7.2×10^{1}	9.2×10^{2}	6.0×10^{1}

THPC= Total Heterotrophic Plate Count
TCC= Total Coliform Count
TSC= Total Staphylococcus Count
TSSC= Total Salmonella Shigella Count

coli (23%), *Shigella* spp. (20%), *Klebsiella* spp. (17%), *Staphylococcus aureus* (13%), *Pseudomonas* spp. (10%) and *Proteus* spp. (10%). Tooth brushing produced a significant drop in bacterial counts. Total heterotrophic bacterial count reduced to 3.2 × 10 1 - 6.0 × 10 4 CFU/g while total coliform count ranged from 1.6 × 10 1 - 5.0 × 10 3 CFU/g. Similarly, the overall staphylococcal count was lowered to the range of 6.0 × 10 2 – 4.2 × 10 3 CFU/g. The total Salmonella-Shigella count was in the range of 4.0 × 10 1 – 6.5 × 10 3 CFU/g. After brushing only *Klebsiella* spp., *Pseudomonas* spp. and *Staphylococcus aureus* were isolated. *Klebsiella* spp. and *Pseudomonas* spp. each contributed for 35% while *Staphylococcus aureus* accounted for 30%.

The reduction in bacterial load and variety following brushing indicates the importance of oral hygiene practices in regulating the proliferation of microbes in the mouth cavity [9]. Mechanical disruption of dental plaque and removal of food debris that fuels bacterial development is achieved by tooth brushing. Some organisms may survive after brushing, probably due to incorrect brushing procedures, bacterial resistance to mechanical removal or recontamination from contaminated toothbrushes [17].

The oral cavity is a complex microbial environment, which is of great importance for dental and systemic health. oral health is very much dependent on the maintenance of healthy indigenous microflora on the teeth, gums and oral mucosal surfaces[18]. Oral microbiome composition is regulated by several factors including age, nutrition, environmental exposures, antibiotic use and personal cleanliness [19, 20].

Isolation of enteric organisms such *Escherichia coli*, *Shigella* spp., and *Klebsiella* spp. may indicate inadequate oral hygiene habits, fecal-oral contamination, or contamination from incorrectly cleaned toothbrushes. This is in agreement with the findings of [21] who observed that the oral cavity can serve as a reservoir for Enterobacteriaceae that can be transmitted to susceptible persons through saliva. Dietary habits and frequent intake of sugary foods and beverages can also lead to increased bacteria colonisation and dental biofilm production [22].

Indeed, the study highlights the critical role of proper oral hygiene in reducing oral microbial burden and preventing oral health complications such as halitosis, dental caries, and periodontal

Table 6: Colonial and Morphological Characteristics of Bacterial Isolates after Brushing.

Sample	Colour	Shape	Surface	Arrangement	Probable organism
1	Yellow	Round	Glassy	Cocci in clusters	*Staphylococcus aureus*
2	Pink	Irregular	Smooth	Rod	*Proteus spp*
3	Pink	Raised growth	Smooth and dry	Rods in singles	*Eschericia coli*
4	Pink	Raised growth	Slimy	Short Rods	*Klebsiella spp*
5	Greenish	Irregular	Smooth	Rods	*Pseudomonas spp*
6	Pale	Raised growth	Smooth	Rod	*Shigella spp*

Table 7: Morphological appearances and biochemical properties of isolated bacteria from the oral cavity after brushing.

Isolates	Bacteriological tests			Biochemical tests										Probable organism
	Gram reaction test	Cellular arrangement	Motility test	Catalase test	Citrate test	Indole test	Oxidase test	Coagulase test	Voges Proskuer test	Methyl red test	Glucose test	Lactose test	Sucrose test	
1	-	Rods	+	+	+	-	+	-	-	-	-	-	-	*Pseudomonas* spp.
2	-	Rod	+	+	-	+	-	-	-	+	+	+	+	*Escherichia coli*
3	-	Rod	-	+	+	-	-	-	-	+	+	-	-	*Shigella* spp.
4	+	cocci	-	+		-	-	+	+	+	+	+	+	*Staphylococcus aureus*
5	-	Rod	+	+	+	-	-	-	+	-	+	+	+	*Klebsiella* spp.
6	-	Rod	+	+	+	-	-	-	+	-	-	-	-	*Proteus* spp

Table 8: Frequency of occurrence of bacterial isolates from the oral cavity after brushing.

ISOLATES	FREQUENCY	PERCENTAGE (%)
Staphylococcus aureus	4	30
Pseudomonas spp	5	35
Klebsiella spp	5	35
Total	14	100

diseases among university students.

Conclusion

The present study demonstrated that the oral cavities of students are colonized by diverse bacterial species, with significantly higher bacterial loads observed before brushing than after brushing. Tooth brushing effectively reduced both the quantity and diversity of microorganisms present in the oral cavity, confirming its importance in maintaining oral hygiene.

The persistence of some bacterial species after brushing suggests that poor brushing techniques or inadequate oral hygiene practices may still allow certain microorganisms to survive within the oral cavity. The presence of these microorganisms may contribute to oral conditions such as halitosis and periodontal infections.

Based on these findings, students should be encouraged to practice proper and regular oral hygiene, including brushing at least twice daily, maintaining clean toothbrushes, and attending routine dental check-ups. Public health education on oral hygiene should also be intensified within university communities to reduce the prevalence of oral microbial infections and improve overall oral health.

References

1. Loesche W. J. The identification of bacteria in human saliva. Microbial Ecology in Health and Disease. 2019; 6(3): 121-125.
2. Agha-Hosseini F, Mirzaii-Dizgah I and Mirjalili N. Relationship of stimulated saliva 17β-estradiol and oral dryness feeling in menopause. Maturitas. 2019; 62(2): 197-199.
3. Quirynen M, Avontroodt P, Soers C, Zhao H, Pauwels M and van Steenberghe D. Impact of tongue cleansers on microbial load and taste. Journal of Clinical Periodontology. 2019; 36(6): 420-424.
4. Ruszkiewicz J. A, Li S, Rodriguez M. B and Aschner M. "Is Triclosan a neurotoxic agent?". Journal of Toxicology and Environmental Health Part B: Critical Reviews. 2017; 20 (2): 104–117.
5. Ghaffari M, Rakhshanderou S, Ramezankhani A, Noroozi M and Armoon B. Oral health education and promotion programmes: Meta-analysis of 17-year intervention. International Journal of Dental Hygiene. 2018; 16(1): 59–67.
6. Glick M, Williams D. M, Kleinman D. V, Vujicic M, Watt R. G and Weyant R. J. A new definition for oral health developed by the FDI World Dental Federation opens the door to a universal definition of oral health. British Dental Journal. 2016; 221(12): 792.
7. Arweiler N. B, Grelle F, Sculean A, Heumann C and Auschill T. M. Antibacterial effect and substantivity of toothpaste slurries in vivo. Oral Health and Preventive Dentistry. 2018; 16: 175–181.
8. Bernabé E, MacRitchie H, Longbottom C, Pitts N. B and Sabbah W. Birth weight, breastfeeding, maternal smoking and caries trajectories. Journal of Dental Research. 2017; 96(2): 171-178.
9. Cui L, Morris A and Ghedin E. "The human mycobiome in health and disease". Genome Medicine. 2013; 5 (7): 63.
10. Al-Ansari J. M, Boodai H, Al-Sumait N, Al-Khabbaz A. K, Al-Shammari K. F and Salako N. Factors associated with frequent dental visits among adults in Kuwait. Journal of Dentistry. 2016; 34(3): 164-171.
11. Kaur P, Singh S, Mathur A, Makkar D. K, Aggarwal V. P, Batra M, Sharma A and Goyal N. Impact of dental disorders and its influence on self-esteem levels among adolescents. Journal of clinical and diagnostic research. 2017; 11(4): ZC05.
12. Modikoe M. M Reid M and Nel R. Oral health-related knowledge, attitudes and practices of adult patients in Mangaung Metropolitan Municipality, South Africa. South African for dental journal. 2019; 74(4): 180-186.
13. Sharma N, Bhatia S, Sodhi A.S, Batra N. Oral microbiome and health. AIMS Microbiology. 2018; 4(1): 42.
14. Tawakoli P, Sener B and Attin T. Mechanical effects of different Swiss market leading dentifrices on dentin. Swiss Dental Journal. 2015; 125:

1210–1219.

15. Blaggana A, Grover V, Anjali A. K, Blaggana V, Tanwar R, Kaur H and Haneet R. K. Oral health knowledge, attitudes and practice behaviour among secondary school children in Chandigarh. Journal of clinical and diagnostic research. 2016; 10(10): ZC01.
16. Clark-Perry D and Levin L. "Comparison of new formulas of stannous fluoride toothpastes with other commercially available fluoridated toothpastes: A systematic review and meta-analysis of randomised controlled trials". International Dental Journal. 2020; 70 (6): 418–426.
17. Laleman I and Teughels W. The paradigm shift towards prevention and minimal intervention in halitosis management. Oral Health and Preventive Dentistry. 2017; 15(3): 205-210.
18. O'Mullane D. M. "Fluoride and Oral Health". Community Dental Health. 2016; 33 (33): 69–99.
19. Schwiertz A. Microbiota of the human body: implications in health and disease. Switzerland: Springer. 2016; 45.
20. Thema K. L and Singh S. Oral health service delivery in Limpopo Province. South African dental journal. 2017; 72(7): 310-314.
21. West N. X, He T, Zou Y, DiGennaro J, Biesbrock A and Davies M. "Bioavailable gluconate chelated stannous fluoride toothpaste meta-analyses: Effects on dentine hypersensitivity and enamel erosion". Journal of Dentistry. 2021; 105: 103566.
22. Yaegaki K and Coil J. M. Examination, classification, and treatment of halitosis; clinical perspectives. Journal of the Canadian Dental Association. 2019; 66(5): 257-261.
23. Koshimune S, Awano S, Gohara K, Kurihara E and Ansai T. Six-month use of an antiseptic mouthrinse and toothbrushing reduces gingivitis and plaque. Journal of Periodontology. 2019; 74(3): 428-433.
24. Parkinson C. R, Milleman K. R and Milleman J. L. "Gingivitis efficacy of a 0.454% w/w stannous fluoride dentifrice: a 24-week randomized controlled trial". BMC Oral Health. 2020; 20 (1): 89.