Effect of Denture on Oral Microbiome Diversity of Denture-Wearing Older Adult

* Francisca O. **NWAOKORIE,**** Bolanle Oyeyemi **AKINBOBOYE,**** Patricia Adetokunbo **AKEREDOLUA,** *** Mitchelle **DANIA**

*[*Department of Microbiology, Faculty of Basic Medical Sciences, University of Lagos,**Department of Restorative Dentistry, Faculty of Dental Sciences, College of Medicine, University of Lagos,***Department of Medicine, Lagos University Teaching Hospital, Idi Araba Lagos]*

Correspondence
Dr BO Akinboboye
Department of Restorative Dentistry, Faculty of Dental Sciences, College of Medicine, University of Lagos
Email: bakinboboye@unilag.edu.ng

ABSTRACT

Background: Dentures remain a treatment option for tooth replacement, however, their role in defining the oral microbiome is still elusive.

Objective: To evaluate the effect of the insertion of dentures on oral microbiomial profile using metagenomics.

Methods: Baseline denture sonicate was obtained from the denture before insertion, supragingival plaque was collected before and after denture insertion. Metagenomic DNA extraction and next-generation sequencing on the Illumina Miseq platform were all done using standard protocols. Structural composition analysis was done using Quantitative Insights into Microbial Ecology (QIIME).

Results: A total of 9 respondents, five males (55.6%) and four elderly females (44.4%) with a mean age of 69.89±3.79 participated in this study. The five most common species found in the dental plaque before the insertion of dentures were *Stenotrophomonas* (26.06%), Capnocytophaga (9.11%), *Streptococcus* (3.24%), *Unknown* (10.58%) and *Haemophilus Parainfluenza* (8.29%). After insertion and use of dentures for 6 months, the bacteria identified in the dental plaque were *Stenotrophomonas* which *increased in quantity from* 26.06.% to 40.22%. The top five species found on the same denture after insertion were *Pseudomonas veronii* (41.29%) and other similar species of *Pseudomonas* (21%), *Stenotrophomonas* (30.45%), unknown species (in the range of 1.93 to 53.53%), *Methylobacterium adhaesivum* (4.04%) *and Pelomonas* sp.(3.12%). All the species found at baseline on the denture that was positive with microorganisms before insertion were absent on the same denture 6 months after except for *Streptococcus* species that was also present consistently in 4 dentures out of the 9 dentures tested (44.4%). *Streptococcus species* was not one of the top five common species in dental plaque compared to denture plaque (44.4%).

Conclusion: The study observed a relatively diverse microbial community from the dental plaque which increased after insertion of a denture. It is suggested that the microbial load of the denture before insertion might be a risk factor for oral infection in older people.

Keywords: Denture, microbiome, elderly, diversity

Francisca O. Nwaokorie
https://orcid.org/000-0003-4600-478X
Bolanle O. Akinboboye
https://orcid.org/0000-0001-5779-4567
Patricia A Akeredolu
https://orcid.org/
Mitchelle Dania
https://orcid.org/

Received: 4-March, 2024
Revision: 18 June, 2024
Accepted: 21 June, 2024

Citation: Nwaokorie FO, Akinboboye BO, Akeredolua PA, Dania M. Effect of denture on oral microbiome diversity of denture-wearing older adult. Nig J Dent Res 2024; 9(2): 53-62. https://dx.doi.org/10.4314/njdr.v9i2.2

INTRODUCTION

The population of older adults is increasing by the day,[1] likewise there will be challenges of oral care and systemic diseases in these older persons [2]. Oral diseases have been linked with systemic diseases[3] and reports show that the oral cavity is the entrance for bacteria and viruses into the body.[4,5] This suggests the need for professional oral hygiene care and self-oral care in the older population.[6-8] Oral hygiene status is associated with the wearing of dentures[9] and an increased tendency of plaque accumulation, which is a potential reservoir for microorganisms implicated in some systemic diseases.[10,11] The progression of such systemic disease could be fatal in older age groups, residents in nursing homes, older adults with underlying several systemic disorders or those with deterioration of swallowing function, dehydration, and dementia.[12]

Removable dentures or prostheses have a wide scope of applications and they are desired because of their affordability, usefulness as provisional prosthesis, enhancement of good access to hygiene and advantages over biomechanical and practical challenges associated with dental implants.[13] It however has the disadvantages of increased risk of plaque accumulation, caries and periodontal diseases especially if optimum oral hygiene is not maintained.[14] There is a report of increased plaque accumulation and formation of denture plaque on the denture surface,[15] especially on the surface of dentures/dental prosthesis made with polymethylmethacrylate resin which is the most common denture base material used for removable dentures. This material is preferred owing to its advantageous properties such as good aesthetics, dimensional stability, ease of manipulation and repair.[16] Denture plaque is an organized biofilm composed of microorganisms amassed with saliva like dental plaque.[17] Dental plaque is a unique and dynamic biofilm, highly heterogeneous and poly-microbial and has more diverse microbial statistics than denture plaque.[18] Most studies have reported the presence of *Candida species*, *Streptococcus*, Pseudomonas, *Klebsiella*, *Haemophilus*, *Moraxella species a*nd some other respiratory pathogens on wearable dentures pre and post-insertion.[9,19] The understanding of the microbial composition of denture plaque is still incomplete[20] and there is a need to determine the microbial composition. The study, therefore, determined the effects of denture wear on oral microbiome diversity among the elderly in Lagos State, Southwestern Nigeria.

METHODOLOGY

Ethics approval and consent to participate: Responsible conduct of research was employed during the study after obtaining ethical approval with reference no: ADM/DCST/HREC/APP/2715 from the Health Research Ethics Committee of Lagos University Teaching Hospital, before embarking on the research. Permission was granted by the Medical Consultant and Collaborator in this research. Informed consent was obtained from the respondents and the nature of the research was duly explained to them verbally. Strict confidentiality of the responses provided by the respondents was strictly maintained. The study did not interfere with the treatment received while accessing the clinic. All participation was voluntary and subjects were allowed to withdraw at any point during the research.

Study design

The study is a longitudinal study design. For the microbial assessment, the denture/ denture plaque and dental plaque were assessed

Study Site

The study was done at the Prosthodontics Outpatient Clinic of the Restorative Dentistry Department, College of Medicine, University of Lagos and the Department of Medical Laboratory Sciences, College of Medicine, University of Lagos, Lagos State. Samples were collected at the Prosthodontics Outpatient Clinic of the Restorative Dentistry Department. This Restorative Dentistry Department handles all cases of dentures, dental implants, crowns and bridges. Microbial analysis was done at the Department of Medical Laboratory Sciences, College of Medicine, University of Lagos, Lagos State.

Inclusion and exclusion criteria

The study recruited older patients aged 60 years and above, physical presence at LUTH, well-defined gender (either male or female), presence of dental or denture plaques, willingness and informed consent to participate in the study, absence of antibiotics therapy in the last three weeks or one month before enrollment in the study and absence of uncontrolled systemic diseases. On the other hand, those who did not meet the inclusion criteria were excluded from the study.

Sampling technique and population

A total of 9 respondents demanding partial dentures were recruited. Respondents attending the

Prosthodontics Clinic of the Restorative Dentistry Department were randomly drawn using a convenient sampling technique.[21] Respondents were given oral hygiene instructions to follow. They were asked to clean their dentures daily and brush them twice daily throughout the study duration. Participants were reviewed in 6 months and the duration of the study was for 12 months.

Design and administration of questionnaires

A simple open-ended questionnaire was designed with the sole aim of capturing the demographics (gender and age). The administration of the questionnaire was done by a trained medical practitioner (dentist). Only consenting participants who enrolled in the study were administered the questionnaire.

Sample collection and coding of samples

Baseline dental plaque and denture plaque samples were collected from the participants using a standard previously reported technique.[22,23] Supragingival dental plaque collection was done at a specific site with care to prevent contamination on both the anterior and posterior parts of the upper arch. The anterior region was on the labial, palatal surface of the central and lateral incisors on both quadrants. The posterior region was on the buccal, palatal surface of the first molar on both quadrants (using the teeth for determining plaque index).[22] Similarly, denture and dental plaque samples were collected 6 months after insertion of dentures as described previously.[23]

For collection of dental plaque, a periodontal probe was used to collect dental plaque from specific surfaces on the tooth and placed in normal saline in a sterile sample tube. Care was taken not to initiate any gag reflex while collecting the sample. For the collection of denture plaque, dentures were removed from each of the participant's mouths and placed in a sterile bag (Fisher Scientific Loughborough, UK) filled with 50 mL Phosphate-buffered Saline (Sigma-Aldrich, Dorset, UK). It was then placed in a sonic bath (Ultrawave, Cardiff, UK) for 5 minutes to remove biofilm from the surface before returning the dentures to the patient[9]. The denture sonicates after collection were immediately transported in ice to the laboratory for metagenomics DNA extraction. Care was taken to prevent contamination during sample collection in specific locations of the oral cavity as these might be potential confounders. All collected samples were suspended in DNA preservation solution in a 2ml cryovial and stored at -20 $^{\circ}$C before DNA extraction.

The collected samples were coded as DTV_1 for dental plaque first visit, DTV_2 for dental plaque second visit, DRV_1 for denture plaque first visit, DRV_2 for denture plaque second visit. Cases were numbered CA_1 to CA_9. For example, CA_1V_1DT denotes Case 1, first visit dental plaque sample and CA_1V_2DT denotes Case 1 second visit, dental plaque samples. This numbering also applies to denture (DR) samples.

DNA extraction and amplification

Metagenomic DNA was extracted from approximately 2 ml of the collected samples suspended in DNA preservation solution using the standard protocol for ZR Fecal DNA MiniPrep™ D6010 (Zymo Research USA) according to the manufacturer's instructions. The concentration of the extracted DNA was determined using a UV visible spectrophotometer (NanoDrop Mode l3300, Thermo Fisher Scientific, USA). Bacterial DNA was stored in a DNA Elution Buffer at 20°C before further analysis. Only specimens with DNA concentration > 300 µg/µl or the equivalent of 60-100 ng were further analyzed. DNA amplification and sequencing were conducted at Inqaba Biotechnical (Pty) Limited, Pretoria, South Africa. Briefly, genomic DNA samples were PCR amplified using a universal primer pair 27F and 1492R - targeting the V1 -V9 region of the bacterial 16S rRNA gene. The resulting amplicons were barcoded with PacBio M13 barcodes for multiplexing through limited cycle PCR. The resulting barcoded amplicons were quantified and pooled equimolar and an AMPure PB bead-based purification step was performed. The PacBio SMRTbell library was prepared from the pooled amplicons following the manufacturer's protocol and index codes were added. The library quality was assessed on the Qubit@ 2.0 Fluorometer (Thermo Scientific) and Agilent Bioanalyzer 2100 system. Sequencing primer annealing and Polymerase binding were done following SMRTlink Link software protocol to repair the library for sequencing on the PacBio Sequel IIe system (Pacific Biosciences of California, Inc). The library was sequenced on a PacBio HiFi Sequencing system with High Fidelity reads (HiFi) attainable with an accuracy of > 99.9% to define the phylogenetic types (phylotypes) present. Species-level bacterial phylotypes were defined as organisms sharing > 97% nucleotide sequence identity (% ID) in the V4 regions of their 16S rRNA genes.

Bioinformatic analysis

Sequence data were generated to differentiate the dental plaque, denture plaque and throat swab community composition of the individual's sample. The data was edited by trimming low-quality ends from reads in addition to adapter removal. Fast Length Adjustment of Short reads (FLASH)[24] to improve genome assemblies was used to merge paired-end reads that were generated from DNA fragments whose lengths were shorter than the length of reads. As previously reported in our earlier study generated sequence data was used to evaluate the faecal communities from both categories of respondents. To achieve this, the sequence was subjected to quality control by trimming the low-quality ends and removal of adapters.[25] In addition, Fast Length Adjustment of Short reads (FLASH) was used to merge pair-end reads that were generated from DNA with lengths shorter than the lengths of the reads.[26-28] Using the Quantitative Insight into Microbial Ecology (QIIME) Package version 1.70, the generated sequences were used to identify the microbial communities down to species-level operational taxonomic units (OTU).[29]

Statistical analysis

Mean age determination for the participants was carried out using Microsoft Excel spreadsheet 2016. Alpha diversity analysis was done using the abundance at species levels for the various categories of cases. Analysis was done using PAST-PAleontological *Statistical* software.[28] Alpha diversity was carried out using the Shannon and Simpson indices. The abundance (%) top five phyla for each case (first and second visits) for dental, denture and throat microbiomes were transformed into stacked plots while the top species were transformed into pie charts using the 2018 Origin statistical package

RESULTS

The study analysed five males (55.6%) and four elderly females (44.4%) with a mean age of 69.89±3.79.

Table 1: **Sociodemographic and Respiratory Assessment**

Variable	N(%)
Age (years)	
60-65	1(11.1)
66-70	6(66.7)
71-75	1(11.1)
76-80	1(11.1)
Gender	
Male	5(55.6%)
Female	4 (44.4%)
Total	9(100%)

The five most common species found in the dental plaque before insertion of dentures were *Stenotrophomonas* (26.06%), Capnocytophaga (9.11%), *Streptococcus* (3.24%), *Unknown* (10.58%), *Haemophilus Parainfluenza* (8.29%) as shown in Table 2. After insertion and use of dentures for 6 months, the read count of *Stenotrophomonas* was 26.06 to 40.22%, The Unknown species increased from 10.58% to 13.4%. There was no presence of *Streptococcus, Capnocytophaga, and Haemophilus Parainfluenza in the dental plaque* after insertion and use of denture for 6 months. In addition, species like *Chryseobacterium* sp, *Achronobacter* sp and *Veillonella Dispar* sp were not detected before the insertion of the denture but were present in the dental plaque after the insertion of denture plaque in the following order; *Chryseobacterium* (10.89%), *Achronobacter* (6.54%), and, *Veillonella Dispar* (5.73%)

Table 2: Top five species of Dental Plaque in the various groups of participants at the genus level

Species	DTV1(%)	DTV2(%)
Stenotrophomonas	26.06%	40.22%
Unknown	10.58%	13.4%
Capnocytophaga	9.11%	ND
Haemophilus Parainfluenza	8.29%	ND
Streptococcus	3.24%	ND
Chryseobacterium	ND	10.89%
Achronobacter	ND	6.54%
Veillonella dispar	ND	5.73%

*ND- NOT DETECTED

The study also observed a diverse microbial community from the denture plaque before and after insertion (Table 3). The study observed the presence of *Serratia_marcescens* (89.54%), *Streptococcus* sp (3.76%), *Rothia_dentocariosa* (1.03%), *Veillonella_dispar* (1.01%), *Tannerella* sp (0.93%) on one of the denture before insertion. The top five species found after insertion include *Pseudomonas_veronii with about* 41.29 found on only one patient and *Pseudomonas species (21%)* in other participants, *Stenotrophomonas* (30.45), unknown species (1.93 to 53.53%), *Methylobacterium_adhaesivum (4.04%) and Pelomonas* sp.(3.12%). All the species found at baseline on one denture before insertion were absent

on the same denture 6 months after except for *Streptococcus* sp., which was also consistently seen in 4/9 patients (44.4%) after use. In addition, *Streptococcus species* was not one of the top five species in the dental plaque but they were found on denture plaque (44.4%). Other Genera and Species detected are as shown in table 3.

Sequences obtained from this study are registered at The National Center for Biotechnology Information with BioProject number: PRJNA913582 and accessible at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA913582

Table 3: Diversity of bacteria species in Denture Plaque Pre and Post Insertion in the elderly.

SPECIES	*DRV 1 (%	*DRV 2 A(%	DRV2 B (%)	DRV2 C (%)	DRV2 D (%)	DRV2 E (%)	DRV2 F (%)	DRV2 G (%)	DRV2 H (%)	DRVI (%)
Pseudomonas_veronii	0		0	41.29	0	0	0	0	0	0
Pseudomonas sp.	0		21.0	0	0	0	0	21	0	0
Stenotrophomonas	0		0	30.45	0	0	0	0	0	0
Unknown	0		11.56	6.67	0	5.33	53.53	11.56	5.45	1.93
Methylobacterium_adhaesivum	0		0	4.04	0	0	0	0	0	0
Pelomonas sp.	0		0	3.12	0	0	0	0	0	0
Agrobacterium sp	0		0	0	26.3	0	0	0	0	0
Microbacterium_chocolatum	0		0	0	16.02	0	0	0	0	0
Pseudomonas_nitroreducens	0		0	0	12.81	0	0	0	0	0
Chryseobacterium sp.	0		0	0	12.78	12.92	0	0	20.21	0
Psychrobacter sp.	0		0	0	7.62	0	0	0	0	0
Stenotrophomonas_maltophilia	0		0	0	0	23.93	0	0	0	0
Bradyrhizobium sp.	0		0	0	0	6.82	0	0	0	0
Lutrolibacter sp.	0		0	0	0	4.96	0	0	0	0
Serratia_marcescens	89.54		0	0	0	0	0	0	0	0
Streptococcus sp.	3.76		9.99	0	0	0	2.35	9.99	4.53	0
Rothia_dentocariosa	1.03		0	0	0	0	0	0	0	0
Veillonella_dispar	1.01		0	0	0	0	0	0	0	0
Tannerella sp.	0.93		0	0	0	0	0	0	0	0
Streptococcus_alactolyticus	0		0	0	0	0	2.94	0	0	0
KD1	0		0	0	0	0	2.35	0	0	0
Flavobacterium sp.	0		0	0	0	0	1.76	0	0	0
TG5	0		15.63	0	0	0	0	15.63	0	0
Caulobacter	0		3.89	0	0	0	0	3.89	0	0
Ralstonia	0		0	0	0	0	0	0	58.11	0
Mycobacterium	0		0	0	0	0	0	0	1.41	0
Neisseria	0		0	0	0	0	0	0	0	66.98
Porphyromoas	0		0	0	0	0	0	0	0	11.83
Neisseria_subflava	0		0	0	0	0	0	0	0	2.29
Haemophilus_parainfluenza	0		0	0	0	0	0	0	0	2.18

*** DRV1-The only denture sonicate whose microbial species were detected at baseline**
***DRV2 A-DRV2 I denotes individual second visit denture plaque of the 9 participants.**
*** DRV2 A did not amplify**

Furthermore, alpha diversity (Shannon and Simpson) showed relatively higher diversity at the species level after insertion.

Dental plaque first visit values for the diversity analysis gave values that ranged from 34 to 103, 1081 to 9772, 1.141 to 2.782 and 0.499 to 0.900, respectively for taxa counts, species diversity count, and Shannon and Simpson indices. On the other hand, the second dental plaque visit gave values that were 94 to 124, 8163 to 9497, 2.289 to 2.533 and 0.825 to 0.836, respectively for taxa counts, species diversity count, Shannon and Simpson indices. The values for denture samples are: for the first visit since only one sample successfully amplified were 74, 9497, 0.600 and 0.1974, respectively for taxa counts. For the second visit, the values ranged from 39 to 129, 157 to 9876, 1.522 to 3.179 and 0.535 to 0.908, respectively for taxa counts, species diversity count, and Shannon and Simpson indices.

DISCUSSION

Denture has been linked to the formation of plaques and the presence of potential respiratory pathogens in the oral cavity. Modern sequencing approach is unveiling the diversity of bacterial phylotypes associated with dentures in health and disease. This prosthetic device is frequently used by older patients as a tooth replacement option because of its affordability.[6] Over the years, studies have shown that microbial-induced pneumonia with subsequent respiratory distress is one of the major causes of morbidity and mortality in the elderly. Our findings analyzed differences and highlighted the oral microbiome diversity of denture-wearing older adults. The study focused on analyzing the bacterial species present on denture or denture plaque after fabrication before and after insertion in both male and female denture wearers. Although our sample size was small the occurrence of these species did not vary with gender and all elderly populations studied were predisposed to bacterial colonization. The five most common species found in dental plaque before the insertion of dentures were *Stenotrophomonas*, Capnocytophaga, *Streptococcus*, and *Haemophilus parainfluenza*. This is in agreement with other findings.[30,31]

Like in most metagenomic studies, there is an array of *Unknown* species, therefore this finding will require a comprehensive novel analysis to establish their identity.[32] The *Stenotrophomonas*, species were also predominated after insertion and use of dentures for 6 months. This shows that these species could play a major role in the oral microbiome of denture wearing elderly population.

In 2012 it was classified as an emerging multidrug-resistant global opportunistic human pathogen[33] *Stenotrophomonas sp* is implicated in bacteremia, respiratory tract infection, skin and soft tissue infection, and endocarditis. It causes respiratory infection in immunocompromised individuals.[31] It belongs to the same phylum Pseudomonadota as *Pseudomonas. Stenotrophomonas maltophilia is a* co-colonizer with *Pseudomonas aeruginosa. In addition, it has the potential to form biofilm* on surfaces including Teflon, glass, plastics and host tissue.[34]

This implies that surfaces such as acrylic and materials for flexible dentures are potential media and this might predispose the denture wearer to respiratory infection. It is a priority bacterial species of pathogenic potentials in dental prosthesis and dental unit water lines.[30]

Although analysis of the dental plaque showed that species like *Streptococcus, Capnocytophaga, and Haemophilus Parainfluenza were* present in the denture before insertion, they were not found after the insertion and use of this denture for 6 months in the supragingival dental plaque. *Pseudomonas aeruginosa*, *Haemophilus influenzae Bacterium*, and *Moraxella catarrhalis* are respiratory pathogens[19] that have been isolated from dentures[9] and are reported to occur as a reservoir for respiratory pathogens. Similar genera and species were found in the denture and dental plaques assessed in this study.

In addition, species like *Chryseobacterium* sp, *Achronobacter* sp and *Veillonella dispar* were not detected on the surface of the denture before insertion but were present in the dental plaque after wearing the dentures for six months. This shows that the presence of the denture in the mouth may have contributed to their habitation and sustainable colonization of the gingival sites. This may be possible because the presence of dentures in the mouth may trigger conditions that may support the growth of these species. Moreover, *Veillonella Dispar* is strictly commensal oral anaerobes and the presence of these acrylic dentures may have contributed to a reduction in oxygen level thus supporting their growth.[35]

The study observed the presence of *Serratia_marcescens* (89.54%), *Streptococcus* species (3.76%), *Rothia_dentocariosa* (1.03%), *Veillonella_dispar* (1.01%), *Tannerella* species (0.93%)

on one denture before insertion. These species may be on the denture as contaminants. To support this, studies have shown that *Serratia_marcescens* is a bacterial contaminant associated with nosocomial pneumonia.[36] It can form biofilms and stay long in an environment including disinfectant solution and prosthetic devices.[36] A similar report has been documented for *Streptococcus* species.[37] Although filamentous *Rothia_dentocariosa* is previously seen as a bacteria species of known low virulence, recent findings have shown their involvement in oral and non-oral infection due to their ability to induce the production of human cytokines.[38] This is similar to *Veillonella_dispar* which is described as having low virulence potential.[35] *Tannerella* species were found in a very low count. They are well-known oral anaerobic pathogens that have been found in low abundance in the oral microbiome of Japanese older adults,[39] with chronic periodontitis. Their presence on the denture before insertion is of concern because they are classified as oral pathogens. Interestingly, the diversity of the bacterial species differs after six months of wearing the denture as the top five species found on the same denture after insertion were *Pseudomonas veronii* and other species of *Pseudomonas*. Others were *Stenotrophomonas*, unknown species, *Methylobacterium adhaesivum and Pelomonas* species. Thus, all the species found at baseline on the denture before insertion was absent on the same denture six months after except for *Streptococcus* species. This is the first study to report the presence of *Pseudomonas veronni* in the oral cavity of the Nigerian population. This is a new strain of *Pseudomonas* identified and the taxonomy proposed in 1996 by Elomari and his coworkers.[40] This bacterium can degrade polyethene, a material commonly used in denture composite reinforcement[41]. Further studies are required to establish if these species are capable of degrading dentures used in our environment.

Streptococcus species are known bacteria in the oral microflora. The study could not establish the source of streptococcus species found on dentures before insertion and those found after denture use. Their presence on dentures can pose a source of health risk. The use of dentures has been reported to be associated with the formation of denture plaque on the surface of the denture and a change in the microbial composition of dental plaque.[42] This was observed in this study as there was an observable presence of denture plaque, dental plaques and an increase in the diverse microbial community of the dental plaque after six months of denture wearing. There was an obvious change in the composition of denture and dental plaque with resultant bacterial biodiversity on the denture and in the overall oral microbiome.

This study suggests that the microbial load of dentures before and post-insertion might be a risk factor for oral infection in the older population and there is a need for improvement in oral and denture hygiene. In addition, the denture surface seems to be a potential conducive habitat for *Streptococcus* species and its persistence on the denture plaque, but the inability to detect these species on the dental plaque of older adult patients studied needs further investigation with a larger population. This would help to support the hypothesis that the microbial load of the denture before insertion might be a risk factor for oral infection in older adults. This would also justify the need for an intervention to ensure that dentures are kept sterile and free of microbial load before insertion, especially in older adults.

CONCLUSION

This is the first study to highlight the oral microbiome of denture wearers in Nigeria and has provided evidence that Microbial diversity increases with denture wearing and might be a source of potential pathogens for the elderly if oral hygiene is not maintained. The use of dentures resulted in the formation of denture plaque, with a change in the composition of denture and dental plaque with resultant biodiversity in the oral microbiome.

Study limitations

The study used a small number. Although the huge microbial data obtained per sample per individual showed broader diversity, the small sample may have affected the percentage variable on demographics (sex and age) and also limited the detection of other species. The study did not include the microbiome of other microbes like fungi and viral species known to be found on denture wearers. The need for further studies to attend to this study's limitations cannot be overemphasized. -

Availability of data and materials: Data generated in this study are contained in this manuscript. Supplementary sequence data are accessible at https://www.ncbi.nlm.nih.gov/bioproject/PRJNA913582.

Authors' contributions: This work was carried out in collaboration with all authors. Authors FON and BOA designed the study, collected the data and wrote a

draft of the manuscript, authors BOA, PAA and GD recruited and examined the patients, author FON and BOA performed the laboratory experiments and BOA performed- the clinical studies. FON and provided technical advice and revised the manuscript. All authors read and approved the final manuscript.

Source of Support
The research was sponsored By the University of Lagos Research Grant (CRC 2019/14).

Conflict of interests
None declared

REFERENCES

1. Perpetua Lum Tanyi, Pelser André & Peter Mbah | Kar-wai Tong (Reviewing editor). Care of the elderly in Nigeria: Implications for policy, Cogent Soc. Sci 2018, 4:1, DOI: 10.1080/23311886.2018.1555201
2. Griffin SO, Jones JA, Brunson D, Griffin PM, Bailey WD. Burden of oral disease among older adults and implications for public health priorities. Am J Public Health. 2012; 102:411-418. doi:10.2105/AJPH.2011.300362
3. Kapila YL. Oral health's inextricable connection to systemic health: Special populations bring to bear multimodal relationships and factors connecting periodontal disease to systemic diseases and conditions. Periodontol 2000. 2021; 87:11-16. doi: 10.1111/prd.12398. PMID: 34463994; PMCID: PMC8457130.
4. Imai K, Iinuma T, Sato S. Relationship between the oral cavity and respiratory diseases: Aspiration of oral bacteria possibly contributes to the progression of lower airway inflammation. Jpn Dent Sci Rev. 2021; 57:224-230.
5. Scannapieco FA, Shay K. Oral health disparities in older adults: oral bacteria, inflammation, and aspiration pneumonia. Dent Clin North Am. 2014; 58(4):771-782. doi: 10.1016/j.cden.2014.06.005. Epub 2014 Jul 22. PMID: 25201541.
6. Akinboboye B. O, Ogunyemi A. O, Ladi-Akinyemi T. W. Oral Health Status And Service Utilization Among A Group Of Rural Older Nigerians. Afr J. Oral Health, 2018; 8: 30-34.
7. Gluhak C, Arnetzl GV, Kirmeier R, Jakse N, Arnetzl G. Oral status among seniors in nine nursing homes in Styria, Austria. Gerodontology. 2010; 27:47-52. doi: 10.1111/j.1741-2358.2009.00281.x. Epub 2009 Mar 26. PMID: 19371391.
8. Gaszynska E, Szatko F, Godala M, Gaszynski T. Oral health status, dental treatment needs, and barriers to dental care of elderly care home residents in Lodz, Poland. Clin Interv Aging. 2014; 9:1637-1644. doi: 10.2147/CIA.S69790. PMID: 25284997; PMCID: PMC4181440.
9. O'Donnell LE, Smith K, Williams C, Nile CJ, Lappin DF, Bradshaw D, Lambert M, Robertson DP, Bagg J, Hannah V, Ramage G. Dentures are a Reservoir for Respiratory Pathogens. J Prosthodont. 2016; 25:99-104. doi: 10.1111/jopr.12342. Epub 2015 Aug 10. PMID: 26260391.
10. Liu C, Cao Y, Lin J, Ng L, Needleman I, Walsh T, Li C. Oral care measures for preventing nursing home-acquired pneumonia. Cochrane Database Syst Rev. 2018; 9:CD012416. doi: 10.1002/14651858.CD012416.pub2. PMID: 30264525; PMCID: PMC6513285.
11. Bassim CW, Gibson G, Ward T, Paphides BM, Denucci DJ. Modification of the risk of mortality from pneumonia with oral hygiene care. J Am Geriatr Soc. 2008; 56:1601-1607.
12. Bukleta MS, Bukleta D, Selmani M, Kuhar M. Frequency of Complete and Removable Partial Denture Treatment in the Primary Health Centres in Three Different Regions of Kosovo from 2002 to 2013. Zdr Varst. 2019 Jun 26;58:104-111
13. Yeung C, Yu OY, Lam WYH, Leung KCM, Wong AWY, Chu CH. Improving Esthetics of Removable Partial Dentures Using Palatal Retentive Arms. Clin Cosmet Investig Dent. 2020; 12:391-397.
14. McKenna G, Jawad S, Darcey J. Functionally Orientated Tooth Replacement for Older Patients. Prim Dent J. 2020; 3:40-43.
15. Kusama, T., Aida, J., Yamamoto, T. *et al.* Infrequent Denture Cleaning Increased the Risk of Pneumonia among Community-dwelling Older Adults: A Population-based Cross-sectional Study. Sci Rep. 2019; 9: 13734
16. Ikeya K, Iwasa F, Inoue Y, Fukunishi M, Takahashi N, Ishihara K, Baba K. Inhibition of denture plaque deposition on complete dentures by 2-methacryloyloxyethyl phosphorylcholine polymer coating: A clinical study. J Prosthet Dent. 2018; 119(1):67-74.

17. Iwawaki Y, Matsuda T, Kurahashi K, Honda T, Goto T, Ichikawa T. Effect of water temperature during ultrasonic denture cleaning. J Oral Sci. 2019; 61:140-145.
18. Mark Welch JL, Rossetti BJ, Rieken CW, Dewhirst FE, Borisy GG. Biogeography of a human oral microbiome at the micron scale. Proc Natl Acad Sci. 2016; 113: E791–800.
19. Shi B, Wu T, McLean J, Edlund A, Young Y, He X, Lv H , Zhou X. *et al*. The denture-associated oral microbiome in health and stomatitis. mSphere. 2016; e00215–e00216
20. O'Donnell LE, Robertson D, Nile CJ, Cross LJ, Riggio M, Sherriff A, Bradshaw D, Lambert M, Malcolm J, Buijs MJ, Zaura E, Crielaard W, Brandt BW, Ramage G. The Oral Microbiome of Denture Wearers Is Influenced by Levels of Natural Dentition. PLoS One. 2015; 10(9):e0137717.
21. Andrade C. The inconvenient truth about convenience and purposive samples. Indian J Psychol Med. 2021; 43:86–88. https://doi.org /10.1177/0253717620977000
22. Hegde S, Chatterjee E, Rajesh KS, Arun Kumar MS. Obesity and its association with chronic periodontitis: A cross-sectional study. J Educ Health Promot. 2019; 8:222. doi: 10.4103/jehp.jehp_40_19.
23. Mihalow D M, Tinanoff N. The influence of removable partial dentures on the level of streptococcus mutans in saliva. J Prosthet Dent. 1988; 59:49 -51.
24. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011; 27:2957-63. doi: 10.1093/bioinformatics/btr507.
25. Janto M, Iurcov R, Daina CM, Neculoiu DC, Venter AC, Badau D, Cotovanu A, Negrau M, Suteu CL, Sabau M, Daina LG. Oral Health among Elderly, Impact on Life Quality, Access of Elderly Patients to Oral Health Services and Methods to Improve Oral Health: A Narrative Review. J Pers Med. 2022; 12:372. doi: 10.3390/jpm12030372
26. Rocha EP, Francisco SB, Del Bel Cury AA, Cury JA. Longitudinal study of the influence of removable partial denture and chemical control on the levels of Streptococcus mutans in saliva. J Oral Rehab. 2023; 30:131-138
27. Magoč T, Salzberg SL. FLASH: fast length adjustment of short reads to improve genome assemblies. Bioinformatics. 2011; 27(21):2957 - 2963. doi: 10.1093/bioinformatics/btr507.
28. Hammer, Øyvind, Harper, David A.T., and Paul D. Ryan, 2001. Past: Paleontological Statistics Software Package for Education and Data Analysis. Palaeontologia Electronica, vol. 4, issue 1, art. 4: 9pp., 178kb. http:www//palaeoelectronica.org/2001_1/past/issue1_01.htm
29. Kuczynski J, Stombaugh J, Walters WA, González A, Caporaso JG, Knight R. Using QIIME to analyze 16S rRNA gene sequences from microbial communities. Curr Protoc Bioinformatics. 2011; 10:10.7.1-10.7.20. doi: 10.1002/0471250953.bi1007s36. PMID: 22161565; PMCID: PMC3249058.
30. Liaqat I, Sabri AN. Effect of biocides on biofilm bacteria from dental unit water lines. Curr. Microbiol. 2008; 56:619–624
31. Karolina Wiatrak, Tadeusz Morawiec, Rafał Rój, Anna Mertas, Agnieszka Machorowska-Pieniążek, Patryk Kownacki, Marta Tanasiewicz, Małgorzata Skucha-Nowak, Stefan Baron, Tomasz Piekarz, Maciej Wrzoł, Mateusz Bogacz, Jacek Kasperski, Iwona Niedzielska, "Oral Health of Patients Treated with Acrylic Partial Dentures Using a Toothpaste Containing Bee Product", Evid-Based Complementary Alt Med. 2017, Article ID 4034179:1-12
32. Thomas, A.M., Segata, N. Multiple levels of the unknown in microbiome research. BMC Biol. 2019; 17, 48. https://doi.org/10.1186/s12915-019-0667-z
33. Brooke JS. Stenotrophomonas maltophilia: an emerging global opportunistic pathogen. Clin Microbiol Rev.2012; *25:2*–41. https: //doi.org/ 10 .1128/CMR.00019-11
34. Jucker BA, Harms H, Zehnder AJB. 1996. Adhesion of the positively charged bacterium Stenotrophomonas (Xanthomonas) maltophilia 70401 to glass and Teflon. J. Bacteriol. 178:5472–5479
35. Zhang Shi-Min, Huang Shir-Ly (2023). The Commensal Anaerobe Veillonella dispar Reprograms Its Lactate Metabolism and Short-Chain Fatty Acid Production during the Stationary Phase. American Society for Microbiology Journal. 11;2.:doi: 10.1128/spectrum.03558-22
36. Abbas, H. A., & Elsherbini, A. M. Silencing the nosocomial pathogen *Serratia marcescens* by

glyceryl trinitrate. Afr. J. Health Sci. 2018; *18:* 1–10. https://doi.org/10.4314/ahs.v18i1.2

37. Nair VV, Karibasappa GN, Dodamani A, Prashanth VK. Microbial contamination of removable dental prosthesis at different interval of usage: An in vitro study. J Indian Prosthodon Soc*.2016; 16*:346–351. https://doi.org /10.4103/ 0972-4052.176536
38. Fatmah Alshatti1, Rahaf Almutairi1, Shareefa Alkandari1, Asma Haneef1, Radhika Bhardwaj1 et al. Bioinformatics Analysis of the Rothia dentocariosa Proteome and Assessment of the Proinflammatory Potential of Biofilm and Planktonic Cells. Open Dent J. 2023; 17 e187421062211300.DOI: 10.2174/18742106-v16-e221130-2022-71.
39. Toyama N, Ekuni D, Yokoi A, Fukuhara D, Islam MM, Sawada N, Nakashima Y, Nakahara M, Sumita I, Morita M. Features of the oral microbiome in Japanese elderly people with 20 or more teeth and a non-severe periodontal condition during periodontal maintenance treatment: A cross-sectional study. Front Cell Infect Microbiol. 2022; 12:957890. doi: 10.3389/fcimb.2022.957890. PMID: 36275030; PMCID: PMC9582337.
40. Elomari M, Coroler L, Hoste B, Gillis M, Izard D, and Leclerc, H. "DNA relatedness among Pseudomonas strains isolated from natural mineral waters and proposal of Pseudomonas veronii sp. nov." Int. J. Syst. Bacteriol. 1996; 46:1138-1144.
41. Malik, S.; Maurya, A.; Khare, S.K.; Srivastava, K.R. Computational Exploration of Bio-Degradation Patterns of Various Plastic Types. *Polymers* 2023; *15*, 1540. https://doi.org/10.3390/polym15061540
42. Nair VV, Karibasappa GN, Dodamani A, Prashanth VK. Microbial contamination of removable dental prosthesis at different interval of usage: An *in vitro* study. J Indian Prosthodont Soc. 2016; 16: 346-351. doi: 10.4103/0972-4052.176536. PMID: 27746598; PMCID: PMC5062147.