Famurewa et al. *Afr. J. Clin. Exper. Microbiol.* 2025; 26 (2): 135 - 143 https://www.afrjcem.org

African Journal of Clinical and Experimental Microbiology. ISSN 1595-689X Apr 2025; Vol.26 No.2
AJCEM/2378. https://www.ajol.info/index.php/ajcem

Original Article **Open Access**

Bacterial etiology of spreading odontogenic infection in southwest Nigeria using the 16S rRNA next generation sequencing technique

[1,2,3]Famurewa, B. A., [1,2]Uwanibe, J. N., [1,2]Olawoye, I. B., [2]Eromon, P., [3]Aregbesola, S. B., [3]Oginni, F. O., [1,2]Happi, C. T., and *[1,2]Folarin, O. A.

[1]Department of Biological Sciences, College of Natural Sciences, Redeemer's University, PMB 230, Ede, Osun State, Nigeria
[2]African Center of Excellence for the Genomics of Infectious Diseases (ACEGID), Redeemer's University, PMB 230, Ede, Osun State, Nigeria
[3]Department of Oral and Maxillofacial Surgery, Obafemi Awolowo University Teaching Hospitals Complex, Ile-Ife, Osun State, Nigeria
*Correspondence to: folarino@run.edu.ng; ORCID No: 0000-0001-7283-2920

Abstract:

Background: Genomics surveillance and characterization of pathogens have enabled prompt and accurate diagnosis, for improved management and control of infectious diseases. This study aimed to identify bacteria associated with spreading odontogenic infections (SOIs) among patients visiting the Dental Center of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife, Nigeria, by sequencing 16S rRNA gene of the bacteria.
Methodology: This was a cross-sectional study of 15 participants with clinically confirmed SOIs. Pus samples were obtained from the participants and stored at -80^0 C until ready for analysis. DNA was extracted from the pus samples using the Quick-DNA™ Miniprep Plus Kit. Polymerase chain reaction (PCR) was used to amplify the V1-V9 regions of the 16S rRNA gene. Successfully amplified samples were cleaned up and next generation sequencing (NGS) technique was used to sequence the bacterial 16S rRNA gene. Sequence data were analyzed using Geneious Prime version 2021.2.2 which used Ribosomal Database Project Tools to assign genus and higher-level taxonomy.
Results: Multiple genera of bacteria were detected in individual sample. The detected and identified bacteria belonged to *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, *Proteobacteria*, *Tenericutes* and *Spirochaetes* phyla. Detected predominant bacterial genera were *Streptococcus, Prevotella*, *Peptostreptococcus, Parvimonas* and *Porphyromonas*. Some novel bacteria identified include *Legionella*, *Taonella*, *Ferrovibro*, *Holdemania*, and *Limnobacter*.
Conclusion: Bacteria detected in this study include previously reported bacteria associated with SOIs and novel bacteria, with preponderance of anaerobes.

Keywords: Spreading odontogenic infection; 16S rRNA; Next Generation Sequencing (NGS); Oral microbiota

Received Dec 26, 2024; Revised Jan 10, 2025; Accepted Jan 12, 2025

Étiologie bactérienne de la propagation de l'infection odontogène dans le sud-ouest du Nigeria à l'aide de la technique de séquençage de nouvelle génération de l'ARNr 16S

[1,2,3]Famurewa, B. A., [1,2]Uwanibe, J. N., [1,2]Olawoye, I. B., [2]Eromon, P., [3]Aregbesola, S. B., [3]Oginni, F. O., [1,2]Happi, C. T., et *[1,2]Folarin, O. A.

[1]Département des Sciences Biologiques, Collège des Sciences Naturelles, Université du Rédempteur, PMB 230, Ede, État d'Osun, Nigéria
[2]Centre Africain d'Excellence en Génomique des Maladies Infectieuses (ACEGID), Université du Rédempteur, PMB 230, Ede, État d'Osun, Nigeria
[3]Département de Chirurgie Buccale et Maxillo-Faciale, Complexe des Hôpitaux Universitaires de l'Université, Obafemi Awolowo, Ile-Ife, État d'Osun, Nigéria
*Correspondance à: folarino@run.edu.ng; ORCID No: 0000-0001-7283-2920

Resumé:

Contexte: La surveillance génomique et la caractérisation des agents pathogènes ont permis d'établir un diagnostic rapide et précis, afin d'améliorer la prise en charge et le contrôle des maladies infectieuses. Cette étude visait à identifier les bactéries associées à la propagation des infections odontogènes (SOI) chez les patients visitant le Centre dentaire du Complexe des hôpitaux universitaires Obafemi Awolowo (OAUTHC), à Ile-Ife, au Nigeria, en séquençant le gène de l'ARNr 16S de la bactérie.
Méthodologie: Il s'agissait d'une étude transversale portant sur 15 participants présentant des troubles de l'information cliniquement confirmés. Des échantillons de pus ont été prélevés chez des participants atteints de SOI et stockés à -80^{0}C jusqu'à ce qu'ils soient prêts à être analysés. L'ADN a été extrait d'échantillons de pus à l'aide du kit Quick-DNA™ Miniprep Plus. La réaction en chaîne par polymérase (PCR) a été utilisée pour amplifier les régions V1-V9 du gène de l'ARNr 16S. Les échantillons ont été amplifiés et nettoyés avec succès. Une technique de séquençage de nouvelle génération a été utilisée pour séquencer le gène bactérien de l'ARNr 16S. Les données de séquence ont été analysées à l'aide de la version 2021.2.2 de Geneious Prime, qui a utilisé les outils de projet de base de données ribosomique pour attribuer le genre et la taxonomie de niveau supérieur.
Résultats: Plusieurs genres de bactéries ont été détectés dans un échantillon individuel. Les bactéries détectées et identifiées appartenaient aux embranchements *Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Proteobacteria, Tenericutes* et *Spirochaetes*. Les genres bactériens prédominants détectés étaient *Streptococcus, Prevotella, Peptostreptococcus, Parvimonas* et *Porphyromonas*. Parmi les nouvelles bactéries identifiées, citons Legionella, Taonella, Ferrovibro, Holdemania et Limnobacter.
Conclusion: Les bactéries détectées comprennent des organismes bactériens précédemment signalés associés à la SOI et de nouvelles bactéries avec une prépondérance d'anaérobies.

Mots-clés: Propagation de l'infection odontogène; séquençage du gène de l'ARN 16S; Séquençage de nouvelle génération (NGS); Microbiote buccal

Introduction:

Spreading odontogenic infections (SOIs) are severe form of odontogenic infections that have extended to involve the deeper tissues of the head and neck region typically through the lateral surface of the alveolar bone (1). Poorly treated and untreated dentoalveolar infections will almost always progress into SOIs. It is a mixed infection involving mostly bacteria of the normal oral microbiota that become opportunistic pathogens. Common clinical presentations are swelling and pain over the anatomic space involved in the head and neck region, restricted mouth opening, dysphagia and fever (2).

Late presentation and/or delayed appropriate interventions often result in poor outcomes such as airway obstruction/respiratory distress, prolonged hospitalization with/without intensive care admission, orbital cellulitis, cervicofacial necrotizing fasciitis, cavernous sinus thrombosis, brain abscess, sepsis and death. Systemic co-morbidities such as diabetes mellitus, chronic liver disease, malnutrition and any condition that impairs host immunity are associated with complications and prolonged hospital stay (2-4).

Traditionally, the putative bacterial pathogens of SOIs are identified by culture of clinical specimens (mostly pus) and usually followed by antibiotics sensitivity testing in microbiology laboratory. This approach is time consuming, especially when fastidious, slow-growing oral pathogens are involved, and can only detect organisms that can be cultured. Even with recent advances in culture techniques, about one third of oral bacteria are still uncultured (5).

Molecular biology techniques such as polymerase chain reaction, cloning and sequence analysis of bacterial 16S rRNA genes have been used to identify some bacteria associated with SOIs and detect antibiotic-resistant strains which could enhance clinical outcomes by facilitating development and implementation of rational surgical and supportive treatment protocols (6,7).

Böttger et al., (8) sequenced the 16S rRNA gene by next generation sequencing (NGS) platform to identify bacterial organisms in the pus samples of Caucasian patients with severe odontogenic infections. The study concluded that 16S rRNA gene metagenomics detected more bacteria than the conventional culture dependent method and also confirmed bacteria in previous culture-negative samples. There is paucity of published report on molecular identification of bacteria associated with SOIs in Nigeria. Considering the high diagnostic yield of 16S rRNA gene sequencing by high throughput NGS platform and its likely clinical benefits on the overall management of patients with SOIs, this study aimed to identify bacteria associated with SOI in Nigerian patients by sequencing bacterial 16S rRNA gene using the NGS technique.

Materials and method:

Study design and participants:

This was a cross-sectional study of a total of 15 participants with clinically confirmed spreading odontogenic infections (SOIs) who were enrolled into the study, with the aim of identifying bacteria associated with SOI through 16S rRNA gene sequencing.

Ethical consideration:

The study was approved by the Ethics

and Research Committee (Protocol no: ERC/2021/05/12) of the Obafemi Awolowo University Teaching Hospitals Complex (OAUTHC), Ile-Ife Osun State, Nigeria. Written informed consents were obtained from all confirmed patients with SOI visiting the Dental Center of OAUTHC, Ile-Ife before enrollment into the study. All procedures in the study were in compliance with the ethical standards of the institutional and national research committee and with the 1964 Helsinki Declaration and its later amendments.

Sample collection:

Pus was aseptically collected by sterile needle aspiration using disposable 5ml syringe with a 21G needle through the povidone iodine prepared mucosa (for intraoral swellings) or skin overlying facial and upper neck swellings. Sampling site was determined by swelling with maximum fluctuance intraorally and extraorally. The pus samples were transferred to a labelled Eppendorf vial containing 1ml of sterile phosphate buffered saline (PBS) and transported to the African Center of Excellence for Genomics of Infectious Diseases (ACEGID), Redeemer's University Ede, Osun State Nigeria on ice packs. All samples were stored at –80°C until molecular assay was conducted at the ACEGID laboratory.

DNA extraction from pus samples:

DNA was extracted using Quick-DNA™ Miniprep Plus Kit (Zymo Research Corporation) according to the manufacturer's instructions. A final volume of 50 µL of the DNA was eluted into a clean labelled microcentrifuge tube and store at -20°C until use for the analysis.

Polymerase chain reaction of 16S rRNA gene:

The universal primers used for PCR targeted the conserved V1-V9 regions of the 16S rRNA gene were 27F (5'-AGA GTT TGA TCC TGG CTC AG-3') and 1492R (5'-AAG GAG GTG ATC CAG CCG CA-3') forward and reverse primers respectively. This amplification was done in a final reaction volume of 20 µL using PuReTaq™ Ready-To-Go PCR Beads that were reconstituted with 12.5 µL of double distilled water, 1.25 µL each of the forward and reverse primers (each primer at a concentration of 0.1µM) and 5 µL of extracted DNA sample (at concentration of 5 ng/µL). The amplification was done in the Eppendorf Mastercycler PRO S 6325 Thermal Cycler set at appropriate cycling conditions as previously described (7).

Sequencing of 16S rRNA gene amplicons:

Samples of 9 patients with severe SOI with or without concomitant systemic disease were selected for sequencing of the 16S rRNA gene. The amplified products were cleaned up using QIAquick® PCR & Gel Cleanup Kit, and libraries of the amplicons were prepared according to protocol for PulseNet Nextera XT library prep and run setup for the Illumina MiSeq (9). The NGS technique was used to sequence the bacterial 16S rRNA gene using the Illumina MiSeq (Illumina, San Diego, CA) sequencing system (9). The sequence data for this study were submitted to the SRA database (accession number: PRJNA926479) and are available at: https://www.ncbi.nlm.nih.gov/sra/PRJNA926479

Bioinformatics analysis of the sequence data:

Illumina MiSeq sequence data were uploaded into the Geneious Prime version 2021.2.2 (https://www.geneious.com) (10). Data were cleaned by trimming, merging and length filtering to eliminate PCR and sequencing errors, and chimeric sequences before analysis by a web-based 16S Biodiversity tool. Merged sequence reads were clustered into operational taxonomic units (OTUs) at the 98% similarity cut off point. The platform uses Ribosomal Database Project (RDP) Tools (11) version 2.12 which assigned taxonomy (from domain to genus) and bootstrap confidence-estimate to each sequence by comparing them to sequences on the 16S rRNA database.

The interactive krona chart of bacterial diversity based on the assigned bacterial taxonomy was generated by Krona version 2.0 (12) for each sequenced sample. Detected bacterial genera were compared with the expanded Human Oral Microbiome Database (13). This was done to ascertain if these organisms are previously reported resident oral microbiota or exogeneous pathogenic organisms.

Statistical analysis:

Bacterial diversity in the samples was determined by alpha diversity indices such as Shannon and Chaol 1 while between sample diversity was assessed by Bray-Curtis similarity index (14,15).

Results:

Demographics and clinical profile of study participants:

Overall, 15 participants with clinically confirmed SOIs were enrolled into the study. The mean age of the study participants was 51.8 years (range: 27-84 years). Eight of the participants were females. Twelve participants (80.0%) presented with no underlying co-morbidity while three participants had hypertensive heart disease and diabetes mellitus (Table 1).

Results of PCR, 16S rRNA gene sequencing and Bioinformatics analysis:

The PCR amplification of the V1-V9 region of the bacterial 16S rRNA gene was successful in 14 (93.3%) of the 15 pus samples. Based on the clinical presentations (severity of infection and presence of systemic dis-

ease) of the participants, 9 (64.3%) of the 14 amplified samples were successfully sequenced using Illumina MiSeq sequencing platform. The total number of sequence reads was 23,925,665 after trimming, merging and length filtering, with a median read of 2,545,659 (Table 2).

Table 1: Demographics and clinical profiles of study participants with spreading odontogenic infections

Patient code	Gender	Age (years)	Systemic disease	Sampling site
01	Female	60	Hypertensive heart disease	Maxillary vestibule
03	Male	38	Nil	Submandibular space
04	Male	45	Nil	Submandibular space
05	Male	31	Nil	Buccal space
06	Male	65	Nil	Submandibular space
07	Male	38	Nil	Buccal space
11	Male	27	Nil	Maxillary vestibule
12	Female	80	Hypertensive heart disease	Maxillary vestibule
15	Female	84	Diabetes mellitus	Maxillary vestibule
02	Female	45	Nil	Mandibular vestibule
08	Female	58	Nil	Submandibular space
09	Female	65	Nil	Maxillary vestibule
10	Female	35	Nil	Mandibular vestibule
13	Female	57	Nil	Maxillary vestibule
14	Male	50	Nil	Maxillary vestibule

Table 2: Valid 16S rRNA gene sequence reads and operational taxonomic units (OTUs)

Patient code	Sample ID	Total sequenced read	Total OUT
01	OIF1	4,290,898	43,090
06	OIF2	4,673,600	7,852
07	OIF3	4,270,008	53,231
05	OIF4	2,416,339	1,602
11	OIF5	4,676,708	12,552
04	OIF6	1,281,138	86,286
12	OIF7	2,545,659	13,677
03	OIF8	2,004,437	1,044,797
15	OIF9	1,766,878	1,025,678

ID: Identification code; OTU: Operational Taxonomic Unit

The abundance of detected operational taxonomic units (OTUs) is represented by radial slices of the Krona charts and the hue (red to green) highlights the average confidence value for each segment. Low confidence is shown as red and it increases to green with a higher confidence for taxonomic classification.

All the nine samples revealed the presence of a wide range of bacteria as shown in the Krona charts for samples OIF1 (Fig 1) and OIF2 to OIF9 (Supplementary Figs 1-8) (https://afrjcem.org/supplementary-materials/).

Detected bacteria and diversity analysis:

The average number of bacteria found in the 9 sequenced samples was 269,209 (Range: 585-1,154,363). Detected bacteria in the 9 samples belonged to at least 7 phyla, namely *Firmicutes*, *Bacteroidetes*, *Fusobacteria*, *Actinobacteria*, *Proteobacteria*, *Tenericutes* and *Spirochaetes* phyla.

The different abundant bacterial genera detected in the samples are presented in Fig 2 with *Streptococcus, Prevotella*, *Peptostreptococcus Parvimonas* and *Porphyromonas* genera as the most predominant bacteria in the pus samples. Expectedly, there were more anaerobic than aerobic bacteria in the pus samples.

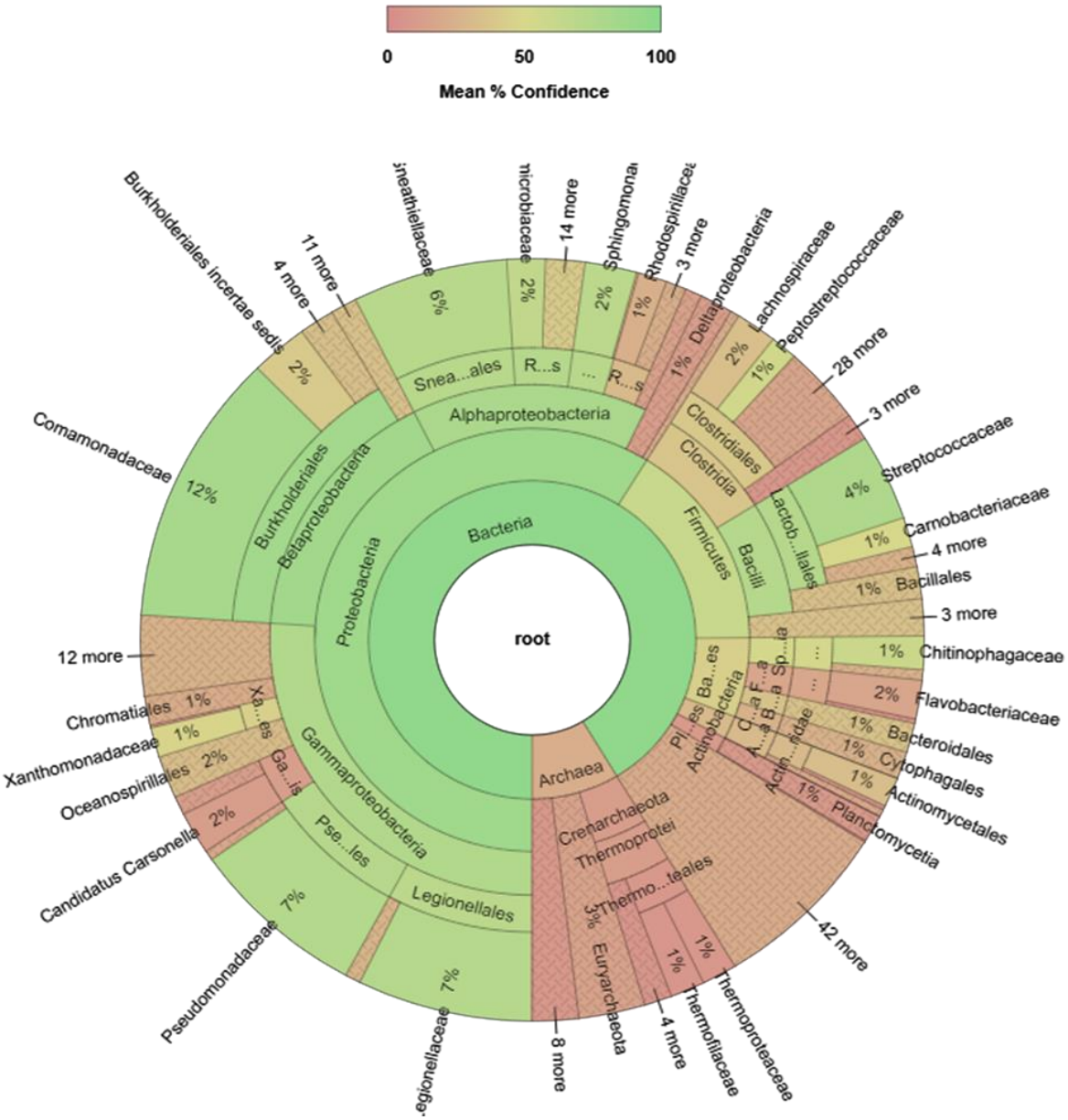

Fig 1: Krona chart of sample OIF1
Full names of truncated legends/labels: Sneathiellaceae, Hyphomicrobiaceae, Sphingomonadaceae, Rhodospirallaceae, Legionellaceae

The Shannon diversity index was highest in sample OIF8 (2.183), followed by OIF1 (2.097) and lowest in OIF5 (1.770). The values of Chao1 index revealed that diversity was highest in OIF1 (16.0), followed by OIF2 and OIF8, and lowest in OIF5 and OIF9 (Table 3). The mean alpha diversity indices of intraoral samples were 1.9495 (Shannon index) and 11.125 (Chao 1 index). Conversely, mean alpha diversity indices of extraoral samples were 2.0348 (Shannon index) and 10.55 (Chao 1 index).

For beta diversity, bacterial genera composition was similar between OIF4 and OIF9 (Bray-Curtis similarity index of 0.79), followed by OIF5 and OIF6, then OIF1 and OIF7 (Fig 3). Pairwise comparison of beta diversity indices of extraoral with intraoral samples showed 100% similarity between six pairs of intraoral and extraoral samples (Supplementary Table 1). Supplementary Table 2 showed 15 bacterial genera detected but were not known to be associated with SOIs nor part of resident oral microbiota when compared with publicly available oral microbiome database (https://afrjcem.org/supplementary-materials/).

Table 3: Alpha diversity indices of individual sample microbiota

Sample ID	Sampling site	Shannon index	Chao 1 index
OIF1	Intraoral/Maxillary vestibule	2.097	16.00
OIF2	Extraoral/Submandibular space	2.047	11.00
OIF3	Extraoral/Buccal space	1.903	10.50
OIF4	Extraoral/Buccal space	1.927	10.25
OIF5	Intraoral/Maxillary vestibule	1.77	9.00
OIF6	Extraoral/Submandibular space	2.064	10.00
OIF7	Intraoral/Maxillary vestibule	2.075	10.50
OIF8	Extraoral/Submandibular space	2.183	11.00
OIF9	Intraoral/Maxillary vestibule	1.856	9.00

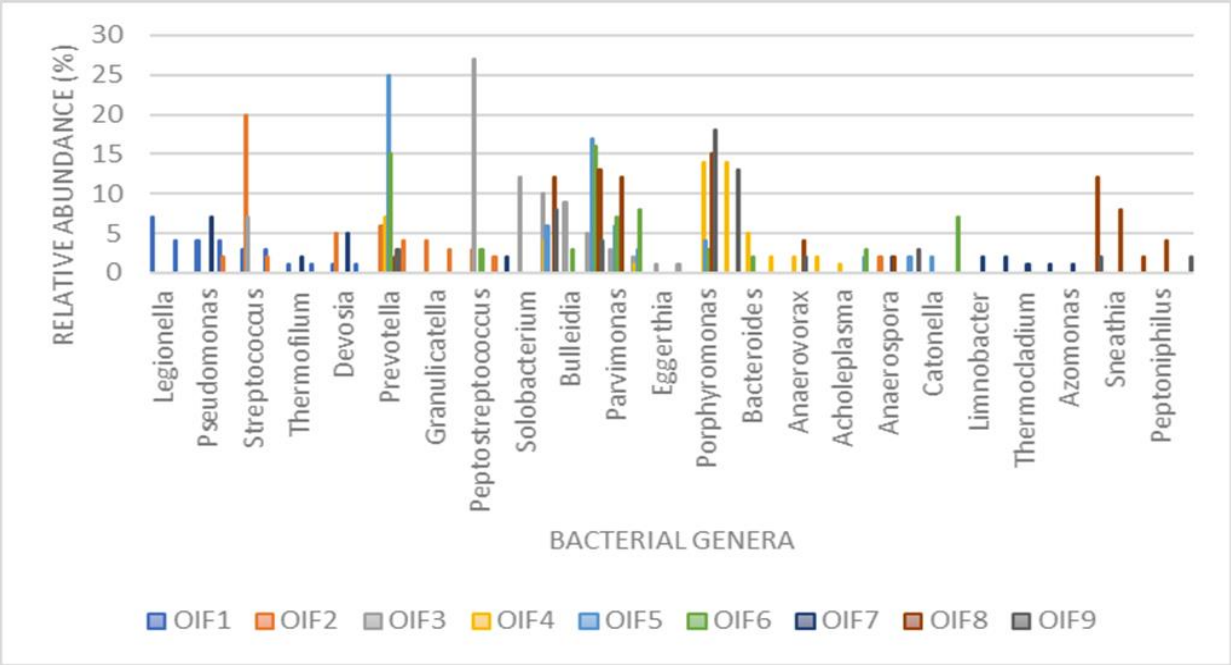

Fig 2: Clustered bar chart showing different abundant bacterial genera in pus samples
Bacterial genera with less than 1% abundance were excluded

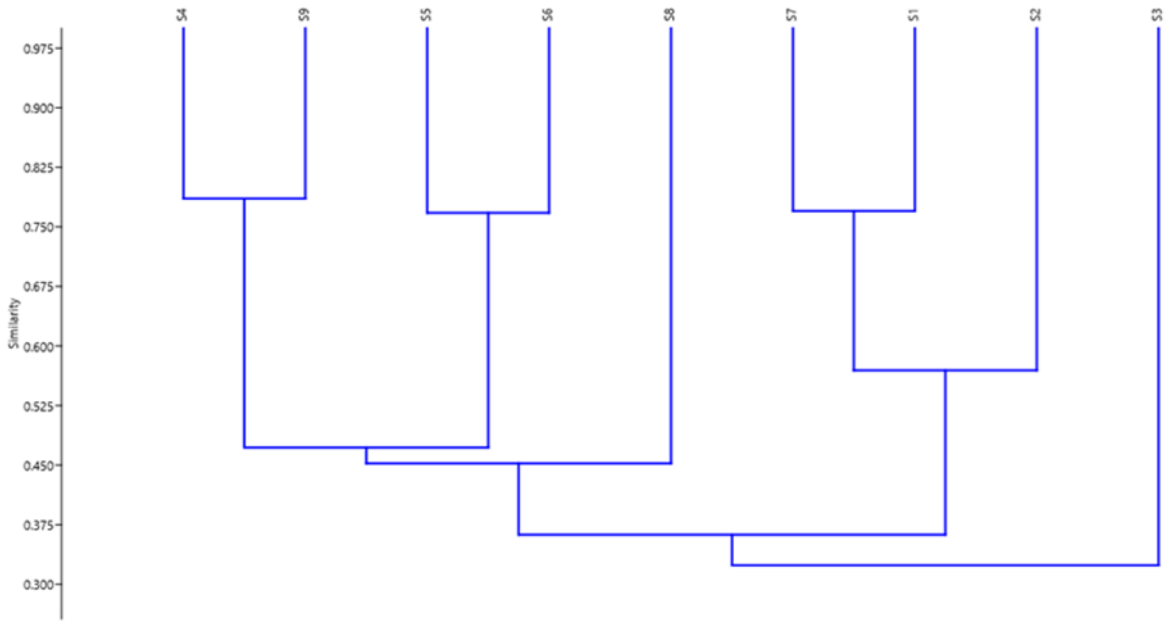

Fig 3: Dendrogram showing the Bray-Curtis similarity of bacterial genera in nine pus samples*
*S1=OIF1; S2=OIF2; S3=OIF3; S4=OIF4; S5=OIF5; S6=OIF6; S7=OIF7; S8=OIF8; S9=OIF9

Discussion:

This study was undertaken to identify bacteria associated with SOIs in Southwest Nigeria by sequencing 16S rRNA gene in order to comprehensively determine the bacterial etiology of SOI in the study population. The spectrum of bacteria detected and identified in this study belonged to seven phyla (Firmicutes, Bacteroidetes, Fusobacteria, Actinobacteria, Proteobacteria and Spirochaetes) to which previously reported bacteria species associated with odontogenic abscesses are classified (16). This shows that the phylum level biodiversity of this study is broader than similar studies on SOIs that used lower throughput molecular methods such as Sanger sequencing and nested PCR.

Furthermore, *Streptococcus, Prevotella*, *Peptostreptococcus, Parvimonas* and *Porphyromonas* genera were found to be the most predominant bacteria detected in the pus samples. This is similar to the reports of Bottger and colleagues (8) in Germany where *Prevotella*, *Porphyromonas*, *Fusobacterium*, *Veillonella* and *Parvimonas* genera were the most prevalent organisms after 16S rRNA gene sequencing in 48 patients with severe odontogenic infection. This trend is typical of the microbial pathogenesis of odontogenic infections. Specifically, *Prevotella intermedia*, *Prevotella nigrescens*, *Porphyromonas endodontalis*, and *Fusobacterium nucleatum* have been reported as the most important pathogenic anaerobes implicated in the pathogenesis of orofacial odontogenic infections because of their virulence factors and synergistic relationship with other bacteria (17).

Bacteriology of SOIs involved predominantly anaerobes, with aerobic bacteria in the early phase of infections. Aerobic bacteria serve as initiators of the infectious process, preparing the local environment for anaerobic bacterial invasion as the local tissue condition turns to a more hypoxic state that encourages anaerobic bacterial growth but inhibits aerobic bacterial growth (18,19). This microbial succession was validated in the present study as anaerobes were prominent among the bacterial genera detected and identified in all the samples. Since the bacterial profile of SOI is polymicrobial (with preponderance of anaerobes), the first line antibiotic therapy should address these organisms with immediate surgical intervention to drain abscess and/or decompress cellulitis.

Accurate identification of microrganisms implicated in SOI is critical in patients' timely and efficient management. This guides the prescription of antibiotic therapy as a potent adjunct to the surgical management of SOI. Appropriate non-surgical treatment along with antibiotics therapy is particularly important in immunocompromised and medically compromised patients with SOI (20). The treatment goal is to prevent or minimize rapid spread of infection to contiguous areas and septic complication (21).

Plethora of bacterial organisms were identified at the genus level in this study. This is enormously diverse and overwhelming in terms of counts compared to the limited number of identified bacterial species associated with SOIs as reported by Flynn et al., (6) and Walia et al., (21) who used Sanger sequencing technique and culture method respectively. Diagnostic yield of 16S rRNA gene sequencing by high throughput NGS technologies have

significantly expanded the knowledge of the bacterial diversity associated with predominantly bacterial odontogenic infections (22).

Analysis of bacterial diversity based on the metrics of Shannon and Chao1 indices showed that pus samples OIF1 and OIF8 have high microbial diversity than other samples. Intraoral samples had more diversity (Chao 1 index) than extraoral samples. Conversely, intraoral samples had lower mean Shannon index, that measured species richness and evenness. With regards to beta diversity, there were more than 70% similarity in the bacterial composition of six out of the nine samples. This is probably because most odontogenic infections are caused by opportunistic resident oral microbiota (18).

There is dearth of normal oral 16S rRNA derived microbiome data in Nigeria and indeed Africa (23,24). Accordingly, we compared our data with the expanded Human Oral Microbiome Database (13). Following this, there were 15 bacterial genera which to the best of our knowledge, have not been previously associated with SOIs nor are normal oral microbiota. The detection of these novel organisms may be attributed to the sensitivity and wider coverage (deep sequencing of 16S rRNA gene) of the molecular technique used in this study (22). Furthermore, race and diets of study participants could be responsible for this finding as reported by Yang et al., (25).

Apart from the small sample size, this study is also limited by the fact that there was no oral microbiome analysis from individuals with no SOIs as comparative or control group. This would have enabled us to objectively determine whether detected bacteria were part of the resident oral microbiota in the studied population or exogenous bacteria. However, majority of the detected bacteria in this study have been reported to be opportunistic pathogens implicated in SOIs and other odontogenic infections (6-8).

In conclusion, our study provides important information on the bacteriology of SOIs in the study population using 16S rRNA gene NGS technique. We recommend further study with adequate sample size, and age and gender matched control group to validate our findings and assign taxonomy to identified organisms up to the species level.

Acknowledgements:

The authors acknowledge support of the funders and would also like to appreciate the study participants.

Contributions of authors:

BAF and OAF were involved in the study conceptualization and design; BAF, SBA and FOO were involved with participants' recruitment and clinical sample acquisition; BAF, JNU and PE were involved in molecular experiment and investigation; IBO and BAF were involved with analysis and interpretation of sequence data; CTH was responsible for funding acquisition; OAF and CTH were involved with project administration; OAF was responsible for project supervision; BAF wrote the original draft; BAF, OAF and IBO reviewed and edited the manuscript. All authors read and approved the manuscript submitted for publication.

Source of funding:

The study was supported by grants from the National Institute of Allergy and Infectious Diseases (https://www.niaid.nih.gov), NIH-H3Africa (https://h3africa.org) (U01HG007480 and U54HG007480) and the World Bank grants (project ACE-019 and ACE-IMPACT).

Conflict of interest:

No competing interest is declared.

Data availability:

The dataset of this study is available from the corresponding author on reasonable request. The sequence data for this study were submitted to the SRA database (accession number: PRJNA926479) and are available at https://www.ncbi.nlm.nih.gov/sra/PRJNA926479

References:

1. Lopez-Gonzalez, E. Vitales-Noyola, M., González-Amaro, A. M., et al. Aerobic and anaerobic microorganisms and antibiotic sensitivity of odontogenic maxillofacial infections. Odontology. 2019; 107:409-417. https://doi.org/10.1007/s10266-019-00414-w
2. Adeosun, P. O., Fatusi, O. A., and Adedeji, T. A. Assessment of severity of illness and monitoring response to treatment of odontogenic space infection using serum prealbumin. J Maxillofac Oral Surg. 2019; 18: 106–111. https://doi.org/10.1007/s12663-018-1105-4
3. Weise, H., Naros, A., Weise, C., Reinert, S., and Hoefert, S. Severe odontogenic infections with septic progress – a constant and increasing challenge: a retrospective analysis. BMC Oral Health. 2019; 19:173. https://doi.org/10.1186/s12903-019-0866-6
4. Bali, R.K., Sharma, P., Gaba, S., Kaur, A., and Ghanghas P. A review of complications of odontogenic infections. Natl J Maxillofac Surg. 2015; 6:136-143. https://doi.org/10.4103/0975-5950.183867
5. Wade, W., Thompson, H., Rybalka, A., Vartoukian, S. Uncultured Members of the Oral Microbiome. J Calif Dent Assoc. 2016; 44: 447-456.
6. Flynn, T. R., Paster, B. J., Stokes, L. N., Susarla, S. M., and Shanti, R. M. Molecular methods for diagnosis of odontogenic infections. J Oral Maxillofac Surg. 2012; 70:1854-1859. https://doi.org/10.1016/j.joms.2011.09.009
7. Riggio, M. P., Aga, H., Murray, C. A., et al. Identification of bacteria associated with spreading odontogenic infections by 16S rRNA gene sequencing. Oral Surg Oral Med Oral Pathol Oral

Radiol Endod. 2007; 103:610-617. https://doi.org/10.1016/j.tripleo.2006.08.009
8. Böttger, S., Zechel-Gran, S., Schmermund, D., et al. Clinical relevance of the microbiome in odontogenic abscesses. Biology (Basel). 2021; 10: 916; doi: 10.3390/biology10090916
9. Centers for Disease Control and Prevention. Laboratory standard operating procedure for PulseNet Nextera XT Library Prep and Run Setup for the Illlumina MiSeq. Code PNL32. (2015). https://www.cdc.gov/pulsenet/pdf/pnl32-miseq-nextera-xt.pdf (Accessed February 1st, 2016)
10. Kearse, M., Moir, R., Wilson, A., et al. Geneious Basic: an integrated and extendable desktop software platform for the organization and analysis of sequence data. Bioinformatics. 2012; 28: 1647–1649. https://doi.org/10.1093/bioinformatics/bts199
11. Cole, J. R., Wang, Q., Fish, J. A., *et al*. Ribosomal Database Project: data and tools for high throughput rRNA analysis. Nucl Acids Res. 2014; 42: D633-D642. https://doi.org/10.1093/nar/gkt1244
12. Ondov, B. D., Bergman, N. H., Phillippy, A. M. Interactive metagenomic visualization in a Web browser. BMC Bioinform. 2011; 12: 385. https://doi.org/10.1186/1471-2105-12-385
13. Escapa, F. I., Huang, Y., Chen, T., et al. Construction of habitat-specific training sets to achieve species-level assignment in 16S rRNA gene datasets. Microbiome 2020; 8: 65. https://doi.org/10.1186/s40168-020-00841-w
14. Kim, B. R., Shin, J., Guevarra, R., et al. Deciphering diversity indices for a better understanding of microbial communities. J Microbiol Biotechnol. 2012; 27: 2089–2093. https://doi.org/10.4014/jmb.1709.09027
15. Ricotta, C., and Podani, J. On some properties of the Bray-Curtis dissimilarity and their ecological meaning. Ecol Complex. 2017; 31:201–205. https://doi.org/10.1016/j.ecocom.2017.07.003
16. Siqueira, J. F., and Rocas, I. N. Microbiology and treatment of acute apical abscesses. Clin Microbiol Rev. 2013; 26:255–273. https://doi.org/10.1128/cmr.00082-12
17. Stefanopoulos, P. K., and Kolokotronis, A. E. The clinical significance of anaerobic bacteria in acute orofacial odontogenic infections. Oral Surg Oral Med Oral Pathol Oral Radiol Endod. 2004; 98:398-408. https://doi.org/10.1016/j.tripleo.2004.01.016
18. Ogle, O. E. Odontogenic infections. Dent Clin N Am. 2017; 61: 235–252. https://doi.org/10.1016/j.cden.2016.11.004
19. Zirk, M., Buller, J., Goeddertz, P. et al. Empiric systemic antibiotics for hospitalized patients with severe odontogenic infections. J Craniomaxillofac Surg. 2016; 44:1081-1088. https://doi.org/10.1016/j.jcms.2016.05.019
20. Flynn, T. R., Shanti, R. M., Levi, M. H., Adamo, A. K., Kraut, R. A., and Trieger, N. Severe odontogenic infections, part 1: prospective report. J Oral Maxillofac Surg. 2006; 64:1093–1103. https://doi.org/10.1016/j.joms.2006.03.015
21. Walia, I. S., Borle, R. M., Mehendiratta, D., and Yadav, A. O. Microbiology and antibiotic sensitivity of head and neck space infections of odontogenic origin. J. Maxillofac. Oral Surg. 2014; 13: 16–21. https://doi.org/10.1016/j.jcms.2017.07.013
22. Wade, W. G., and Prosdocimi, E. M. Profiling of Oral Bacterial Communities. J Dent Res. 2020; 99:621-629. https://doi.org/10.1177/0022034520914594
23. Anukam, K. C., and Agbakoba, N. R. A comparative study of the oral microbiome compositions of healthy postmenopausal, premenopausal, and prepubertal Nigerian females, using 16S rRNA metagenomics methods. Niger J Clin Pract. 2017; 20:1250-1258. https://doi.org/10.4103/njcp.njcp_32_17
24. Araújo, V., Fehn, A. M., Phiri, A., Wills, J., Rocha, J., and Gayà-Vidal, M. Oral microbiome homogeneity across diverse human groups from southern Africa: first results from southwestern Angola and Zimbabwe. BMC Microbiol. 2023; 23: 226. https://doi.org/10.1186/s12866-023-02970-2
25. Yang, Y., Zheng, W., Cai, Q., et al. Racial differences in the oral microbiome: data from low-income populations of African ancestry and European ancestry. mSystems. 2019; 4: e00639-19. doi:10.1128/mSystems.00639-19