Oral Bacteriome Compositions Identified by 16S rRNA Metagenomics in a Randomly Selected "Healthy" Nigerian Male and Female Subjects

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ABSTRACT

Background: There is a dearth of information on the core oral bacteriome compositions of healthy Africans especially Nigerians, mostly due to the non-existence of apt molecular techniques.
Objectives: In this study, we sought to determine the core oral bacteriome compositions of 'healthy' Nigerian males and females.
Methods: Oral samples were collected from nineteen adult subjects comprising 11 females and 8 males. DNA was extracted and 16S rRNA V4 region amplified using pattern barcoded primers prior to sequencing with the Illumina MiSeq program. Quantitative Insights into Microbial Ecology (QIIME) pipeline was used for 16S rRNA identification. The core genera were defined as taxa

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present in all subjects and over 2.0% in abundance, while core species defined as taxa found in at least 17/19 samples and over 0.1% in abundance.

Results: Overall, 111 genera and 151 species representing 14 phyla were identified from the 19 subjects. *Firmicutes* (43.6%) were the most abundant phyla followed by *Proteobacteria* (33.62%), *Bacteroidetes* (9.67%), *Actinobacteria* (8.48%), *Fusobacteria* (4.31%) and others. The most abundant genera were *Streptococcus* (27.28%) followed by *Haemophilus* (14.95%), *Neisseria* (9.67%), *Veillonella* (7.22%), *Gemella* (5.77%), *Rothia* (3.11%), *Prevotella* (3.03%), *Porphyromonas* (2.94%), *Lautropia* (2.86%), *Corynebacterium* (2.74%), and *Leptotrichia* (2.61%). The most abundant core species identified were *Haemophilus parainfluenzae* (29.31%), followed by *Haemophilus influenzae* (6.91%), *Lautropia sp* TeTO (6.82%), *Porphyromonas catoniae* (6.0%), *Streptococcus thermophilus* (5.23%), *Actinobacillus porcinus* (4.80%), *Rothia dentocariosa* (3.32%), *Rothia mucilaginosa* (2.95%), *Neisseria elongata* (2.66%), and *Streptococcus gordonii* (2.55%).

Conclusions: The genera and species-level core oral bacteriome identified could be used as a reference for comparison with larger population studies.

Keywords: Core; oral bacteriome; microbiome; human; mouth; 16S metagenomics; Africans.

1. INTRODUCTION

The use of 16S rRNA metagenomics sequencing has revolutionised the identification of bacterial organisms occupying body sites that could not have been cultured with conventional methods. The oral cavity is "home" to over 1000 bacterial species, of which a large number are still uncultivable thus comprising what is now known as oral bacteriome [1]. Oral bacteriome has recently been referred to as the bacterial constituent of the microorganisms that inhabit the oral cavity and their collective genomes [2].

The Human Microbiome Project (HMP), under the auspices of the National Institutes of Health (NIH) was launched in 2008 with the cardinal objective and mandate to sequence the microbiome of healthy human subjects (http://commonfund.nih.gov/hmp). The study revealed that different body sites showed approximately a ten-fold difference in estimated microbial richness, with stool samples having the highest estimated richness, followed by the mouth, throat, and gums, then by the skin, nasal and vaginal sites [3].

Contemporary studies on healthy individuals have shown that the predominant oral microbiota was mostly consistent [4], which were generally nonpathogenic and considered to be commensal in the human oral cavity [5]. The consistent microbiota has been fitted into five phyla such as *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria*. Over 30 genera including *Streptococcus, Rothia, Neisseria, Prevotella, Haemophilus, Fusobacterium, Porphyromonas, Veillonella, Granulicatella, Gemella, Leptotrichia,* *Actinomyces, Aggregatibacter, Lautropia*, and *Capnocytophaga*, have been repeatedly described among the most common and/or abundant genera in oral samples and have been implicated as predominant in the oral cavity of healthy individuals [6,7].

However, some species such as *Porphyromonas gingivalis, Treponema denticola, Tannerella forsythia, Fusobacterium nucleatum, Prevotella intermedia*, and *Aggregatibacter actinomycetemcomitans* are frequently isolated from dental plaques in periodontal patients. These species were found in biofilms and appear to play a role in the development of periodontal disease [8]. There is mounting evidence that some species are regarded as members of a 'common' core human oral bacteriome. For example, *Streptococcus mitis* [9], *Rothia mucilaginosa, Rothia dentocariosa* and *Haemophilus parainfluenzae* [10]. A recent controversial study demonstrated a significant association between ethnic affiliation and the composition of the oral microbiome, suggesting that ethnicity is a critical determinant of oral microbial colonisation [11]. Some core species such as *Veillonella parvula, Neisseria flavescence/subflava, P. melaninogenica, Fusobacterium periodonticum, Fusobacterium nucleatum ss polymo rphum, Granulicatella adiacens, Gemella haemolysans, Lautropia mirabilis, Granulicatella elegan, Rothia aeria, Actinomyces odontolyticus, Porphyromonas catoniae, Streptococcus sanguinis*, and *Neisseria sicca* have also been identified in Arab population and in other populations as being core species associated with health [12]. While most of the human microbiome studies, including oral bacteriome where done in the Western and

Asian hemisphere, little or no data exists on the oral bacteriome composition of Africans especially Nigeria, which has over 25% of the African population. It should be noted that knowledge of the oral bacteriome composition is a *sine qua non* in the determination of microbial deviation in oral diseased conditions and has the potential to provide an insight for treatment plans and future investigations. In this study, the main objective is to determine the oral bacteriome of randomly selected healthy Nigerians and to ascertain whether some genera and species are exclusively associated with males and females within the sampled populations.

2. METHODS

2.1 Sample Collection and Data Analysis

Demographic data and oral health or disease history were randomly collected from the subjects by administering structured questionnaires mostly about the oral health and disease history. The subjects were recruited based on the following criteria; absence of recent antibiotic use in the last two months prior to sample collection, no steroid contraceptives, no recent periodontal treatment, no diabetes (with the exception of one), human immunodeficiency virus infection or pregnancy. Oral samples were collected from 19 adult subjects, comprising of 11 females and 8 males, after informed consent. Oral samples were self-collected following uBiome® sample collection instructions. Briefly, participants were instructed to use a sterile swab to transfer a small amount of buccal material into a vial containing a lysis and stabilization buffer that preserves the DNA for transport at ambient temperatures. Samples were lysed using bead-beating, and DNA was extracted by a guanidine thiocyanate silica column-based purification method. PCR amplification of the 16S rRNA genes was performed with primers containing universal primers amplifying the V4 variable region (515F: GTGCCAGCMGCCGCGGTAA and 806R: GGACTACHVGGGTWTCTAAT) [13]. Bacterial DNA was extracted and 16S rRNA V4 region amplified using custom barcoded primers prior to sequencing with the Illumina MiSeq platform. Sequencing was performed in a pair-end modality on the Illumina NextSeq 500 platform rendering 2 x 150 bp pair-end sequences [14].

2.2 Sequence Analysis

Sample sequence reads were demultiplexed using Illumina's BCL2FASTQ algorithm. Reads were filtered using an average Q-score > 30.

The raw paired-end sequence FASTQ reads were imported into MG-RAST pipeline for a quality check (QC). Quantitative Insights into Microbial Ecology (QIIME) pipeline was used for 16S rRNA recognition. Sequences were pre-screened using QIIME-UCLUST algorithms for at least 97% identity to ribosomal sequences from the following RNA databases.

Reads passing all above filters were aligned using 100% identity over 100% of the length against a hand-curated database of target 16S rRNA gene sequences and taxonomic annotations derived from version 123 of the SILVA database [15]. In addition, Operational Taxonomic Unit (OTU) picking was done at 97% identity against the RDP, LSU and SSU databases. Microbial taxonomy was generated from the non-rarefied OTU table. Distribution of taxonomic categories at different levels of a resolution was done using the ribosomal RNA similarities to entries in the Refseq protein database. The core genera were defined as taxa present in all subjects and over 2.0% in abundance, while core species is defined as taxa found in at least 17/19 samples and over 0.1% in abundance. Species diversity score was calculated based on the inverse Simpson's index. Scores range from 0 to 10, with 10 being the most diverse.

2.3 Ethics Approval

This study was carried out in accordance with the recommendations of the Faculty of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State ethics committee, with written and informed consent from all subjects. All subjects gave written and informed consent in accordance with the Declaration of Helsinki.

3. RESULTS

3.1 Sequence Characteristics

On average the data sets generated 45,612 high-quality sequences per sample totaling a mean of 6,797,368 base-pairs (bp) with an average length of 149 bps (149 ± 13 bp). Post QC produced an average base-pair count of 274,373 bp, post sequence count of 1,97, mean sequence length of 140 ± 25 bp and mean Guanine-Cytosine (GC) percent of 55 ± 3%. About 60% of the samples had on average 2,115 sequences (4.64%) that failed to pass the QC pipeline, while the remaining 40% had on average 2064 sequences (4.52%) that failed the QC pipeline.

3.2 Oral Bacteriome Compositions

Overall, 111 genera and 152 species representing 14 phyla were identified from the 19 subjects as shown in Fig. 1. Thirteen (13) phyla were identified from 11 female subjects while eleven phyla were found from the 8 male subjects. Generally, in both males and females *Firmicutes* (43.6%) were the most abundant phyla followed by *Proteobacteria* (33.62%), *Bacteroidetes* (9.67%), *Actinobacteria* (8.48%), *Fusobacteria* (4.31%), Spirochaetes (0.18%), *Candidatus Saccharibacteria* (0.04%), *Tenericutes* (0.03%), *Synergistetes* (0.02%), *Acidobacteria* (0.01%) and *Streptophyta, Chloroflexi, Chlamydia, Cyanobacteria* were less than 0.01% respectively. The phylum *Chloroflexi* was not detected in female subjects, while *Streptophyta, Chlamydia,* and *Cyanobacteria* were not detected in the male subjects.

At the genera level, out of 111 genera, 103 were found in females while 88 were detected in males. Twenty-three (20.7%) genera were exclusively identified in females, while 8 (7.2%) genera were detected exclusively in males and shown in Table 1.

The most abundant core genera in both subjects which constituted 96.39% of the total sequence reads were *Streptococcus* (27.28%) followed by *Haemophilus* (14.95%), *Neisseria* (9.67%), *Veillonella* (7.22%), *Gemella* (5.77%), *Rothia* (3.11%), *Prevotella* (3.03%), *Porphyromonas* (2.94%), *Lautropia* (2.86%), *Corynebacterium* (2.74%), *Leptotrichia* (2.61%), *Capnocytophaga* (2.33%), *Granulicatella* (2.30%), *Actinomyces* (2.14%), *Actinobacillus* (1.98%), *Fusobacterium* (1.42%), *Aggregatibacter* (0.86%), *Bergeyella* (0.69%), *Campylobacter* (0.41%), *Alloprevotella* (0.37%), *Centipeda* (0.28%), and others. Comparatively, there was no significant difference between females and males on the relative abundance of the taxonomic genera (*P*=0.9269). However, some genera such as *Neisseria, Veillonella, Porphyromonas, Lautropia, Corynebacterium, Leptotrichia* and *Capnocytophaga* appear to be slightly higher in females than males, while *Streptococcus, Gemella, Rothia*, and *Actinobacillus* are slightly higher in males (Fig. 2).
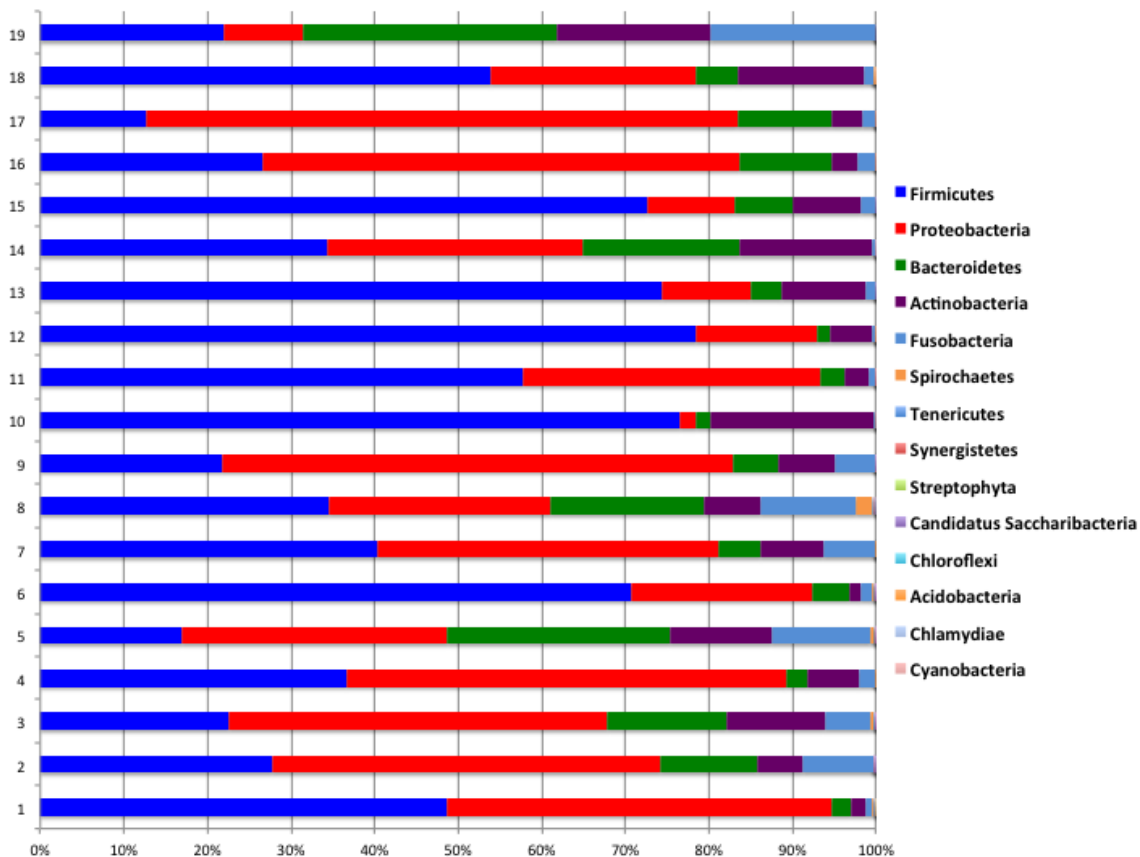

Fig. 1. Relative abundance of the phyla taxa represented as 100% stacked bar chart
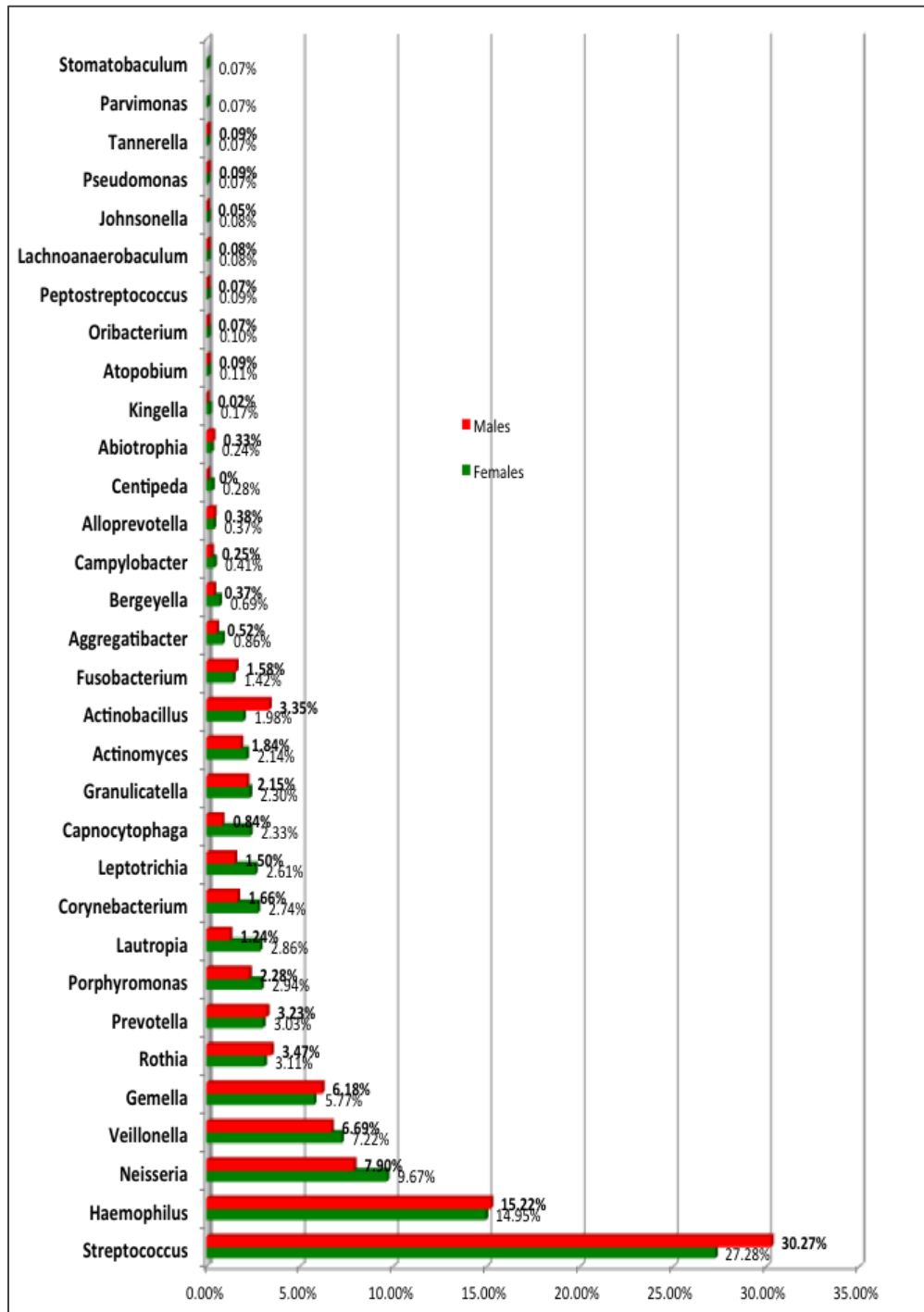
Fig. 2. Core genera identified in both subjects showing percentage in abundance

Interestingly, the participant with diabetes (Number 19 in Fig. 1) has a higher proportion of *Capnocytophaga* (28.8%), as the most abundant followed by *Leptotrichia* (18.15%), *Corynebacterium* (13.13%), *Streptococcus* (10.52%), *Veillonella* (5.46%), *Haemophilus* (5.07%), *Actinomyces* (4.44%), *Campylobacter* (3.56%), *Centipeda* (2.24%), and *Fusobacterium* (1.58%).

At the species taxonomic level, out of 151 species, 130 were found in females, of which 30 (19.9%) were exclusive. The males were colonized by 121 species out of which 21

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(13.9%) were exclusively detected as presented in Table 2.

Table 1. Exclusive genera found in both females and males

Females	Males
Anaerococcus	*Altererythrobacter*
Anoxybacillus	*Blautia*
Arthrobacter	*Citrobacter*
Brevibacterium	*Desulfobulbus*
Bulleidia	*Kluyvera*
Candidatus Cardinium	*Mannheimia*
Chlamydia	*Simonsiella*
Dermabacter	*Stenotrophomonas*
Eggerthia	
Facklamia	
Faecalibacterium	
Fastidiosipila	
Finegoldia	
Intestinimonas	
Kocuria	
Peptoniphilus	
Propionivibrio	
Roseburia	
Slackia	
Sneathia	
Synergistes	
Trichococcus	
Weissella	

The most abundant core species identified in both gender were *Haemophilus parainfluenzae*

(29.31%), followed by *Haemophilus influenzae* (6.91%), *Lautropia sp* TeTO (6.82%), *Porphyromonas catoniae* (6.0%), *Streptococcus thermophilus* (5.23%), *Actinobacillus porcinus* (4.80%), *Rothia dentocariosa* (3.32%), *Rothia mucilaginosa* (2.95%), *Neisseria elongata* (2.66%), *Streptococcus gordonii* (2.55%), *Leptotrichia hongkongensis* (2.48%), *Granulicatella adiacens* (2.07%), *Aggregatibacter segnis* (1.75%), *Fusobacterium periodonticum* (1.64%), *Corynebacterium durum* (1.40%), *Capnocytophaga sputigena* (0.82%), *Prevotella nanceiensis* (0.76%), *prevotella nigrescens* (0.75%), *Bergeyella sp* AF14 (0.48%), *Capnocytophaga sp* oral taxon 329 (0.35%), *Actinomyces dentalis* (0.35%), *Prevotella oris* (0.24%), *Fusobacterium nucleatum* (0.21%) and *Actinomyces massiliensis* (0.16%).

Comparatively, there was no significant difference between females and males on the relative abundance of the taxonomic species (*P*=0.9380) colonising the oral cavity of the tested population. Nevertheless, some individual species, such as *Haemophilus parainfluenzae, Haemophilus influenzae*, *Actinobacillus porcinus*, *Rothia dentocariosa* and *Granulicatella adiacens* were found in higher proportion in males than in females. In the same vein, *Lautropia sp.* TeTO, *Streptococcus thermophilus, Rothia dentocariosa, Streptococcus gordonii* and *Leptotrichia hongkongensis* were higher in females than in males (Fig. 3).

Table 2. Exclusive species detected in both females and males

Female	Male
Actinomyces oris	*Actinobacillus delphinicola*
Actinomyces sp. oral taxon 448	*Actinomyces meyeri*
Arthrobacter albus	*Actinomyces sp.*
Bacteroides coprocola	*Blautia faecis*
Bacteroides zoogleoformans	*Capnocytophaga sp. oral strain A47ROY*
Brevibacterium massiliense	*Dialister micraerophilus*
Brevibacterium paucivorans	*Eikenella sp. MDA2346-4*
Bulleidia extructa	*Enterobacter ludwigii*
Capnocytophaga sp.	*Klebsiella pneumoniae*
Corynebacterium argentoratense	*Lactobacillus mucosae*
Corynebacterium canis	*Lactobacillus rhamnosus*
Corynebacterium epidermidicanis	*Lactobacillus salivarius*
Corynebacterium mustelae	*Megasphaera elsdenii*
Corynebacterium sp. NML 97-0186	*Mycoplasma falconis*
Dialister succinatiphilus	*Neisseria meningitidis*
Eggerthia catenaformis	*Prevotella amnii*

Female	Male
Facklamia hominis	*Prevotella bivia*
Faecalibacterium prausnitzii	*Rothia sp. 3_1/4V*
Gemella sp. 1754-94	*Scardovia inopinata*
Lachnoanaerobaculum umeaense	*Selenomonas sputigena*
Leptotrichia buccalis	*Simonsiella muelleri*
Mycoplasma lipophilum	
Neisseria bacilliformis	
Prevotella sp. oral taxon 299	
Roseburia inulinivorans	
Slackia exigua	
Sneathia sanguinegens	
Streptococcus anginosus	
Streptococcus constellatus	
Veillonella dispar	

The phylogenetic interactive tree of the 26-core species in both males and females are represented in Fig. 4.

However, it remains to be determined if the exclusive species detected in females and males, correlates with any physiological functions. For example, three *Lactobacillus* species were detected exclusively in males, while five *Corynebacterium* species and other genera were found only in the female subjects (Table 2).

The inverse Simpson's Diversity Index (SDI) on average was 7.87±1.03. However, the female population tested appears to have more bacteriome diversity with mean SDI of 8.09, while males mean inverse SDI was 7.57.

4. DISCUSSION

There is a growing interest in deciphering the human microbiome compositions of all ethnic nationalities. We hereby present the oral bacteriome (microbiome) compositions of both males and females from the sampled population in Nigeria. To the best of our knowledge, this is the first study from the Eastern part of Nigeria, describing the oral bacteriome, employing Next Generation Sequencing (NGS) especially 16 S rRNA metagenomics platform targeting V-4 variable region for bacterial classifications of the oral microbial compositions. Although not statistically significant, the female population tested appears to have more diversity and relatively higher proportion of the phylum *Firmicutes*, *Proteobacteria*, *Bacteroidetes*,

Actinobacteria and *Fusobacteria* than the male's subjects sampled.

It remains to be determined if there are other factors that may affect oral microbial diversity in African populations. However, our previous study suggested that age may play a role [16], while oral environmental changes, such as high-sugar diet, low-pH, smoking, and fluoride use, may affect oral microbial diversity [8]. Few studies have demonstrated that microbial relative abundances are similar either with enzymatic or mechanical bead approach [17].

Our study is consistent with previous findings indicating the predominant microbiome in healthy individuals [4], which include five major phyla; *Firmicutes*, *Proteobacteria*, *Actinobacteria*, *Bacteroidetes*, and *Fusobacteria*. A total of 14 phyla were identified from the study population, which is very close and consistent with the number of phyla (15 in total) found to date in the Human Oral Microbiome Database. (http://www.homd.org). The detection of *Streptophyta*, an unranked plant clade that includes all land plants and some green algae observed in the female subjects is not very clear considering the close evolutionary relationship between chloroplasts and cyanobacteria [18]. Chloroplast sequences have been previously reported in modern dental plaque [1] and were observed at variable, but generally very low, frequencies in archaeological dental calculus. It appears that such sequences may indicate the presence of chloroplast DNA, and therefore possible dietary components in the female population sampled.
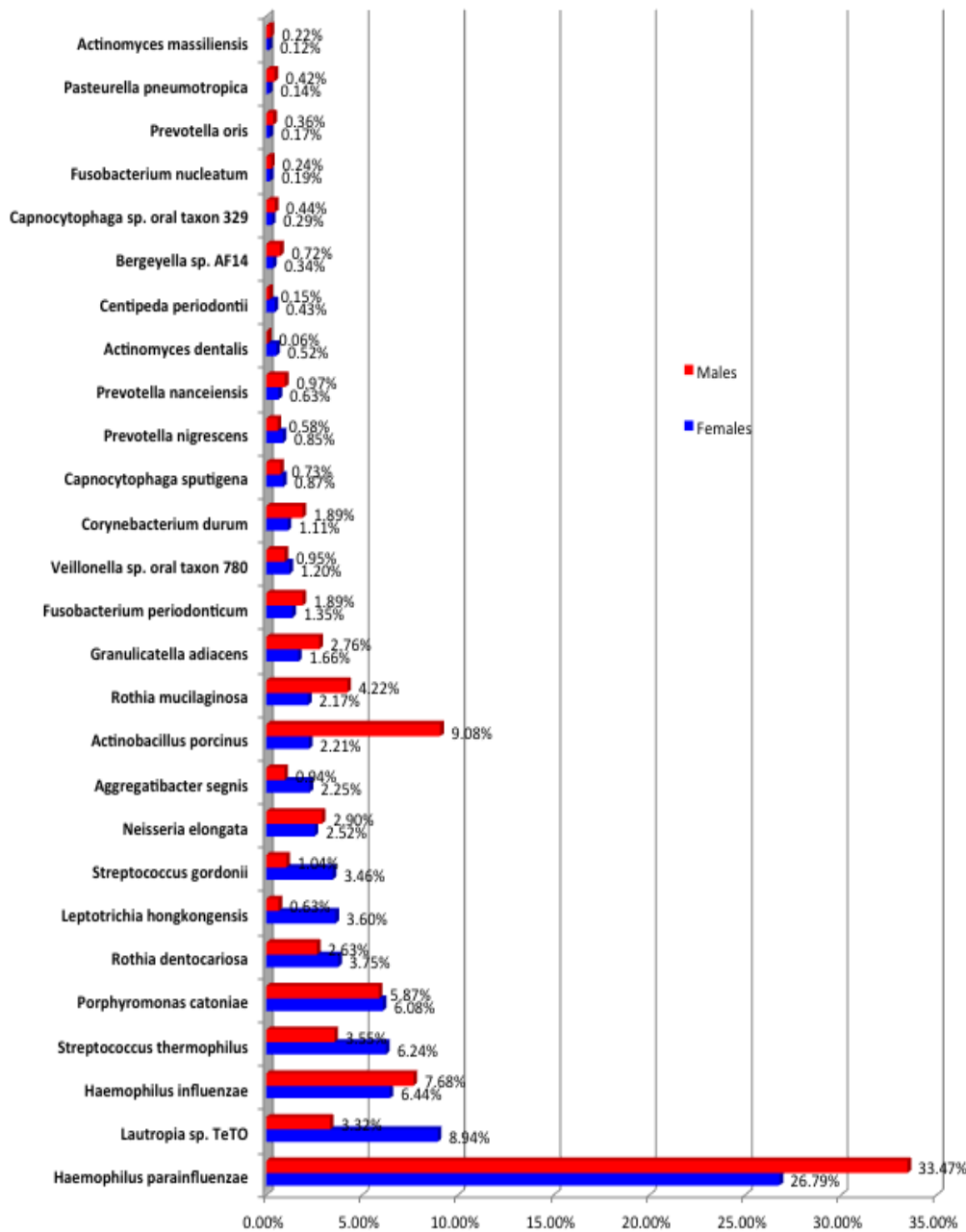
Fig. 3. Core species identified in both subjects, showing percentage in abundance

Taking cognizance of the core genera from this study, which was defined as taxa present in all subjects and over 2.0% in abundance, it appears that the genera are consistent with healthy oral microbiota in the study population. The core genera include *Streptococcus* followed by *Haemophilus*, *Neisseria*, *Veillonella*, *Gemella*, *Rothia*, *Prevotella*, *Porphyromonas*, *Lautropia*, *Corynebacterium*, *Leptotrichia*, *Capnocytophaga*, *Granulicatella*, and *Actinomyces*. In contrast, a recent study from the Arab population shows that *Streptococcus* and *Rothia* were the most abundant (27.7 and 13.8% of the sequences, respectively) followed by *Neisseria, Prevotella, Haemophilus, Fusobacterium, Porphyromonas* and *Veillonella* constituting together nearly 80% of all reads [12]. The identification of *Lautropia* as a core genus in the studied population is notable in that *Lautropia mirabilis* has been found in the oral cavities of HIV infected and uninfected children [19] and it has previously been isolated in the human mouth [20]. However, it remains to be determined whether *Lautropia sp* TeTO found in this study has any clinical relevance.
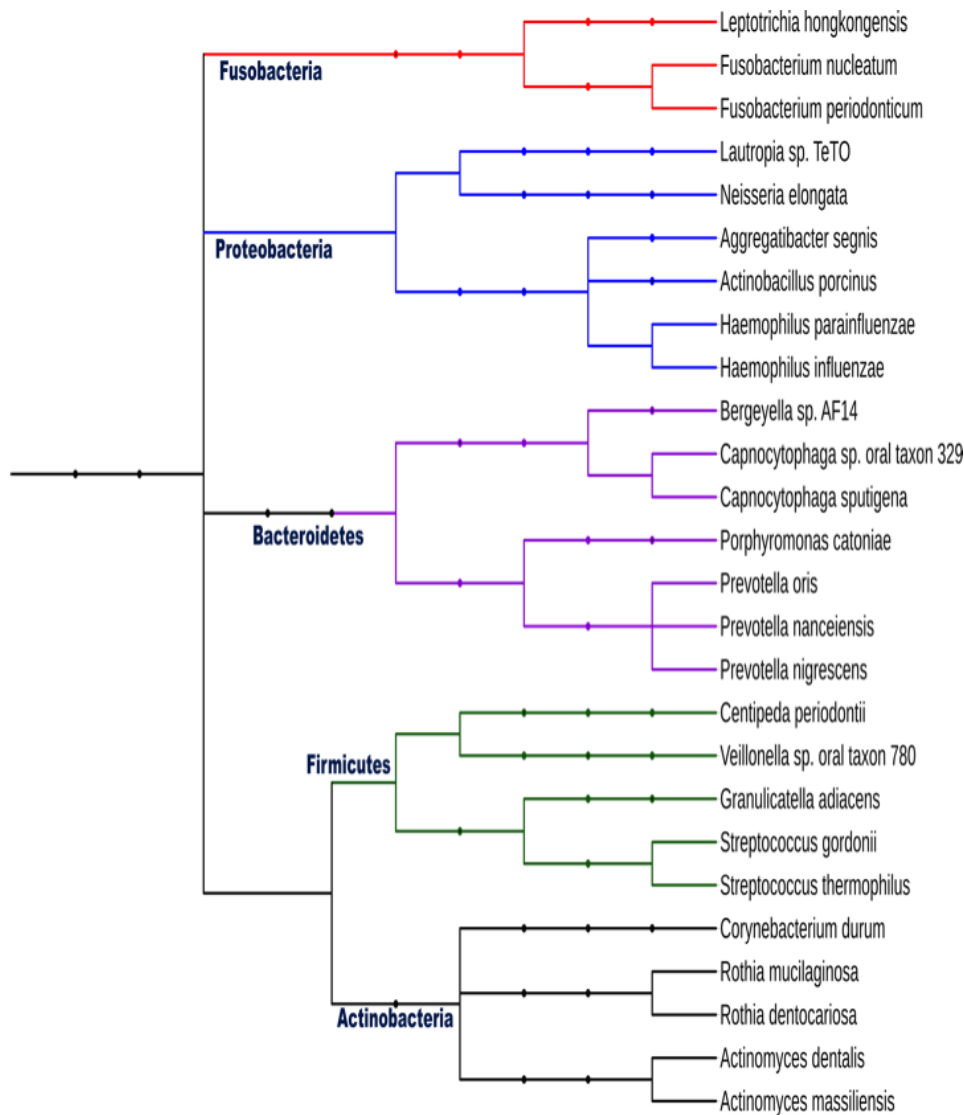
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Fig. 4. Phylogenetic relationship of the core species in both subjects determined using iterative tree of life (iTOL) software

We used stringent criteria to establish core species bacteriome in this study. Of the 151 species identified in this study 27 species met our criteria as core species in both male and female of the study population. *Haemophilus parainfluenzae* ranked first in this study followed by *Actinobacillus porcinus* and *Haemophilus influenza*e in males, while *Lautropia sp* TeTO ranked second in the females. Previous studies showed that *Haemophilus parainfluenzae* has been identified as a core and abundant species in association with health [9,10,3].

Other core species from this study include *Porphyromonas catoniae*, *Streptococcus thermophilus*, *Actinobacillus porcinus*, *Rothia dentocariosa*, *Rothia mucilaginosa*, *Neisseria elongata*, *Streptococcus gordonii*, *Leptotrichia hongkongensis*, *Granulicatella adiacens*, *Aggregatibacter segnis*, *Fusobacterium periodonticum*, *Corynebacterium durum*, *Capnocytophaga sputigena*, *Prevotella nanceiensis*, *prevotella nigrescens*, *Bergeyella sp* AF14, *Capnocytophaga sp* oral taxon 329, *Actinomyces dentalis*, *Prevotella oris*, *Fusobacterium nucleatum* and *Actinomyces massiliensis*.

It should be noted that *Haemophilus parainfluenzae* ranked first as a core species with the highest proportion in the study population. In contrast, *Streptococcus mitis*

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ranked first in the Arab study and was the most abundant core species on average, which is consistent with previous reports of the normal oral bacteriome [21,22]. Two subjects (one male and one female) were colonized by *Pyramidobacter piscolens*, a species that belongs to the phylum *Synergistetes*. The genus *Pyramidobacter* comprises of strains that are anaerobic, non-motile, asaccharolytic bacilli that produce acetic and isovaleric acids and minor to trace amounts of propionic, isobutyric, succinic, phenylacetic acids as end products of metabolism and growth is stimulated by addition of glycine [23].

5. CONCLUSION

To our knowledge, this is the first comparative oral metagenomics study between male and female subjects in Nigeria. On average, oral bacteria consistent with other published studies colonized the study population. It remains to be determined if the exclusive genera and species detected in both females and males have any role to play in health and diseases.

The genera and species-level core oral bacteriome from this Nigerian populations could be used as a reference in comparison with oral diseased conditions and future investigations involving a larger sample size.

ETHICAL APPROVAL AND CONSENT

This study was carried out in accordance with the recommendations of the Faculty of Health Sciences, Nnamdi Azikiwe University, Nnewi Campus, Anambra State ethics committee, with written and informed consent from all subjects. All subjects gave written and or verbal informed consent in accordance with the Declaration of Helsinki.

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COMPETING INTERESTS

Authors have declared that no competing interests exist.

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