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Research Paper

Evaluation of Oral Bacterial Microbiota Profile among Outpatients and Asymptomatic Volunteers In Kaduna Metropolis, Kaduna, Nigeria.

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ABSTRACT
The human microbiome is the ecological community of commensal, symbiotic and pathogenic microorganisms that share human body space. These micro-organisms, though are essential in maintaining general health, yet also capable of initiating diseases. This study aims at isolation, identification and determination of antibiogram profile of bacterial isolates from oral cavities of hospital outpatients and asymptomatic volunteers in parts of Kaduna metropolis, Nigeria. Ninety-seven oral samples comprising of 48 outpatients and 49 asymptomatic volunteers were collected from Yusuf Dantsoho Memorial Hospital, Tudun Wada, Kaduna; Nigerian Defense Academy Hospital, Mando, Kaduna; and asymptomatic volunteers within Kaduna metropolis were recruited for the study. Oral rinse was collected, innoculated on Nutrient agar, Blood agar, Mannitol salt agar and Mac-Conkey agar, then incubated at 37^0C for 24 hours. Gram staining, biochemical test and 16SrRNA gene sequencing were carried out for identification and characterization of the bacterial isolates. Antibiogram profile of the bacterial isolates was carried out using Disc Diffusion Method (DDM). A total of 97 bacterial isolates were obtained. The most prevalent bacteria species in the oral cavity was Streptococcus species (48:49.5%), and the least was Enterococcus faecalis (6:6.2%).
KEYWORDS: *Microbiome, Oral Cavity, Antibiogram Profile, Bacterial Isolates, Disc Diffusion Method, Gene Sequencing, Gram staining.*

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I. INTRODUCTION

Microorganisms aid in the fight against disease, improve nutrition, protect against infection, and regulate metabolism. Microbial diversity is caused by anatomic site, aggregate function, and complexity of bacterial communities, which may be related to an individual health, genotype, diet hygiene, age, gender, ethnicity, geographical region, and risky behaviors (smoking and drinking) of the host (Blaser and Falkow, 2009; Costello *et al.,* 2009). A healthy microbiome is distinguished by its high diversity and ability to adapt to change under physiological stress (Lloyd-Price *et al.,* 2016). So far, our understanding of the microbiome as a component of health and disease has stemmed from an appreciation of the microbiome's multiple metabolic and physiological functions. These include: energy harvesting via nutrient extraction and fermentation of indigestible food substances, synthesis of key substances such as vitamin B12, vitamin K, neurotransmitters such as serotonin, gut barrier (mucosa) maintenance, infection protection, systemic immunity, and autoimmune disease protection (Calafiore *et al.,* 2012). Contrary to popular belief, healthy people frequently carry low levels of pathogens or disease-causing bacteria in their various body sites; however, these pathogenic bacteria do not cause a problem because these body sites are protected by a strong defense of good commensal microbes (Cass, 2019).

Even in the absence of disease, microbiomes exhibit a high level of interpersonal diversity. Qin *et al.* (2010). This frequently impairs the ability to identify simple microbial constituents or dysbioses that cause disease or reflect a diseased state. The oral cavity is a major gateway to the human body, it has two main types of surfaces for microbial colonization: non-shedding surfaces (teeth) and shedding surfaces (mucosa), including gingival crevices, tongue, hard palate, soft palate, cheeks, and lips. The oral microbiota contributes to the host's primary benefits, such as cardiovascular system regulation, gastrointestinal regulation, immunological priming,

suppression of excessive pro-inflammatory responses, and colonization by exogenous microbes (Phil *et al.,* 2015).

Bacteria have been considered the dominant part of the microbiome in man. Common oral bacteria include *Streptococcus mutans*, *Porphyromonas gingivalis*, *Staphylococcus* and *Lactobacillus* are common oral bacteria (Dzidic *et al.,* 2018). The major constituent of oral microbiota is *Streptococcus mutans*, it is usually a component of dental plaque (Gomez *et al.,* 2017). Untreated infection caused by *Porphyromonas gingivalis* a periodontal pathogen in the mouth can cause teeth to fall off gum (Maoyang *et al*., 2019). Other examples of bacteria found in the mouth belong to eubacteria, *Leptotrichia, Prevotella, Treponema, Actinomyces*. Majority of bacteria are explicit to certain sites. Bacteriophages are also part of the oral microbiota (Wang *et al.,* 2016). During all stages of life, the type of phage present in the mouth remains constant (Dudek *et al.,* 2017).

Oral viruses have gene that maybe involved in pathogenic roles of their host bacteria, most oral viruses are lysogenic and live in harmony with their host, they may be important in shaping the microbial diversity of the oral cavity. Another peculiarity is that the viral communities of the mouth are highly personalized, even more personalized than the bacterial communities when analyzed with 16SrRNA sequencing (Abeles and Pride 2014). Archaea were originally considered as primitive form of life that thrives in extreme environments. However, high numbers of methane-producing archea (methanogens) have now been detected in the oral cavity (Olsen, 2016). He *et al.* (2014), reported oral archea from the genera *Methanobrevibacter*, *Methanobacterium*, *Methanosarcina*, and *Methanosphaera* and order *Thermoplasmatales.* The primary species discovered is *Methanobrevibacter oralis*. Archaea were found in saliva, periodontitis, infected root canals, peri-implantitis, and pericoronitis (Faveri *et al*., 2011; Mansfield *et al*., 2012; Bringuier *et al*., 2013). Also, *Streptococcus gordonii* glycosyltransferase promotes biofilm interaction with *Candida albicans* (Ricker *et al.,* 2014).

Maoyang *et al*., (2019) reported that about 700 species of microorganism reside in the mouth. Bacteria, fungi and viruses are among several microorganisms that exist in the mouth (Segata *et al*., 2012). Type of bacteria mainly found in the mouth belong the phylum *Firmicutes*, *Bacillus*, *Proteobacteria* and *Actinomycetes* (Mark *et al*., 2016). The most important fungi in the mouth are *Candida* species (Baker *et al*., 2017). Diseases caused by microorganisms found in the mouth include, caries, periodontics disease, endodontic infections, alveolar osteitis and tonsillitis. A number of systemic diseases have been linked to oral bacteria (Craves *et al*., 2018), including cardiovascular diseases (Bryan *et al*., 2017), stroke, preterm birth, diabetes, pneumonia, rheumatoid arthritis (AR) (Chen *et al*., 2018) and digestive diseases (Ray, 2017). Numerous species, such as *Streptococcus* and *Veillonella*, are commonly found in healthy individuals. Gram-positive cocci are common in healthy people, accounting for more than 20% of the oral microbiome on average (Aas *et al*., 2005; Keijser *et al.,* 2008 Colombo *et al.,* 2009; Nasidze *et al.,* 2009; Zaura *et al.,* 2009; Bik *et al.,* 2010; Lazarevic *et al.,* 2012; Simon-Soro *et al.,* 2013).

Studies on the composition of human microbiome in Nigeria is very limited and there is little awareness and/or knowledge about the human microbiome amongst residents within Kaduna (North-west Nigeria) metropolis. Considering the vital role played by the host microbiota in modulating health and scarcity of information on oral microbiota composition among residents within Kaduna metropolis, analyzing the bacteria composition of the oral and nasal cavities, and determination of the antibiogram profile of isolates, would help to better understand the microbiome. Hence, this research was aimed at assessing the antibiotic profile of oral bacteria isolated from hospital outpatient and asymptomatic volunteers within Kaduna metropolis, Nigeria.

II. MATERIALS AND METHOD

Study design

This is a cross-sectional study design

Study population

The participants recruited for the study were adult outpatients of Yusuf Dantosho Memorial Hospital, Tudun Wada, Kaduna State and Nigerian Defence Hospital Mando, Kaduna State as well as asymptomatic volunteers within Kaduna metropolis.

Ethical approval

Ethical approval was obtained from Kaduna State Ministry of Health Research Ethics Committee before the commencement of the study.

Inclusion criteria

Consenting adults, asymptomatic volunteers and outpatients (Yusuf Dantsoho Memorial Hospital Tudun Wada and Nigerian Defense Hospital Mando) within Kaduna metropolis, who were not on antibiotic therapy in the preceding 2weeks were randomly selected for the study.

Exclusion criteria

Patients and volunteers less than 18years, those with history of antibiotics therapy in the preceding 2 weeks were excluded from the study.

Consent

Informed consent was obtained from each participant. The purpose and nature of the study, as well as the method of sample collection was properly explained to them. Afterwards, participants were required to voluntarily complete the consent form in their handwriting and endorsed by their signature as proof of willingness to provide sample for the test. Participants were then assured of confidentiality.

Sample Collection and Processing

Oral rinse specimens were collected, as described by Sedgley *et al.*, 1994; White *et al.*, 2004 and Yan *et al*., 2008, by giving participant sterile water, which was used to gaggle the mouth for 1 minute, content of the mouth was expectorated into a sterile screwed cap container, properly covered and labelled. The specimens were then transported to the laboratory in a sealed plastic bag within 2hours of collection. Samples were transported to the laboratory as soon as possible and processed on the same day.

Sample analysis

Oral rinse samples were properly mixed by shaking the container properly and then streaked using a sterile inoculating wire loop on Blood agar, Mannitol Salt agar, Nutrient agar and MacConkey agar plates and were incubated at 37^0C for 24 hours.

Identification of bacterial isolates

Macroscopy and microscopy as described by Ochei and Kolhatkar (2016), were adopted for identification of bacterial isolates. Morphological characteristics of colonies such as the shape, size, elevation, pigmentation, opacity and margin were noted and recorded. Gram staining procedure was demonstrated to reveal their shapes and arrangement. Biochemical characteristics were determined by carrying out, Mannitol fermentation test, catalase test, motility test, coagulase test and indole test as outlined by Sagar, (2018).

Catalase test: Using a sterile wooden stick a small amount of colony growth was transferred onto the surface of a clean, sterile, dry, grease-free glass slide, followed by one or two drops of 3% H_2O_2. Following that, the slide was examined to determine whether or not oxygen bubbles evolved. Observations and outcomes were meticulously documented.

Coagulase test: For coagulase test, the slide test method was used.

A drop of physiological saline was added to each end of a clean sterile dry grease-free slide, and a sterile loop was used to emulsify a portion of the isolate colony in each drop to make a suspension. A drop of human plasma was also added to one of the suspensions, and it was softly mixed. The organism's clumping indicated a positive result, whereas non-clumping indicated a negative result. Observations and outcomes were recorded accordingly.

Mannitol fermentation test: A loopful of cells were aseptically transferred to a sterile tube of phenol red Mannitol broth from a pure culture of inoculum obtained from the isolated organisms. The inoculated tube was incubated for 24 hours at 37°C. Observations and outcomes were duly documented.

Methyl red test: A loopful of cells from an axenic culture of bacteria isolate 22 hours suspected to be *Escherichia coli* was lightly inoculated in a broth medium, aerobically incubated at 37°C for 24 hours, and 1ml of broth was aliquoted into a clean test tube after 24 hours incubation. The remaining broth was re-incubated for another 24 hours before adding 3 drops of methyl red indicator to the aliquot. Observations and outcomes were duly documented.

Indole test: Peptone water broth was prepared in test tubes and autoclaved at 15lbs/inch 2 pressure for 15 minutes. The broth was inoculated with one loopful of bacteria cells sample isolate of test organism and tube labeled with the name of the organism and incubated at 37^oC for 36 hours. Following proper incubation, 6 drops of Kovac's reagent was added to the tube, touching the wall of the tube, and the tube was rolled between the palms to mix the reagent through the culture. The tube was then allowed to stand for a while and the development of cherry red color at the surface of the media was observed. Observation and result were duly recorded.

Motility test: Touching a colony of a young culture growing on agar medium with a straight sterile needle the medium was then stabbed in the center down to about half its depth, incubated at 37°C, and examined daily. Observation and outcome were documented.

Determination of antibiotic susceptibility pattern of bacterial isolates

Antibiotic susceptibility pattern of the bacterial isolates was determined using commercially prepared antibiotics disc of known concentration marketed by Maxi Nigeria Limited according to the modified Kirby-Bauer disc diffusion technique as described by Cheesebrough, (2006) and CLSI, (2009). The antibiotics used were as follows Ciprofloxacin (10µg), Perfloxacin (10µg), Ofloxacin (10µg), Sparfloxacin (10µg) Amoxacillin (30µg), Streptomycin (30µg), Gentamycin (10µg), Augmentine (10µg) Cefriaxone (20µg) Septrin (30µg), Ampicillin

(30µg), Erythromycin (10µg) and Chloramphenicol (30µg). Control strains for each test isolate was used to ascertain the performance of the method. Zones of growth inhibition around each of the disk were carefully measured (to the nearest millimeter), recorded and interpreted and isolates reported as Sensitive, Intermediate or Resistant.

Isolation and purification of DNA

Isolation and purification of bacteria DNA was carried out using kits (Bioscience inc.), and following manufacturer instructions: Using a sterile wire loop, cells were scraped from an axenic culture of some selected isolates obtained during the study. Each was placed in a 1.5ml tube with 400µl of lyses buffer and 100µl of proteinase K. After 40 minutes on a heat block at 55°C, 400µl of phenol chloroform (1:1) was added to the lysate and vortexed briefly before spinning in a microcentrifuge at 13000rpm for 10 minutes to separate the phases. The upper layer was carefully removed with a pipette for each isolate and transferred to a new 1.5ml tube. 400µl of chloroform was added to each tube and vortexed briefly before spinning in a microcentrifuge at 13000rpm for 5 minutes to separate the phases. The upper layer was carefully removed with a pipette and transferred to a new 1.5ml tube. In each tube, equal volumes of 100% ethanol and 40µl of 3M sodium acetate were added, mixed by inverting the tube several times, and incubated overnight. After an overnight incubation, tubes were spun in a refrigerated centrifuge at 13000rpm in the same orientation for 20 minutes. Ethanol was removed, and 400µl of 70% ethanol was added to each tube before spinning at 15000rpm for 5 minutes at 4°C.The tubes were then spun at 15000rpm for another 30 seconds to remove any remaining traces of ethanol. Tubes were left open to allow residual ethanol to evaporate before being labeled and refrigerated for further analysis.

Polymerase chain reaction

The table below shows the primer sequence used for polymerase chain reaction

Table 3.1 Primer sequence used for polymerase chain reaction

F24	(5'-GAG TTT GAT YMTGGCTCA3')
Y36	(5- GAAGGAGGTGWTCCA DCC 3')

Test samples were reconstituted with 13µl biomix (deoxynucleosides, $Mgcl_2$, buffer,Taq polymerase) 1µl forward primer, (F24 5′-GAG TTT GAT YMTGGCTCA-3′),1µl reverse primer, (Y36 5′-GAAGGAGGTGWTCCA DCC-3′) and 5µl extracted DNA, from selected bacterial isolates labelled numerically as 1, 2, 3, 4, 5, and 6 respectively, these were then placed in a thermocycler (Invitrogen Carlsbad, CA) with cycling condition set as follows, 94°C for 5minutes (predenaturation), 94°C for 30 seconds (denaturation), 50°C for 40seconds (annealing), 72°C for 1minutes (extension). 30 cycles were performed, followed by 72°C for 4minutes (final extension).

Gel electrophoresis

Agarose powder of (1.5g) was dissolved in 100ml of Tris acetate EDTA (TAE) buffer, and the solution was completely dissolved in a boiling water bath before cooling in a water bath set at 50°C. Gel casting tray was prepared by taping the ends of the gel chamber and placing the comb in the gel tray. 5µl of ethidium bromide (Invitrogen Carlsbad, CA) was added to the cooled gel and poured into the gel tray, which was allowed to cool for 20 minutes at room temperature. DNA and molecular markers were loaded onto gel, electrophoresed at 1.5V for 50 minutes, and bands were photographed using an ultraviolet (UV) trans-illuminator (Invitrogen Carlsbad, CA).

Gel extraction protocol

Fragments of DNA were cut out from gel and weighed (a gel slice of 100mg approximately equals 100l), 3 gel volume buffer added and incubated at 60°C for 8 minutes while also tapping the bottom of the mixture tube every 3 minutes to ensure gel completely melts, then 1 gel volume isopropanol was added to each mixture and mixed. Mixtures were transferred to a DNA mini column with collection tubes, centrifuged for 50 seconds at 11,000rpm, flow through was discarded, and collection tubes were replaced. To the mini column 500µl DNA wash buffer was added, also added was 400µl of 70% ethanol, centrifuged at 11,000rpm for 1minute at room temperature. Flow through discarded. Empty column centrifuged at 11,000rpm for 2 minutes to remove residual ethanol, columns were placed in clean 1.5ml microcentrifuge tubes. Elution buffer 30µl was

added to each column and incubated at room temperature for 1minute, centrifuged at 11000rpm for 1 minute. The eluate reapplied to the column, eluted one more time to enhance DNA yield.

DNA cleanup for sequencing reaction

For each sample, a 1.5ml sterile tube was labeled, the sequencing mixture was transferred into a DNA binding column, spun for 1 minute at 11000rpm, the flow through was discarded, and the column was replaced. 500µl of wash buffer added to the column, which was allowed for 5 minutes at room temperature before spinning at 11000rpm for 1 minute, after which the flow through was discarded and the column was replaced (this process was repeated). For 2 minutes, the empty column spun at 14000rpm, collection tubes discarded, and columns placed in new collection tubes. 2µl of sample loading solution was added to each column, and columns spun at 14000rpm for 1 minute. Re-eluting the flow through. **Gene sequencing**

Applied Biosystems dye terminator cycle sequencing kit with a quick start kit was used. 7µl deionized water, 3µl DNA template, 2µl primers, and 8µl quick start master mix were added to each 2.0ml tube. The thermal cycling program (96°C for 20 seconds: extension, 50°C for 20 seconds: annealing, for 30 cycles, and then 60°C for 4 minutes final extension) was used in the sequencing machine.

Sequence alignment (Blast)

Chromograms were visually inspected for read quality and length. Results of poor quality were discarded. BLAST was used to compare the sequences to all bacterial sequences in GenBank (Basic Alignment Search Tool).

Data analysis

Data generated was entered into Microsoft Excel. Statistical analysis was carried out using SPPS statistics Software package (Version 21.0). Data obtained were statistically analyzed using the Generalized Linear Model, Turkey method, and unpaired T-test. The level of significance was determined at 95%. P values < 0.05 were considered significant.

IV. RESULTS

Distribution of bacteria microbiota from oral cavities

Results presented on Table 4.1a shows that a total of 97 bacteria belonging to 5 genera were isolated from the oral cavities of both asymptomatic and outpatient volunteers during this study. The most predominant genus of bacteria found in the oral cavities of volunteer samples was *Streptococcus* species (48:49.5%) while the least was *Enterococcus faecalis* (6:6.2%). The turkey test revealed *Streptococcus* species had the highest mean *(*24.0^{A}), and *Enterococcus faecalis* (3.0^{c}) had the lowest. There was no significant difference (P>0.05), in the volunteer category (hospital outpatients and asymptomatic volunteers).

Table 4.1a: Distribution of bacteria isolated from oral cavities

Isolates	Asymptomatic volunteers	Outpatient volunteers	Total
***Streptococcus* species**	25	23	48
***Lactobacillus* species**	10	11	21
Staphylococcus aureus	06	08	14
Escherichia coli	05	03	08
Enterococcus faecalis	03	03	06
Total	**49**	**48**	**97**

Note: Grouping information using Turkey method at 95.0% confidence interval.

Gram staining and biochemical analysis of oral bacterial isolates recovered from volunteers (asymptomatic and outpatient)

Table 4.2a shows bacteria strains belonging to 5 genera obtained from the oral cavities of both asymptomatic and outpatient volunteers during the study. The results obtained from the biochemical tests showed that bacteria belonging to the genera of *Streptococcus*, *Lactobacillus*, *Staphylococcus*, *Enterococcus* and *Escherichia* were obtained from the oral cavities of asymptomatic and outpatient volunteers that participated.

Table 4.2a: Gram staining and biochemical analysis of some oral bacterial isolates recovered from volunteers (outpatients and asymptomatic)

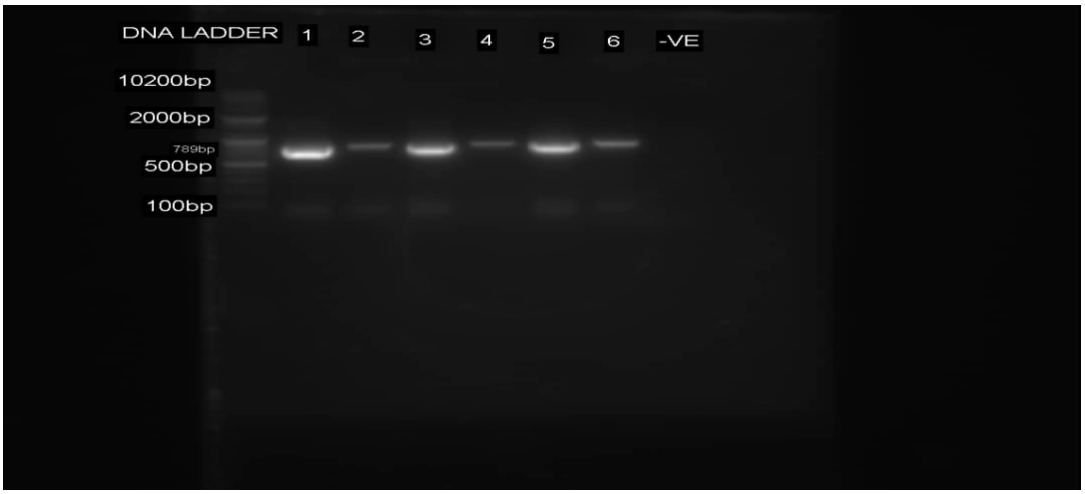
Code	Grams reaction	Catalase	Coagulase	Methyl Red	Oxidase	Urease	Citrate	Indole	Motility	Mannitol	Lactose	Probable organism
OU1	GPR	-	-	-	-	+	-	-	-	+	-	*Lacto.* sp
OU2	GPC	+	+	-	-	+	+	-	-	+	+	*S. aureus*
OU3	GPC	-	-	-	-	+	+	-	-	-	-	*Strept.* sp
OU4	N/A	-	-	-	-	-	-	-	-	-	-	*Candida.* sp
OU5	GPC	-	-	-	-	-	-	-	-	-	-	*Strept.* sp
OU6	GPR	-	-	-	-	-	-	-	-	+	-	*Lacto.*sp
OU7	GPC	-	-	-	-	-	-	-	-	-	-	*Strept. Sp*
OH1	GPC	+	+	-	-	+	+	-	-	+	+	*S. aureus*
OH2	GPC	+	+	-	-	+	-	-	-	+	+	*S. aureus*
OH3	GPC	-	-	-	-	-	-	-	-	-	-	*Strept.* sp
OH4	GPR	-	-	-	-	-	-	-	-	+	-	*Lacto. sp*
OH5	GPC	-	-	-	-	-	-	-	-	+	-	*E.faecalis*
OH6	GPC	-	-	-	-	-	-	-	-	-	-	*Strept.* sp
OH7	GPC	-	-	-		-	-	-	-	-	-	*Strept. sp*

Table 4.1c: Isolation rates of bacteria recovered from volunteer samples of oral and nasal cavities

Isolates	Number of isolates	Isolation rate (%)
Staphylococcus aureus	49	33.8
***Streptococcus* species**	49	33.8
***Lactobacillus* species**	21	14.5
Staphylococcus epidermidis	12	8.3
Escherichia coli	8	5.5
Enterococcus faecalis	6	4.1
Total	**145**	**100**

PCR amplification of some selected bacterial isolates from oral and nasal cavities

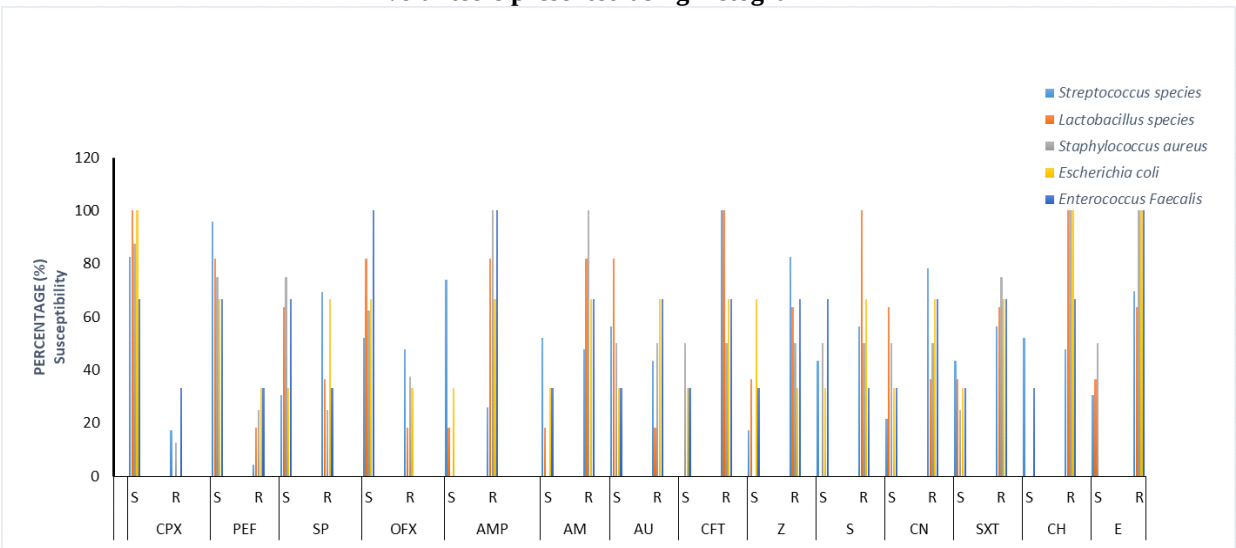
Plate I: shows result of DNA amplification of 6 selected bacterial isolates obtained during the study. The expected band size for 16SrRNA bacterial genes was 789bp



Antibiotic susceptibility pattern of bacterial isolates recovered from oral cavities of outpatient volunteers

Fig 4.1a shows antibiotic susceptibility pattern of bacterial isolates recovered from oral cavities of outpatient volunteers. Isolates obtained displayed variable susceptibility to antibiotics used as follow, *Streptococcus* species from outpatient volunteers showed highest sensitivity to fluoroquinolones (65.2%), while highest resistance to cephalosporine (91.3%) was recorded. Isolates of *Lactobacillus* species obtained from outpatient volunteers were most sensitive to fluoroquinolones (81.8%), resistance to phenicols (100%) and cephalosporine (81.8%) was observed. Isolates of *Staphylococcus aureus* recovered from outpatient volunteers, were most sensitive to fluoroquinolones (75%), while highest resistance to penicillin, phenicol and macrolide (100%) was recorded. Isolates of *Escherichia coli* obtained from outpatient volunteers, showed highest sensitivity to fluoroquinolones (66.7%), while highest resistance to macrolides (100%) and phenicol (100%) was observed. Isolates of *Enterococcus feacalis* showed highest sensitivity to fluoroquinolones (66.7%) and aminoglycoside (66.7%), while highest resistance to macrolide and phenicol (100%) was recorded.

Fig 4.1a: Antibiotic susceptibility pattern of bacterial isolates recovered from oral cavities of outpatient volunteers presented using histogram

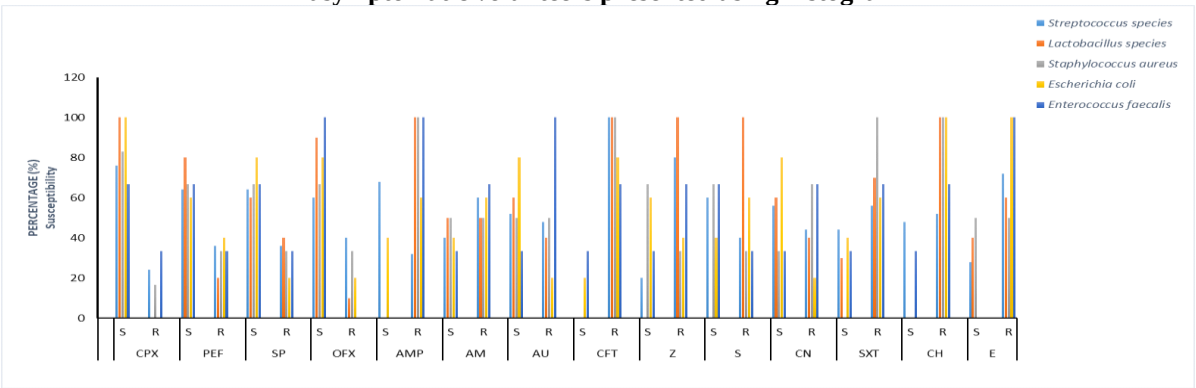


Note: CPX- Ciprofloxacin, PEF- Perfloxacin, SP- Sparfloxacin, OFX- Ofloxacin, AMP- Ampicillin, AM- Amoxicillin, AU- Augumentin, CFT-Ceftazidime, Z- Cefriazone, S- Streptomycin, CN- Gentamycin, SXT- Septrin, CH- Chloramphenicol, E- Erythromycin, S-Sensitivity, R- Resistance.

Antibiotic susceptibility pattern of bacterial isolates recovered from oral cavities of asymptomatic volunteers

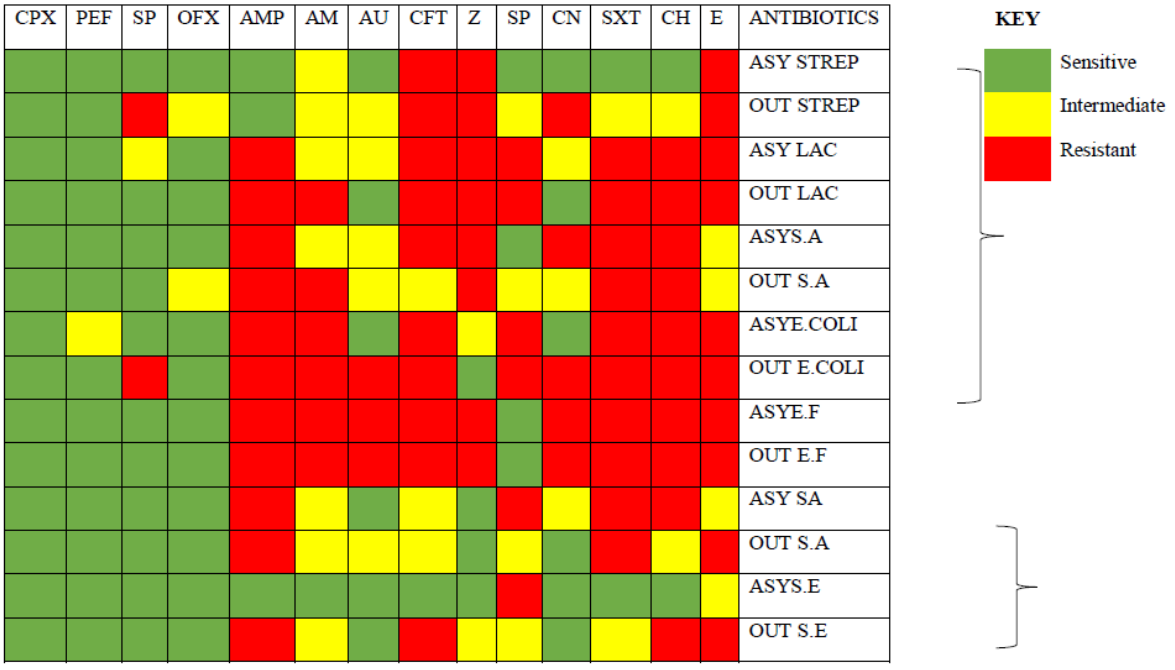
Fig 4.1b shows antibiotic susceptibility pattern of bacterial recovered from asymptomatic volunteers. Isolates obtained displayed variable susceptibility to antibiotic used as follows, *Streptococcus* species the most prevalent from asymptomatic volunteers showed highest sensitivity to fluoroquinolones (66%), while highest resistance to cephalosporine (90%) was recorded. Isolates of *Lactobacillus* species from asymptomatic volunteers were majorly sensitive to fluoroquinolones (82.5%), while highest resistance to phenicol and cephalosporine (100%); aminoglycoside and penicillin (75%) was recorded. *Escherichia coli* also isolated from asymptomatic volunteers were highly sensitive to fluoroquinolones (80%) and augumentine (80%), while highest resistance to phenicol and macrolides (100%) was observed. *Staphylococcus aureus* isolated from asymptomatic volunteers were highly sensitive to fluoroquinolones (70.8%), while highest resistance to sulfanomide and phenicol (100%); penicillin (75%) was observed**.** *Enterococcus faecalis* isolates recovered from asymptomatic volunteers were highly susceptible to fluoroquinolones (75%), while highest resistance to macrolide (100%) and penicillin (83.4%) were recorded.

Fig4.1b: Antibiotic susceptibility pattern of bacterial isolates recovered from oral cavities of asymptomatic volunteers presented using histogra



Note: CPX- Ciprofloxacin, PEF- Perfloxacin, SP- Sparfloxacin, OFX- Ofloxacin, AMP- Ampicillin, AM- Amoxicillin, AU- Augumentin, CFT-Ceftazidime, Z- Cefriazone, S- Streptomycin, CN- Gentamycin, SXT- Septrin, CH- Chloramphenicol, E- Erthroymcin, S-Sensitivity, R-Resistance.

Figure 4.3: Colour graphical representation of the response of individuals bacterial isolates to antibiotics used during the study



Note: CPX- Ciprofloxacin, PEF- Perfloxacin, SP- Sparfloxacin, OFX- Ofloxacin, AMP- Ampicillin, AM- Amoxicillin, AU- Augumentin, CFT-Ceftazidime, Z- Cefriazone, S- Streptomycin, CN- Gentamycin, SXT- Septrin, CH- Chloramphenicol, E- Erythromycin, STREP- *Streptococcus* species, LACT – *Lactobacillus* species, E.COLI – *Escherichia coli*, E. F- *Enterococcus faecalis*, S. A-*Staphylococcus aureus*, S.E – *Staphylococcus epidermidis*

Colony morphology, Gram staining and biochemical analysis of oral bacterial isolates recovered from outpatient volunteers

Code	Appearance on MA	Appearance on BA	Appearance on NA	Appearance on MSA	Grams reaction	Catalase	Coagulas	Methyl	Oxidase	Urease	Citrate
OU1	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
OU2	No growth	β-Haemolytic colonies	Large, circular smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+
OU3	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	+	+
OU4	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU5	N/A	N/A	N/A	N/A	N/A	-	-	-	-	-	-
OU6	Flat dry pink colonies (lactose fermenting)	β-Haemolytic colonies	white circular colonies	No growth	GNR	+	-	+	-	-	+
OU7	No growth	β-Haemolytic colonies	Large, circular smooth	Large Yellow	GPC	+	+	-	-	+	+

			colonies	colonies, yellow zones								
OU8	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU9	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU10	No growth	β-Haemolytic colonies	Large, circular smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+
OU11	No growth	β-Haemolytic colonies	Large, circular smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+
OU12	N/A	N/A	N/A	N/A	N/A	-	-	-	-	-	-
OU13	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	-	-
OU14	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU15	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	-	-
OU16	Flat dry pink colonies (lactose fermenting)	β-Haemolytic colonies	white circular colonies	No growth	GNR	+	-	+	-	-	+
OU17	Tiny pink colonies	ɤ-Haemolytic colonies	Shiny convex colonies	Small colonies, ferment mannitol	GPC	-	-	-	-	-	-
OU18	Non lactose fermenting	α-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU19	No growth	β-Haemolytic colonies	Large, circular smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+
OU20	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
OU21	Non lactose fermenting	α-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU22	No growth	β-Haemolytic colonies	Large, circular smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+
OU23	Flat dry pink colonies (lactose fermenting)	β-Haemolytic colonies	white circular colonies	No growth	GNR	+	-	+	-	-	+

OU24	Non lactose fermenting	β-Haemolytic colonies	Creamy pin point colonies	No growth	GPC	-	-	-	-	-	-
OU25	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
OU26	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU27	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU28	No growth	β-Haemolytic colonies	Large, circular smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+
OU29	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU30	Tiny pink colonies	ɣ-Haemolytic colonies	Shiny convex colonies	Small colonies, ferment mannitor	GPC	-	-	-	-	-	-
OU31	No growth	β-Haemolytic colonies	Large, circular smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+
OU32	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU33	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU34	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
OU35	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU36	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
OU37	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
OU38	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU39	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU40	Non lactose fermenting	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-
OU41	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	+	-
OU42	Tiny pink colonies	ɣ-Haemolytic colonies	Shiny convex colonies	Small colonies, ferment mannitor	GPC	-	-	-	-	-	-
OU43	Non lactose	β-Haemolytic colonies	Circular pin point	No growth	GPC	-	-	-	-	-	-

	fermenting			colonies											
OU44	Non	β-Haemolytic colonies	Circular pin point	No growth	GPC	-	-	-	-	-	-				
	Key:														
	OU- unhealthy (outpatient); *S. aureus- Staphylococcus aureus;*														
	GPC- Gram positive cocci; *Strept.* spp- *Streptococcus* species;														
OU45	GNR-Gram negative rod; *E. coli- Escherichia coli;*													-	- -
	GPR- Gram positive rod; *E. faecalis- Enterococcus faecalis;*														
	MA-MacConkey Agar *Lacto.* spp- *Lactobacillus* species.														
	BA-Blood Agar NA-Nutrient Agar														
OU46	MSA-Mannitol Salt Agar													-	- -
OU47														-	+ -
OU48														-	- -
OU49	[illegible] lactose fermenting	[illegible]	[illegible]	colonies	[illegible]									-	- -
OU50	Non lactose fermenting	ɤ-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-				

Colony morphology, Gram staining and biochemical analysis of oral bacterial isolates recovered from asymptomatic volunteers

Code	Appearance on MA	Appearance on BA	Appearance on NA	Appearance on MSA	Grams Reactio	Catalas	Coagul	Methyl Red	Oxidas	Urease	Citrate	Indole	Motilit	Mannit		Probable Organism
OH1	No growth	β-Haemolytic colonies	Large smooth colonies	Large Yellow colonies	GP C	+	+	-	-	+	+	-	-	+	+	*S. aureus*
OH2	No growth	β -Haemolytic colonies	Large smooth colonies	Large Yellow colonies	GP C	+	+	-	-	+	-	-	-	+	+	*S. aureus*
OH3	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GP C	-	-	-	-	-	-	-	-	-	-	*Strept.* sp
OH4	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GP R	-	-	-	-	-	-	-	-	+	-	*Lacto.* sp
OH5	Tiny deep colonies	β-Haemolytic colonies	Convex colonies	Small colonies	GP C	-	-	-		-	-	-	-	+	-	*E.faecalis*
OH6	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GP C	-	-	-		-	-	-	-	-	-	*Strept.* sp
OH7	No growth	α-Haemolytic colonies	Circular pin point colonies	No growth	GP C	-	-	-		-	-	-	-	-	-	*Strept.* sp
OH8	No growth	α -Haemolytic colonies	Circular pin point colonies	No growth	GP C	-	-	-	-	-	-	-	-	-	-	*Strept. sp*
OH9	N/A	N/A	N/A	N/A	GP R	-	-	-	-	-	-	-	-	-	-	*Unknown*
OH10	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GP R	-	-	-	-	-	-	-	-	+	-	*Lacto.* sp
OH1	Flat lactose fermenting	β-Haemolytic	Small circular	No growth	GN	+	-	+	-	-	-	+	+	+	-	*E. coli*

1	colonies	colonies	white colonies		R												
OH12	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	-	-	-	-	+	-		*Lacto.* sp
OH13	No growth	β -Haemolytic colonies	Large smooth colonies	Large Yellow colonies	GPC	+	+	-	-	+	+	-	-	+	-		*S. aureus*
OH14	No growth	α -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH15	No growth	β-Haemolytic colonies	Large smooth colonies	Large Yellow colonies	GPC	+	+	-	-	+	+	-	-	+	+		*S. aureus*
OH16	Flat lactose fermenting colonies	β-Haemolytic colonies	Small circular white colonies	No growth	GNR	+	-	+	-	-	-	+	+	+	-		*E. coli*
OH17	No growth	α -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH18	No growth	ɤ-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH19	No growth	β-Haemolytic colonies	Large smooth colonies	Large Yellow colonies, yellow zones	GPC	+	+	-	-	+	+	-	-	+	-		*S. aureus*
OH20	No growth	ɤ -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH21	No growth	β-Haemolytic colonies	Small circular white colonies	No growth	GPR	-	-	-	-	-	-	-	-	+	-		*Lacto* sp
OH22	Tiny deep colonies	β-Haemolytic colonies	Convex colonies	Small colonies	GPC	-	-	-	-	-	-	-	-	+	-		*E. faecalis*
OH23	No growth	ɤ -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.*sp
OH24	Flat lactose fermenting colonies	β-Haemolytic colonies	Small circular white colonies	No growth	GNR	+	-	+	-	-	-	+	+	+	-		*E. coli*
OH25	No growth	ɤ -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.*sp
OH26	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.*sp
OH27	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH28	No growth	β-Haemolytic colonies	Large smooth colonies	Large Yellow colonies	GPC	+	+	-	-	+	+	-	-	+	+		*S. aureus*
OH29	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH30	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH31	No growth	ɤ -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH32	No growth	ɤ -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept* .sp
OH33	No growth	β-Haemolytic colonies	Small circular white colonies	No growth	GPR	-	-	-	-	-	-	-	_	+	-		*Lacto.* sp
OH34	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	--	-	-	-	-	-	+	-		*Lacto.* sp
OH35	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	-	-	-	-	-	_		*Lacto.* sp
OH36	Flat lactose fermenting colonies	β-Haemolytic colonies	Small circular white colonies	No growth	GNR	+	+	+	-	-	-	+	+	+	-		*E. coli*

OH37	No growth	α-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH38	Flat lactose fermenting colonies	β-Haemolytic colonies	Small circular white colonies	No growth	GPC	-	-	-	-	-	-	-	-	+	-		*E. faecalis*
OH39	No growth	α -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH40	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH41	No growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH42	Flat lactose fermenting colonies	β-Haemolytic colonies	Small circular white colonies	No growth	GNR	+	-	+	-	-	-	+	+	+	-		*E. coli*
OH43	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	-	-	+	-	+	-		*Lacto.* sp
OH44	NoX growth	β-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH45	No growth	α-Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH46	No growth	α -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept* sp
OH47	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	-	-	-	-	+	-		*Lacto.* sp
OH48	No growth	β-Haemolytic colonies	Small circular colonies	No growth	GPR	-	-	-	-	-	-	-	-	+	-		*Lacto.* sp
OH49	No growth	α -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp
OH50	No growth	α -Haemolytic colonies	Circular pin point colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-		*Strept.* sp

Key:

OH- Oral Healthy (asymptomatic); ***S. aureus***- *Staphylococcus aureus;* ***Strept.*** **sp** - *Streptococcus* species;
GPC- Gram Positive Cocci; ***E. coli***- *Escherichia coli;****Candida. sp***- *Candida* species
GNR- Gram Negative Rod; ***E. faecalis***- *Enterococcus faecalis;*
GPR-Gram Positive Rod;*Lacto. sp*- *Lactobacillus* species;
MA-MacConkey Agar
BA-Blood Agar
NA-Nutrient Agar
MSA-Mannitol Salt Agar

Antibiogram profile of bacterial isolates from oral cavity of outpatient volunteers

Organism	*Strept species*				*Lactobacillus species*				*S. aureus*				*E. coli*					
	S		R		S		R		S		R		S		R		S	
Antibiotics	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%	N	%
Isolates	23	100			11	100			8	100			3	100			3	
CPX	19	82.6	14	17.4	11	100	-	-	7	87.5		12.5	3	100		-	2	
PEF	22	95.7	1	4.3	9	81.8	2	18.2	6	75	12	25	2	66. 7	1	33.3	2	
SP	7	30.4	16	69.3	7	63.6	4	36.4	6	75	2	25	1	33.3	2	66. 7	2	
OFX	12	52.2	11	47.8	9	81.8	2	18.2	5	62.5	3	37.5	2	66. 7	1	33.3	3	
AMP	17	74	6	26	2	18.2	9	81.8	-	-	8	100	1	33.3	2	66. 7	-	
AM	12	52.2	11	47.8	2	18.2	9	81.8	-	-	8	100	1	33.3	2	66. 7	1	
AU	13	56.5	10	43.5	9	81.8	2	18.2	4	50	4	50	1	33.3	2	66. 7	1	
CFT	-	-	23	100		-	11	100	4	50	4	50	1	33.3	2	66. 7	1	
Z	4	17.4	19	82.6	4	36. 4	8	63.6	-	-	4	50	2	66. 7	1	33.3	1	
S	10	43.5	13	56.5		-	11	100	4	50	4	50	1	33.3	2	66. 7	2	
CN	5	21.7	17	78.3	7	63.6	9	.36.4	4	50	4	50	1	33.3	2	66. 7	1	
SXT	10	43.5	23	56.5	4	36. 4	8	63.6	2	25	6	75	1	33.3	2	66. 7	1	
CH	12	52.2	11	47.8		-	11	100	-	-	8	100	-	-	3	100	1	
E	7	30.4	16	69.6	4	36.4	8	63.6	4	50	8	100	-	-	3	100	-	

CPX- Ciprofloxacin
PEF- Perfloxacin
SP- Sparfloxacin
OFX- Ofloxacin
AMP- Ampicillin
AM- Amoxacillin
Au- Augmentin
CFT- Ceftazidine
Z- Ceftriazone
S- Streptomycin
CN- Gentamycin
SXT-Septrin
CH-Chloramphenicol
E-Erythromycin
N- Number of isolates
S- sensitivity
R-resistance

Antibiogram profile of bacterial isolates from oral cavity of asymptomatic volunteers

Organism	*Strept. Species*				*Lactobacillus Species*				*S. aureus*				*E.coli*				*E.faecalis*			
	S		R		S		R		S		R		S		R		S		R	
Antibiotic	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**	**N**	**%**
Isolates	25	100	0	0	10	100	0	0	6	100	0	0	5	100	0	0	3	100	0	0
CPX	19	76	5	24	10	100	-	-	5	83	1	16.7	5	100	-	-	2	66. 7	1	33.3
PEF	16	64	9	36	8	80	2	20	4	66.7	2	33.3	3	60	2	40	2	66. 7	1	33.3
SP	16	64	9	36	6	60	4	40	4	66.7	2	33.3	4	80	1	20	2	66. 7	1	33.3
OFX	15	60	10	40	9	90	1	10	4	66.7	2	33.3	4	80	1	20	3	100	-	-
AMP	17	68	8	32	-	-	10	100	-	-	6	100	2	40	3	60	-	-	3	100
AM	10	40	15	60	5	50	5	50	3	50	3	50	2	40	3	60	1	33.3	2	66. 7
AU	13	52	12	48	6	60	4	40	3	50	3	50	4	80	1	20	1	33.3	2	66.7
CFT	-	-	25	100	-	-	10	100	-	-	6	100	1	20	4	80	1	33.3	2	66. 7
Z	5	20	20	80	-	-	10	100	4	66.7	2	33.3	3	60	2	40	1	33.3	2	66.7
S	15	60	10	40	-	-	10	100	4	66.7	2	33.3	2	40	3	60	2	66. 7	1	33.3
CN	14	56	11	44	6	60	4	40	2	33.3	4	66.7	4	80	1	20	1	33.33	2	66.7
SXT	11	44	14	56	3	30	7	70	-	-	6	100	2	40	3	60	1	3.3	2	66.7
CH	12	48	13	52	-	-	10	100	-	-	6	100	-	-	5	100	1	33.3	2	66.7
E	7	28	18	72	4	40	6	60	3	50	3	50	-	-	5	100	-	-	3	100

Key:

CPX- Ciprofloxacin
PEF- Perfloxacin
SP- Sparfloxacin
OFX- Ofloxacin
AMP- Ampicillin
AM- Amoxacillin
Au- Augmentin
CFT- Ceftazidine

Z- Ceftriazone S- Streptomycin CN- Gentamycin SXT-Septrin
CH-Chloramphenicol E-Erythromycin N- Number of isolates
S- sensitivity
R-resistance

V. DISCUSSION

Discussion

The human microbiome is an ecological community of commensal, symbiotic, and pathogenic microorganisms that live in and on the human body (Lederberg and McCray, 2001). These microorganisms are essential for maintaining general health, but also able to initiate diseases (Zarco *et al*., 2012). Oral and nasal health are essential to the overall health and wellbeing of an individual.

According to our findings, *Streptococcus* species is the most abundant species in the oral cavity, a total of (48:48%) *Streptococcus* species were isolated in both group of the oral cavities, this is in agreement with previous report by Amortoso *et al*. (2003) that *Streptococcus* species is frequently recovered from the oral cavity, and make up almost 50% of culturable flora of the tongue and Saliva. These oral streptococci are beneficial to the host as they produce molecules that are inhibitory to pathogenic species, some pathogenic streptococci residing in the oral cavity can gain access to the bloodstream and cause systemic infections such as endocarditis (Abranches *et al.*, 2008).

Lactobacillus species found in food such as yoghurt, cheese, coffee and a wide variety of fermented food was also isolated (21:21%), from the oral cavities of participants during the study. This could be attributed to type of food consumed by these individuals and the ability of *Lactobacillus* in fermented food to colonize the oral cavity. In a literature review of the ecology of *lactobacillus* in the oral cavity Bussher *et al*. (1999), noted that *L. acidophilus* and *L. casei* present in yoghurts are able to adhere to the enamel and colonize the oral cavity. *Lactobacillus* species found in caries lesions are a major contributor to caries progression, serve as major reservoir to the gastrointestinal (GI) tract (Caufield *et al.,* 2015).

Staphylococcus aureus a normal flora of the nasopharynx and nose, is generally not considered to form part of the oral cavity, were isolated (14:14%) from oral cavities of participants, this could be attributed to poor hygiene (such as blowing nose with bare hands and eating with same hands without proper washing), also sniffing back mucous from the nose into the mouth. Jackson *et al*. (1999) reported (24%) *Staphylococcus aureus* in the mouth, contrary to a review by Christine, (2019) which found about 1000 (>19%) in a population of 5005 to be *S. aureus*, this could be as a result of difference in the sample size considered in both study and the dental health status of the study participants. *Staphylococcus aureus* can be pathogenic when found outside the nasal region. It can cause infections under certain circumstance such as presence of an open wound or an underlying health condition such as diabetes. Improved level of oral hygiene can reduce the spread of *Staphylococcus aureus* into the oral cavity.

A total of (8:8%) of *Escherichia coli,* a Gram-negative motile bacteria naturally found in the intestinal tract, was recovered from both asymptomatic and outpatient volunteers during the study, this confirms the presence of *E. coli* in the mouth as also reported by Zawadzki *et al.* (2016), in a study which detected *E. coli* in oral cavities of patients with systemic diseases. *E. coli* in the mouth is an indication of poor sanitation by subjects from whom they were isolated. Faecal-oral transmission is the main avenue through which pathogenic strains of *E. coli* cause disease. It is responsible for a wide range of hospital and community onset infection affecting patients with normal immune system as well as those with pre-existing conditions (Pitout, 2012). *Escherichia coli* may cause urinary tract infections and respiratory infections.

Prevalence of *Enterococcus faecalis* has been reported to be relatively low in healthy individuals i.e 1-20% (Sedgley *et al*., 2006), (6:6%) of isolates from oral cavities of volunteers (asymptomatic and outpatient) during this study was *Enterococcus faecalis*. The presence of *E. faecalis* in subjects maybe attributed to the resilient nature and ability of *E. faecalis* to survive a wide assemblage of hostile conditions, as they can persevere in the environment for long duration (Van Tyne and Gilmore, 2014).

Although *E. faecalis* is not considered to be part of healthy oral flora (Aas *et al*., 2005), it has been associated with common dental disease such as periodontis, peri-implantis and caries (Kouldhi *et al*., 2011; Dahlen *et al*., 2012; Rams *et al*., 2012). *Enterococcus faecalis* has been regularly found in re-infected, root canal-treated teeth in prevalence ranging from 30%-90% of the cases (Enitan *et al*., 2020).

There was significant difference in the number and type of bacterial isolated from outpatient individuals compared to asymptomatic individuals in the volunteer category (P>0.05). This is an indication that state of health may be a determinant factor to colonization of the oral cavity either by commensal bacteria, opportunistic bacteria, and/or pathogenic bacteria. Blaser and Falkow (2009) in an essay also noted that various changing pattern such as diet, environmental factors, state of health of an individual and more could influence human microbiota.

Antibiotic susceptibility profile analysis of oral isolates

All isolates from the oral cavity revealed variable sensitivity to the antibiotics tested in the study. *Streptococcus* isolates from the oral cavity of asymptomatic volunteers and outpatient volunteer showed highest sensitivity to fluoroquinolones (65.6%) similar to pattern obtained from studies by Okesola and Ige, (2008), although *Streptococcus* isolates in the study were most resistant to cephalosporines (90.7%), studies by Enitan *et al*. (2020) to assess hygiene practices on the composition of oral microbiota and antibiotic profile of pathogens, *Streptococcus* isolates showed most resistant to penicillin and ofloxacin respectively.

Escherichia coli isolated from oral cavities of asymptomatic volunteers were most susceptible to fluoroquinolones (73.4%). In a study reported by Obi *et al.* (2004), *Escherichia coli* strains isolated from HIV patients and their drinking water showed over 90% susceptibility to fluoroquinolones and aminoglycoside, but contrary to studies by Amin *et al.* (2009) and Akpan *et al*. (2011) which reported that *E. coli* was majorly sensitive to penicillin. Sensitivity to quinolones as observed in this study is an essential finding; these pharmaceuticals are drugs for treatment of infections caused by Gram-negative rods because they inhibit DNA replication of bacterial cell and are thus wide spectrum antibacterial agent. Resistance to Phenicols and macrolides could be attributed to antibiotics abuse or misuse.

Staphylococcus aureus strains isolated from the oral cavity of asymptomatic volunteers were highly susceptible to fluoroquinolones (72.9%), this is similar to report by Manikandan and Amsath. (2013) of the susceptibility of *S. aureus* strains from patients with respiratory tract infection to fluoroquinolones. *Staphylococcus aureus* from the oral cavities showed resistance to penicillin (87.5%). Swati *et al*. (2019), reported *S. aureus* resistance to penicillin this could be attributed to production of β-lactamase by *S. aureus,* and also misuse or abuse of this agent.

Strains of *Enterococcus faecalis* isolated from oral cavities of volunteers (outpatient and asymptomatic), were sensitive to fluoroquinolones (75%) and aminoglycoside (66.7%), susceptibility to aminoglycoside was also reported by Kouldhi *et al.* (2011). *Enterococcus faecalis* strains were highly resistant to penicillins (83.4%) and macrolides (100%). Resistance to macrolides has also been reported by Anderson *et al*. (2016). Resistance to these agents could be attributed to inability of these agents to synthetize the biofilm which protects *enterococci* (a group which *E. faecalis* belongs), from host immune response and antibiotics.

Generally bacterial isolates from oral cavities of volunteers in the study were most sensitive to fluroquinolones (72%). These bacterial agents inhibit DNA replication and are effective against Gram-positive and Gram-negative bacteria. High resistance to phenicols (96.9%), macrolides (92.6%), cephalosporines (90.8%) and penicillin (83.4%) was observed, this could be attributed to abuse or inappropriate use of these bacterial agent, which is more prevalent in low economic nations like Nigeria, than in developed countries. There was significant difference in the number and type of bacterial isolated from outpatient individuals compared to asymptomatic individuals in the volunteer category considered during the study, ($P>0.05$), which suggests that health status of an individual may be a determinant of bacterial microbiota colonization of oral and nasal cavities.

There was no significant difference ($P<0.05$) in the pattern of response of antibiotic susceptibility to bacterial isolates obtained from oral cavities (asymptomatic and outpatient volunteers) considered during the study.

VI. Conclusion

The oral cavity of volunteers in Kaduna metropolis are largely colonized by *Streptococcus* species. The oral cavity sometimes harbors *Staphylococcus aureus*, *Escherichia coli* and *Enterococcus faecalis* these opportunistic and pathogenic bacteria species are capable of initiating diseases in immune compromised individual or during an imbalance of the oral bacteria microbiota.

There was no significant difference ($P<0.05$) in the pattern of response of antibiotic susceptibility to bacterial isolates obtained from samples of hospital outpatients and asymptomatic volunteers considered during the study.

VII. Recommendations

Following the outcome of this research, it is therefore recommended that There is need for health sector to educate or enlighten the public on proper use of antimicrobial agent, this will minimize antibiotic resistance as a result of abuse or inappropriate use. Individuals are also admonished to take personal hygiene, hygiene as top priority. Doctors should rely more on the laboratory results in prescribing antibiotics. Most bacteria species are not culturable, but all bacteria can be captured using Nextgen sequencing, future studies using Nextgen sequencing will help to better understand the human bacterial microbiota.

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