Determination of Antibiogram Profile of Nasal Bacterial Isolates from Hospital Outpatients and Asymptomatic Volunteers in Kaduna Metropolis, Nigeria.

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Abstract
The human body is surrounded by a variety of distinct habitats, each of which harbors a complex microbial community with distinct phylogenetic and functional gene composition. These micro-organisms are essential in maintaining general health, yet also capable of initiating diseases. This study aims at Isolation, Identification and Determination of Antibiogram Profile of Nasal Bacterial Isolates from Hospital Outpatients and Asymptomatic Volunteers in Kaduna Metropolis, Nigeria. A total number of Fourty-eight (48) nasal samples comprising of (25 outpatients and 23 asymptomatic volunteers) were collected from outpatients at Yusuf Dantsoho Memorial Hospital, Tudun wada; Nigerian Defence Academy Hospital, Mando and asymptomatic volunteers were recruited for the study. Nasal swabs were collected, innoculated on Nutrient agar, Blood agar, Mannitol salt agar and MacConkey agar and 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. A total of 48 bacterial isolates comprising of Staphylococcus aureus (35), Staphylococcus epidermidis (12) and Streptococcus species (1) were obtained from the nasal cavities of asymptomatic and outpatient volunteers during this study. The most abundant bacteria found in the nasal cavities was S. aureus (35:72.9%), and the least was Streptococcus species (1:2.1%). Turkey test revealed that S. aureus recorded the highest mean (17.5^A), while Streptococcus species (0.5^B) had the least. There was no significant difference $(P>0.05)$, in the volunteer category (hospital outpatients and asymptomatic volunteers). Generally, bacterial isolates obtained showed variable susceptibility to antibiotics used, isolates showed highest sensitivity to Fluoroquinolone (72%), and exhibited a substantial resistance to antibiotics such as, Phenicols (96.9%), Macrolides (92.6%), cephalosporine (90.8%) and penicillin (83.4%). which suggests that health status of an individual may influence bacterial microbiota colonization of nasal cavities.

Key Words: *bacterial microbiota, Antibiogram profile, gene sequencing, nasal cavity.*

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I.   Introduction
Bacterial cells outnumber the number of cells in each human. Microbial cells are found in and on the human body (Kathryn and James, 2012). 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). Several diseases such as diabetes (type 1 and 2), asthma, autism, allergies, inflammatory bowel disease and cancer have been associated with an imbalance in the microbiome (Backhed *et al*., 2012; Garrett, 2015; Hsiao *et al*., 2013; Trompette *et al*., 2014).

The human body is surrounded by a variety of distinct habitats, each of which harbors a complex microbial community with distinct phylogenetic and functional gene composition (Human Microbiome Project Consortium, 2012). The nasal cavity has been viewed as one such habitat from an anatomical perspective, the familiar outward appearance of the nasal cavity belies a complicated internal network of passages leading to the lungs and paranasal sinuses (Jones, 2001). Although it is most conventionally recognized for its olfactory function, the nose is an important part of the respiratory and immune systems. As a portal into the respiratory tract, the nasal cavity both conditions and filters inhaled air. Smaller particulate matter, including bacteria are trapped in a flowing mucous blanket covering the nasal mucosa deeper in the nose (Cohen, 2006). Antimicrobial compounds such as lysozyme, lactoferin and secretory IgA are present in mucous, and along with mucousal immune cells, contribute to the innate immune system (Ooi *et al.,* 2008). The presence of microbes in the sticky

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milieu is an important component of immune priming. The nasal cavity has long been thought to be a clinically important microbial habitat, especially for *Staphylococcus aureus*. *S. aureus* colonization screening campaigns have resulted from efforts to remove this species from the nasal vestibule (Lowy, 1998).

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 respiratory tract microbiome is divided into two parts: the upper respiratory tract microbiome, which includes the nose, nasal cavity, sinuses, mouth, and larynx, and the lower respiratory tract microbiome, which includes the lungs (trachea, bronchi, bronchioles, and alveoli) (Bassis *et al.,* 2014). *Firmicutes, Actinobacteria, Proteobacteria, Bacteriodetes,* and *Fusobacteria*, which include species from *Streptococcus Neisseria, Gemella, Corynbacterium, Alloiococcus,* and *Haemophilus,* commonly dominate the Upper Respiratory Tract (Charlson *et al.,* 2011; Allen *et al.,* 2014; Bassis *et al.,* 2014; Botero *et al.,* 2014; Dickson *et al.,* 2015b; Tarabichi *et al.,* 2015).

Every human is home to a diverse microbial community that lives in discrete body habitats, and these microbiomes vary greatly in terms of composition and function (Olsen, 2016). The local environment and biology of each body habitat influence the richness of microbes at a body site as well as the genetic diversity of microbiomes (Kathryn and James 2012). Blaser and Falkow, (2009) also noted that microbial diversity varies by anatomic sites, and the complexity and aggregate functions of bacterial communities may correlate with an individual health status, genotype, diet, hygiene, gender, ethnicity, geographical region and risky behaviours (smoking and drinking). Stearns *et al*. (2015) discovered similarities in the microbiomes of all Canadian populations. *Firmicutes*, *Actinobacteria*, *Proteobacteria, Bacteroidetes*, and *Fusobacteria* phyla dominate the upper respiratory tract, which includes species from the *Streptococcus*, *Neisseria*, *Gemella*, *Corynbacterium*, *Alloiococcus*, and *Heamophilus* genera (Gupta *et al.,* 2017).

The microbiome of the anterior nares of healthy adults has been observed to be dominated by three phyla: *Actinobacteria, Firmicutes* and *Proteobacteria* (Bassis *et al.,* 2014). The middle meatus of 28 healthy adults was found to have a diverse bacterial community, with the most abundant microorganisms being *Staphylococcus aureus*, *Staphylococcus epidermidis*, and *Propionibacterium acnes* (Ramakrishnan *et al.,* 2013). The adult nasal microbiome in healthy people is better characterized than the adult nasal microbiome in disease states. 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. Hence, deepen our perception of human health and importantly contribute to more effective ways to deploy traditional treatments. This research was aimed at assessing the antibiotic profile and some characteristics of nasal bacteria isolated from hospital outpatient and asymptomatic volunteers within Kaduna, (North-west, Nigeria) metropolis.

II.   Materials And Methods

The study area

This study was conducted in Kaduna metropolis, Kaduna, located in the north-west geopolitical zone of Nigeria and lies between latitude $10^0 20'$ N and $10^0 33'$ longitude $70^0 45'$ E and $7^0 75'$ E. The state shares boarder with Kano, Kastina and Zamfara state to the north, Plateau and Bauchi state to the east, Nasarawa state and the Federal Capital Territory (FCT to the south and Niger state to the west). Kaduna state occupies $46, 053 Km^2$ and has a population of more than 5million according to the 2016 census.

Study design and population

This is a cross-sectional study was carried out among adult hospital outpatients (Yusuf Dantsoho Memorial Hospital, Tudun Wada and Nigerian Defense Hospital Mando) and asymptomatic volunteers in some parts of Kaduna metropolis.

Ethical approval

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

Informed 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

Nasal swab samples were collected using sterile swab stick from both nares of consented participants, then placed into its container properly covered and labelled. The nasal specimens were transported to the laboratory in a sealed plastic bag within 2hours of collection.

Sample analysis

Nasal swab sticks were streaked directly on Mannitol salt agar, Blood agar and Nutrient 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°C 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 acertain 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

An 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

Raw data obtained from the study 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.

III. Results

Distribution of bacterial isolates from nasal cavities

A total of 48 bacterial isolates comprising of *Staphylococcus aureus* (35)*, Staphylococcus epidermidis* (12) and *Streptococcus* species (1) were obtained from the nasal cavities of asymptomatic and outpatient volunteers during this study (Table 4.1). The most abundant bacteria found in the nasal cavities was *S. aureus* (35:72.9%), and the least was *Streptococcus* species (1:2.1%). Turkey test revealed that *S. aureus* recorded the highest mean (17.5[A]), while *Streptococcus* species (0.5[B]) had the least. There was no significant difference (P>0.05), in the volunteer category (hospital outpatients and asymptomatic volunteers).

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

Table 4.2 shows bacterial strains belonging to 2 genera obtained from the nasal cavities of both asymptomatic and outpatient volunteers during the study. The results obtained from the biochemical tests showed bacteria belonging to the genera of *Staphylococcus* and *Streptococcus* were obtained from the oral cavity of asymptomatic and outpatient volunteers.

Table 4.1: Distribution of bacterial isolates from nasal cavities according to type of volunteers

Nasal cavity	Asymptomatic volunteers	Outpatient volunteers	Total
Staphylococcus aureus	16	19	35
Staphylococcus epidermidis	07	05	12
Streptococcus **species**	-	01	01
Total	**23**	**25**	**48**

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

Table 4.2: Gram staining and biochemical analysis of some nasal bacterial isolates recovered from volunteers (asymptomatic and outpatient)

Code	Grams reaction	Catalase	Coagulase	Oxidase	Urease	Citrate	Indole	Motility	Methyl Red	Mannitol	Lactose	Probable Organism
NU1	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NU2	GPC	-	-	-	-	-	-	-	-	-	-	*Strept.* sp
NU3	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NU4	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NU5	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NU6	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*
NU7	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH1	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 2	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 3	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 4	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*
NH 5	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 6	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 7	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*

Key:

NU-Nasal unhealthy   +-Positive

--Negative   NH-Nasal healthy   GPC-Gram positive cocci

PCR amplification of some selected bacterial isolates from the study

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
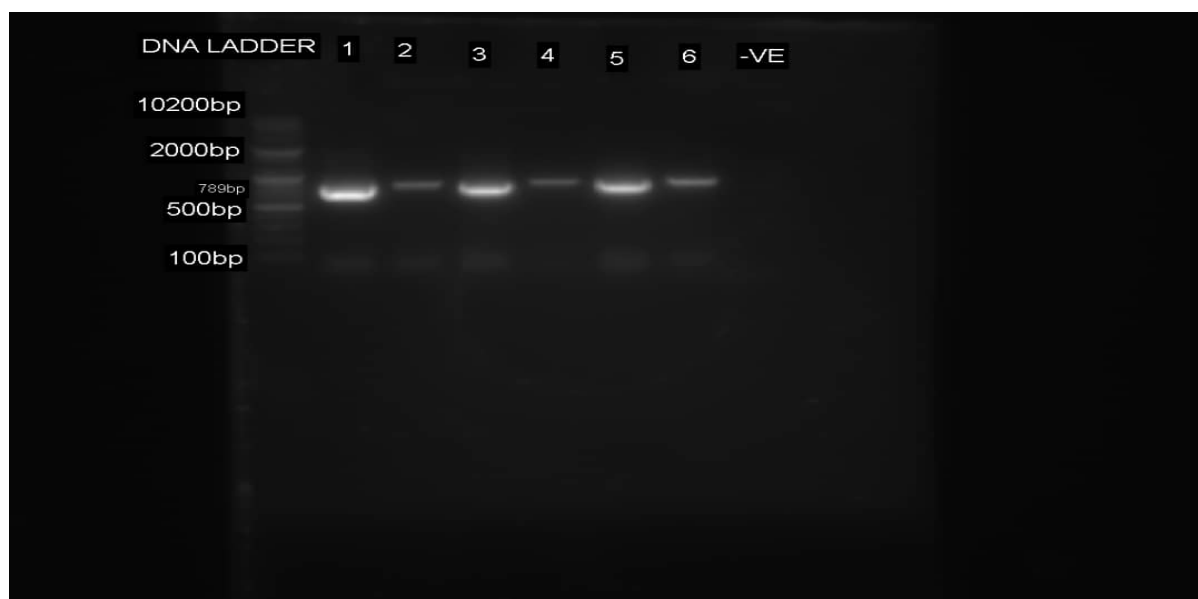

Plate I: Representative gel for PCR amplification of DNA extracted from some selected bacterial isolates from oral and nasal cavities showing the bands for 16SrRNA bacteria genes (789bp). DNA ladder (10200bp) DNA size marker; (Lane 1 - *Streptococcus* species, Lane 2-*Stapylococcus aureus*, Lane 3-*Lactobacillus* species, Lane 4-*Enterococcus faecalis)* from oral cavity, Lane 5 and 6-*Staphylococcus aureus* from nasal cavity, -ve- negative control.

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

The antibiotic susceptibility pattern of bacterial isolates from outpatient volunteers is shown in Fig. 4.2a. Bacterial isolates obtained displayed variable susceptibility to antibiotic used as follows, *S. aureus* isolates showed highest sensitivity to fluoroquinolones (94.7%), while highest resistance to macrolides (68.4%) was recorded.

Isolates of *S. epidermidis* from outpatient volunteer were highly susceptible to fluoroquinolones (90%) and augumentine 80%), while highest resistance to macrolide (80%) was recorded.
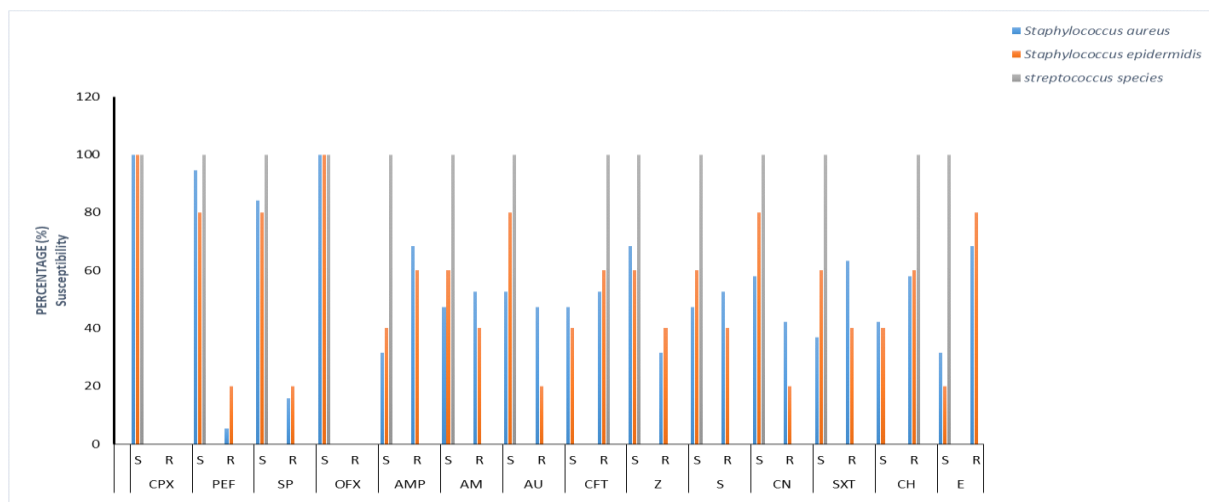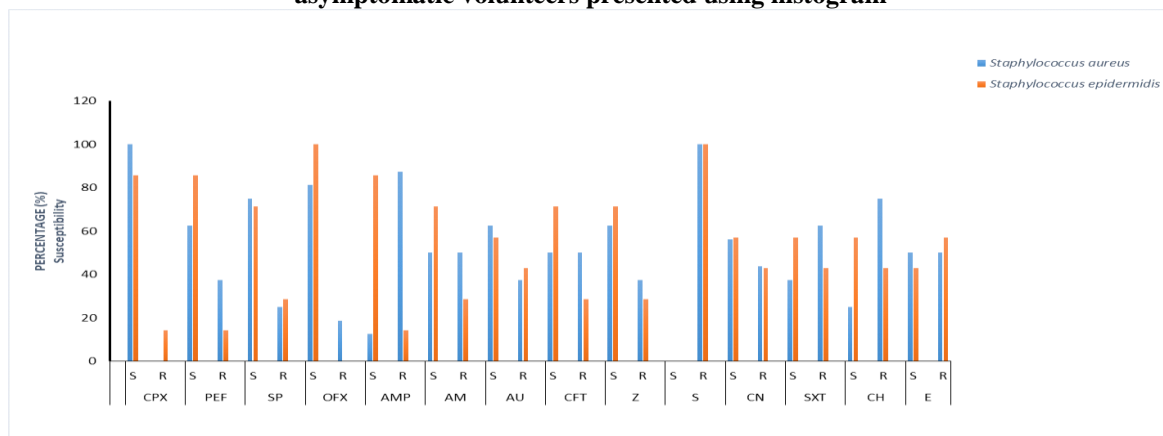

Note: CPX- Ciprofloxacin, PEF- Perfloxacin, SP- Sparfloxacin, OFX- Ofloxacin, AMP- Ampicillin, AM-Amoxicillin, AU- Augumentin, CT-Ceftazidime, Z- Cefriazone, S- Streptomycin, CN- Gentamycin, SXT-Septrin, CH- Chloramphenicol, E- Erthroymcin, S-Sensitivity, R-Resistance.

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

The antibiotic susceptibility pattern of bacterial isolates recovered from nasal cavities of asymptomatic volunteers is shown in Fig 4.2b. Bacterial isolates obtained displayed variable susceptibility to antibiotic used as follows, *S. aureus* showed highest sensitivity to fluoroquinolones (79.7%), while highest resistance to phenicol (75%) was recorded.
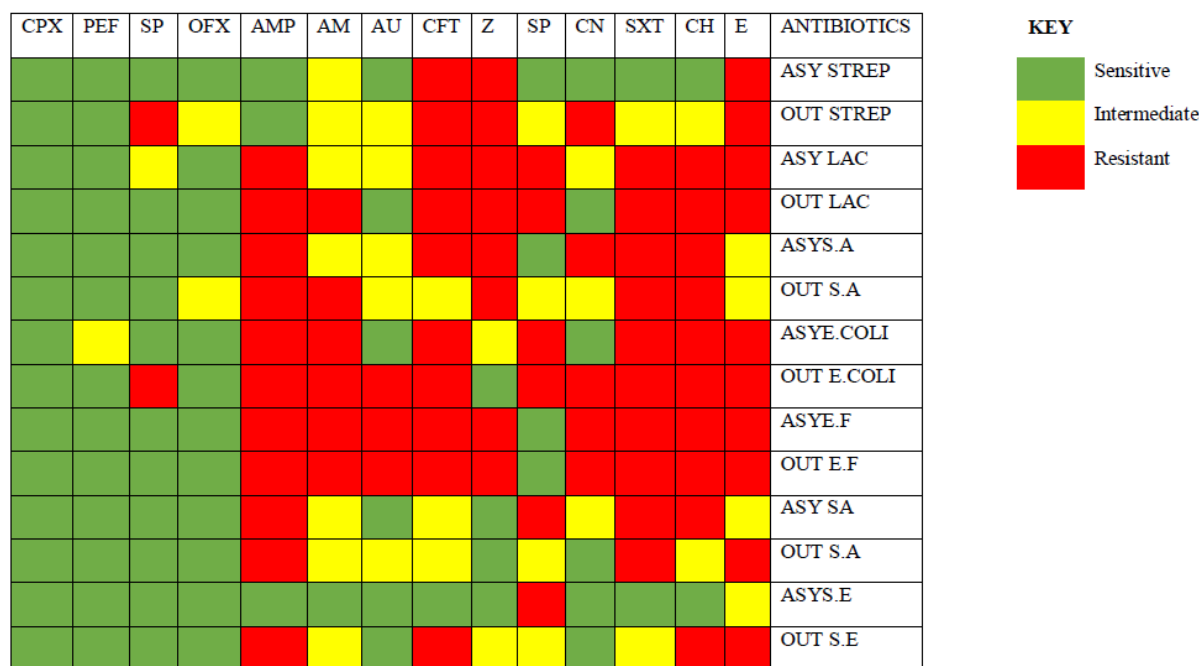
Staphylococcus epidermidis isolated from asymptomatic volunteers were highly sensitive to fluoroquinolones (85.7%), while highest resistance to aminoglycoside (71.5%) was recorded.

Fig 4.2b: Antibiotic susceptibility pattern of bacterial isolates recovered from nasal cavities of asymptomatic 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- Erthroymcin, S-Sensitivity, R-Resistance.

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

CPX	PEF	SP	OFX	AMP	AM	AU	CFT	Z	SP	CN	SXT	CH	E	ANTIBIOTICS
														ASY STREP
														OUT STREP
														ASY LAC
														OUT LAC
														ASYS.A
														OUT S.A
														ASYE.COLI
														OUT E.COLI
														ASYE.F
														OUT E.F
														ASY SA
														OUT S.A
														ASYS.E
														OUT S.E

KEY

- Sensitive
- Intermediate
- Resistant

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*

IV. Discussion And Conclusion

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). Nasal health is essential to the overall health and wellbeing of an individual.

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.

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).

All isolates from the Nasal cavity showed varying sensitivity to the antibiotics tested in the study. Isolates of *S. aureus* from the nasal cavities of volunteers (asymptomatic and outpatient) were highly sensitive to fluoroquinolones (87.3%); this supports previous findings by Rijal *et al*. (2008) in a study, that reported *S. aureus* isolates obtained from school children, as sensitive to fluoroquinolones, Ciprofloxacin (93.8%); (Onanuga and Onalapo, 2008) in a study also reported susceptibility of *S. aureus* obtained from healthy women in Zaria to fluoroquinolones. susceptibility to these agents could be attributed to wide spectrum antibacterial nature of these agents to *S. aureus*. Highest resistance of *S.aureus* to penicillin (80%) was recorded, this has been widely reported for *S. aureus* from various healthy subjects. Onanuga and Onalapo, (2008) also reported resistance to penicillin, this could be due to effect of β-lactamase produced by *S. aureus,* it may also be as a result of unchecked use which favour increasing number of resistant strains due to selection pressure (Okeke *et*

al., 1999). Resistance to phenicol (75%) could also be attributed to misuse or abuse of antibiotics common in developing countries.

Staphylococcus epidermidis isolated from nasal cavities of volunteers (asymptomatic and outpatient) were highly susceptible to fluoroquinolones (87.9%) and aminoglycosides (74.2%). There was no significant difference ($P<0.05$) in the pattern of response of antibiotic susceptibility to bacterial isolates obtained from nasal samples (asymptomatic and outpatient volunteers) considered during the study

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

Code	Appearance on MA	Appearance on BA	Appearance on NA	Appearance on MSA	Grams reaction	Catalase	Coagulase	Oxidase	Urease	Citrate	Indole	Motility	Methyl Red	Mannitol	Lactose	Probable Organism
NU1	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S.aureus
NU2	No growth	β-haemolytic colonies	Large circular colonies	No growth	GPC	-	-	-	-	-	-	-	-	-	-	Strept.sp
NU3	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S.aureus
NU4	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S.aureus
NU5	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S.aureus
NU6	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	S. epid
NU7	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU8	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU9	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU10	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	S. epid
NU11	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	S.epid
NU12	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU13	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU14	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU15	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU16	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	S. epid
NU17	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU18	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU19	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU20	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	S. epid
NU21	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU22	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU23	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU24	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus
NU25	No growth	ɤ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	+	-	+	+	-	-	-	+	+	S. aureus

Key:

NU=	Nasal Unhealthy (outpatient);		*S. epid* -	*staphylococcus epidermidis;*
GPC=	Gram Positive Cocci;		*Strept.* sp -	*Streptococcus* species.
S. aureus-	*Staphylococcus aureus;*			
MA-	MacConkey Agar			
BA-	Blood Agar			
NA-	Nutrient Agar			
MSA-	Mannitol Salt Agar			

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

Code	Appearance on MA	Appearance on BA	Appearance on NA	Appearance on MSA	Gram reaction	Catalase	Coagulase	Oxidase	Urease	Citrate	Indole	Motility	Methyl Red	Mannitol	Lactose	Probable Organism
NH1	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 2	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 3	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 4	No growth	ɣ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*
NH 5	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 6	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 7	No growth	ɣ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*
NH 8	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 9	No growth	ɣ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*
NH 10	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 11	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 12	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 13	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 14	No growth	ɣ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*
NH 15	No growth	ɣ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-	-	+	*S. epidermidis*
NH 16	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*

NH 17	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 18	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies yellow zones	GPC	+	+	-	+	+	-	-	-	+	+	*S. aureus*
NH 19	No growth	ϒ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	-	-	-	-		+	*S. epidermidis*
NH 20	No growth	ϒ-haemolytic colonies	Large circular colonies	Red colonies	GPC	+	-	-	+	_	_	_	_		+	*S. epidermidis*
NH 21	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-		+	*S. aureus*
NH 22	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-		+	*S. aureus*
NH 23	No growth	β-haemolytic colonies	Large circular colonies	Yellow colonies	GPC	+	+	-	+	+	-	-	-		+	*S. aureus*

Note: NH=Nasal healthy (asymptomatic);*S. aureus- Staphylococcus aureus;*
 GPC=Gram Postive Cocci; *S. epidermidis- Staphylococcus epidermidis*
MA-MacConkey Agar
BA-Blood Agar
NA-Nutrient Agar
MSA-Mannitol Salt Agar

Antibiogram profile of bacterial isolates recovered from nasal cavity of asymptomatic volunteers

Organism	*S. aureus*				*S. epidermidis*			
	S		R		S		R	
Antibiotics	N	%	N	%	N	%	N	%
Isolates	16	100			7	100		
CPX	16	100	-	-	6	85.7	1	14.3
PEF	10	62.5	6	37.5	6	85.7	1	14.3
SP	12	75	4	25	5	71.4	2	28.6
OFX	13	81.3	3	18.7	7	100	-	-
AMP	2	12.5	14	87.5	6	85.7	1	14.3
AM	8	50	8	50	5	71.4	2	28.6
AU	10	62.5	6	37.5	4	57.1	3	42.9
CFT	8	50	8	50	5	71.4	2	28.6
Z	10	62.5	6	37.5	5	71.4	2	28.6
S	-		16	100	-	-	7	100
CN	9	56.3	7	43.8	4	57.1	3	42.9
SXT	6	37.5	10	62.5	4	57.1	3	42.9
CH	4	25	12	75	4	57.1	3	42.9
E	8	50	8	50	3	42.9	4	57.1

Key:
CPX-   Ciprofloxacin
PEF-   Pefloxacin
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 nasal cavity of outpatient volunteers

Organism	S. aureus				S. epidermidis				Strept. Species			
	S		R		S		R		S		R	
Antibiotics	N	%	N	%	N	%	N	%	N	%	N	%
Isolates	19	100			5	100			1	100		
CPX	19	100	-	-	5	100	-	-	1	100	-	-
PEF	18	94.7	1	5.3	4	80	1	20	1	100	-	-
SP	16	84.2	3	15.8	4	80	1	20	1	100	-	-
OFX	19	100	-	-	5	100	-	-	1	100	-	-
AMP	6	31.6	13	68.4	2	40	3	60	1	100	-	-
AM	9	47.4	10	52.6	3	60	2	40	1	100	-	-
AU	10	52.6	9	47.4	4	80	1	20	1	100	-	-
CFT	9	47.4	10	32.6	2	40	3	60		-	1	100
Z	13	68.4	6	31.6	3	60	2	40	1	100	-	-
S	9	47.4	10	52.6	3	60	2	40	1	100	-	-
CN	11	57.9	8	42.1	4	80	1	20	1	100	-	-
SXT	7	36.8	12	63.2	3	60	2	40	1	100	-	-
CH	8	42.1	11	57.9	2	40	3	60		-	-	-
E	6	31.6	13	68.4	1	20	4	80	1	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.   Conclusion

The nasal cavities of volunteers in some parts of Kaduna metropolis are largely colonized by *Staphylococcus aureus* respectively. Generally, bacterial isolates from nasal cavities of volunteers in the study were most sensitive to fluroquinolones (72%), this bacterial agent 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 samples of (hospital outpatients and asymptomatic volunteers) considered during the study.

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