Prevalence and Antibiogram of Microbiome of Selected Body Parts from Students of Rivers State University, Nigeria

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Abstract: The human microbiome has been said to play an important role in disease development and overall health of the host which is affected by the different practices of the individuals ranging from the abuse of drugs, use of certain cosmetics, poor hygiene and a lack of preventive measure for infection which tend to alter the normal state and function of the microbiome. This study was carried out to investigate the prevalence of microbial isolates and the antibiotic susceptibility pattern of these isolates from the nose, armpit and ear of students in selected faculties of Rivers State University. A total of 30 samples were collected at random using sterile swab sticks from selected body parts of students, including male and female and subjected to standard microbiological methods. A total of 42 isolates with the following genera *Staphylococcus* spp*, Corynebacterium*spp*, Klebsiella* spp*, Haemophilus* spp and *Moraxella* spp were isolated. *Staphylococcus* spp has the highest prevalence (69%: 47.62%: 25%) in both males and females in the armpit, nose and ear respectively. This was followed by *Corynebacterium* spp with a prevalence of (19.05%; 23.08%: 0%) from the nose and armpit respectively with no occurrence from the ear. *Haemophilus* spp had the least prevalence, and occurred only in the ear samples. The organisms were more prevalent in females (50%) than in males (16.7%). The Results of the susceptibility pattern showed that *Moraxella catarrhalis, Haemophilus* spp and *Klebsiella pneumonia* were 100% resistant to Ampiclox. Generally, all the organisms were highly susceptible to Levofloxacin (100%)>Gentamycin (100%) >Azithromycin (100%) and Ofloxacin (100%). Indiscriminate use of antibiotics should be discouraged and be personal hygiene encouraged.
Keywords: Antibiogram, Body parts, Microbiome, Prevalence, Students

INTRODUCTION

The human body accommodates about 10 trillion to 100 trillion microorganisms, greatly outnumbering the number of human cells (Turnbaugh *et al.,* 2007). The microbes and their genomes located within a particular area are defined as the microbiome. Microbiomes are made up of "helpful" bacteria that live with us and within us. Microbiomes can be found in all different types of hosts, such as plants, animals and humans. Some microorganisms that colonize humans are commensal, meaning they co-exist without harming humans and others have a mutualistic relationship with their human hosts.(Sherwood *et al*., 2013).

The human microbiome has been said to play an important role in disease development and the overall health of the host (Cho and Blaser, 2012; Arrieta *et al.,* 2014). The imbalance of microbial composition can potentially affect inflammatory conditions, including inflammatory bowel disease, obesity, and allergic disease (De-Gruttola *et al*., 2016) The human microbiome is the aggregate of all microbiota that reside on or within human tissues and biofluids along with the corresponding anatomical sites in which they reside (Marchesi and Ravel, 2015), including the skin, mammary glands, placenta, seminal fluid, uterus, ovarian follicles, lungs, upper airways, oral mucosa, conjunctiva, biliary tract, gastrointestinal tract, ear (Such as *Staphylococcus epidermidis*, *Turicellaotitidis, Alloiococousotitis,Pseudomonas aeruginosa, Corynebacterium, Staphylococcus aureus,* and *Streptococcus saprophyticus*) and armpit (Such as *Corynebacterium*, firmicutes and *Staphylococcus*). It consists of about a thousand different bacterial species. The term human microbiome is sometimes used to refer to the collective genomes of resident microorganisms and most of these microorganisms are highly resistant to many antibiotics which is a global posing serious threat to public health (Sherwood *et al.,* 2013).

The bacterial communities in humans live in areas with external exposure andthese microbial communities are involved in the healthy growth of the body, in protecting the body from invaders by preventing attachment and penetration of pathogenic microorganisms, in helping digestions, and also in regulating the microbiome (Sherwood *et al.,* 2013).

Microbiome is dynamic and some changes may occur during our growth with early development, environmental factors such as the foods that are eaten, the environment in which we live, the people and animals that interact with us, or medicines taken, such as antibiotics especially in response to disease(Lozupone *et al.,* 2012) all contribute to the alteration of the microbiomes of the body and hence making the study of microbiome important to justify the dearth of information on the microbiome of the body and the susceptibility pattern of the organisms to antibiotics. Hence, this study is aimed at studying the prevalence and antibiogram of the microbiome of selected body parts from students of the Rivers State University, Nigeria.

MATERIALS AND METHODS

Description of the study area

This study was carried out in five (5) faculties viz; Faculty of Engineering, Humanities, Law, Management and Sciences of Rivers State University, Nkpolu-Oroworukwu. This study area was chosen due to the population density of the faculty where the rate of drug abuse is high among students.

Sample Collection

A total of 30 samples were collected at random from three body parts with ethical approval from Rivers State Ministry of Health (Armpit, Ear and Nose)using sterile swab stick from males and females under hygienic conditions from the selected facultiesin Rivers State University. The samples were transported aseptically to the Department of Microbiology Laboratory in Rivers State University for bacteriological analysis.

Microbiological analysis

The samples were inoculated onto already prepared sterile Mannitol Salt agar, Blood agar andMacConkey agar plates, using streak plate technique. The plates labelled properly and incubated at 37^{o}C for 24hours.

Characterization and Isolation of the Test Organism

After 24hours, distinct colonies were picked from theplates and characterized morphologically and sub-cultured onto prepared nutrient agar plates and incubated at 37^{o}C, for 24hours to obtain pure cultures of the organisms and further characterized microscopically and gram stained(Taylor, 2008).

Preservation of pure culture

The pure cultures were stored in sterile 3 ml of 10% (v/v) glycerol suspension at -4^{o}C as a cryopreservative agent to prevent the damage of the pure cultures during drying for further analysis.

Identification of the Bacterial Isolates

Identification of the organism were further carried out through conducting series of biochemicaltests such as Oxidase, Catalase, Coagulase, Citrate Utilization, Methyl red, Indole, Voges Proskauer and sugar fermentation tests to confirm the test organisms(Cheesbrough,2006).

Antibiotic susceptibility testing

The antimicrobial susceptibility profiles of the bacterial isolates to conventional antibiotics were determined using the Kirby-Bauer disk diffusion method on sterile Mueller-Hinton agar. Standardization of the bacterial isolates was carried out by adjusting to 0.5 McFarland turbidity standards containing x10^{8} cells. The swab is deepened into the bacterial suspension and streaked over the surface of the agar plates, rotating the agar plate 60º each time to ensure even distribution of the inoculum.

The plates were left to air dry for 3–5 min. Conventional antibiotics disk impregnated withCefuroxime (CXM) 30ug, Amoxicillin Clavulanate (AUG) 30ug, Cefotaxime (CTX) 25ug, Imipenem-Cilastatin (IMP) 10/10ug, Ofloxacin (OFX) 5ug, Gentamycin (GN) 10ug, Nalidixic acid (NA) 30ug, Nitrofurantoin (NF) 300ug, Levofloxacin (LBC) 5ug, Ceftriaxone Sulbactarm (CRO) 45ug, Ampiclox (ACX) 10ug, Cefixime (ZEM) 5ug, Azithromycin (AZN) 15ug, Erythromycin (ERY; 15ug), and Ciprofloxacin (CIP) 5ugwere aseptically placed on the surface of the inoculated agar plate with sterile forceps. Each disk was pressed down to ensure full contact with the surface of the agar. The plates were then incubated for 24 hours at 33 to 35ºC in an inverted position. The zones of inhibition were measured in millimetre (mm) using a meter rule and compared to (CLSI, 2017).

Determination of Multiple AntibioticResistance (MAR) indexes

Multiple antibiotic resistance is the resistance of bacterial isolates to three or more antibiotics (Osundiya *et al.,* 2013). Multiple antibiotic resistance (MAR) index was ascertained for each isolate by using the formula MAR = a/b, where ''a'' represent the number of antibiotics to which the test isolates depicted resistance and ''b'' represent the total number of antibiotics to which the test isolate has been evaluated for susceptibility (Krumperman, 1985).

Data analysis

Statistical Package for Social Sciences (SPPSS) version 22 was used to analyse the data obtained from the measurement of the zones of inhibition. Descriptive statistics were used to summarize all data obtained. (Bewick*et al*., 2004).

RESULTS

This study revealed as shown in Fig. 1 that the highest occurrence of *Klebsiella* spp(50%: 33.3%) and *Haemophilus* spp(50%: 16.7%) were observed in females and males respectively. This was followed closely by *Staphylococcus* spp(0%: 33.3%)in females and males respectively, and *Moraxella catarrhalis* had the least prevalence (0%:16.7%) in females and males in the ear samples.

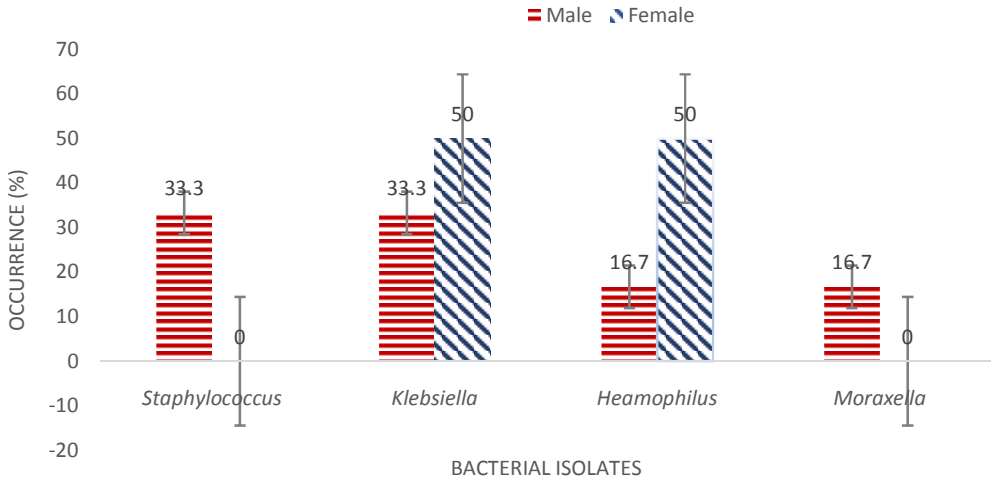

Fig.1: Prevalence of the bacterial organism in Ear samples among males and females.

The result as shown in Fig. 2. revealed that the highest occurrence of *Staphylococcus* spp(60%: 36.6%) was recorded in females and males respectively. This was followed closely by *Moraxella catarrhalis*(0%: 36.6%) and *Corynebacterium diphtheriae*(20%:18.18%) in females and males respectively, and *Klebsiella* spp had the least occurrence (20%: 9.09%) in females and males respectivelyin the nose samples and *Haemophilus* spp were completely absent from the nose samples.

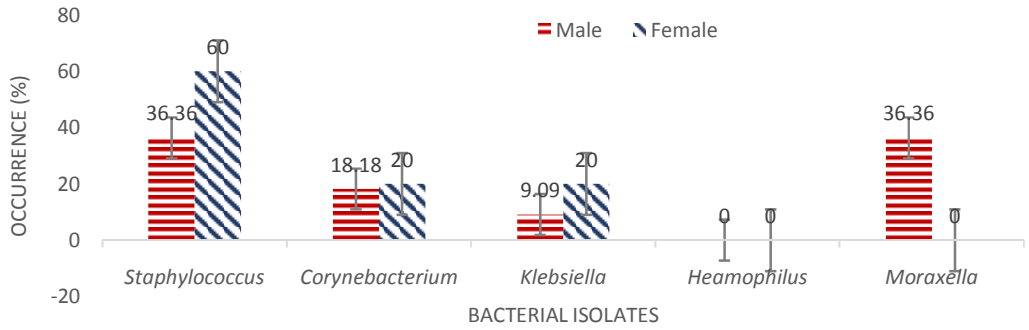

Fig. 2: Prevalence of the bacterial organism in Nose samples among males and females.

The result as shown in Fig. 3. revealed that the highest prevalence was observed in *Staphylococcus* (85.71%: 50%) in males and females respectively. This was followed closely by *Corynebacterium diphtheria* (33.33%:14.29%) and *Moraxella catarrhalis* had the least prevalence (16.69%: 0%) in females and males respectively in the armpit samples. *Klebsiella* and *Haemophilus* were completely absent in the armpit samples.

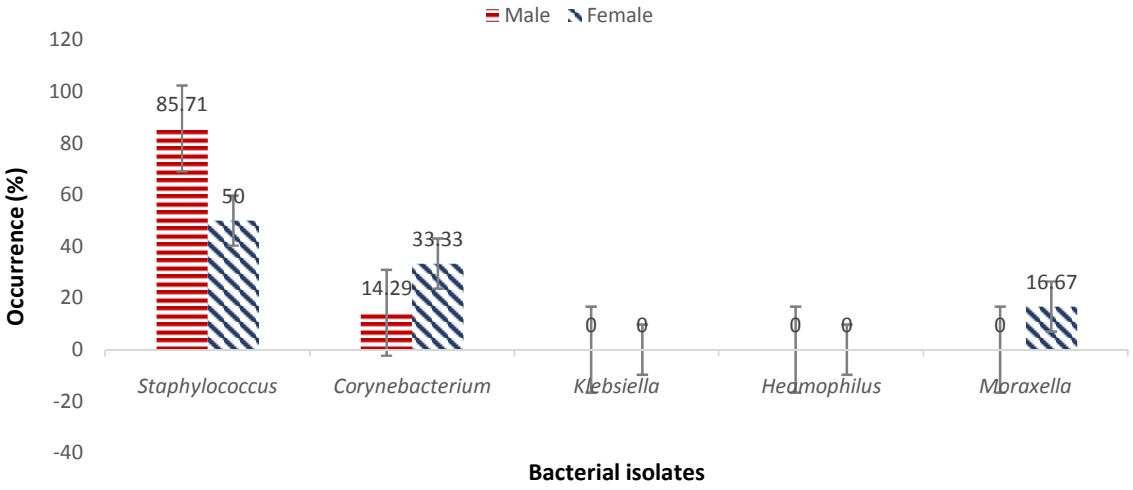

Fig. 3: Prevalence of the bacterial organism in Armpit samples among males and females.

Generally, as seen in Fig. 4: that out of 42 isolates from the ear, nose and armpit, *Staphylococcus* spp (69%: 47.62%: 25%)had the highest prevalence followed closely by *Klebsilella pneumonia* (37.5%: 14.29: 0%),*Corynebacterium diptheriae* (23.08%: 19.05%: 0%), *Moraxella* spp (19.05%: 12.5%: 7.69%) and *Haemophilus*spp (25%: 0%: 0%) had the least occurrence for Armpit, Nose and Ear respectively. *Corynebacterium diptheriae* was completely absent in ear samples, *Klebsilella* spp was absent in armpit samples while *Haemophilus* spp is absent from nose and armpit samples.

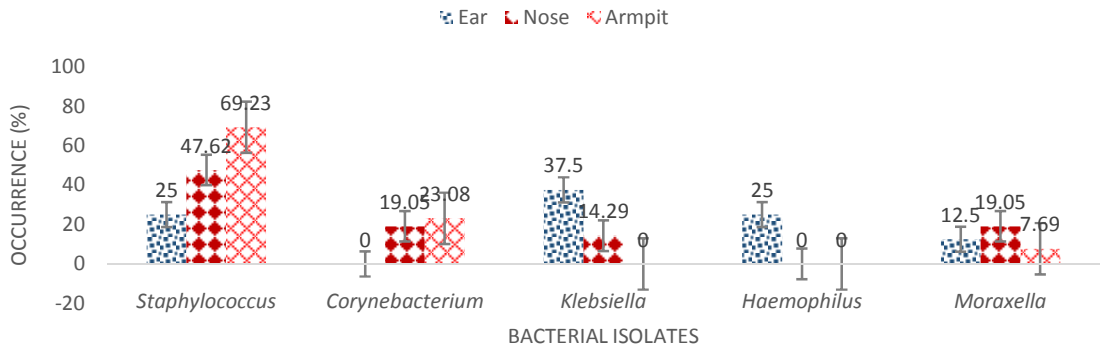

Fig. 4: Prevalence of the bacterial organism in Nose, Ear and Armpit samples among males and females in the Rivers State University.

The sensitivity test result as shown in Table 1. revealed that the *Staphylococcus* isolates were highly resistant to Cefuroxime, Cefixime, Imipenem-Cilastatin and Erythromycin (100%) and susceptible to Levofloxacin and Gentamicin (100%), Azithromycin and Ofloxacin (95.2), Gentamycin (87.5%) and Ciprofloxacin (90.5%).

Further, results of the antibiogram of *Corynebacterium* spp as presented in Table 2. Showed 100% resistance to Cefuroxime, Imipenem-Cilastatin and Amoxicillin Clavulanate and susceptible to Azithromycin (100%), and Gentamicin (85.7%).

Table 1: Susceptibility pattern of S*taphylococcus* sppisolated from ear, nose and armpit of students in the Rivers State University

Antibiotics (Conc.)	Resistant (%)	Intermediate (%)	Susceptibility (%)
Cefuroxime (30ug)	21(100)	0(0.00)	0(0.00)
Amoxicillin Clavulanate (30ug)	16(76.2)	5(23.8)	0(0.00)
Cefotaxime (25ug)	13(61.9)	8(38.1)	0(0.00)
Ceftriaxone Sulbactarm (45ug)	1(4.8)	16(76.2)	4(19.0)
Cefixime (5ug)	21(100)	0(0.00)	0(0.00)
Levofloxacin (5ug)	0(0.00)	0(0.00)	21(100)
ImipenemCilastatin (10/10ug)	21(100)	0(0.00)	0(0.00)
Azithromycin (15ug)	1(4.8)	0(0.00)	20(95.2)
Ofloxacin (5ug)	0(0.00)	1(4.8)	20(95.2)
Erythromycin (15ug)	21(100)	0(0.00)	0(0.00)
Gentamicin (10ug)	0(0.00)	0(0.00)	21(100)
Ciprofloxacin (5ug)	0(0.00)	2(9.5)	19(90.5)

The result of susceptibility of *Klebsiella* spp as shown in Table 3 revealed that *Klebsiella* spp were completely resistant to Cefuroxime, Amoxicillin Clavulanate, Cefotaxime, Ceftriaxone Sulbactarm, Cefixime, Imipenem-Cilastatin, Nalidixic acid, Nitrofurantoin and Ampiclox (100%) and completely susceptible Ofloxacin and Levofloxacin (100%).

Table 2: Susceptibility pattern of *Corynebacterium* spp isolated from ear, nose and armpit from students in the Rivers State University

Antibiotics (Conc.)	Resistant (%)	Intermediate (%)	Susceptibility (%)
Cefuroxime (30ug)	7(100)	0(0.00)	0(0.00)
Amoxicillin Clavulanate (30ug)	7(100)	0(0.00)	0(0.00)
Cefotaxime (25ug)	1(14.3)	6(85.7)	0(0.00)
Ceftriaxone Sulbactarm (45ug)	1(14.3)	6(85.7)	0(0.00)
Cefixime (5ug)	4(57.1)	3(42.9)	0(0.00)
Levofloxacin (5ug)	5(71.4)	2(28.6)	0(0.00)
ImipenemCilastatin (10/10ug)	7(100)	0(0.00)	0(0.00)
Azithromycin (15ug)	0(0.00)	0(0.00)	7(100)
Ofloxacin (5ug)	1(14.3)	2(28.6)	4(57.1)
Erythromycin (15ug)	2(28.6)	5(71.4)	0(0.00)
Gentamicin (10ug)	1(14.3)	0(0.00)	6(85.7)
Ciprofloxacin (5ug)	2(28.6)	0(0.00)	5(71.4)

Table 3: Susceptibility pattern of *Klebsiella* spp isolated from ear, nose and armpit samples

Antibiotics (Conc.)	Resistant (n %)	Intermediate (n %)	Susceptibility (n %)
Cefuroxime (30ug)	6(100)	0(0.00)	0(0.00)
Amoxicillin Clavulanate (30ug)	6(100)	0(0.00)	0(0.00)
Cefotaxime (25ug)	6(100)	0(0.00)	0(0.00)
Ceftriaxone Sulbactarm (45ug)	6(100)	0(0.00)	0(0.00)
Cefixime (5ug)	6(100)	0(0.00)	0(0.00)
Levofloxacin (5ug)	0(0.00)	0(0.00)	6(100)
Imipenem-Cilastatin (10/10ug)	6(100)	0(0.00)	0(0.00)
Ofloxacin (5ug)	0(0.00)	0(0.00)	6(100)
Gentamicin (10ug)	4(66.7)	2(33.3)	0(0.00)
Nalidixic acid (30ug)	6(100)	0(0.00)	0(0.00)
Nitrofurantoin (300ug)	6(100)	0(0.00)	0(0.00)
Ampiclox (10ug)	6(100)	0(0.00)	0(0.00)

The result of susceptibility of *Moraxella* spp as shown in Table 4 revealed that *Moraxella* spp was resistant to Cefixime and Ampiclox (100%) and highly susceptible to Cefuroxime, Imipenem-Cilastatin, Ofloxacin, Gentamycin and Nitrofurantoin (100%).

Table 4: Susceptibility pattern of *Moraxella* spp isolated from ear, nose and armpit samples

Antibiotics	Resistant (%)	Intermediate (%)	Susceptibility (%)
Cefuroxime (30ug)	0(0.00)	0(0.00)	6(100)
Amoxicillin Clavulanate (30ug)	0(0.00)	6(100)	0(0.00)
Cefotaxime (25ug)	0(0.00)	6(100)	0(0.00)
Ceftriaxone Sulbactarm (45ug)	0(0.00)	6(100)	0(0.00)
Cefixime (5ug)	6(100)	0(0.00)	0(0.00)
Levofloxacin (5ug)	0(0.00)	6(100)	0(0.00)
ImipenemCilastatin (10/10ug)	0(0.00)	0(0.00)	6(100)
Ofloxacin (5ug)	0(0.00)	0(0.00)	6(100)
Gentamicin (10ug)	0(0.00)	0(0.00)	6(100)
Nalidixic acid (30ug)	0(0.00)	6(100)	0(0.00)
Nitrofurantoin (300ug)	0(0.00)	0(0.00)	6(100)
Ampiclox (10ug)	6(100)	0(0.00)	0(0.00)

The result of susceptibility of *Haemophilus* spp as shown in Table 4 revealed that *Haemophilus* spp was resistant to Cefixime and Ampiclox (100%) and susceptible to Cefuroxime, Imipenem-Cilaststin, Ofloxacin, Gentamicin and Nitrofurantoin (100%) (Table 5).

Table 5: Susceptibility pattern of *Haemophilus*spp isolated from ear, nose and armpit

Antibiotics	Resistant (%)	Intermediate (%)	Susceptibility (%)
Cefuroxime (30ug)	0(0.00)	0(0.00)	6(100)
Amoxicillin Clavulanate (30ug)	0(0.00)	6(100)	0(0.00)
Cefotaxime (25ug)	0(0.00)	6(100)	0(0.00)
Ceftriaxone Sulbactarm (45ug)	0(0.00)	6(100)	0(0.00)
Cefexime (5ug)	6(100)	0(0.00)	0(0.00)
Levofloxacin (5ug)	0(0.00)	6(100)	0(0.00)
ImipenemCilastatin (10/10ug)	0(0.00)	0(0.00)	6(100)
Ofloxacin (5ug)	0(0.00)	0(0.00)	6(100)
Gentamicin (10ug)	0(0.00)	0(0.00)	6(100)
Nalidixic acid (30ug)	0(0.00)	6(100)	0(0.00)
Nitrofurantoin (300ug)	0(0.00)	0(0.00)	6(100)
Ampiclox (10ug)	6(100)	0(0.00)	0(0.00)

The results of the Multiple Antibiotic Resistance (MAR) index of the bacterial isolated in this study as shown in Table 6 revealed that the percentage of isolates with MAR index ≥ 0.2 was 100%.

Table 1: MAR Indices of the bacterial isolates

MAR Index	*Klebsiella* (N=6)	*Haemophilus* (N=2)	*Moraxella* (N=6)
0.2	0(0.00)	0(0.00)	6(100)
0.3	0(0.00)	1(50)	0(0.00)
0.4	0(0.00)	1(50)	0(0.00)
0.5	0(0.00)	0(0.00)	0(0.00)
0.6	0(0.00)	0(0.00)	0(0.00)
0.7	0(0.00)	0(0.00)	0(0.00)
0.8	6(100)	0(0.00)	0(0.00)

DISCUSSION

Microbiomes are helpful and contribute to the healthiness of the body by acting as a defense to prevent the infiltration of pathogenic microorganism into the different parts of the body (Sherwood *et al.,* 2013). The high prevalence of *Klebsiella*sppand *Haemophilus* spp from the ear indicate that these organisms are normal microbiome of the ear but factors such as consistent use of water and ear swabs could reduce the population of the organisms as well as antibiotic properties of the ear swab (Cho and Blaser 2014). This is in line with the work of Suzuki *et al*. (2015) where they found high prevalence of *Klebsiella* spp, *Haemophilus* spp and *Staphylococcus* speciesand the relative number of *Moraxella* from the ear samples (Fig. 1). Study has reported that the nasal microbiome of healthy humans is primarily composed of *Corynebacterium* spp, *Staphylococcus* spp and *Moraxella* spp (Whelan, 2014) which is revealed in this study why this organism has a higher prevalence. The high occurrence of *Staphylococcus* in females is as a result of compositional perturbations as also reported by Bassis *et al*. (2014) (Fig. 2). *Staphylococcus* spp has the highest prevalence from the different part of the body because of the role it plays in these areas of the body such as the reduction of malodour formation in the armpit, prevent the penetration of harmful organisms into the nasal cavity and inhibition of organism associated with ear wax (Callewaert*et al*., 2013). (Fig. 3). *Staphylococcus* spp*, Corynebacterium diphtheria* and *Moraxella catarrhalis* are normal microbiome of the armpit contributing to malodour formation not only the bacteria on the epidermis layer, but especially those living in the sweat glands, sweat pores and hair follicles which play a pivotal role in themalodour development and *Corynebacterium diphtheriae* is a major contributor (Callewaert *et al*., 2017). To treat malodour, females indulge in an extensive use of deodorant, antiperspirants as well as some environmental factors, which contribute to the prevalence of the organisms in females than in males (Callewaert *et al*., 2017). Li *et al*., 2019 also found these organisms from armpit and more prevalent in males (Fig. 4).

Antibiotics as well as intranasal corticosteroids are used, combining antimicrobial and anti-inflammatory properties for the infections associated with the organisms from the different body part of the body. *Staphylococcus* spp and *Corynebacterium* spp have been resistant to the β-Lactam drugs due to their pathogenic properties, production of the β-lactamases enzyme which binds to β-Lactam drugs as its original substrate as well as other factors like possession of genes that conferred resistance to the antibiotics and increased affordability of the antibiotics (Bernstein, 2013).

This result is in line with work done by Ramakrishnan, *et al*. (2018) which also revealed that these organisms were mostly resistant to first-generation cephalosporin and susceptible to most of the aminoglycosides. These organisms that are susceptible to the antibiotics used in this study is an implication of malodor formation and bridge to the first line of defense mechanism that contribute to the resistance to invading pathogenic organisms. It is of great significance to know that MAR index values greater than 0.2 indicate high risk of antibiotic resistance were these antibiotics are often used (Davis *et al*., 2016; Krumperman, 1985)). It also follows that 100% of the bacteria isolated in this study shows multiple resistances to antibiotics, due to the indiscriminately usageof these antibiotic as a result of infections caused by these organisms.

CONCLUSION

This study reveals a high prevalence of *Staphylococcus* spp*, Corynebacterium, Moraxella and Klebsiella* species which are the major causes of infections associated with the ear, nose and armpit as well as its normal microbiota. These organisms also colonize these areas of the body through poor personal hygiene and contact with contaminated surfaces. The antibiogram from this study reveal that the organisms were highly resistant to the first-generation Cephalosporin and Gentamicin. Ofloxacin, Levofloxacin, Azithromycin and Nitrofurantoin can be the drug of choice for the treatment of infections due to these organisms from the parts of the body studied. Advocating for personal hygienic practices and indiscriminate use of antibiotics should be discouraged.

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