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Research Article

Nasal colonization and antibiotic sensitivity pattern of bacterial isolates among healthy students from Delta State

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Abstract: ***Background**:* This research work was carried out to evaluate the antibiotic sensitivity profile of nasal bacterial isolates from Delta State University students. ***Materials and Methods:*** To determine this, nose swabs were obtained from two hundred (200) students in site 2 and site 3 of Abraka campus of Delta State University and were immediately cultured and identified using bacteriological methods according to the laid down procedure. Antibiotic sensitivity test (AST) was performed to determine the sensitivity pattern of the nasal isolates. ***Results and Discussions**:* Of the 200 nose swabs collected, 94 distinct colonies were obtained and analyzed. Fifty (54) were identified to be Gram-positive organisms, of which 19 (41.5%) were identified to be Staphylococcus aureus , 13 (13.8%) to be *Stahpylococcus epidermis and 2 (2.1%) to be Streptococcus species while 40 isolates were identified to be* Gram-negative organisms, (of which 3(3.2%) were identified to be *Esherichia coli*, 1 (1.06%) to be *Proteus species*, 5(5.32%) to be *Enterobacter spp*, 24 (25.553%) to be *Citrobacter spp*, and 7(7.45%) to be *Klebsiella species*. Amongst the Gram-positive antibiotics susceptibility was highest with the Streptomycin, levofloxacin, ampiclox, Chloramphenicol and rifampicin, but lowest with ciprofloxacin and erythromycin. Amongst the Gram –negative antibiotics, susceptibility was highest with Streptomycin, Ofloxacin, Trimethoprim/ sulmethoxazole and perfloxacin but lowest with augmentin and nalidixic acid. ***Conclusion:*** It should be noted that the presence of different species in the nares is in accordance with the fact that microorganisms are ubiquitous meaning it can be found everywhere in nature.

Keywords: Antibiotics, Nasal, sensitivity and Abraka.

INTRODUCTION

The nose is one of the few openings of the body through which bacteria and other microbes go into the body and the nasal passage is important for filtering the air that we breathe in thereby preventing tiny foreign particles or micro-organisms from entering into the body. The nares are predominantly an aerobic environment for bacteria to live in (Wilson M, 2005). World Health Organization (WHO, 2014) has defined antibiotic resistance as micro-organisms that are resistant to antimicrobial agents that were once capable of treating an infection by same micro-organism. Resistance is a property of microbes, not the person or other organisms infected by the microbes.

Antibiotic resistance can likewise be defined in accordance to the Centre of Disease Control (CDC, 2019) as capability of microorganisms to resist the actions of drugs in that the microorganisms are not killed, and their growth is not inhibited. Although some persons experience greater risk than others, nobody can completely avoid the risk of antibiotic resistant infections. Infections with resistant organisms are difficult to treat requiring costly and sometimes toxic alternatives. Antibiotic resistance is a progressively more problematic concern that leads to death of millions. As resistance to antibiotic becomes prevalent there is larger need for alternative treatments. The Call for new antibiotic therapies has been issued, although there is ongoing decrease in the number of drugs that are approved. A World Health Organization (WHO) report released April, 2014 stated “this serious threat is no longer a prediction for future, it is happening right now in every region of the world and has the potential to affect any one of any age, in any country. Antibiotic resistance is now a major threat to public health. There have been increasing public calls for global collective action to address the threat including a proposal for an international treaty on antibiotic antimicrobial resistance, (Hoffman et al, 2015).

Most research on the microbiota of nose and throat habitats has focused on carriage of one or a few pathogens. There is an increased knowledge of the composition of the complex bacterial communities in

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which these pathogens reside would provide new insights into why some individuals become colonized with pathogens, while others do not. Indeed, in the nostril microbiota of participants, there was prevalence of the *Staphylococcaceae* family (*Firmicutes*), whose members include important pathogens, and the *Corynebacteriaceae* and *Propionibacteriaceae* families (both *Actinobacteria*), whose members are more commonly benign commensals. An improved understanding of competitive bacterial colonization will increase our ability to define predispositions to pathogen carriage at these sites and the subsequent risk of infection (Katherine et al, 2010).

The aim of this study is to determine the micro-organisms that make up the normal flora of the nares (nostrils) and evaluate their in-vitro antibiotic susceptibility pattern among students of Delta State University, Abraka.

MATERIALS AND METHODS:

Bacteriological Media:

Nutrient Agar, Nutrient Broth, Mannitol Salt Agar, Macconkey Agar, Mueller Hinton Agar and Peptone Water.

Antibiotics

Ciprofloxacin, norfloxacin, gentamycin, amoxicillin, streptomycin, rifampicin, erythromycin, chloramphenicol, ampiclox, levofloxacin, ofloxacin, augmentin, cephalexin, nalidixic acid, ampicillin and septrin.

METHODS

Ethical Consideration

Before samples were collected, information regarding the study was explained to the participants after approval was sought.

Collection of nose swabs.

The isolates were obtained from the nares of 200 students within the age bracket (18-30) in sites 2 and 3 of Delta State University, Abraka. Sterile swab sticks were given to each of the subjects and were used to swab both nostrils thoroughly.

Bacteriology

The identification and biochemical tests were carried out on the isolates obtained, some of which include Gram staining, Catalase test, Coagulase test, Motility test, Indole test, and Fermentation test (Cheesbrough M, 2006).

Preparation of isolates

The test organisms were inoculated into freshly prepared nutrient broth and were incubated for 24 hours at 37°C.

Antibiotic sensitivity testing

Antibiotic sensitivity testing was carried out using the Kirby-Bauer disc diffusion method. The pour plate method used involves the following procedures;1ml of the test organism was measured with a sterile syringe and released into the culture plate.19mls of Mueller-Hinton agar was then released into the culture plate and was mixed properly by rocking gently. The agar was left to solidify and with the aid of sterile forceps, the antibiotic discs were placed gently without piercing the agar. The culture plates were then incubated at 37°C for 24 hours. The zones of inhibition of each antibiotic was measured and recorded appropriately (Cheesbrough M, 2006).

ANALYSIS

Data obtained were analyzed using the general linear model procedure.

RESULTS

Of the 200 nose swabs collected and cultured, a total 94 distinct isolates were obtained of which 54(57.45%) were Gram-positive and 40(42.55%) were Gram-negative organisms.

The isolates after being identified using the various biochemical tests are *Staphylococcus aureus* {39 (41.5%)}, *Staphylococcus epidermidis* {13 (13.83%)}, *Staphylococcus spp.* {2 (12%)}, *Escheria coli {3 (3.19%)}, Proteus spp. {1 (1.06%)}, Citrobacter spp.* {24 (25.53%)}, *Enterobacter spp.*, {5 (5.32%)} and *Klebsiella spp.* {7 (7.45%)}.

Table 1. Frequency of All Nasal Isolates Obtained

BACTERIA	FREQUENCY	PERCENTAGE
Staphylococcus aureus	39	41.5
Staphylococcus epidermidis	13	13.83
Streptococcus spp.	2	2.12
Proteus spp.	1	1.06
Escherichia coli	3	3.19
Enterobacter spp.	5	5.32
Citrobacter spp.	24	25.53
Klebsiella pneumonia	7	7.45
Total	94	100

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Furthermore, the sensitivity patterns of all the isolates to the antibiotic used are depicted in Table 2. (Gram-positive isolates), and Table 3 (Gram-negative isolates) showing whether they are Susceptible, moderately susceptible (Intermediate) or Resistant. Tables 2 and 3 shows the proportion of susceptible gram-positive and gram-negative isolates in their percentages respectively and also shows the Mean Susceptibility of the Isolates (MSI) and the Susceptibility Pattern of the Isolates to Antibiotics (SPIA). *Staphylococcus aureus* was the most prevalent bacteria which showed susceptibility to all the antibiotics but showed highest susceptibility to Streptomycin, Levofloxacin and Ampiclox. Other gram-positive isolates showed susceptibility in similar patterns. *Citrobacter spp.* Was next to *Staphylococcus aureus* in their percentage respectively, and also shows the Mean Resistance of the Isolates (MRI) and the Resistance Pattern of the Isolates to Antibiotics (RPIA). The gram-positive isolates except *Streptococcus spp.* (showed no resistance) showed the highest resistance to Amoxicillin, while the gram-positive isolates showed highest resistance to Nalidixic acid, Ceporex, and Ampicilin.

Table2: Proportion of the Sensitivity of Gram-Positive Isolates in Percentage

ORGANISMS	S %	NB %	CH %	CPX %	E %	LEV %	CN %	APX %	RD %	AMX %	MSI %
Staphylococcus epidermidis (38)	14 (36.8)	15 (39.5)	28 (73.7)	17 (44.7)	15 (39.5)	20 (52.6)	35 (92.1)	-	-	27 (71)	171 (45)
Staphylococcus aureus (14)	5 (35.7)	4 (28.6)	10 (71.4)	8 (57.1)	6 (42.8)	7 (50)	13 (92.8)	-	-	5(35.7)	58 (41.4)
Streptococcus spp. (2)	1 (50)	1 (50)	2 (100)	0 (0)	0 (0)	0 (0)	2 (100)	-	-	2 (100)	8 (40)
SPIA	20	20	40	25	21	27	50	-	-	34	

NOTE: S – Susceptible ; I – Intermediate; R – Resistant; S – Streptomycin; NB – Norfloxacin; CH – Chloramphenicol; CPX – Ciprofloxacin; E – Erythromycin; LEV – Levofloxacin; CN – Gentamicin; APX – Ampiclox; RD – Rifampicin; AMX - Amoxicillin

Table 3: Proportion of the Sensitivity of Gram-Negative Isolates in Percentage.

ORGANISMS	S %	SXT %	CPX %	AU %	CN %	PEF %	NA %	OFX %	CEP %	PN %	MSI
Escherichia coli (3)	0 (0)	0 (0)	1 (33.3)	1 (33.3)	1 (33.3)	1 (33.3)	0 (0)	1 (33.3)	1 (33.3)	1 (33.3)	7 (23.3)
Citrobacter spp. (24)	5 (20.8)	5 (20.8)	10 (41.7)	5 (20.8)	12 (50)	3 (12.5)	0 (0)	6 (25)	7 (29.2)	6 (25)	59 (24.6)
Enterobacter aerogenes (5)	1 (20)	0 (0)	1 (20)	1 (20)	3 (60)	1 (20)	0 (0)	3 (60)	2 (40)	2 (40)	14 (28.0)
Klebsiella spp. (7)	0 (0)	0 (0)	0 (0)	1 (14.3)	5 (71.4)	2 (28.6)	0 (0)	2 (28.6)	1 (14.3)	1 (14.3)	12 (17.1)
Proteus (1)	0 (0)	0 (0)	0 (0)	1 (100)	1 (100)	0 (0)	0 (0)	1 (100)	1 (100)	1 (100)	5 (50)
SPIA	6	5	12	9	22	7	0	13	12	11 ()	

NOTE: MSI – Mean Susceptibility of Isolates; SPIA – Susceptibility pattern of Isolates to Antibiotics; S – Streptomycin; SXT – Septrin; CPX – Ciprofloxacin; AU – Augumentin; CN – Gentamycin; PEF – Perfloxacin; NA – Nalidixic acid; OFX – Ofloxacin; CEP – Ceporex; PN – Ampicillin

DISCUSSION

The presence of micro-organisms of different species in the nares is in accordance with the fact that micro-organisms are ubiquitous (i.e. they are found everywhere) in nature. Subsequent biochemical examination of morphologically distinct colonies confirmed as shown in Table 1 showing the frequency and percentage of all the isolates obtained including the

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gram-positive and gram-negative isolates. The highest proportion of isolates are in this order, *Staphylococcus aureus* {39 (41.5%)}, *Citrobacter spp.* {24 (25.53%)}, and *Staphylococcus epidermidis* {13 (13.83%)} *Klebsiella spp.* {7)7.45%)}, *Enterobacter spp.*, {5 (5.32%)}, *Escherichia coli* {3 (3.19%)}, *Streptococcus spp. {2 (2.12%)}, and Proteus spp.* {1 (1.06%)}, which is in accordance with the finding of (Anie et al, 2017).

This study evaluates the prevalence and sensitivity pattern of gram-positive and gram negative nasal isolates obtained from students of Delta State University, Abraka and has shown that the nares are heavily colonized by pathogenic micro-organisms but however, the normal flora of the nares (nostrils) can be influenced by factors such as Age, Sex, Stress, Nutrition, and Environment. Health condition (immune activity), and Antibiotics and Diet of the individual (Rasmussen et al, 2000).

It has been estimated that 20% of the human population are long-term carriers of *Staphycococcus aureus*, which can be found as part of the normal skin flora and in interior nares of the nasal passage (Cole et al, 2001). The elevated level of colonization of the nose by *S. aureus* as seen in this study has equally been reported by other researchers (Appelbaum P C, 2007).

This study has therefore identified the most prevalent (dominant) micro-organism to be *Staphylococcus aureus* (41.5%) a pathogenic gram-positive organism, unlike the findings of survey (El-Mahmood et al,2010; Anie et al, 2017). The ability of the nasal passage to harbor *Staphycococcus aureus* results from a combination of a weakened or defective host immunity and the bacteria’s ability to evade host innate immunity (Quinn et al, 2007). The presence of *Staphycococcus aureus* (a pathogenic organism) in the nares is not desirable as it’s capable of causing infections and these infections usually occur when the organisms successfully break through the body’s defense system. When the infections eventually occurs, the antibiotics with highest susceptibility to the organisms should be used to combat the infections and should be prescribed in the appropriate manner for maximum effective antibiotic therapy. Microspically, *Staphycococcus aureus* and other gram-positives appeared as cluster of cocci, it also fermented mannitol causing a yellow change, showed beta hemolysis when grown on blood agar plates, was both catalase and coagulase positive and this correlates with the findings (Ryan et al 2004). *S. epidermidis* strains from healthy adult nares show that there are many kinds of the organism in each individual (O’Gara et al, 2001).

Citrobacter species are the next most prevalent (dominant) organism in the nares unlike the findings of Anie et al, 2017 and they are differentiated by their ability to convert tryptophan to indole, ferment lactose, use malonate ,ferment mannitol with the production of gaseous H_2S, (Chen et al, 2002) and are motile using their peritrichous flagella (Knirel et al 2002).Microscopically, *Citrobacter species* and other gram-negatives appeared as chains of rods, some of them fermented all sugars while other could not ferment all the sugars sued, but all showed positive to catalase test.

Antibiotic sensitivity testing (AST) is very important to the physicians because it guides them in choosing the right drug in the appropriate dose for the treatment of difficult-to-treat infections in patients (Levinson W, 2010). The results gotten from the test are used to deduce an effective antimicrobial therapy for patients with bacterial infections.

From the results Tables 2 and 3 showing the proportion of the sensitivity of isolates to the antibiotics, most strains of all the identified micro-organisms were susceptible, others were moderately susceptible (or intermediate) while others were resistant to the antibiotic. For the gram-positives, all isolates showed susceptibility to all antibiotics but showed highest susceptibility to streptomycin (94.4%), levofloxacin (77.8%), ampiclox (77.8%) chloamphenicol (74.1%), and rifampicin (70.3%), however, most of the isolates showed highest resistance to amoxicillin (45%), [7] susceptibility was medium with gentamicin (63%) and norfloxacin (63%) and susceptibility was lowest with ciprofloxacin (42.6%) and erythromycin (38.9%) and this contrary to the findings of (Ifeanyiet al., 2013).

For the gram-negatives, all isolates also showed a reasonable susceptibility to all the antibiotics used but susceptibility was highest with streptomycin (75%) and ofloxacin (70%), while resistance was highest with nalidixic acid (62.5%), ceporex (52.5% and ampicilin (47.5%), susceptibility was medium with perfloxacin (45%), trimethoprim/sulphamethxazole (45%), ciprofloxacin (27.5%), and gentamicin (27.5%), and susceptibility was lowest with augumentin (20%) and nalidixic acid (10%). From the results stated above (Table 2), streptomycin, ofloxacin, levofloxacin, ampiclox and chloramphenicol showed highest susceptibility to all isolates and should be included as first line drugs in antibiotic therapies involving nasal infections. Nalidixic acid on the other hand showed resistance to all the isolates which shows that it’s a very bad choice for inclusion in antibiotic therapy. However, the moderately susceptibility to antibiotics such as ciprofloxacin, gentamicin, norfloxacin, perfloxacin and trimethoprim/sulphamethoxazole can also be included in antibiotics therapies involving nasal infections but should be used in higher doses or in combination with other moderately susceptibility antibiotics to produce the best therapeutic outcomes.

It has been suggested that, at least in terms of direct relevance to the care of patients with infection, AST may be the single most important activity performed in the clinical microbiology laboratory. AST results are often used to dictate specific management for individual patients, AST data is used to drive empiric antimicrobial therapy, and finally, formulary decisions in some cases are made based on AST results from the laboratory (Gary V D, 2011).

CONCLUSION

From all the results gathered from this study, it can be concluded that *Staphylococcus aureus* (94.4%) and *Citrobacter spp.* (25.53%) are the most dominating bacteria that make up the normal floral of the nares. The antibiotics that showed highest susceptibility should be used instead to bring about effective therapeutic outcomes.

Antibiotic sensitivity is therefore very important when the need for choosing the best antibiotic therapy arises as it assists the physicians in selecting the most effective antibiotics for infections thereby producing the best therapeutic outcomes.

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