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PREVALENCE OF MULTI DRUG RESISTANT *STAPHYLOCOCCUS AUREUS* ISOLATED FROM NASAL ORIFICE OF STUDENTS

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ABSTRACT
Staphylococcus aureus cause illness by preformed toxin production as well as by infecting both local tissues and systemic circulation. Microbiological status of nasal isolates and their multiple drug resistance (MDR) pattern were determined. Two hundred nasal swabs were collected from volunteer students of the Federal University of Technology (FUTO), Owerri, Imo State, Nigeria. Swabs were subjected to routine microbiological analysis using standard methods. Identities of the bacterial isolates were confirmed with reference to standard laboratory manual. Pure cultures of standardized bacterial isolates were screen for antibiotic susceptibility using standard oxoid disc. Multi drug resistance was determine for *Staphylococcus aureus* and *Staphylococcus saprophyticus.* Percentage occurrence of bacteria isolated from male and female students are *Micrococcus luteus* (5.62% and 2.66%), *Staphylococcus saprophyticus* (24.59% and 21.73%), *Bacillus cereus* (5.37% and 7.76%), *Staphylococcus aureus* (46.84% and 41.91%), *Enterococcus faecalis* (16.86% and 23.95%) and *Corynebacterium* species (1.41% and 2.00%) respectively. *Enterococcus faecalis* is highly susceptible while *Bacillus cereus* and *Corynebacterium* species are moderate in resistance to the antibiotics. The percentage of MDR *Staphylococcus aureus* is significantly high, 94.5% in males and 89.0% in females compared to *Staphylococcus saprophyticus* which recorded 5.5% and 11.0% in males and females respectively. The bacterial isolates are common in the environment and the frequent habit of picking of nose could further facilitates their spread, especially *Staphylococcus aureus* with reported cases of resistance and pathogenicity.

KEYWORDS: *Staphylococcus aureus*, nasal swabs, multi-drug resistance.

INTRODUCTION
Staphylococcus aureus is part of the skin flora found in the nose and skin. About 20 percent of the human population are long term carriers of *S. aureus* (Kluymans *et al*., 1997; Paul *et al*., 1882; Tuo *et al*., 1995). It is the most common species of staphylococci to cause *Staphylococcus* infections such pimples, impetigo, boil (furuncles), cellulites, folliculitis, carbuncles, Staphylococcal Scalded Skin Syndrome (SSSS) and abscesses, to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis (Willey *et al*., 2002). It is still one of the five most causes of nosocomial infections (Tuo *et al*., 1995).

Attempts to control these diseases by chemotherapy through the use of antimicrobial agents particularly antibiotics have resulted in increased incidence of resistance to these agents (Levy, 2004). *S. aureus* is resistant to B-lactam antibiotics, aminoglycoside and macrolides (Atkinson and Lorian, 1984; Maple *et al*., 1989) and carries a wide range of multi antibiotics resistant genes plasmids, which can be exchanged and spread among different species of Staphylococci (Nelhart *et al*., 1988).

The multi-resistant determinants can be transferred to new bacterial hosts making it more difficult in developing countries such as Nigeria where antimicrobial drugs are readily available to consumers across the counter with or without prescription leading to misuse with the associated high incidence of antibiotic resistance among the Staphylococci (Weese, 2010; Cuny *et al*., 2015; Fluit, 2012; Johnson, 2011). Community strains of *Staphylococcus aureus* are usually resistant to a variety of different antibiotics including vancomycin (Aubry-Daman *et al*., 1998; Shakibaie *et al*., 2002).

The bacteria are transported on the hands of health care workers, who may pick them up from a seemingly healthy patiently carrying a benign or commensal strain of *S. aureus* and, then pass it on to the next patient being treated. *Staphylococcus aureus* is wide spread in the nasal orifice and the fingers are vehicles in its dissemination as it is constantly in contact with the interior part of the nose. The risk associated with this

involuntary attitude is high as unclean hands have been frequently implicated in the spread of disease causing organisms through foods. *S. aureus* colonizes the nose and abrasion, lesion or wound in these parts may migrate into the body or blood and cause infections. The presence of multi drug resistant *S. aureus* in the nose (nasal orifice) poses a threat to human existence.

This research reported on the prevalence of multi drug resistance *Staphylococcus aureus* isolated from nasal orifice of students in Federal University of Technology, Owerri, Imo State, Nigeria.

MATERIALS AND METHODS

Collection of samples

A total of two hundred (200) nose swabs were collected from the students with moistened sterile swab sticks and swirl in 9 ml of freshly prepared peptone water to resuscitate the organisms (Sharma, 2000). This was kept on the bench for 4 h until turbidity is produced.

Inoculation and identification of Bacteria

One milliliter (1 ml) nose swab suspension was dispersed in 9 ml of sterile physiological saline. Ten-fold dilution method was used by transferring 1ml from each tube until the required dilution was obtained. One – tenth milliliter (0.1 ml) of broth was transferred into freshly prepared surface dried Nutrient Agar and Mannitol Salt Sgar. Inocula were spread evenly and incubated at 37^0C for 48 h (Sharma, 2000). Pure cultures of bacteria isolated were characterized and identified using standard methods (Sharma, 2000; Beishir, 1987; Sneath *et al.*, 1986; Buchanan and Gibbon, 2000; Cheesbrough, 2000).

Antibacterial susceptibility of Bacterial isolates

Test isolates were sub-cultured twice on Nutrient Agar and incubated at 37^0C for 24 hrs. Suspension equivalent to 0.5 McFarland standards was prepared and streaked on Mueller-Hinton Agar surface using a sterile swab stick and evenly distributed over the surface of the plate by a rotational streak at angles of 60 degrees. Commercial antibiotic (Oxoid) discs were then placed on the surface of the inoculated plates at a distance of 40 mm to each other thereby obtaining a total of 5 discs per plate. The plates were incubated for 48 hrs at 37°C. Zone of inhibition was measured and recorded in millimeter with a transparent meter rule (Sharma, 2000).

RESULT

Table 1 shows the colonial characteristics of bacteria isolated from nasal cavities. The isolates were characterized based on some cultural and microscopic morphologies such as gram reaction, spore formation and ability to demonstrate motility in the presence of flagella. Pigmentation, elevation, shapes were also used in the characterization.

The biochemical and carbohydrate test carried out on the isolates is shown in Table 2. Production of enzymes and the ability to utilize substrate is the main characteristic of the biochemical test.

The isolates identified are, *Staphylococcus aureus, Bacillus cereus Corynebacterium, Micrococcus luteus, Enterococcus faecalis* and *Staphylococcus saprophyticus.*

Table 1: Colonial characteristics of Bacteria isolated from the nasal cavity.

Colonial characteristics	Grams reaction	Motility	Spore formation	Probable identity of isolates
Smooth moist and shiny golden yellow on nutrient agar and yellow colonies on mannitol salt agar	Gram positive cocci predominantly in clusters, few in pairs and tetrads	-	-	*Staphylococcus* sp
Smooth moist and shiny low convex colonies on nutrient agar and light pink colonies on mannitol salt agar	Gram positive cocci in clusters	-	-	*Staphylococcus* sp
Large dull and dry serrated flat colonies on nutrient agar	Gram positive rods in short chains	+	+	*Bacillus* sp
Small circular smooth moist and shiny low convex yellow colonies	Gram positive predominantly in tetrads and few in pairs and clusters	-	-	*Micrococcus* sp
Small smooth moist and shiny cream colonies	Gram positive cocci in chains	-	-	*Enterococcus* sp
Cream colonies umbonate shaped	Gram positive pleomorphic	-	-	*Corynebacterium* sp

Table 2: Biochemical and Carbohydrate Fermentation of Bacterial isolates.

CAT	OXI	COAG	IN	MR	VP	CIT	URS	NO_3	GLU	SUC	LAC	MAL	XYL	Identity of isolates
+	-	-	-	+	-	+		+	-	-	-	-	-	*Micrococcus luteus*
+	+	-	-	+	-	+	-	-	+	+	-	-	-	*Staphylococcus saprophyticus*
+	-	-	-	-	+	-	+	+	+	+	+	+	-	*Staphylococcus aureus*
+	-	-	-	-	+	+	-	+	+	+	-	-		*Corynebacterium* sp
-	-	-	-	+	-	+	-	-	+	+	+	-	-	*Enterococcus faecalis*
+	-	-	-	-	+	+	-	+	+	-	-	+	-	*Bacillus cereus*

CAT, catalase; OXI, oxidase; COAG, coagulase; IN, indole; MR, methyl red; VP, voges Proskaeur, CIT, citrate, URS, urease production; No_3; nitrate reduction; GLU, glucose; SUC, sucrose; LAC, lactose; MAL, maltose; XYL, xylose.

The result of antibiotic sensitivity test carried out on the isolates is shown on Table 3. *Enterococcus faecalis* is susceptible to all the test antibiotics except chloramphenicol and streptomycin. *Staphylococcus aureus* is resistant to all the antibiotics except gentamycin, amoxycilin and oflaxacin. *Bacillus cereus* and *Corynebacterium* sp are susceptible to four out the nine antibiotics tested on them.

The prevalence ratio of the bacterial isolates from the nasal cavity of both male and female students is shown in Table 4. *Staphylococcus aureus* is predominant in both male (46.84%) and female (41.91%) students while *Corynebacterium* sp is the least with male (1.41%) and female (2%) students.

The percentage of multidrug resistant of *Staphylococcus* species is shown in Table 5. *S. aureus* shows 94.5% resistance for the male and 89% for the female. Resistant *S. saprophyticus* represents 5.5% and 11% for the male and female students respectively.

Table 3: Antibiotic susceptibility test of bacterial isolates (ZOI mm).

Bacterial isolates	Gen	Pef	Cot	CPX	Ery	AMX	Ofl	Str	Chl
Staphylococcus aureus	10	0	0	0	0	8	14	0	0
Staphylococcus saprophyticus	12	0	8	8	0	14	0	10	8
Bacillus cereus	0	10	14	14	0	0	12	0	0
Corynebacterium sp	0	18	16	14	0	0	14	0	0
Enterococcus faecalis	14	14	12	16	0	12	18	0	0

Gen, Gentamycin; Pef, Pefloxacin; Cot, Cotrimoxazole; Cpx, Ciprofloxacin; Ery, Erythromycin; Amx, Amoxycilin; Ofl, Ofloxacin; Str, Streptomycin; Chl, Chloramphenicol.

Table 4: Percentage occurence of bacterial isolates in the nasal cavity of Male and Female students (x= 200).

Bacterial isolates	Male	Female
Micrococcus luteus	21 (5.62%)	12 (2.66%)
Staphylococcus saprophyticus	105 (24.59%)	98 (21.73%)
Bacillus cereus	23 (5.37%)	35 (7.76%)
Corynebacterium sp	6 (1.41%)	9 (2.00%)
Staphylococcus aureus	200 (46.84%)	189 (41.91%)
Enterococcus faecalis	72 (16.86%)	108 (23.95%)
Total number of isolates	427	451

Table 5: Percentage of multiple drug resistant *Staphylococcus* species in the nasal cavity of Male and Female Students (x=200).

Bacterial isolates	Male (%)	Female (%)
Staphylococcus aureus	189 (94.5)	178 (89)
Staphylococcus saprophyticus	11(5.5)	22 (11)

Figures in parenthesis represent percentage of MDR *Staphylococcus* species.

DISCUSSION

One of the most persistent problems faced by health care services worldwide is the increasing prevalence of multidrug resistance. This resistance is widely recognized as a major public health threat and the problem is compounded by a constant diminishing of the number of new antibiotics entering the clinical practice. *Staphylococcus aureus* is a noteworthy human pathogen, conceivably ready to contaminate any tissue of the human body, causing life threatening and debilitating infection (Tuo *et al*., 1995). *S. aureus* also colonize and taint the human body including the nose, the skin and even the human system.

This study indicates that *Staphylococcus aureus* isolated from the nasal cavity of FUTO students was relatively

high. However, this is not surprising since the organism is part of the normal flora of the human body (Prescott *et al*., 2002; Willey *et al*., 2002). *Staphylococcus aureus* is predominant compared to other bacterial isolates, recording 46.84% in male and 41.91% in female. The percentage occurrence of other isolates are *Staphylococcus saprophyticus* (24.59%), *Micrococcus luteus* (5.62%), *Enterococcus faecalis* (16.86%), *Bacillus cereus* (5.37%) and *Corynebacterium* sp (1.41%) for male and *Staphylococcus saprophyticus* (21.73%), *Micrococcus luteus* (2.66%), *Enterococcus faecalis* (23.95%), *Bacillus cereus* (7.76%) and *Corynebacterium* sp (2.00%) for female.

Bacillus cereus, Staphylococcus aureus, Staphylococcus saprophyticus, Micrococcus luteus, Enterococcus faecalis and *Corynebacterium* species isolated from the nasal cavity are organisms frequently isolated in the environment (from inanimate objects such as door handles, shuttle handles, knobs of toilets doors, cosmetics, chairs. handsets, tables, keys, soil water and air), clinical samples (skin, wound, faeces, urine) and as nosocomial (Tuo *et al*., 1995; Neely and Maley, 2000; Prescott *et al*., 2002; Otter and French, 2009). Tuo *et al*. (2009) reported that *Staphylococcus aureus* is among the five leading causes of nosocomial infection.

The pathogenicity of some of the isolates have been extensively discussed (Willey *et al*., 2002). *Staphylococcus aureus* have been implicated in infections such pimples, impetigo, boil (furuncles), cellulites, folliculitis, carbuncles, Staphylococcal Scalded Skin Syndrome (SSSS) and abscesses, to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome (TSS), bacteremia and sepsis (Chamber, 2001; Quinn and Cole, 2007; Sing, 2008; Prescott *et al*., 2002;Willey *et al*., 2002; Salyers and Whitt, 2002; Cenci- Goga, 2003; Ryan and Ray, 2004; Fluit, 2012; Smith, 2015; Cuny *et al*., 2015).

The percentage of MDR *Staphylococcus aureus* is significantly high, 94.5% in males and 89.0% in females compared to *Staphylococcus saprophyticus* which recorded 5.5% and 11.0% in males and females respectively. This result is worrisome considering reported cases of *Staphylococcus aureus* in human and animal pathogenesis (CDPC, 2002; Cenci-Goga, 2003.

The excessive and indiscriminate use of antibiotics and transfer of resistance genes could account for one of the major causes of resistance to multiple antibiotics by *S. aureus* (Neely and Maley, 2000; Aubry-Daman *et al*., 1998; Chambers, 2001; Shakibaie *et al*., 2002; Becker *et al*., 2003; Chigbu and Ezeronye, 2003; Morgan, 2008; Cosgrove *et al*., 2009; English and Gaur, 2010; Johnson, 2011). Multidrug resistance of *Stapylococcus aureus* including Methicillin-resistant *Staphylococcus aureus* (MRSA) is on the increase (Olowe *et al*., 2007; Cimolai, 2008; Sing, 2008; Weese, 2010; Waters *et al*., 2011; Cuny *et al*., 2015; Smith, 2015). While vancomycin is currently the drug of choice for infections caused by multidrug resistant *S. aureus* especially MRSA, there is a growing increase of resistance to the drug (Tiwari and Sen, 2006; Gardeta and Tomasz, 2014; McGuinness *et al*., 2017). It is however important that alternatives could be sought and other non-glycosidic drugs such as the fluoroquinolones, may be considered to reduce the over dependence on vancomycin to curb MDR and reduce the use of neurotoxic antibiotics (Gade and Qazi, 2013).

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