Virulence factors and Antibiotic Resistant *Staphylococcus* spp from the Anterior Nares of Apparently Healthy Undergraduate Students in Uyo

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ABSTRACT

The occurrences of virulence factors, betalactamase and antibiotic resistant *Staphylococcus* spp from the anterior nares of apparently healthy undergraduate students were determined using various bacteriological agar, chromogenic agent (nitrocephin) and disc diffusion technique. Of the 120 *Staphylococcus* spp isolated, 84 (70.0 %) were *Staphylococcus aureus*, while 36 (30.0 %) were coagulase negative (CoN)-*Staphylococcus* spp. Highest number of *S. aureus* and CoN-*Staphylococcus* spp were obtained in aged groups ≤ 20 yrs and 21-25 yrs, respectively. The results showed that 50 (59.5 %) *S. aureus* produced *α*-haemolysin, 26 (30.9 %) showed *β*-haemolysis, while 16 (44.4 %), 14 (38.9 %) and 6 (16.7 %) of CoN- *Staphylococcus* spp showed *α, β* and γ haemolysis, respectively. Of the 84 *S. aureus* isolated, 32 produced β-lactamase, 70 produced deoxyribonuclease and 10 produced thermonuclease. Lipolytic activities with clearing zones ranging from 4.0 mm to 8.5 mm were observed in 16 (19.0 %) of *S. aureus* and 6 (16.7 %) of CoN-*Staphylococcus* spp. The results showed that between 27.8 % and 33.3 % *S. aureus* and CoN-*Staphylococcus* spp were resistant to gentamycin, ampicillin and nobactracin. Moderately high resistant to penicillin, streptomycin and chloramphenicol were also observed among the isolates. Of the 12 (35.7 %) methicillin resistant coagulase negative *Staphylococcus* spp (MRCoNS) isolated, 4 were obtained from males, and 8 from females with no statistical

difference at $P \geq 0.05$ in the occurrence of MRCoNS. Eight (8) resistance patterns were observed in *S. aureus*, while five (5) in CoN-*Staphylococcus* spp. The Multiple Antibiotic Resistant Index of *S. aureus* and CoN-*Staphylococcus* spp ranged from 0.1 to 0.7 and 0.2 to 0.6, respectively. Routine screenings of nasal colonization of anterior nares among the students should be carried out and strategies to control spreads of multi-drug resistant strains should be adopted.

Key Words: *Staphylococcus*, Virulence, Resistant, Anterior nares, Betalactamase

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INTRODUCTION

The *Staphylococcus* belongs to the *Staphylococcaceae* family and order *Bacillales*. Among the microorganisms belonging to this family, *Staphylococcus aureus*, a facultative anaerobic, Gram-positive bacterium, is the most common species and has been highly studied because of its potential pathogenicity against human and animals (Kluytmans, 1997; Cole *et al*., 2001). It is frequently part of the flora found particularly in the anterior nares of the nasal passages and on skin with about 20% of human populations being long-term carriers of *S. aureus* (Kluytmans, 1997; Cole *et al*., 2001). *S. aureus* is recognized as an important bacterial pathogen contributing towards hospital infection globally. *S. aureus* causes localized infection spreading into the blood stream and more that 500,000 patients in American hospitals contract a staphylococcal infection (Espersen, 1995; Lowy, 1998; Bowersox, 1999). *S. aureus* can cause

skin infections such as pimples, impetigo, boils (furuncles), scalded skin syndrome, abscesses , pneumonia, endocarditis and bacteremia (Prescott *et al*., 2008; Akinjogunla and Enabulele, 2010). Coagulase negative *Staphylococcus* spp, a commensal of the skin can cause severe infections in immune-suppressed patients and those with central venous catheters. Most developed countries have reported an increase in colonization and infection in hospitalized patients caused by CoN- *Staphylococcus* spp, while there are scanty data on infection caused by this organism in developing countries (Akinjogunla and Enabulele, 2010).

The ability of the *Staphylococcus* spp to cause disease is closely related to the host susceptibility, such as immune system response, predisposing conditions or accidental factors (trauma, injury) (van-Belkum *et al*., 2009). *Staphylococcus* spp, like some other micro-organisms produce some virulence factors such as haemolysins, hyaluronidase, deoxyribonuclease, thermonuclease, lipase, staphylokinase and betalactamase (for drug resistance) (Talaro and Talaro, 2002). These virulence factors may be encoded on chromosomal DNA, bacteriophage DNA, plasmids, or transposons (Johnson *et al*., 1997; Akinjogunla and Enabulele, 2010).

Treatment of infections caused by *S. aureus* has become more problematic since the development of antimicrobial resistant *S. aureus* (Boyce, 1994). The emergence of methicillin resistant *S. aureus* (MRSA) is a worldwide problem in clinical medicine (Rich 2005). MRSA strains, first identified in 1960s in England, were first observed in the United States in the mid 1980s. Healthy individuals may carry MRSA asymptomatically for periods ranging from a few weeks to many years and patients with compromised immune systems are at a significantly greater risk of symptomatic secondary infection. Irum *et al*. (2013) reported the occurrence of coagulase-negative staphylococci (MRCoNS) in a tertiary care hospital. Methicillin resistance in *Staphylococcus* spp is due to the acquisition of an altered penicillin-binding protein, encoded by

the *mec* A gene (Ito *et al*., 2001). The *mec A* gene is found in both MRSA and MRCoNS isolates (Eady and Cove, 2003). Infections caused by multidrug and methicillin resistant *S. aureus* result in increased morbidity, mortality; health care costs as well as lengths of hospital stay (Harbarth *et al*., 1998, Harbarth *et al*., 1999; Rubin *et al*., 1999). The use of surveillance cultures such as anterior nares and perineum greatly improves the detection of MRSA colonization compared to clinical cultures alone (Coello *et al*., 1994). The anterior nares is the most frequent site of MRSA colonization, with a single culture from this site having a sensitivity of approximately 85 % (Sewell *et al*., 1993; Coello *et al*., 1994). Although, nasal carriage of *Staphylococci* spp has been reported and suggested as a source of infections in some parts of our country, there are still scanty reports on colonization rates of *Staphylococci* spp among the students in tertiary institutions from our region. Thus, there is need to determine the colonization rates, virulence factors and evaluate the antibiotic susceptibility of *Staphylococci* spp in the anterior nares of students in a tertiary institution.

MATERIALS AND METHODS

Collection of Samples and Bacteriology

One hundred and twenty (120) nasal samples were obtained from apparently healthy undergraduate students of the University of Uyo under aseptic conditions using sterile swabs moistened with sterile nutrient broth. These moistened swabs were inserted into the anterior nares and rotated gently before inoculated onto nutrient broth for 4-6 hrs and later inoculated onto plates of Mannitol Salt Agar (MSA). The plates were incubated aerobically for 24 hrs at

37 °C. After overnight incubation, the plates were examined for fermentation of mannitol indicated by colour change from red to yellow around each colony. The organisms on the positive plates were subcultured onto nutrient agar plates, incubated aerobically for 24 hrs at 37 °C, streaked onto nutrient agar slants and further speciated by conventional laboratory techniques including Gram staining and biochemical tests (catalase, coagulase, citrate utilization, indole, methyl red, gelatin hydrolysis, Vogues-Proskauer, sucrose, maltose, lactose and glucose).

Detection of Deoxyribonuclease (DNase) Producing *Staphylococcus* spp

The DNase agar plates were spot inoculated with *Staphylococcus* spp and incubated for 48 hrs at 37 ^{o}C. After incubation, the growth on the surface of the agar was flooded with 1N hydrochloric acid. Clear zones around the colonies showed the production of DNase enzymes.

Detection of Thermonuclease (TNase) Producing *Staphylococcus* spp

Production of thermonuclease by *Staphylococcus* spp was determined using the method described by Hawkey and Lewis (1989). Toluidine blue-deoxynucleic acid agar plates were spot inoculated with *Staphylococcus* spp and incubated for 24 hrs at 37 ^{o}C. After incubation, formation of a pink halo around the colonies indicated the production of thermonuclease.

Detection of Lipase Producing *Staphylococcus* spp

Preliminary screening of lipase producing *Staphylococcus* spp was carried out using tributyrin agar (The composition of tributyrin agar medium (per liter) is 5 g peptone, 3 g yeast extract, 10 ml tributyrin and 15 g agar). The agar plates were spot inoculated with *Staphylococcus* spp and incubated for 48 hrs at 37 ^{o}C. Clear zones observed at the end of the incubation period around the colonies indicated the production of lipase (lipolysis).by the isolates.

Detection of Capsule Producing *Staphylococcus* spp

With the use of a sterile wire loop, a colony was emulsified in sterile distilled water to make a thin smear on a clean scratch and grease free slide. The smear was air dried, stained with crystal violet for 5-7 mins. The stain was washed off with 20 % copper sulphate and air-dried. The smear was then examined microscopically with the 100 x oil immersion objective and bacterial capsule appeared as faint blue-violet zones around dark-blue bacterial cells.

Determination of Haemolysin Producing *Staphylococcus* spp

The production of haemolysins by *Staphylococcus* spp**.** was determined using Columbia blood agar base (Oxoid, UK) supplemented with 5 % sheep blood. The *Staphylococcus* spp were streaked onto blood agar plates and incubated for 24 hrs at 37 ºC. The presence of greenish colouration halos around the colonies indicated production of α-haemolysin, while complete clear zone indicated production of β-haemolysin.

Determination of Beta-lactamase Producing *Staphylococcus* spp

The production of β-Lactamase enzymes was determined using Nitrocephin. The *Staphylococcus* spp were inoculated into sterile nutrient broth and incubated at 37 ^{0}C for 24 hrs. Two drops of nitrocephin solution were added for colour change within 30 mins. The β-lactamase production was inferred when the broth turned red within 30 mins of addition of the reagent.

Antibiotic Susceptibility Testing

In vitro susceptibility of the *Staphylococcus* spp to antibiotics was determined using Kirby-Bauer disk-diffusion (Bauer *et al*., 1996). Approximately 0.1 ml of *Staphylococcus* spp prepared directly from an overnight agar plate adjusted to 0.5 McFarland Standard was inoculated using sterile pipette onto each of the Petri dishes containing Mueller-Hinton Agar (MHA). The discs containing the following antibiotics: CN: Gentamycin;

CEF: Ceftriaxone; S: Streptomycin; NB: Nobactracin; CH: Chloramphenicol; E: Erythromycin; APX: Ampicillin; CTX: Cefotaxime; CPX: Ciprofloxacin; LEV: Levofloxacin; PEN : Penicillin (Oxoid, UK) were aseptically placed on the surfaces of the culture plates with a sterile forcep and gently pressed to ensure even contact. The plates were incubated at 37 °C for 18-24 hrs. Zones of inhibition after incubation were observed and the diameters of inhibition zones were measured in millimeters (mm) using a ruler. The interpretation of the measurement was made according to the manufacturer's standard zone size interpretative manual.

Determination of Multiple Antibiotic Resistance Index (MAR)

Staphylococcus spp. that were resistant to three or more antibiotics were taken to be multiple antibiotic resistant and the multiple antibiotic resistance (MAR) index was determined using the formula MAR = *x/y,* where *x* was the number of antibiotics to which test isolate displayed resistance and *y* was the total number of antibiotics to which the test isolates has been evaluated for sensitivity (Krumpermann, 1983; Ehinmidu, 2003).

Phenotypic Detection of Methicillin Resistant *Staphylococcus* spp

Methicillin resistant *Staphylococcus* spp isolated from the anterior nares were detected phenotypically using 1 µg oxacillin disc (Oxoid, UK). Approximately 0.1 ml of *Staphylococcus* spp prepared directly from an overnight agar plate adjusted to 0.5 McFarland Turbidity Standard (NCCLS, 2004) was inoculated onto plate containing Mueller-Hinton Agar (MHA). Commercially available oxacillin disc (Domstell Limited, Lagos) was placed on the plate of Mueller-Hinton agar and incubated aerobically at 37 ^{o}C for I8 - 24 hrs. Zone of inhibition (≤ 12 mm) was interpreted as methicillin resistant, while inhibitory zone (≥ 13 mm) was interpreted as methicillin sensitive.

RESULTS

The morphological and biochemical tests of *Staphylococcus aureus* and CoN- *Staphylococcus* spp isolated from anterior nares of undergraduate students are shown in Table 1. One hundred and twenty (120) *Staphylococcus* spp were isolated from the anterior nares of the undergraduate students. Of these, 84 (70.0 %) were identified as *Staphylococcus aureus,* while 36 (30.0 %) were CoN-*Staphylococcus* spp. Highest numbers of *S. aureus* were obtained in aged groups ≤ 20 yrs, while aged groups 21-25 yrs had the highest numbers of CoN-*Staphylococcus* spp (Table 2). The percentage occurrences of *S. aureus* in relation to sex showed that males and females in aged group ≤ 20 yrs were 80.0 % and 70.0 %, respectively, while in aged groups 21-25 yrs, 26-30 yrs and ≥ 31 yrs were 66.7 % and 76.9 %; 80.0 % and 57.1 % ; 66.7 % and 33.3%, respectively (Table 2). There was no significant difference (P>0.05) in the distribution of *S. aureus* and CoN-*Staphylococcus* spp between males and females. The percentage occurrences of CoN-*Staphylococcus* spp in relation to sex are also shown in Table 2.

The results showed that 50 (59.5 %) *S. aureus* isolated produced *α*-haemolysins, 26 (30.9 %) showed *β*-haemolysis, while 8 (9.5 %) showed no lysis of the red blood cell (γ-haemolysis). Only 16 (44.4 %), 14 (38.9 %) and 6 (16.7 %) of CoN- *Staphylococcus* spp showed *α, β* and γ haemolysis, respectively. Of the eighty-four *S. aureus* obtained, thirty-two produced β-lactamase, seventy produced DNase and ten produced TNase (Table 3). Only 16 (19.0%) of *S. aureus* and 6 (16.7%) of CoN- *Staphylococcus* spp showed lipolytic activity with clearing zones ranging between 4.0 mm to 8.5 mm. None of the CoN-*Staphylococcus* spp showed capsule production, while the percentage occurrences of CoN-*Staphylococcus* spp isolated according to enzyme production are also shown in Table 3.

The results showed that between 28.6 % and 30.9 % of the *S. aureus* were resistant to gentamycin, ampicillin and nobactracin, while between 27.8 % and 33.3% of the CoN-*Staphylococcus* spp were resistant to gentamycin, penicillin ampicillin, ciprofloxacin, chloramphenicol and nobactracin. Moderately high resistant to penicillin, streptomycin and chloramphenicol were observed in *S. aureus* isolated from the anterior nares. However, all the CoN-*Staphylococcus* spp were sensitive to cefotaxime (Table 4). The percentage occurrences of methicillin sensitive *S. aureus (*MSSA), methicillin resistant *S. aureus* (MRSA) and methicillin resistant CoN-*Staphylococcus* spp (MRCoNS) isolated in relation to sex are shown in Table 5. The results showed that there was no statistical difference at $P > 0.05$ in the occurrence of MRSA between males and females.

The varied antibiotic resistant patterns of *S. aureus* and CoN-*Staphylococcus* spp are shown in Table 6. The results showed that eight (8) resistance patterns were observed in *S. aureus,* while five (5) in CoN-*Staphylococcus* spp. Of the 36 antibiotic resistant *S. aureus* isolated, 6 (16.7 %) had resistant pattern of CN-APX-CEF-S-NB-CH-E, while 1 (2.8 %) showed resistant pattern of APX-PEN-CPX-LEV-E. Resistant patterns (CN-APX-CH-NB-CPX-LEV) and (PEN-S-CEF) were obtained in 30.8% and 38.5 % of CoN-*Staphylococcus* spp, respectively (Table 6).

The Multiple Antibiotic Resistant (MAR) Index of *S. aureus* and CoN-*Staphylococcus* spp ranged from 0.1 to 0.7 and 0.2 to 0.6, respectively. The results showed that 22 (26.2%), 6 (7.1%), 12 (14.3%) and 12 (14.3%) *S. aureus* had MAR Index of 0.1, 0.2, 0.3 and 0.7, respectively, while 10 (27.8%) CoN-*Staphylococcus* spp had MAR index of 0.3 and 0.6 each (Table 7).

Table 1: Morphological and Biochemical Characteristics of *Staphylococcus* spp isolated from Anterior Nares

Morphology / Biochemical Tests	*Staphylococcus aureus*	CoN-*Staphylococcus* spp
Shape	Coccoid in cluster	Coccoid in cluster
Gram Staining	+ve	+ve
Catalase	+ve	+ve
Coagulase	+ve	-ve
Indole	-ve	-ve
Citrate	+ve	+ve
Methyl red	+ve	+ve
Vogues-Proskauer	-ve	-ve
Gelatin hydrolysis	-ve	-ve
Mannitol	A	A
Sucrose	A	A
Maltose	A	A
Lactose	A	A
Galactose	A	A
Glucose	A	A

Keys: +ve positive; -ve: negative

Table 2: Age / Sex Distribution of *Staphylococcus* spp in the Anterior Nares of the Undergraduate Students

		No of Samples	No (%) of Occurrences	
Age (Yrs)	Sex	Collected	*S. aureus*	CoN-*Staphylococcus* spp
≤ 20	M	20	16 (80.0)	4 (20.0)
	F	20	14 (70.0)	6 (30.0)
21-25	M	18	12 (66.7)	6 (33.3)
	F	26	20 (76.9)	6 (23.1)
26-30	M	10	8 (80.0)	2 (20.0)
	F	14	8 (57.1)	6 (42.9)
≥ 31	M	6	4 (66.7)	2 (33.3)
	F	6	2 (33.3)	4 (66.7)
	Total	120	84 (70.0)	36 (30.0)

Keys: CoN: Coagulase negative; M : males; F: females

Table 3: Betalactamase Production and Virulence Factors of *Staphylococcus* spp from the Anterior Nares

	Haemolysin			β-lactamase	DNase	TNase	Lipase	Capsule Formation
	α (%)	β (%)	γ (%)	No (%)	No (%)	No (%)	No (%)	No (%)
S. aureus (N=84)	50 (59.5)	26 (30.9)	8 (9.5)	32 (38.1)	70 (83.3)	10 (11.9)	16 (19.0)	4 (4.8)
CoN-*S* spp (N=36)	16 (44.4)	14 (38.9)	6 (16.7)	10 (27.8)	6 (16.7)	2 (5.5)	6 (16.7)	0 (0.0)
Total (N=120)	66 (55.0)	40 (33.3)	14 (11.7)	42 (35.0)	76 (63.3)	12 (10.0)	22 (18.3)	4 (3.3)

Keys: CoN: Coagulase negative; α: Alpha; β: Beta; γ: Gamma; DNase: Deoxyribonuclease; TNase: Thermonuclease

Table 4: Antibiotic Susceptibility of *Staphylococcus* spp isolated from the Anterior Nares

	Staphylococcus aureus (N=84)			CoN-*Staphylococcus* spp (N=36)		
	Sensitive	Intermediate	Resistant	Sensitive	Intermediate	Resistant
Antibiotics	No (%)	No (%)	No (%)	No (%)	No (%)	No (%)
Gentamycin	44 (52.4)	14 (16.7)	26 (30.9)	16 (44.4)	8 (22.2)	12 (33.3)
Ampicillin	52 (61.9)	8 (9.5)	24 (28.6)	18 (50.0)	8 (22.2)	10 (27.8)
Penicillin	30 (35.7)	10 (11.9)	44 (52.4)	18 (50.0)	6 (16.7)	12 (33.3)
Streptomycin	60 (47.6)	6 (7.1)	18 (45.2)	22 (61.1)	10 (27.8)	4 (11.1)
Ciprofloxacin	54 (62.3)	12 (14.2)	18 (21.4)	20 (55.6)	6 (16.7)	10 (27.8)
Nobactracin	58 (69.0)	0 (0.0)	26 (30.9)	18 (50.0)	6 (16.7)	12 (33.3)
Ceftriaxone	54 (62.3)	18 (21.4)	12 (14.2)	18 (50.0)	2 (5.6)	16 (44.4)
Erythromycin	50 (59.5)	14 (16.7)	20 (23.8)	20 (55.6)	12 (33.3)	4 (11.1)
Cefotaxime	64 (76.2)	16 (19.0)	4 (4.8)	26 (72.2)	10 (27.8)	0 (0.0)
Chloramphenicol	44 (52.4)	6 (7.1)	34 (40.5)	24 (66.7)	2 (5.5)	10 (27.8)
Levofloxacin	66 (78.6)	10 (11.9)	8 (9.5)	24 (66.7)	4 (11.1)	8 (22.2)

Key: CoN: Coagulase negative

Table 5: Occurrence of Methicillin Sensitive and Methicillin Resistant *Staphylococcus* spp in Relation to Sex

Sex	No. of *S. aureus*	No (%) of Occurrences MRSA	MSSA	No. of C0N *Staphylococcus* pp	No. (%) of Occurrences MRCoNS	MSCoNS
Male	40	12 (30.0)	28 (70.0)	14	4 (28.6)	10 (71.4)
Female	44	18 (41.0)	26 (59.0)	22	8 (36.4)	14 (63.6)
Total	84	30 (35.7)	54 (64.3)	36	12 (33.3)	24 (66.7)

Keys: MSSA: Methicillin sensitive *Staphylococcus aureus;* MRSA: Methicillin resistant *Staphylococcus aureus;* MRCoNS Methicillin resistant coagulase negative *Staphylococcus* spp; MSCoNS: Methicillin sensitive coagulase negative *Staphylococcus* spp

Table 6: Antibiotic Resistant Patterns of *Staphylococcus* spp from the Anterior Nares

Bacterial Isolates	Resistant Patterns	Number of Occurrence	Percentage of Occurrence
S. aureus	CN-APX-CEF-S-NB-CH-E	12	16.7
	CN-PEN-CH-NB-CPX	10	13.9
	APX-PEN-CPX-LEV-E	2	2.8
	CPX-E-LEV	6	8.3
	CH-APX	6	8.3
	CTX	22	30.6
	PEN-CH-S	6	8.3
	APX-CN-PEN-CTX-NB	4	5.6
	Total	72	100
CoN-*Staphylococcus* spp	CN-APX-CH-NB-CPX-LEV	8	30.8
	PEN-S-CEF	10	38.5
	PEN-CN-E-CEF-NB	2	7.7
	CN-APX-CH-NB-CPX-E	2	7.7
	S-CEF	4	15.4
	Total	26	100

Keys: CoN: Coagulase negative; CN: Gentamycin; APX: Ampicillin; CEF: Ceftriaxone; S: Streptomycin; NB: Nobactracin; CH: Chloramphenicol: E: Erythromycin; CTX: Cefotaxime; CPX: Ciprofloxacin; LEV: Levofloxacin; PEN: Penicillin

Table 7: Multiple Antibiotic Resistant (MAR) Index of *Staphylococcus* spp. from the Anterior Nares

Multiple Antibiotic Resistance Index (MAR)	*Staphylococcus aureus* No. (%)	CoN-*Staphylococcus* spp. No. (%)
0.1	22 (26.2)	-
0.2	6 (7.1)	4 (11.1)
0.3	12 (14.3)	10 (27.8)
0.4	16 (19.0)	-
0.5	-	2 (5.6)
0.6	-	10 (27.8)
0.7	12 (14.3)	-

Key: CoN: Coagulase negative

DISCUSSION

The human anterior nares are known to be the principle habitat of both commensals and opportunistic pathogens such as *Staphylococcus aureus*, *Staphylococcus epidermidis* and *Moraxella* spp (Wos-Oxley *et al*., 2010). *S. aureus* colonizes the moist squamous epithelium in the anterior nares of about 20% of the human population and is a transient resident of another 60% (Gordon and Lowy, 2008). Nasal colonization is an important risk factor for the pathogenesis of infection (Kluytmans *et al*., 1997; Cole *et al*., 2001; Peacock *et al*., 2001). The *S. aureus* can be transmitted to nares by contaminated hands and from surfaces where it can survive for months (Kluytmans *et al*., 1997). Nasal carriage has a crucial function as a source of invasive infections in both community and hospital settings (von-Eiff *et al*., 2001; van-Belkum *et al*., 2009). Of the 120 *Staphylococcus* spp isolated from the anterior nares in 36 (30.0 %) were CoN-*Staphylococcus* spp. The occurrence of CoN-*Staphylococcus* spp in this study confirmed the previous results of Silva *et al.* (2001). Isolation of *S. aureus* and CoN-*Staphylococcus* spp from the anterior nares in this study is also in agreement with Nakamura *et al*. (2002) and Wertheim *et al*. (2005). There was age-wise and sex-wise distributions of *Staphylococcus* spp in the anterior nares of the subjects in this study. The highest *S. aureus* colonization of anterior nares was obtained among the subjects aged ≤ 20 yrs and lowest in subjects aged ≥ 31 yrs and this agrees with the reports of Lee *et al*. (2009) who reported significant variations in *S. aureus* nasal carriage rates with age. The differences in colonization of anterior nares by *Staphylococcus* spp have been attributed to host factors such as host immunity, age, gender and environmental factors (García-Rodríguez and Fresnadillo-Martínez, 2002).

Carriage of Methicillin resistant *S. aureus* (MRSA) or Methicillin sensitive *S. aureus* (MSSA) varies in different geographical areas (Sa-Leao *et al*., 2001; El- Jalil *et al.*, 2008; Odu and Okonko, 2012). In this study, less than 40.0 % of the *S. aureus* isolated were methicillin resistant and this corroborates the previous reports of Odu and Okonko (2012) who reported that 37.5 % of the *S. aureus* isolated from apparently healthy students of University of Port Harcourt were MRSA. Silva *et al.* (2001) showed the occurence of Methicillin resistant coagulase negative *Staphylococcus* spp (MRCoNS) from nasal flora of healthy humans and this study confirms it as 12 (33.3 %) MRCoNS were obtained.

The pathogenicity of both *S. aureus* and coagulase negative *Staphylococcus* spp comes from their production of impressive repertoire of virulence factors that promote colonization of hosts' tissues, bacterial spread in tissue and inhibition of phagocyte engulfment (Schroder *et al*., 2006; Corrigan *et al*., 2007). In this study, 70 (83.3 %) *S, aureus* and 6 (16.7 %) CoN-*Staphylococcus* spp were DNase positive. The production of DNase by *S. aureus* isolated from anterior nares in this study is in concordance with Finegold and Baron (1986). Prescott *et al*. (2008) has also revealed that one of the virulence factors produced by *Staphylococcus aureus* is DNase. Deoxyribonucleases (DNases) produced by micro-organisms are extracellular endonucleases that cleave DNA, yielding a high concentration of oligonucleotide (Talaro and Talaro, 1996; Prescott *et al*., 2008). Detection of 5.5 % TNase producing CoN-*Staphylococcus* spp in this study is in line with the results of Turkyilmaz and Kaya (2005). Of the 36 CoN-*Staphylococcus* spp isolated, 16 (44.4 %) produced α-heamolysins while 14 (38.9 %) produced β-heamolysins. Productions of haemolysin by CoN-*Staphylococcus* spp have been reported by Cunha *et al., (*2006); Akinjogunla and Enabulele, (2010); Azih and Enabulele, (2013). Prevalence of *β*-haemolytic *S. aureus* strains in this study is in agreement with other research

papers (Aarestrup *et al*., 1999; Larsen *et al*., 2002). Lipolytic activities of 16 (19.0 %) *S. aureus* with clearing zones ranging between 4.0 mm to 8.5 mm were observed. Isolation of *S. aureus* with lipolytic activity in our study confirms the previous reports of Odeyemi and Aderiye (2013). The number of lipolytic CoN-*Staphylococcus* spp 6 (16.7 %) obtained in our study was very low, compared with the results of Saising *et al.* (2012) who reported that 85 (100 %) of CoN-*Staphylococcus* spp isolated produced lipase enzymes. β-lactamase production by *Staphylococci* spp is the recognized mechanism of resistance to β-lactamase antibiotics, such as penicillin and cephalosporins (Ekrem and Meltem, 2006). Although, high beta-lactamase producing *S. aureus* was obtained in this study but the value obtained was lower the than 83.7 % by Odugbemi *et al*. (1995) and 86.2 % by Kesah *et al.* (1997). The results showed that 32 (38.1 %) *S. aureus* and 10 (27.8 %) CoN-*Staphylococcus* spp from anterior nares possessed β-lactamase enzyme that hydrolyzed the β-lactam bond of the β-lactamase antibiotics used, thereby making these antibiotics inactive.

Among the eleven drugs used in this study, cefotaxime and levofloxacin were the highly effective drugs against the *Staphylococci* spp isolated from the anterior nares. The high sensitivity of the *Staphylococci* spp to cefotaxime is in constrast with the results of Adamu *et al*. (2010). Thus, suggesting that most of the *Staphylococci* spp isolated were not producing cephalosporinase but penicillinase as high resistant to penicillin were recorded. The sensitivity of 78.6 % *Staphylococci* spp to levofloxacin is an indication that this fluoroquinolones have not been abused in this environment. *S. aureus* were moderately sensitive to streptomycin and erythromycin and this is in agreement with previous reports of Ehinmidu (2003) and Olayinka *et al. (*2004). A study done by Onanuga and Temedie (2011) showed the nasal carriage of multi-drug resistant *S. aureus* in healthy inhabitants of Amassoma in Niger Delta region of Nigeria and

this study corroborate the findings. The emergence of multi-drugs resistant *S. aureus* (MDRSA) strains, has posed a challenge in the treatment of staphylococcal infection (Ekramul *et al*., 2011).

Conclusively, routine screenings of nasal colonization of anterior nares among the undergraduates students should be carried out; awareness on the implications of misuse and abuse of drugs and strategies on spreads of multi-drug resistant strains should be adopted and also epidemiological studies of nasal carriage of these organisms in the entire university community is consequently recommended.

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