



RESEARCH PAPER

Antibiotic Susceptibility Studies of *Staphylococcus aureus* Isolated from Anterior Nares of Students of Ibrahim Badamasi Babangida University Lapai, Niger State, Nigeria

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ABSTRACT

The antibiotic susceptibility studies of *Staphylococcus aureus* isolated from anterior nares of 70 students of Ibrahim Badamasi Babangida University, Lapai was carried out. The samples were analysed using Nutrient agar and Mannitol salt agar. The isolates were Gram stained, identified and classified using biochemical tests. Antimicrobial susceptibility test was carried out on each isolates using disc diffusion method. The antibiotics used include, Ciprofloxacin, Norfloxacin, Gentamycin, Amoxil, Streptomycin, Rifampicin, Erythromycin, Chloramphenicol, Ampiclox and Levofloxacin. *Staphylococcus aureus*, *Staphylococcus hemolyticus*, and *Staphylococcus epidermidis* were the common bacteria isolated in the study area. The rate of isolation of *Staphylococcus aureus* was higher among students of 21-25 years 37(52.9%). It was showed that female students were found to be the carriers of *Staphylococcus aureus* 24(34.3%) than male students with 19(27.1%). *Staphylococcus aureus* isolates was susceptible to Erythromycin, Gentamycin and Levofloxacin but resistant to Ciprofloxacin, Norfloxacin, Amoxil, Streptomycin, Rifampicin, Chloramphenicol and Ampiclox antibiotics. The present study revealed that Gentamycin, Erythromycin and Levofloxacin can be used as a drug of choice for empirical treatment of disease cause by *Staphylococcus aureus*. The bacterium could be called Ciprofloxacin, Norfloxacin, Amoxil, Streptomycin, Rifampin and Chloramphenicol Staphylococcal resistance.

Keywords: Antibiotic, Susceptibility, Resistance, *Staphylococcus aureus*

INTRODUCTION

Antibiotics are also known as antibacterial agents that either destroy or inhibit the growth of microorganism (Waskman, 1947). Antibiotic was described as any substance produced by a microorganism that is antagonistic to the growth of other microorganism in high dilution (Waskman, 1947). With advances in medical chemistry, most modern antibacterial are semisynthetic modifications of various natural compounds (Von *et al.*, 2006).

Examples are the beta-lactam antibiotics, which include the penicillin (produced by fungi *Penicillium*), the cephalosporin, and the carbapenems. Compounds that are still isolated from living organisms are the aminoglycosides, whereas other antibacterial-for example, the sulphonamides, the quinolones, and the

oxazolidinones are produced solely by chemical synthesis (Von *et al.*, 2006).

Staphylococcus aureus is a Gram- positive coccal and facultative anaerobic bacterium that belongs to the Firmicutes Family. *S. aureus* is a catalase positive and nitrate reduction and is frequently found in the nose, human respiratory tract and on skin (Kluytmans *et al.*, 1997). Although *S. aureus* is the common pathogen responsible for skin infections example (boils), respiratory disease (sinusitis), and food poisoning (Ryan and Ray, 2004)

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Staphylococcus aureus is an important pathogen of humans and animals and is implicated in a wide variety of infections. Apparently normal humans and animals harbour *S. aureus* but become virulent when the immune mechanisms are compromised. It is a pathogen of greater concern because of its virulence (Chambers, 2005). *S. aureus* can cause range of illnesses, from minor skin infections such as pimples, impetigo, boils, cellulites, folliculitis, carbuncles, scalded skin syndrome and abscesses to life threatening diseases such as pneumonia, meningitis, osteomyelitis, endocarditis, toxic shock syndrome, bacteraemia, sepsis and hospital acquired infections (Bowersox, 1999).

An estimated 20% of the human population are long term carriers of *S. aureus* which can be found as part of the normal skin flora and in the nostril (Cole *et al.*, 2001). *Staphylococcus aureus* is a normal inhabitant of the healthy lower reproductive tract of women (Senok *et al.*, 2009; Hoffman, 2012). Carriage of *Staphylococcus aureus* appears to play a key role in the epidemiology and pathogenic infection. The ecological niches of *Staphylococcus aureus* are found in the anterior nares. The anterior nares have been shown to be the main reservoir of *S. aureus* in both adults and children.

The *S. aureus* is transmitted to nares by contaminated hands and from surfaces where it can survive for months (Kluytmans *et al.*, 1997). Nasal carriage of *S. aureus* acts as endogenous reservoir for clinical infections in the colonized individual but also as a source of cross-colonization for community spread. The spread of colonization occur especially in close contact areas like schools, pre-schools or households probably by the contaminated hands and surfaces (Peacock *et al.*, 2003).

The individuals colonized with *S. aureus* tend to have a complicated clinical course from a disease originating from their endogenous *S. aureus* (Kluytmans *et al.*, 1997). The complicated clinical course results from increasing resistance in *S. aureus* isolates and also because the bacteria can cause deep-seated infections and sepsis (Kluytmans *et al.*, 1997; Grundmann *et al.*, 2006). The difficulties in treating these deep-seated infections and sepsis require urgent measures to prevent further

spread of MRSA and VRSA (Kluytmans *et al.*, 1997; Grundmann *et al.*, 2006).

The carriage of *Staphylococcus aureus* is an important source of nosocomial infection and community-acquired methicillin-resistant *S. aureus* (MRSA). Although *S. aureus* can be present on the skin of the host, a large proportion of its carriage is through the anterior nares of the nasal passages (Kluytmans *et al.*, 1997). The ability of the nasal passages to harbour *S. aureus* results from a combination of a weakened or defective host immunity and the bacteria's ability to evade host innate immunity (Quinn and Cole), resistant community acquired *Staphylococcus aureus* to vancomycin.

METHODOLOGY

Study area

The study was carried out at Ibrahim Badamasi Babangida University campus, Lapai, Niger State, Nigeria, with the students from different level and locations from the period of July to August 2014. The research was conducted with the students of the university on the matter of *Staphylococcus aureus* isolated from anterior nares. The nares are located at the anterior inferior aspect of the face below orbital sockets.

Study population

The study population was university campus based, in which the target population were seventy (70) students, 30 males and 40 females students , aged 15-35 years. Ethical approval: students consent was sought before taken samples.

Preparation of culture media

The Nutrient agar and Mannitol salt agar used for this study were prepared according to their respective manufacturer's prescription or directives.

Preparation of Mannitol salt agar

Fifty five point five gram (55.5g) of the Mannitol salt agar powder was dissolved in 500ml of distilled water. The agar was thoroughly mixed, after which was sterilized in an autoclave at 121°C for 15 minutes. The sterile media was placed in a water bath at 42°C, cooled out; the molten agar was poured into sterile Petri-dishes under aseptic conditions and allowed to solidify on a level laboratory bench.

Preparation of Nutrient agar

Fourteen gram (14g) of nutrient agar powder was dissolved in 500ml of distilled water, heated for 10 minutes to allow homogenization. The homogenized nutrient agar was autoclaved at 121°C for 15 minutes. The sterilized molten agar was placed in a water bath at 42°C and the cooled out; the molten agar was poured into sterile Petri-dishes under aseptic conditions and allowed to solidify on a level laboratory bench.

Sample analysis

The swabs were inoculated onto Mannitol salt agar (MSA) and Nutrient agar (NA) plates and incubated for 24 hours at 37°C for isolation of *Staphylococcus aureus*; pure isolates on the plates were transferred onto MacConkey bottles, incubated for 24 hours at 37°C.

Characterization of bacterial isolate

The bacteria isolates were characterized based on morphology and biochemical test, the organisms were identified by comparing their characteristics with those of known described by Cowan (1974).

Gram staining

Gram staining was carried out on typical colonies from the solid media after preparing a dried fixed smear from the plate as follow:

Smear making

A grease pencil was used to write sample number on a clean grease slide. A colony was emulsified in normal saline on clean grease-free slide with the use of a sterilized wire loop until a thin smear of about 15-20mm was made. Slides were left in a safe place to air dry. Slides smears were fixed by passing them three times rapidly over a Bunsen burner flame. The slides were allowed to cool before staining.

Gram staining

The slides were stained with crystal violet for 60 seconds and washed with water. Lugol's iodine was applied for 60 seconds and then washed with clean water. The smear were rapidly decolorized for few seconds with acetone-alcohol and then washed immediately with clean water. The slides were then counter-stained with neutral red for 60 seconds and then washed off with water. Back of the slides were blotted dry with cotton wool and place on a draining rack

for the smear to air dry. Smears were examined microscopically with oil immersion objective for the presence of bacteria.

Biochemical test

Biochemical test was carried out on the test isolates.

Catalase test

The bacteria culture was emulsified on a slide and 3% hydrogen peroxide (H₂O₂) drop was added to it, effervescence of oxygen indicated the presence of catalase enzyme in the organism.

Coagulase test

A drop of distilled water was placed on a slide; a small portion of the culture was taken and emulsified on a slide, a drop of plasma was added to know which colour indicate or evidence indicate positive or negative result.

Antimicrobial susceptibility test

Antimicrobial susceptibility test was carried out on the isolate using disc diffusion method. Nutrient agar plates were dried at 37°C in an incubator and appropriately labelled for each organism tested.

Preparation of inoculums

Using a sterile inoculating loop, five colonies of *Staphylococcus aureus* were touched. The organism was suspended in 2ml of sterile saline. The saline tube was mixed to create a smooth suspension. The turbidity of this suspension was adjusted to a 0.5 McFarland standard by adding more organisms when the suspension was too light or diluted with sterile saline when the suspension was heavy. A 0.5 McFarland standard was prepared by adding 0.5ml aliquot of 0.048M BaCl₂ to 99.5 ml of 0.18M H₂SO₄ with constant stirring to maintain a suspension. This organism suspension was used within 15 minutes of preparation.

Inoculation of the Nutrient agar plate

A sterile swab was dipped into the inoculums tube. The swab was rotated against the side of the tube using firm pressure in order to remove fluid. The Nutrient agar plate was inoculated by streaking the swab three times (3x) over the entire agar surface, the plate was rotated approximately 60° each time to ensure an even distribution of the inoculums. The plate was

rinsed with the swab to pick up any excess liquid. The plate was allowed to sit at room temperature for 5 minutes leaving the lid slightly ajar, for the surface of the agar plate to dry.

Placement of antibiotic discs

The multiple antibiotic discs called Gram-positive antibiotics were used by placing them on the agar plates using sterile forceps aseptically on the vicinity of the flame. A sterilized forceps was used to touch discs on the plate to ensure complete contact with agar surface. Care was taken to ensure that the disc was not moved once it has contacted the agar surface. After all the disc were placed, the lid was replaced, the plates inverted and incubated at 37°C for 24 hours.

Measuring zone sizes

Zones sizes were measured after incubation period to the nearest millimetre using a ruler. All measurements were made with the unaided eye while viewing the back of the petridish. Growth up to the edge of the disc was reported as a zone of 0mm (resistant).

RESULTS

A total number of 70 students were amassed for the prevalence of nasal carriage of *Staphylococcus aureus* at Ibrahim Badamasi Babangida University, Lapai, Niger State. Students' age groups related to number of nasal carriage isolates was shown in Table1 and Table2 former was specific for *Staphylococcus aureus*, while the later represent the general bacterial isolates from various age groups. In Table2, out of 70 students investigated, there were 43 *Staphylococcus aureus* isolates (61.40%), all of which were found in student age of 15-30 years and even none *Staphylococcus*

aureus was also found in student age of 15-30 years.

In comparison to the total isolates, table3 shows that out of 70 students investigated, there were 63 bacterial isolates (90%) distributed also among age group of 15-30 years. Also from these two tables, the rate of isolation of *Staphylococcus aureus* and other bacterial isolates was highest among students age group of 21-25 years.

Students' gender related to the number of isolates was shown in table 4 and table 5. The former represent *Staphylococcal* isolates, while the later represent the general bacterial isolates in this work. In table 4, out of 43 *Staphylococcus aureus* isolates (61.4%) from 70 students investigated, 19 students were males (27.10%) and 24 students were females (34.39%). In comparison to the total bacterial isolates, table 5 shows that out of 70 students investigated, there were 63 bacterial isolates, out of which 24 students were male (34.30%) and 39 students were females (55.70%).

The type of bacterial isolates according to various age groups was shown in table 6. The most frequent isolates were *Staphylococcus aureus* 29(46.00%), followed by *Streptococcus pneumoniae* 14(22.20%), *Staphylococcus hemolyticus* 11(17.50%), *Staphylococcus epidermis* 05 (7.90%), and *Streptococcus pyogenes* 04(6.40%).

Table 7 shows the in-vitro antibiotic susceptibility patterns of *Staphylococcus aureus* and other bacterial isolates. The most effective antibiotics among all the 43 *Staphylococcus aureus* were Levofloxacin, Gentamycin, and Erythromycin. All 43 samples of *Staphylococcus aureus* were resistant to Ciprofloxacin, Norfloxacin, Amoxil, Streptomycin, Rifampicin and Chloramphenicol

Table1: Number of samples collected from anterior nares of 70 students of Ibrahim Badamasi Babangida University, Lapai, Niger State- related their age group from July to August, 2014.

Age group (year)	No of samples from male students (%)	No of samples from female students (%)	Total (%)
15-20	03 (4.30)	08 (11.40)	11 (15.70)
21-25	24 (34.30)	29 (41.40)	54 (77.10)
26-30	03 (4.30)	03 (4.30)	05 (7.10)
31-35	00 (0.00)	00 (0.00)	00 (0.00)
Total (%)	30 (42.90)	40 (57.10)	70 (100)

Table 2: Students age group related to 43 *Staphylococcal* isolates from anterior nares of 70 students of Ibrahim Badamasi Babangida University, Lapai, Niger State, from July to August, 2014.

Age group (year)	No of Staphylococcus positive (%)	No of Staphylococcus Negative	Total (%)
15-20	05 (7.10)	06 (8.60)	11 (15.70)
21-25	37 (52.90)	16 (22.90)	53 (75.70)
26-30	01 (1.40)	05 (7.10)	06 (8.60)
31-35	00 (0.00)	00 (0.00)	00 (0.00)
Total (%)	43 (61.40)	27 (38.60)	70 (100)

Table 3: Students age group related to 63 bacteria isolates, from anterior nares of 70 students of Ibrahim Badamasi Babangida University, Lapai, Niger State from July to August, 2014.

Age group (year)	No of bacteria positive (%)	No of bacteria Negative	Total (%)
15-20	05(10.00)	01(1.40)	08 (11.40)
21-25	52 (74.30)	06 (8.60)	58 (82.90)
26-30	04 (5.70)	00 (0.00)	04 (5.70)
31-35	00 (0.00)	00 (0.00)	00 (0.00)
Total (%)	63 (90)	07 (10)	70 (100)

Table 4: Students gender related to 43 *Staphylococcal* isolates from anterior nares of 70 students of Ibrahim Badamasi Babangida University, Lapai, Niger State from July to August, 2014.

Isolated organism	Students gender		Total (%)
	Male (%)	Female (%)	
15-20	05 (7.10)	06 (8.60)	11 (15.70)
21-25	37 (52.90)	16 (22.90)	53 (75.70)
26-30	01 (1.40)	05 (7.10)	06 (8.60)
31-35	00 (0.00)	00 (0.00)	00 (0.00)
Total (%)	43 (61.40)	27 (38.60)	70 (100)

Table 5: Students gender related to 63 bacterial isolates from anterior nares of 70 students of Ibrahim Badamasi Babangida University, Lapai, Niger State from July to August, 2014.

Bacteria isolates	Students gender		Total (%)
	Male (%)	Female (%)	
Bacteria positive	24 (34.30)	39 (55.70)	63(90.00)
Bacteria Negative	06 (8.60)	01 (1.40)	07(10.00)
Total (%)	30(48.90)	40 (57.10)	70 (100)

Table 6: Distribution of 63 bacteria isolates among various age group of 70 students of Ibrahim Badamasi Babangida University, Lapai, Niger State from July to August, 2014.

Bacteria isolates	Age group (year)				Total (%)
	15-20	21-25	26-30	31-35	
<i>Staphylococcus aureus</i>	04(6.30)	22(34.90)	03(4.80)	00(0.00)	29(46.00)
<i>Staphylococcus hemolyticus</i>	01(1.60)	10(15.90)	00 (0.00)	00(0.00)	11(17.50)
<i>Staphylococcus epidermis</i>	00(0.00)	05(7.90)	00(0.00)	00(0.00)	05(7.90)
<i>Staphylococcus pneumoniae</i>	02(3.20)	11(17.50)	01(1.60)	00(0.00)	14(22.20)
<i>Staphylococcus pyogens</i>	00(0.00)	04(6.30)	00(0.00)	00(0.00)	04(6.30)
Total (%)	07(11.10)	52(82.50)	04(6.40)	00(0.00)	63(100)

Table 7: Antibiotic susceptibility pattern of three *Staphylococcal* and two *Streptococcal* species isolated from anterior nares of 70 students of Ibrahim Badamasi Babangida University, Lapai, Niger State.

Bacteria isolates	Zone of inhibition (mm)										
	Disc	CPX	NB	CN	AML	S	RD	E	CH	APX	LEV
	potency	10 μ g	10 μ g	10 μ g	10 μ g	30 μ g	20 μ g	30 μ g	30 μ g	20 μ g	20 μ g
<i>Staphylococcus aureus</i>		00	00	05	00	00	00	2.0	00	00	11
<i>Staphylococcus hemolyticus</i>		00	00	00	12	00	00	10	07	00	10
<i>Staphylococcus epidermis</i>		9.0	00	00	00	00	00	00	00	00	00
<i>Streptococcus pneumoniae</i>		12	08	12	11	00	00	00	13	00	07
<i>Streptococcus pyogens</i>		00	06	00	12	00	10	00	00	09	09

Key: μ g= Microgram

%= Percentage symbol

CPX_{10 μ g}= Ciprofloxacin

CN_{10 μ g}=Gentamycin

S_{30 μ g}= Streptomycin

E_{30 μ g}= Erythromycin

APX_{20 μ g}=Ampiclox

mm= Millimetre

00= Resistance

NB_{10 μ g}=Norfloxacin

AML_{10 μ g}= Amoxil

RD_{20 μ g}= Rifampicin

CH_{30 μ g}= Chloramphenicol

LEV_{20 μ g}= Levofloxacin

DISCUSSION

This study aimed to determine the antibiotic susceptibility pattern of nasal carriage of *Staphylococcus aureus* within healthy students in Ibrahim Badamasi Babangida University, Lapai, Niger State, which is the first study made in this specific population and region.

Staphylococcus aureus is a well-known pathogen with an alarmingly increasing level of developing resistance to most antimicrobial agents (Lowy *et al.*, 1998). *Staphylococcus aureus* is an important pathogen associated with nosocomial and community-acquired infection. Nasal colonisation by *Staphylococcus aureus* has been considered an important risk factor for infections that could threaten a carrier's life (Mason *et al.*, 2001).

The study showed an overall prevalence of 46% of *S.aureus* in the nostril of the volunteers which is in support with Pant and Rai's findings that revealed higher *S. aureus* nasal colonisation rate (43.8%) in staffs of teaching hospital in Nepal (Pant and Rai, 2007). Also, Chigbu and Ezeronye, (2003) reported 50% nasal colonization in both hospital and non-hospital subjects, a case study in Abia State of Nigeria. In contrary, the findings of Kuehnert *et al.* (2006) and Uemura *et al.* (2004) reported a nasal colonisation in healthy adults of 32.4% and

36.0% in United State of America and Japan respectively.

These variations may be attributed to the characteristics of the population under study. This study's high prevalence of nasal carriage of *S. aureus* further supports the fact that the anterior nares remains a principal reservoir of this organism and there is need to eliminate its virulent strains because of their involvement in most severe community and hospital associated *S. aureus* infections in colonized individuals (Wenzel and Perl, 1995; Smith *et al.*, 1999).

The prevalence of *Staphylococcus aureus* nasal colonisation is 46.0% in present study. The highest rate of *S. aureus* nasal colonisation is within the age range of 21-25 (77.1%). The study made here, suggested that factors associated with nasal carriage include; sharing of bathing soap, presence of acne on the face, being on hospital admission, over use of drug, lack of knowledge of *Staphylococals* infection and overcrowding, which is in line with the studies in the developed world which suggest factors associated with community acquired-methicilin resistance *Staphylococcus aureus* carriage which include; prior antibiotic usage, contact with health care facility, poor socio-economic conditions and overcrowding (Rihn *et al.*, 2005; CDC, 2004).

It was shown that female students were found to be the carrier of *Staphylococcus aureus* 24 (34.3%) than male students with 19 (27.1%). This is in line with Odu and Okonko, (2012) findings where the female students were also found to be carriers with (46.0%) than males (18.0%). In contrast, the presence of *Staphylococcus aureus* in the nares was greater in male carriers (40.9%) than in the females (31.9%) (Aida *et al.*, 2010). This varies because social and economic circumstance is different for Nigeria and Mexico.

In the antimicrobial susceptibility test of the organisms isolated from anterior nares, Ten antimicrobial agents were used which include Ciprofloxacin, Norfloxacin, Gentamycin, Amoxil, Streptomycin, Rifampicin, Erythromycin, Chloramphenicol, Ampiclox and Levofloxacin. Some bacteria showed high susceptibility to some antibiotics, while some are intermediate and some were resistant. *Staphylococcus aureus* were susceptible to gentamycin, erythromycin, and levofloxacin. *Staphylococcus hemolyticus* were susceptible to amoxil, erythromycin, chloramphenicol and levofloxacin, while *Staphylococcus epidemidis* were susceptible to only ciprofloxacin. *Streptococcus pneumoniae* were susceptible to ciprofloxacin, norfloxacin, gentamycin, amoxil, chloramphenicol and levofloxacin. Also, *Streptococcus pyogens* were susceptible to norfloxacin, amoxil, ampiclox and levofloxacin.

This susceptibility test result showed levofloxacin to be the most effective agent. All bacterial /isolates were resistant to streptomycin, except *Streptococcus pyogens* which were resistant to rifampicin. The resistance observed could be due to irrational use of antibiotics for conditions that may not clinically indicate their use and over-the-counter sale of antibiotics in pharmacies without prescription by authorized practitioners. However, these help some of the bacteria that have potential mechanism of acquired resistant genes to become resistance to these antibiotics.

Conclusion

The results emphasize the need to discourage antibiotic's abuse in order to prevent the return of the pre-antibiotic era. It also supports the need to implement strategies for elimination of nasal carriage of *S. aureus*, so as to prevent severe

multi-drug resistant *S. aureus* infections in our environment.

Recommendation

There is need for much larger epidemiological studies in community and development of strategies to control spreads of multi-drug resistant strains. Infective control and laboratory personnel should implement active surveillance for detection of resistant strains. Also there should be need to provide more campuses in order to avoid overcrowding which brings about spread of resistant *Staphylococcus aureus* in Ibrahim Badamasi Babangida University, Lapai, Niger

REFERENCES

- Aida, H.P., Teresita, S.E. and Jaime, B.M. (2010). Characterization and persistence of *Staphylococcus aureus* strains isolated from the anterior nares and throat of healthy carriers in Mexican. *J. Clin.Microbiol.*48(5):1701-1705.
- Chambers, H.F. (2005). Community-associated MRSA-resistance and virulence converge. *New Engl. J. Med.* 352:1485-1487.
- Chambers, H.F. (1997). Methicillin resistance in *Staphylococci*: molecular and biochemical basis and clinical implications. *Clinical Microbiology Reviews*10:781–791.
- Chigu, C.O. and Ezeronye, O.U. (2003).Antibiotic resistant *Staphylococcus aureus* in Abia State of Nigeria. *Afr. J. Biotech.* 2(10):37
- Grundmann, H., Aires-de-Sousa, M., Boyce, J., and Tiemersma, E. (2006). Emergence and resurgence of Methicillin-resistant *Staphylococcus aureus* as a public-health threat. *Lancet.* 368(9538):874-885.
- Hidayat, L.K., Hsu,D.I.,Quist,R., Shriner, K.A. and Wong-Beringer, A. (2006). High-dose vancomycin therapy for methicillin-resistant *Staphylococcus aureus* infections: efficacy and toxicity. *Arch. Intern. Med.* 166:2138–2144.
- Kluytmans, J., Van- Belkum, A. And Verbrugh, H. (1997). Nasal carriage of *Staphylococcus aureus*: epidemiology, underlying mechanisms, and associated risks. *Clin. Microbiol. Rev.*10(3):505-520.

- Kuehnert, M.J., Deanna, K. and Hill, H.A. (2006). Prevalence of *Staphylococcus aureus* Nasal colonization in the United States. *The Journal of Infectious Diseases*.193:172–179.
- Lowy, F.D. (1998). *Staphylococcus aureus* infections. *N Engl. J. Med.* 339(8):520-532.
- Lowy, F.D. (2003). Antimicrobial resistance: the example of *Staphylococcus aureus* *J. Clin. Invest.* 111:1265-1273.
- Mason, W.S., Blevins, J.S., Wibowo, N., Ojha, N. and Smeltzer, M.S. (2001). Multiplex PCR protocol for the diagnosis of *Staphylococcal* infection. *J. Clin.Microbiol.*39:3332-3338.
- Odu, N.N. and Okonko, I.O. (2012).Nasal carriage and antibiotics susceptibility of *Staphylococcus aureus* in healthy students of University of Port Harcourt Rivers State, Nigeria. *New York Science Journal* 5(7):56-61.