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Airefetalor AI
[1] Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Uromi, Nigeria
[2] Lassa Fever Enable Study CEPI/ISTH Irrua, Nigeria
[3] Department of Community Medicine, Irrua Specialist Teaching Hospital (ISTH) Irrua, Nigeria

Iyevhobu KO
A) Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Uromi, Nigeria
B) Lassa Fever Enable Study CEPI/ISTH Irrua, Nigeria

Abinokhauno SO
Quality Assurance Department, R-Jolad Hospital, Lagos State, Nigeria

Omolumen LE
Department of Chemical Pathology, Ambrose Alli University, Ekpoma, Nigeria

Ken-Iyevhobu BA
St. Kenny Research Consult, Ekpoma, Nigeria

Correspondence
Iyevhobu KO
A) Department of Public Health, National Open University of Nigeria, Uromi Community Study Centre, Uromi, Nigeria
B) Lassa Fever Enable Study CEPI/ISTH Irrua, Nigeria

Nasal carriage of *Staphylococcus aureus* and its antibiotics susceptibility isolated from secondary school students

Airefetalor AI, Iyevhobu KO, Abinokhauno SO, Omolumen LE and Ken-Iyevhobu BA

Abstract
Staphylococcus aureus is ubiquitous and may be a part of human flora found in the axillae, the inguinal and perineal areas, and the anterior nares. This study was undertaken to evaluate the nasal carriage of *Staphylococcus aureus* and its susceptibility to ascertain commonly used antibiotics in Ujoelen secondary school students, Ekpoma Edo State. The study population comprises of Ujoelen Secondary School students within the age range of 11-16 years with a number of 40 males and 40 females. A total of eighty (80) nasal swabs from Ujoelen secondary school students were used in the study. Eighty subjects were enrolled for this study without any sign of illness. Samples were taken by cotton swab from the nasal cavity and properly labelled with subjects' name, sex, age, and serial number were used for sample collection. Nasal swabs were collected in good light vision from subjects bending their heads backward to collect the specimens deep down the anterior passages using a sterile swab stick. Both right and left nostrils were used. The swab sticks were carefully returned to their sterile containers, sealed with adhesive tape and labelled accordingly. Collected specimens were taken to the laboratory where bacteriological analyses were carried out immediately. Out of the eighty (80) swabs sticks sampled, thirty (30) yielded growth out of which twenty-eight (28) are *Staphylococcus aureus* and the other two (2) were *Streptococcus* species. Out of the eighty (80) nasal swab sticks sampled, Forty (40) Male and Female were sampled each. Out of the sampled male, 17 (42.5%) were positive to *Staphylococcus aureus* and out of the 40 female sampled, 11 (27.5%) were positive for *Staphylococcus aureus.* The percentage prevalence of the male samples from the total number of samples collected in the study was higher (21.25%) than that of the female samples (13.75%) in the study. The current findings clearly highlighted the significance of implementation of efficient quality control systems in areas of direct contact with children in ill health and future research addressing effective methods for sustained eradication of Staphylococcal nasal carriage. In conclusion, a relatively high prevalence rate of *Staphylococcus aureus* in nasal carriage was recorded among the investigated among secondary school students in Ujoelen Secondary School students, Ekpoma, Edo State.

Keywords: Nasal, Carriage, *Staphylococcus aureus,* antibiotics, secondary school, students

Introduction
Staphylococcus aureus is ubiquitous and may be a part of human flora found in the axillae, the inguinal and perineal areas, and the anterior nares. Von *et al.,* (2001) [24] described 3 patterns of carriage: those who always carry a strain, those who carry the organism intermittently with changing strains, and a minority of people who never carry *Staphylococcus aureus* (Bayer *et al.*, 1998; Momoh *et al.*, 2012) [2, 18]. Persistent carriage is more common in children than in adults (Iwase *et al.,* 2010) [10]. Nasal carriers may be divided into persistent carriers with high risk of infection and intermittent or non-carriers with low risk of infection (Blot *et al.,* 2002) [3]. Persistent nasal carriage depends on host genetic determinants (Liu *et al.,* 2005) [16]. *Staphylococcus* is a versatile microorganism which is an important hospital and community pathogen (Neely and Maley, 2000) [20]. Direct invasion through breaks in the skin or mucus membrane leads into the production of superficial local infections such as folliculitis, furuncles and abscesses. Antibiotic treatment of these infections has become difficult as multidrug resistance is a common feature in *Staphylococcus aureus* (Francois and Schrenzelg 2008) [9]. Some enzymes such as penicillinase produced by *Staphylococcus aureus* is responsible for its resistance to penicillin group of antibiotics (Foley and Perret, 2006) [8]. Penicillinase positive *Staphylococcus aureus* infections are currently being treated using penicillinase resistant drugs such as methicillin,

oxacillin and nafcillin. *Staphylococcus aureus* has been shown to develop resistance to these antibiotics as well (Shafiei *et al.*, 2011; Abinokhauno *et al.*, 2018; Iyevhobu *et al.*, 2022b) [22, 1, 13]. Penicillinase producing *Staphylococcus aureus* are relatively methicillin-resistant and has an increased resistance pattern of *Staphylococcus aureus* to antibiotics use, which has relatively increases resistance and failure of antibiotics used in conventional treatment and management of *Staphylococcus aureus* infections (Iyevhobu *et al.*, 2022b) [13].

Although *Staphylococcus aureus* infections were historically treatable with common antibiotics, emergence of drug-resistant organisms is now a major concern (Deleo and Chambers, 2009; Liebowitz, 2009) [6, 15]. Staphylococci are gram positive cocci of uniform size, occurring characteristically in groups but also singly and in pairs. They are non-motile and non-capsulated (Cheesbrough, 2000) [5]. *Staphylococcus aureus* is the most medically important member in terms of pathogenicity of the group (Ochei and Kolhatkar, 2000) [21].

Staphylococcus is present in the nose of 30% of healthy people and may be found on the skin. It causes infection most commonly at sites of lowered host resistance, such as damaged skin or mucous membrane (Humphrey, 2007; Iyevhobu *et al.*, 2022a,b) [12, 13]. Although 50 – 60% of patients with MRSA are merely colonised (i.e. they carry the bacteria but do not have symptoms or an illness), serious infections such as those involving the blood stream, respiratory tract and bones or joints do occur (Humphrey, 2007). *S. aureus* causes boils, pustules, styes, impetigo, infections of wounds (cross-infections), ulcers and burns, osteomyelitis, mastitis, septicaemia, meningitis, pneumonia and pleural empyema. Also, toxic food poisoning (rapid onset, no fever), toxic shock syndrome and toxic skin exfoliation (Chessbrough, 2000).

Mannitol salt agar is a useful selective medium for recovering *S. aureus* from faecal specimens when investigating staphylococcal food poisoning. It can also be used to screen for nasal carriers. *S. aureus* ferments mannitol and is able to grow on agar containing 70 – 100g/l sodium chloride. Mannitol salt agar containing 75g/l sodium is recommended particularly for isolating MRSA strains (Cheessbrough, 2000; Momoh *et al.*, 2012) [5, 18]. On mannitol salt agar, *S. aureus* produces yellow colonies (Ochei and Kolhatkar, 2000) [54]. The MRSAs are usually sensitive to vancomycin (Ochei and Kolhatkar, 2000) [21]. Flucloxacillin and chloxacillin are used to treat β-lactamase (penicilinase) producing staphylococci. Vacomycin is often needed to treat MRSA infections. Antibacterial resistance to penicillin may occur due to the β-lactamase production, cell membrane alterations reducing antibiotic uptake (gram negative bacteria), or changes in the penicillin-binding protein as occurs with MRSA (Cheesbrough, 2000; Iyevhobu *et al.*, 2021) [5, 11]. There is no effective immunisation with toxoids or bacterial vaccines for preventing the spread of *S. aureus* (Levinson and Jawetz, 2002) [14].

Healthy carriers are potential source of *Staphylococcus aureus* infection and spread to other body sites as well as to other individuals. *Staphylococcus aureus* have been found frequently as aetiologic agent of a variety of human infections. Centre for disease control (CDC) reported *Staphylococcus aureus* as primary source of infections, which could be transferred from individual to another. The organism also elaborates toxins that can cause specific diseases or syndromes and likely participate in the pathogenesis of staphylococcal infection. Enterotoxin-producing strains of *S aureus* cause one of the most common food-borne illnesses (food poisoning) (Momoh *et al.*, 2012) [18]. The most common presentation is acute onset of vomiting and watery diarrhea 2-6 hours after ingestion. The symptoms are usually self-limited. The cause is the proliferation of toxin-producing organisms in uncooked or partially cooked food that an individual carrying the staphylococci has contaminated (Matthews *et al.*, 1997; Iyevhobu *et al.*, 2022a) [17, 12].

Staphylococcus aureus has been found frequently as an aetiological agent of a variety of human infections. Methicillin/resistance and penicillinase producing strain are potential source of nosocomial infections in patients and healthcare workers (Abinokhauno *et al.*, 2018; Iyevhobu *et al.*, 2022b) [1, 13]. There seems to be a steady rise of MRSA isolates resistance to commonly used antibiotics like Sulbatam and Vacomycin (Abinokhauno *et al.*, 2018) [1]. Centre for disease control (CDC) reported MRSA as primary source of nosocomial infections, which could be transferred from patients to patients, patients to health workers, health workers to health workers and health workers to patients. Failure of antibiotics activities in treatment of *Staphylococcus aureus* infections is increased due to resistance, a defining characteristics of penicillinase producing *Staphylococcus aureus* (Iyevhobu *et al.*, 2022a) [12]. This study was undertaken to evaluate the nasal carriage of *Staphylococcus aureus* and its antibiotics susceptibility in Secondary school students, Ekpoma, Edo State.

Materials and Methods

Area of Study

This study was carried out in Ujoelen secondary school in Ekpoma, the administrative headquarters of Esan West Local Government Area of Edo State, Nigeria. The area proper lies between latitudes $6^0$43' and $6^0$45' North of the Equator and longitudes $6^0$6' and $6^0$8' East of the Greenwich Meridian (Aziegbe, 2006). Ekpoma has a population of 127,718 (NPCN, 2012), majority of which are civil servants, traders, businessmen/women, transporters, farmers, teachers/lecturers and students by occupation (Aziegbe, 2006). Ekpoma is made up of many quarters including Eguare, Iruekpen, Emaudo, Ujoelen, Ihumudumu, Illeh, Uke, Uhiele, Ujemen, Ukpenu, Idoa, Ukhun, Egoro, Emuhi, Igor and Idumebo (Aziegbe, 2006). Ujoelen is considered in this study.

Study Population/ Size

The study population comprises of Ujoelen Secondary School students within the age range of 11 - 16 years with a number of 40 males and 40 females. A total of eighty (80) nasal swabs from Ujoelen secondary school students were used in the study.

Ethical Approval

This was obtained from the university ethics committee. Also, permission and consent was sought from the headmaster of the secondary school by presenting a letter of recommendation from my department. The aim and objectives, economic importance and benefits of the study to the subjects and society were well stated also the subjects consent and ascent were sought, only those students that gave their consent and ascent were enrolled.

Collection of Samples

Eighty subjects were enrolled for this study without any sign

of illness. Samples were taken by cotton swab from the nasal cavity and properly labelled with subjects' name, sex, age, and serial number were used for sample collection. Nasal swabs were collected in good light vision from subjects bending their heads backward to collect the specimens deep down the anterior passages using a sterile swab stick. Both right and left nostrils were used. The swab sticks were carefully returned to their sterile containers, sealed with adhesive tape and labelled accordingly. Collected specimens were taken to the laboratory where bacteriological analysis was carried out immediately.

Sample Analysis/Methods

The sample analysis was done in the Microbiology Laboratory of St. Kenny Research Consult, Edo State for bacteriological examination. Swabs were cultured on Manitol salt agar (MSA) and sub cultured on Nutrient agar (for antibiotics sensitivity) and incubated at 37°C. Different biochemical tests were applied; catalase test, Oxidase test and coagulase test. Microorganisms were recognized on the basis of macroscopic, microscopic and differential tests.

Method for detection of *Staphylococcus aureus*

The colonies that were yellow pigmented or cream white (Cheesbrough, 2000) [5] were sub-cultured onto mannitol salt agar and selected for catalase (using H_2O_2) and coagulase tests (using plasma). Mannitol fermenting and slide coagulase positive isolates were identified as *Staphylococcus aureus*.

Antibiotic Sensitivity Test

Antibiotic discs such as Erythromycin, Gentamycin, Streptomycin, Ciprofloxacin, Ampicillin, Septrin, Zinnacef, Amoxycilin and Rocephin (manufactured by Abtek Biologicals Ltd) were used to test the susceptibility of *Staphylococci aureus* isolates obtained. The test isolates were inoculated into sterile peptone water broth. The antibiotic discs were placed aseptically on the seeded plate. They were incubated at 37^0C for 24hours and examined for zones of inhibition. The zones of inhibition were measured in millimetres and recorded. Antibiotic zones less than 10mm in diameter were recorded as been resistant (R) by the organism while those with diameters of 10mm and above were recorded as sensitive (S)

Statistical Analysis

The collected data was expressed as Frequency and percentage. Comparison of qualitative variables was made using chi-square test. In all cases studied, the difference having $p<0.05$ were considered statistically significant using interactive calculation Chi square tool software (version 18).

Result

The present study evaluates the nasal carriage of *Staphylococcus aureus* and its antibiotics susceptibility in Ujoelen secondary school students, Ekpoma, Edo State. Based on standard bacteriological analytical methods used in the investigation of 80 samples of nasal swab, the following results were arrived at.

Table 1 Distribution of *Staphylococcus aureus* among Studied population. Out of the eighty (80) swabs sticks sampled, thirty (30) yielded growth out of which twenty-eight (28) were *Staphylococcus aureus* and the other tw0 (2) are *Streptococcus* species.

Table 2 Distribution of *Staphylococcus aureus* among Studied Subjects in Relation to Sex. Out of the eighty (80) nasal swab sticks sampled, forty (40) Male and Female were sampled each. Out of the sampled male, 17 (42.5%) were positive to *Staphylococcus aureus* and out of the female samples, 11 (27.5%) were positive to *Staphylococcus aureus.* The percentage prevalence of the male samples from the total number of sample collected in the study was higher (21.25%) than that of the female samples (13.75%) in the study.

Table 3 shows the Distribution and Percentage Prevalence of *Staphylococcus* species among Studied Subjects with respect to Age. From the study students of age thirteen (13) had the highest percentage prevalence of *Staphylococcus aureus* while those of age eleven (11) had the lowest percentage prevalence.

Table 1: Distribution of *Staphylococcus aureus* among Studied

Sample Type	Number Sampled	Number of Growth	Number of *Staphylococcus aureus*	Other bacteria Isolated
Nasal Swab	80	30	28	2

Table 2: Distribution of *Staphylococcus aureus* among Studied Subjects in Relation to Sex

Sex	Number of Sample	*Staphylococcus aureus* isolated (%)	Total Percentage Prevalence of Infection
Male	40	17 (42.5)	21.25
Female	40	11 (27.5)	13.75
Total	80	28	35.00

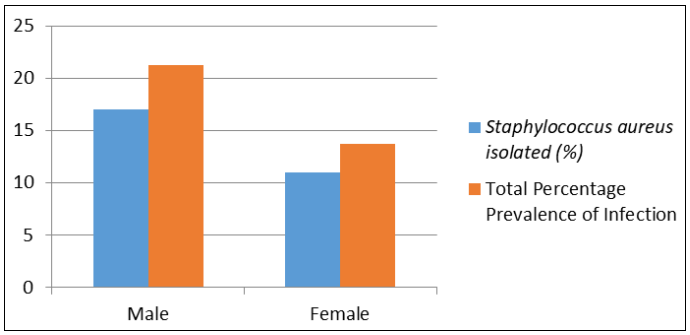

Fig 1: Distribution of *Staphylococcus aureus* (%) and total percentage prevalence of infection.

Table 3: Distribution and Percentage Prevalence of *Staphylococcus* species among Studied Subjects with respect to Age

Age	Number of Samples Collected	Number of *Staphylococcus aureus* (%)	Total Percentage Prevalence (%)
11	5	3 (60)	3.75
12	15	5 (33.3)	6.25
13	15	6 (40)	7.5
14	15	5 (33.3)	6.25
15	15	5 (33.3)	6.25
16	15	4 (26.6)	5.0
Total	80	28	35.00

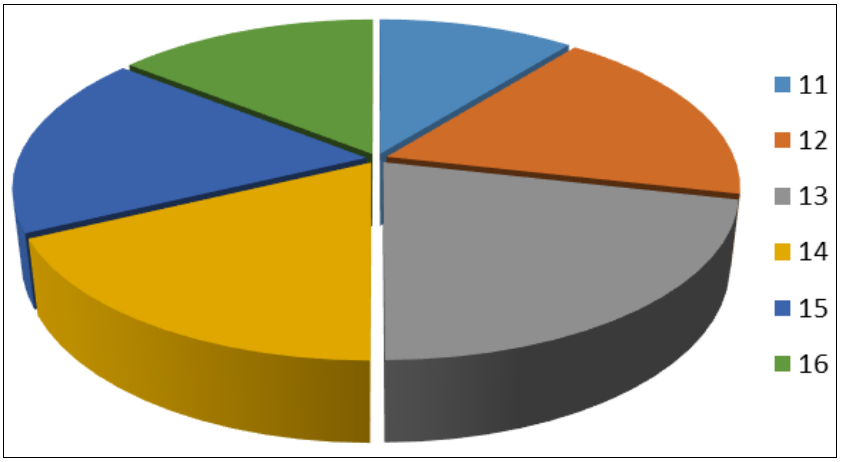

Fig 2: Total Percentage Prevalence (%) of *Staphylococcus* species among Studied Subjects with respect to Age

Table 4: Antibiotics Susceptibility rate of *Staphylococcus aureus* Isolates

Antibiotics	Frequency	Percentage Susceptibility (%)
Erythromycin	20/28	71.4
Gentamycin	24/28	85.1
Streptomycin	14/28	50
Ampiclox	22/28	78.5
Rifampicin	24/28	85.1
Ciproflox	22/28	78.5
Amoxil	28/28	100
Levofloxacin	24/28	85.1
Norfloxacin	22/28	78.5
Chloramphenicol	8/28	28.5

Table 5: Cultural Characteristics and Biochemical Analysis of Bacterial Isolates

Organism	Cultural characteristics				Biochemical analysis							
	Shape	Elevation	Consistency	Colour	Gram	Catalase	Coagulase	Indole	Motility	Oxidase	Citrate	Urease
Staphylococcus aureus	Cocci in cluster	Convex	Moist	Golden yellow	+	+	+	_	_	_	+	+
Streptococcus spp	Cocci in chains	Convex	Moist	Shinny greyish white	+	_	_	_	_	_	_	_

Key: + = Positive; - = Negative

4. Discussion

Staphylococcus species are regional flora of the skin and mucus membrane of the body, certain species have been found frequently as aetiological agents of a variety of human and animal infections. The most common among these infections are the superficial supportive infection caused by *Staphylococcus aureus*. Infection can lead to life threatening conditions and disease spectrum which includes abscesses, septicemia, osteomyelitis, endocarditis and cellulitis, pneumonia, in addition to various toxin mediated diseases as toxic shock syndrome and staphylococcal food poisoning. The variety of such spectrum of clinical manifestations is mostly dependent on the numerous virulence factors produced by each strain (Vasconcelos and da Cunha, 2010) [23]. The ingestion of the preformed toxins produced by *Staphylococcus aureus* (enterotoxigenic strains) in food often results to the development of food poisoning.

Findings from this investigation indicate a significant (P< 0.05) distribution of *Staphylococcus aureus* of 17(21.25%) prevalence from male subjects with the highest occurrence when compared to the female subjects 11(13.75%) which is in agreement with investigation reported by Mous-tafa *et al.*, (2013) [19] of 10.5% nasal carriage of *Staphylococcus aureus* in women. The findings from this study in relation to area of study, was not in agreement with findings report by Eke *et al.*, (2015) [7], with a wide variation of 60% prevalence from 100 nasal swab analysis of primary school children in Ekpoma. The results of the findings of this study was higher than that discovered by Iyevhobu *et al*. (2022b) [13] were they had chi-square 30 (15%) *Staphylococcus aureus* prevalence with the highest occurrence of 24 (30%) from skin swabs and 6 (7.5%) from nasal swabs. In a study carried out by Iyevhobu *et al*. (2022a) [12], shows the

prevalence of *Staphylococcus aureus* among food handlers and restaurant workers, samples collected from anterior nasal nares of subject with high prevalence of 24(16%) Staphylococcus aureus to that of the skin sample of 6(7.5%). From the total of 67 (22%) bacteria infected subject which was comparatively significant (P>0.05) with X2Cal 18.4, P-value 0.000.

Previous studies have shown that gender, age, marital status or level of education had no significant effect with respect to the nasal carriage of *Staphylococcus aureus* (Eke *et al.*, 2015) [7]. This study has revealed that nasal nares of secondary school students harbour *Staphylococcus aureus* 28(35%). Prevalence and distribution of *Staphylococcus aureus* in relation to gender showed lesser occurrence in female 11(13.75%) than males 17(21.25%). This was not in agreements with the findings by Eke *et al.*, (2015) [7], which reported male to have higher prevalence than the females in Ekpoma and Iyevhobu *et al*. (2022a) [12] which reported high occurrence of Staphylococcus aureus in female food handlers and workers of 17 (56.6%) from females and 13 (43.3%) from males co-workers with no statistical significant difference (P>0.05) distribution of *Staphylococcus aureus* (X2cal=1.663, p-value=0.435). The disparity of this report may be due to the types of gender of the subjects who consented more to participate as at that time of study.

The sensitivity pattern of *Staphylococcus aureus* isolated from this study had high susceptibility to Amoxil, Gentamycin, Rifampicin, Levofloxacin, Norfloxacin, Ciprofloxacin, Ampiclox and Erythromycin and intermediate to Streptomycin, and resistant to Chloramphenicol which is in agreement with the study reported by Eke *et al.*, (2015) [7] and Iyevhobu *et al*. (2022a) [12] who shows the sensitivity pattern of Staphyloccus aureus isolated from food handlers and restaurant workers. Staphyloccus aureus had various degree of sensitivity to antibiotics used and was sensitive to Gentamycin (70%), Zennacef, (93%), Rocephin (80%), Ciprofloxacin (93%), intermediate sensitivity to Septrin (53%), Streptomycin, and resistant to Erythromycin (60%), Amoxacilin (36) and Ampiclox (17%). From this research it has been revealed that nasal nares harbours *Staphylococcus aureus* which are probable source of the enterotoxigenic stains causing boils, impertigo, pimples etc observed in children these days.

Conclusion

From all the organisms known to cause skin and nasal infections, *Staphylococcus aureus* is the most prevalent among them that is easily isolated. It colonizes the skin and mucosal surfaces of healthy individuals. Evidence from the results obtained have shown that the nasal nares have high carrying capacity of *Staphylococcus aureus*.

In conclusion, a relatively high prevalence rate of *Staphylococcus aureus* in nasal carriage was recorded among the investigated among secondary school students in Ujoelen Secondary School students, Ekpoma, Edo State. Moreover, 10% of the investigated carriers harboured *Staphylococcus aureus* in their anterior nares increasing the likelihood of transmission of the pathogen. These findings resurges the imperative need for protective measures by public school among their students.

Finally, the current findings clearly highlighted the significance of implementation of efficient quality control systems in areas of direct contact with children in ill health and future research addressing effective methods for sustained eradication of Staphylococcal nasal carriage.

Conflict of interest

The authors declare no conflicts of interest. The authors alone are responsible for the content and the writing of the paper.

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