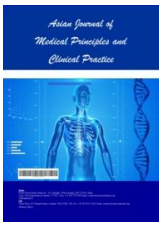

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Isolation, Molecular Identification and Plasmid Profiling of *Staphylococcus* Species from Nasal Carriage of University Some Students of Rivers State University, Port Harcourt, Nigeria

U. Oparaodu[1*] N. N. Odu[2] and G. A. Uzah[2]

[1]ENT Department, College of Medical Sciences, Rivers State University Port Harcourt, Nigeria.
[2]PAMO University of Medical Science, Rivers State, Nigeria.

Authors' contributions

This work was carried out in collaboration among between authors. Both authors read and approved the final manuscript.

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ABSTRACT

Nasal carriage with *Staphylococcus* species is a common risk factor for invasive infections, indicating the necessity to monitor prevalent strains, This study was aimed to isolation, molecularly identify, and profile the plasmid of *Staphylococcus* species from the nasal carriage of some University students of Rivers State University, Port Harcourt, Nigeria. Fifty (50) Nasal swab samples were collected from the anterior nares of some students of Rivers State University using sterile cotton swabs. The samples were examined for the presence of *Staphylococcus* species using standard bacteriological methods by streaking on mannitol salt agar and incubated for 24 hours at 37.2^0C. Identification and plasmid proofing of the isolated *Staphylococcus* species were carried out using genomics studies. The results showed that 48 out of the 50 samples examined were positive *Staphylococcus* species which includes *Staphylococcus aureus, Staphylococcus gallinarum*, *Staphylococcus simulans* , *Staphylococcus sciuri,* and *Staphylococcus Camosus,* this indication that *Staphylococcus* species does not only colonize sick or individuals with the sign of infection but also healthy individual, The results of plasmid profiling of the five *Staphylococcus*

**Corresponding author: E-mail: nodu@pums.edu.ng;*

species revealed that all the species posses plasmid which is capable to enhance their resistance to the commonly used antibiotics The presence of these *Staphylococcus* species is a serious public concern because even though only *S. aureus* is the only known human pathogen others could be opportunistic pathogens. Therefore continuous surveillance and improvement of personal hygiene standards among students is highly recommended such as hand washing with soap and warm water since *Staphylococcus* species can quickly be spread among students.

Keywords: Isolation; Staphylococcus; nasal; carriage; plasmid; and molecular.

1. INTRODUCTION

The adult nasal microbiota differs between individuals, but species belonging to *Corynebacterium*, *Propionibacterium*, and *Staphylococcus* genera are the most abundant [1-3]. In a study conducted on the nasal microbiota of 178 adults, 88.2% were *Corynebacterium* carriers, 83.7% *Propionibacterium* acnes carriers, and 90.4% *Staphylococcus epidermidis* carriers [4]. Proportional abundance varied considerably between individuals [5]. The health status may influence the nasal microbiota and vice versa. In a study involving healthy and hospitalized individuals, healthy adults harbored nares microbiota dominated by Actinobacteria *(*mainly *Propionibacterium* and *Corynebacterium sp.)* whereas patients' microbiota were dominated by *S. aureus* and *S. epidermidis*. *S. aureus* colonization was negatively associated with the presence of other bacteria including *S. epidermidis* [1] Such counterweight effect between bacteria could be as a result of interdependent activation- inhibition mechanisms as reviewed by Krismer et al. [6]. In fact, some bacterial species are capable of secreting anti-staphylococcal molecules modulating *S. aureus* abundance, For instance, in vitro production of H_2O_2 by *Streptococcus pneumoniae* can be bactericidal on *S. aureus* [7]. Recently, an in vitro and the human study demonstrated that lugdunin, a non-ribosomal synthesized bioactive compound produced by *Staphylococcus lugdunensis*, can prevent S. aureus nasal colonization via a bactericidal effect [8].

Nasal carriage with *Staphylococcus* species is a common risk factor for invasive infections, indicating the necessity to monitor prevalent strains, particularly in the vulnerable pediatric, and student population.

Staphylococcus aureus is a gram-positive commensal bacterium of humans causing a wide variety of diseases ranging from simple skin and soft tissue infections to severe life-threatening septicaemic conditions [9]. It is a pathogen of greatest concern because of its inherent capacity to cause resistance, cause severe infections by production of different types of toxins and its ability to adapt to different environmental conditions [10,4]. *Staphylococcus aureus is* considered as the leading cause of nosocomial infections. The primary mode of transmission of *S. aureus* is by direct skin-to-skin contact and also by indirect contact with contaminated objects or surfaces [3] An important character of *S. Aureus* is its ability to colonize the skin and mucous membrane of the anterior nares, gastro-intestinal tracts, perineum, the genitourinary tracts and pharynx [11], in healthy individuals without any symptoms [12]. Nasal carriage of S*taphylococcus* species especially *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection. The ecological niches of *s. aureus* are the anterior nares. In healthy subjects, over time, three patterns of the carriage can be distinguished: about 20% of people are persistent carriers, 60% are intermittent carriers, and approximately 20% almost never carry *s. aureus*. The molecular basis of the carrier state remains to be elucidated. In patients who repeatedly puncture the skin and patients with human immunodeficiency virus (HIV) infection, increased carriage rates are found. The carriage has been identified as an important risk factor for infection in patients undergoing surgery, those on hemodialysis or those with HIV infection and aids, those with intravascular devices, and those colonized with MRSA.

The anterior nares of the nose are the primary reservoir for replication and spread to other body sites. The organism is in a carrier state in the anterior nares and can remain so without causing infections for weeks or months. According to Odu and Okonko [13]

The association between the nasal carriage of *S. aureus* and subsequent infection has been comprehensively established. It has been shown

that nasal carriers of *S. aureus* have an increased risk of acquiring an infection with this pathogen. The anterior nares of the nose are the main reservoir of *S. aureus* in both adults and children [10], though there is a paucity of information about the various species that are associated with the anterior nares. Therefore, this study was aimed at Isolation, Molecular identification, and plasmid profiling of *Staphylococcus* species from nasal carriage of University Students of Rivers State University, Port Harcourt, Nigeria.

2. MATERIAL AND METHODS

2.1 Study Area

The study was conducted in the Department of Microbiology of Rivers State University located at Nkpolu-Oroworukwo in Port Harcourt, the capital of Rivers State, Nigeria.

2.2 Sample Collection

Nasal swabs were collected from the anterior nares of 50 students using sterile cotton swabs (Improswab, Guangzhou, China) moistened with normal saline. Both nostrils were sampled one at a time using the same swab by rotating gently against the inner surface swabs were placed in Stuart's transport media (in-house made) (Oxoid, Basingstoke, UK) and transported to the Microbiology Laboratory at Rivers State University for processing within eight hours of collection.

2.3 Isolation of *Staphylococcus* Species

The method adopted by Odu and Okonko [13] was employed for the isolation of *Staphylococcus* species. Each Nasal swab sample was inoculated by streaking on Mannitol salt agar plates (Oxoid, Basingstoke, UK), the plates were incubated at 37.2°C and examined for growth after 24–48 hours. for *Staphylococcus* species isolated were initially screened based on their cultural morphology on Mannitol salt agar.

2.4 Identification of the Isolates

The isolates were primarily identified using some basic biochemical reactions such as Gram's staining, catalase test (positive), indole test, methyl red test, Voges-Proskauer test, citrate utilization test, urease test, sugar fermentation test, and nitrate reduction test (positive). A sugar fermentation test was performed using seven sugars which included fructose, galactose, sucrose, arabinose, Raffinose, lactose, and trehalose.

2.5 Molecular Identification

2.5.1 DNA extraction (using ZR bacterial DNA miniprep)

The bacterial DNA from two milliliters (2ml) of bacterial cells in Luria Bertani broth (LB) were extracted using ZR bacterial DNA Miniprep Manufactured by Zymo research cat number: D6005 following the Manufacturer's analytical procedures.

2.5.2 DNA quantification

The extracted genomic DNA was quantified using the Nanodrop 1000 spectrophotometer. The software of the equipment was launched by double-clicking on the Nanodrop icon. The equipment was initialized with 2 µl of sterile distilled water and blanked using normal saline. Two microlitres of the extracted DNA were loaded onto the lower pedestral; the upper pedestral was brought down to contact the extracted DNA on the lower pedestral. The DNA concentration was measured by clicking on the "measure button and the concentration of the extracted genomic DNA was displayed on a computer screen [14].

2.5.3 16S rRNA amplification

The 16s rRNA genes of the isolates were amplified using the universal primers on an ABI 9700 Applied Biosystems thermal cycler at a final volume of 25 microlitres for 35 cycles. The PCR mix was made up of 12.5µL of Taq 2X Master Mix from New England Biolabs (M0270); 1µL each of 10µM 16SrRNA gene forward primer (16SF GTGCCAGCAGCCGCGCTAA) and reverse primer (16SR: AGACCCGGGAACGTATTCAC); 3µL of DNA template and then made up with 7.5µL Nuclease free water.

2.5.4 Cycling conditions for 16SrRNA gene

Initial denaturation at 94˚C for 5 mins, followed by 36 cycles of denaturation at 94˚C for 30 sec, annealing at 56˚C for 30 secs and elongation at 72˚C for 45 sec. Followed by a final elongation step at 72˚C for 7 minutes. The product was resolved on a 1% agarose gel at 120V for 20 minutes and visualized on a blue light transilluminator [15].

2.5.6 Sequencing

The PCR products were purified using absolute ethanol and then sequences on ABI 3500 Genetic Analyser (Thermofisher, USA), and all analysis were performed using Bio-Edit sequence editor, and organisms were identified by aligning isolate sequences with those on NCBI via BLAST tool.

2.5.7 Plasmid extraction

The plasmid was extracted using the Zyppy plasmid mini-prep extraction kit manufactured by Zymo Research Inc, the USA was used according to the manufacturer's procedure.

2.5.8 Gel electrophoresis

Plasmids were separated by electrophoresis in 1% agarose (Sigma Aldrich, USA) at a voltage of 4.5 V/cm; the buffer used was TAE (Tris-AcetateEDTA) for 3 hours. Following electrophoresis, the gels were stained for 15 minutes with ethidium bromide solution (1.0 µg/ml EtBr in 0.5 x TrisAcetate-EDTA (TAE)), and then observed under UV light. The image was registered and analyzed using Quantity One software, version 4.1 14]

3. RESULTS AND DISCUSSION

3.1 Occurrence *Staphylococcus* Species

Out of the total number of fifty samples screened for the presence of *Staphylococcus* species among students, 48(96%) were positive of *Staphylococcus* species. Table 1 gives the frequency and percentage of occurrence of *Staphylococcus* species according to age of students. The study showed that there was no significant difference at $P<0.05$ between age groups for carriage rates of *Staphylococcus* species. The year group within 17 – 22 recorded 100% while the Year group within 28 – 33 recorded 83% among the population studied (Table 1). Frequency *Staphylococcus* species among the healthy and students that shown signs of respiratory infection are presented in Fig. 1. The results revealed that out of the 35 nasal swab samples from the healthy students 33 were positive of *Staphylococcus* species while all the 15 nasal swab samples from students that developed sign of infection were positive.

The results of prevalence of *Staphylococcus* species obtained in the study have proven the report of Pathak et al*.,* [10] that the anterior nares of the nose have been serving as the main reservoir of *S. aureus* in both adults and children. Odu and Okonko [13], also reported Nasal carriage of Staphylococcus species especially *S. aureus* has an increased risk of developing infections. *Staphylococcus aureus* carriage appears to play a key role in the epidemiology and pathogenesis of infection because carriage often proceeds to infection.

The high frequencies of *Staphylococcus* species recorded from healthy students (33 out of 35 Nasal swab samples) is an indication that *Staphylococcus* species does not only colonize sick or individuals with the sign of infection but also healthy or individuals our findings is in line with the report of Peacock et al*.,* [6], who observed that There is increasing evidence that community-acquired methicillin-resistant *S. aureus* (CA-MRSA) is spreading among healthy individuals, especially students.

3.2 Distribution and Percentage Occurrence of the *Staphylococcus* Isolates

The results of distribution and percentage occurrence of the *Staphylococcus* species from the nasal carriage among the students revealed shows that from the conventional and genomics identification, five different species of *Staphylococcus* which includes *Staphylococcus aureus* (30%), *Staphylococcus gallinarum* (26%), *Staphylococcus simulans* (20%), *Staphylococcus sciuri* (12%) and *Staphylococcus Camosus* (12%) were isolated and identified in this study with varied percentage of occurrence as showed in the parenthesis. The result showed that *Staphylococcus aureus* recorded the highest percentage of 30% followed by *Staphylococcus gallinarum* which had 26%, *Staphylococcus sciuri, and Staphylococcus Camosus* had the lowest percentage of 12% respectively as presented in Fig. 2. The evolutionary history of the *Staphylococcus isolated* in this study was inferred using the Neighbour-Joining method and the bootstrap consensus tree inferred was from 500 replicates. The evolutionary distances were computed using the Maximum Composite Likelihood method and are in the units of the number of base substitutions per site. There were a total of 1190 positions in the final dataset (Fig. 3).

Table 1. Frequency and Percentage Occurrence of Staphylococcus species according to age of subjects

Age Group year	Number Tested	Number of Positive Sample
17 - 22	38	38(100%)
28- 33	12	10 (83.0%)
Total	**50**	**48 (96%)**

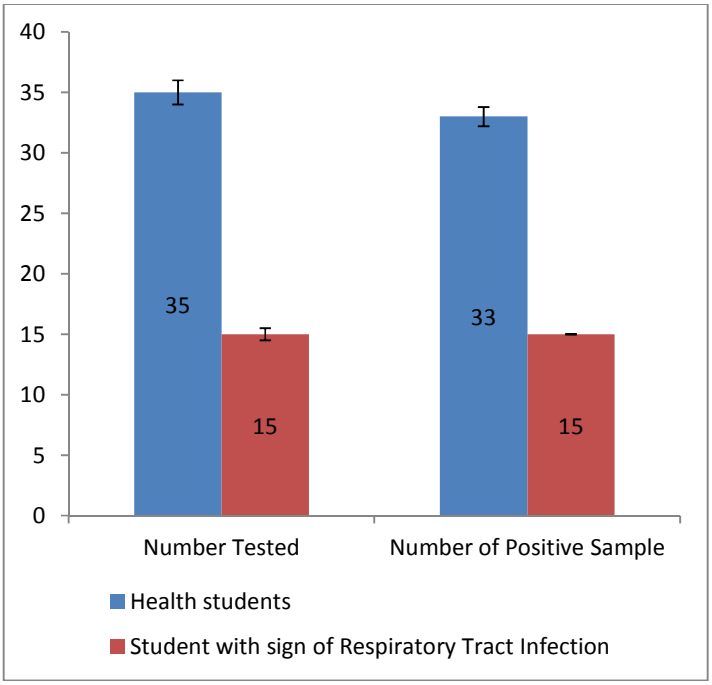


Fig. 1. Frequency Occurrence of Staphylococcus species according to age of subjects

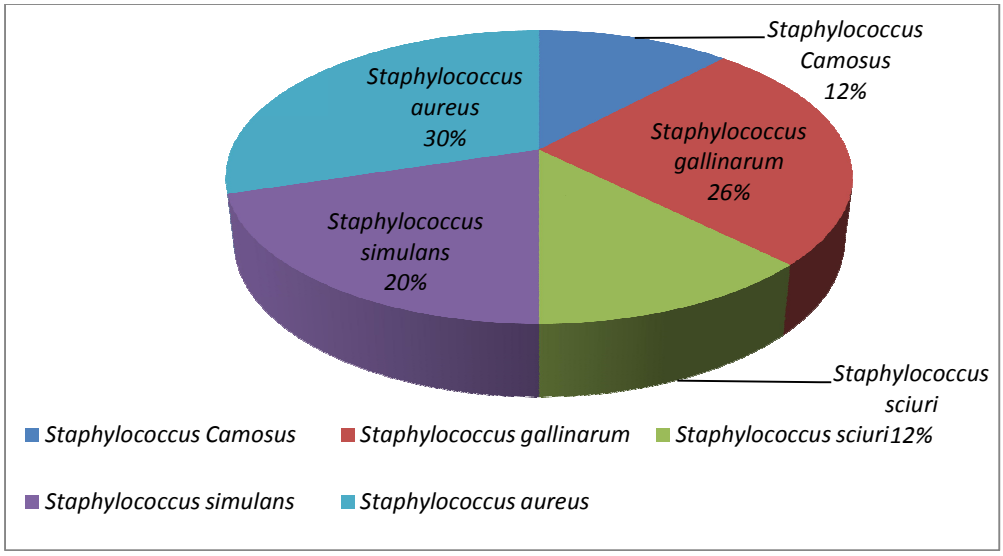


Fig. 2. Percentage occurrence of the *Staphylococcus* species

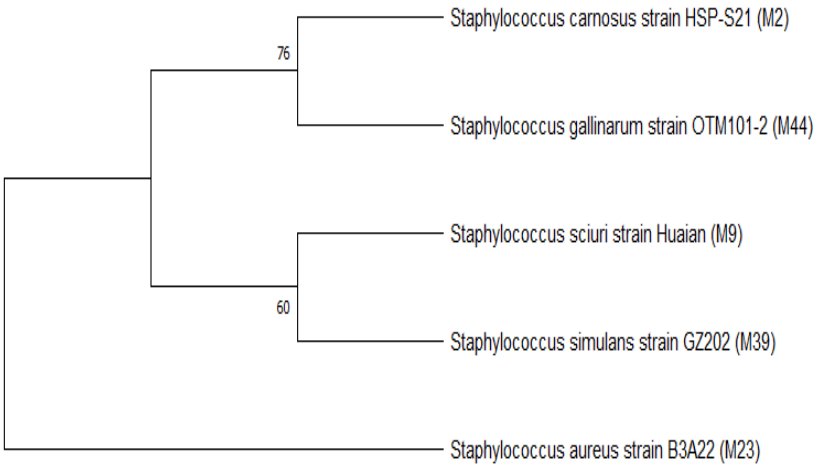
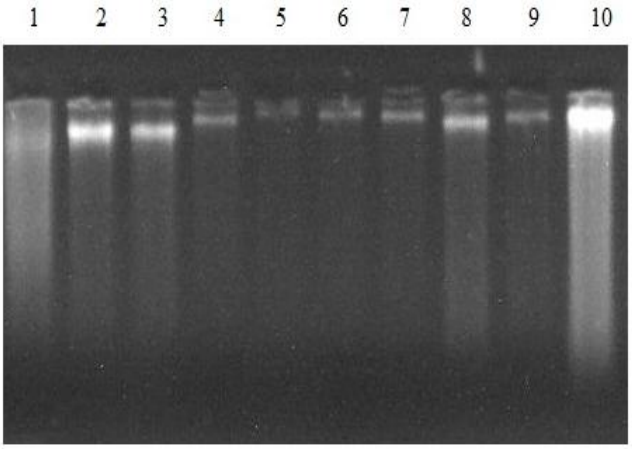


Fig. 3. Evolutionary relationships of *Staphylococcus* sp. taxa



Gel image showing DNA at genomic and Plasmid level respectively. Lane 1-5 shows DNA at genomic lavel while lane 6-10 shows DNA at Plasmid level.

Plate 1. Result of plasmid profile

The percentage of *Staphylococcus aureus* isolated from the nose swabbed of students is a serious public health threat because of its pathogenicity and epidemiology among the populace especially students. *S. aureus* has long been recognized as one of the most important bacteria that cause disease in humans. It is the leading cause of skin and soft tissue infections such as abscesses (boils), furuncles, and cellulitis.

The various *Staphylococcus* species isolated and identified in this study were subjected to further analysis for the determination of the presence of plasmid which is an extrachromosomal genetic element that occurs in many bacterial strains. Plasmids are circular deoxyribonucleic acid (DNA) molecules that replicate independently of the bacterial chromosome. They are not essential for the bacterium but may confer a selective advantage. The results showed all the five species of the Staphylococcus species identified in this study poses plasmid as showed-hown in plate 1.

The MAR index is a good tool for health risk assessment which identifies if isolates are from a region of high or low antibiotic use. A MAR index

of 0.2 indicates a 'high-risk' source of contamination. The result of MAR index obtained showed 0.7 which indicated a high level of commonly use antibiotics among students.

3.3 Discussion

A total of Fifty (50) swap samples from students of Rivers State University were analyzed for the isolation of *Staphylococcus* species associated with the nostrils of the students in years one and two of the Department f Microbiology of the University, 48 of samples out the 50 samples were positive of *Staphylococcus* species. The high frequency of occurrence and prevalence of *Staphylococcus* species recorded in this study is significantly higher than the previous reports by Chigbu and Ezeronye [16] and the reported by Kuehnert [17,18]. respectively. The higher percentage of *Staphylococcus* species recorded in this study may be attributed to the characteristics of the population under study. This study's high prevalence of nasal carriage of *Staphylococcus* species further supports the fact that anterior nares remains a principal reservoir of this organism and there is a need to eliminate its virulent strains because of their involvement in most severe community and hospital *S. aureus* infection in a colonized individual.

Genomic identification of the *Staphylococcus* species revealed that the following *Staphylococcus* which includes *Staphylococcus aureus, Staphylococcus gallinarum*, *Staphylococcus simulans*, *Staphylococcus sciuri* and *Staphylococcus Camosus* are associated with Nostril of both healthy and unhealthy individuals. The presence of these *Staphylococcus* species is a serious public concern because even though only *S. aureus* is only known human pathogen others could be opportunistic pathogens.

Staphylococcus sciuri was first described by Kloos and colleagues in [19] and is considered one of the most ancestral and dispersed staphylococcal species, with a wide range of habitats that includes the skin of several animals as well as environmental reservoirs, such as soil, sand, water, and furniture The impressive colonizing capacity of this species may result from its broad range of biochemical.

Coagulase-negative staphylococci (CoNS), once an umbrella term for normal skin microbiota, are increasingly implicated in hospital-acquired infections. Quick, easy, and accurate speciation of CoNS via matrix-assisted laser desorption ionization time-of-flight mass spectrometry was recently validated [20] Integrating this technology into hospital laboratories has allowed multiple members of the genus to surface as potential human pathogens [20], the of report a case of *Staphylococcus simulans* causing skin and soft tissue infection to alert dermatologists to this emerging Coagulase-negative *staphylococci* pathogen is on record. *S. simulans* is a CoNS and a well-established animal pathogen affecting cows, sheep, goats, and horses. It is commonly implicated in bovine mastitis. Reports of *S simulans* as the sole pathogen in human infections are rare; however, we hypothesize that our patient may have acquired infection from his farm and regular handling of animals, an identifiable risk factor. *S simulans* has also been implicated in osteoarticular infections, native valve endocarditis, and diabetic osteitis with diabetes and prosthetic joints identified as additional risk factors. Previously classified as rare opportunistic agents, *Staphylococcus lugdunensis* and *Staphylococcus schleiferi* are additional CoNS with emerging pathogenicity [21]. Limited data exist explaining how these species are gaining pathogenicity, but shared virulence factors with *Staphylococcus aureus* have been documented in infectious animal isolates, including staphylococcal enterotoxins, tissue necrosis cytotoxin.

4. CONCLUSION AND RECOMMENDA-TIONS

The high prevalence of *Staphylococcus species* (96%) isolated from nose of students between the age and isolation of five different species of Staphylococcus species namely: *Staphylococcus aureus, Staphylococcus gallinarum*, *Staphylococcus simulans* , *Staphylococcus sciuri,* and *Staphylococcus Camosus* in this study had proven that *Staphylococcus* species especially *aureus* is a normal flora of the anterior nares of the nose of both adults and children, this revealed that there serious public health risk among students especially the spread of Staphylococci infection. Therefore continuous surveillance and improvement of hygiene standards among students is highly recommended such as handwashing with soap and warm water since Staphylococcus species are quickly spread by nose picking.

CONSENT AND ETHICAL APPROVAL

As per international standard or university standard guideline participant consent and

ethical approval has been collected and preserved by the authors.

DISCLAIMER

The company name used for this research is commonly and predominantly selected in our area of research and country. There is absolutely no conflict of interest between the authors and company because we do not intend to use this company as an avenue for any litigation but for the advancement of knowledge. Also, the research was not funded by the company rather it was funded by personal efforts of the authors.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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