Nasal Carriage of Staphylococcus aureus in People Living with Human Immunodeficiency Virus (HIV) And Tuberculosis Patients In Lagos, Nigeria.

O. A Babatunde¹, O. O. Akinnola², A. David³ and E. A. Omonigbehin⁴.

Department of Biological Sciences, Covenant University, Ota, Ogun State, Nigeria¹, ², ⁴
Nigerian Institute of Medical Research, Yaba, Lagos, Nigeria³.
Corresponding Author: O. A Babatunde

Abstract: Staphylococcus aureus is one of the leading human pathogens responsible for a wide range of infections in susceptible host. Nasal carriage has been identified as the major risk factor causing varying degrees of infections in immunosuppressed individuals. This study evaluated the prevalence of nasal carriage of S. aureus individuals with HIV, TB and HIV/TB co-infection, antibiotic susceptibility pattern of the S. aureus isolates and the presence of mec A and virulence gene were also evaluated. This study was conducted at the DOT and HIV clinics of the Nigeria Institute of Medical Research (NIMR) between November 2018 and April 2019. A total of 250 nasal swab samples were collected from the anterior nares of the participants. The result showed a prevalence rate of 18.8% nasal carriage. 32 (21.3%) S. aureus isolates was obtained from 150 HIV, 11 (15%) from 70 TB and 4 (13.3%) from 30 HIV/TB co-infected individuals. Antimicrobial susceptibility to S. aureus showed (100%) sensitivity to Augmentin, ciprofloxacin 77% and (9%) sensitivity to Cotrimoxazole. Mec A gene was detected in 12 (25.5%) while Lukf-PV was detected in 1 (2.12%) of the total S. aureus isolates.

Risk factors associated with nasal carriage include recent hospitalization, frequent use of antibiotics and surgical procedures. The detection of methicillin resistance in S. aureus isolates has serious implications in the treatment of S. aureus infections in these vulnerable individuals.

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I. Introduction

Staphylococcus aureus is one of the commonest human pathogens, capable of causing a wide range of nosocomial and community acquired infections in susceptible hosts¹, ¹¹, ²¹. The organism resides in the anterior nares and nasal colonization has been identified as a risk factor due to its ability to cause both community and hospital acquired infections², ¹⁵. S. aureus is a commensal on the skin but can take up pathogenic role especially in immunosuppressed/susceptible hosts where it becomes a leading cause of pyogenic infections². S. aureus colonizes the anterior nares and an estimated 20% of humans permanently carry this organism, 60% are transient carriers and about 20% do not harbor S. aureus³. The skin serves as a defense against the development of staphylococcal infections but any break in the skin especially in immunosuppressed individuals, allows the organism gain entrance into the body system thereby causing infections ranging from skin and soft tissue infections (SSTI)¹³, ¹⁵, ¹⁷. S. aureus is highly virulent because it is endowed naturally with a number of virulent factors. Also, the organism has the ability to acquire antibiotic resistance thereby making treatment difficult¹³, ¹⁵. S. aureus has long been recognized as an important pathogen in human disease and an important pathogen in patients with HIV infection. Despite multiple reports on the severity, morbidity and mortality of S. aureus infections, the factors predisposing patients infected with HIV to S. aureus infections have not been well studied³.

Nasal colonization is an important risk factor for various S. aureus infections and studies have shown that nasal carriage is higher among human immunodeficiency virus (HIV) infected patients than in healthy individuals⁶, ⁴, ¹⁴. The African continent is the most affected with the highest burden of HIV and TB infections globally¹², ¹⁵. 68% percent are domiciled in this part of the world and this accounts for 12% of world population¹⁶. Onanuga and Temedie (2011) reported high nasal carriage of methicillin resistant Staphylococcus aureus among HIV patients. Due to an increasing number of infections caused by methicillin-resistant S. aureus (MRSA) strains, therapy has become problematic. Methicillin resistant Staphylococcus aureus (MRSA) is capable of causing infections in all individuals but infections in those with HIV are serious due to the lowered immunity¹, ⁸, ⁹. Nasal carriage of S. aureus is a major risk factor for staphylococcal infection compared to individual who do not carry S. aureus¹⁴. High incidence of S. aureus has also been reported among hospitalized patients with tuberculosis and HIV co-infection in sub-Saharan Africa which may increase

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risk of MRSA colonization and infection. Clinical manifestations ranged from superficial to systemic disease conditions and are responsible for high morbidity and mortality rate. The aim of this study was to investigate the prevalence of nasal carriage of S. aureus in individuals with HIV and TB, and to provide an insight into antibiotic sensitivity pattern of this organism.

II. Material and Methods

This study was carried out on patients of the Directly Observed Therapy and HIV clinics, Clinical Science Department, Nigeria Institute of Medical Research (NIMR), Yaba, Lagos from November, 2018 to April, 2019. A total of 250 adult subjects (both male and female) of aged ≥ 18 year, were included in the study. Study design: cross-sectional study Study location: This is a research institute based study done in the Directly Observed Clinic and HIV clinic, Clinical Science Department, NIMR, Yaba, Lagos, Nigeria. Study Duration: November 2018 to April 2019. Sample size: 250 patients Sample size calculation: Since the prevalence of Staphylococcus aureus nasal carriage in Lagos state for previous study was 27%, according to Olalekan. The samples size was estimated using Fisher’s formula:

\[ n = \frac{z^2 p (1 - p)}{d^2} \]

Where;

\( n \) = sample size
\( z \) = level of confidence according to the standard normal distribution (for a level of confidence of 95%, \( z = 1.96 \), for a level of confidence of 99%, \( z = 2.575 \))
\( p \) = known characteristic or prevalence about the population \( q = 1 - p \) (probability of failure)
\( d \) = tolerated margin of error

NB: Where prevalence is 27%, \( p = 0.27 \), \( q = 1 - 0.27 = 0.73 \). Hence;

\[ n = \frac{(1.96)^2 \times 0.27(1-0.27)}{(0.05)^2} \approx \frac{3.8416 \times 0.27 \times 0.73}{0.0025} = \frac{0.7572}{0.0025} = 249.87 \]

According to this, a minimum sample size of 250 is needed for this study.

Subjects and selection method: The study population was drawn from subjects who have been confirmed positive for HIV and Tuberculosis and are receiving treatment at DOT and HIV clinics of NIMR. Clinical Science Department. The sample size obtained for this study was 150 for HIV, 70 for TB and 30 for HIV and TB Co-infection.

Inclusion criteria: patients with established case of HIV, TB, male or female, aged ≥ 18 years who are receiving treatment at NIMR clinic and willing to sign an informed consent form.

Exclusion criteria: lack of willingness to sign the informed consent form.

Procedure methodology

Ethical approval was obtained from Institutional Review Board (IRB) of the Nigeria Institute of Medical Research, Yaba, Lagos. Written informed consent was obtained from all participants prior to enrollment into the study.

A total of two hundred and fifty nasal swabs were taken from the anterior nares of HIV, TB and HIV/TB patients using sterile cotton wool swab moistened in distilled water. The swab sticks were inserted into the patient’s nostrils, gently swirled and rotated in both anterior nares to carefully sample the mucosa. The nasal swabs were carefully placed in the swab stick containers and immediately transported to the microbiology Laboratory for analysis. The nasal swabs were inoculated aseptically on mannitol salt agar plates incubated at 37°C for 24 hours. Mannitol fermenting colonies were sub cultured on blood agar and incubated at 37°C overnight to check for characteristic β-hemolysing. Presumptive identification of S. aureus was on the basis of colony morphology, Gram staining, biochemical tests such as catalase and tube coagulase. Genotypic identification was done by amplifying a sequence of the nuc gene which encodes the thermostable nuclease of S. aureus. The isolates were then sub cultured on nutrient agar slants and stored at 4°C. The genes coding for methicillin resistance and virulence were amplified by polymerase chain reaction.

Antimicrobial susceptibility testing

Antibiotic susceptibility testing was carried out by the Kirby-Bauer disk diffusion method to test for the susceptibility of the S. aureus isolates to Gentamycin (10µg), Erythromycin (15µg), Ciprofloxacin (5µg), Ampicillin (10µg), Cefotaxime (30µg), Ceftriaxone (30µg), Augmentin (10µg), Oxacillin (1µg), Vancomycin (5µg) and Trimethoprim/sulfamethoxazole (5µg). Results were interpreted according to Clinical and Laboratory Standards Institute (CLSI) 2017.

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Statistical analysis

All data were entered into the Microsoft excel sheet. Statistical analysis was carried out using the Chi-square from statistical package for social sciences (SPSS) version 20. A p value of ≤0.05 was considered statistically significant.

III. Results

Table 1 shows the prevalence of *S. aureus* among the study participants. Of the Two hundred and fifty nasal swabs obtained from the HIV, TB and HIV/TB patients, *S. aureus* was isolated from 47 (18%) of the total number of samples collected. These include 32 (21.3%) from 150 HIV patients, 11 (15%) from 70 tuberculosis patients and 4(13.3%) from TB/HIV patients.

<table>
<thead>
<tr>
<th>Patients</th>
<th>Number of Nasal swabs collected</th>
<th>Number of Isolates</th>
<th>Percentage (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>HIV</td>
<td>150</td>
<td>32</td>
<td>21.3</td>
</tr>
<tr>
<td>TB</td>
<td>70</td>
<td>11</td>
<td>15.7</td>
</tr>
<tr>
<td>HIV/TB Co-infection</td>
<td>30</td>
<td>4</td>
<td>13.3</td>
</tr>
<tr>
<td><strong>TOTAL</strong></td>
<td><strong>250</strong></td>
<td><strong>47</strong></td>
<td><strong>18.8</strong></td>
</tr>
</tbody>
</table>


Figure 1 shows the antibiotic susceptibility pattern of *S. aureus* strains to the different antibiotics tested. The *S. aureus* isolates were sensitive to Augmentin (100%), Ciprofloxacin (77%), Gentamycin (64%), Cefotaxime (57%), Erythromycin (44%), Oxacillin (24), Ceftriaxone (23), Ampicillin (16%) and Cotrimoxazole (9%). Antimicrobial susceptibility to *S.aureus* showed (100%) sensitivity to Augmentin and the highest resistance was obtained against Cotrimoxazole (9%).

![Antibiotic susceptibility and resistance pattern of S. aureus isolates](image)

Figure 1: Antibiotic susceptibility and resistance pattern of *S. aureus* isolates

Figure 2 shows the detection of antibiotic resistance and virulence genes in the *S. aureus* isolates. All the strains were screened for carriage of antibiotic resistance (*Mec A*) and virulence (*LuKF-PV*) genes. Out of the 47 strains screened for these genes, 12(25.5%) were positive for *mec A* while 1 (2.12%) was positive *LuKF-PV* gene.
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IV. Discussion

*S. aureus* is a commensal and opportunistic pathogen capable of causing a wide range of human diseases leading to high mortality and morbidity in tropical Africa. Over the past decades, there has been an increase in the rate of infections caused by MRSA all over the world. The results of this study showed a total percentage prevalence of 18.8% in the anterior nares of the 250 study participants. The 21.3% nasal carriage of *S. aureus* in HIV patients in this study is in agreement with an earlier study conducted in Nigeria by Olalekan et al. who obtained 29% nasal carriage. The 18.8% nasal carriage of *S. aureus* in HIV/TB patients obtained in this study is higher than 8% result of Beverly et al. from the research in Ghana. The high colonization rates could be due to factors such as frequent contact with healthcare workers both in community settings and frequent exposure to antibiotics leading to a greater likelihood of being colonized with resistant strains. Similarly, the level of hygienic disposition of a populace according to Pathak et al. could be a factor determining the rate of nasal carriage of MRSA among HIV patients. This study identified frequent usage of antibiotics, recent hospitalization and direct contact with hospital workers as the major predisposing factor with colonization of the anterior nares by MRSA. This is in agreement with results of previous studies. The commonly known predisposing factor to nasal colonization of *S. aureus* are history of hospitalization, surgical procedures, intravenous drug users, alcoholism and living with health worker as reported by Ayepola et al.

The antibiotic susceptibility testing showed that all the *S. aureus* isolates were susceptible to Augmentin (100%) while cotrimoxazole showed the least susceptibility of 16% and 9% respectively. This finding is in agreement with the research of Olalekan et al. which reported 92% resistance to cotrimoxazole. The high resistance to cotrimoxazole may be due to the fact that most of the HIV patients receive cotrimoxazole as prophylaxis against secondary infections. Despite the result of this finding, the administration of cotrimoxazole as prophylaxis should be encouraged in these patients because the benefit has been established in previous studies. Augmentin is an antibiotic that contains amoxycillin and clavuacid, which is non-antibiotic. Clavuacid inhibits beta-lactamase enzyme and prolongs the antibacterial activity of the amoxicillin component of Augmentin even among the penicillinase producing bacteria. The 100% sensitivity to Augmentin agrees with the work from Nigeria which reported 19.7% resistance to Augmentin. The resistance to cotrimoxazole in this study and the previous studies is an indication of the abuse of this drug. Cotrimoxazole is an antibiotic that could easily be bought from several patent and pharmaceutical shops without a prescription. This is not the same for Augmentin because of its relatively high cost and therefore, Augmentin is rarely abused unlike cotrimaxagole that is very cheap. The 100% sensitivity of the isolates to Augmentin indicates that the drug might be effective in the management of MRSA infections although, the in-vitro sensitivity pattern does not always translate to in-vivo activity. Nevertheless, Augmentin should be considered along with the global accepted drugs like vancomycin and muciporin in the management of staphylococcal infections.

The presence of *mec* A gene (25.5%) and lukF-PV gene (2.10%) in the *S. aureus* isolates in this study is quite significant compared with the work conducted in India with 13.6% detection of *mec* A gene in the *S.
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Staphylococcus aureus isolates. MRSA has become a global health challenge because of its negative consequence on antibiotics treatment and ability to easily acquire resistance gene thereby making treatment difficult. Mec A encodes the penicillin binding proteins called PBPs which has a low affinity for beta-lactams which replaces the binding site of penicillin, thereby causing resistance to methicillin and other related beta-lactam antibiotics. The significance of the (25.5%) mec A gene underscores the seriousness of MRSA in this environment since MRSA has been implicated in nosocomial and community infections, it is evident that resistance to a wide range of antibiotics is likely to be on the increase considering that infections associated with MRSA are difficult to treat mainly as a result of resistance to a host of antibiotics. The detection of PVL in this study is clinically significant. PVL is a known cytotoxin which destroys leukocyte, causes degeneration of the tissue resulting into skin and soft tissue infections (SSTI) and it is a major virulent factor of S aureus. The implication of the detection in HIV patient management is to sensitize clinicians on the early detection of SSTI in HIV patients receiving treatment for S. aureus infection.

V. Conclusion

Molecular characterization of S. aureus for mec A gene should be made compulsory at State and Federal owned clinical laboratories. This would enhance the detection and a detailed knowledge of the prevalence of MRSA and antibiotic options for the management of staphylococcal infections in individuals at risk. Similarly, a coordinated synergy involving primary, secondary and tertiary hospitals in the country should be implemented with a view to eliminating or reducing nasal carriage of S. aureus in individuals or groups to avoid multi-drug resistant S. aureus infections.

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References


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