Nasal Carriage of *Staphylococcus aureus* and Associated Risk Factors among Students in a Nigerian University

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Abstract

Nasal carriage of *Staphylococcus aureus* has been associated with subsequent infection and transmission within the hospital and community settings. This study was carried out to evaluate the carriage rates of *S. aureus* in a University student population and describe risk factors associated with the carriage. Two-hundred and seventy-seven nasal samples were obtained from healthy volunteer students and screened for *S. aureus* by standard microbiological techniques. Antibiotic susceptibility testing was conducted on the bacterial isolates by the disk diffusion technique. A questionnaire was conducted with each student to acquire demographic and risk factor information. One hundred and fifty-seven (56.7%) isolates were identified as *S. aureus*. Antibiotic resistance was highest for cloxacillin (91%), ceftazidime (71%), cotrimoxazole (23%), erythromycin (20%) and oxacillin (16%). Risk factors such as hospitalization in the past 12 months, recent skin infection and participation in sports were significantly associated with carrier status. High resistance to certain antibiotics observed in this study shows that nasal colonization could serve as a reservoir of antibiotic resistant strains within the community.

Keywords: Nasal Carriage; *Staphylococcus aureus*; Antibiotic Susceptibility Testing; Risk Factors; Antibiotic Resistant Strains

Introduction

Staphylococcus aureus, one of the most frequently occurring community and hospital-associated pathogens, causes infectious diseases including mild skin infection to systemic infections [1]. Approximately 20 - 30% of the global population is persistently colonized with *S. aureus* in the anterior nares [2] and approximately 10 to 40% of people tested as outpatients or on admission are nasal carriers of *S. aureus* [3]. Nasal carriage (colonization) of *S. aureus* has been identified as a major risk factor for subsequent infections as carriers act as reservoirs for the pathogen assisting its spread in the community. Certain risk factors have been associated with nasal carriage of *S. aureus* and they include sex, occupation, age groups, ethnicity, hospitalization, nasal abnormalities, genetic makeup, immunological status, repeated needle injections, hormonal status in women, recent hospitalization, insulin dependent and non-insulin dependent diabetes mellitus, haemodialysis, HIV status, *S. aureus* skin infections, nose picking and administration of multiple antibiotics [4,5]. The spread of colonization occurs especially in close contact areas like schools probably by contaminated hands and surfaces [6]. An understanding of the risk factors for carriage of *S. aureus* is crucial to understanding the potential for invasive infections and transmission of diseases caused by this pathogen. This is of importance because the increasing resistance of *S. aureus* to various antibiotics has been known to complicate the treatment of diseases caused by it [7]. In addition, the emergence and spread of methicillin resistant *S. aureus* (MRSA) has aggravated the situation [8]. The purpose of this study was to investigate the incidence of nasal carriage of *S. aureus* among University students, risk factors for nasal carriage and antimicrobial susceptibility pattern of the *S. aureus* isolates to selected antimicrobial agents

Materials and Methods

Sample and Data Collection

Two-hundred and seventy-seven nasal samples were obtained from male and female undergraduate students who were not showing symptoms of any infection. After informed consent was obtained, a self-administered questionnaire [9] was given to each participant to collect information pertaining to demographics, contact with or exposure to potential *S. aureus* carriers, medication history, intravenous drug use and involvement in sporting activities. This was followed by a single screening nose swab for each volunteer using sterile, moistened cotton swabs.

Isolation of Bacteria

Nasal swabs were inoculated directly onto on mannitol salt agar (MSA), a selective medium for the isolation of *S. aureus* and the MSA plates were incubated at 37°C for 48 hours. Mannitol-fermenting colonies that were yellow were selected from the MSA plates and subcultured on blood agar and incubated at 37°C overnight to check for characteristic β -haemolysis. Further identification was carried out with Gram staining, catalase and coagulase tests. The staphylase test (Oxoid) was used to detect the presence of clumping factor and to confirm *S. aureus*. The isolates were stored on slant nutrient agar at 4°C and were used for antibiotic susceptibility testing.

Antimicrobial Susceptibility Testing

The disk diffusion method was used to test the susceptibility of *S. aureus* isolates as recommended by the Clinical Laboratory Standards Institute guidelines (CLSI, 2011) to the following antibiotics: ceftazidime, cloxacillin, gentamicin, ceftriaxone, erythromycin,

cephalexin, cotrimoxazole, amoxicillin/clavulanate and oxacillin. The susceptibility test was performed on Mueller-Hinton agar (Oxoid) and plates were incubated at 35°C. Results were interpreted according to the CLSI guidelines.

Statistical Analysis

Statistical analyses was conducted using SPSS for Windows, version 15.0. Potential predictors of colonization were analysed using the one sample t- test. A p value of < 0.05 was considered statistically significant.

Results

Of the 277 samples screened, 157(56.7%) were positive for *S. aureus* with 82 (52.2%) obtained from males and 75 (47.8%) from females (Table 1). The students within the age range of 16 - 18 years accounted for a greater percentage of carriage (48.4%). The antimicrobial susceptibility pattern of 157 *S. aureus* isolates showed the rate of resistance was highest for cloxacillin (91%); ceftazidime (71%) and cotrimoxazole (23%) (Table 2). The lowest resistance was to gentamicin (2%). Sixteen percent of the isolates were resistant to oxacillin (methicillin). In response to the questionnaire 75 (47.1%) of the carriers reported having had a boil/skin infection in the past 12 months, 75 (47.1%) had been hospitalized at least once in the past 12 months, 4 (2.5%) had undergone surgery in the past 12 months, 14 (8.9%) had worked in a healthcare facility in the past 12 months, 92 (58.6%) had used antibiotics in the past 3 months, 17 (10.8%) were asthmatic and 120 (76.4%) had actively participated in athletics in the past 12 months (Table 3). Hospitalization in the past 12 months, involvement in athletics and recent skin infection were significantly associated with carrier status. All other risk factors were not statistically significant.

Subjects	Frequency	Percentage (%)
Age (years)		
16 - 18	76	48.4
19 - 21	61	38.9
22 - 24	18	11.5
Sex		
Male	82	52.2
Female	75	47.8

Table 1: Prevalence of *S. aureus* among volunteer students.

Antibiotics	Frequency	Percentage (%)
Erythromycin	32	20.4
Cephalexin	22	14
Cotrimoxazole	36	22.9
Amoxicillin/Clavulanate	31	19.7
Ceftazidime	111	70.7
Cloxacillin	143	91.1
Gentamicin	3	1.9
Ceftriaxone	29	18.5
Oxacillin	24	16

Table 2: Antibiotic resistance pattern of *S. aureus* isolates (n = 157).

Risk factors	Frequency	Percentage (%)
Ethnicity		
Yoruba	50	31.2
Igbo	22	14
Edo	8	5.1
Others	77	49.7
History of Boil/Skin infection in past 12 months	75	47.1
Patient in the hospital in the past 12 months	75	47.1
Had surgery in the past 12 months	4	2.5
Worked in a healthcare facility in the past 12 months	14	8.9
Used antibiotics in the past 3 months	92	57.3
Asthmatic	17	10.8
Participated in sports in the past 12 months.	120	75.8

Table 3: Risk factors associated with carriage of *S. aureus*.

Discussion and Conclusion

The results of this study showed an overall prevalence of 56.7% of *S. aureus* in the nostrils of the volunteer students. The prevalence rate observed is in line with previous reports of 80% isolation of *S. aureus* [10] but significantly higher than previous reports of 32.4% [11]; 39% [12] and 33.3% [13]. A higher carriage rate was observed in male students in line with another study which reported a significant difference between sex for carriage [6,14]. However, gender had no effect on carriage rate as reported by Adesida., *et al* [4]. These variations in carriage rates may be attributed to the characteristics of the population under study, the quality of sampling and culture techniques [15]. This study's high prevalence of nasal carriage of *S. aureus* further supports the fact that 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 [3].

The susceptibility tests showed cloxacillin to be the least effective agent with 91% bacterial resistance. Another study reported 100% resistance [16]. The low resistance observed to augmentin (amoxicillin/clavulanate) (20%) may be due to the presence of clavulanic acid (a competitive inhibitor of beta-lactamases) coupled with their low level of misuse in the community [13]. The outcome of this study indicated a very high rate of sensitivity (97%) to gentamicin. This observed result is in line with previous results of high sensitivity of nasal isolates of *S. aureus* to gentamicin [6,17]. Gentamicin is a less commonly used antibiotic as it is a parenterally administered drug making it more difficult to misuse or abuse. The 23% resistance observed for cotrimoxazole is significantly lower than the reported resistance of 32.5% by Onanuga and Temedie [13]. However 100% sensitivity to cotrimoxazole was reported by Eke., *et al* [14]. In this study, the strains of *S. aureus* showed varied resistance to the third generation Cephalosporins; Cephalexin, Ceftazidime and Ceftriaxone with the highest

resistance to Ceftazidime (71%). Resistance to methicillin in this study was 16% which is higher than 7.4% MRSA colonization rate earlier reported [9] but lower than 38.5% reported by Eke., *et al* [14].

The commonly known risk factors of *S. aureus* colonization are usually healthcare-associated, including hospital admission, recent surgery, intravenous drug use, and working in a healthcare environment. The results of this study revealed that participation in athletics, recent boil/skin infection, and recent hospitalization may be associated with nasal carriage of *Staphylococcus aureus*. A previous study found that only hospitalization in the past 12 months was significantly associated with the risk of being a *S. aureus* carrier Rhode., *et al.* [9] and in another male gender, age $\geq$ 23 years, and not taking antibiotics in the past year was associated with carriage [1]. Nasal carriage of *S. aureus* has been known to vary across geographical locations. In Africa, carriage rates of 13.0% has been reported in Tunisia [18]; 29% in Gabon [19] and 18.3% in Kenya [20].

The present study has certain limitations. Firstly, the population studied may not be representative of the student population. Secondly, the study was conducted only in a higher institution and the findings may not represent the larger community. Further studies need to be carried out with a higher sample population. In conclusion, this study clearly reports a high prevalence of nasal colonization with multi-drug resistant *S. aureus*. It also shows the necessity of investigating the epidemiology of nasal carriage of *S. aureus* and its susceptibility to antibiotics so as to establish effective infection control measures. It also supports the need to implement strategies for elimination of nasal carriage of *S. aureus* in groups at risk so as to prevent multi-drug resistant *S. aureus* infections within the population.

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