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Carriage of Drug-Resistant *Staphylococcus aureus* in the Anterior Nares of a Healthy Student Population

Kome Otokunefor[1*], Martina Emeonye[1] and Glory Odion[1]

[1]Department of Microbiology, Faculty of Science, University of Port Harcourt, P.M.B. 5323, Port Harcourt, Nigeria.

Authors' contributions

This work was carried out in collaboration between all authors. Author KO designed the study, managed the analyses of the study, managed the literature searches, wrote the protocol and wrote the first draft of the manuscript. Authors ME and GO were primarily responsible for the lab work. All authors read and approved the final manuscript.

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ABSTRACT

Aims: This study set out to explore *Staphylococcus aureus* nasal carriage rates in University student populations and determine possible effects of consistent contact with hospital environment, on carriage levels.
Introduction: Nasal carriage of *S. aureus* (the second most common human pathogen isolated in the clinical laboratory) has long been recognized as a major risk factor for the development of infection. Few studies have however focused on exploring nasal carriage rates in University student populations.
Methodology: Anterior nares of 140 University students (70 medical and 70 non-medical) were analyzed for the carriage and antibiotic resistance patterns of *S. aureus* using Kirby Bauer disc diffusion method.
Results: Overall, 46 (32.9%) *S. aureus* nasal carriers were detected, with higher carriage rates observed in medical students (38.6% versus 27.1%). Overall rates of resistance to isolates were 100% for augmentin, 100% for cloxacillin, 97.8% for erythromycin, 93.5% for ceftazidime, 84.8% for

**Corresponding author: E-mail: kome.otokunefor@uniport.edu.ng;*

cefuroxime, 73.9% for ceftriaxone, 69.6% for gentamicin, and 6.5% for ofloxacin. Majority of isolates (41, 89.1%) were multidrug resistant.

Conclusion: This study contributes to the relatively limited epidemiological data on an important pathogen. It provides a worrisome picture of high carriage of MDR isolates. Further studies are needed to provide more data, explore possible risk factors and design control measures.

Keywords: Antimicrobial resistance; nasal carriage; Staphylococcus aureus.

1. INTRODUCTION

Carriage of *S. aureus* has long been recognized as one of the risk factors for the development of staphylococcal infections [1-3], particularly in hospital settings. This organism is the 2nd most common human pathogen and is associated with a wide variety of infections. It is a leading cause of hospital acquired infection and has recently been associated with severe cases of infection in healthy persons in the community. *S. aureus* may be carried on various moist body sites such as the armpits and inguinal regions. Though the anterior nares are thought to be the most common carriage site [4,5]. Carriage of *S. aureus* may be classed as persistent, intermittent or transient [6]. Carriage rates in healthy adults however varies with 20% of the population persistent carriers, 60% intermittent carriers, while 20% never carry the organism [7] Humans are therefore an important reservoir for staphylococcal infections. Reports have linked a reduction in specific *S. aureus* infections to a reduction in carriage [2,8,9]. Furthermore, a link was noted between colonizing and infecting strain, with up to 80% of cases of staphylococcal bacteremia in colonized cases found to be caused by the carriage stain [10,11]. With the increase in infections caused by methicillin resistant strains of *S. aureus*, the association of these strains with multidrug resistance [12] and the negative effect this has on therapy options, strategies to reduce the prevalence of staphylococcal infections became essential. Recognition, isolation and decolonization of individuals carrying these organisms have been shown to be one of the significant activities affecting the reduction in MRSA outbreaks in the UK [13].

Previously, majority of studies on *S. aureus* carriage focused on hospital-associated personnel, but with the relatively recent reports of outbreaks of severe staphylococcal infections in the community [14], the focus of studies has also included the community. This study was therefore aimed at assessing the carriage rates of *S. aureus* and MDR-*S. aureus* in a population of students in a tertiary institution and exploring if consistent contact with a hospital environment would have any impact on carriage rates.

2. MATERIALS AND METHODS

2.1 Study Population

Nasal samples were collected from University students following informed consent, using a sterile pre-moistened swab as described by Okamo and colleagues 2016 [15]. Samples were immediately transported to the laboratory.

2.2 Sample Processing

Sample processing was carried out within two hours and involved aseptic inoculation of nasal swab sample unto mannitol salt agar. Following a 24 hour incubation at 37℃, suspected characteristic *S. aureus* colonies were subcultured to nutrient agar plates. Isolates were then identified as *S. aureus* using standard microbiological methods (mannitol fermentation, Gram reaction, catalase and coagulase test) [16]. Chi-square test was used for statistical analysis (EPI info V3.5.).

2.3 Antibiotic Resistance Screening

Susceptibility testing of isolates was carried out using the standard Kirby Bauer disc diffusion method [17] on Mueller Hinton agar. A commercial multidisc was used (Abtek Biologicals Ltd, USA) comprising of Augmentin (AUG), cloxacillin (CXC), erythromycin (ERY), ceftazidime (CAZ), cefuroxime (CRX), ceftriaxone (CTR) gentamicin (GEN) and ofloxacin (OFL). Organisms were classed as susceptible or resistant using the NCCLS 2000 standard [18], based on the zones of inhibition observed. Finally, multidrug resistant (MDR) organisms were defined as organisms resistant to 4 or more drug classes, and diversity levels defined based on the antibiogram pattern as previously described [19].

3. RESULTS AND DISCUSSION

3.1 Results

Of the 140 nasal swabs collected in total, 70 were from clinical medical students based at the University teaching hospital and 70 from non-medical students. The results showed that 47 (33.6%) of these were from male students and 93 (66.4%) from female students. As a whole, nasal carriage of *S. aureus* was detected in 32.9% (46/140) of the test populations. This rate varied between the subset populations, with higher carriage levels among the medical students (38.6% versus 27.1%). This difference was however not statistically significant (P = .15).

The results of the antibiotic susceptibility testing showed high levels of resistance to augmentin (100%), cloxacillin (100%), erythromycin (97.8%), ceftazidime (93.5%), cefuroxime (84.8%), ceftriaxone (73.9%) and gentamicin (69.6%). Most isolates (93.5%) were however susceptible to ofloxacin.

Rates of resistance were similar (< 10% difference) between both population subsets for most antibiotics (Fig. 1). For cefuroxime, gentamicin and ofloxacin however, resistance rates varied by more than 10%. While higher resistance rates against cefuroxime and ofloxacin were observed in *S. aureus* from hospital based students (96.3% versus 68.4% and 11.1% versus 0% respectively), a different trend was noticed for gentamicin. In this case, resistance rates were higher in *S. aureus* from non-hospital based students (89.5% versus 55.6%).

As a whole, majority of isolates in this study (89.1%, 41/46) were resistant to more than 4 drug classes i.e. multidrug resistant (Fig. 2). The degree of multidrug resistance however varied. More non-hospital *S. aureus* isolates were resistant to 4 out of the 6 drug classes, while none of the non-hospital *S. aureus* isolates was resistant to all 6 drug classes.

A total of nine drug resistance patterns were observed in this study (Table 1). Three profiles were common to both study subsets. And though the number of profiles per subset differed, both subsets had almost identical levels of diversity (0.81 versus 0.80).

3.2 Discussion

Despite the important role of *S. aureus* nasal carriage as a risk factor for the development of infection both in community and healthcare settings, only a few studies have explored nasal carriage of *S. aureus* in various populations in Rivers State, Nigeria [20]. Several studies have however explored *S. aureus* carriage in various parts of Nigeria, with a few focused specifically on carriage in tertiary student population [21-24], and others focused specifically on MRSA carriage [23-26]. Results of this study showed a 32.9% *S. aureus* nasal carriage rate among healthy Nigerian students. These results were much lower than previously reported Nigerian carriage levels of 59%, 60%, 100%, and 56.3% from Calabar [27], Ekpoma [28], Uturu [29] and Owerri [30], respectively. They also differed from nasal carriage rates of 41.0%, 51.5% and 53.1% reported from Germany, Ethiopia and Italy respectively [31-33]. The rates were however within previously reported nasal carriage ranges of 9.1% to 57.8% noted in a recent review [34] and similar to carriage levels observed in healthy populations both within and outside Nigeria [4, 20,22,35,36]. Also, they were the same as reported by a recent Burkina Faso study [37]. Variations in carriage rates could be a function of geographical location, infection control policies (or lack of) and possible exposure levels [38].

Table 1. Drug resistance patterns of *S. aureus* isolates

Antibiotic resistance patterns	Number of *S. aureus* isolates	
	Medical students	Non-medical students
AUG-CTR-CXC-ERY	1	-
AUG-CAZ-CRX-CXC-ERY	2	2
AUG-CAZ-CRX-CXC-ERY-GEN	4	4
AUG-CAZ-CRX-CTR-CXC-GEN	1	-
AUG-CAZ-CRX-CTR-CXC-ERY	9	-
AUG-CAZ-CTR-CXC-ERY-GEN	-	6
AUG-CRX-CTR-CXC-ERY-GEN	-	2
AUG-CAZ-CRX-CTR-CXC-ERY-GEN	7	5
AUG-CAZ-CRX-CTR-CXC-ERY-GEN-OFL	3	-

Key: Augmentin (AUG), cloxacillin (CXC), erythromycin (ERY), ceftazidime (CAZ), cefuroxime (CRX), ceftriaxone (CTR), gentamicin (GEN) and ofloxacin (OFL).

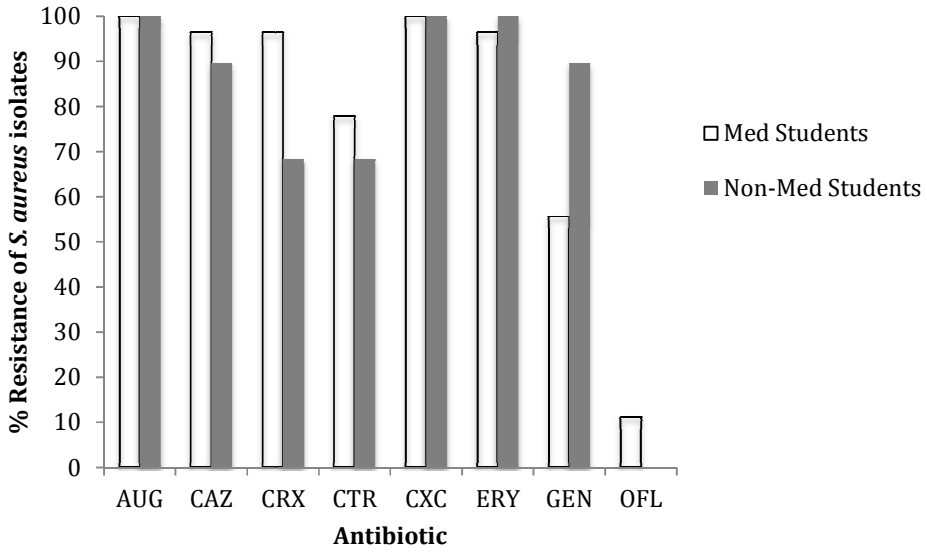

Fig. 1. Antibiotic resistance profile of *S. aureus* nasal carriage isolates (Med = Medical)

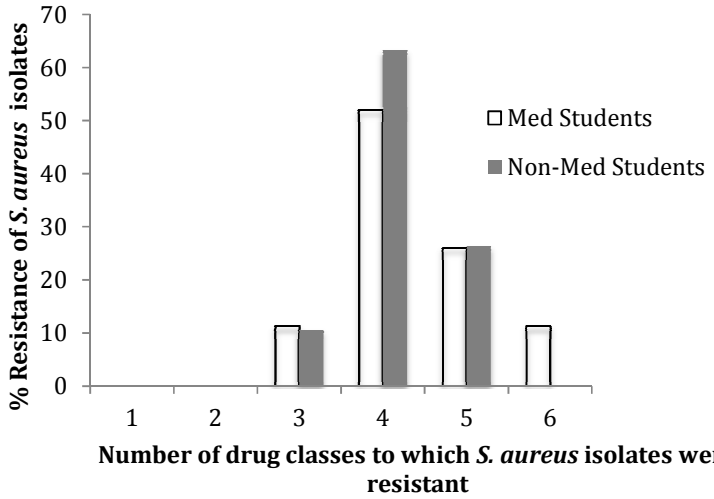

Fig. 2. Multidrug resistance pattern of nasal carriage *S. aureus* isolates (Med = Medical)

Hospital environment has long been noted as a major risk factor for the acquisition of *S. aureus* in the anterior nares. Hence, we hypothesized that the medical students would have higher levels of carriage due to the higher exposure levels to the hospital environment. Findings from this study noting a higher level of *S. aureus* nasal carriage in medical students in constant contact with the hospital environment, appear to support this hypothesis. This trend of higher rates of *S. aureus* carriage in individuals associated with hospital environments has previously been reported. Lennox and colleagues in a 2014 study noted a higher carriage level in nurses (80%) than that of the general public (56%). Several studies even went further and assessed carriage of *S. aureus* in various cadres of hospital staff. These noted higher levels of carriage in staff more closely involved in-patient care [27,39]. On the other hand though, a number of previous studies [15,40,41], noted similar levels of carriage between hospital and non-hospital individuals (22.3% versus 19.9%, 25.4% versus 26.1%; 27.5% versus 24.0%).

Some of these studies however noted that while the rate of nasal carriage did not vary, organisms from hospital individuals exhibited higher levels of antibiotic resistance rates [39]. In this study, very high levels of resistance were observed to most of the antibiotics tested. Similar very high levels of resistance have previously been reported [21,42,43]. The most recent of these, reported these very high levels of resistance from nasal carriage of *S. aureus* isolates in an elderly population. While rates of resistance in this study, were generally, unexpectedly similar between both subsets of students (Fig. 1), perhaps pointing at the interplay of multiple factors which could affect the development of drug resistance in bacteria, there were however striking minor differences. Key among this was the lower rate of resistance to gentamicin seen with *S. aureus* isolates from hospital-based students. In the early 2000, a Belgian publication noted the emergence of gentamicin-susceptible strains of MRSA, which was subsequently linked with the emergence of a new clone of *S. aureus* isolates [44]. Similarly, a 2011 study noted that community acquired strains of MRSA (CA-MRSA) belonging to the CC22 MLST clonal complex were typically resistant to gentamicin perhaps pointing to a possible link between susceptibility pattern to specific antibiotics and *S. aureus* clone. While it has been noted that storage of gentamicin-resistant *S. aureus* isolates for long periods of time at room temperature may result in the spontaneous development of gentamicin-variants [45], isolates in this study were tested for antibiotic susceptibility immediately following identification, thereby eliminating the need for storage.

All this therefore points to the need for further typing studies to be carried out on these isolates to provide a clearer picture of the epidemiology of *S. aureus* in this locale and help inform control strategies. While the MDR and antibiogram patterns provide some discrimination perhaps indicative of isolate relatedness, this is not sufficient to provide useful typing information.

Finally, a high degree of multidrug resistance was noted in this study (Fig. 2). This is particularly worrisome with regards to the hospital-based students, considering their potential for interaction with individuals with reduced/compromised immune systems. Perhaps, this strongly highlights the need for 'search and destroy' strategy instituted in Europeans countries [46] especially as this effectively formed the basis for decline in MRSA particularly in hospital environments.

4. CONCLUSION

This study provides epidemiological data on one of the two most common organisms associated with bacterial infections in the clinical setting, and an emerging problem in the community. Though essential, the information provided here is only a bit of the puzzle and while indicative of a major public health situation, points at the need for even more research with regards to carriage, drug resistance and influencing factors.

While this study provides useful data on a representative population, it is limited in its scope and more studies are essential to provide a complete epidemiological picture.

CONSENT

All authors declare that informed consent was obtained from healthy volunteers for publication of this paper.

ETHICAL APPROVAL

All authors declare that this study was approved by the appropriate ethical committee and therefore performed in accordance with the ethical standards contained in the 1964 Declaration of Helsinki.

COMPETING INTERESTS

Authors have declared that no competing interests exist.

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