

Nasal carriage and antibiotics susceptibility of *Staphylococcus aureus* in healthy students of University of Port Harcourt, Rivers State, Nigeria

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ABSTRACT: One hundred nasal swabs were collected from 50 males and 50 females students of University of Port Harcourt were examined for *Staphylococcus aureus* using standard bacteriological methods. Thirty-two percent of the 100 samples were found to be carrying *Staphylococcus aureus* in their nasal cavity. This study showed that there was a significant difference between sex for carriage rate of *S. aureus* [18.0% vs. 46.0%, $P < 0.05$]. The study also showed that there was no significant difference between age groups for either carriage rates of *S. aureus* (35.3% vs. 25.0%, $P > 0.05$). It showed that Methicillin susceptible *S. aureus* (MSSA) [62.5%] was most predominant over Methicillin resistant *S. aureus* (MRSA) which was 37.5% and that there was a significant difference between carriage rate of MRSA and MSSA [37.5% vs. 62.5%, $P < 0.05$].

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1. INTRODUCTION

There is increasing evidence that community acquired *Staphylococcus aureus* infections are spreading among healthy children (Pathak et al., 2010). *Staphylococcus aureus* has long been recognized as an important pathogen in human disease (Kluytmans et al., 1997). *Staphylococcus aureus* infections are associated with considerable morbidity and, in certain situations, mortality (Wenzel and Perl, 1995). *Staphylococcus aureus* is a common pathogen responsible for community as well as hospital-associated infections (Wertheim et al., 2005; Pathak et al., 2010). The infections caused by *S. aureus* have clinical range from minor skin infections to severe life threatening infections (Lowy, 1998; Pathak et al., 2010). There is increasing evidence that community acquired methicillin-resistant *S. aureus* (CA-MRSA) is spreading among healthy individuals, especially children (Peacock et al., 2003; Pathak et al., 2010).

The association between the nasal carriage of *S. aureus* and subsequent infection has been comprehensively established in a variety of clinical settings, in particular, patients undergoing haemodialysis and continuous ambulatory peritoneal dialysis (CAPD), and in patients undergoing surgery (Wenzel and Perl, 1995). Postoperative wound infections are associated with a high degree of morbidity and represent an important medical issue (Wenzel and Perl, 1995; Herwaldt, 1998).

For the last fifty years, the nose has been intermittently recognized and targeted as a source of

Staphylococcus aureus causing surgical site infection (Casewell et al., 1998). Due to an increasing number of infections caused by methicillin-resistant *S. aureus* (MRSA) strains, therapy has become problematic (Kluytmans et al., 1997). Until recently, eradication of *S. aureus* nasal carriage by various topical and systemic agents had proved unsuccessful (Wenzel and Perl, 1995).

It has been shown that nasal carriers of *S. aureus* have an increased risk of acquiring an infection with this pathogen. The nose is the main ecological niche where *S. aureus* resides in human beings, but the determinants of the carrier state are incompletely understood (Wertheim et al., 2005). The anterior nares have been shown to be the main reservoir of *S. aureus* in both adults and children (Pathak et al., 2010). The *S. aureus* is transmitted to nares by contaminated hands and from surfaces where it can survive for months (Kluytmans et al., 1997; Pathak et al., 2010). Nasal carriage of *S. aureus* acts as endogenous reservoir for clinical infections in the colonized individual but also as a source of cross-colonization for community spread (Pathak et al., 2010). Healthy individuals have a small risk of contracting an invasive infection caused by *S. aureus*, but they can be carriers of the organism (Foster, 2004). The spread of colonization occur especially in close contact areas like schools, pre-schools or households (Peacock et al., 2003), probably by the contaminated hands and surfaces (Pathak et al., 2010).

Nasal colonization with *S. aureus* plays pivotal role in the increasing prevalence of resistant community acquired *S. aureus* infections worldwide (Pathak et al., 2010). Carriage of *S. aureus* appears to play a key role in the epidemiology and pathogenesis of infection (Kluytmans et al., 1997). The ecological niches of *S. aureus* are the anterior nares. In healthy subjects, over time, three patterns of carriage can be distinguished: about 20% of people are persistent carriers, 60% are intermittent carriers, and approximately 20% almost never carry *S. Aureus* (Kluytmans et al., 1997).

Worldwide, the increasing resistance of this pathogen to various antibiotics complicates treatment of *S. aureus* infections (Wertheim et al., 2005). Effective measures to prevent *S. aureus* infections are therefore urgently needed (Wertheim et al., 2005). Elimination of carriage has been found to reduce the infection rates in surgical patients and those on hemodialysis and CAPD. Elimination of carriage appears to be an attractive preventive strategy in patients at risk (Kluytmans et al., 1997). The increasing appearance of epidemic methicillin-resistant *S. aureus* (MRSA) in the 1980s rekindled interest in these (largely overlooked) studies, when the elimination of nasal carriage by topical mupirocin proved pivotal for the control of MRSA in Northern Europe and elsewhere (Casewell et al., 1998). Thus, decolonization of the nares may prevent *S. aureus* infections and the attendant complications (Herwaldt, 1998). A regular surveillance system is important in ensuring quality of patient care (Pathak et al., 2010).

The incidence of community-acquired and hospital-acquired *S. aureus* infections has been rising with increasing emergence of drug-resistant strains called methicillin-resistant *S. aureus* (MRSA) (Fluit et al., 2001; Deresinski et al., 2005; Mainous et al., 2006). MRSA is an established pathogen in most health care facilities. Previously limited to hospitals, MRSA infections have been increasingly reported in the community (Naimi et al., 2003; Nguyen et al., 2005; Harbarth et al., 2005; Ma et al., 2005; Ochoa et al., 2005; Mainous et al., 2006). Because many clinical infections arise from spread from a healthy carrier, an understanding of the risk factors for carriage of *S. aureus* is crucial to understanding the potential for invasive infections and transmission of MRSA; however, most surveillance of *S. aureus* and MRSA has focused on individuals with invasive infections rather than on an entire population (Harbarth et al., 2005; Ma et al., 2005; Ochoa et al., 2005; Kuehnert et al., 2005; Mainous et al., 2006).

A variety of studies have examined community prevalence of nasal carriage of *S. aureus* in diverse

sub-populations, such as adult outpatients, health care workers, college students, and injection drug users (Wertheim et al., 2004; Bischoff et al., 2004; Bassetti et al., 2004; Eveillard et al., 2004; Mainous et al., 2006). The prevalence of *S. aureus* ranges from 20% to 45%, with an estimate of MRSA colonization from 10 community surveillance studies of 1.3% (Salgado et al., 2003; Mainous et al., 2006). Few studies, however, have focused on which individuals are most likely to be colonized with *S. aureus* and which are most likely to specifically have MRSA (Mainous et al., 2006). Therefore, prevention of staphylococcal infections has become more important. The aim of the study was to assess the prevalence of and the factors associated with nasal carriage of *S. aureus* and its antibiotic sensitivity pattern among healthy students of Microbiology, University of Port Harcourt, Nigeria.

2. MATERIALS AND METHODS

2.1. SAMPLES COLLECTION

Swabs were obtained from one hundred students of microbiology, University of Port Harcourt using sterile, dry cotton wool swab stick and swabs were dipped in sterile saline solution to moisten the cotton wool. The swabs were immediately placed in Stuart's transport medium and transported to the Medical Microbiology laboratory for analysis.

2.2 ISOLATION OF PURE CULTURE

The nasal swabs were inoculated onto MacConkey agar (MCA), blood agar (BA) and mannitol salt agar (MSA). The plates were incubated at 37°C for 24 and 48h respectively. Colonies identifiable as discrete on the MCA, MSA and BA were carefully examined macroscopically for cultural characteristics.

2.3. IDENTIFICATION OF ISOLATES

All isolates were subjected to various morphological characterization and gram stained to determine their gram reaction. Biochemical tests were carried out as described by Jolt *et al.* (1994) to determine the identity of the bacteria isolates with reference to Bergey's Manual of Determinative Bacteriology. The isolates were identified by comparing their characteristics with those of known taxa, as described by Jolt *et al.* (1994) and Cheesbrough (2006).

2.4. ASSESSMENT OF MSSA AND MRSA

S. aureus isolates were screened for methicillin resistance by the disk diffusion method of the National Clinical and Laboratory Standards Institute (CLSI, 2006). Overnight cultures from *S. aureus* were plated on Mueller-Hinton agar, and a 1- μ g oxacillin disk was placed on the inoculated plate. Zone diameters were measured and recorded after a 24-hour incubation at 37°C; the results were classified as sensitive (\geq 13

mm), intermediate (11–12 mm), or resistant (≥ 10 mm).

2.5. ANTIBIOTICS SENSITIVITY TO OTHER ANTIBIOTICS

Antibiotic sensitivity tests were performed using Kirby Bauer's disc diffusion method according to performance standards of CLSI (2006). The panel of antibiotics tested included those that are recommended by CLSI or are commonly used locally in empirical treatment of *S. aureus* infections. Susceptibility testing was done and results are presented for the following most important antibiotics: co-trimoxazole, ampicillin, cloxacilin, erythromycin, ciprofloxacin, streptomycin, tetracycline, and gentamicin. For both MSSA and MRSA, we defined multi-drug resistant (MDR) isolates as those resistant to 3 different antibiotics i.e., co-trimoxazole, ciprofloxacin and erythromycin (Pathak et al., 2010).

2.6. DATA ANALYSIS

The carriage rates of *Staphylococcus aureus* was calculated by using subjects with positive samples as numerator and the total numbers of subjects enrolled in this study as denominator. The data generated from this study were presented using descriptive statistics. The data was subjected to Fisher's Exact Test for comparison of proportions to determine any significant relationship between carriage rate, age and gender. Confidence level was set at $p = 0.05$. Prevalence of *S. aureus* and MRSA were estimated with 95% confidence intervals. All the variables were adjusted for age and sex. A complete case series analysis was used. The independent variables included were: sex (males versus females), age group (20 to 24 years versus 25 to 29 years), and education of the parents (educated versus illiterate). Chi-square tests were used to test for statistical significance (5%).

3. RESULTS ANALYSIS

3.1. Carriage rate of *S. aureus*

Table 1 show the carriage rate (prevalence) of *S. aureus* in males to be 18.0 while females had 46.0%. This study showed that there was a significant difference between sex for carriage rate of *S. aureus* [18.0% vs. 46.0%, $P < 0.05$] (Table 1).

Table 1: Frequency of isolation of *Staphylococcus aureus* according to sexes of subjects

Sex	No. Tested	No. Positive (%)
Males	50	9(18.0)*
Females	50	23(46.0)*
Total	100	32(32.0)

Key: * = Significant ($P < 0.05$)

Table 2 gives the frequency of isolation of *Staphylococcus aureus* according to age of subjects. The study showed that there was no significant difference between age groups for carriage rates of *S. aureus* (35.3% vs. 25.0%, $P > 0.05$) among the population studied (Table 2).

Table 2: Frequency of isolation of *Staphylococcus aureus* according to age of subjects

Age groups (years)	No. Tested	No. Positive (%)
20-24	68	24(35.3)**
25-29	32	8(25.0)**
Total	100	32(32.0)

Key: ** = No significant ($P > 0.05$)

3.2. Frequency of occurrence of *Staphylococcus aureus*

Table 3 shows the frequency of occurrence of *Staphylococcus aureus* isolated from healthy students of University of Port Harcourt, Nigeria. It showed that Methicillin susceptible *S. aureus* (MSSA) [62.5%] was most predominant over Methicillin resistant *S. aureus* (MRSA) which was 37.5% (Table 3). It showed that there was a significant difference between carriage rate of MRSA and MSSA [37.5% vs. 62.5%, $P < 0.05$].

Table 3: Frequency of occurrence of *Staphylococcus aureus*

<i>Staphylococcus aureus</i> isolates	No. (%)
Methicillin resistant <i>S. aureus</i> (MRSA)	12(37.5)*
Methicillin susceptible <i>S. aureus</i> (MSSA)	20(62.5)*
Total	32(100.0)

Key: * = Significant ($P < 0.05$)

3.3. Antibiotic sensitivity pattern

Table 4 shows the antibiotics sensitivity and resistance profile of the *S. aureus* isolates. It showed that the percentage sensitivity ranged from 21.3% to 87.5% while percentage resistance ranged from 12.5% to 68.7% (Table 4). The in-vitro antibiotic sensitivity pattern of twenty isolates of methicillin susceptible *S. aureus* (MSSA) is shown in Table 4. Resistance to commonly used oral antibiotics, ampicillin (80.0%), cloxacillin (20.0%), ciprofloxacin (60.0%), co-trimoxazole (20.0%), erythromycin (40.0%), gentamycin (40.0%), tetracycline (60.0%) and streptomycin (40.0%) was noted in MSSA isolates. Co-resistance to a combination of one antibiotic with different classes of antibiotics is as follows: ampicillin with ciprofloxacin (60.0%), ampicillin with erythromycin (20.0%), ampicillin with co-trimoxazole (20.0%), cloxacilin with erythromycin (20.0%), ciprofloxacin with tetracycline (60.0%) and

ciprofloxacin with streptomycin (20.0%). Four (20.0%) MSSA isolates were MDR (resistant to 3 different antibiotics i.e., co-trimoxazole, ciprofloxacin and erythromycin).

The in-vitro antibiotic sensitivity pattern of five isolates of methicillin resistant *S. aureus* (MRSA) is also shown in Table 4. The MRSA isolates showed resistance to ampicillin (60.0%), ciprofloxacin (33.3%), co-trimoxazole (66.7%), erythromycin (66.7%), Gentamycin (83.3%), tetracycline (66.7%)

and streptomycin (66.7%). Co-resistance to ciprofloxacin and erythromycin (40.0%), erythromycin and ampicillin (20.0%), ciprofloxacin and Gentamycin (40.0%), ciprofloxacin and tetracycline (60.0%), was noticed. Among the 12 MRSA isolates, 2(16.7%) were MDR. On the overall, higher sensitivity was noted to cloxacillin (87.5%), co-trimoxazole (62.5%), ciprofloxacin (50.0%), erythromycin (50.0%) and streptomycin (50.0%) by both MSSA and MRSA.

Table 4: Antibiotics sensitivity and resistance profile of the *S. aureus* isolates

Isolates	No.	Total number sensitive to Antibiotics tested (%)							
		Ampicillin	Cloxacillin	Ciprofloxacin	Co-trimoxazole	Erythromycin	Gentamycin	Tetracycline	Streptomycin
MSSA	20	4(20.0)	16(80.0)	8(40.0)	16(80.0)	12(60.0)	12(60.0)	8(40.0)	12(60.0)
MRSA	12	6(50.0)	12(100.0)	8(66.7)	4(33.3)	4(33.3)	2(16.7)	4(33.3)	4(33.3)
Overall	32	10(21.3)	28(87.5)	16(50.0)	20(62.5)	16(50.0)	14(43.7)	12(37.5)	16(50.0)
		Total number resistant to Antibiotics tested (%)							
MSSA	20	16(80.0)	4(20.0)	12(60.0)	4(20.0)	8(40.0)	8(40.0)	12(60.0)	8(40.0)
MRSA	12	6(60.0)	0(0.0)	4(33.3)	8(66.7)	8(66.7)	10(83.3)	8(66.7)	8(66.7)
Overall	32	22(68.7)	4(12.5)	16(50.0)	12(37.5)	16(50.0)	18(56.3)	20(62.5)	16(50.0)

4. DISCUSSION

Nasal carriage of *Staphylococcus 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. Numerous studies conducted in different countries and in different populations of patients on dialysis have consistently documented that a large proportion of such patients carry *Staphylococcus aureus* in their nares and that the risk of them becoming infected with their own strains is quite high (Herwaldt, 1998). In 1995, Kluytmans and colleagues demonstrated that nasal carriage of *S. aureus* is a significant risk factor for wound infection after cardiac surgery (Casewell et al., 1998). Studies have been done in adults in intensive care units (Majumder et al., 2001; Anupurba et al., 2003; Saxena et al., 2003; Rajadurai pandi et al., 2006; Pathak et al., 2010) and among patients at high risk of *S. aureus* infection (Chacko et al., 2009; Pathak et al., 2010) but studies on prevalence of nasal carriage and antibiotic susceptibility pattern of *S. aureus* in children are few (Ramana et al., 2009; Chatterjee et al., 2009; Pathak et al., 2010).

The study showed that 32.0% of apparently healthy students of University of Port Harcourt, Nigeria below 30 years of age had *S. aureus* out of which 37.5% were MRSA. Ramana et al. (2009) reported a prevalence of 16.0% for *S. aureus*, of which 19.0% were MRSA among school going children (5 to 15 years) in Narketpally, Andhra Pradesh, India. However, the factors associated with acquisition of *S. aureus* were not evaluated in that study. Other study

by Chatterjee et al. (2009) using polymerase chain reaction (PCR) showed a prevalence of 52.5% for *S. aureus* of which 3.9% were MRSA. They identified living in mud-thatch housing as factor associated with nasal carriage in their study (Pathak et al., 2010).

Nasal carriage of MRSA or MSSA varies in different geographical areas (Madani et al., 2001; Abudu et al., 2001; Sa-Leao et al., 2001; El- Jalil et al., 2008). The 32.0% prevalence reported for *S. aureus* in this study is comparable to the 32.40% reported by Mainous et al. (2006). The prevalence of MRSA among *S. aureus* isolates in the study by Mainous et al. (2006) was 2.58%, for an estimated population carriage of MRSA of 0.84%. The 37.5% reported for MRSA among healthy young students in this study contradicts what previously reported by some authors that while the prevalence of carriage of methicillin resistance is high and increasing in hospital environments (Alghaithi et al., 2000; El- Jalil et al., 2008), that it is rather low among strains colonizing young and healthy members of the community (Sa-Leao et al., 2001; El- Jalil et al., 2008). The study shows that the carriage rate of both MRSA and MSSA in young healthy adults whether exposed to hospital environments or not is higher than that reported by Al-Zu'bi et al. (2004) and El- Jalil et al. (2008). Nevertheless, the carrier rate of MRSA in microbiology students of Uniport in this study is comparable to that of nursing students (38.0%) and higher than that of pharmacy students (18.0%) reported by El- Jalil et al. (2008) in their study. The prevalence of MRSA in some countries is still low. In the Netherlands for example, it is as low as 1.0% (Lytkaenen et al., 2004; El- Jalil et al., 2008).

This study showed that there was a significant difference between sex for carriage rate of *S. aureus* [18.0% vs. 46.0%, $P < 0.05$]. The study showed that there was no significant difference between age groups for carriage rates of *S. aureus* (35.3% vs. 25.0%, $P > 0.05$). The prevalence of colonization with *S. aureus* has previously been shown to be age-dependent (Kluytmans et al., 1997; Peacock et al., 2003; Bogaert et al., 2004; Grundmann et al., 2006; Ciftci et al., 2007; Huang et al., 2007; Pathak et al., 2010). The prevalence varied across different age groups in our study with lower prevalence in the 25-29 years of age although these differences were not statistically significant. Similar finding was reported by (Pathak et al., 2010). In a study by Mainous et al. (2006), among individuals with *S. aureus* isolates, individuals aged 65 years or older had the highest MRSA prevalence (8.28%). The peak of colonization with a respiratory pathogen may be seen at 2-3 years of age (Bogaert et al., 2004; Pathak et al., 2010). During this age a lot of pathogens compete for colonization of the anterior nares; examples are pneumococci, *Haemophilus influenzae*, *Moraxella catarrhalis* and *S. aureus* (Pathak et al., 2010). Bacterial interference, phenomenon by which colonization by one bacterial strain prevents colonization by another strain, plays an important role in establishing or eliminating one bacterial strain over another (Sivaraman et al., 2009; Pathak et al., 2010).

The *S. aureus* isolates in our study show high sensitivity to cloxacillin but have high resistance to ampicillin, gentamycin and streptomycin. This may represent a scenario of a late stage of spread in the community, albeit at a low rate (Salgado et al., 2003; Bartlett, 2008; Pathak et al., 2010). In MRSA isolates, resistance was seen to antibiotics that are important for empirically treating severe infections. These antibiotics include ampicillin, ciprofloxacin, cotrimoxazole, erythromycin, Gentamycin, tetracycline and streptomycin. Resistance to cloxacillin, ciprofloxacin and gentamycin is a cause of concern because of their therapeutic value in treating serious *S. aureus* infections in high-risk patients. However, it is important to note that the present results are of carriage state and not clinical infections (Pathak et al., 2010). The study found multiple resistances to erythromycin and gentamicin in 66.7% and 83.3% of MRSA respectively. However, 66.7% of MRSA isolated in this study were resistant to erythromycin and streptomycin. Resistance to other antibiotics was common in both MRSA and MSSA. This is similar to the findings of previous studies. This agrees with previous studies (Al-Zu'bi et al., 2004; El- Jalil et al., 2008) which also found multiple resistances to

erythromycin and gentamicin in 48% of MRSA. However, 38% of MRSA isolated in the study by El-Jalil et al. (2008) were resistant to erythromycin and lincomycin but not to gentamicin. Resistance to other antibiotics was rather uncommon in both MRSA and MSSA in a study by El- Jalil et al. (2008).

Study of nasal carriage of MRSA is important to the community since carriage plays a key role in the epidemiology and pathogenesis of community associated disease (Pan et al., 2005; Lo et al., 2006; El- Jalil et al., 2008). Risk factors for CA-MRSA carriage are not well understood. Some studies have suggested that recent anti-microbial drug use plays a role in CA-MRSA colonization (Bagget et al., 2004; Ellis et al., 2004; El- Jalil et al., 2008). This study did not test this hypothesis. None of the students was asked about their history of recent anti-microbial therapy. It can be noted that *S. aureus* colonization is generally lower in resource poor countries and varies in the same community over time with increasing rates in the more recent studies. One possible explanation of high nasal carriage of *S. aureus* in resource rich countries could be low rates of exposure to antigens due to better personal hygiene leading to decreased clearing of pathogens in the tested patients (Sivaraman et al., 2009; Pathak et al., 2010). However, transmission of infections caused by these strains is readily established by close contact (Xander et al., 2006; El- Jalil et al., 2008). Furthermore, transmission from humans to animals (Seguin et al., 1999) or from animals to man (Juhász-Kaszanyitzky et al., 2007) may further complicate the epidemiology of these organisms (El- Jalil et al., 2008).

In line with other studies (Mainous et al., 2006; Pathak et al., 2010), even with the aforementioned strengths, our study has some limitations. Because of the small number of students used for this study, we were limited in our ability to identify risk factors for community carriage of MRSA; our ability to identify characteristics of this group beyond age and sex was limited. A second limitation of the study was that it was restricted to the non-institutionalized population. Although this population provides for the broadest assessment of the entire population, carriage of *S. aureus* and MRSA may differ in other settings. Thirdly, we did not measure the minimum inhibitory concentration (MIC), which is the recommended method especially for glycopeptide antibiotics. Fourthly, we did not do confirm MRSA status by doing *mecA* gene due to financial constraints. Lastly, our study is a study conducted in university setting with healthy students from the community. A community based cohort design with sampling of a given individual at various ages would have identified

persistent carriage (carriage over time), which is more important source for community spread.

5. CONCLUSION

Our study may be useful in that our findings point out that relative to younger individuals, older adults are less likely to be colonized with *S. aureus* but, when colonized, are more likely to have MRSA strains. This finding suggests that older adults with suspected staphylococcal infections may need antibiotic coverage against resistant strains. This is the first study among University of Port Harcourt students studying the prevalence of nasal carriage and showed a prevalence of 32.0% for *S. aureus*, 37.5% of which were MRSA. More studies with cohort design are needed to accurately assess the epidemiology of *S. aureus* nasal carriage in various geographical locations in the University. Thus, we must elucidate the mechanisms behind *S. aureus* nasal carriage and infection to be able to develop new preventive strategies (Wertheim et al., 2005). In conclusion, our study confirms the high prevalence of *S. aureus* nasal colonization in the young healthy individuals; however, it also shows that the rate of MRSA carriage remains high. Few demographic or clinical characteristics are related to either *S. aureus* carriage or, more specifically, MRSA carriage. Since *Staphylococcus aureus* is quickly spread by nose picking (hand contamination) continuous surveillance and improvement of hygiene standards among students is highly recommended such as hand washing with soap and warm water. Hands should be washed with soap and warm water. Hands should be washed after using the necessary house (toilet) or any unclean thing to avoid contamination of microorganisms.

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