Nasal Carriage of *Cloxacillin-Resistant Staphylococcus aureus* among University Students in Umudike, Abia State, Nigeria

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Abstract: Strains of *Staphylococcus aureus* resistant to beta-lactamase stable antibiotics such as methicillin, oxacillin and cloxacillin, commonly called Methicillin resistant *Staphylococcus aureus* (MRSA) have become a serious public health concern worldwide. Nasal carriage is a major risk factor for infection with MRSA. In this study, nasal carriage rate of Cloxacillin resistant *S. aureus* was assessed among students of Michael Okpara University of Agriculture, Umudike. Nasal swabs were collected from 50 apparently healthy students and cultured on Mannitol salt agar for isolation of *S. aureus*. Coagulase positive *S. aureus* was isolated from 39 (78%) of the nasal swab specimens. The isolates were tested for their antibiotic susceptibility profile against Cloxacillin and seven other antibiotics using the disc agar diffusion method. Of the 39 *S. aureus* isolates, 32(82.1%) were resistant to Cloxacillin, giving a nasal carriage rate of Cloxacillin-resistant *S. aureus* of 64% among the students. Thirty-seven (94.9%) and 35(89.7%) of the isolates were sensitive to Streptomycin and Gentamycin, respectively. The isolates exhibited various levels of resistance to other antibiotics, ranging from 33% to Chloramphenicol to 69.2% to Cotrimoxazole. The high level of resistance to Cloxacillin in this study should alert the public health authorities to ?he risk of infections with MRSA and the need to institute infection control measures to prevent outbreaks of MRSA related diseases both in healthcare settings and in community.

Key words: Antibiotic susceptibility, Cloxacillin-resistance, nasal carriage, MRSA, *Staphylococcus aureus.*

Introduction

Staphylococcus aureus is a facultative anaerobic Gram-positive bacterium commonly isolated from different parts of human body, particularly the anterior nares, skin and the throat but the nares are the most consistent site of isolation (Stenehjem and Rimland, 2013). *Staphylococcus aureus* can be found on the skin and/or in the nose of about 20-40% of healthy individuals in which it generally causes no harm if it remains on the surface of the skin (Mazzulli, 2017). However, certain strains of the bacterium possess virulence factors that enable them to cause a plethora of diseases ranging from mild skin infections such as boiis, furuncles, impetigo to more invasive and sometimes life threatening systemic infections like pneumonia, septic arthritis, endocarditis, osteomyelitis and sepsis (Shen *et al*., 2013, Ekundayo and Ndubuisi, 2015).

Soon after Penicillin was introduced in the 1940s, a strain of *S. aureus* producing the enzyme penicillinase (beta- lactamase) which destroys the beta-lactam core of the antibiotic emerged. To combat penicillin-resistant *S. aureus*, semi-synthetic class of Penicillins, resistant to the action of beta-lactamases was developed. The first of this class of antibiotics was Methicillin.

However, shortly after the introduction of Methicillin, strains resistant to it were reported in 1961(Barber,1961).Methicillin-resistant *Staphylococcus aureus* (MRSA) strains are resistant to all members of the beta-lactam antibiotics including the semi-synthetic ones like Oxacillin, Amoxycillin, Cloxacillin and Nafcillin. Although Oxacillin was chosen as the agent of choice for antibiotic susceptibility testing of staphylococci since early 1990 because Methicillin ceased to be in clinical use in the USA, the MRSA continues to be used to describe *Staphylococcus aureus* resistant to Oxacillin (CLSI, 2007).

In Nigeria, Oxacillin discs are not commonly available but the commercial kits of antibiotic discs used for susceptibility testing contain Cloxacillin which is in the same class. It is therefore important to highlight the susceptibility of *S. aureus* isolates to Cloxacillin as a way of monitoring the presence of MRSA in the community.

The Methicillin-resistant *Staphylococcus aureus* (MRSA) strains constitute the greatest public health threat. Infections caused by MRSA were originally prevalent in the hospital environment and are referred to as Hospital Associated MRSA (HA-MRSA). Infections due to MRSA have however moved into the community and are called Community associated MRSA (CA-MRSA) infections. Nasal carriage of MRSA is one of the most important risk factors for CA-MRSA (David and Daum, 2010). In this study, we investigated the nasal carriage rate of Cloxacillin - resistant *Staphylococcus aureus* among the students of Michael Okpara University of Agriculture, Umudike to assess the risk of infections with MRSA in the community.

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Materials and Methods

Specimen Collection and Culturing

In this study, one nasal swab was collected from each of 50 students of MOUAU who gave informed consent. The study participants consisted of 35 males and 15 females selected randomly. A sterile cotton-tipped swab moistened by dipping in sterile normal saline and pressing out excess fluid against the inner side of the container, was used to collect the specimen. Each participant was given a moistened swab and instructed how to collect the specimen by inserting the tip of the swab into both anterior nares, one at a time, and rolling it gently against the inner surface. The swab specimen was inserted into the labelled sleeve and taken immediately to the laboratory for bacteriological examination.

The swab specimen was inoculated by streak plate method on Mannitol salt agar [MSA] (Hardy Diagnostics, Inc, USA). The inoculated plates were incubated aerobically at 37°C for 24h.

Isolation of *Staphylococcus aureus*

Using a sterile wire loop, cells from one yellow or creamed coloured colonies on MSA plate was sub-cultured on nutrient agar and incubated at 37°C for 24h. Smear was prepared from each subculture and subjected to Gram staining and examined microscopically. Catalase test was conducted on Gram-positive cocci. Coagulase test was carried out on catalase positive isolates to identify *Staphylococcus aureus*.

Antibiotic susceptibility testing

The antibiotic susceptibility testing was done by Kirby-Bauer disk diffusion method according to the Clinical Laboratory Standards Institute (CLSI), M02-A11 guidelines (CLSI, 2012). A suspension of *S. aureus* was prepared by picking 4-5 colonies from nutrient agar plate and mixing in 4ml of normal saline in sterile bijou bottle. The turbidity of the bacterial suspension was adjusted to tube No. 0.5 McFarland standard (about 10^8 CFU/mL). Using sterile swab stick, 0.1mL of the bacterial suspension was spread plated onto the surface of Mueller-Hinton agar plates. The antibiotic discs were placed aseptically on the agar plates and the plates were incubated aerobically at 37°C for 16-18h. The diameter of zone of inhibition around the antibiotic discs was measured in millimetres using a transparent plastic ruler.

The susceptibility of the bacteria to the antibiotics tested was categorized into sensitive (S), intermediate(I) or resistant (R) according to the interpretive criteria of the Clinical Laboratory Standards Institute M100-S24 (CLSI, 2014) based on the diameter of zone of inhibition. The reference diameters of zone of inhibition are as follow: Cloxacillin (S: ≥22mm, R: ≤21mm); Cotrimoxazole (S: ≥16mm, I: 11-15mm, R: <10mm); Erythromycin (S: >23mm, I: 14-22mm, R: <13mm); Gentamycin (S: ≥15mm, I: 13-14mm, ≤12mm); Augmentin (S: ≥18mm, I: 14-17mm, R: <13mm); Streptomycin (S: >15mm, I: 13-14mm, R: ≤12mm); Tetracycline (S: ≥19mm, I: 15-18mm, R: <14mm); Chloramphenicol (S: >18mm, I: 13-17mm, R: <12mm).

Statistical analysis.

Differences in the rates of nasal carriage of *S. aureus* and Cloxacillin Resistant *S. aureus* between male and female students were compared using the Pearson's Chi-Square test; $p \leq 0.05$ was considered statistically significant.

Results

A total of fifty (50) nasal swabs were collected from external nares of students in MOUAU for this study. Out of the 50 nasal swabs, 39 (78%) produced colonies of coagulase positive *S. aureus*. The distribution of *S. aureus* isolates among male and female students is presented in Table 1. Twenty-six (52%) isolates were from males while 13 (26%) were from females. There was a significant difference between the number of isolates of *S. aureus* from the nasal passage of male and female students ($p \leq 0.05$).

The antimicrobial susceptibility profile of *S. aureus* isolates is shown in Table 2. Thirty-eight (97.4%) of the *S. aureus* isolates were resistant to Cloxacillin as they produced diameter of zone of inhibition less than 22mm which is the breakpoint for sensitivity to Cloxacillin. Only one (2.6%) isolate was sensitive to the antibiotic and the rest. The nasal carriage rate of Cloxacillin-resistant *S. aureus* was therefore 76%. Twenty-two (28) were isolated from males and 10 isolates were from female (data not shown in table 2) . There was no significant difference between the number from the male and the female ($p > 0.05$).

Thirty seven of the 39(94.9%) isolates were sensitive to Streptomycin and 32(82.1%) were sensitive to Gentamycin with just 2(5.1%) resistant to the antibiotic. The resistance level of the isolates to Augmentin was 51.3%. The resistance level of the isolates to Cotrimoxazole (Trimethoprim/Sulphamethoxazole) and Erythromycin was 69.2% and 61.5%, respectively.

Table 1: Incidence of nasal carriage of S. aureus among Students of MOUAU.

Sex	No of Swab Specimens Examined	No with *S. aureus*
Male	35(75%)	26(52%)
Female	15(15%)	13(26%)
Total	50(100%)	39(78%)

Table 2: Antimicrobial susceptibility profile of *S. aureus* isolates from nasal passages of MOUAU Students.

Antimicrobial Agents	Disk Potency (ug)	№ (%) Sensitive (S)	№ (%) Intermediate (I) sensitivity	№ (%) Resistant (R) Isolates
Cloxacillin	10	1(2.6)	-	38(97.4)
Cotrimoxazole	1.25/23/75	8(20.5)	4(10.3)	27(69.2)
Erythromycin	15	4(10.3)	11(28.2)	24(61.5)
Gentamycin	10	35(89.7)	2(5.1)	2(5.1)
Augmentin	20/10	20(51.3)	4(10.3)	15(38.5)
Streptomycin	30	37(94.9)	2(5.1)	0(0%)
Tetracycline	30	19(48.7)	4(10.3)	16(41.0)
Chloramphenicol	30	19(48.7)	7(18.0)	13(33.3)

[a] Interpretive criteria according to Clinical and Laboratory Standards Institute (CLSI_M100-S24)
Cloxacillin (S: ≥22mm, R: ≤21mm); Cotrimoxazole (S: ≥16mm, I: 11-15mm, R: <10mm); Erythromycin (S: >23mm, I: 14-22mm, R: <13mm); Gentamycin (S: ≥15mm, I: 13-14mm, ≤12mm); Augmentin (S: ≥18mm, I: 14-17mm, R: <13mm); Streptomycin (S: >15mm, I: 13-14mm, R: ≤12mm); Tetracycline (S: ≥19mm, I: 15-18mm, R: <14mm); Chloramphenicol (S: >18mm, I: 13-17mm, R: <12mm)

Discussion

Staphylococcus aureus nasal carriage rate of 78% found in this study is higher than 56.4% found by Lamikanra *et al.* (1985), in a survey of Nigerian students from primary school to university in Western Nigeria. This is much higher than the commonly reported 20-40% nasal colonization of human population by *S. aureus* (van Belkum *et al.*, 2009). Although, nasal colonization with *S. aureus* is not necessarily an infection, it is a major risk factor for infection (Shen *et al.*, 2013).

Strains of *S. aureus* resistant to Oxacillin are traditionally termed Methicillin resistant *S. aureus* (MRSA). Such strains are resistant to all the members of the semi-synthetic beta-lactamase resistant antibiotics, including Cloxacillin (CLSI, 2014). Much discussion in literature has focused on MRSA and there is little reference to Cloxacillin (Barber,1961, Baptiste *et al.*, 2005, CLSI, 2007). However, it is Cloxacillin that is commonly available and often included in imported antibiotic susceptibility testing panel such as Abtek™ (Abtek Biologicals Ltd, UK) multidisc susceptibility testing ring commonly used in Nigeria. In this study, we found a nasal carriage rate of 64% for Cloxacillin-resistant *S. aureus*. In contrast to nasal carriage rate of MRSA, reported by others, this is relatively high. Prates *et al.* (2010) reported MRSA nasal carriage rate of 5.8% in Brazil and Reta *et al.* (2015) reported a rate of 13.8% in Ethiopia. The findings of our study are however consistent with that of Onolitola *et al.* (2007) who reported a prevalence rate of 72% among healthy adults in Zaria, Northern Nigeria. Onanuga and Temedie (2011) reported a prevalence of 47.6% of MRSA in Amassoma in Niger Delta, Southern Nigeria.

The antibiotic susceptibility profile of the Cloxacillin-resistant *S. aureus* isolates show that the isolates were highly susceptible to Streptomycin (94.9%), Gentamicin (89.7%) and moderately susceptible to Augmentin (51.3%). The isolates exhibited high resistance to 3 antibiotics, Cloxacillin (97.4%), Cotrimoxazole (69.2%) and Erythromycin (61.5%). The isolates exhibited moderate level of resistance to Tetracycline (41%) and Chloramphenicol (33.3%). In contrast Shittu *et al.* (2011), reported that 55% of their clinical isolates of *S. aureus* from different geographical locations in Nigeria were resistant to Tetracycline and 72% to Trimethoprim /Sulphamethoxazole (Cotrimoxazole).

The results of this study apply to one institution and this may limit the generalization of the findings to other settings. However, the results may suggest the need to conduct multicentre studies with larger number of participants in the future. We could not perform genetic analysis of the isolates due to lack of facility for such studies. Genetic studies of the resistance genes would have facilitated the comparison of the isolates with those from other studies. Our findings however highlight the need for future study which should include molecular characterization and a larger sample size.

Conclusion

In conclusion, we found a high rate of nasal carriage of Cloxacillin-resistant *S. aureus* among students of Michael Okpara University Agriculture, Umudike. Since resistance to Cloxacillin can be taken as a surrogate for resistance to Oxacillin or Methicillin, there is need for public health actions to institute infection control measures including programme of antimicrobial resistance surveillance for Cloxacillin-

resistant *S. aureus* or MRSA, dissemination of resistance information to healthcare professionals, education on the risk for MRSA infections as well as programmes of decolonization of patients who need to be admitted into hospitals.

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