

# Antimicrobial Evaluation of Methicillin-Resistant *Staphylococcus Aureus* Nasal Carriage amongst Healthy Students in Agbor, Delta State, Nigeria

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## Abstract

The antibacterial susceptibility of Methicillin resistant *Staphylococcus aureus* isolated from the nostrils of students of College of Education Agbor, Nigeria was investigated. Three hundred (300) specimens was collected from 150 male and 150 female students and cultured on appropriate bacteriological media. The bacterial isolates (*S. aureus*) were identified by standard biochemical tests. The MRSA was determined using Oxacillin antibiotic disk. Antibiotic susceptibility of the isolates was performed according to Clinical Laboratory Standard testing Institute (CLSI) guidelines. Of the 300 nasal swab samples collected and screened, a total 218 (72.7%) of the isolates were found to be *S. aureus* based on morphology and biochemical tests. The incidence rate from female to male individuals were 103 (68.6%) and 115 (76.6%) respectively. The prevalence rate/colonization of MRSA among healthy individuals in the community of the *S. aureus* isolated was 56 (18.7%). The antibiotic resistant pattern of the MRSA isolates was: Amoxicillin 30 (54%) > Streptomycin 25 (45%) > Amoxicillin-clavulanic acid 14 (25%), > Erythromycin 13 (23%) > Chloramphenicol 12 (21%), > Co-trimoxazole 10 (18%), > Ofloxacin 8 (13%), > Ciprofloxacin 9 (16%) > Gentamicin 5 (9%). The MRSA isolates showed multiple drug resistance to the beta-lactam and other commonly used antibiotics.

Keywords: MRSA; Nasal; Antibiotics; Nigeria; Resistance

## Introduction

*Staphylococcus aureus* is a species of bacteria commonly found on the skin and or in the noses of healthy people [1]. The nose is one of the few openings that bacteria have direct access to get inside the body. Thus the nose and nasal passages can be perfect environments for some bacteria. *S. aureus* has been recognized as one of the most important

bacteria pathogen significantly contributing to hospital and community-acquired infections all over the world [2]. It is the most frequently isolated pathogen causing bloodstream infections, skin and soft tissue infections, and pneumonia. Diseases caused by *S. aureus* include folliculitis, boil, furunculosis, scalded skin syndrome, conjunctivitis, paronychia, mastitis, and toxic shock syndrome [3]. Attempts to control these diseases through the use of antimicrobial agents particularly antibiotics have led to increased prevalence of resistance to these agents [4,5]. The *S. aureus* diseases and their high mortality were greatly reduced with the use of penicillin in the early 1940s [1]. This success was short-lived with the emergence of Penicillin Resistant *Staphylococcus aureus* (PRSA) producing beta-lactamase [1]. The beta-lactamase enzyme inactivates the penicillin antibiotic. Methicillin, a beta-lactamase-resistant beta-lactam, provided new treatment options for PRSA infections. However, the emergence of Methicillin-Resistant *Staphylococcus aureus* (MRSA) that is cross resistant to all beta-lactams thwarted the treatment options for staphylococcal infections. MRSA can colonize healthy people at a lower rate, about 1- 8%, and represents a potent and increasingly prevalent risk factor for subsequent *S. aureus* infections [6]. MRSA has evolved resistance not only to beta-lactam antibiotics, but also to several classes of antibiotics. MRSA plays crucial role in diseases acquisition from the community and there has been a global increase in the number of infections caused by MRSA [1,7]. However, data concerning the frequency of nasal carriage of MRSA in the Agbor community is not known. Thus this study was designed to determine the prevalence and antibacterial susceptibility profile of MRSA nasal carriage among healthy students of Agbor college of Education. This we hope may influence antibiotic-use decisions/policies.

## Methods

### Collection and purification of bacteria isolates

Strains of *Staphylococcus aureus* were isolated from nostrils of 150 male and 150 female students of college of education Agbor, Delta state, Nigeria using sterile swab sticks. The swabs

were immediately inoculated on mannitol- salt agar (Oxoid, England) and incubated for 24 h. Colonies which caused fermentation of mannitol were isolated, Gram-stained and examined microscopically. Thereafter, all Gram-positive cocci in clusters were stored in an agar slant at 4°C for further identification/characterization.

### Identification of bacterial Isolates

All Gram-positive cocci isolates that were in clusters and that fermented mannitol were subjected to standard biochemical characterization tests for *Staphylococcus aureus* [8].

### Phenotypic detection of MRSA using the oxacillin disc

Susceptibility of all the *S. aureus* isolates was conducted by means of the agar screening method on Muller-Hinton agar using Oxacillin sensitivity disc. The *S. aureus* isolates were standardized to 0.5 Mcfarland standards and were inoculated aseptically onto the nutrient agar plates. The plates were incubated for exactly 24 hours at 37°C.

### Antibiotic susceptibility testing (AST)

The antibiotic resistance pattern of the isolates was determined against nine antibiotics using Kirby – Bauer disc – diffusion method (1966) following the CLSI (2010) guidelines. Briefly, the isolates were grown in Nutrient broth at 37°C for 24h. Two loopfuls (0.08 ml) of the suspension of each isolate, standardized by matching with 0.5 MCF were inoculated into 20 ml of sterile molten agar in 10 cm diameter Petri dishes and mixed. The plates were allowed to set and the antibiotic sensitivity disc was aseptically placed on their surfaces. The plates were incubated at 37°C for 24 h and the resultant Inhibition Zone Diameters (IZDs) measured and recorded. These were then interpreted as susceptible, intermediate and resistant according to standard specifications of CLSI.

## Results and Discussion

Nasal carriage of *S. aureus* represents a potent and increasingly prevalent risk factor for subsequent *S. aureus* infection as strains of Community-Associated (CA)-MRSA that cause infections in healthy people have been detected [9]. In Agbor Delta state, no data exist concerning nasal carriage of MRSA. Thus it is important to determine the incidence of *S. aureus* and MRSA nasal carriage as this can influence antibiotic-therapy decisions. Out of three hundred nasal swab samples collected and screened, a total 218 (72.7%) of the isolates were found to be *S. aureus* based on morphology and biochemical tests. The prevalence rate from female to male individuals were 103 (68.6%) and 115 (76.6) respectively as shown in table 1. There was no observed significant difference in colonization rate of *S. aureus* between the male and female group (P0.05). This observation is line with the findings of Ajoke et al. [1] in Jos, North-central Nigeria and Okwu et al.

[10] in Okada, South west Nigeria. They reported that sex is not a remarkable determinant in *S. aureus* colonization.

**Table 1:** Frequency of isolation of *S. aureus* from healthy community individuals in college of Education Agbor

Source	Number sampled	Number of samples collected	Number	percentage (%)
Male	150	150	103	68.6
Female	150	150	115	76.6
TOTAL	300	300	218	72.7

Table 2 shows the prevalence rate of MRSA colonization among healthy individuals in community of the *S. aureus* isolated was 56 (18.7%). Colonization has been recognized as an important step in the chain of events that leads to *S. aureus* infections. Individuals are first colonized, invaded and infected. Thus, colonization with *S. aureus* is a major risk factor for staphylococcal infections [11-13]. The colonization rate (18.7%) recorded in our study is different from the reports of Onanuga et al. in a study conducted in Zaria, Northern Nigeria. They recorded a higher rate of MRSA Colonization (69%) among healthy women. MRSA was detected using oxacillin (30 µg) disc which has high efficiency to detect MRSA as an alternative to PCR in resource constrain areas [1,10].

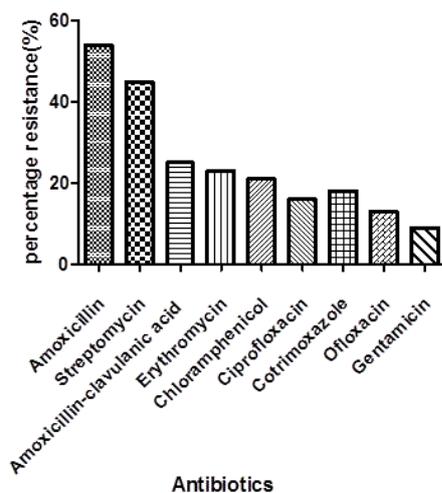
**Table 2:** Prevalence of MRSA among the healthy students

Source	MRSA Number	Percentage (%)
Male	30	53.6
Female	26	46.4
TOTAL	56	100%

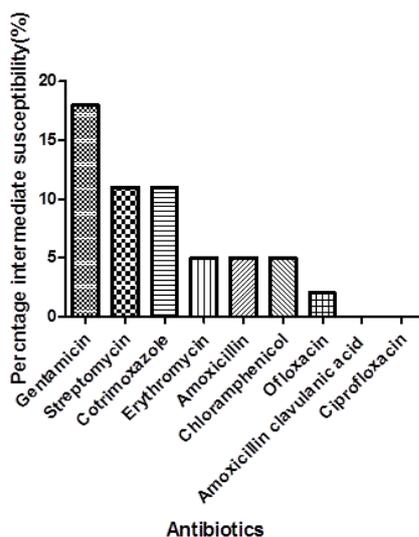
From Figure 1 the antibiotic resistant pattern of the MRSA isolates was: Amoxicillin 30 (54%) > Streptomycin 25 (45%) > Amoxicillin-clavulanic acid 14 (25%), >Erythromycin 13 (23%) >Chloramphenicol 12 (21%),> Co-trimoxazole 10 (18%),>Ofloxacin 8 (13%),> Ciprofloxacin 9 (16%) > Gentamicin 5 (9%). The MRSA isolates recorded a high resistance to Amoxicillin and Streptomycin among all the tested antibiotics. The highest level of resistance was observed to the amoxicillin, a penicillin.

This is in agreement with the previous reports of Onanuga et al. [14], Rosina and Estifanos, [15], Nkwelang et al. [16] and Rasamiravaka et al. [7]. This observation is related to common prescribing of the penicillin antibiotics [13]. It should however be noted that Amoxicillin-clavulanic acid (Augmentin®) recorded a better effect than amoxicillin against the MRSA isolates. This shows that the resistance of MRSA isolates to amoxicillin is due to β-lactamase inactivation [17]. The clavulanic acid present in the Amoxicillin –clavulanic acid combination (Augmentin®) offers protection to the β-lactam chemical ring nucleus present in the amoxicillin moiety to enhance the activity of Amoxicillin. Several studies have reported that in *S.*

*aureus* there are  $\beta$ -lactamase enzymes-producing strains [18-21]. In this study most of the isolates were susceptible to Gentamicin (96%) Ofloxacin (86%) and Ciprofloxacin (84%). The sensitivity profile of the MRSA isolates to the flouroquinolones and gentamicin is in line with a previously published work [1]. The high susceptibility was attributed to absence of genes conferring resistance among the isolates (Figure 2). The high sensitivity to the non-beta-lactam antibiotics supports the recommendation of Onanuga that non-beta-lactam antibiotics are preferred drugs for the treatment of Community-Acquired (CA) MRSA infections [22,23].



**Figure 1:** Antibiotic resistance patterns of the MRSA isolates



**Figure 2:** Percentage intermediate susceptibility profile of the MRSA isolates

## Conclusion

The study has established prevalence of MRSA among healthy subjects in the institution. The MRSA isolates showed multiple drug resistance to the beta-lactam, Amoxicillin and other commonly prescribed antibiotics: Spectromycin, cotrimoxazole and Erythromycin. Therefore, there is an urgent need to reassess policies on antibiotics use.

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