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Comparison of Prevalence and Risk Factors for Methicillin-Resistant *Staphylococcus aureus* in Two Tertiary Institutions, Southern Nigeria.

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Abstract
Methicillin-resistant *Staphylococcus aureus* (MRSA) is an incessant public health challenge with a rising trend in community infection among students. This study compared the prevalence and risk factors for MRSA nasal colonization among students in two tertiary institutions in Southern (east and west) Nigeria. One hundred nasal swab samples were collected from healthy university students and questionnaire aided in the retrieval of demographic and possible risk data. *Staphylococcus aureus* isolates were identified using morphological and biochemical approach. MRSA was detected phenotypically using both cefoxitin test and double disk synergy test. The antibiotic susceptibility test was done on the MRSA isolates against 9 antibiotics. A total of 18% (18/100) and 6% (6/100) *Staphylococcus aureus* and MRSA isolates was detected respectively. Of which, 8% (4/50) and 4% (2/50) MRSA strains was found in south eastern and south western Nigeria respectively (p = 0.036). The mean age of the students was 21.4 years ± 1.81. Overall, the age-group and gender with the highest MRSA colonization was 20-24 years and females respectively. Significant association was found between MRSA colonization and regular hospital visitation, touching of nose/face, and use of antibiotics without doctor's prescription (P < 0.05) in both institutions. A total of 100% of the isolates were resistant to clotrimazole. High prevalence (67%) of multi-drug resistance was observed. The prevalence of MRSA in both institutions had significant difference; with the risk factors: regular hospital visitation, touching of nose/face and indiscriminate use of antibiotics among the students.

Key Words: Methicillin-resistant *Staphylococcus aureus*, Students, Tertiary institution, Nigeria

Introduction

The *Staphylococcus* genus are gram-positive cocci and belong to the family *Micrococcaceae* (Daniel and Daum, 2010).They are well-known bacterium of increasingly resistance to the most commonly used antimicrobial agents (Agbakoba *et al*., 2020). Methicillin-resistant *Staphylococcus aureus* (MRSA) was originally described in 1960s. They have been globally reported in nosocomial infections with fatal diseases such as severe sepsis, pneumonia, toxic shock syndrome, necrotizing fasciitis, endocarditis, and osteomyelitis (Abroo *et al*., 2017). Methicillin-resistant *Staphylococcus aureus* are a group of gram-positive bacteria; *S. aureus* that are genetically different. Studies have shown that they emerged as a result of acquisition of antibiotic resistance genes encoding for resistance to beta-lactam antibiotics and all types of penicillin as well as methicillin. Furthermore, MRSA strains has reported multi-drug resistance (Akujobi *et al*., 2013; Ebenebe *et al*., 2014)

Staphylococcus aureus, an important pathogen in human disease is frequently isolated in hospitals and communities in Nigeria. Owing to the constant rise in the number of infections by MRSA strains, treatment has become challenging. The anterior nares (upper respiratory tract) which is the ecological niches of *S. aureus* contributes to the epidemiology and pathogenesis of MRSA infection. (Foster, 2017;

Ondusko and Nolt, 2018). The individual carriers are a major contributor to the spread of this pathogens as they are at risk of infections (Akujobi *et al*., 2013). Consequently, monitoring the incidence of MRSA has become imperative.

Tertiary-school students are often exposed to infectious agents owing to their seemingly care-free lifestyle. They are more likely to be colonized by MRSA due to their communal lifestyle and frequent visit to the medical centre. The risk factor for MRSA among young adults and teenagers include previous skin infection, previous hospitalization, and/or recent contact with medical personnel, poor hygiene, contact with a MRSA carrier (Shamshul *et al*., 2016).The nasal site is a biome for most pathogenic bacteria found in hospital especially *S. aureus*. Nasal colonization of these bacteria remains an endogenous reservoir for infections and also a source of cross-infection, thus promoting community spread (Turner *et al*., 2019). Isolation of MRSA in tertiary institutions is a potential threat to the gradual spread of these bacteria in the student's community.

Nasal *S. aureus* has been linked to soft tissue infections in the community (Foster, 2017) and hospital infections such as bacteraemia (Guo *et al*., 2020). Antimicrobial drug resistance in *S. aureus*, particularly multidrug-resistant strains, is a serious global concern that places a significant burden on health-care facilities (Kock *et al*., 2010). Most community acquired *S. aureus* infections have been attributed to anterior nares, with recent finding reporting nasal colonization as a source of bacteraemia. Nasal colonization in students has been linked to the incidence of *S. aureus* infection especially MRSA (Abdelmalek *et al*., 2022), thus the need for this study.

Materials and Methods

Study design and Sample size

This study; cross-sectional was done between April 2021 to June 2022. One hundred nasal swab samples (50 samples each) were collected from randomly selected university students across various life science departments in two different institutions- Nnamdi Azikiwe University, Awka, (South East) and Caleb University Lagos, (South West) Nigeria. The Departments included: Medical Laboratory Science, Medical Rehabilitation, Microbiology, and Biochemistry. Sample size was determined using statistical methods with estimated prevalence of 50% (Bolarinwa, 2020).

Ethical approval

The study's ethical approval was received from the Health Ethics Committee of the Faculty of Health Science and Technology, Nnamdi Azikiwe University. The Consent of the subjects was obtained prior to the study.

Inclusion and Exclusion Criteria

Inclusion Criteria

I. Students in Life sciences departments of Nnamdi Azikiwe University and Caleb University, Nigeria.
II. Apparently healthy students of both genders.

Exclusion Criteria

I. Students in non-life sciences departments.
II. Students who have Staphylococcal infection such as pneumonia, sepsis or wound infection at the time of the study.
III. Students who have received either intra-nasal antibiotic treatment or anti-staphylococcal antibiotics 4 weeks prior to this study.

Finally, a questionnaire served as a tool to retrieve demographic and risk factor data among the included participants.

Identification of *Staphylococcus aureus* isolates

Identification was done using cultural methods, Microscopy (Gram- staining) and Biochemical tests. The biochemical tests included - coagulase test, catalase test, urease test, haemolysis test, motility test and sugar fermentation test. Cultural method involved cultivation of *S. aureus* with Mannitol Salt Agar (MSA) containing beef extract and protease peptone [Hi-Media]. After incubation, the plate was examined for appearance, size, colour, and other morphology of the colonies.

Phenotypic detection of methicillin-resistant *S. aureus*.

Firstly, use of cefoxitin antibiotics was used. Resistance of the isolates to the third generation

cephalosporin, Cefoxitin (30 µg), served as marker for the MRSA detection according to methods by Abdulhadi (2017). The zone of inhibition was reported in millimeters according to the clinical and laboratory standards Institute guideline. Secondly, double disc synergy test was further employed. This test was carried out to detect the production of extended spectrum beta-lactamase (ESBL) by members of *Staphylococcus aureus.* The test was carried out by using a disc of amoxicillin-clavulanate (30µg) along with the third generation cephalosporin; ceftazidime (30µg) and cefotaxime (30µg) according to methods by Ezeanya *et al*. (2017).

Antibiotic Susceptibility Testing

The disk diffusion method was used for the following antibiotics such as Ceftazidime (30 µg), Cefotaxime (30µg), Imipenem (10 µg), Ceftriaxone (30µg), Clotrimazole (50 µg), Tetracycline (30µg), Erythromycin (15 µg), Amoxicillin-Clavulanic acid (30µg) and Gentamicin (10 µg). A colony of the test organism prepared with peptone water. The turbidity was equivalent to 0.5 McFarland standard. The suspension was swabbed on Mueller Hinton agar plate. For an average of 18 hours, the inoculated plate was incubated. After incubation, the zone of inhibition was assessed as recommended by the Clinical and Laboratory Standard Institute (CLSI, 2020).

Statistical Analysis

The Statistical package for the Social Sciences (SPSS) 21.0 was used. Descriptive analysis of number/ percentage was used for data variables obtained. Chi-square and fisher's exact test were used for comparison and to establish association between predisposing factors and MRSA prevalence; $p < 0.05$ was considered significant.

Results

Comparison of Prevalence of *S. aureus* and MRSA in both institutions

The prevalence of *S. aureus* nasal colonization among the students in both institutions was 18% (18/100). The prevalence of 16% (8/50) was found among the students in south west while 20% (10/50) among the students in south east. The overall prevalence of MRSA in both institutions was 6% (6/100). There was a significant difference in the prevalence of MRSA in both institutions; $p = 0.037$ (Table 1).

Table 1: Comparison of prevalence of *Staphylococcus aureus* and MRSA in the two institutions.

Variable	Group (%)		p-value
	SW	SE	
Staphylococcus aureus			
Negative	42 (84.00)	40 (80.00)	0.674
Positive	8 (16.00)	10 (20.00)	
MRSA			
Negative	6 (12.00)	6 (12.00)	0.036
Positive	2 (4.00)	4 (8.00)	

Key: SE-south east; SW-south west; **P*-value less than or equal to 0.05 is considered significant

Characteristics of the study population associated with MRSA colonization

A total of 100 students with 50 males and 50 females' nasal swabs was collected for detection of *Staphylococcus aureus* and subsequently MRSA. The mean age of the students was 21.4 years ± 1.81. The age distribution of the study participants revealed that 62% were between the ages of 20 – 24 years (Figure 1). However, in terms of age distribution with gender, majority of the male students (56%) and females students (56%) were between the ages of 20-24 years whereas; the female students (36%) and the male students (24%) were between the ages of 15-19 years. While, the female students (8%) and the male (17%) were between the ages of 25-29 years. Only the male students (3%) were found to be between the ages of 30 years and above. In this present study, MRSA was isolated from nasal swabs of randomly selected life science students from various departments (Medical Laboratory Science, Medical Rehabilitation, Microbiology and Biochemistry). The age-group (20-24 years), had the highest MRSA colonization. Similarly, the gender (female) had the highest MRSA colonization. Among the departments, MRSA colonization was found in students of Medical Laboratory Science and Microbiology. The relationship between MRSA colonization rate with age-group, gender and departments is shown in table 2.

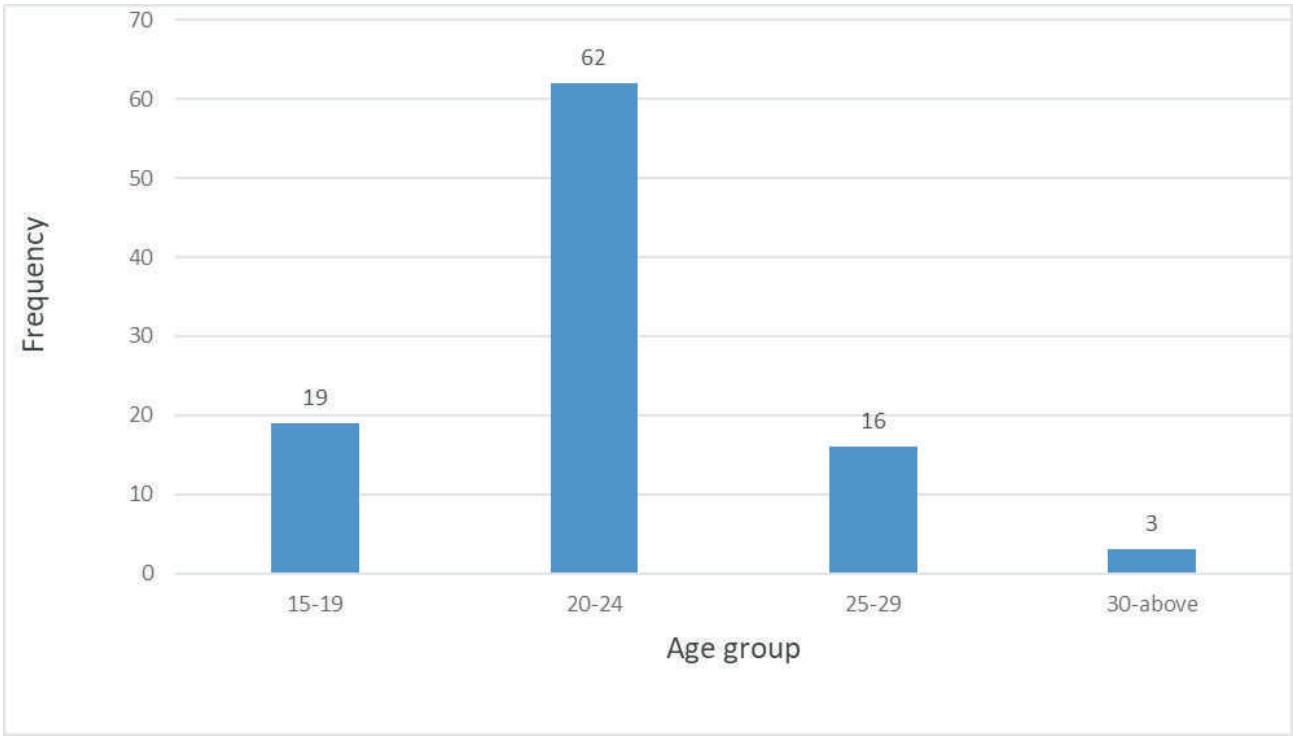

Figure 1. A bar column bar showing the age distribution of the study participants.

Table 2: Association between MRSA and the socio-demographics of the participants

Socio-demographics	Colonization rate (%)		χ^2 value	p-value
	SW	SE		
Age group				
15-19	1 (50.00)	1 (25.00)	5.91	0.116
20-24	1 (50.00)	3 (75.00)		
25-29	0 (0.00)	0 (0.00)		
30-above	0 (0.00)	0 (0.00)		
Gender				
Female	1 (50.00)	3 (75.00)	2.60	0.171
Male	1 (50.00)	1 (25.00)		
Department				
Medical Laboratory Science	0 (0.00)	4 (100.00)	1.57	0.628
Medical Rehabilitation	0 (0.00)	0 (0.00)		
Microbiology	2 (100.00)	0 (0.00)		
Biochemistry	0 (0.00)	0 (0.00)		

Key: SW-South west; SE-South east, χ^2 value = Chi- square value

Association of risk factors with MRSA colonization in the study population

A statistically significant association was found between variables: regular hospital visitation, habitual touching of nose/face and indiscriminate use of antibiotics with MRSA nasal colonization ($p < 0.05$) as shown in table 3. In both institutions, the variables with the highest MRSA nasal colonization were habitual touching of nose/face (χ^2=0.16; p = 0.019) and indiscriminate use of antibiotics (χ^2=0.0532; p = 0.023).

Table 3. Association between risk factors and isolation of MRSA in both institutions

Variables	Positive colonization rate		χ^2 value	p-value
	SW	SE		
Touching of nose and face	2	4	0.16	0.019
Keeping of long nails	0	1	0.0182	0.893
Regular hand washing	1	4	2.71	0.607
Regular hospital visitation	0	4	8.90	0.031*
Use of antibiotics without prescription	2	4	0.0532	0.023*

Key: SE- South east; SW-South west; χ^2 value = Fisher exact, **P*-value less than or equal to 0.05 is considered significant.

Antimicrobial susceptibility result

Multi-drug resistant isolates were found to be 67%. The distribution of resistance to the antibiotics were: Clotrimazole (100%), followed by Amoxicillin- Clavulanic acid (50%), Imipenem (50%), Erythromycin (50%), and the least was Gentamicin (11%). The antibiotics with the highest level of susceptibility observed was Gentamicin (89%) (Figure 2).

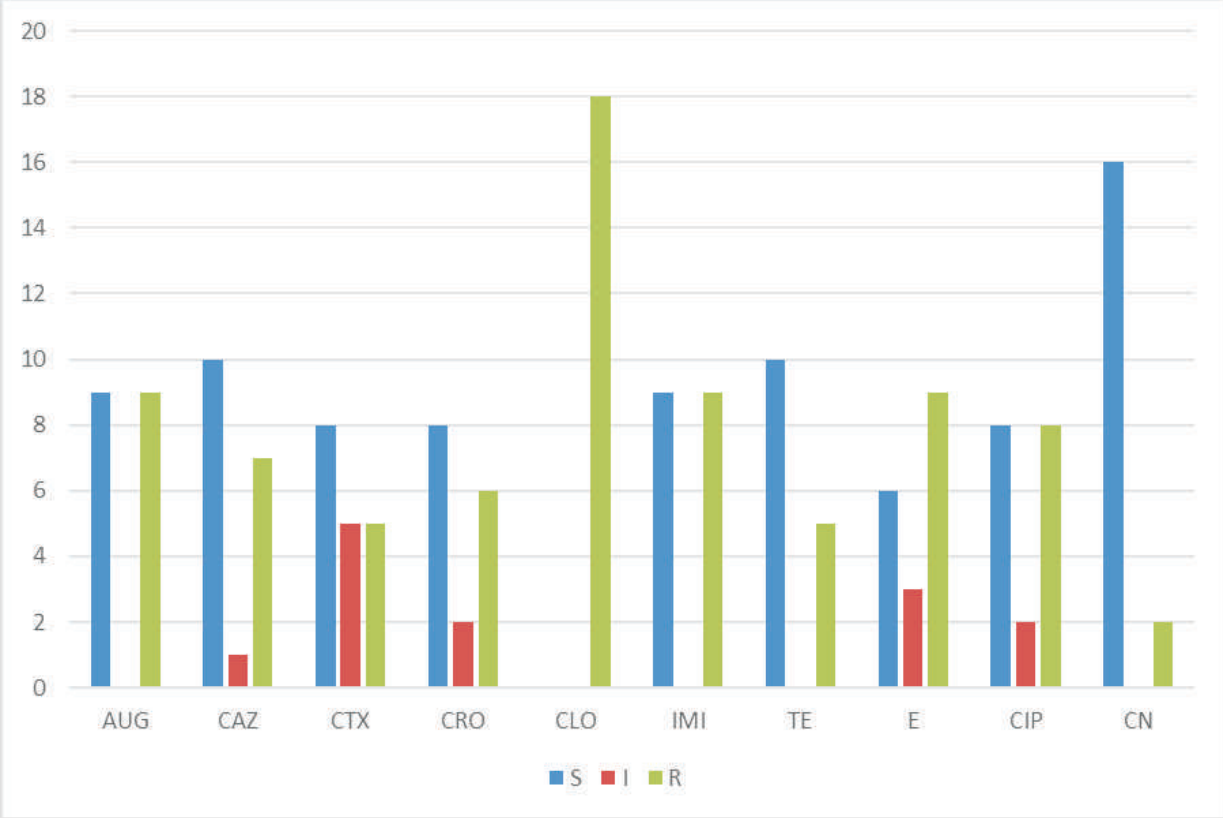

Figure 2. Frequency of the antimicrobial susceptibility testing of the isolates in this study

Keys: S- Susceptible, I- Intermediate, R- Resistance, AUG: Amoxicillin-Clavulanic acid, CAZ: Ceftazidime, CTX: Ceftaxime, IMI: Imipenem, CRO: Ceftriaxone, CLO: Clotrimazole, TE: Tetracycline, E: Erythromycin, CN: gentamicin.

Discussion

According to this present study, both institutions had recorded prevalence of MRSA isolates from the nasal swabs of apparently healthy students. Issues related to MRSA have become an increasing threat to the general population in recent years. First and foremost, the overall prevalence of *Staphylococcus aureus* among the study population in both institutions was 18% (16% and 20% per institution). This is somewhat similar to study conducted in Taiwan, Iran and Nepal with reported 19.3%, 19.6% and 15% respectively in a student community (Chang *et al*., 2016; Shamshul *et al*., 2016; Abroo *et al*., 2017). A higher prevalence of 27%, 35%, and 40.5% have been reported among university students in Saudi Arabia, India, and Jordan respectively (Baag *et al*., 2017; Albusayes *et al*., 2019; Abdelmalek *et al*., 2022). In Nigeria, there have been limited data on the prevalence of *Staphylococcus aureus* nasal colonization among university students most especially in the life science field. In a recent study by Ifediora and Enya (2022), a prevalence of 42% *Staphylococcus aureus* among 100 apparently healthy students of a tertiary institution in Nigeria was reported. The difference with our data could be due to the study population (clinical students) who has regular contact with patients and staff of the tertiary care hospital. Likewise, studies by Anueyiagu *et al*. (2020) with reported prevalence of 51.9% *S. aureus* among 130 healthy clinical students in Jos, Nigeria.

Here, a prevalence of 6% MRSA was found among the study population in both tertiary institutions. Moreover, the prevalence for individual institution was 8% and 4% for Nnamdi Azikiwe University (South East) and Caleb University (South West) respectively. There has been variation in reported prevalence of MRSA among apparently healthy college students globally (Zakai, 2015). However, our

study with 4% prevalence corroborates with reports from Jordan and Nepal (Al-Tamimi *et al*., 2018; Sharma, 2021). In our study, MRSA was significantly more in students from south eastern compared to students from south western, Nigeria. Consequently, there was a statistically significant difference in MRSA nasal prevalence in both tertiary institutions ($p = 0.036$). This substantiates a systematically reviewed study by Abdoli *et al*. (2020) with reported prevalence of 0-26% for MRSA prevalence. Our study provides the first comparative study from Nigeria on this subject matter; to the best of our knowledge.

The nasal prevalence of MRSA was more in females compared to the males; likewise the age group 20 -24 years. This is a correlation with other studies in Jordan, Nepal and Saudi Arabia (Zakai, 2015; Sharma, 2021; Abdelmalek *et al*., 2022). Females within the ages of 20-24 years are majority nurtures or caregivers; they are most likely the ones to provide care to a sick relative or friend. As regards the departments, MRSA was found to be more prevalent in Medical Laboratory Science and Microbiology with 4 and 2 MRSA isolates respectively. This significantly differs from other reports (Okamo *et al*., 2016; Ajani *et al*., 2020) as they reported on clinical students in practice. *Staphylococcus aureus* is a major gram-positive bacteria that is readily isolated in microbiology laboratories across the world and with the rising trend in antimicrobial resistance; no wonder MRSA is prevalent.

A few possible risk factors were identified in our study. Touching of nose and face, regular hospital visitation and indiscriminate use of antibiotics were found to have a significant association with MRSA colonization with p values= 0.019, 0.031 and 0.023 respectively. This deviates from studies in Saudi Arabia and Europe (Kock *et al*., 2010; Aljeldah, 2020). Although, touching of nose and face was found to be a potential predisposing factor among college students in New South Wales (Yen *et al*., 2015).

Overall, 100% of the isolates demonstrated resistance to clotrimazole. Similarly, to other studies globally among apparently healthy individuals (Abdelmalek *et al*., 2022). Furthermore, 67% of the isolates were found to be multi-drug resistant. The increasing drug resistance of MRSA has led to the exploration of alternative control options (Akharaiyi *et al*., 2021; Ezeanya-Bakpa *et al*., 2021). Multi-drug resistance was observed with amoxicillin-clavalanate (50%), ceftazidime (39%), cefotaxime (28%), ceftriaxone (44%), cefoxitin (67%), clotrimazole (100%), imipenem (50%), tetracycline (28%), erythromycin (50%), ciprofloxacin (44%) and gentamicin (11%). Extended spectrum beta lactamase was detected in 60% of *Staphylococcus aureus. This is in accordance with reports by Guo et al. (*2020). Subsequently, justifies the high resistance observed against third generation cephalosporin (Akujobi *et al*., 2012). This production of the enzyme; beta-lactamases by *S. aureus* and the unregulated availability and use of these antimicrobial agents could result to misuse and exertion of more selection pressure on the microbial strains (Akujobi and Ezeanya, 2013; Okeke *et al*., 2019) thus, the high resistance profile observed.

Conclusion

The present study established the prevalence of MRSA among tertiary-school students. The compared prevalence in both tertiary institutions had a significant difference, although similar risk factors were found.

Acknowledgment

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