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Antibiogram of Methicillin- Resistant *Staphylococcus aureus* Isolates among Healthy Human Subjects in Oleh, South-Southern Nigeria

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A B S T R A C T

Methicillin-resistant *Staphylococcus aureus* (MRSA) is one of the major causes of hospital-acquired infections worldwide. It is widespread in communities and prevalent in hospital and even amongst the livestock.The mortality and morbidity amongst patients with infections associated with MRSA are usually high owing to increasing resistance to several antibiotics. The resistant pattern of MRSA has not been clearly defined in Oleh. This study was designed to determine the incidence and antibiotics resistant pattern of MRSA from the healthy students in Oleh campus, south-southern Nigerian locality. Methods: Three hundred (300) specimens were collected and appropriate procedures were followed for identification and susceptibility testing. The MRSA was determined using Oxacillin antibiotic disc. Of the 300 nasal swab samples collected and screened, a total 164 (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 78(57 %) and 87 (52 %) respectively. The incidence rate of MRSA and colonisation among healthy individuals in the community was 15.7% (47/300) and 28.7 % (47/164) of the *S. aureus* isolates. The antibiotic resistant pattern of MRSA isolates was ciprofloxacin ~ ofloxacin ~ amoxicillin clavulanate > gentamicin,> co-trimoxazole > chloramphenicol > erythromycin > streptomycin >amoxicillin Although, multi-drug resistant strains of *S. aureus* have been identified in this study, infections caused by *S aureus* infections could be treated with flouroquinolones and Gentamicin.

Introduction

Staphylococcus aureus is one of the common Gram positive bacterial pathogens significantly contributing to hospital and community-acquired infections globally (Ugwu *et al.*, 2016). Frequent use of antibiotics has modified *S. aureus* antibiotic susceptibility to methicillin and related antibiotics such that strains that were methicillin-sensitive (MSSA) have become Methicillin-resistant *Staphylococcus aureus* (MRSA (Rosina *et al.*,2017). A strain of *S. aureus* is said to be MRSA when is resistant to a methiccilin, oxacillin and vancomycin (Ike *et al.*, 2016). MRSA started as hospital-acquired, but has reached a pandemic status and now it is community-acquired and

livestock-acquired with a consequent potential epidemiological shift in staphylococcal infections (Akerele *et al.*, 2015, Kejela and Bacha, 2013, Som and Chatterrjel 2013). Antibiotics misuse, prolonged hospitalization and nasal carriage of MRSA are recognized risk factors for MRSA acquisition and spread (Oomen *et al.*, 2010, Wertheim 2004). MRSA can colonize healthy people at a reduced rate (< 8%) and represents a potent and prevalent risk factor for subsequent *S. aureus* infections (Ugwu *et al.*, 2016, Som and Chatterjel 2013). Nasal carriers of MRSA are susceptible to wound infections, increased the likelihood of invasive disease and occasionally toxic shock syndrome (Ugwu *et al.*, 2016,Ilejela *et al.*, 2017, Okwu *et al.*, 2009, Ugwu *et al.*, 2009). MRSA plays crucial role in community - diseases acquisition and there has been a global increase in the number of MRSA infections [(Okwu *et al.*, 2012, Som and Chatterjel,2013).Many infections that are difficult to treat are attributed to MRSA and is known to contribute to extended periods of hospitalization, high costs of treatment, as well as mortality rate (Ugwu *et al.*,2016,Ike et *a;l.*, 2016) Epidemiological data in the human population suggest that there is a rise in CA-MRSA carriage(Som and Chatterjel 2013).

CA-MRSA is a significant public health burden and it is endemic in most countries [Som and Chatterjel 2013, Moellering 2006, Skov 2012). In developing settings e.g. Nigeria, CA-MRSA poses a serious therapeutic challenge. The mechanism of their emergence as well spread in the community is sparsely documented (Cheesbrough 2004). Thus, in order to implement strategies for the effective containment of MRSA carriage, it is necessary to characterize isolates at the community level. This will provides useful baseline information as CA-MRSA colonization has been reported as one of the risk factors for MRSA dissemination (Ugwu *et al.*, 2016). Data concerning the frequency of nasal carriage of MRSA in the Ole community is not known. Therefore, we designed this study to evaluate the prevalence and antibiotic susceptibility profile of MRSA among healthy tertiary Students in Oleh, South-southern Nigeria.

Materials and Methods

Sample collection/purification, Isolation and identification of Test Bacteria

This study was conducted in Delta University State University Oleh campus. The nasal samples were obtained with sterile swab sticks and Individual swab was adequately labelled and was immediately inoculated on mannitol- salt agar procured from Oxoid (England) and incubated for18-24 h at 37^0 C. Pure culture of each isolate was obtained by streak plate technique. Identification of isolates were by colony morphology, Gram staining,catalase test and coagulase test (Ugwu *et al.*, 2016). Thereafter, isolates that were positive to Gram staining, catalase and coagulase tests were considered as *S. aureus* [Ugwu *et al.*, 2016, Ike *et al.*, 2016 Cheesbrough 2004].

Phenotypic screening of *S.aureus* for Methicillin resistance using the Oxacillin disc

Susceptibility of the *S.aureus* isolates was done by means of the agar diffusion method on Muller Hinton agar using Oxacillin (1mcg) sensitivity disc. The isolates were standardized to 0.5 Mcfarland standards and suspension of which was aseptically inoculated on the Muller Hinton agar plates. The plates were incubated for 18-24 hours at 37°C.Therefater, the zones of inhibition were measured and interpreted using to Clinical laboratory standard institutes (CLSI) breakpoint [CLSI,2016]

Phenotypic screening of VRSA using the Vancomycin disc

This was done by agar diffusion method on Muller Hinton agar using Vancomycin (30mcg) sensitivity disc The standardized suspension of the isolates was inoculated aseptically onto the Muller Hinton agar plates and Vancomycin (30mcg) sensitivity disc placed aseptically on the surfaces. The plates were incubated for 24 hours at 35-37 °C. Then the zones of inhibition were recorded and interpreted [CLSI, 2016].

Antibiotic susceptibility testing (AST)

The antibiogram of the isolates was determined against some commonly used antibiotics using disc diffusion method following the CLSI guidelines. The antibiotic discs contained the following antibiotics and concentrations; Gentamycin l0μg; Amoxicillin 30μg; Vancomycin,Co-trimoxazole Ciprofloxzcin l0μg; Ofloxacin: Streptomicin30μg; Co-trimoxazole 30μg; Erytbromycin l0μg. Isolates were classified as either resistance or intermediate or sensitive according to the CLSI (2016)

Results and Discussion

Table 1 shows the incidence of *S. aureus* among males and females of the tertiary institution in Oleh locality in Delta State. Characterization studies revealed that 66.7% (64/300) of the three hundred (300) nasal samples screened, were to be *S. aureus*. The incidence of *S. aureus* among female to male individuals were 52 (%) and 57 (%) respectively. The incidence rate of MRSA colonization among healthy subjects in the studied community of the *S. aureus* isolated was 15.7% (47/300) (as shown in Table 2.

The antibiotic susceptibility pattern of MRSA is shown in Table 3. Ciprofloxacin, ofloxacin and Amoxicillin-clavulanic acid had the best anti-staphylococcal activities, with five (5) isolates resistant to these agents, while 42 isolates were susceptible. Seven (7) isolates were resistant to gentamicin while 40 isolates were susceptible to it. Eight (8) of the isolates were resistant to amoxicillin clavulanate antibiotic while 39 isolates were susceptible. Co-trimoxazole has 10 isolates resistant to it, while 37 MRSA isolates were susceptible. Twenty isolates were resistant to chloramphenicol, while 27 isolates were susceptible. For erythromycin, 23 isolates were resistant, while 24 isolates were susceptible. Greater number of the isolates was resistant to streptomycin with 27 isolates resistant to it while 20 isolates were susceptible. Similarly, amoxicillin had the highest number of isolates (37) resistant to it, while only 10 isolates were susceptible. Summarily, the pattern of susceptibility of antibiotic to MRSA was as follows: ciprofloxacin ~ ofloxacin ~ amoxicillin clavulanate > gentamicin,> > co-trimoxazole > chloramphenicol > erythromycin > streptomycin >amoxicillin

There have been reports of Strains of CA-MRSA that cause infections in healthy people and CA-MRSA infections are on the increase in various regions and countries (Ugwu *et al.,* 2016). CA-MRSA continues to be a major global health challenge due to the presence of multiple drug resistant encoding genes (Ugwu *et al.,* 2016, Ike *et al.,* 2016, Onanuga *et al.,* 2005). We are not aware of any published data on the nasal carriage of MRSA in Oleh campus of Delta state Nigeria.

This study evaluated the prevalence as well as the antibiotic susceptibility profile of MRSA among healthy tertiary Students in Oleh, South-southern Nigeria.

The carrier rate of *S. aureus* in this study was 54.7% (164/300) made up of 28.7% (male) and 26% (female).

Table.1 Frequency of isolation *S aureus* among healthy students of DELSU Oleh

Source	Number sampled	Number of *S.aureus* Isolated	Percentage (%)
Male	150	86	57
Female	150	78	52
Total	300	164	55

Table.2 Incidence of MRSA among healthy students of DELSU Oleh

SOURCES	Frequency of MRSA	Percentage (%)
Male	24	51.1
Female	23	48.9
Total	47	100

Table.3 Antibiotic Susceptiblity pattern of MRSA isolates from nasal swabs of healthy students of DELSU, Oleh

Antibiotic	Number Resistant		Number Susceptible	
	Number(n)	Percentage (%)	Number(n)	Percentage (%)
Amoxicillin	37	78.8	10	21.3
Amoxicillin-Clavulanic acid	5	10.6	42	89.4
Chloramphenicol	20	42.6	27	57.5
Ciprofloxacin	5	10.6	42	89.4
Co-trimoxazole	10	21.3	37	78.7
Erythromycin	23	48.9	24	51.1
Gentamicin	7	14,9	40	85.1
Ofloxacin	5	10.6	42	89.4
Streptomycin	27	57.5	20	42.6

Colonization rate of *S. aureus* between the sex was not statistically significant (P>0.05). Similar no statistical significant difference in colonization rate of *S. aureus* between the male and female category have been reported in Agbor (South-southern Nigeria), in Jos, (North-central Nigeria), in Okada, South west Nigeria (Ugwu *et al.*,2016 Okwu *et al.*, 2012 Ajoke *et al.*, 2012) and in Riyadh, Saudi Arabia (Alaklobi *et al.*, 2015). The studies all maintained that sex is not a notable determinant in *S. aureus* colonization. The incidence rate of MRSA nasal colonization among healthy individuals in the studied community of was 15.7%. This rate is similar to 18.7% recorded by our team among Healthy Students in Agbor, another South-southern Nigerian locality (Ugwu *et al.*, 2016). However, the rate of CA-MRSA in this locality is a concern because: (i) Colonization has severally been reported to be a significant aspect of the events that leads to *S. aureus* infections and transmission. Subjects are first colonized, invaded and are finally infected [Ugwu *et al.*,2016 (Alaklobi *et al.*, 2015,Ugwu *et al.*, 2015] (ii) CA-MRSA has been reported to be more virulent than the hospital counterpart (Braga *et al.*,2014, Mahde *et al.*,2017, Ugwu *et al.*,2013). Virulence factors such as production of

toxins, possession of antibody inactivating cell-surface proteins as well as high transmission rates are the hallmarks of CA-MRSA strains (Nwafal *et al.,* 2015). The ability of CA-MRSA to spread from person to person is well established and occurs frequently in closed populations (schools or sport facilities) (Nwafal *et al.*,2015, Van *et al.*,2013).

The antibiotic susceptibility pattern of MRSA was as follows: ciprofloxacin ~ ofloxacin ~ amoxicillin clavulanate > gentamicin,> > co-trimoxazole > chloramphenicol > erythromycin > streptomycin >amoxicillin.

This pattern is in line with similar studies from Nigeria as well as other countries as high level of resistance to Amoxicillin, chloramphenicol and erythromycin have previously been reported ([Okwu *et al.,* 2012, Ugwu *et al.,* 2015). The increasing prevalence of multidrug resistant CA-MRSA is of potential epidemiological threat with clinical and pharmacological implications. The resistant genes can be transferred to other potential pathogens (Ugwu *et al.,* 2013)

The observed improved activity of the Amoxicillin-Clavulanic acid over Amoxicillin against the MRSA is worthy of note. The weakness associated with Amoxicillin class is related to their β-lactam chemical ring nucleus which has been compromised by activities of β-lactamase. The enhancement of the activity of Amoxicillin in the Clavulanic acid -complex is thus attributed to the protection by Clavulanic acid (Ugwu *et al.,* 2009). The observed high MRSA susceptibility to flouroquinolones (Ciprofloxacin, Ofloxacin), and gentamicin in this study is line with other published studies [Ajoke *et al.,* 2012, Ugwu *et al.,* 2015] Research, has shown that Agents which produce their antibacterial effect through inhibition of protein synthesis and DNA gyrase are active against staphylococcal infections (Ugwu *et al.,* 2015).

The rate of CA-MRSA carriage among healthy subjects in Oleh was within the range reported regionally and nationally. Protein synthesis and DNA gyrase inhibitors should be considered in subjects with suspected moderate to severe *S. aureus* infections and for empiric MRSA coverage.

We equally recommend that there should be increased use of disinfectants, promotion of proper sanitary measures as well as improved and frequent hand washing practices in the institution as part of Staphylococcal infection control measures in the institution.

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