American Journal of Science and Technology
2015; 2(1): 18-21
Published online November 30, 2014 (http://www.aascit.org/journal/ajst)
ISSN: 2375-3846

Keywords
Antimicrobial Susceptibility,
Pathogenic *Staphylococci*,
Ca-MRSA,
Nigeria

Received: November 06, 2014
Revised: November 19, 2014
Accepted: November 20, 2014

Community acquired methicillin resistant *Staphylococcus aureus* (ca-MRSA) carriage amongst tertiary school students

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Citation
Iroha Ifeanyichukwu, Ejikeugwu Chika, Nwakaeze Emmanuel, Oji Anthonia, Afiukwa Ngozi, Udu-Ibiam Esther. Community Acquired Methicillin Resistant *Staphylococcus aureus* (Ca-MRSA) Carriage Amongst Tertiary School Students. *American Journal of Science and Technology.* Vol. 2, No. 1, 2015, pp. 18-21.

Abstract
Methicillin resistant *Staphylococcus aureus* (MRSA) started making wave soon after the introduction of methicillin (a more potent drug than penicillin) into clinical medicine in the early 1960's. Methicillin, a beta-lactam drug was introduced for clinical use following the resistance of pathogenic bacteria to penicillin. Though implicated in some nosocomial infections, strains of *S. aureus* that are multidrug resistant known as community-acquired MRSA (ca-MRSA) now occur in the community and these are now widespread especially in places where antibiotics are used for other non-clinical purposes such as in livestock production and animal husbandry. This study evaluated the occurrence of ca-MRSA amongst tertiary school students in Ebonyi State, southeast of Nigeria. Swab stick specimens were aseptically collected from the nares, ears and skin of 20 consenting students, and these were analyzed based on standard bacteriological techniques. Antimicrobial susceptibility studies was carried out in line with the CLSI guideline using the Kirby-Bauer disk diffusion method and ca-MRSA isolates was phenotypically confirmed using a double disk diffusion technique based on detecting reduced susceptibility to oxacillin (1 µg) disk. Antimicrobial susceptibility test result showed that the pathogenic *S. aureus* isolated in this study showed reduced susceptibility to ceftazidime (100 %), cefoxitin (100 %), cefuroxime (80 %), ampicillin (33.3 %) and oxacillin (75 %). However, they were susceptible to erythromycin and clindamycin. The carriage rate of ca-MRSA in this study was 75 %. The expansion of ca-MRSA strains in the community poses public health issues due to their multidrug resistant profile. Proper monitoring of ca-MRSA strains in both the community and hospital environment is critical to forestalling any disease outbreak due to them. And this should be followed with antibiotic stewardship in both the community and hospital environment to preserve the efficacy of available drugs.

1. Introduction

Staphylococcus aureus is non-sporulating, non-motile Gram positive bacteria carried asymptomatically on the body of humans as normal microflora, and they cause a plethora of pathological conditions in humans including skin infections, meningitis, pneumonia,

arthritis, endocarditis and osteomyelitis amongst others (Michael *et al*., 2010 and Madigan *et al*., 2009). Methicillin, the first β-lactamase-resistant-penicillin is a β-lactam antibiotic that is stable to β-lactamase enzymes produced by *S. aureus* and other Gram negative bacteria (Denyer *et al*., 2004), but the efficacy of this potent agent has been put to jeopardy by the emergence of pathogenic *S. aureus* strains resistant to the antibiotic (i.e. methicillin). Methicillin resistant *Staphylococcus aureus* (MRSA) is defined as a strain of *S. aureus* that is resistant to methicillin (Terry *et al*., 2011). MRSA started making rounds in the health sector and became a global public health issue in 1961 when the first strain of *S. aureus* that resisted the actions of methicillin was reported in the UK, thus making it the first report of MRSA in the world (Michael *et al*., 2010 and Sae *et al*., 2010). MRSA have since emerged and spread as a serious worry in human medicine because of their multiple antibiotic resistant profiles; and they occur worldwide with varying prevalence's (Sae *et al*., 2010; Frazee *et al*., 2005; Coelho *et al*., 2011; Terry *et al*., 2011). Community-acquired methicillin resistant *Staphylococcus aureus* (ca-MRSA) strains are those strains of *S. aureus* that are not acquired from a healthcare setting, but rather emerged from the community and without identified risk factors that allowed hospital-associated methicillin-resistant *S. aureus* (HA-MRSA) which is nosocomial in origin to emerge (Coelho *et al*., 2011; Terry *et al*., 2011). According to Coelho *et al* (2011), ca-MRSA differs genetically from HA-MRSA, and they are also less-resistant to non-β-lactam antibiotics than the Ha-MRSA strains which emerged from hospital settings. Ca-MRSA which has been a cause of concern in people who have no previous invasive procedures or had been hospitalized before was first reported in the early 1980's (Frazee *et al*., 2005), and they can now be found even in healthy persons causing varying rates of morbidity. This study is aimed at determining the frequency of ca-MRSA carriage amongst students of a tertiary institution in Abakaliki metropolis, Ebonyi state, Nigeria.

2. Materials and Methods

Sample collection: Sterile swab sticks in polypropylene tubes and moistened with sterile physiological saline was used to swab the skin surface, ears and nares of 20 consenting healthy students who were included in the study. Collected samples were transported immediately to the Microbiology Laboratory Department of Ebonyi State University Abakaliki, Nigeria for further processing.

Bacteriological analysis: Swabs containing the specimen were first suspended in Tryptone soya broth (Oxoid, UK) and incubated at 37^{o}C for 18-24 hrs. Loopful of bacterial growth were plated onto nutrient agar (Oxoid, UK), blood agar and Mannitol salt agar (Oxoid, UK) and incubated at 37^{o}C for 24 hrs for the isolation of pathogenic *Staphylococcus aureus*. Suspect colonies were subcultured onto Mannitol salt agar plates and purified on nutrient agar plates. Bacterial isolates were identified according to standard microbiology identification techniques (Cheesbrough, 2004). *S. aureus* is Gram positive cocci, catalase positive and coagulase positive.

Susceptibility testing: All isolated *S. aureus* bacteria were evaluated for antimicrobial susceptibility testing by the Kirby-Bauer disk diffusion method as per the clinical laboratory standard institute (CLSI) guidelines. Susceptibility studies were carried out on Mueller-Hinton (MH) agar plates already inoculated with the test organisms. Single disks of ampicillin (10 µg), erythromycin (15 µg), clindamycin (2 µg) cefoxitin (30 µg), cefuroxime (30 µg) and ceftazidime (30 µg) [procured from Oxoid, UK] were aseptically placed on each of the culture plates, and then incubated at 37^{o}C for 24 hrs.

Detection of MRSA: MRSA strains were detected by the Kirby-Bauer disk diffusion method according to a previously used methodology (Suleiman *et al*., 2012; Frazee *et al*., 2005). Briefly, test bacteria were aseptically swabbed on MH agar plates and oxacillin (1 µg) disk was placed on the plate(s) and incubated at 37^{o}C for 24 hrs. Zones of inhibition ≤ 13 mm for the oxacillin antibiotic against the test isolate phenotypically infer MRSA.

3. Result

Twenty *Staphylococcus aureus* bacteria were recovered from the swab specimens collected from 20 consenting healthy students at a tertiary institution in Ebonyi State Nigeria. Table 1 shows the biochemical reaction of the *S. aureus* bacteria isolated in this study. Table 2 shows the number of *S. aureus* bacteria isolated from the different sample collection sites (skin, ear and nares). Ear swab produced the highest number of *S. aureus* bacteria (45 %), and this was followed by swabs from the nares (30 %) and skin (25 %) respectively. The antimicrobial susceptibility test results of the *S. aureus* bacteria are shown in Table 3. Cefoxitin was the least effective antibiotic tested as none of the *S. aureus* bacteria was susceptible to the agent. The *S. aureus* bacteria were also resistant to ceftazidime and cefuroxime. The number of *S. aureus* bacteria resistant to oxacillin is shown in Table 4.

Table 1. *Biochemical reaction of the test isolates*

Organism	Gram staining	Coagulase test	Catalase test
S. aureus (n=20)	+ve	+	+

Table 2. *Number of S. aureus isolated from the different specimens*

Bacteria	Skin n (%)	Nares n (%)	Ear n (%)
S. aureus (n=20)	5 (25)	6 (30)	9 (45)

Table 3. *Percentage susceptibility profile of the S. aureus bacteria*

Drugs (µg)	Skin (n=5)		Nares (n=6)		Ear (n=9)	
	*S n(%)	*R n(%)	*S n(%)	*R n(%)	*S n(%)	*R n(%)
Clindamycin (2)	5 (100)	0 (0)	5 (83.3)	1 (16.7)	7 (77.8)	2 (22.2)
Erythromycin (5)	4 (80)	1 (20)	5 (83.3)	1 (16.7)	8 (88.9)	1 (11.1)
Ampicillin (2)	3 (60)	2 (40)	4 (66.7)	2 (33.3)	8 (88.9)	1 (11.1)
Cefoxitin (30)	0 (0)	5 (100)	0 (0)	6 (100)	0 (0)	9 (100)
Ceftazidime (30)	0 (0)	5 (100)	1 (16.7)	5 (83.3)	0 (0)	9 (100)
Cefuroxime (30)	1 (20)	4 (80)	3 (50)	3 (50)	5 (55.6)	4 (44.4)

*S=Susceptible, *R=Resistant, n=number of isolates

Table 4. *Oxacillin resistance profile of S. aureus bacteria*

Bacteria	Oxacillin (1 µg)	
	Resistant	Susceptible
S. aureus (n=20)	15 (75)	5 (25)

4. Discussion

Staphylococcus aureus bacteria are a member of the normal flora of the human body, and they are mainly found on the skin, in the nares and ears. Community-acquired methicillin resistant *Staphylococcus aureus* (ca-MRSA) is largely responsible for most community infections related to pathogenic *S. aureus*. This study was aimed at evaluating the carriage frequency of ca-MRSA amongst students at a tertiary institution as a panacea to elucidate the spread of the pathogen in the community. Several studies have reported the prevalence of ca-MRSA from various sources including livestock, animals, meat products and even from the human skin from around the world but none of these studies reported the carriage rate of ca-MRSA amongst healthy students (Michael *et al*., 2010; Suleiman *et al*., 2012; Terry *et al*., 2011; Lo *et al*., 2007; Akinjogunla *et al*., 2010; Nkang *et al*., 2009; Onanuga *et al*., 2005). All the twenty swab specimens collected from the nares, ears and skin of the consenting students produced *S. aureus* isolates that were pathogenic in nature (Tables 1 and 2). Ear swab specimens produced 9 (45 %) *S. aureus* isolates while the skin and nares swabs produced 5 (25 %) and 6 (30 %) *S. aureus* isolates respectively. The results of our antimicrobial susceptibility testing showed high level resistance amongst the *S. aureus* isolates especially to ceftazidime (100 %), cefoxitin (100 %), cefuroxime (80 %) and ampicillin (33.3 %). Clindamycin and erythromycin were the most potent antibiotics against the *S. aureus* isolates (Table 3). However, this result is in contrast to a similar work done in Taiwan where community-acquired methicillin resistant *S. aureus* (CA-MRSA) showed high level of resistance to clindamycin (100 %) and erythromycin (100 %) (Lo *et al*., 2007). The high resistance of pathogenic *S. aureus* to some commonly available antibiotics as obtainable in our study compares to other studies (Suleiman *et al*., 2012; Nkang *et al*., 2009; Onanuga *et al*., 2005). Out of the twenty *S. aureus* isolates tested for susceptibility to oxacillin (1 µg) in this study, only 5 (25 %) isolates were susceptible to the drug (Table 4). Thus, the carriage rate of pathogenic *S. aureus* (that are community-acquired and resistant to methicillin and/or oxacillin antibiotics) in this study was 75 %. In Northern Taiwan, nasal carriage of community-acquired methicillin resistant *Staphylococcus aureus* (as reported in our study) has been reported among kindergarten attendees (Lo *et al*., 2007); and several other studies have also reported similar carriage rates of ca-MRSA in the community and even amongst dogs (Coelho *et al*., 2011; Sae *et al*., 2010; Suleiman *et al*., 2012; Onanuga *et al*., 2005). MRSA strains could be extremely dangerous when transmitted to critically ill-patients due to the multidrug profile of the pathogen (as obtainable in this study). The spread of methicillin resistant *S. aureus* (MRSA) in both the hospital environment and community should be monitored through proper antibiotic usage policies and proper detection of such multidrug resistant bacteria in the laboratory.

In conclusion, our study has presumptively reported the occurrence of ca-MRSA bacteria amongst students of a tertiary institution in this part of the world. It is critical to monitor the emergence of the pathogen so as to forestall their spread within the community. Further molecular studies are required to characterize by genotypic techniques the genes responsible for the ca-MRSA occurrence in this region.

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