

Prevalence and Antibiotic Susceptibility Patterns of Methicillin-Resistant *Staphylococcus Aureus* (MRSA) Isolated from Healthy Inhabitants of Uturu Rural Communities, Abia State, Nigeria

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Abstract

The antimicrobial resistance ability and extraordinary virulence of community acquired methicillin-resistant *Staphylococcus aureus* which allow it infect healthy persons are major medical issues worldwide. A total of 84 (nose and ear swabs, urine) samples were collected from healthy individuals and screened for *Staphylococcus aureus* using standard microbiological techniques. Susceptibility testing of the isolates to oxacillin and to other conventional antimicrobial sensitivity discs of some antibiotics readily accessible in the study area was done using the discs diffusion method. *Staphylococcus aureus* was grown from 69(82.1%) samples while 51(60.7%) of the cultures were methicillin-resistant. The 51(60.7%) community acquired methicillin-resistant *Staphylococcus aureus* isolates showed a percentage resistance pattern which included 100% resistance to ampicillin followed by amoxicillin (64.7%)> vancomycin (35.3%)> erythromycin (19.6%)> ceftriaxone (17.6%)> gentamycin (13.7%)> ciprofloxacin (11.8%)> ofloxacin (7.8%). High resistance to vancomycin (35.3%) was recorded in the study area. Ofloxacin was the best antibiotic of choice in the treatment of disorders associated with community acquired methicillin-resistant *Staphylococcus aureus* in the study area. Other antibiotics such as gentamycin, and ciprofloxacin proved to be potent in the management of MRSA infections. Ten isolates of methicillin-resistant *Staphylococcus aureus* obtained showed multidrug resistance to at least 4 antibiotics tested and this necessitates caution in the prescription of antibiotics without proper indication. The high prevalence of methicillin-resistant *Staphylococcus aureus* in the study area is of great public health importance and calls for effective measures including public enlightenment to discourage indiscriminate use of antimicrobials.

Keywords: Community acquired methicillin resistant *Staphylococcus aureus*, prevalence, antibiotics susceptibility pattern, ofloxacin, Nigeria.

1. Introduction

Staphylococcus aureus (*S. aureus*), a spherical aerobic Gram-positive, catalase positive, non-motile, non-spore forming coccus (Abdalla et al., 2012; Haque et al., 2011; Taiwo, 2009), is an opportunistic pathogen in human (Abdalla et al., 2012; Corvaglia et al., 2010) and animal (Hague et al., 2011; Shearer et al., 2011). The pathogen is responsible for broad spectrum of human and animal diseases ranging from skin infections (Corvaglia et al., 2010) to such severe diseases as pneumonia (den Heijer et al., 2013; Shen et al., 2013), endocarditis (Pai et al., 2013; Dar et al., 2006), osteomyelitis (Orji et al., 2012; Hague et al., 2011), septicaemia and enterocolitis (Al-Anazi, 2009) such that infections involving antibiotic resistant strain may impact on human health (Perveen et al., 2013). Humans are a natural reservoir for *S. aureus*, and asymptomatic colonization is far more common than infection. Colonization of the nasopharynx, perineum, or skin, particularly if the cutaneous barrier has been disrupted or damaged, may occur shortly after birth and may recur anytime thereafter (Olowe et al., 2007).

A major issue in the treatment of *S. aureus* infection with methicillin is the presence of methicillin-resistant *S. aureus* (MRSA) strain (Rajadurai et al., 2006) which may also be referred to as multidrug-resistant *S. aureus* or oxacillin resistant *S. aureus* (ORSA) (Hena and Sudha, 2011). The term “methicillin-resistant *S. aureus*” (MRSA) refers to those strains of *S. aureus* that have acquired resistance, whether in the community or in the hospital, to the antibiotics: methicillin, oxacillin, nafcillin, cephalosporins, imipenem, and/or other beta-lactam antibiotics (Hena and Sudha, 2011; Al-Anazi, 2009). *S. aureus* especially methicillin-resistance *S. aureus* (MRSA) is relatively ubiquitous and is the cause of many community infections (Peters et al., 2013; Okwu et al., 2012; McDougal et al., 2010; Olowe et al., 2007; Yamamoto et al., 2006), endemic and epidemic nosocomial

colonization (Hena and Sudha, 2011; Moussa et al., 2011) and infections (Al-Baidani et al., 2011; Olowe et al., 2007).

Community acquired MRSA (CA-MRSA) infections which were first described in small series of adult and paediatric patients presenting skin and soft tissue infections (SSTIs), pneumonia, or bacteremia have become a significant public health threat (Peters et al., 2013), in Nigeria and abroad. Since 1930, the epidemiology of *S. aureus* has changed dramatically, and methicillin-resistant *S. aureus* (MRSA) has reached epidemic levels in both hospital and community settings (Stenehjem and Rimland, 2013). CA-MRSA usually differs in several ways from typical health-care-associated MRSA (HA-MRSA). They typically carry the smallest staphylococcal cassette chromosome *mec* (SCC*mec*) types IV and V, that are resistant to fewer antimicrobial agents, and are associated to the presence, and enhanced expression of specific virulence factors (Gouveia et al., 2013). The epidemiological success of CA-MRSA strain is believed to stem from combination of antibiotic resistance fitness at low cost with extraordinary virulence, allowing these strains infect otherwise healthy individuals and spread sustainably in the population (Perveen et al., 2013). The resistance to antibiotics in MRSA is due to the presence of *mecA* gene on the mobile genetic element, termed the staphylococcal cassette chromosome (SCC) (Corvaglia et al., 2010), which expresses a novel cell wall synthesizing enzyme, penicillin-binding protein 2A (PBP2A) with low affinity for all β -lactams (Al-baidani et al., 2011; Perwiaz et al., 2007; Brown et al., 2005). The *mecA* gene complex also contains insertion sites for plasmids and transposons that facilitate the acquisition of resistance to other antibiotics (Orji et al., 2012; Hena and Sudha, 2011).

Individual plasmid mediated resistance in MRSA isolates (Al-Mohana et al., 2012) and plasmid carried by resistant isolates (Kenedy et al., 2010; Dar et al., 2006; Yamamoto et al., 2006), have been studied. At present, MRSA has become an endemic pathogen worldwide (Prakash et al., 2007) and has also become multidrug resistant (Khadri and Alzohairy, 2010), with most isolates exhibiting resistance to both quinolones (Abdalla et al., 2012) and aminoglycosides (Khadri and Alzohairy, 2010). Vancomycin resistant *S. aureus* is not widely seen even though a low level resistance to vancomycin is being reported (Orji et al., 2012; Zorgani et al., 2006). MRSA is of concern not only because of its resistance to methicillin but also because it is generally resistant to many other chemotherapeutic agents (Olowe et al., 2007), second- and third-line antimicrobial resistance is a growing concern (Al-Baidani et al., 2011). Therefore, the aim of the present study was to evaluate the prevalence and antibiotic susceptibility patterns of MRSA isolated from the healthy inhabitants of Uturu rural communities, Abia State, Nigeria as well as find out the suitable drug for the treatment of disorders due to these organisms.

2. Materials and Methods

2.1 Sample Collection

A total of 84 pathological specimens (28 specimens for each; nose and ear swabs, urine) from otherwise healthy inhabitants of Uturu communities were collected with the use of sterile swab sticks and universal bottles. The specimens were labeled accordingly with the first initial of the site of specimen collection (N for nose, E for ear and U for urine). Each specimen was streaked on mannitol salt agar (MSA), nutrient agar (NA) and incubated at 37°C for 24 hours at the Department of Plant Science and Biotechnology Laboratory, Abia State University, Nigeria.

2.2 Bacterial Identification

Colonies that appeared white or yellow or cream-coloured on MSA plates with yellow surrounding (indicative of fermentation) were considered as *S. aureus*. These colonies were sub-cultured to obtain pure cultures from which stock cultures were immediately prepared. Isolates were further identified as *S. aureus* by testing their ability for DNase production on agar plates. Gram stain was performed and isolates were identified by their ability to produce catalase, coagulase and oxidase enzymes then identification was finally confirmed by Bergey's manual (Breed et al., 1957) according to Al-Jumaily et al. (2012).

2.3 Oxacillin Susceptibility Testing

The oxacillin discs used were procured from Oxoid, Germany. The antimicrobial susceptibility profile of the isolates was determined using the disc diffusion technique as described by Orji et al. (2012). All discs used were prepared by MAYO diagnostic laboratory, Nigeria in accordance with the clinical and laboratory standard institute (CLSI) recommendations (CLSI, 2007).

With a sterile wire loop, few colonies of each of the isolates were separately but aseptically emulsified in 5 ml of sterile peptone water to a turbidity corresponding to 0.5 McFarland standards (corresponding to approximately 10^8 cfu/ml). Then 0.5 ml of each inoculum was dispensed unto the surface of dried Mueller Hinton agar (MHA) plate using sterile Pasteur pipette (one for each inoculum). These were spread evenly on the agar surface with sterile swab sticks (one for each inoculum). The excess inocula were discarded into a disinfectant jar. The inoculated plates were kept on the bench for 3 minutes to dry. The oxacillin disc (1 μ g) was then aseptically placed centrally on the inoculated plates using a sterile forceps.

The preparations were incubated aerobically for 24 hours at 35°C. The diameter of zone of inhibition produced

by each of the discs was measured, recorded and the isolates were classified as resistant (≤ 10 mm) or sensitive (≥ 13 mm) based on the standard interpretative chart as recommended by the Clinical and Laboratory Standard Institute (CLSI, 2010; CLSI, 2007).

2.4 Susceptibility Testing of MRSA Isolates to other Conventional Antibiotics

The following antimicrobial sensitivity discs were used: ampicillin (10 μ g), ciprofloxacin (5 μ g), ofloxacin (5 μ g), erythromycin (15 μ g), gentamicin (10 μ g), vancomycin (30 μ g), amoxicillin (10 μ g), and ceftriaxone (30 μ g), to determine the susceptibility profile of all the methicillin-resistant strains using the disc diffusion assay as described by Orji et al. (2012). The antibiotic concentration of each disc used was as recommended by Clinical and Laboratory Standard Institute (CLSI, 2007).

With a sterile wire loop, few colonies of each of the isolates were emulsified in 5 ml of sterile peptone water to a turbidity corresponding to 0.5 McFarland standard. Then 0.5 ml of each of the inoculum was dispensed onto the surface of dried Mueller Hinton agar plate using sterile Pasteur pipette (one for each inoculum). These were then spread evenly on the agar surface with sterile swab sticks (one for each inoculum). The excess inocula were discarded into a disinfectant jar. The plates were kept on the bench for 3 minutes to dry. The various discs were placed on the inoculated plates at 25 mm away from one another and 15 mm away from the edge of the plates aseptically using a sterile forceps.

The preparations were incubated aerobically for 24 hour at 35°C. The diameter of the zone of inhibition produced by each of the discs was measured, recorded and interpreted based on the standard interpretative chart as recommended by the Clinical Laboratory Standard Institute (CLSI, 2007).

3. Results and Discussion

Pathological specimens from 84 healthy individuals were examined and amongst them, 69(82.1%) were positive for *S. aureus* and 51(60.7%) were methicillin (oxacilin)-resistant (table 1). The prevalence of *S. aureus* and CA-MRSA isolates amongst the inhabitants of uturu rural communities was estimated to be 82.1% and 60.7% respectively. A similar high prevalence of MRSA has been noted by Orji et al. (2012), Scott et al. (2011) and Onanuga et al. (2005). INSAR (2013) reported the overall MRSA prevalence in their study to be 42% in 2008 and 40% in 2009 and Gonsu et al. (2013) reported a prevalence of 40.6%, both of which are lower when compared to the result of this study. The high prevalence in this study could be due to living in crowded household and having household members of MRSA-colonized persons which are unique characteristics of most rural communities in developing countries and have been found to increase the risk of becoming colonized by MRSA (Shen et al., 2013).

The MRSA isolates subjected to antibiotic susceptibility test in the present study revealed that the isolates have also developed resistance to other antibiotics tested. The CA-MRSA isolates showed a maximum of 100% resistance to ampicillin which was followed by amoxicillin (64.7%)> vancomycin (35.3%)> erythromycin (19.6%)> ceftriaxone (17.6%)> gentamycin (13.7%)> ciprofloxacin (11.8%)> ofloxacin (7.8%) (see table 2). The highest level of resistance was observed in ampicillin and amoxicillin, which agreed favourably with the reports of Okwu et al. (2012) and Nkwelang et al. (2009). This high resistance may be due to the expression of *mecA* gene or as a result of the thickening of the cell walls of the isolates (Zer et al., 2009).

The high sensitivity 64.7% observed in this study in favour of vancomycin is at variance with the finding of Onanuga et al. (2005), who reported low sensitivity of 11%. However, our result supports the work of Orji et al. (2012) who reported 60% sensitivity, and is closely related to the reports of INSAR (2013), Perveen et al. (2013), Al-Mohana et al. (2012) and Hague et al. (2011) who all reported 100% susceptibility to vancomycin. Thus, this antibiotic has been in use in the treatment of infections caused by MRSA. The low resistance (13.7%) reported in gentamycin in this study could be due to the absence of the plasmid pUB10 encoding resistance for aminoglycoside (gentamycin) in the field isolates. This result is closely related to the reports of Al-Mohana et al. (2012) who reported 27% resistance but, at variance with the work of khadri and Mohammed (2010) and Perwaiz et al. (2007) who reported 65% and 93% resistance to gentamycin in isolates of MRSA respectively. Ciprofloxacin with the resistance rate as low as 11.8% in this study supports previous work which proposed that it should be used as an alternative therapy for MRSA infections (Pai et al., 2013). However, the result contradicts the reports of Gupta et al. (2013) who reported ciprofloxacin to be ineffective (with resistance rate of 99%) against MRSA and Tenguria et al. (2013) who also reported 83% resistance to ciprofloxacin in a study conducted in India. Ofloxacin (with sensitivity of 80.4%) appeared to be the best antibiotic of choice for the treatment of CA-MRSA infections associated with the inhabitants of Uturu rural communities. The reason could be due to the relatively new and expensive nature of ofloxacin in most rural communities. Ofloxacin is not commonly prescribed or purchased easily over the counter in chemist stores accessible to rural dwellers as it is with most other antibiotics in rural communities.

Orji et al. (2012), defined multidrug resistant as resistance to four or more of the antibiotics tested. Thus, 10 (100%) of the CA-MRSA isolates showed multidrug resistance to the antibiotic used in this study as shown in

table 3. This result is closely related to the findings of Okwu et al (2012), who reported 13 (100%) of the MRSA isolates in their study to be resistant to more than 4 antibiotics. The level of multidrug resistance shown by MRSA from the healthy inhabitants in this study is of great public health significance. These CA-MRSA strains cause serious skin and soft tissue infections, necrotizing pneumonia, and sepsis in healthy children (Saravanan et al., 2013). About 60% of the MRSA isolates were resistant to 4, and 6 antibiotics; 20% were resistant to 5, and 7, and while 20% were resistant to 8 antibiotics used. None of the isolates was fully sensitive to all antibiotics tested. This result is in agreement with that of Okwu et al. (2012) who reported 46.2% of MRSA to be resistant to 6 antibiotics; 23.1% to be resistant to 7; 23% to be resistant to 5 and 7.7% to 8 antibiotics. The emergence of multidrug resistant bacteria is attributed to inappropriate prescription, self-medication and indiscriminate use of antibiotics. These observations confirmed the postulation that healthy members of the community are the highest reservoir of antimicrobial resistant bacteria (Okwu et al., 2012).

The resistance rate in this study is not as high as the values reported in other studies, but there was a marked increase in resistance patterns in terms of the availability (common use) of the antibiotics used. These results are in line with similar studies from Nigeria as well as other countries (Mahmood et al., 2010; Beyene and Abdisa, 2005), and in conformity with previous observations that most isolates of MRSA are resistant to a large number of commonly prescribed antibiotics (Okwu et al., 2012).

4. Conclusion

The degree of resistance or sensitivity of MRSA towards commonly used antibiotics is recognized to be diverse from region to region and ofloxacin had the highest antibacterial effect on the MRSA isolates from the region of this study. Treatment of MRSA infections should be based on in vitro susceptibility testing of the isolates. Ofloxacin promises to be the best antibiotic for the treatment of disorders associated with MRSA in the study area.

The prevalence of MRSA is higher in the study area and this should necessitate caution in the prescription of antibiotics without proper indication. Multidrug resistance in the isolates to antimicrobials was observed indicating the presence of strong selective pressure from the use of antibiotics in this environment. This is of immense concerns to health workers and officials and calls for effective measures including public enlightenment to promote rational use of antibiotics and to discourage their indiscriminate use.

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Table 1: Distribution of *S. aureus* and MRSA in the pathological specimens.

Specimen	n=69*		n=51‡	
	No <i>S. aureus</i>	% <i>S. aureus</i>	No MRSA	%MRSA
Urine	14	50	9	64.3
Ear swab	27	96.4	23	85.2
Nose swab	28	100	19	67.9

*Total prevalence (%) = 82.1

‡Total Prevalence (%) = 60.7

N = 84

Table 2: Antibiotic susceptibility patterns of CA-MRSA isolates

n = 51

Antibiotic	Resistant (%)	Intermediate (%)	Sensitive (%)
Ampicillin	100	-	-
Ciprofloxacin	6 (11.8)	12 (23.5)	33 (64.7)
Ofloxacin	4 (7.8)	6 (11.8)	41 (80.4)
Erythromycin	10 (19.6)	18 (35.3)	23 (45.1)
Gentamycin	7 (13.7)	4 (7.8)	40 (78.4)
Vancomycin	18 (35.3)	-	33 (64.7)
Amoxycillin	33 (64.7)	-	18 (35.3)
Ceftriaxone	9 (17.6)	29 (56.9)	13 (25.3)

Table 3: Prevalence of multidrug resistance amongst 10 CA-MRSA isolates

Parameter	Frequency of multidrug resistance	Percentage
Fully sensitive	0	0
Resistance to 4 agents	3	30
Resistance to 5 agents	1	10
Resistance to 6 agents	3	30
Resistance to 7 agents	1	10
Resistance to 8 agents	2	20

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