Detection and phenotypic characterization of methicillin-resistant *Staphylococcus aureus* from clinical and community samples in Abakaliki, Ebonyi State, Nigeria

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Abstract

Objective: The objective of this study was to isolate and phenotypically characterize methicillin-resistant *Staphylococcus aureus* (MRSA) from clinical and community samples in Abakaliki, Nigeria.

Methods: A total of 709 clinical (303) and community samples (406) samples were obtained for this study. MRSA isolates were detected using Kirby-Bauer disc diffusion method with the inclusion of 1 μg oxacillin and cefoxitin antibiotic disc. The isolates were screened for the β-lactamase production using nitrocefin sticks.

Results: A total of 44 MRSA isolates were obtained from the samples with prevalence frequency of 22.6 % and 20.8 % from clinical and community samples respectively. The clinical isolates were completely resistant (100 %) to ceftazidime, tetracycline and penicillin. Gentamicin and ciprofloxacin were the most effective antibiotics against the clinical and community isolates respectively with a susceptibility frequency of 63.2 % and 80 %. Exactly 38.1 % and 24.2 % of the clinical and community *S. aureus* isolates were positive for beta-lactamase production respectively. The HA-MRSA and CA-MRSA isolates had MARI values within the range of 0.3 to 1.0.

Conclusion: This present findings of multi-drug resistance MRSA is very worrisome as it further highlights the pressing need to keep a strict watch on MRSA emerging from this study area.

Keywords: MRSA, beta-lactamase, antibiotics, multidrug resistance, clinical samples, community samples.

Introduction

Globally, antibiotic resistance has become a growing concern in the public health sector. This is because resistance often results in treatment failure, which can have serious consequences especially in critically ill patients[1]. Resistant bacteria may spread and create broader infection control problems, both within healthcare institutions and in the community. Multiple antibiotic resistant *Staphylococcus aureus* are major threats to patients care, owing to their stubborn intransigence to chemotherapy and disinfection[2]. Prolonged therapy with methicillin may lead to the development of low-level resistance that compromise therapy, but may not be detected by routine susceptibility testing methods used in hospital laboratories[3]. *S. aureus* has been implicated in a wide range of infections ranging from acute to chronic infections such as boils, bacteriuria, osteomyelitis, pneumonia, endocarditis, meningitis, septicaemia and arthritis. This organism is a leading cause of human bacterial infection worldwide and is endemic in hospitals and communities[4]. The bacterium is frequently found on the skin and anterior nares of healthy individuals. However, it predominates in surgical wound infections with prevalence ranging from 4.6% to 54.4%[5]. Most Staphylococcal infections are associated with serious community-acquired and nosocomial diseases which arise often in individuals with predisposing risk factors such as haemodialysis or surgery. It causes superficial,

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deep-skin, soft tissue infections, endocarditis and bacteremia with metastatic abscess formation and a variety of toxin–mediated diseases including gastroenteritis, staphylococcal scalded skin syndrome, toxic shock syndrome, meningitis, septicaemia and arthritis[6]. The emergence of antimicrobial resistance makes the effective management and treatment of infections caused by methicillin-resistant *Staphylococcus aureus* difficult. This study, is therefore, designed to evaluate the prevalence of methicillin-resistant *Staphylococcus aureus* in Abakaliki, Ebonyi State.

Materials and methods
Sample collection
A total of seven hundred and nine samples were collected for this study. Three hundred and three were clinical samples [wound, pus, urine, high vaginal swab (HVS) (41), ear swab, sputum and semen of patients visiting Federal Teaching Hospital Abakaliki (FETHA I and II) and Mile Four General Hospital, Abakaliki while 406 [(nasal and ear swabs] were community samples. Wound, pus, HVS and ear swab samples were collected using sterile swab sticks while urine, semen and sputum samples were collected using sterile specimen bottles. In the same way, the four hundred and six (406) community samples (nasal and ear swabs) were collected from apparently healthy tutors (121), artisans (62), secondary school students of Abakaliki high school (153) and petty traders from Kpirikpiri Market (70). The Hospital patients' group encompasses individuals who are at greater risk of becoming infected by this opportunistic pathogen. These individuals are generally older, have chronic underlying illnesses, and require more frequent interactions with healthcare facilities; all of which predispose them to more serious infections. The Community group includes individuals who, in general, are otherwise healthy. They are usually not predisposed by age or underlying illness to these infections, but predisposed by specific activities and community interactions that place them at an increased risk for infection contraction. The collected samples were immediately transported to the Department of Applied Microbiology Laboratory unit of Ebonyi State University, Abakaliki for bacteriological analysis.

Culturing, isolation, characterization and identification of the isolates
The clinical and community samples were aseptically inoculated on Mannitol Salt broth (Oxoid, UK) and in-

cubated at 37[0]C for 48 hours. A loopful of the inoculated mannitol salt broth was later streaked on mannitol salt agar (MSA, Oxoid, UK) and incubated at 37[0]C for 24 hours. The plates were observed for creamy golden colonies typical of *S.aureus*. Purified colonies were later cultured on sheep blood agar. Colonies displaying beta-haemolysis on sheep blood agar were presumptively identified as *S. aureus*. These suspected *S. aureus* isolates were further characterized using conventional/standard microbiology techniques such as colony morphology, Gram-staining, catalase test, motility test and other biochemical tests which include oxidase test, indole test, H2S production test, Voges-Proskauer test, methyl red test, sugar fermentation test, coagulase test and *Staphylococcus lactase* agglutination assay as described by[7,8,9].

Antibiotic susceptibility test
The susceptibility patterns of isolated *S. aureus* isolates were determined by the Kirby and Bauer disc diffusion method as recommended by CLSI[10]. Each of the isolate wasstandardized to 0.5 McFarland equivalent and aseptically inoculated on prepared Muller-Hinton agar (Oxoid, UK) plates using sterile swab stick. The inoculated plates were allowed to stand for 10-15 min. Antibiotic impregnated discs namely penicillin G (10 μg), tetracycline (30 μg), gentamicin (30 μg), nitrofurantoin (300 μg), erythromycin (15 μg), ciprofloxacin (5 μg), ceftazidime (30 μg), sulphamethoxazole (25 μg) and clindamycin (2 μg) (Oxoid, UK) were placed on the inoculated plates using sterile forceps. The plates were incubated at 37[0]C for 24 h after which the zones of inhibition around each disc were measured to the nearest mm with a metre rule, recorded and interpreted according to the Clinical Laboratory Standard Institute (CLSI) guidelines[10].

Detection of methicillin resistant *Staphylococcus aureus* (MRSA)
This was done using Kirby Bauer disc diffusion method according to Clinical and Laboratory Standard Institute (CLSI) guidelines[10]. A Mueller-Hinton agar plate was prepared according to its manufacturer's specification. Colonies of the isolated bacteria were suspended in 5 ml of nutrient broth. The turbidity of the broth culture was adjusted to 0.5 McFarland standard, which approximately equals 1.5 x 108 CFU/ml. Standardized inoculum was swabbed onto the prepared Mueller-Hinton agar plate. After at least 3 min, antibiotic discs impregnated with ox-

acillin and cefoxitin were placed on Mueller-Hinton agar plate for MRSA detection. The plate was then incubated at 37⁰C for 24 hours. Inhibition zone diameter was measured to nearest millimeter and interpreted according to CLSI guidelines.

Beta-Lactamase detection using Nitrocefin stick
This was done to detect the production of beta-lactamases by the *S. aureus* isolates. Before inoculation, the nitrocefin stick was allowed to cool to room temperature. A drop of distilled water was used to moderately moisten the tip of the nitrocefin stick. After this, the colour coded end of the stick was used to touch the colony by making sure that the reagent on the brown tip of the stick was rotated to pick mass of the cells. The colour coded tip

was observed after 5 minutes for pink-red colour development to show the production of beta-lactamase.

Determination of multiple antibiotic resistance index (MARI)
Multiple antibiotic resistance indices (MARI) of the S. aureus isolates were calculated using the technique described by Christopher et al.[11] and Subramani et al.[12]. This was calculated as the number of antibiotics to which the tested isolate was resistant to (a), divided by the total number of antibiotics that was tested on the isolates (b).
MARI = Number of antibiotics to which the tested isolate was resistant to (a) / Total number of antibiotics tested (b)

Results

Table 1: Clinical samples collected from Federal Teaching Hospital Abakaliki (FETHA I)

Sample source	No. of samples collected	No. of *S. aureus* isolated	No. of MRSA isolated
Wound swab	21	11	4
Urine	33	6	1
High vaginal swab	11	2	0
Sputum	7	2	2
Ear swab	17	9	2
Pus	7	2	0
Semen	21	4	0
TOTAL	**117**	**36 (30.8 %)**	**9 (25 %)**

Sample source	No. of samples collected	No. of *S. aureus* isolated	No. of MRSA isolated
Wound swab	10	7	2
Urine	35	7	2
High vaginal swab	13	4	1
Sputum	8	2	0
Ear swab	10	5	1
Pus	9	4	1
Semen	18	2	0
TOTAL	**103**	**31 (30.1 %)**	**7 (22.6 %)**

Table 3: Clinical samples collected from mile four general hospital Abakaliki

Sample source	No. of samples collected	No. of *S. aureus* isolated (%)	No. of MRSA isolated
Wound swab	5	3	1
Urine	31	4	0
High vaginal swab	17	3	0
Sputum	20	3	0
Ear swab	10	4	2
Pus	0	0	0
Semen	0	0	0
TOTAL	83	17 (20.5 %)	3 (17.7 %)

Table 4: Samples collected from apparently healthy individuals in the community

Occupation	Ear Swab		Nasal Swab		Total	
	No of Samples	No of *Staph.* Isolated	No of Samples	No of *Staph.* Isolated	Sample	Isolate
Tutors	66	25	55	19	121	44
Artisans	32	13	30	9	62	22
Students	73	18	80	14	153	32
Petty Traders	38	12	32	10	70	22
Total	**209**	**68**	**197**	**52**	**406**	**120 (29.6 %)**

Table 5: Prevalence of MRSA in Hospital and community isolates

Sample source	No. of isolate tested	FETHA I	FETHA II	Mile Four	No. positive
			MRSA		
Hospital	84 (27.7 %)	9 (47.4 %)	7 (36.8 %)	3 (15.8 %)	**19 (22.6 %)**
Community	120 (29.6 %)				**25 (20.8 %)**

Table 6: Occupational distribution of population with community acquired methicillin resistant *staphylococcus aureus* (CA-MRSA)

Occupation	Swab	Ear M	Ear F	Nasal Swab M	Nasal Swab F	Total M	Total F	Total
Tutor	2	4	2	3	4	7		11
Artisan	3	1	1	2	4	3		7
Petty Trader	1	2	0	0	1	2		3
Student	0	2	1	1	1	3		4
TOTAL		**6**	**9**	**4**	**6**	**10**	**15**	**25**
			15	**10**				

Key: M = Male   F = Female

Table 7: Beta-lactamase detection in Hospital and community isolates

Source	No of isolates tested	Beta-lactamase positive	Beta-lactamase negative
Hospital	84	32	52
Community	120	29	91
Total	**204**	**61**	**143**

Discussion

Staphylococcus aureus has long been recognized as the cause of a variety of infectious diseases ranging from mild conditions like soft tissue infections, to severe life threatening debilitation such as endocarditis[13]. Infections caused by methicillin-resistant *S. aureus* (MRSA) represent a growing public health problem and a challenge for health care institutions[14]. Regardless of serious efforts employed to control antibiotic resistant bacteria by aggressive infectioncontrol methods, antibiotic resistant *Staphylococci*, especially MRSA, has become the most frequent cause of hospital and community acquired infections worldwide[15,16].

In this study, 36 (30.8 %) *S. aureus* isolates were obtained from the 117 clinical samples collected from FETHA I. Nine (25 %) out of these isolates were identified as MRSA (Table 1). In FETHA II, 31 (30.1 %) S. aureus isolates were obtained from the 103 clinical samples collected from patients. Seven (22.6 %) of these isolates were identified as MRSA (Table 2). In Mile Four Hospital, 17 (20.5 %) *S. aureus* isolates were obtained from 83 clinial samples. Three (17.7 %) of the isolates were identified as MRSA (Table 3). One hundred and twenty (29.6 %) *S. aureus* isolates were obtained from the 496 community samples (nasal and ear swabs) collected from tutors, artisans, students and petty traders (Table 4). This study is in agreement with the research conducted in Osogbo, South-western Nigeria by Olowe et al.[17] who reported the isolation of 42.9 % S. aureus from Staphylococci isolates in Osogbo, South-western Nigeria. Nineteen (22.6 %) isolates were confirmed to be MRSA out of the 84 *S. aureus* isolated from the clinical samples (wound, pus, urine, HVS, ear swab, sputum and semen) (Table 5). Similarly, 25 (20.8 %) community-associated methicillin resistant S. aureus (CA-MRSA) were detected from the 120 Staphylococcus aureus isolates obtained from the community samples of nasal and ear swabs (Table 5). The distribution of MRSA isolated from the three major hospitals in Abakaliki showed that the highest prevalence frequency of MRSA was found in FETHA I [9 (47.4 %)].

This was closely followed by FETHA II [(7 (36.8 %)] and Mile Four General Hospital [(3 (15.8 %)] being the least (Table 5). The highest number of MRSA was isolated from wound samples (7); followed by ear swabs (5), urine (3) and sputum (2). HVS and pus recorded the least number of MRSA as they each had one isolate. There was no MRSA isolate from semen samples (Tables 1, 2 and 3). This is similar tothe report by Olowe et al.[17] who reported that wound samples had the highest prevalence of MRSA. Many researchers have reported an increase in the incidence of MRSA most of which originated from wounds[18]. CA-MRSA was most predominant among tutors (11). The overcrowded nature of tutors' staff rooms and irrational use of antibiotics might possibly be the reason for the high prevalence of MRSA among them. This is because overcrowded living places have been seen as one of the risk factors for CA-MRSA[19]. The least number of CA-MRSA was observed among petty traders (Table 6). CA-MRSA isolates were more prevalent in ear swabs than in nasal swabs (Table 6).This result is in concord with the work of Falagas et al. in South Africa[20]. They reported MRSA prevalence of 23 %. The same researchers later reported a slightly higher prevalence frequency of 24 % and 20 % for HA-MRSA and CA-MRSA respectively in another research they conducted in the same area some years later. Similar observation was also made by Borg et al. who recorded MRSA prevalence rate of 18 % in a research they conducted in 4 hospitals in Tunisia[21]. The present research recorded lower prevalence rate when compared with the result of Olowe et al.[17] who recorded a higher MRSA prevalence frequency of 47.7 %. Another study conducted in South-western Nigeria is also in agreement with the report of this present study. The research reported a HA-MRSA prevalence rate of 20.23 % where 70 out of 346 S. aureus isolates were methicillin resistant22. The CA-MRSA prevalence rate of 20.8 % reported in this work is similar to that reported in another research conducted by Lucianne et al.[23] who reported a CA-MRSA prevalence of 27.8 %.

The MRSA isolates (HA-MRSA) obtained from the clinical samples in the hospitals were multidrug resistant as they were resistant to at least two classes of antibiotics (Figure 1). All the HA-MRSA isolates were completely resistant (100 %) to ceftazidime, tetracycline and penicillin. This was closely followed by ciprofloxacin (94.7 %), nitrofurantoin (89.5 %), clindamycin (89.5 %) and erythromycin (78.9 %). The most effective antibiotic against the HA-MRSA isolates was gentamicin as 63.2 % of the isolates were susceptible to this antibiotic (Figure 1). This is in agreement with the work of Ibrahim et al.[24] where antibiotic susceptibility results of *S. aureus* showed absolute

resistance (100 %) against ampicillin, and high resistance against cefotaxime (81 %), while resistance frequencies to ceftriaxone, erythromycin, ciprofloxacin, trimethoprim, gentamicin, levofloxacin and clindamycin were 59 %, 59 %, 41 %, 41 %, 35 %, 23 % and 18 %, respectively. The susceptibility frequency of MRSA isolates to gentamicin in this study is similar to the findings of Tiwari et al.[25] who reported a high susceptibility frequency of MRSA isolates to gentamicin. The CA-MRSA isolates were also multidrug resistant. All the HA-MRSA isolates were completely resistant (100 %) to ceftazidime, sulphamethoxazole and penicillin. This was closely followed by gentamicin (80 %), nitrofurantoin (80 %), clindamycin (80 %)nd tetracycline (60 %). Interestingly, ciprofloxacin, and erythromycin were the most effective antibiotics against the CA-MRSA in contrast to the HA-MRSA in which gentamicin was the most effective antibiotic (Figure 2). These resistances might result from inappropriate prescriptions due to lack of standard treatment guidelines[26]. In developing countries, an estimated 50 % of those who need antimicrobials do not have access to them due to cost. Sometimes, these drugs are not taken by patients as prescribed and some people indulge in self-medication[27]. Relentless exposure of bacterial strains to a large number of β-lactams has induced active and continuous production and mutation of β-lactamases in these bacteria, thus expanding their activity even against the newly developed β-lactam antibiotics. Thirty-two (32) MRSA (or HA-MRSA) isolates out of the 84 clinical samples of *S. aureus* isolates were positive for beta-lactamase production while 29 CA-MRSA isolates were positive for beta-lactamase production (Table 7). This is in agreement with the findings of Terry et al.[28] in South-East Nigeria who reported that 124 (64 %) of their *S. aureus* isolates were able to produce β-lactamase enzyme by changing colour from yellow to red on the addition of nitrocefin solution. Production of β-lactamase in *S. aureus*is reported to have been consistently high in Nigeria[29]. They reported that 70-80 % of *S. aureus* isolates produced β-lactamase. Other researchers reported such high β-lactamase prevalence[30,31]. The spread of β-lactamase genes had been enhanced by their integration within mobile genetic elements such as plasmids and transposons which facilitate the rapid transfer of genetic materials between microbes[32]. Multidrug resistant bacteria have created therapeutic problems especially to healthcare providers and, its consistent emergence will only cause hazard to the public health[33]. The HA-MR-

SA isolates had MARI values within the range of 0.7 to 1.0. These high MARI values depict the high resistances of these isolates to antibiotics (Table 8). The MARI values for the CA-MRSA ranged from 0.3 to 0.7. These values are also high but lower than the MARI values for HA-MRSA which exhibited higher antibiotic resistance potential (Table 9).

Our study is in agreement with the report of Subramani and Vignesh[34] who revealed that all the *S. aureus* isolates in their study had very high MARI values of above 0.2. This indicates that the bacterial isolates may have been exposed to several antibiotics and the selective pressure of the antibiotics used in the management of bacterial infections could be the main reason for resistance apart from the bacteria acquiring the resistance gene through mutation or interspecies gene transmission[35].

Conclusion

This study shows that misuse of antibiotics could possibly lead to a change in microbial resistance characteristics, causing treatment failure and increase in the cost of infection control. This report further reveals that MRSA isolates are present in some major hospitals in Abakaliki and within the community in Abakaliki, Ebonyi State, Nigeria. Gentamicin, ciprofloxacin and erythromycin are still effective against MRSA infections. Obviously, if the infections caused by MRSA are not controlled, it will spread to larger population of people in the community thereby causing a very serious public health problem especially among the immunocompromised. Therefore, there is urgent need to keep a strict watch on MRSA emerging from this research study area.

Conflict of interest statement

The authors declare that there are no conflicts of interest

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