World Journal of Pharmaceutical and Life Sciences
WJPLS
www.wjpls.org
SJIF Impact Factor: 4.223

PHENOTYPIC DETECTION OF METHICILLIN RESISTANCE IN PATHOGENIC *STAPHYLOCOCCUS AUREUS* BY DISK METHOD

Orinya Chinedu[1], Iroha Ifeanyichukwu[1], Ejikeugwu Chika[1]*, Iroha Chidinma[2], Nwakaeze Emmanuel[1], Ajah Moniaque[3], Ugwu Emmanuel[1]

[1]Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, Abakaliki, P.M.B 053, Ebonyi State, Nigeria.
[2]Department of Pharmacy, Federal Teaching Hospital, P.M.B 102, Abakaliki, Ebonyi State, Nigeria.
[3]Cancer Screening Unit, Federal Teaching Hospital, P.M.B 102, Abakaliki, Ebonyi State, Nigeria.

***Corresponding Author: Ejikeugwu Chika**
Department of Applied Microbiology, Faculty of Sciences, Ebonyi State University, Abakaliki, P.M.B 053, Ebonyi State, Nigeria.

Article Received on 20/02/2017 **Article Revised on 12/03/2017** **Article Accepted on 03/04/2017**

ABSTRACT

Methicillin resistant *Staphylococcus aureus* (MRSA) is defined as a strain of *S.aureus* that is resistant to methicillin, a semi-synthetic antibiotic that was introduced into clinical medicine for the treatment of bacterial infections that defied the actions of penicillin. This study determined the frequency of MRSA isolates from clinical samples of 100 individuals (inclusive of in-patients and out-patients) who sought medical care in a tertiary hospital in Abakaliki, Ebonyi state, Nigeria. Clinical samples were aseptically collected from the anterior nares of the participants using sterile swab sticks, and these samples were bacteriologically analyzed using standard microbiology techniques. Antimicrobial susceptibility testing was carried out by the Kirby-Bauer disk diffusion technique using standard antibiotic disks, and MRSA isolates was identified using disk diffusion technique. Out of the 100 clinical samples, a total of 33 (75%) MRSA isolates was recovered from female patients while 41 (73.2%) MRSA isolates was recovered from male participants. The carriage rate of MRSA among the in-patients and out-patients was 69.2 % and 75.2 % respectively. MRSA isolates was recovered from all the age-groups of the participants. However, more MRSA isolates was recovered from participants at the age of 6-10 yrs (86.7 %) and 11-16 yrs (80 %). The isolated pathogenic *S. aureus* from both the in-patients and out-patients were highly resistant to more than 50 % of the tested antibiotics especially to ampicillin, cefotaxime, cefoxitin, bacitracin, oxacillin, ertapenem, amikacin and vancomycin. Conclusively, this study reports the occurrence of MRSA strains amongst in-patients and out-patients of adolescence age group, and it was revealed that MRSA is harboured by both patients within the hospital environments and those coming from the community. The emergence and spread of antibiotic resistant pathogens including MRSA strains should be kept under check through accurate and prompt monitoring, evaluation, and detection of resistant pathogens from suspect specimens in both the community and hospital environment.

KEYWORDS: MRSA, Antibiotic Resistance, *Staphylococcus aureus*, Pneumonia, Nigeria.

INTRODUCTION

Pneumonia caused by pathogenic *Staphylococcus aureus* that are methicillin resistant have been recently reported across the world as an important cause of nosocomial- and community-acquired infections.[1,2,3] *Staphylococcus aureus* (which is notorious in evolving resistance to antibiotics) accounts for many hospital- and community-acquired infections including skin infections, bacteraemia, and pneumonia. Methicillin-resistant *Staphylococcus aureus* (MRSA) still remains an important nosocomial and community-acquired pathogen because of its multidrug resistant nature which gives them the innate/acquired ability to evade the onslaught of antibiotics. *Staphylococcus aureus* has evolved from a once susceptible pathogen to methicillin resistant (MRSA), penicillin resistant, low-level vancomycin resistant (VISA), and high-level vancomycin resistant (VRSA) bacterial strain. Methicillin resistant *Staphylococcus aureus* (MRSA) are strains of pathogenic *Staphylococcus* that are resistant to a wide variety of beta-lactam antibiotic including methicillin, for which they are named.[4] MRSA became a global public health issue of discourse in 1961 when the first *Staphylococcus aureus* strain that showed resistance to the actions of methicillin was reported in UK.[5,6] This marked the first report of MRSA in the world; and this followed the introduction of methicillin into clinical medicine in order to effectively manage bacterial infections caused by

bacteria known to be resistant to penicillin. Their emergence and spread is a serious worry in human medicine because of their multiple antibiotic resistance nature. MRSA carries genes that make pathogenic *Staphylococcus aureus* insensitive to methicillin, a potent class of antibiotic in clinical medicine.[7,8] The acquisition of *mecA* gene confers on pathogenic *Staphylococcus aureus* strain the ability to show total resistance to all β-lactam antibiotics including penicillin, cephalosporins, and other non-beta-lactam drugs like gentamicin.[9] Methicillin resistant *Staphylococcus aureus* can cause infection in either the community or hospital environment, and thus the two major types of MRSA are community-acquired MRSA and hospital-acquired MRSA – which cause infection in the community and hospital settings respectively.[10] This present study presumptively detected the occurrence of MRSA-producing strains from clinical samples in a tertiary hospital.

MATERIALS AND METHODS

Sampling and study design: The clinical samples used in this study were collected from in-patients and out-patients attending the paediatrics unit of a tertiary hospital in Abakaliki metropolis, Ebonyi State Nigeria after obtaining ethical clearance from the Ethics and Research Committee of the same hospital. A total of 100 individuals who presented clinical signs of bacterial pneumonia infection were included in this study.

Sample collection: Samples was aseptically collected from the anterior nares (nostrils) of each participant in the study using sterile swab sticks; and each of the collected samples was returned to their respective containers, labeled and transported to Microbiology Laboratory Unit of Ebonyi State University, Abakaliki, Nigeria for bacteriological analysis.

Isolation and identification of pathogenic *Staphylococcus aureus*: Each of the collected samples was inoculated onto 5 ml nutrient broth (Oxoid, UK) and incubated for 18-24 hrs overnight at 37^{o}C; and a loopful of the turbid culture was aseptically transferred onto mannitol salt agar (MSA) plates, and incubated at 37^{o}C for 18-24 hrs. *S. aureus* was identified using standard microbiology identification techniques, and the organism produces yellowish colonies on MSA.[10,11]

Antimicrobial susceptibility testing: This was carried out by the Kirby-Bauer disk diffusion technique using single antibiotic disks including ampicillin, amikacin, ofloxacin, erythromycin, amoxicillin, ertapenem, vancomycin, amoxycillin-clavulanic acid, cefotaxime, cefoxitin, oxacillin, gentamicin and mupirocin (Oxoid, UK). Briefly, suspension of the test organism (adjusted to 0.5 McFarland turbidity standards) was aseptically swabbed onto freshly prepared Mueller-Hinton (MH) agar plates, and each of the antibiotic disks was aseptically placed on the inoculated MH agar plates. All susceptibility test plates was incubated at 37^{o}C for 18-24 hrs, and the inhibition zone diameters (IZDs) was measured as per the CLSI guidelines.[12,13]

Phenotypic determination of MRSA isolates: MRSA isolates was phenotypically screened and confirmed using disk diffusion technique. To screen for and phenotypically confirm methicillin resistance in the pathogenic *S. aureus* isolates, cefoxitin disk and oxacillin disk was used. The pathogenic *S. aureus* isolates (adjusted to 0.5 McFarland turbidity standards) were subjected to cefoxitin disk (30 µg) and oxacillin disk (5 µg) on MH agar plates, and incubated at 37^{o}C for 18-24 hrs. After incubation, the zones of inhibition was measured, recorded and interpreted as per the guidelines of the Clinical Laboratory Standards Institute (CLSI).[13,14,15]

RESULTS

This study evaluated the occurrence of methicilin resistant *Staphylococcus aureus* from clinical samples of both in-patients and out-patients in a tertiary hospital in Abakaliki, Ebonyi State, Nigeria. Table 1 shows the prevalence of MRSA isolates amongst male and female individuals. Out of the 44 female patients, only 33 (75 %) individuals were positive for MRSA while 41 male participants were found to be positive for MRSA out of 56 male patients included in the study (Table 1). Table 2 shows the frequency of MRSA from both in-patients and out-patients. MRSA isolates was recovered in higher amounts from clinical samples of out-patients (75.2 %) than from samples of in-patients (69.2 %). The distribution of MRSA isolates according to the age of the participants in this study is shown in Table 3.

Table 1: Carriage rate of methicillin resistant *S. aureus* among male and female individuals.

Sex	Number of Participants	MRSA Positive n (%)
Females	44	33 (75)
Males	56	41 (73.2)

Table 2: Frequency of MRSA among in-patients and out-patients.

Participants	Number of Participant	MRSA Positive n (%)
In-patients	26	18 (69.2)
Out-patients	74	56 (75.2)

Table 3: Carriage Rate of MRSA among Different Age Groups of the participants.

Age group	Number of Participant	MRSA Positive	Percentage (%)
0-5	70	49	70.0
6-10	15	13	86.7
11-16	15	12	80
Total	100	74	236.7

Table 4 shows the antimicrobial susceptibility profile of the *S. aureus* isolates to some commonly used antibiotics. The pathogenic *S. aureus* isolates from both the in-patients and out-patients showed varying rates of susceptibility and resistance to the tested antibiotics. *S. aureus* from in-patients were found to be highly resistant to ampicillin (100 %), amoxicillin (100 %), cefotaxime (100 %), cefoxitin (94. 4 %), cloxacillin (100 %), bacitracin (100 %), oxacillin (100 %), mupirocin (100 %) and vancomycin (100 %). However, the *S. aureus* from the out-patients were found to be less resistant to the tested antibiotics when compared to *S. aureus* isolates from in-patients (Table 4).

Table 4: Antimicrobial susceptibility profile of *S. aureus* isolates from in-patients and out-patients

Antibiotics	Inpatients		Outpatient	
	Sensitive (%)	Resistance (%)	Sensitive (%)	Resistance (%)
Amikacin	11 (61.1)	7 (38.9)	14 (25)	42 (75)
AMC	1 (5.5)	17 (94.4)	2 (3.6)	54 (96.6)
Ampicillin	0 (0)	18 (100)	0 (0)	56 (100)
Amoxicillin	0 (0)	18 (100)	1 (1.7)	55 (98.2)
Cefotaxime	0 (0)	18 (100)	0 (0)	56 (100)
Cefoxitin	1 (3.8)	18 (94.4)	2 (3.6)	54 (96.6)
Cloxacillin	0 (0)	18 (100)	0 (0)	56 (100)
Ertapenem	9 (50)	9 (50)	4 (7.1)	52 (92.9)
Erythromycin	2 (11.1)	17 (88.9)	10 (17.9)	46 (82.1)
Gentamicin	12 (66.7)	4 (22.2)	35 (63.7)	21 (37.5)
Bacitracin	0 (0)	18 (100)	2 (3.6)	54 (96.4)
Ofloxacin	10 (55.6)	8 (44.4)	25 (44.6)	31 (55.4)
Oxacillin	0 (0)	18 (100)	3 (5.3)	54 (94.6)
Mupirocin	0 (0)	18 (100)	11 (19.6)	45 (80.4)
Vancomycin	0 (0)	18 (100)	0 (0)	56 (100)

AMC = Amoxicillin-clavulanic acid

DISCUSSION

Pneumonia caused by pathogenic *Staphylococcus aureus* and strains of *S. aureus* that are methicillin resistant is on the horizon in both the community and hospital environments. Methicillin-resistant *Staphylococcus aureus* otherwise known as MRSA still remains an important nosocomial and community-acquired pathogen because of its multidrug resistant nature which gives them the acquired ability to evade the onslaught of many antibiotics used in clinical medicine. This study determined the frequency of MRSA isolates from clinical samples of 100 individuals (inclusive of in-patients and out-patients) who sought medical care in a tertiary hospital in Abakaliki, Ebonyi state, Nigeria. The isolated pathogenic *S. aureus* isolates showed varying levels of susceptibility to the tested antibiotics. A high level of resistance of the pathogenic *S. aureus* from in-patients was observed to amoxicillin (100 %), cefotaxime (100 %), cefoxitin (94. 4 %), cloxacillin (100 %), bacitracin (100 %), oxacillin (100 %), mupirocin (100 %) and vancomycin (100 %). However, the pathogenic *S. aureus* isolates from the out-patients were found to show less susceptibility to a greater number of the antibiotics used especially to amoxicillin, gentamicin, cefoxitin, oxacillin, mupirocin, ampicillin, ertapenem, erythromycin and vancomycin. The high resistance of pathogenic *S. aureus* to the tested antibiotics in this study is in agreement with the work of Suleiman *et al.*[16] and Huang *et al.*[17] – who reported in their study that pathogenic *S. aureus* especially those with genes for resistance to methicillin are multidrug resistant in nature. The carriage rate of MRSA amongst the participants was highest amongst the female patients (75 %) than in the male participants who had a carriage rate of 73 %. Higher prevalence of MRSA isolates as reported in this study have also been described elsewhere.[18,19] However, the carriage rate of MRSA amongst the female and male patients in this study is higher than the rate reported by Adcock *et al.*,[20] – who showed in their study that MRSA was isolated at the rate of 42 %. Also in a similar work, Gorak *et al.*,[21] also reported a lesser prevalence rate of MRSA amongst their study participants (40.6%). The carriage rate of MRSA amongst the in-patients and out patients in this study varied. A higher carriage rate

of MRSA was observed amongst the out-patients (75.7 %) included in this study when compared to the in-patients (69.2 %). MRSA isolates was recovered from participants of all age group included in this study, but participants in the age group of 6-10 yrs showed higher carriage rate of MRSA (86.7 %) than participants in the age group of 11-16 yrs (80 %). This is also in agreement to the study of Balta *et al.*,[22] and Bogaert *et al.*,[23] – who showed higher carriage rate of MRSA in people of younger age. According to Kamana *et al.*, and previous reports,[4,5,6,24] the current antibiotic resistance phenomenon across the globe is an indication of the indiscriminate use of antibiotics for both human and non-human purposes. Thus, the effective usage of antibiotics in both the community and hospital environments is important to assuage the growing prevalence of antibiotic resistance across the globe.

ACKNOWLEDGEMENT

This work was supported in part by a grant from the Nigeria's Tertiary Education Trust Fund (TETFund); Grant No: EBSU/TETFund/IBR/2015/03.

REFERENCES

1. Warshawsky, B, Hussain Z, Gregson D, Alder R, Austin M, Brugschwaiger D. Hospital- and community-based surveillance of methicillin-resistant Staphylococcus aureus: previous hospitalization is the major risk. Infect Control Hosp Epidemiol. 2000; 21: 724–727.
2. Shopsin B, Mathema B, Martinez J, Ha E, Campo ML, Fierman A. Prevalence of methicillin-resistant Staphylococcus aureus in the community. J Infect Dis. 2000; 182: 359–62.
3. Kuehnert MJ, Hill HA, Kupronis BA, Tokars JI, Solomon SL, Jernigan DB. Methicillin-resistant Staphylococcus aureus hospitalizations United States. Emerg. Infect Dis. 2005; 11: 868-872.
4. Terry AOA, Ogbolu DO, Akorede E, Onemu OM, Okanlawon BM. Distribution of mecA gene amongst Staphylococcus aureus isolates from southwestern Nigeria. Afr. J. Biomed. Res, 2011; 9-16.
5. Michael ZD, Robert SD. Community-Associated Methicillin Resistant Staphylococcus aureus: Epidemiology and Clinical Consequences of Emerging Epidemic. Clinical Microbiology Review. 2010; 23(3): 616-687.
6. Sae T, Kyoko KA, Takashi S, Keiichi H. Origin and Molecular Evolution of the Determinant of Methicillin Resistance in Staphylococci. Antimicrobial Agents Chemotherapy. 2010; 54(10): 4352-4359.
7. Otter JA, French GL. Molecular epidemiology of community-associated methicillin-resistant Staphylococcus aureus in Europe. Lancet Infect Dis. 2010; 10(4): 227-39.
8. Pinho MG, de Lencastre H, Tomasz A. An acquired and native penicillin-binding protein cooperate in building the cell wall of drug-resistant Staphylococci. Proc Natl Acad Sci USA. 2001; 98(19): 10886-91.
9. Nakou A, Woodhead M, Torres A. MRSA as a cause of community-acquired pneumonia. Eur Respir J. 2009; 34: 1013–1014.
10. Amini R, Abdulamir AS, Ling BP, Jahanshiri F, Hematian A, Zargar M, Sekawi Z, Jalilian FA. Isolation and Identification of Methicillin-Resistant Staphylococcus aureus from keys of College Students Using Different Detection Methods. British Biotechnology Journal. 2012; 2(1): 13-25.
11. Cheesbrough M. Biochemical tests to identify bacteria. In: District Laboratory Practice in Tropical Countries. 2nd edition. Cambridge University Press, UK. 2000; 178-187.
12. Ike B, Ugwu MC, Ikegbunam MN, Nwobodo D, Ejikeugwu C, Gugu T, Esimone CO. Prevalence, Antibiogram and Molecular Characterization of Comunity-Acquired Methicillin-Resistant Staphylococcus aureus in AWKA, Anambra Nigeria. The Open Microbiology Journal. 2016; 10: 211-221.
13. Clinical Laboratory Standard Institute, CLSI. Performance standards for antimicrobial disk susceptibility test. Fifteenth informational supplement, CLSI document M100-S15. Wayne, PA. USA. 2011.
14. Sasirekha B, Usha MS, Amruta AJ, Ankit S, Brinda N, Divya R. Evaluation and comparison of different phenotypic tests to detect methicillin resistant Staphylococcus aureus and their biofilm production. International Journal of Pharm Tech Research. 2012; 4(2): 532-541.
15. Salmenlinna S, Vuopio-Varkila J. Recognition of two groups of methicillin-resistant Staphylococcus aureus strains based on epidemiology, antimicrobial susceptibility, hypervariable-region type, and ribotype in Finland. J Clin Microbiol. 2001; 39: 2243–2247.
16. Suleiman AB, Umoh VJ, Kwaga JKP, Shaibu SJ. Prevalence and antibiotic resistance profiles of methicillin resistant Staphylococcus aureus (MRSA) isolated from bovine mastitis milk in Plateau state, Nigeria. International Research Journal of Microbiology. 2012; 2(8): 264-270
17. Huang YC, Chou LH, Su RI, Lien TY. Methicillin resistant Staphylococcus aureus colonization and its association with infection among infants hospitalized in neonatal intensive care units. Paediatrics. 2006; 118: 469-474.
18. Summiya N, Seema I, Afia Z. Evaluation of prevalence of low and high level Mupirocin resistance in Methicillin Resistant Staphylococcus aureus isolates at tertiary care hospital. J Pak Med Assoc. 2011; 61(6): 519-521.
19. Gorak E, Yamada S, Brown J. Community-acquired methicillin-resistant Staphylococcus aureus in hospitalized adults and children without known risk factors. Clin Infect Dis. 1999; 29: 797–800.

20. Adcock PM, Pastor P, Medley F, Patterson JE, Murphy TV. Methicillin-resistant Staphylococcus aureus in two child care centers. J Infect Dis. 1998; 178: 577–580.
21. Gorak E, Yamada S, Brown J. Community-acquired methicillin-resistant Staphylococcus aureus in hospitalized adults and children without known risk factors. Clin Infect Dis. 1999; 29: 797–800.
22. Balta B, Derbie F. Nasal carriage of methicillin resistant Staphylococcus aureus. Ethiop J. Health Sci, 2003; 13: 108-109.
23. Bogaert A, Van B, Sluijter M. Colonization by streptococcus pneumonia and Staphylococcus aureus in helthy children. Lancet, 2004; 1871-1772.
24. Kamana KV, Mohanty SK, Wilson CG. Staphylococcus aureus colonization of the anterior nares of school going children Indian. J. Pediatr. 2009; 76(8): 813-816.