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Original Research Article

Prevalence of methicillin-resistant *S. aureus* (MRSA) among apparently healthy students in Afikpo, Ebonyi State, Nigeria

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This study was carried out to determine the prevalence of community-acquired methicillin resistant *Staphylococcus aureus* (CA-MRSA) among apparently healthy students of Akanu Ibiam Federal Polytechnic Unwana, Afikpo, Ebonyi State, Nigeria. A total of 200 nasal swab samples were collected from apparently healthy students using sterile swab sticks and these were screened for the presence of *S. aureus* colonization using standard microbiology techniques. MRSA positive bacteria were detected phenotypically using oxacillin disk (1 µg). Antibiogram was determined by the disk diffusion method using ampicillin, cephalexin, clindamycin, ciprofloxacin, gentamicin, ofloxacin, perfloxacin, sparfloxacin, cefotaxime and vancomycin as per the guidelines of CLSI. Out of the 200 nasal swab samples, 76 (38.0 %) isolates were isolated. MRSA positive bacteria were only detected in 33 (43.4 %) of the *S. aureus* isolates. High level of resistance was recorded amongst the MRSA positive bacteria to some commonly used antibiotics especially cefotaxime (100 %), ampicillin (100 %), clindamycin (9.0 %) and vancomycin (12 %). antibiotics. This study reported a high prevalence of MRSA bacteria amongst apparently healthy students. The irrational use of antibiotics especially in the community and without prescription spurs the development of resistance strains of bacteria. It is vital to detect antibiotic resistant bacteria (including MRSA bacteria) from both environmental and clinical samples in order to sustain the gains of antimicrobial chemotherapy.

Key words: Community acquired infections, MRSA, Resistance, Nigeria

INTRODUCTION

Methicillin**,** the first β-lactamase-resistant-penicillin is a β-lactam antibiotic that is stable to β-lactamase enzymes produced by pathogenic *S. aureus* and other Gram negative bacteria (Denyer et al., 2004). It was introduced into clinical medicine in the early 1960's as a substitute to penicillin which was before then made less-efficacious by β-lactamase enzymes produced by pathogenic bacteria (Michael et al., 2010). Nowadays, strains of *S. aureus* that are resistant to methicillin (a potent bacterial cell wall inhibitor) is now found in both the hospital and community settings. These newer beta-lactamases that hydrolyze methicillin are known as methicillin-resistant *S. aureus* (MRSA). MRSA isolates are strains 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 and Robert, 2010; Sae et al., 2010). They have since emerged and spread as a serious public health issue

because of their multiple antibiotic resistant patterns which limits the choice for therapeutic options in bacterial infections caused by MRSA strains. According to Brook et al., (2010), MRSA harbour genes that render pathogenic *S. aureus* insensitive to methicillin, and the acquisition of mecA gene (the gene that defines MRSA) by *S. aureus* strain confers total resistance on the pathogen to β-lactam antibiotics including penicillin, clindamycin, erythromycin, tetracycline and cephalosporin (Otter et al., 2010). The gene, mecA codes for penicillin-binding-protein (PBP2a) – which confers on MRSA isolates the ability to be multiply resistant (Pinho et al., 2001). Community-acquired MRSA (CA-MRSA) and hospital-acquired MRSA (MRSA) now exist, and both are responsible for a variety of bacterial infections in humans. 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-acquired MRSA (HA-MRSA) which is nosocomial in origin to emerge. Risk factors for the acquisition of HA-MRSA include long term antibiotic usage, long hospitalization, direct contact with an infected or colonized individual, intravenous drug use, and regular exposure to clinical specimens without adequate preventive measures, crowded and unhygienic living conditions, compromised immune system and underlying chronic illness amongst others (Akande, 2010). This study evaluated the frequency of MRSA amongst students of a polytechnic in Abakaliki, Nigeria.

MATERIALS AND METHODS

Specimen

A total of 200 nasal swab samples were collected from the nares of apparently healthy students of Akanu Ibiam Federal Polytechnic, Unwana, Afikpo, Ebonyi State, Nigeria. Ethical clearance was obtained from the school authorities prior to the collection of samples from the participating students. The nasal swab samples were randomly collected from the students by swabbing there nares with sterile swab sticks moistened in physiological saline. All swab sticks were returned to their containers; and the samples were taken to the microbiology laboratory of Ebonyi State University, Abakaliki where they were analyzed by bacteriological methods.

Bacteriology

Each of the nasal swab samples were cultured on mannitol salt agar (MSA), blood agar (BA) and chocolate agar (CA) plates (Oxoid, UK); and the plates were incubated at 37^{o}C for 24 hrs. The isolates on each of the plates were subcultured onto MSA, BA, CA and nutrient agar plates to get pure cultures. Characteristic isolates of *S. aureus* on the agar plates were isolated and identified by standard microbiological tests including coagulase test, catalase test, DNase, test, sugar fermentation test and Gram staining (Cheesbrough, 2004).

Susceptibility studies

The antimicrobial susceptibility pattern of the isolates was determined by the disk diffusion method in line with the guideline of the Clinical Laboratory Standard Institute (CLSI) guidelines (CLSI, 2005). Briefly, all the confirmed *S. aureus* isolates were aseptically swabbed on Mueller-Hinton agar plates (Oxoid, UK), and antibiotic disks were placed on each of the plates at a distance of 15 mm. The plates were incubated at 37^{o}C for 24 hrs; and zones of inhibition were recorded according to the CLSI criteria, and recorded to the nearest millimeter (mm) as was previously described (Taiwo et al., 2004; Shittu and Johnson, 2006). The antibiotic disks used were single disks of oxacillin (1 µg), ampicillin (10 µg), cephalexin (30 µg), clindamycin (2 µg), ciprofloxacin (5 µg), gentamicin (10µg), ofloxacin (5 µg), perfloxacin (5 µg), sparfloxacin (5 µg) cefotaxime (30 µg) and vancomycin (30 µg). Antibiotic disks were procured from Oxoid Ltd (Oxoid, UK).

Detection of MRSA isolates

All confirmed *S. aureus* isolates was subjected to beta-lactamase test using Nitrocefin sticks from Oxoid Limited (Oxoid, UK) to determine if they express beta-lactamase enzymes. Beta-lactamase producing *S. aureus* was confirmed on those organisms that changed the colour of the strip from yellow to red. MRSA positive isolates were detected by the method of Ghamba et al (2012). Methicillin resistance in the confirmed *S. aureus* bacteria was determined by the disk diffusion method using 1 µg of oxacillin disk (Oxoid, UK). *S. aureus* isolates that showed or produced a zone of inhibition less than or equal to 14 mm were phenotypically confirmed as MRSA positive bacteria.

RESULTS

Out of the 200 nasal swab samples recruited for this present study, 76 isolates (representing 38.0 % of the total sample) were confirmed as *S. aureus*. More isolates of *S. aureus* were recovered from male students than female students (Table 1).

All the isolated *S. aureus* were positive for beta-lactamase production using the Nitrocefin stick for beta-lactamase production. Table 1 also shows the rate of isolation of MRSA positive bacteria from the nasal swab samples. Out of the 76 isolates of *S. aureus* obtained from the 200 nasal swab samples, 33 (43.4 %) isolates of *S. aureus* were methicillin resistant.

The result of antimicrobial susceptibility studies conducted on all confirmed MRSA positive bacteria isolates

Table 1. Prevalence of MRSA among the isolated *S. aureus*

Sex	No. sampled	No (%) of *S. aureus*	No (%) MRSA negative	No (%) MRSA positive
Male	100	40 (20.0)	23 (30.3)	18 (23.7)
Female	100	36 (18.0)	20 (26.3)	15 (19.7)
Total	200	76 (38.0)	43 (56.6)	33 (43.4)

Table 2. Result of susceptibility of MRSA positive bacteria to antibiotics

Antibiotics (µg)	Resistance	Intermediate	Susceptible
	No (%)	No (%)	No (%)
Ampicillin (10)	33 (100)	0 (0.0)	0 (0.0)
Cefotaxime (30)	33 (100)	0 (0.0)	0 (0.0)
Clindamycin (2)	3 (9.0)	0 (0.0)	30 (91.0)
Ciprofloxacin (5)	10 (30.0)	3 (9.1)	20 (61.0)
Gentamicin (10)	6 (18.0)	0 (0.0)	27 (82.0)
Ofloxacin (5)	10 (30.0)	1 (3.0)	22 (67.0)
Perfloxacin (5)	13 (39.0)	1 (3.0)	19 (58.0)
Sparfloxacin (5)	9 (27.0)	1 (3.0)	23 (70.0)
Vancomycin (30)	4 (12.0)	0 (0.0)	29 (88.0)

are shown in Table 2. The MRSA positive bacteria were resistant to ampicillin (100 %), cefotaxime (100 %), clindamycin (91.0 %), and vancomycin (88.0 %). Resistance was also recorded for perfloxacin (39.0 %), ofloxacin (30.0 %), ciprofloxacin (30.0 %), sparfloxacin (27.0 %) and gentamicin (18.0 %).

DISCUSSION

The development of resistance in bacteria is one of the mechanisms of natural adaptation to the presence of an antimicrobial agent that inhibits susceptible organisms and selects for the resistant ones. Under continued selection pressure, the selected resistant organisms multiply and spread to other geographic locations as well as to other microbes by transfer of resistance genes (Levy and Marshall, 2004). Selection of resistant strains occurs so rapid for some bacteria that clinical usefulness of the antibiotics is lost within a 5-year period (Bush, 2004). The emergence and spread of microbes that are resistant to cheap and effective first-choice drugs has become a common occurrence. Sensitivity to oxacillin disk (1 µg) and the presence of PBPs (which was detected by latex agglutination test kit from Oxoid, UK) in the confirmed isolates of *S. aureus* were used to confirm MRSA positive bacteria. Out of the 76 isolates of *S. aureus* investigated for methicillin resistance in this study, only 33 (43.4 %) isolates were phenotypically confirmed to be MRSA. This is very high compared to previous studies conducted on the same issue both within and outside Nigeria. The prevalence rates of community-acquired MRSA as reported by some researchers in different parts of Nigeria were 34.7 %, 36.4 %, 43 % and 49.1 %; and these reports are in accordance with ours in which the prevalence rate of MRSA from the nasal swab samples were 43.4 % (Taiwo et al., 2004; Azeez et al., 2008; Ikeh, 2003; Olayinka et al., 2005). In Imo state, southeast Nigeria, Amadi et al. (2013) reported a prevalence rate of 27 % isolates of *S. aureus* that were methicillin resistant.

However, this is lower than the 43.4 % prevalence of *S. aureus* isolates that were resistant to methicillin in our study. In earlier studies carried out in Japan and the Republic of Korea, MRSA prevalence rate was reported to be 54 % and 70 % respectively (Lotus et al., 1995; Woojoo and Seunchill, 1999); and this was higher than the prevalence recorded in our study. The differences observed in our results with that of previously conducted studies (as per the prevalence rate of MRSA) might be related to the abuse of antimicrobial agents in Unwana, Afikpo, Ebonyi State where the study was conducted as wells as possible poor personal hygiene amongst the students under study. The high prevalence of MRSA bacteria in this present day study (43.4 %) could be attributed to the irrational use of antibiotics (particularly beta-lactam drugs) in this part of the world; and people often resort to self medication as a way of meeting some of their primary health care needs. In some cases; physicians resort to blind treatment prior to getting the susceptibility test result of their sick patients; and this phenomenon coupled with the acquisition of antibiotics over-the-counter (OTC) even without a doctor's prescription encourages pathogenic bacteria (including *S. aureus*) to develop resistance to these drugs over time. Higher rates of community-acquired methicillin resistant *S. aureus* (as obtainable in this study) have also been previously reported both within and outside Nigeria. The

MRSA positive isolates were resistant to some commonly used antibiotics at varying rates. The highest resistance of *S. aureus* isolates that were methicillin resistant was recorded against vancomycin, ampicillin, cefotaxime and clindamycin. The increasing prevalence of MRSA bacteria and their associated wide spectrum of resistance to some commonly used antibiotics (as reported in this present day study) are of public health concern; and this calls for effective measures (inclusive of public enlightenment to promote rational use of antibiotics and detection of MRSA bacteria from clinical and environmental samples) in order to contain the emergence and spread of such pathogens. A national policy on the controlled use of antibiotics and their restriction for non-clinical usages (especially drugs meant for human medicine) in animal production, livestock production and other veterinary purposes is urgently needed to sustain the shelf life of available antimicrobial agents.

Conclusion

In conclusion, a high prevalence of *S. aureus* isolates was observed in this study; and the organisms were also found to be resistant to some commonly used antibiotics especially cefotaxime, ampicillin, vancomycin and clindamycin which are all beta-lactam drugs. The result of this presumptive study is a clue to understanding the level of antimicrobial resistance in this region especially as it relates to MRSA positive bacteria. Continued monitoring and surveillance of antimicrobial resistance in both the community and hospital environment is therefore advocated to contain the situation.

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