ORIGINAL ARTICLE

Prevalence and Pattern of Methicillin Resistant *Staphylococcus Aureus* in a Tertiary Healthcare Facility in Nigeria

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ABSTRACT

Background: Currently, there is high level of antimicrobial resistance among microorganisms circulating in hospital environment as evidenced by microbial isolates from inpatients and organisms causing nosocomial infections. This trend is on the increase consequently there is prolong hospital stay, increased hospital bills, and increased morbidity and mortality. The widespread use of antimicrobial agents such as the β-lactam antibiotics has contributed to the emergence of Methicillin Resistant *Staphylococcus aureus*(MRSA); which has become a "hard nut to crack "in terms of treatment today.

Objective: To determine the prevalence and pattern of Methicillin Resistant *Staphylococcus aureus*(MRSA) in clinical specimens sent to clinical microbiology laboratory for analysis and to determine the vancomycin sensitivity of the MRSA isolates.

Design: The study was conducted at the University of Benin Teaching Hospital (UBTH) in Nigeria. This was a prospective, systemic microbiological study of clinical specimens whereby sequential Staphylococcal isolates were identified and confirmed by using standard methods and antibiotics susceptibility test was performed using Kirby-Bauer disc diffusion method adhering to the Clinical Laboratory Standards Institute interpretive criteria. Methicillin Resistant *Staphylococcus aureus* was detected using a rapid latex agglutination test -MRSA Screen (Denka-Seiken, Tokyo- Japan).

Results: In our study specimens, the prevalence of MRSA was 42.7% and vancomycin had an outstanding susceptibility of 100% for all our MRSA isolates.

Conclusion: The result of our findings call for regular surveillance of hospital infections and monitoring of antibiotic susceptibility pattern of MRSA and other pathogens periodically. This is essential for the formulation of antibiotic usage policies which in turn will have an impact on the control of hospital acquired infections such as MRSA.

INTRODUCTION

Resistance to antibiotics has been on the increase for the past decades[1], consequently some antibacterial agents are no longer useful for the treatment of bacterial infections. It is also of concern that an increasing number of bacteria are becoming resistant to more than one class of antibiotics.[1]The escalating resistance poses a threat in the management and control of infectious diseases and this may jeopardize much of the successes and progress made so far in the past century. Methicillin Resistant *Staphylococcus aureus* (MRSA) is responsible for several difficult to treat infections in humans worldwide.[2,3]

A major problem with the MRSA is that they develop resistance not only to the β- lactam group of antibiotics but also resistant to some other classes of antibiotics as well.[4,5]Infected and colonized patients in hospitals mediate the dissemination of MRSA strains, and hospital staff is the main source of transmission. This leads to serious endemic and epidemic outbreak of MRSA infection.[6]In Nigeria, the widespread use of antibiotics had led to high levels of resistance in bacterial isolates from patients with nosocomial infections.[7-10]

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There has also been a recent increase in resistance to gentamicin and variable susceptibility to other non-β-lactam antibiotics such as tetracycline, trimethoprim, erythromycin and ciprofloxacin.[11-13] Antibiotic resistance mechanisms and the prevalence of MRSA isolates from clinical samples have been studied extensively.[13] Community Acquired -MRSA is traditionally distinguished from hospital-acquired MRSA in several ways. It predominantly affect patients in community rather than hospital settings, is typically susceptible to multiple antibiotic classes and skin/soft tissue infections are the most common clinical manifestations. However, as the prevalence of CA-MRSA increases it is also becoming an important cause of nosocomial infections. Outbreaks and clusters have occurred which indicate certain risk factors including skin-to-skin contact, compromised skin surfaces, crowded living conditions and poor hygiene. USA300 is the predominant CA-MRSA strain in the united states.[14]

It is important to know the prevalence of MRSA in any environment especially in health care facilities, because of public health concerns and the threat posed.

MATERIALS AND METHODS

The study was conducted at the clinical microbiology laboratory of the UBTH, Nigeria from September 2013 to November, 2014.

Staphylococcus aureus strains were obtained from different clinical samples (pus, urine, blood, body fluids, sputa and wound swabs). Pus specimens were specimens collected in either sterile universal containers or syringes from sites such as breast abscess, empyema and intra-abdominal abscess. The swabs are mainly from post-operational wounds and skin ulcers. The body fluids were cerebrospinal fluid, ascetic fluid, pleural fluid and Joint fluid aspirates. Multiple *Staphylococcus aureus* isolates from same patient were excluded. The *Staphylococcus aureus* isolates were identified by gram positive cocci morphology, catalase positivity, DNase test using Oxoid CM 321 and mannitol salt fermentation (Oxoid, Melbourne, Australia) according to the method described by Kloos[15] and Hacbarth[16].Other test included positive coagulase reaction using rabbit plasma.

MRSA Screen Test /Antibiotic Susceptibility Test: MRSA screening test was employed to screen for methicillin resistance. It is a rapid slide latex agglutination assay for the detection of PBP-2a with sensitivity of about 98.5% and specificity 100%.[17] It took 5 minutes to complete a test. The method involved extraction of PBP-2a from suspensions of colonies and detection by agglutination with latex particles coated with monoclonal antibodies to PBP-2a.Antibiotic susceptibility testing was done using agar diffusion and broth micro-dilution(for vancomycin) methods as recommended by Bauer et al[18] and the Clinical and Laboratory Standards Institutes (CLSI)[19]using Oxoid Mueller Hinton agar (Difco Laboratories, Detroit, Mich. U.S.A). The antibiotics used for the susceptibility testing include: cefuroxime (CF)30µg, vancomycin(VAN)5µg-(Mayne Pharma Warwickshire, UK); tetracycline (TET)30µg, gentamicin (GN)10µg, ciprofloxacin (CIP)5µg, erythromycin(ERY)10µg, ofloxacin(OFL)30µg, and ampicillin(AMP)30µg. The suspension for inoculation was prepared from the colonies of an overnight growth on nutrient agar. One to two colonies was suspended in 0.5ml of sterile saline and the turbidity was adjusted to 0.5 McFarland's standard. A sterile swab dipped into the suspension and pressed against the side of the bottle to remove excess fluid. Mueller-Hinton agar plate was inoculated with the swab. Antibiotic disks were placed on the agar plate. The plates were incubated at a temperature of 37^{oC} for 18-24 hours. The diameters of zones of clearance were measured and the results interpreted accordingly. (*Staphylococcus aureus* ATCC 25923 was the control strain used).

RESULTS

A total of 3510 specimens were analysed, comprising of 439(12.5%) wound swabs, 1240(35.9%) blood culture, 497(14.2%) sputa, 856(24.4%) urine, 187(5.3%) pus, and 27(7.7%) body fluids (Table 1). Five hundred and twenty two *Staphylococcus aureus* were isolated from the specimens. Two hundred and ninety two(56%) from wound swabs; blood 18(3.5%); sputa 13(2.5%); urine 5(1%); pus 183(35%) and body fluids 11(2%) (Figure 1).Table 2 shows that 1667 specimens were from males, with 253 *Staphylococcus aureus* isolated and 109 being methicillin resistant *Staphylococcus aureus* (MRSA); whereas 1843 specimens came from females with 269 *Staphylococcus aureus* isolated and 114 MRSA.Antibiotic susceptibility pattern of the MRSA

shows 100% resistance to cefuroxime, tetracycline, ampicillin, gentamicin, erythromycin, and ciprofloxacin. However, it was 99% for ofloxacin because two of the isolates show intermediate resistance. Vancomycin has 100% susceptibility for all the MRSA isolates with zones of inhibition greater than 15mm and the minimum inhibitory concentrations ranges from 0.25to 2.0 µg/ml (Table 3& 4).

Table 1: Distribution of clinical specimen from section of the hospital

	Wound Swab	Blood	Sputum	Urine	Pus	Body Fluids	Total
I.C.U.	35	93	-	51	13	-	192
Children	28	292	77	58	8	211	675
Medical	39	512	103	384	70	60	1168
Surgical	171	122	-	67	42	-	402
Gynae/ obstetrics	16	200	16	287	11	-	530
Chest clinic	-	32	391	9	-	-	342
Orthopaedic	150	9	-	-	42	-	201
	439	**1,260**	**497**	**856**	**187**	**271**	**3,510**
	(12.5%)	**(35.9%)**	**(14.2%)**	**(24.4%)**	**(5.3%)**		**(7.7%)**

Table 2: Sex distribution in number of collected specimens, isolated *S.aureus* and MRSA

SEX	NO. OF SPECIMENS 3510)	(n= NO. OF S. aureus (n=522)	NO. OF MRSA (n=223)
M	**1,667 (47.5%)**	**253 (48.4%)**	**109 (49%)**
F	**1843 (52.5%)**	**269 (51.6%)**	**114 (51%)**

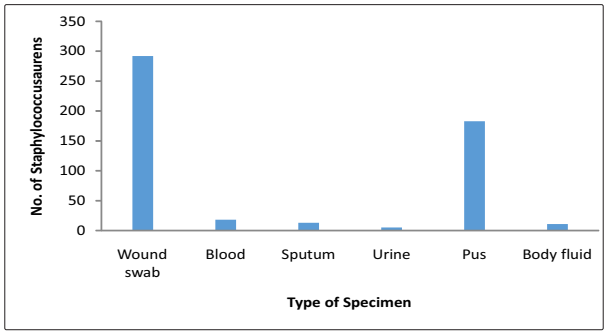
Table 3: Antibiotics resistance pattern of the MRSA isolates

NO. OF ISOLATES	PERCENTAGE (%) RESISTANT TO:							
	CF	TET	AMP	GN	ERY	OFL	CIP	VAN
223	**100**	**100**	**100**	**100**	**100**	**99**	**100**	**00**

Table 4: Minimum inhibitory concentration of vancomycin to MRSA isolates from clinical specimens

MRSA isolates from clinical specimens (N=223)	Minimum inhibitory concentration of vancomycin to MRSA isolates(µg/ml)
-	0.06
-	0.13
6	0.25
39	0.50
71	1.00
107	2.00
-	4.00
-	8.00
-	16.0

Fig 1: Distribution of *S. aureus* isolate from different specimen collected



DISCUSSION

Methicillin resistant *Staphylococcus aureus* (MRSA) is a major nosocomial isolate in health care facilities which is responsible for high morbidity and mortality rate. Sources of MRSA include infected patients, asymptomatic colonized hospital staff and hands of health care workers attending to MRSA positive patients in intensive care units.[20] Bowers et al.,[21]reported MRSA latex agglutination test as a reliable and rapid detection test from both broth pure culture and selective media as well as being a reliable alternative to *mec*A PCR for the definitive diagnosis of MRSA.[22] Supporting this view is the report of Atoum et al.,[22]who found *mec*A negative strains being methicillin resistant and *mec*A positive

strains being methicillin sensitive.[20]The result of this study shows that our MRSA isolates exhibits 100% multidrug resistance not only to the beta-lactam antibiotics but also to the macrolides and 5-fluoroquinolones. MRSA is known to possess several drug resistant genes in a single plasmid, each with its own resistance markers. A bacterial cell may carry more than one plasmid with resistance and resistance development in *Staphylococcus aureus* dates back to 1940s.[23]Our result also revealed excellent performance by vancomycin as all the isolated MRSA were sensitive to it; which suggestthat none of the isolated MRSA possesses van A gene that codes for vancomycin resistance. This finding is similar to that of Jan et al[24] who isolated strains of MRSA with reduced susceptibility to vancomycin in Japan in 1997 and similar trend have since been described in the United States, France, Hongkong, China and South Korea. In a similar study, out of a total of 52 multidrug resistant isolates by Yah et al.[7]in a tertiary hospital in Benin City, Nigeria in 2003, none is resistant to vancomycin and methicillin.[7]In this study the prevalence rate of MRSA is 42.7%.

CONCLUSION

The outcome of this study showed that MRSA has a prevalence rate of 42.7% in the University of Benin Teaching Hospital, Benin City- Nigeria; while all our MRSA have susceptibility of 100% to vancomycin. We advocate for regular surveillance of hospital infections and monitoring of antibiotic susceptibility pattern of MRSA and other pathogens periodically as this is essential for the formulation of antibiotic usage policies which in turn will have animpact on the control of infections especially hospital associated infections. We will also advise that clinicians consider obtaining cultures and antibiotic susceptibility reports to enable them modify empirical therapies to provide coverage for MRSA.

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REFERENCES

1. Piddock LJV. Multidrug resistance efflux pumps- not just for resistance. *Rev. Microbiol*2006;4:629-636.
2. Dominique S.Blanc. Epidemiology of methicillin resistance: results of a nation-wide survey in Switzerland.*Swiss Med Wkly* 2002;132:223-229.
3. Tiwari HKM, Das AK, Sapkota D, Sivarajan K, Pahwa VK. Methicillin Resistant *Staphylococcus aureus*: Prevalence and antibiogram in a tertiary hospital in western Nepal. *J. Infect DevCtries* 2009;3:681-684.
4. Udaya SC, Harish BN, Umesh KPM, et al. Prevalence of methicillin resistant *Staphylococcus aureus* in JIPMER hospital. *Indian J. Med Microbiol* 1997;15:137-138.
5. Muralidharan S. Special article on methicillin resistant *Staphylococcus aureus*. *J AcadClinMicrobiol* 2009;11:15-16.
6. Rajaduraipandi K, Mani KR, Panneerselvam K, Mani M, Bhaskar M, Manikandan M. Prevalence and antimicrobial susceptibility pattern of methicillin resistant *Staphylococcus aureus*: A multicenter study. *Indian J Med Microbiol*.2006;24:34-38.
7. Yah SC, Enabulele IO, Eghafona NO. The screening of multidrug resistance (MDR) susceptibilities of *Staphylococcus aureus* and *Staphylococcus epidermidis* to methicillin and vancomycin in teaching hospitals in Nigeria.*Sudan JMS*2007;2(4):257-262.
8. Kesah C, Redjeb SB, Odugbemi TO et al. Prevalence of methicillin resistant *Staphylococcus aureus* in eight African countries and Malta. *ClinMicrobiol Infect* 2003 Feb;9(2):153-156.
9. Obaseki-Ebor EE, Akerele JO and Ebea PO. A survey of antibiotic outpatient prescribing and antibiotic self-medication.*J AntimicrobChemother* 1987;20:759-763.
10. Okeke I, Lamikanra A. Quality and bioavailability of tetracycline capsules in a Nigerian semi-urban community. *Intern J Antimicrobial Agents* 1995;5:245-250.
11. Ronald NJ, Helio SS, Thomas RF et al. Selection of a surrogate agent (vancomycin and Teicoplanin) for initial susceptibility testing of Dalbavancin: results from international antimicrobial surveillance program. *Journal of Clinical Microbiology*2006;44:2622-2625.

12. Tenover FC, Lancaster MV, Hill BC et al. Characterization Staphylococci with reduced susceptibilities to vancomycin and other glycopeptides. *ClinMicrobiol* 1998;36:1020-1027.
13. Ahonkhai I and Monye AC. In-vitro effect of ciprofloxacin on methicillin resistant isolates of *Staphylococcus aureus*. *Journal of Med Laboratory Sciences* 1996;5:68-72.
14. Moran GJ, Krishnadasan A, Gorwitz RG, et al. Methicillin-resistant S. aureus infections among patients in the emergency department. *New Engl J Med* 2006; 355: 666-74.
15. Kloos WE, Schleifer KH. Simplified scheme for routine identification of human*Staphylococcus* species.*Journal ClinMicrobiol* 1975;1(1):82-84.
16. Harbarth CJ, Chambers HF. Methicillin – resistant Staphylococci: detection methods and treatment of infections. *Antimicrobiol Agents and Chemother* 1989;33:995-999.
17. Cavassini M, Wenger A, Jaton K, Blanc DC, and Bille J. Evaluation of MRSA-Screen, a simple anti-PBP 2a slide latex agglutination kit, for rapid detection of methicillin resistance in *Staphylococcus aureus*. J ClinMicrobiol 1999 May; 37(5): 1591-4.
18. Bauer AW, Kirby WM, Sherris JC. Antibiotics susceptibility testing by a standard single disk method.*Am J ClinPatho 1997*;45:493-496.
19. Performance standards for antimicrobial susceptibility testing, wayne, PA. 17TH informational supplement. 2007. *Clinical and Laboratory Standards Institute* (CLSI); pp. M100-S17.
20. Costantini M, Donisi PM, Turrin MG, Diana L. Hospital acquired infection surveillance and control in intensive care services: Result of an incidence study. *Eur J Epidemiol* 1987;3:347-355.
21. Bowers KM, Wren MWD, Shetty NP. Screening for methicillin resistance in Staphylococcus aureus and coagulase negative Staphylococci: an evaluation of three selective media and Mastlex-MRSA latex agglutination.*Br J Biomed Sci* 2003;60:71-74.
22. Atoum MF, Akel H, Battiki MN. Comparison of PCR and disc diffusion method in detecting methicillin resistance among staphylococcus species from nosocomial infections.*Saudi Med J* 2003;24:1410-2.
23. Mehta AP, Rodrigue C, Seth K, et al. Control of methicillin Staphylococcus aureus in a tertiary healthcare centre: A five year study. Indian J Med Microbiol 19998; 16: 31-4.
24. Jan MB, John DT, and SENTRY APAC participants. High prevalence of oxacillin-resistant Staphylococcus aureus isolates from hospitalized patients in Asia-Pacific and South Africa: Results from antimicrobial surveillance program, 1999. *Antimicrob Agent Chemother* 2002; 46(3): 879-881.