The screening of multi-drug resistance (MDR) susceptibilities of *Staphylococcus aureus* and *Staphylococcus epidermidis* to methicillin and vancomycin in teaching hospitals in Nigeria

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Abstract

Background: In Nigeria, the widespread use of antibiotics had led to high levels of resistance among bacterial isolates from patients with nosocomial infections. This had led to prolonged hospital stay and antibiotic therapy, especially β-lactam antibiotics that predispose patients to acquisition of methicillin -resistant *Staph. aureus* (MRSA) and coagulase negative resistant staphylococci.

Objective: to evaluate the resistant pattern of multi-drug resistant strains of 80 clinical *Staph. aureus,* 22 environmental *Staph. aureus*, 30 clinical *Staph. epidermidis* and 12 environmental *Staph. epidermidis* to methicillin and vancomycin from teaching hospitals in Nigeria.

Material and Methods: The *Staphylococcus* species were identified and confirmed by gram-positive positive reaction, tested for mannitol salt fermentation and DNase production. The organisms were confirmed to be *Staph. aureus* and *Staph. epidermidis* by the tube coagulase test. The antibiotics susceptibility patterns were determined both by overnight broth-micro-dilution and agar disk diffusion methods.

Results: The isolates were resistant to ampicillin, followed by penicillin, tetracycline, erythromycin and gentamicin but to a lesser extent were sensitive to ciprofloxacin. All the multi-drug resistant (MDR) *Staphylococcus* species were 100% sensitive to vancomycin and methicillin with a minimum inhibition concentration (MIC) breakpoint $< 4\mu g/ml$ to vancomycin and MIC $< 5\mu g/ml$ to methicillin on Mueller Hinton agar supplemented with 2%NaCl.

Conclusion: The results indicated that methicillin and vancomycin are still very potent antibiotics against staphylococcal infections in Nigeria.

Key Words: MDR Staphylococcus, methicillin and vancomycin.

Introduction

In Nigeria, the widespread use of antibiotics had led to high levels of resistance among bacterial isolates from patients with nosocomial infections [19-20,15]. This had led to prolonged hospital stay and antibiotic therapy, especially β-lactam antibiotics that predispose patients to acquisition of methicillin -resistant *Staph. aureus* (MRSA) and coagulase negative resistant staphylococci. Methicillin resistant strains that emerged by late 1980s have become increasingly present as nosocomial pathogens. The medical community was again relieved when vancomycin a glycoprotein was discovered that added effective therapy to all strains of methicillin resistant *Staph. aureus*. Nevertheless vancomycin resistant strains of coagulase–negative staphylococci were also a cause of concern[16,7,14,18]. Added to these concerns were observations that vancomycin resistant enterococci isolates or epidemics in some U.S. hospitals were becoming increasingly prevalent in critical care units[5,4] and high level vancomycin resistance were experimentally transferred from Enterococcus faecalis to *Staph. aureus* in both in- vitro and in vivo- models[4,7]. Strains of *Staph. aureus* and gram negative organisms resistant to vancomycin and other antimicrobial agents including quinolones are endemic already in numerous hospitals and health care institutions leaving only a few effective and costly antimicrobials for the treatment of patients infected with these pathogens[8]. In Nigeria, there has been a recent increase in resistant to gentamicin and variable susceptibility to other non-ß-lactam antibiotics, namely tetracycline, trimethoprim, erythromycin and ciprofloxacin [1,22,24]. In this study we investigated both the broth-micro-dilution and agar disk diffusion methods on multi-drug resistant on both hospital environment and long term clinical isolates of *Staph. aureus* and *Staph. epidermidis* from some selected teaching hospitals in Nigeria to ascertain their level of resistance to methicillin and vancomycin.

Methods

Bacterial strains and selection of isolates for analysis: One hundred and forty four multi-drug resistant *Staph. aureus* and *Staph. epidermidis* from some selected teaching hospitals in Nigeria were obtained and examined for their antibiotics susceptibility profiles to methicillin and vancomycin. These isolates include 80 multi-drug resistant clinical *Staph. aureus* strains, 22 multi-drug resistant environmental *Staph. aureus* strains and 30 multi-drug resistant clinical *Staph. epidermidis* strains and 12 multi-drug resistant environmental *Staph. epidermidis* strains. The clinical isolates were obtained randomly from routine specimens from different infected sites

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(urine, wounds, and diarrheal stool) of prolonged hospitalized patients between May 2003 and October 2004. The environmental isolates were obtained from the teaching hospitals wards (air) by the suck-let sampler method. The teaching hospitals were; University of Benin Teaching Hospital (UBTH), Benin City, Edo State- Southern Region of Nigeria; Nnamdi Azikiwe Teaching Hospital (NAUTH), Nnewi, Anambra State – Eastern Region of Nigeria; Ahmadu Bello University Teaching Hospital (ABUTH), Zaria – Kaduna State – Northern Region of Nigeria and University College Hospital (UCH), Ibadan, Oyo State- Western Region of Nigeria.

Bacterial identification: All *Staph. aureus* and *Staph. epidermidis* strains were identified primarily by routine laboratory procedures [9] and confirmed to be *Staph. aureus* and *Staph. epidermidis* by gram-positive cocci morphology, catalase-positive reaction were tested for mannitol salt fermentation (Oxoid, Melbourne, Australia) and DNase production on agar plates (Oxoid CM321). Clumping factor was detected by using rabbit plasma. Organisms were confirmed to be *Staph. aureus* and *Staph. epidermidis* by the tube coagulase test

Antibiotic Sensitivity Testing: The antibiotics susceptibility patterns were determined both by overnight broth-micro-dilution and agar disk diffusion methods as recommended by Bauer et a[12] and National Committee for Clinical Laboratory Standard [17] using Oxoid- Mueller Hinton agar (Difco Laboratories, Detroit, Mich). The following antibiotics were used to screen for the resistance of the isolates; ampicillin (AM) 30µg, gentamicin (GN) 10µg, tetracycline (TE) 30µg, ciprofloxacin (CIP) 5µg, erythromycin (E) 10µg, Penicillin (PN) 30µg (Optun Laboratories Nig Ltd., Nigeria), methicillin 5µg (Bristol Meyers Squib) and vancomycin (VAN) 5µg (Mayne Pharma Warwickshire -UK). The inocula were prepared directly from an over night agar plate. Investigation of bactericidal activity were done by measuring the zone of inhibition with standard measuring procedures according to NCCLS,[17] after incubation at 30 - 350C for 24 hours. *Staphylococcus* strains that showed resistance to three or more classes of antibiotics were titled as 10 multi drug resistant (MDR), and were further preserved for other analyses. The fully sensitive strains of the organisms were discarded.

Agar Dilution tests of methicillin and vancomycin: The minimum inhibition concentration (MIC) of methicillin (MET) 500mg (Bristol Meyers Squib Hampshire- England) and vancomycin (VAN) 500mg (Mayne Pharma Warwickshire -UK) was determined by agar dilution method, according to the guidelines of NCCLS. [17] Colonies of each strain from an over night growth were transferred to sterile saline. The suspension were adjusted to a 0.5 McFarland standard, diluted 1:10, and inoculated on Mueller Hinton agar (Difco Laboratories, Detroit, Mich) plates supplemented with 2% NaCl wt/vol. The plates were incubated at 30 - 350C for 24 hours.

Results

The comparative antibiotic susceptibility profiles of the 80 multi-drug resistant clinical *Staph. aureus*, 22 multi-drug resistant environmental *Staphylococcus aureus* and 30 multi-drug resistant clinical *Staph. epidermidis* and 12 multi-drug resistant environmental *Staph. epidermidis* were shown in Tables 3a to 3d. All the MDR isolates were sensitive to methicillin and vancomycin, but were resistant to ampicillin, followed by penicillin, tetracycline, erythromycin, gentamicin and ciprofloxacin. The results showed that both isolates were highly resistant to ampicillin and penicillin from all the teaching hospitals with resistant ranged of 40% to 71%. The isolates also had a resistant range of 00% to 17% to ciprofloxacin, although environmental *Staph. epidermidis* were 00% resistant to ciprofloxacin. Only *Staph. epidermidis* from UBTH as shown in Table 1 had a resistance of 17% to ciprofloxacin. The resistance pattern varied among the gentamicin, erythromycin and tetracycline as shown in Tables 3a to 3b. The minimum inhibitory concentration (MIC) range was 0.5µg/ml to 5µg/ml with a MIC break point of < 4µg/ml for both isolates as shown in Tables 3c and 3d. All the 144 MDR *Staph. aureus* and *Staph. epidermidis* were considered to be susceptible to methicillin and vancomycin according to published NCCLS guidelines. None of the isolates had a MIC > 5µg/ml

Discussion

The 144 MDR isolates in this report were described as sensitive isolates to methicillin and vancomycin. This contradicts other reports from Nigeria and some other African countries.[1, 15]. Since all the strains were sensitive to methicillin and vancomycin, the study therefore suggested that none of the strains contained vanA or mecA genes respectively. The rate of resistance continues to reduce from the earlier reports [15], the

Table3a: Percentage (%) Occurrence of multi drug Resistant *Staphylococcus* and coagulase negative *Staphylococcus* from some selected Teaching Hospitals in Nigeria to methicillin and vancomycin from clinical sources.

Region/Isolates	Percentage resistant							
	AM	PN	TE	E	CIP	GN	MET	VAN
Southern region								
S. aureus (N=28)	57%	50%	32%	21%	07%	14%	00%	00%
S. epidermidis (N=12)	50%	67%	42%	25%	17%	17%	00%	00%
Eastern Region								
S. aureus (N=20)	40%	60%	35%	30%	10%	20%	00%	00%
S. epidermidis (N=6)	67%	50%	33%	17%	00%	17%	00%	00%
NORTHERN REGION								
S .aureus (N=14)	64%	50%	43%	21%	14%	21%	00%	00%
S. epidermidis (N=7)	71%	57%	43%	15%	00%	00%	00%	00%
WESTERN REGION								
S. aureus (N=18)	67%	39%	67%	39%	17%	17%	00%	00%
S. epidermidis (N=5)	50%	67%	33%	16%	00%	17%	00%	00%

KEY: UBTH= University of Benin teaching Hospital (Southern Region), NAUTH = Nnamdi Azikiwe UniversityTeaching Hospital (Eastern Region), ABUTH= Ahmadu Bello University Teaching Hospital (Northern Region) and UCH= University Collge Hospital (Western Region).

Table3b: Percentage (%) Occurrence of multi drug Resistant *Staphylococcus* and coagulase negative *Staphylococcus* from some selected Teaching Hospitals in Nigeria to methicillin and vancomycin from environment

Isolates	Percentage Resistant							
	AM	PN	TE	E	CIP	GN	MET	VAN
S. aureus (N=22)	68%	81%	55%	23%	09%	18%	00%	00%
S. epidermidis (N=12)	42%	58%	75%	33%	00%	17%	00%	00%

Table 3c: Minimum Inhibitory Concentration (MIC) of *Staphylococcus aureus* and *Staphylococcus epidermidis* to methicillin from some selected teaching hospitals in Nigeria

Source/Isolates		Strains for which methicillin MIC (µg/ml) was tested											
		0.5µg	1µg	2µg	3µg	4µg	5µg	6µg	8µg	10µg	12µg	14µg	
Clinical/*S. aureus*	(N=80)	1	7	55	16	2	-	-	-	-	-	-	-
Environment/*S. aureus*	(N=22)	4	12	3	3	-	-	-	-	-	-	-	-
Clinical/*S. epidermidis*	(N=30)		2	9	12	6	1	-	-	-	-	-	-
Environment/*S. epidermidis*	(N=12)	1	2	6	1	1	1	-	-	-	-	-	-

Table 3d: Minimum Inhibitory Concentration (MIC) of *Staphylococcus aureus* and *Staphylococcus epidermidis* to Vancomycin from some selected teaching hospitals in Nigeria

Source/Isolates		Strains for which methicillin MIC (µg/ml) was tested									
		0.5µg	1µg	2µg	3µg	4µg	5µg	6µg	8µg	10µg	12µg
Clinical/ *S. aureus*	(N=80)		2	10	17	34	17	-	-	-	-	-
Environment/*S. aureus*	(N=22)		-	3	1	15	3	-	-	-	-	-
Clinical/*S. epidermidis*	(N=30)		1	2	5	19	3	-	-	-	-	-
Environment/ *S. epidermidis*	(N=12)	-	1	4	6	1	-	-	-	-	-	

isolates according to the present study were all sensitive to both methicillin and vancomycin. The current studies also found that methicillin, oxacillin and vancomycin were not at all among the commonly prescribed antibiotics in teaching hospital in Nigeria[25]. However, cloxacillin with similar mode of action was very rare in circulation as compared to commonly prescribed ampicillin, penicillin, aminoglycosides and quinolones[23].

Despite the fact that a MIC = 4μg/mL was defined as susceptible by NCCLS standards[17], it is still considered to be at the borderline of resistance. S. aureus strains that are methicillin or oxacillin resistant and have a MIC of vancomycin ≥4 μg/mL should be suspected for decreased susceptibility to vancomycin and should be considered for additional testing strategies because of the possible sub-population heterogeneity of S. aureus isolates with these MIC results [6,22]. Our results indicate that methicillin and vancomycin are still very potent antibiotics against *Staph. aureus* and *Staph. epidermidis* infections. According to Jan et al[10], strains of MRSA with reduced susceptibility to vancomycin were isolated in Japan in 1997 and have since been described in the United States, France, Hong Kong, China, and Korea. Their findings as well had no strains of vancomycin intermediate *Staph. aureus* despite having three sites in Japan, consistent with the suggestion that these strains are still relatively rare. The isolation of these strains in an area of high endemicity indicates the need for continuous surveillance of antibiotic resistance of *Staphylococcus* species and the rationalization of antibiotic in clinical set up.

Conclusion

The results indicated that methicillin and vancomycin are still very potent antibiotics against *Staph. aureus* and *Staph. epidermidis* infections. Therefore the cry of methicillin-resistant ***Staph. aureus*** (MRSA) that was first identified in the United Kingdom in 1961 11 and since then assumed increasing importance internationally as a cause of both nosocomial and community-acquired infections should not be the case in Nigeria for now.

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