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OCCURRENCE OF METHICILLIN AND VANCOMYCIN RESISTANT *STAPHYLOCOCCUS AUREUS* IN UNIVERSITY OF ABUJA TEACHING HOSPITAL, ABUJA, NIGERIA.

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ABSTRACT

The susceptibility of clinical isolates of *Staphylococcus aureus* from a hospital to seven antibiotics; namely ofloxacin, vancomycin, oxacillin, erythromycin ampicillin and gentamicin was examined. The isolates were recovered from wound, skin, urine, blood, vaginal, cerebrospinal fluid and ear infections. After confirmation as *S. aureus* through gram stain and biochemical tests, the antibiogram of each isolate was determined using the disk diffusion method. A total of 214 *S. aureus* isolates were examined of which 28 (13.1%) were resistant to methicillin. Of these 25% were sensitive to ofloxacin, 85.7% to vancomycin, 10.7% to erythromycin 0% to ampicillin and gentamicin. Four (14.3%) of the Methicillin resistant isolates were also resistant to vancomycin and all other antibiotics. There was a significant difference in the sensitivity pattern between inpatient isolates and outpatient isolates in this study (p <0.05). This study established the presence of methicillin resistant *Staphylococcus aureus* (MRSA) as well as VRSA in this locality and hence the need to implement measures that will limit the dissemination of these strains in the hospital and the community.

INTRODUCTION

Staphylococcus aureus is a gram-positive pathogen that causes a wide range of infections including life-threatening ones. *S. aureus* causes bloodstream infections, skin and soft tissue infections as well as pneumonia [1]. Rates of *S. aureus* infection have increased during the past 2 decades [2]. Bacteremia due to *S. aureus* has been reported to be associated with very high mortality rates [3]. Methicillin-resistant *Staphylococcus aureus* (MRSA) has occurred in many countries since its discovery in 1961 [4].
The emergence of antibiotic-resistant strains, particularly MRSA is recognized as very serious health problem because of difficulties in combating these strains [5].

Infections with antibiotic-resistant organisms generally result in higher morbidity and mortality rates than are similar infections with antibiotic-susceptible strains [6].
In recent years, clinicians have been concerned by the increased frequency of MRSA infections [7]. This resurging MRSA problem seems to be based on the lack of potent therapeutic agents having an unequivocal cell-killing effect, and thus capable of eliminating MRSA from the patient's body [8]. MRSA strains are often resistant to multiple antibiotics and pose serious challenges in both hospitals and the community [9]. MRSA infections are associated with significant increases in mortality, longer hospital stays and higher inpatient costs compared to patients with methicillin susceptible *S. aureus* [10,11]. Initially MRSA was considered a nosocomial problem, however community-associated methicillin-resistant *S. aureus* (CMRSA) has become the most frequent cause of skin and soft tissue infections [12].

Since the emergence of MRSA, vancomycin has been the most reliable therapeutic agent against infections caused by methicillin-resistant *Staphylococcus aureus* (MRSA). However, in 1997, the first clinical isolate of *Staphylococcus aureus* with reduced susceptibility to vancomycin was reported, [13] and by 2001 Vancomycin resistant *S. aureus* (VRSA) strains had been isolated in USA, France, Korea, South Africa, and Brazil [8]. A certain group of *S aureus*, designated hetero-VRSA, frequently generate VRSA upon exposure to vancomycin, and are associated with infections that are potentially refractory to vancomycin therapy [8].
The present study sought to determine the prevalence of MRSA and VRSA and hence the extent of the problem in clinical isolates of *S. aureus* since there is a paucity of published data in this locality.

MATERIALS AND METHODS

S. aureus isolates were obtained from various clinical samples at the Microbiology Laboratory University of Abuja Teaching Hospital and National Hospital from April 2010 to June 2011. For each specimen of a patient only one positive isolate was included in the study. Specimens were categorized as inpatients or outpatient using the definition of nosocomial infection as a localized or systemic condition that results from adverse reaction to the presence of an infectious agent not present or incubating at the time of admission to the hospital according to the Centers for Disease Control and Prevention [14]. *S. aureus* strains were identified by colony morphology, pigmentation and growth on Mannitol salt agar (Merk), Gram stain, catalase activity, and slide coagulase tests. Using a 0.5 McFarland-equivalent suspension of organisms inoculated on a Mueller-Hinton agar plate (Oxoid, Basingstoke,). Isolates were tested using antibiotic discs obtained from Oxoid, Basingstoke. The isolates were tested for methicillin resistance using oxacillin discs (1 µg) and for vancomycin resistance using vancomycin discs (5 µg) as recommended by Andrews [15]. Susceptibility of the isolates to other antibiotics namely; gentamicin (10 µg), Ofloxacin (5 µg), erythromycin (5 µg),

ampicillin (25 µg) was also determined as recommended by Andrews [15].

Statistical Analysis

Chi-square (χ^2) test was performed using The Primer of Biostatistics soft ware (McGraw-Hill version 4.0). It was used to analyze data from resistance of inpatient and outpatient MRSA and MSSA strains to antibiotics to determine if there were significant differences. *P* values < 0.05 were considered significant.

RESULTS

The total number of *S. aureus* strains examined was 214. Of these 33(15.4%) originated from the wound, 30(14%) from urine, 85(39.7%) from the blood, 10(4.7%) from the vagina, 6(2.8%) from hair scalp, 6(2.8%) from ear infections, 42(19.6%) from the skin and 2(0.9%) from cerebrospinal fluid. The total number of isolates from inpatients was 114 while 100 were from outpatients.

Out of the total number of 214 isolates tested, 28 were MRSA and of these 4 were also VRSA. 20 of the MRSA were from inpatients and 8 MRSA were from outpatients. Vancomycin resistance was not detected in any of the outpatient isolates. The highest number of MRSA was detected in blood isolates (11) followed by wound (7), 4 were detected in urine and skin isolates whilst 2 were detected in cerebrospinal fluid. Generally the number of MRSA detected in inpatients was higher than in outpatients. In terms of proportion of number of MRSA detected to the total number of isolates examined for each type of specimen, cerebrospinal fluid specimens had the highest value with all two isolates being MRSA i.e. 100%. This was followed by wound swabs in which 7 out of 33 (21%) were MRSA, urine samples with 4 out 30 (13.3%), blood with 11 out of 85 (12.9%) and skin swabs 4 out 42(9.5%0) respectively. MRSA was not detected from vaginal swab, hair scalp and ear infections samples. As earlier stated out of the 28 MRSA isolates, 4 were also VRSA. These originated from cerebrospinal fluid, wound and blood samples. One out of the 4 MRSA isolated from wound was also a VRSA, 1 out of 2 from cerebrospinal fluid was VRSA while 2 out of 10 of blood were MRSA.

Apart from oxacillin and vancomycin other antibiotics tested against the isolates were erythromycin, gentamicin, ampicillin and ofloxacin.

TABLE 1: DISTRIBUTION OF MRSA, MSSA AND VRSA STRAINS ACCORDING TO THEIR ORIGIN.

Specimen	MRSA (VRSA)		MSSA(VRSA)		Total, n (%)
	inpatient	outpatient	Inpatient	outpatient	
Wound	4(1)	3(0)	14(0)	12(0)	33(15.4)
Urine	2(0)	2(0)	16(0)	10(0)	30(14)
blood	10(2)	1(0)	50(0)	24(0)	85(39.7)
Vaginal	0(0)	0(0)	6(0)	4(0)	10(4.7)
hair scalp	0(0)	0(0)	0(0)	6(0)	6(2.8)
ear infections	0(0)	0(0)	2(0)	4(0)	6(2.8)
Skin	2(0)	2(0)	6(0)	32(0)	42(19.6)
Cerebrospinal fluid	2(1)	0(0)	0(0)	0(0)	2(0.9)

Values in brackets indicate the number of VRSA in a particular group out of the total.

TABLE 2 ANTIBIOTIC RESISTANCE PATTERNS IN MRSA AND MSSA ISOLATES TO SELECTED ANTIBIOTICS

ANTIBIOTIC	MRSA					MSSA				
	Inpatient Isolates		Outpatient isolates		Total resistance, n (%)	Inpatient isolates		Outpatient Isolates		Total resistance, n (%)
	Sensitive	Resistant	Sensitive	Resistant		Sensitive	Resistant	Sensitive	Resistant	
Erythromycin	2	18	1	7	25(89.3)	60	34	79	13	47(25.3)
Ofloxacin	4	16	3	5	21(75.0)	79	15	71	21	36(19.4)
Vancomycin	16	4	8	0	4(14.3)	94	0	92	0	0(0)
Ampicillin	0	20	0	8	28 (100)	0	94	2	90	184(98.9)
Gentamicin	0	20	0	8	28 (100)	23	71	30	40	111(59.6)

Approximately fourteen percent of MRSA isolates were also resistant to vancomycin, 75% to ofloxacin, 89.3% to erythromycin and 100% to ampicillin and gentamicin respectively. Among the MSSA none (0%) was resistant to vancomycin, 19.4% was resistant to ofloxacin, 25.3% to erythromycin, 59.6% to gentamicin and 98.9% to ampicillin respectively. The difference in resistance of inpatient and outpatient MRSA strains to other antibiotics was significantly different (p <0.05). Similarly, the difference in resistance of inpatient and outpatient MSSA strains to other antibiotics was also significantly different (p <0.05).
In all, three of VRSA were not susceptible to any of the other antibiotics used in the study while one was susceptible to ofloxacin.

DISCUSSION

MRSA has caused problems in most hospitals worldwide and increasing numbers have been reported in a number of countries. There have been significant increases in methicillin resistance in clinical strains of *S. aureus* isolates between 1999 and 2002 in European countries, particularly Belgium, Germany, Ireland, the Netherlands and the United Kingdom [16]. MRSA prevalence varied widely, from less than 1% in northern Europe to greater than 40% in Southern and Western Europe [16].

In this study 13.1% of *S. aureus* isolates were resistant to methicillin. This figure is less than 20.6% and 47.8% reported from Southwestern Nigeria [17, 18] and 69% reported in Zaria northern Nigeria [19]. The low rate of MRSA in this study may be due to low level of abuse of antibiotics in this locality by both health practitioners and in the community since emergence of resistant strains has been largely due to antibiotic abuse. Moreover MSSA strains were largely susceptible to other antibiotics and none was resistant to vancomycin.

Most of the MRSA isolates were also resistant to other antibiotics. The presence of *mec* A gene complex which specifies the production of an abnormal penicillin binding protein PBP2a that has a decreased affinity for binding β-lactam antibiotics results in resistance to methicillin and also to all β-lactams including penicillins and cephalosporins also contains insertion sites for plasmids and transposons that facilitate acquisition of resistance to other antibiotics. Thus, cross-resistance to non-β-lactam antibiotics such as erythromycin, clindamycin, gentamicin, co-trimoxazole and ciprofloxacin is common [7].

MRSA was higher in inpatients expectedly because of established MRSA risk factors, such as recent hospitalization, surgery, residence in a long-term care facility, receipt of dialysis, or presence of invasive medical devices [20]. Detection of MRSA from outpatients warrants more investigation because community associated MRSA (CA-MRSA) has emerged in the community with clinical, epidemiologic, and bacteriologic characteristics distinct from healthcare-associated MRSA [21,22,23]. CA-MRSA isolates also tend to be resistant to fewer antimicrobial classes, possess different toxin genes, and carry a different type of the gene complex known as staphylococcal cassette chromosome *mec* (SCC*mec*), which contains the *mec* A methicillin-resistance gene [24, 25]. The resistance of outpatient isolates to different antibiotics appeared to be less than those from inpatients but this remains to be confirmed by larger studies because of the limited data from the present study. The higher susceptibility pattern of MSSA to antibiotics seems to confirm the tendency of MRSA to acquire resistance genes to other antibiotics.

Vancomycin resistance was observed in 4 isolates and only one of which was susceptible to any other antibiotics. The reported prevalence rate of VRSA in southern parts of Nigeria range from 0% to 6% among clinical isolates in agreement with the present study [17,18]. A prevalence rate 57.7% has also been reported in Zaria northern Nigeria [26] and in another study in non clinical isolates a prevalence rate 89% was reported [19]. The difference in rates of vancomycin resistance is probably reflects differences in levels of over prescription and abuse in different parts of the country This study as well as others might however have underestimated vancomycin resistance because of the inability of routine antimicrobial susceptibility test methods to detect heterogeneous vancomycin-intermediate *Staphylococcus aureus* (hVISA) and vancomycin-intermediate *Staphylococcus aureus* VISA detected in different continents [27-30]. Vancomycin resistant strains are a source of concern because until recently, vancomycin was the only uniformly effective treatment for staphylococcal infections particularly MRSA. Resistance to vancomycin severely limits treatment options as seen in this study.

Although the incidence of MRSA and VRSA observed in this study has not reached epidemic proportions, it is still very important to put in measures to contain their spread and continue surveillance both in healthcare facilities and in the community. The spread of these pathogens in the community can result in skin infections and, less commonly, invasive infections among otherwise healthy adults and children in the community.

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