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

Akanbi 1, B. O. & Mbe 1, J. U.

1Department of Biological Sciences, PMB 117, University of Abuja.

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 gentamycin. 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 outpatients 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 (Merck), 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 (1 µg), and vancomycin (10 µg) was determined using vancomycin discs (5 µg) as recommended by Andrews [15].
ampicillin (25 µg) was also determined as recommended by Andrews [15].

Statistical Analysis
Chi-square ($\chi^2$) test was performed using The Primer of Biostatistics software (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.

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

<table>
<thead>
<tr>
<th>Specimen</th>
<th>MRSA (VRSA)</th>
<th>MSSA(VRSA)</th>
<th>Total, n (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>inpatient</td>
<td>outpatient</td>
<td>Inpatient</td>
</tr>
<tr>
<td>Wound</td>
<td>4(1)</td>
<td>3(0)</td>
<td>14(0)</td>
</tr>
<tr>
<td>Urine</td>
<td>2(0)</td>
<td>2(0)</td>
<td>16(0)</td>
</tr>
<tr>
<td>blood</td>
<td>10(2)</td>
<td>1(0)</td>
<td>50(0)</td>
</tr>
<tr>
<td>Vaginal</td>
<td>0(0)</td>
<td>0(0)</td>
<td>6(0)</td>
</tr>
<tr>
<td>hair scalp</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>ear infections</td>
<td>0(0)</td>
<td>0(0)</td>
<td>2(0)</td>
</tr>
<tr>
<td>Skin</td>
<td>2(0)</td>
<td>2(0)</td>
<td>6(0)</td>
</tr>
<tr>
<td>Cerebrospinal fluid</td>
<td>2(1)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
</tbody>
</table>

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

<table>
<thead>
<tr>
<th>ANTIBIOTIC</th>
<th>MRSA</th>
<th>MSSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Inpatient isolates</td>
<td>Outpatient isolates</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>2</td>
<td>18</td>
</tr>
<tr>
<td>Ofloxacin</td>
<td>4</td>
<td>16</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>16</td>
<td>4</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>0</td>
<td>20</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0</td>
<td>20</td>
</tr>
</tbody>
</table>
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 (SCCmec), 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-resistant-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.

**REFERENCES**


