An In Vitro Study on Methicillin and Other Antimicrobial Agents Against Staphylococcus Aureus, 1994–1996

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ABSTRACT

Staphylococcus aureus infections remain a threat to both immunocompetent and immunocompromised patients despite advances in antibacterial therapy. An in vitro antimicrobial susceptibility study was conducted at the Lagos University Teaching Hospital on clinical isolates of S. aureus over a period of three years, 1994 - 1996. Beta-Lactamase production was detected using a chromogenic cephalosporin method (Nitrocefin, Oxoid) and antibiotic susceptibility testing was performed by E-test (AB Biodisc, Solna, Sweden) including reference strains. A total of 152 strains of S. aureus were analysed.

Eighty-six percent of the 152 isolates produced Beta-Lactamase, 50.4% were high level penicillinase producers, while 47.2% produced low level penicillinase. Approximately 53% of the strains were sensitive to methicillin and 33% to Penicillin G. There was 100% susceptibility to vancomycin over the three year period. 96.6% to rifampicin, 92.2% to tetracycline, 89.6% to clindamycin, 71.8% to amoxicillin-clavulanic acid, 71.7% to erythromycin, 64% to cotrimoxazole, and below 50% in gentamicin and tetracycline.

The MIC90 values for vancomycin, clindamycin, rifampicin and tetracycline were all within the susceptible breakpoints, thus these drugs could be used empirically. For the other antibiotics tested such as erythromycin, tetracycline, augmentin, septrin and gentamicin, susceptibility cannot be assumed in vitro efficacy should validate their therapeutic option.

Key Words: Staphylococcus aureus, beta-lactamase, susceptibility patterns.

INTRODUCTION

Staphylococci are important pathogens in nosocomial and community-acquired infections. Those organisms affect both immunocompetent and Immunocompromised patients often resulting in high morbidity, mortality and complication rates. World-wide, S. aureus is increasingly implicated in endocarditis particularly in intravenous drug abusers, wound, skin and soft tissue infections, bacteremia, toxic shock syndrome, septic shock syndrome, pulmonary infections, osteomyelitis/septic arthritis and prosthetic joint infections (1,2).

Despite advances in antibacterial therapy, serious staphylococcal infections are still a significant clinical problem. The development of resistance, first to penicillin, then to methicillin and many other drugs, and most recently to quinolones has limited therapeutic choices available for staphylococcal infections (3). The objectives of this study were to determine the incidence of beta-lactamase production and in vitro activities of methicillin and many other antimicrobial agents against clinically significant isolates of S. aureus.

MATERIALS AND METHODS

Collection and Processing of Specimens

Routine clinical specimens made up of swabs, urine, sputa, pus, peritoneal fluid and blood were collected from patients at the Lagos University Teaching Hospital between 1994 and 1996. These samples were processed in the Bacteriology Research Laboratory of the Department of Medical Microbiology and Parasitology, College of Medicine, University of Lagos and Lagos University Teaching Hospital. All suspected staphylococcal isolates were identified by standard methods (4). Ninety-six isolates of S. aureus were obtained from sputum and pus, 47 from blood cultures, and 9 from urine samples. Thirty-nine, 70 and 35 strains were analysed in 1994, 1995 and 1996 in that order.

Beta-Lactamase Detection

Beta-Lactamase production was tested using a chromogenic cephalosporin method (Nitrocefin, Oxoid) according to the manufacturer's instructions. Phenotypes of resistance to Beta-lactamase were determined by Minimum Inhibitory Concentration (MIC) pattern analyses of Penicillin G and Oxacillin (5).

Antimicrobial Susceptibility Testing

The MICs of some antimicrobial agents against the clinical isolates were determined by E-test (AB Biodisc, Solna, Sweden). The E test methodology has been described (6). Mueller-Hinton agar was used for testing but was however supplemented with 4% NaCl when testing for methicillin resistance. Methicillin resistance was detected by oxacillin E-testing. Results were interpreted according to the MIC interpretive criteria of the National Committee for Clinical Laboratory Standard (7).

Reference Strains

Staphylococcus aureus ATCC 25923 and Escherichia coli ATCC 25922 were used.

RESULTS

The overall incidence of low and high level penicillinase producing strains of S. aureus was 98.2% during the study period. The percentage of low level penicillinase producers rose from 55.5% in 1994 to 61.6% in 1996 (47.3% for the three-year period). However, 50.4% of the 131 Beta-lactamase producers detected with high level penicillinase producers (Table 1).
Results of the antimicrobial susceptibility testing are shown in Table I. There was 100% susceptibility to vancomycin over the three year period, with an MIC90 value of 3 μg/ml. The isolates were also highly susceptible to rifampin (95.5%), fusidic acid (95.2%) and clindamycin (90.6%). The MICs at which 90% of the strains were inhibited by these drugs fell within the susceptible breakpoints. Although percentages of susceptibility were between 60 and 70% for colistimethate and amoxycillinclavulanate (augmentin), the MIC90 values were out of the susceptible breakpoints. For commonly used antibiotics like gentamicin and tetracycline, percentages of susceptibility were below 50, with an MIC90 range of 64 – 256 μg/ml.

The percentage of susceptibility to Penicillin G ranged from 5% in 1994 to 0% in 1996. Over the three years, 57% of S. aureus strains were sensitive to oxacillin (methicillin). It is noteworthy that 3(2.3%) of the 131 B-lactamase producing strains were sensitive to both penicillin and oxacillin (Salvia strains).

Table I
Betalaactamase activity and Phenotypes of Resistance in B-lactam antibiotics in Staphylococcus aureus isolates

<table>
<thead>
<tr>
<th>Year</th>
<th>Total No. tested</th>
<th>No. (%) Positive</th>
<th>No. (%) producing Low level Penicillinase</th>
<th>No. (%) producing High level Penicillinase</th>
<th>No. (%) of Salvage strains</th>
</tr>
</thead>
<tbody>
<tr>
<td>1994</td>
<td>30</td>
<td>3(29.5)</td>
<td>1(33.3)</td>
<td>1(33.3)</td>
<td>3(3.3)</td>
</tr>
<tr>
<td>1995</td>
<td>78</td>
<td>6(88.9)</td>
<td>0(0)</td>
<td>0(0)</td>
<td>0(0)</td>
</tr>
<tr>
<td>1996</td>
<td>35</td>
<td>3(85.7)</td>
<td>1(28.6)</td>
<td>1(28.6)</td>
<td>0(0)</td>
</tr>
<tr>
<td>1994-1996</td>
<td>152</td>
<td>13(86.2)</td>
<td>6(46.7)</td>
<td>6(46.7)</td>
<td>3(2.3)</td>
</tr>
</tbody>
</table>

Table II
Antimicrobial Susceptibility Profile of Staphylococcus aureus strains, 1994-1996.

<table>
<thead>
<tr>
<th></th>
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<th></th>
<th></th>
<th></th>
</tr>
</thead>
<tbody>
<tr>
<td>Oxacillin</td>
<td>43.2</td>
<td>50.0</td>
<td>61.8</td>
<td>51.7</td>
<td>0.016 – &gt; 32</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Penicillin G</td>
<td>5.1</td>
<td>5.8</td>
<td>0.0</td>
<td>3.3</td>
<td>0.023 – &gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Amoxicillin</td>
<td>71.0</td>
<td>72.3</td>
<td>61.8</td>
<td>71.8</td>
<td>0.064 – &gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Clavulanic acid</td>
<td>32.3</td>
<td>40.2</td>
<td>40.0</td>
<td>41.4</td>
<td>0.032 – &gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>23.1</td>
<td>30.0</td>
<td>NT</td>
<td>27.3</td>
<td>0.025 – &gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>84.1</td>
<td>89.2</td>
<td>82.9</td>
<td>87.7</td>
<td>0.023 – &gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>81.0</td>
<td>91.0</td>
<td>91.4</td>
<td>92.4</td>
<td>&lt; 0.016 – &gt; 48</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Chloramphenicol</td>
<td>88.7</td>
<td>98.7</td>
<td>97.1</td>
<td>98.7</td>
<td>&lt; 0.016 – &gt; 48</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Rifampicin</td>
<td>87.5</td>
<td>84.4</td>
<td>91.4</td>
<td>90.2</td>
<td>&lt; 0.016 – &gt; 256</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>100</td>
<td>0.016 – &gt; 32</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Trimethoprim</td>
<td>64.1</td>
<td>62.7</td>
<td>68.6</td>
<td>64.4</td>
<td>0.75 – &gt; 4</td>
<td>&gt; 256</td>
</tr>
<tr>
<td>Sulphamethoxazole</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
<td>NT</td>
</tr>
</tbody>
</table>

NT = Not tested
MIC90 = The Minimum concentration of antibiotic at which 90% of the bacterial strains were inhibited.

DISCUSSION
Staphylococcal infections are prevalent in various communities and healthcare institutions both in developed and developing countries(2). Staphylococcus is known to have a remarkable genetic versatility which allows for adaptation to the presence of antibiotics such that many strains can be multiresistant to several classes of drugs(3). The present study was designed to highlight the current antimicrobial susceptibility pattern of Staphylococcus aureus in order to guide clinicians as to the choice of antimicrobial agents.

An overall incidence of 662% of beta-lactamase producers was recorded for S. aureus strains at LUTH from 1994 to 1996. Also, a high rate of 70.6% of beta-lactamase producers among S. aureus strains has been previously reported in Nigeria by Rosimi et al.(7). Beta-lactamase production by Staphylococcus is the recognized mechanism of resistance to Penicillin G, as such the high incidence of beta-lactamase production by S. aureus isolates accounts for the high resistance to Penicillin G obtained in this study. Today, 70-90% of Staphylococcus strains are beta-lactamase producers and, consequently, resistant to Penicillin G. V. and the aminos, carboxy and azetidinocillin(9).

In Staphylococcus, methicillin is known to be an inhibitor phenomenon that extends to all beta-lactams and other commonly used antibiotics. As such, infections caused by methicillin-resistant Staphylococcus are highly problematic, and their frequency varies geographically, from hospital to hospital and over time(8). In this study, 46% of S. aureus isolates tested were methicillin-resistant, these results are consistent with reports in the United States and Italian hospitals where methicillin-resistant rates in S. aureus
range from about 10% to 50%. On the contrary, only 0.1 - 0.3% of S. aureus strains are resistant to methicillin in Scandinavian hospitals (9) and only 3% in Somali hospitals (2).

The glycopeptide drugs, vancomycin and teicoplanin, are the antibiotics of choice for treating infections caused by methicillin-resistant S. aureus (MRSA) since these organisms are always resistant to other commonly used antimicrobials. Fortunately, vancomycin resistance is still not a problem in this environment, as there was 100% susceptibility to vancomycin recorded in this study with very low MIC90 values. The advent of penicillinase-producing staphylococci soon after the introduction of Penicillins as therapeutic agents led to rapid development of new drugs in the 1950s, one of which was vancomycin. However, vancomycin was only infrequently used from the late 1970s following the worldwide spread of MRSA, and methicillin-resistant S. epidermidis (MRSE). To date, there are no published reports available on resistance to glycopeptides in MRSA, although low level resistance to glycopeptides has been found in methicillin-sensitive strains and coagulase-negative Staphylococci (13). Methicillin-resistance was detected by oxacillin E-testing; Huang et al. (11) and Novak et al. (12) found a high level of correlation between the E test and reference methodology (oxacillin disk diffusion, broth microdilution) for the detection of methicillin-resistant staphylococci.

The results obtained in this study have shown that vancomycin, rifampin, clindamycin and fusidic acid could be used for empirical therapy in life-threatening conditions. Susceptibility cannot be assumed for the other antibiotics tested, namely augmentin, erythromycin, gentamicin, tetracycline and cepham. In vitro efficacy is needed to validate the therapeutic usage.

REFERENCES


