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Susceptibility at high inoculum of penicillin-resistant Staphylococcus aureus to six cephalosporins

Andres Suarez, MD; Ramon del Busto, MD; Edward L. Quinn, MD, and Donald Pohlod, MD*

Six cephalosporins (currently available for clinical use or undergoing clinical trials) were tested by tube dilution method against high inoculum (10^7 colony forming units) of 37 strains of penicillin resistant Staphylococcus aureus. 3.1 μg/ml of cephalothin inhibited 100% of strains while higher concentrations of cefoxitin, cephapirin, cefamandole, cephalexin and cephradine were required. The percent of strains with minimal inhibitory concentration higher than 3.1 μg/ml ranged between 10.8% (cefoxitin) and 100% (cephradine). These findings support the observation that cephalothin is less susceptible to inactivation than the other cephalosporins. Although the exact clinical implication of these findings has not been established, cephalothin might be the cephalosporin of choice for treatment of severe infections caused by penicillin resistant Staphylococcus aureus.

THE usefulness of the cephalosporins as alternate agents in the treatment of staphylococcal endocarditis is well established. However, several cases have been reported of clinical and bacteriological failure when treating Staphylococcus aureus endocarditis with cephaloridine or cefazolin despite the in vitro susceptibility of the staphylococci to these antibiotics by conventional tests. Burgess and Evans reported a patient in whom bacteremia persisted after five days of treatment with cephaloridine despite presence of 12.5 μg/ml of the antibiotic at its lowest concentration. Further study of the organism showed that although the minimal inhibitory concentration (MIC) of cephaloridine when tested with a small inoculum was 0.12 μg/ml, it rose to 4 μg/ml when a high inoculum was used. Other in vitro studies have indicated that the susceptibility of penicillin resistant S. aureus to cephaloridine is markedly dependent on the size of the inoculum used for the test. In some cases a greater than 100 fold increase in the MIC was observed when the inoculum was increased from 10^4 to 10^7 colony forming units/ml while the MIC of cephalothin showed only a 10 fold increase as maximum. It was also shown that inactivation by Beta lactamase was responsible for these findings.

Quinn et al reported a series of ten patients with penicillin resistant S. aureus endocarditis who were treated with intra-
muscular cefazolin. The MIC's of all ten strains, as determined with standard inoculum of approximately 10^5 colony forming units/ml, ranged between 0.2 and 0.8 μg/ml of cefazolin. One patient failed to respond to treatment and had positive blood cultures while cefazolin was being given. Further study of the organism infecting this patient showed that the MIC increased to 15 μg/ml with a high inoculum (10^7 colony forming units/ml). This marked inoculum effect was not observed for strains of staphylococci from patients who responded favorably to cefazolin. It was also shown that the strain that showed the marked inoculum effect (or was "resistant" when tested at high inoculum) was able to inactivate cefazolin in vitro.

Based on the above mentioned studies, it is clear that the activity of different cephalosporins against penicillin resistant S. aureus is related to their susceptibility to inactivation by a Beta lactamase. This is reflected by higher MIC's at high inoculum and may be associated with therapeutic failure. The present study was designed to determine the frequency with which high concentrations of cephalosporins (currently available and under clinical trial) will be required to inhibit penicillin-resistant strains of S. aureus.

Material and methods

We studied 37 strains of S. aureus isolated from patients at the Henry Ford Hospital. Except for their resistance to penicillin G and their susceptibility to methicillin, these strains were unselected. Susceptibilities to the different cephalosporins were studied by determining the MIC using the standard macro tube dilution method. Standard antibiotic powders were provided by the following laboratories: cephadine by Smith, Kline and French Laboratories, Philadelphia, Pennsylvania; cefoxitin by Merck, Sharp and Dohme, Rahway, New Jersey; sodium cephaeirin by Bristol Laboratories, Syracuse, New York; and sodium cephalothin, cephalaxin monohydrate and cefamandole lithium by Eli Lilly and Company, Indianapolis, Indiana. The culture media used was trypticase soy broth (BBL). The inoculum consisted of 1 ml of a 10^-1 dilution of an 18-hour culture of the strain being studied. Bacterial plate counts using antibiotic free media were performed for all strains in order to determine the exact size of the inoculum which was later found to range from 0.4x10^7 to 3x10^7 colony forming units. Each strain was simultaneously tested against all antibiotics. MIC was defined as the minimum concentration of antibiotic necessary to inhibit growth after 18 hours of incubation.

Results

Our results are summarized in Figure 1. It can be seen that only 3.1 μg/ml or less of cephalothin was required to inhibit 100% of strains tested. On the other hand, higher concentrations of cefoxitin, cephapirin, cefamandole, cephalaxin and cephradine, in that order, were required for the same purpose.

In Table 1 the population of bacteria has been divided in two groups: those strains inhibited by 3.1 μg/ml or less of the various antibiotics and those strains inhibited by 6.2 μg/ml or more. We chose 3.1 μg/ml as the dividing point because when tested with conventional inoculum (10^5 colony forming units/ml) this concentration of cephalothin, cefoxitin, cefamandole, cefazolin and cephalaxin inhibited growth of 100% of strains of penicillin resistant S. aureus isolated from patients with endocarditis and tested in our laboratory. The table shows that all strains were inhibited by 3.1 μg/ml of cephalothin whereas 6.2 μg/ml or more was required to inhibit 10.8% of strains with cefoxitin, 24.3% of strains with cephaeirin, 40.5% of strains with cefamandole, 72.9% of strains with cephalaxin and 100% of strains with cephradine.

Discussion

When the in vitro activity against penicillin resistant Staphylococcus aureus is
Susceptibility of S. aureus to six cephalosporins

studied by the tube dilution method using a high inoculum (10⁷ colony forming units/ml), our results indicate that a lower concentration of cephalothin is required to inhibit the growth of 100% of strains. For at least 10.8% and as much as 100% of strains, higher concentrations of the other cephalosporins are required to inhibit growth.

The definition of susceptibility or resistance of an organism to a given antibiotic implies not only results of in vitro susceptibility testing but the correlation of these results with those of pharmacokinetic studies and clinical trials with the drug in question. Therefore, the conclusion that a given antibiotic is better or worse than the other based only on data of their activity in vitro is not valid. However, the design of our study and the interpretation of its results are based on clinical and bacteriological evidence indicating that failure of a cephalosporin in the treatment of endocarditis due to penicillin

Table I

<table>
<thead>
<tr>
<th>MIC*</th>
<th>Cephalothin No.</th>
<th>Cephalothin %</th>
<th>Cefoxitin No.</th>
<th>Cefoxitin %</th>
<th>Cephapirin No.</th>
<th>Cephapirin %</th>
<th>Cefamandole No.</th>
<th>Cefamandole %</th>
<th>Cephalexin No.</th>
<th>Cephalexin %</th>
<th>Cephradine No.</th>
<th>Cephradine %</th>
</tr>
</thead>
<tbody>
<tr>
<td>3.1 μg/ml or less</td>
<td>37</td>
<td>100%</td>
<td>33</td>
<td>89.2%</td>
<td>28</td>
<td>75.7%</td>
<td>22</td>
<td>59.5%</td>
<td>10</td>
<td>27.1%</td>
<td>0</td>
<td>0%</td>
</tr>
<tr>
<td>6.2 μg/ml or more</td>
<td>0</td>
<td>0%</td>
<td>4</td>
<td>10.8%</td>
<td>9</td>
<td>24.3%</td>
<td>15</td>
<td>40.5%</td>
<td>27</td>
<td>72.9%</td>
<td>37</td>
<td>100%</td>
</tr>
</tbody>
</table>

* MIC — minimal inhibitory concentration determined with high inoculum (10⁷ colony forming units/ml)

Number and percent of strains of Staphylococcus aureus inhibited by 3.1 μg/ml or less and 6.2 μg/ml or more of the six cephalosporins tested.
resistant *S. aureus* might be related to high MIC of the antibiotic when tested at high inoculum. This reflects the susceptibility of the antibiotic to destruction by Beta lactamase produced by the organisms.⁶,⁹

Our results support the observation that cephalothin is less susceptible to inactivation by Beta lactamase than the other cephalosporins.⁶ This observation has been confirmed recently by studying the inactivation of eight cephalosporin antibiotics in broth culture of growing *S. aureus.*¹²

The exact clinical implications of our findings are not well established. However, at the present state of knowledge, we tend to agree with others¹²,¹³ that, if a cephalosporin is used for the treatment of endocarditis, and perhaps other types of severe infection caused by penicillin resistant *S. aureus*, cephalothin would be the drug of choice. The decreased effectiveness of other cephalosporins for severe infections may depend on their susceptibility to inactivation by the strain of staphylococcus being treated. Such susceptibility to inactivation may be presumed to be significant if the MIC using an inoculum of 10⁷ colony forming units/ml is markedly higher than the MIC using a standard inoculum.

**Acknowledgment**

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**References**