In Vitro Susceptibility of Methicillin-Resistant and Methicillin-Susceptible Staphylococcus aureus Strains to N-Formimidoyl Thienamycin

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A total of 82 clinical isolates of methicillin-resistant Staphylococcus aureus and 21 isolates of methicillin-susceptible S. aureus were studied for in vitro susceptibility to N-formimidoyl thienamycin at incubation temperatures of 30 and 35°C. The disk diffusion test results were correlated with the macrobroth dilution test by means of the error rate-bounded method of analysis. Both methicillin-susceptible and (to a lesser degree) methicillin-resistant strains were generally susceptible to the antibiotic as judged from their minimum inhibitory concentrations. The discrepancy between in vitro results obtained at 30 and at 35°C was not very remarkable. However, tolerance of N-formimidoyl thienamycin was observed in 37% of methicillin-resistant strains and 24% of methicillin-susceptible strains at an incubation temperature of 30°C; at 35°C, the values were 54% (methicillin-resistant strains) and 14% (methicillin-susceptible strains).

Methicillin-resistant (MR) Staphylococcus aureus strains frequently demonstrate resistance to the older and newer cephalosporins (1, 6, 7). Currently, vancomycin remains the only antibiotic to which the microorganisms are consistently susceptible (5). Recently, however, a unique and novel β-lactam agent, N-formimidoyl thienamycin (N-F-thienamycin), has shown very promising in vitro activity against gram-positive cocci, including methicillin-susceptible (MS) S. aureus (9, 10, 13).

We studied the in vitro susceptibility of 82 clinical isolates of MR S. aureus and 21 clinical isolates of MS S. aureus (each obtained from a different patient) to N-F-thienamycin. The isolates were identified by conventional methods (8). The minimum inhibitory concentrations (MICs) and the minimum bactericidal concentrations (MBCs) were determined at incubation temperatures of 30 and 35°C. This was done to determine whether (i) antibiotic tolerance to N-F-thienamycin exists and (ii) the in vitro results are affected by the incubation temperature, as they are with methicillin and the cephalosporins (1, 6, 7). N-F-thienamycin powder was supplied by the Merck Institute of Therapeutic Research (Rahway, N.J.). The N-F-thienamycin solution was prepared as suggested by the manufacturer (9), and disks containing 25 μg of the antibiotic were used for the disk diffusion tests. The method of Bauer et al. was used for the disk diffusion studies (4). The MICs were determined by a macrobroth dilution technique with Mueller-Hinton broth (BBL Microbiology Systems, Cockeysville, Md.) by the method of Washington and Sutter (15). An inoculum of 10⁵ colony-forming units per ml was used. One set of tubes was incubated at 30°C, and another was incubated at 35°C. Microorganisms susceptible to concentrations of 8 μg/ml or less were considered susceptible, and those requiring higher concentrations for inhibition were considered resistant. This break-off point has been recommended by the manufacturer. The MBCs were determined as described by Barry (2). Specifically, 0.01-ml samples were transferred to 6.6% sheep blood agar plates (Clinical Standards, Torrance, Calif.) with 0.01-ml calibrated Eppendorf pipettes (Brinkman Instruments, Inc., Westbury, N.Y.). The MBC was defined as the lowest concentration of antibiotic resulting in 99.9% kill. A lethal effect was attributed to those concentrations of antibiotics that contained no more than one colony from the sample. An isolate was reckoned as tolerant if the MBC was 32 times the MIC or higher (11, 14). Control strains of S. aureus (ATCC 25923) and Staphylococcus epidermidis (AT 12228) were routinely included every time the tests were performed.

An attempt to draw a regression line analysis was made by standard methods correlating the disk diffusion and macrobroth dilution results (3). An r value of −0.51, however, was obtained at incubation temperatures of both 30 and 35°C.
FIG. 1. Correlation of the results of N-F-thienamycin disk diffusion susceptibility tests with results of macrobroth dilution susceptibility tests performed at 35°C incubation temperature. The error rate-bounded scheme of analysis was used. Symbols: ●, MR isolates; ▲, MS isolates; ZR, resistant zone; ZS, susceptible zone.

when both MR and MS S. aureus were studied together. When MR S. aureus was studied separately, the r values were −0.53 at 30°C and −0.51 at 35°C. These values were suboptimal for constructing a regression line. Therefore, the error rate-bounded method of analysis was used.

FIG. 2. Correlation of the results of N-F-thienamycin disk diffusion susceptibility tests with results of macrobroth dilution susceptibility tests performed at 30°C incubation temperature. The error rate-bounded scheme of analysis was used. See legend to Fig. 1 for symbols.
TABLE 1. Susceptibility of S. aureus isolates to N-F-thienamycin

<table>
<thead>
<tr>
<th>Incubation temp (°C)</th>
<th>Effective drug concn (μg/ml) against:</th>
<th>82 MR isolates</th>
<th>21 MS isolates</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC₅₀</td>
<td>MIC₉₀</td>
<td>MBC₅₀</td>
</tr>
<tr>
<td>30</td>
<td>0.90</td>
<td>6.25</td>
<td>19.0</td>
</tr>
<tr>
<td>35</td>
<td>0.40</td>
<td>2.40</td>
<td>12.5</td>
</tr>
</tbody>
</table>

* MIC₅₀ and MIC₉₀, MBCs of 50 and 90%, respectively, of the isolates tested; MBC₅₀ and MBC₉₀, MBCs of 50 and 90%, respectively, of the isolates tested.

This study was supported by the Merck Institute for Therapeutic Research, Rahway, N.J.

We thank Jean Lloyd and Beverly McRoy for their excellent secretarial help.

LITERATURE CITED