Interpretive Criteria for Testing Susceptibility of Staphylococci to Mupirocin

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Received 13 September 1996/Returned for modification 5 December 1996/Accepted 10 February 1997

To determine appropriate mupirocin susceptibility testing interpretive criteria, 177 staphylococci were tested by agar dilution, disk diffusion, and E test. E test was found to be a reliable method for determining susceptibility of staphylococci to mupirocin. The agar dilution and disk diffusion results were plotted on a scattergram, and error rates were calculated. No errors were found with susceptibility breakpoints of ≤4 μg/ml (MIC) and ≥14 mm (zone diameter).

Mupirocin is a topical antibiotic that is used for the treatment of impetigo due to staphylococci and streptococci (11) and for the eradication of *Staphylococcus aureus* nasal colonization. It has been suggested that nasal isolates for which mupirocin MICs were elevated (8 to 256 μg/ml) may be amenable to mupirocin therapy (4), and studies are currently being conducted to further characterize appropriate colonization breakpoints (10). Several breakpoints have been used to determine the mupirocin susceptibility of infecting isolates. In initial clinical trials, a MIC breakpoint of ≤4 μg/ml, and a corresponding zone diameter breakpoint of ≥18 mm with a 5-μg mupirocin disk, were used to define susceptibility. However, investigators have reported false resistance with this zone diameter breakpoint (2, 6). Fuchs et al. (3) and Barry et al. (1) suggested a MIC breakpoint of ≤2 μg/ml and a corresponding zone diameter breakpoint of ≥14 mm for the 5-μg mupirocin disk. Because no mupirocin-resistant staphylococci were available for those two studies, those authors suggested that the reliability of the 5-μg mupirocin disk for the detection of mupirocin-resistant staphylococci needed to be determined.

The primary purpose of this study was to determine the interpretive criteria for mupirocin susceptibility testing of infection isolates of staphylococci by using isolates for which there was a range of mupirocin MICs. An additional purpose of this study was to determine if MICs determined by the mupirocin E test resulted in interpretational errors with the proposed breakpoints.

(Results of this study were presented in part at the 35th Interscience Conference on Antimicrobial Agents and Chemotherapy, San Francisco, Calif., 1995.)

**Bacterial isolates.** A total of 177 staphylococci (167 *S. aureus* isolates and 10 coagulase-negative staphylococci) for which there was a range of mupirocin MICs were selected for this study. They included methicillin-resistant, methicillin-susceptible, β-lactamase-positive, and β-lactamase-negative isolates.

Staphylococci for which mupirocin MICs are in the range of 1 to 8 μg/ml are uncommon. To increase the number of isolates for which the MICs were near the proposed mupirocin MIC breakpoint, 10 *S. aureus* isolates for which the MICs were approximately 0.25 μg/ml were serially passaged with the mupirocin E test. To encourage an increase in MIC, an inoculum equivalent to a 1 McFarland standard was used, as opposed to the 0.5 McFarland standard recommended by the manufacturer. Following the first exposure to the mupirocin E test strip, growth around the zone of inhibition was used to make the suspension for the next passage. This process was repeated until the mupirocin MICs were within the range of 1 to 8 μg/ml.

**Susceptibility testing.** All isolates were tested by the disk diffusion, agar dilution, and E test methods. Disk testing and agar dilution MIC determinations were conducted according to National Committee for Clinical Laboratory Standards (NCCLS)-recommended procedures (7, 8). A 5-μg mupirocin disk (BBL, Cockeysville, Md.) was used for disk diffusion testing. MICs were determined by agar dilution with Mueller-Hinton agar (BBL) and lithium mupirocin reference powder (SmithKline Beecham, Philadelphia, Pa.). All organisms were tested by the E test (AB Biodisk, Solna, Sweden) method according to the recommendations of the manufacturer.

*S. aureus* ATCC 25923 was used for quality control; the acceptable zone diameter range was 22 to 27 mm, and the acceptable MIC range was 0.12 to 0.5 μg/ml.

For the development of disk diffusion interpretive criteria for testing a single genus or species, NCCLS document M23-T2 (9) recommends testing 100 to 300 isolates. Guidelines for determining breakpoints with a scattergram state that the proposed zone size should be adjusted until the minimum numbers of false-susceptible (very major errors) and false-resistant (major errors) disk diffusion results are obtained. When evaluating a large collection of unselected clinical isolates, very-major-error rates should be less than 1.5% and major errors should occur for less than 3% of all isolates (9). When a more selective collection of isolates is chosen, very major errors should be calculated with the number of resistant strains as the denominator and major-error rates should be calculated with the total number of susceptible isolates as the denominator. The NCCLS recommends that both types of error rates be presented.

The scattergram depicting the mupirocin agar dilution MICs versus the 5-μg-disk inhibitory-zone diameters for the 177 staphylococci tested is shown in Fig. 1. By using the error-rate-bounded method (5), the proposed susceptibility breakpoints were ≤4 μg/ml (MIC) and ≥14 mm (zone diameter). The correlation coefficient was 0.76, calculated by Spearman’s rank correlation test. There were no errors when these breakpoints were applied to the data.

With the clinical-trial breakpoints of ≤4 μg/ml and ≥18 mm, five isolates were determined to be resistant by disk diffusion
but susceptible by agar dilution. This corresponded to a major-error rate of 2.8%, with the total number of isolates as the denominator in calculations, which was within the NCCLS-recommended error rate of 3%. However, when the number of susceptible isolates was used as denominator, the major-error rate was 3.8%. No very major errors occurred with the \( \#4 \) mg/ml and \$18-mm breakpoints.

The \( \#2 \) mg/ml and \$14-mm breakpoints proposed by Barry et al. (1) and Fuchs et al. (3) are also depicted in Fig. 1. Use of these breakpoints resulted in a major-error rate of 0% and a very-major-error rate of 2.3% in this study. Four isolates were determined to be susceptible by disk diffusion and resistant by agar dilution, which was outside the NCCLS-recommended rate of 1.5% for very major errors. Four isolates were determined to be susceptible by disk diffusion and resistant by agar dilution, which was outside the NCCLS-recommended rate of 1.5% for very major errors. When the number of resistant isolates was used as the denominator, the very-major-error rate was 8.7%. Mupirocin-resistant staphylococci were not available at the time Fuchs et al. and Barry et al. conducted their studies, so less clinically relevant organisms were used to represent resistant isolates. The error rates achieved by applying the three sets of criteria to the scattergram are summarized in Table 1.

The results obtained with E test correlated well with the agar dilution MICs, with a correlation coefficient of 0.87 by Spearman's rank correlation test. The MICs for 41% of the total number of isolates tested were identical by E test and agar dilution, for 97% they were within 1 dilution, and for 100% they were within 2 dilutions. The E test did not give any false-resistant or false-susceptible results. For the 19 isolates for which agar dilution MICs were near the breakpoint, i.e., 2 to 8 \( \mu \)g/ml, the E test endpoint was easy to determine and the MICs for 11 of them as determined by agar dilution and E test were identical. In this study, E test was found to be a reliable method for the determination of mupirocin susceptibility.

In conclusion, the following breakpoints are proposed for mupirocin susceptibility testing of infecting isolates: in dilution tests, mupirocin MIC breakpoints for susceptible and resistant strains should be set at \( \#4 \) and \$8 mg/ml, respectively, and in tests with the 5-\( \mu \)g disk, zone diameter breakpoints for susceptible and resistant strains should be set at \$14 and \#13 mm, respectively.

We thank Carol Kauffman for providing bacterial isolates for this study and Robert Gagnon for assistance with statistical data.

**REFERENCES**


