Quality Control Criteria for Testing the Susceptibility of Anaerobic Bacteria to Meropenem

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Reference values for quality control of in vitro susceptibility tests with meropenem against anaerobic bacteria were determined in a multinational study by the approved National Committee for Clinical Laboratory Standards agar dilution method for the four quality control strains. The study protocol also included the evaluation of microdilution testing, medium additives, and multiple lots of media. The recommended MIC control ranges for three of the control organisms are as follows: Bacteroides fragilis ATCC 25285, 0.06 to 0.125 μg/ml; Bacteroides thetaiotaomicron ATCC 29741, 0.125 to 0.5 μg/ml; and Eubacterium lentum ATCC 43055, 0.125 to 0.5 μg/ml. The modal MIC for Clostridium perfringens ATCC 13124 was at or below the lowest concentration of meropenem tested, and no values are recommended.

Meropenem is a new carbapenem that has excellent in vitro activity against a wide variety of aerobic and anaerobic organisms. While it is slightly less active than imipenem against gram-positive cocci, it is more active against members of the family Enterobacteriaceae, pseudomonads, and Bacteroides spp. (3). To provide clinical microbiologists with MIC quality control guidelines for use in anaerobic susceptibility testing of meropenem, a multiple-laboratory study was conducted. The study satisfied the requirements outlined by the National Committee for Clinical Laboratory Standards (4). The requirements are for a minimum of 20 determinations for each control strain to be made in each of five laboratories. The reference strains tested were Bacteroides fragilis ATCC 25285, Bacteroides thetaiotaomicron ATCC 29741, Clostridium perfringens ATCC 13124, and a new species, Eubacterium lentum ATCC 43055, which has been recommended as a new control strain (2, 5). The following institutions participated: Sinal Samaritan Medical Center, Milwaukee, Wis.; ICI Pharmaceuticals Group, Division of ICI Americas, Wilmington, Del.; Baptist Medical Center, Jacksonville, Fla.; University of Wisconsin Hospital and Clinics, Madison; and University of Illinois Hospital, Chicago.

The National Committee for Clinical Laboratory Standards reference agar dilution procedure (5) for susceptibility testing of anaerobes was used in all laboratories. Three laboratories used one lot of Wilkins-Chalgren agar medium (7), and two laboratories used a different lot. In addition, two laboratories performed additional testing with sheep blood (5%) as an additive to the agar medium. Broth microdilution testing using the same formulation of Wilkins-Chalgren medium without agar (Anaerobe Broth, MIC: Difco Laboratories, Detroit, Mich.) was conducted simultaneously. All five laboratories used two lots of broth media. Microdilution trays were prepared by a commercial laboratory (MicroTech Medical, Aurora, Colo.). Meropenem laboratory standard powder was provided by ICI Americas. Each of the control strains was tested at least 20 times in each laboratory by each method. Cefoxitin was tested by all laboratories to provide a procedural control. The two Bacteroides species yielded cefoxitin endpoints that were within the control limits defined by the National Committee for Clinical Laboratory Standards for both agar and microdilution testing in all laboratories. E. lentum yielded results in agreement with those described by Barry and Zabransky (2). C. perfringens results were not evaluated in this study.

Table 1 presents the meropenem MIC endpoint determinations for the strains tested. For the B. fragilis control strain, when tested by agar dilution, all 107 MIC determinations fell within a range of 0.06 to 0.25 μg/ml; however, >95% of the endpoints were at 0.06 and 0.125 μg/ml. When tested by microdilution, 191 of the 194 determinations fell at the same two concentrations. When the criteria proposed by Barry et al. (1) are used, a quality control range of 0.06 to 0.125 μg/ml is recommended for this strain. An MIC range of from 0.125 to 0.5 μg/ml was obtained by agar dilution testing with B. thetaiotaomicron. The same range with a slight skewing toward the lower concentration was obtained in microdilution testing. The modal value was clearly 0.25 μg/ml. We therefore recommend that the control limits be from 0.125 to 0.5 μg/ml. With E. lentum, a clear modal value of 0.25 μg/ml was also observed with both agar and microdilution testing. The range for both methods was from 0.125 to 0.5 μg/ml, which is the recommended control limits for this organism. The lowest concentration tested, 0.008 μg/ml, was also the most common determination for C. perfringens; however, the true modal MIC may be at this concentration or at a lower concentration. With seven MICs falling outside the 95% limits proposed by Barry et al. (1), we would not recommend a quality control range at this time. Importantly, these concentrations would probably be below those normally tested in a clinical laboratory and would not serve well for quality control purposes.

Two lots of Wilkins-Chalgren agar were tested in this study, one lot in two laboratories and the second lot in three

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TABLE 1. Number of determinations of susceptibility of anaerobe control strains at specific concentrations of meropenem

<table>
<thead>
<tr>
<th>Strain</th>
<th>Test method</th>
<th>No. of determinations with the following MIC (µg/ml):</th>
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<tbody>
<tr>
<td></td>
<td></td>
<td>&lt;0.008 0.016 0.03 0.06 0.125 0.25 0.5</td>
</tr>
<tr>
<td><em>Bacteroides fragilis</em></td>
<td>AD</td>
<td>55 47 5</td>
</tr>
<tr>
<td>ATCC 25285</td>
<td>MD</td>
<td>1 184 7 2</td>
</tr>
</tbody>
</table>
| *Bacteroides thetaio-
  taomicron* ATCC 29741 | AD          | 8 96 4                                               |
|                         | MD          | 65 129 1                                             |
| *Clostridium perfring-
  ens* ATCC 13124        | AD          | 68 31 7                                              |
|                         | MD          | 188 8                                               |
| *Eubacterium lentum*    | AD          | [14 75 18]                                           |
| ATCC 43055              | MD          | 9 186 1                                              |

*AD, National Committee for Clinical Laboratory Standards reference agar dilution; MD, microdilution. *

* Brackets indicate recommended control range.

other laboratories. Only minimal differences were seen between the lots tested (data not shown). Similarly, two laboratories included blood in the agar dilution testing in an additional 20 determinations. The results (not shown) indicated that there is no effect of blood on the meropenem MICs obtained against these organisms. Two separate lots of broth media were tested by microdilution; all laboratories tested both lots. Again, there were no significant differences between the results obtained with the two lots (data not shown). Interestingly, except for *C. perfringens*, a much narrower distribution of MICs was obtained with all strains by microdilution testing as opposed to agar dilution. Lastly and also importantly, the results obtained from all laboratories were very consistent. The laboratories all reported that the endpoints were easy to read, and trailing endpoints, as has been reported with other beta-lactam antibiotics, were not observed (6).

The consistency of results obtained with the two *Bacteroides* species and *E. lentum* indicate their utility for controlling the testing of meropenem by either agar or microdilution testing. The use of *C. perfringens* for quality controlling the testing of this drug is not recommended.

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LITERATURE CITED