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NOTES

Imipenem Susceptibility Testing with a Commercially Prepared Dry-Format Microdilution Tray

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MICs of imipenem, concurrently generated in commercially prepared microdilution trays containing predried antibiotic dilutions (Sensititre), and in a standard agar dilution assay (as recommended by the National Committee for Clinical Laboratory Standards, Villanova, Pa.), were within ±1 twofold dilution for 94% of 226 bacterial isolates. Imipenem biological activity remained stable over 5 months of tray storage at room temperature against *Pseudomonas aeruginosa* ATCC 27853. Activity of imipenem was shown by microdilution testing with 890 clinical isolates to be high, with only 4% of isolates having MICs of ≥16 μg/ml (in vitro resistance).

The beta-lactam antimicrobial agent imipenem (*N*-formimidoyl thienamycin) has been shown to possess in vitro activity against a wide spectrum of microorganisms, including *Enterobacteriaceae*, *Pseudomonas aeruginosa*, *Staphylococcus aureus*, *Streptococcus faecalis*, and *Bacteroides* species (2–5, 8). This derivative of thienamycin has also been reported to be far less subject to inactivation as a solid and in concentrated solution states than the parent drug (10). Microdilution trays containing imipenem in cation-supplemented Mueller-Hinton broth are described as having stability at −70°C for more than 1 year (MK-0787 susceptibility powder package insert: Merck Sharp & Dohme, Rahway, N.J.). However, Baron and Hindler (1) have reported rapid deterioration of imipenem activity in cation-supplemented Mueller-Hinton broth when stored frozen at −20°C in microdilution trays. Nickolai et al. (9) have likewise reported rapid loss of imipenem activity and problems in the long-term storage of ampicillin, ticarcillin, mezlocillin, piperacillin, azlocillin, and moxalactam at temperatures of −20°C and higher. Therefore, routine susceptibility testing of imipenem (and possibly other beta-lactam agents) using microdilution products in which the antibiotic dilutions are prepared in trays, frozen, and shipped to the user to be further stored frozen is problematic in that clinical laboratories seldom have access to −70°C storage facilities. It was therefore of interest to examine the performance of a microdilution format in which the antibiotic dilutions are predried and stored at room temperature for the testing of imipenem. A commercially prepared predried microdilution tray (Sensititre; GIBCO Laboratories, Lawrence, Mass.) was chosen as a representative of dry format microdilution trays. A total of 226 clinical laboratory isolates representing a variety of bacterial species were selected for concurrent MIC testing against imipenem in Sensititre trays and in a reference agar dilution procedure.

Sensititre microdilution trays were prepared by the manufacturer to contain twofold dilutions of imipenem in concentrations ranging from 0.125 to 16.0 μg/ml for the testing of gram-negative bacilli. A second series of trays was prepared for the testing of gram-positive cocci and contained imipenem in concentrations ranging from 0.06 to 8.0 μg/ml. During the manufacturing process, the antibiotic dilutions in trays were reduced to a dry state. Each tray was sealed with an adhesive cover and packaged into a foil pouch with desiccant. The Sensititre trays were used according to the instructions of the manufacturer to determine MICs. Briefly, several colonies, after overnight incubation, were selected and suspended in saline to match a 0.5 McFarland turbidity standard and diluted 1:1,000 in cation-supplemented Mueller-Hinton broth. Inoculation (and hydration) of the microdilution trays were carried out with a Sensititre Autoinoculator, after which trays were covered with a clear adhesive seal and incubated at 35°C for 18 to 24 h. Visual estimation of MIC endpoints and data recording were made by using a computer-assisted viewer described elsewhere (11). The MIC was the lowest concentration of antibiotic in a dilution series that completely inhibited the growth of the test organism.

Agar dilution assays were carried out according to recent recommendations made by the National Committee for Clinical Laboratory Standards, Villanova, Pa., with unsupplemented Mueller-Hinton agar (6). Imipenem was supplied by Merck Sharp & Dohme, West Point, Pa. In brief, standardized suspensions of test organisms were deposited on the surface of control and antibiotic-containing plates by means of a multiple-prong replicating inoculator such that initial organism concentrations were approximately 10^4 CFU per spot application. MICs were determined after 18 to 24 h of incubation in ambient air at 35°C by selecting the lowest concentration of the antimicrobial agent in the dilution series that completely inhibited growth, disregarding a single colony or a faint inoculum haze as growth. In those instances where direct comparison between agar dilution and microdilution broth results was made, the two susceptibility methods were inoculated concurrently from the same inoculum preparation. *P. aeruginosa* ATCC 27853 was included for quality control purposes on each day of testing and to monitor the stability of the bioactivity of imipenem in the microdilution trays over time.

MIC testing by agar and by microbroth dilution was

TABLE 1. Comparison of imipenem microdilution broth MICs with reference agar dilution results

Organism group (no. tested)	No. of organisms showing following twofold dilution deviations of MDB[a] from AD[b] MICs:						
	>−2	−2	−1	0	+1	+2	>+2
Enterobacteriaceae[c] (148)	2	6	52	63	24	1	0
P. aeruginosa (30	0	3	13	14	0	0	0
Gram-positive cocci[d] (48)	0	0	13	29	5	0	1
% of total	1.0	4.0		94.0		0.5	0.5

[a] MDB, Microdilution broth method (Sensititre)
[b] AD, Agar dilution method (National Committee for Clinical Laboratory Standards).
[c] Also other selected gram-negative bacilli.
[d] *S. aureus*, coagulase-nagative staphylococci, and group D enterococci.

carried out on 178 aerobic gram-negative bacilli and 48 gram-positive aerobic cocci, all obtained from clinical specimens submitted to the laboratory for bacteriologic analysis. Agreement of imipenem microdilution MICs with concurrently run agar dilution MICs are summarized in Table 1. Accepting the customary ±1 twofold dilution deviation of microdilution results as satisfactory agreement, 94% of the results with the *Enterobacteriaceae* (and other selected gram-negative bacilli), 90% of the results with the *Pseudomonadaceae* and 98% of the results with gram-positive cocci were in agreement with the reference agar dilution results.

Given a general agreement approaching 95% of microbroth dilution results with values generated in the reference agar dilution procedure, an examination of imipenem activity was carried out against a wide variety of clinical isolates, using the Sensititre microdilution trays. Isolates included 82 *S. aureus*, 92 coagulase-negative staphylococci, 33 group D enterococci, and 683 isolates of gram-negative bacilli representing 27 species.

Application of the interpretive criteria for susceptibility and resistance suggested for imipenem by the manufacturer and recently by the National Committee for Clinical Laboratory Standards (7) to these 890 isolates showed that 94% of MIC results were ≤4 μg/ml (susceptible), and 2% were 8 μg/ml (intermediate). Only 34, or 4%, of all isolates tested had MICs of 16 μg/ml or greater (resistant). A total of 23 of the resistant isolates were coagulase-negative staphylococci, of which 17 were also resistant to methicillin and a variety of other antimicrobials tested except vancomycin. The remaining imipenem-resistant isolates included group D enterococci (4), *Aeromonas hydrophila* (2), *P. aeruginosa* (4), and *P. maltophilia* (1).

On each day of testing, the MIC of imipenem was determined against *P. aeruginosa* ATCC 27853. A total of 31 repetitive determinations were made, spanning 5 months. In 65% of instances, these values were identical to the median imipenem MIC for the series (2 μg/ml). In 97% of instances, the values fell within ±1 twofold dilution of the median MIC. There was no suggestion during this 5 month period that the biological activity of imipenem in the microdilution trays had deteriorated. In addition, concurrently generated MICs for piperacillin, cefotaxime, and moxalactam (beta-lactam drugs which gave on-scale MIC values with this *P. aeruginosa* strain) indicated no loss in activity over time with these agents in the dry-format trays.

The data from Table 1 suggest that microbroth dilution yields imipenem results comparable to agar dilution in that 94% of microdilution MICs fell within ±1 doubling dilution step of the agar dilution MIC, a variance usually regarded as acceptable in comparing twofold dilution systems (6). The distribution of microdilution MICs tended to shift slightly lower than the corresponding agar values. A degree of subjectivity exists in the reading of either agar or broth dilution systems, and the modest differences between the two methods used in this study appear to be without great significance. The biological activity of imipenem was stable in the dry trays during the 5 months of our observations as shown by repetitive testing with the quality control strain of *P. aeruginosa*. The age of the Sensititre trays at the time of final testing was actually approaching 10 months from manufacture. Therefore, the good correlation with an agar dilution procedure (as recommended by the National Committee for Clinical Laboratory Standards) and the apparent stability of the drug in commercially prepared, dry-format microdilution trays (as represented in this study by the Sensititre system) over 5 consecutive months of testing suggest that dry-format trays are well suited for routine susceptibility testing of clinical isolates against imipenem.

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