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False Resistance to Imipenem with a Microdilution Susceptibility Testing System

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Routine monitoring of antibiotic resistance at Children's Hospital, Boston, detected a dramatic increase in the prevalence of imipenem-resistant strains of *Pseudomonas aeruginosa*. Further studies documented that false resistance to imipenem was due, in part, to the loss of imipenem potency in customized MIC microdilution trays supplied by Sensititre Ltd. (West Sussex, United Kingdom). Recognition of the problem was delayed by use of the quality control standards recommended by the manufacturer, which were higher and broader than those suggested by the National Committee for Clinical Laboratory Standards.

As part of the antibiotic monitoring program at Children's Hospital (CH), we noted an apparent increase in imipenem-resistant *Pseudomonas aeruginosa* isolates from sputum cultures of patients with cystic fibrosis. Whereas the rate of resistance was 10% in 1984, it increased to 24% in 1986 and 33% in 1987, with only 52% of strains being fully susceptible. This prompted an epidemiological investigation which found no evidence for nosocomial transmission. Instead, we discovered that the increase in imipenem resistance was largely an artifact due to faulty performance of our custom-made microdilution MIC trays (Sensititre Ltd., West Sussex, United Kingdom).

In this investigation, all 469 *P. aeruginosa* isolates from patients with cystic fibrosis submitted to our clinical laboratory from September 1987 to March 1988 were tested for susceptibility to imipenem by using the Sensititre microdilution MIC and disk diffusion methods. Disk diffusion testing was performed as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (4). Microdilution MICs were performed according to the instructions of the manufacturer by using custom lots of trays stored at room temperature and used before expiration. Interpretation was done according to NCCLS standards (4, 5). Control strains of *P. aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were included with each assay.

Discrepant disk diffusion and Sensititre MIC results for imipenem were noted in 197 (42%) of the 469 strains tested. Resistance to imipenem by disk diffusion was noted in 13% of the strains, only 4 (<1%) of which were fully or moderately susceptible by MIC testing. In contrast, 76 of the isolates (16%) had MICs of ≥16 mg/ml (resistant), while disk diffusion indicated susceptibility. During this testing period, the MICs for the *P. aeruginosa* ATCC 27853 control strain were within the recommended limits (2 to 16 μg/ml) of Sensititre in 90% of the assays. However, control MICs exceeded those recommended by the NCCLS (1 to 4 μg/ml) (5) by more than 1 dilution in over 90% of all assays. Results for *E. coli* ATCC 25922 were more than 1 dilution outside the NCCLS specifications in only 10% of the assays.

Eleven isolates were selected for further testing, to arbitrate these discrepant results. The strains were tested at CH by using three custom lots of Sensititre MIC trays. The Merck Sharp and Dohme Research Laboratory (MSDRL; Rahway, N.J.) performed disk diffusion testing (4), agar dilution MIC testing (5), and another commercial microdilution MIC test (API Uniscept; Analytab Products, Plainview, N.Y.). MSDRL also tested the strains with Sensititre MIC trays from the same lots (lots 7083 and 7414) used at CH. The Sensititre Systems Group (SSG; Lawrence, Mass.) tested the strains using commercial stock Sensititre microdilution panels. By the disk diffusion, agar dilution, and API Uniscept methods, all 11 isolates were susceptible to imipenem (Table 1). There was close agreement between disk diffusion zone diameters at CH and MSDRL. Likewise, there was a good correlation between the results of the agar dilution and API Uniscept MIC assays performed at MSDRL.

In contrast, Sensititre MICs varied widely. Of the 70 Sensititre MIC determinations on the 11 isolates with five lots of trays (Table 1), values were within the susceptible range for 19 (27%), moderately susceptible for 24 (34%), and resistant for 27 (39%) of the determinations. Of the 11 isolates, 10 fell in the resistant range in at least one assay, and the remaining isolate was moderately susceptible in four assays. Sensititre trays from different lots showed considerable variation. For each of the 11 isolates, the range of MICs spanned at least three dilutions over seven assays performed in three laboratories.

To determine the reason for these discrepant results, the concentration of imipenem in wells of Sensititre trays was determined by high-pressure liquid chromatography (HPLC) (Table 2). Measured concentrations of imipenem averaged 40% of the expected concentrations in customized lots 7083 and 7414 and were disproportionately lower at higher concentrations. Imipenem levels in lot 8305, which was produced under a modified protocol after Sensititre was informed of our preliminary findings, were close to nominal values.

We conclude that the false resistance associated with the use of customized Sensititre MIC trays from 1984 to 1987 was attributable in part to a lower-than-expected concentration of imipenem by the time that the trays were used in the

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TABLE 1. Interlaboratory comparisons of imipenem microdilution MICs, agar dilution MICs, and disk diffusion zone diameters for 11 *P. aeruginosa* strains showing resistance by Sensititre MIC and susceptibility by disk diffusion testing[a]

Isolate	Zone diam (mm) by disk diffusion		MIC (μg/ml)								
					Sensititre lot						
	MSDRL	CH	Agar (MSDRL)	API (MSDRL)	7083 (CH)	7414 (CH)	7334 (CH)	7083 (MSDRL)	7414 (MSDRL)	8063 (SSG)	7274 (SSG)
1	28	20	2	2	16	8	2	ND[b]	4	8	16
2	26	23	1	1	>16	8	4	8/16	16	16	16
3	17	17	1	1	16	8	8	ND	2	16	16
4	23	24	1	1	16	8	32	16	16	8	16
5	27	29	0.5	≤0.5	8	2	1	16	1	4	2
6	24	19	1	2	8	8	4	ND	1	8	8
7	29	29	2	≤0.5	16	8	8	ND	2	8	8/4
8	18	18	2	2	8	8	4	16	8	32	32/64
9	27	26	2	≤0.5	16	8	4	ND	2	16	32
10	23	23	1	≤0.5	16	8	4	ND	8	8	16
11	27	23	1	1	16	16	4	ND	8	4	4

[a] Isolates were tested at three laboratories (MSDRL, CH, and SSG) by the disk diffusion, agar dilution (agar), and microdilution methods with API Uniscept (API) and Sensititre microdilution systems.
[b] ND, Not determined.

laboratory. Although imipenem is stable in solution at −70°C for up to 1 year (7), it can deteriorate rapidly in cation-supplemented Mueller-Hinton broth when it is stored at −20°C in microdilution trays (1). To overcome this problem, trays are made and stored in a dry format and reconstituted at the time of use. Additionally, the stability of imipenem is rapidly compromised when the solution pH is greater than 7 (8). Studies of the role of drying, storage, pH, and divalent cations on the reproducibility of MICs were undertaken at Sensititre Ltd. after the company was notified of our discrepant results, and the resulting lot of MIC trays (lot 8305) had acceptable performance characteristics during initial testing. Nonetheless, it is not possible to attribute the previous invalid MIC results totally to defined factors, such as manufacturing losses and deterioration on storage. These factors explain a one- or two-dilution discrepancy, whereas we documented variations of four to five dilutions when we compared the Sensititre panels with the agar dilution standard.

Hospitals routinely monitor antibiotic resistance trends, as required by the Joint Commission for the Accreditation of Health Care Organizations. We discovered an unanticipated benefit of monitoring: quality control of antibiotic susceptibility testing itself. In this case, recognition of false resistance was delayed because our laboratory relied upon the wide, nonstandard quality control MIC range recommended by the manufacturer for *P. aeruginosa* ATCC 27853. The range of 2 to 16 μg/ml recommended by Sensititre (6) was both higher and broader than that recommended by the NCCLS (5) and others (2), perhaps necessitated by previously unrecognized losses in imipenem activity. Quality control standards which deviate from those recommended by the NCCLS should be used by clinical laboratories with great caution, if at all. Sensititre now accepts the NCCLS limits for *P. aeruginosa* ATCC 27853.

TABLE 2. Concentrations of imipenem in wells of customized lots of Sensititre microdilution MIC panels measured by HPLC

Microdilution tray lot no.	Imipenem concn (μg/ml) found with the following concn (μg/ml) expected:					Avg % of expected value
	16	8	4	2	1	
7083	4.5	3.5	1.8			
	4.6	3.7	2.0			40
7414	5.8	3.2	1.5			
	5.2	3.0	1.6	1.2		40
8305	13.0	6.6	3.2	1.5		
	14.6	8.2	4.1	2.4	1.2	95

[a] Imipenem concentrations in Sensititre wells were measured in duplicate by HPLC at MSDRL as described previously (3). Two trays from each lot were analyzed. Lot 8305 was manufactured under modified conditions, inlcuding tighter pH control, addition of a manufacturing overage to compensate for drying losses, and a doubling of the amount of desiccant in each package. The values shown are within the range of the standard curve.

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