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Imipenem Stability in a Predried Susceptibility Panel

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We performed a 15-month study using 11 clinical strains and 1 control strain (ATCC 27853) of *Pseudomonas aeruginosa* to determine whether changes in the manufacturing process of Sensititre predried panels result in a reliable test of susceptibility to imipenem. MIC and breakpoint susceptibility results remained stable during the manufacturer's recommended shelf life of 18 months and compared well with standard agar disk diffusion and broth macrodilution results. Imipenem concentrations measured by high-pressure liquid chromatography were acceptable through 15 months but declined in the breakpoint panels by approximately 50% at 18 months. Between 9 months and panel expiration, 13 of 141 (9%) of the MIC panel packages had moisture entry, as indicated by pink desiccants, with a resultant loss of imipenem activity of 32 to 100%. It appears that the new manufacturing process produces MIC panels that are reliable for imipenem susceptibility testing until the labeled expiration date, provided that packages containing pink desiccants are not used.

Reliable in vitro testing of the susceptibility of clinical isolates of *Pseudomonas aeruginosa* to imipenem is of paramount importance to good patient care. Unfortunately, imipenem is labile in storage and during incubation at 35°C, making in vitro testing problematic (1). Resistance among clinical isolates of *P. aeruginosa* to imipenem appears to be increasing (5, 7, 9) and must be differentiated from false resistance due to degradation of the compound in commercially prepared dry-format and frozen panels, in solutions stored at room temperature, and as found in previous studies, in commercially prepared predried broth microdilution panels (Sensititre; Radiometer America Inc., Westlake, Ohio) (8, 11). Concern over drug degradation prompted the vendor to change the manufacturing process in an attempt to increase the stability of imipenem in Sensititre panels and to modify the expiration date to limit the shelf life of new panels from 24 to 18 months. In addition to a loss of potency over time, other potential factors affecting a laboratory's ability to monitor the reliability of testing of the susceptibility of organisms to imipenem are variations in lots of Mueller-Hinton (MH) broth, multiple passaging of the control strain of *P. aeruginosa*, and moisture in stored dry-format panels (6, 8, 11). Moreover, the zinc concentration in MH agar has been shown to affect the MIC of imipenem for *P. aeruginosa* (2) and may affect susceptibility testing by other methods. More difficulties have been encountered when testing *P. aeruginosa*, since this organism requires for inhibition higher concentrations of imipenem, closer to the breakpoint (BP) values, than those required by members of the family *Enterobacteriaceae*. Because of earlier reports of the varying performance of Sensititre panels, our objective was to conduct a rigorous study in which direct measurement of imipenem concentrations in the test panels and the effect of the age of the panels on susceptibility results obtained in the microbiology laboratory were used to assess the reliability of the currently available BP and MIC panels during their entire shelf life.

(This work was presented in part at the 93rd General Meeting of the American Society for Microbiology, 1993, Atlanta, Ga. [3].)

Sensititre system. Sensititre is a commercially packaged system for quantitative and qualitative susceptibility testing. Antimicrobial agents at appropriate concentrations are put into wells of a microdilution panel, which is then dried and packaged for storage at room temperature. Each package contains a silica gel desiccant that changes color from blue to pink when moisture is present. The system utilizes a nephelometer for standardizing the inoculum. An autoinoculator dispenses 50 μl of broth containing the inoculum into each well. Following incubation, panels can be read manually or by an autoreader, which measures growth by fluorescence. A computer program converts this measurement to a BP result or MIC (4, 10).

Organisms. Eleven unique clinical isolates of *P. aeruginosa* collected at the Medical Center of Central Massachusetts and one *P. aeruginosa* control strain (ATCC 27853) were stored in multiple copies at −70°C in brain heart infusion semisolid agar (Difco Laboratories, Detroit, Mich.) and subcultured on Trypticase soy agar with 5% sheep blood (BBL) before each testing period. Organisms were selected to include those requiring a range of imipenem MICs from ≤1.0 to 16 μg/ml as determined by the Sensititre method at the time of purchase of the panels and 2.0 to 32 μg/ml as determined by broth macrodilution testing. These organisms (Table 1) were selected so that wells in the MIC panel containing low and higher imipenem concentrations (1.0 to 64 μg/ml) would be biologically assayed and that the performance of new and older panels could be measured for accuracy in correctly detecting both susceptible and resistant strains. Since the BP panels contain only two wells with 4 and 8 μg of imipenem per ml, there are fewer datum points within the range of these panels.

Inoculum preparation. Several colonies from overnight incubation on Trypticase soy agar–5% sheep blood plates at 35°C were suspended in demineralized water to match a 0.5 McFarland turbidity standard. Predried fluorogenic enzyme substrate paper strips were added to cation-supplemented MH broth with *N*-tris(hydroxymethyl)methyl-2-aminoethanesulfonic acid (TES) (Sensititre) and were incubated for 15 min at room temperature. A calibrated pipette was used to transfer 10 μl of

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TABLE 1. Comparison of susceptibility test results for imipenem by using broth macrodilution and Sensititre panels to examine 12 strains of *P. aeruginosa*

Strain	Broth macrodilution imipenem MIC (μg/ml)	Results of Sensititre panels tested in duplicate						
		Imipenem MIC (μg/ml)			BP[a]		BP range[b]	
		At time of purchase	At expiration	Range[b]	At time of purchase	At expiration	Interpretation	MIC (μg/ml)
ATCC 27853	4	≤1, ≤1	2, 2	≤1–4	S, S	S, S	S	≤4
Psa 27	2	≤1, ≤1	≤1, ≤1	≤1–2	S, S	S, S	S	≤4
Psa 20	2	≤1, ≤1	≤1, ≤1	≤1	S, S	S, S	S	≤4
Psa 4	2	≤1, ≤1	2, 2	≤1–2	S, S	S, S	S	≤4
Psa 1	2	≤1, 2	4, 2	≤1–4	S, S	S, S	S	≤4
Psa 14	4	≤1, ≤1	≤1, 2	≤1–4	S, S	S, MS	S–MS	≤4–8
Psa 18	4	≤1, ≤1	2, 2	≤1–2	S, S	S, S	S	≤4
Psa 16	4	≤1, ≤1	4, 4	≤1–4	S, S	S, S	S	≤4
Psa 31	8	4, 4	4, 16	4–16	S, S	MS, MS	S–MS	≤4–8
Psa 9	16	8, 8	16, 16	8–16	MS, MS	R, R	MS–R	8–>8
Psa 21	32	8, 8	4, 32	2–32	MS, MS	R, R	MS–R	8–>8
Psa 25	32	16, 16	32, 32	16–32	R, R	R, R	R	>8

[a] S, susceptible; MS, moderately susceptible; R, resistant.
[b] Includes all results obtained during the 15-month study period.

the inoculum into the MH broth, resulting in a bacterial concentration of 10^5 CFU/ml.

Susceptibility testing procedure. Isolates were tested for susceptibility to imipenem by a standard Bauer-Kirby agar disk diffusion method according to accepted guidelines (6a). Standard broth macrodilution testing was performed using MH broth and imipenem standard powder (946 μg/mg; lot 7372V; expiration, December 1993; Merck Human Health Div., West Point, Pa.). Broth microdilution testing using Sensititre MIC and custom BP panels was performed according to the manufacturer's instructions using a single lot of each type of panel stored at room temperature under usual laboratory conditions. Panels were received 15 months prior to the stated expiration date, 3 months after manufacture, so that testing was done 3, 4, 6, 9, 12, 15, and 18 months after the date of panel manufacture. Panels with the desiccant of the desired blue color were inoculated with the Sensititre autoinoculator and were incubated at 35°C in room air for 18 to 24 h. The 12 isolates were run in duplicate by different technologists and interpreted both by the Sensititre autoreader and visually with a mirrored-light view box. A difference of 1 twofold MIC dilution or one panel well was considered acceptable performance in comparison of susceptibility results.

Assay of imipenem concentrations. At study time points between 4 and 18 months, the concentration of imipenem at each dilution in each type of panel was assayed by high-pressure liquid chromatography (HPLC) (Merck Manufacturing Div., Elkton, Va.). Imipenem was reconstituted with phosphate buffer, pH 6.8, and concentrations were determined after incubation for 1 h at room temperature. Assay results were determined with an imipenem reference standard in a pH 6.8 buffer matrix.

Results. Of the 12 isolates chosen, 9 were susceptible and 3 were resistant to imipenem by Bauer-Kirby testing, and these results were in agreement with the results obtained by broth macrodilution testing. With the Sensititre panels and the autoreader, and comparing the time of panel receipt with the panel expiration date, there was acceptable agreement in test results throughout the time that the panels were stored (Table 1). We observed only one (strain Psa 16) notable (>1 twofold dilution and sustained in duplicate testing) change (MIC rose from ≤1 to 4 μg/ml) in the susceptibilities of the 12 *P. aeruginosa* isolates to imipenem. Test-to-test variability (greater than 1-tube-dilution difference in MIC) was observed among both the susceptible and resistant strains and occurred as frequently between tests done at the same time point as between those done at different time points. The control strain tested within the desired range in both the MIC and BP panels

TABLE 2. Labeled versus measured (within 1 h of reconstitution) concentrations of imipenem in Sensititre panels tested by HPLC

Plate	Imipenem concn (μg/ml)						
	Labeled	Measured at indicated time (mo) after plate manufacture:					
		4	6	9	12	15	18[a]
MIC	64	71.0	66.4	66.4	59.3	88.2	61.2
	32	39.0	36.4	36.0	33.3	34.3	27.4
	16	16.7	16.8	16.4	13.7	15.4	10.8
	8	8.0	8.3	8.1	6.4	7.8	5.3
	4	3.9	4.2	3.9	3.2	4.5	3.5
	2	1.8	1.8	1.8	1.7	2.2	2.2
	1	1.1	1.2	1.2	1.0	1.3	1.5
BP	8	8.4	7.7	5.9	6.4	6.0	4.5
	4	3.8	3.5	3.6	3.1	2.9	2.0

[a] Manufacturer's expiration date.

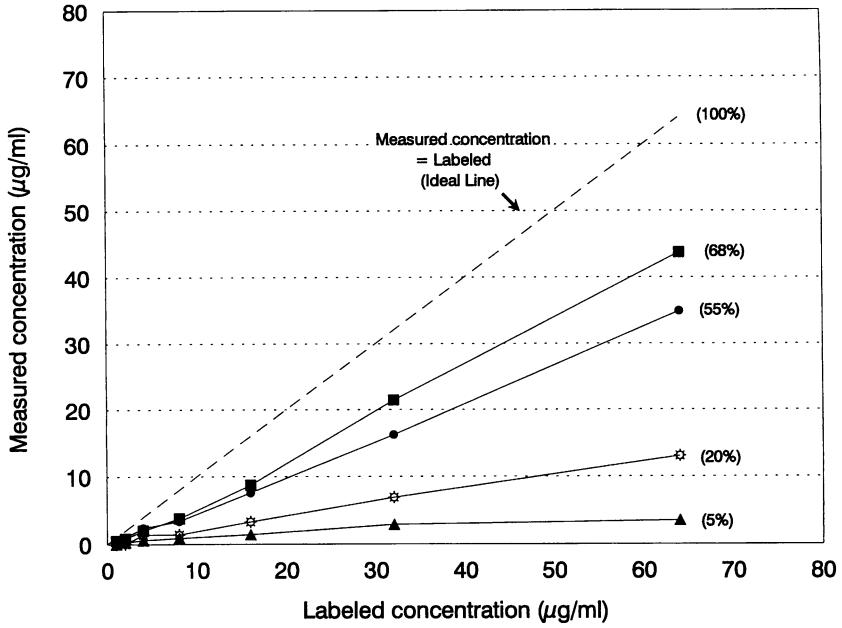

FIG. 1. Comparison of labeled concentration of imipenem with concentration measured by HPLC in wells from panels contained in four different packages with pink desiccants. Different symbols represent different panels. The dashed line represents the ideal in which measured concentration would equal the target concentration. Percentages of ideal are for the 64-μg/ml well.

during the entire panel shelf life, but the MIC increased from ≤1.0 μg/ml at the time of panel purchase to 4.0 μg/ml at 12 and 15 months but then fell to 2.0 μg/ml at panel expiration. Results of duplicate testing at each time point were within 1 twofold dilution in 96.5% of the paired assays. Visually determined MICs agreed with automated readings in 98.6% of the determinations. Very major errors (a resistant organism labeled as susceptible) occurred only when testing strain Psa 21. The MIC for this strain, which was resistant by disk diffusion and broth macrodilution testing, was in the susceptible range in 4 of 14 determinations by the Sensititre system. We cannot explain the phenomenon, but it could be due to instrument variability or a characteristic unique to this strain. Results bore no relationship to the age of the panels.

Imipenem concentrations measured by HPLC correlated with labeled values for MIC panels tested at 1 h after reconstitution (Table 2). HPLC measurements using the BP panels revealed similar results, but at 18 months a 50% decline in imipenem concentration occurred. After storage of the panels for 6 months in our laboratory, pink silica gel desiccants, indicating moisture, were found. Between 9 months and panel expiration, 13 of 141 (9%) of the packaged panels showed pink desiccants and would need to be discarded by a clinical laboratory. HPLC showed a marked decline in imipenem concentration in these panels, ranging from 68 to 5% of labeled concentration in the 64-μg/ml well. A corresponding decrease was seen in all the MIC panel wells, as illustrated in Fig. 1.

Discussion. Since imipenem degrades more rapidly than many other antimicrobial agents, susceptibility testing using both frozen and dry-format broth microdilution panels has been problematic (1, 6, 8, 11). On the basis of our longitudinal testing results, we conclude that the revised Sensititre manufacturing process produces MIC and BP panels that are adequate for testing imipenem susceptibility of *P. aeruginosa* for up to the recommended panel expiration date. There are individual strains in this study that produced variable test results that cannot be explained, but a small number of minor errors are typical when any microdilution susceptibility testing product is evaluated. Despite the low imipenem concentrations found in some wells by HPLC, susceptibility results using the bacterial strains were stable throughout the 18-month period. A 50% loss of activity in HPLC is theoretically equivalent to a difference of 1 twofold dilution in determining an MIC result. Thus, a 50% loss of imipenem activity would be difficult for a clinical laboratory to detect. This loss would be more likely to result in false detection of resistance when testing strains for which MICs are close to the 4.0-μg/ml susceptibility BP.

The number of unusable panels, as indicated by the presence of pink desiccants in the packages, was high (9%) during the last 9 months of the panels' shelf life, suggesting that a change in manufacture or packaging may be necessary to decrease the entry of moisture. Laboratory staff should carefully inspect the desiccant package for a pink color, indicating contamination by moisture, and should not use these panels because of marked degradation of imipenem. In contrast to previous reports using older panels, we found that the currently manufactured panels provide reliable testing results with the control strain of *P. aeruginosa* (ATCC 27853) when stored under usual laboratory conditions for the manufacturer's recommended shelf life.

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