LETTER TO THE EDITOR

External Factors Affecting Imipenem Performance in Dried Microdilution MIC Plates

I am writing in reference to the articles "Pseudoresistance of *Pseudomonas aeruginosa* Resulting from Degradation of Imipenem in an Automated Susceptibility Testing System with Predried Panels" (6) and "False Resistance to Imipenem with a Microdilution Susceptibility Testing System" (4).

On the basis of our extensive research with imipenem, we at Sensititre believe that while slow deterioration is inevitable with imipenem, the widely discrepant results reported by O'Rourke et al. and White et al. may be attributed more to external effects such as those described throughout this letter than to moderate losses of potency during storage.

Initially we could not duplicate the shift in the imipenem susceptibility results obtained by O'Rourke et al. and White et al. As a result of our inability to fully explain the variability between the acceptable results obtained with reserve batches of plates in our hands and the results obtained by White and O'Rourke et al., we worked in conjunction with the manufacturers of imipenem, Merck, Sharp and Dohme, to resolve the problem.

In the interim, we changed our manufacturing process to ensure greater stability of imipenem in our plates. The authors of both these articles (4, 6) were kept informed of the progress of our work and of the interim steps taken to protect the integrity of our product. We also reduced the shelf life of imipenem to 12 months as a safeguard to our customers. In the course of our intensive investigation, additional factors that give a much more complex picture than was described in either publication emerged. It was discovered that the following variables also affect the stability of imipenem.

(i) Moisture. The MIC for the quality control (QC) organism *Pseudomonas aeruginosa* ATCC 27853 was measured by using 166 batches of plates from retention stocks held by the manufacturer. These covered plates stored for up to 3 years. Imipenem MICs for only 1.6% (6 of 374) of the strains were outside the National Committee for Clinical Laboratory Standards QC range of 1 to 4 μg/ml, indicating that the panels performed correctly when the self-indicating desiccant was blue (showing that there had not been ingress of moisture), whereas the MIC results with the small number of plates which had pink silica gel sachets upon opening were aberrant for 53.2% of isolates tested.

(ii) Broth. It has been reported (3) that different lots of Mueller-Hinton broth obtained from different commercial vendors can result in unacceptably high imipenem MICs with the QC strain *P. aeruginosa* ATCC 27853. This was not observed with other QC strains, including *Escherichia coli* ATCC 25922 and *Staphylococcus aureus* ATCC 29213.

TABLE 1. Imipenem MICs obtained by using broths from 2 vendors

Broth	No. of strains with the following MIC (μg/ml):			
	16	8	4	2
A	3	13	0	0
B	0	0	9	3

TABLE 2. Imipenem MICs obtained by using different strains of *P. aeruginosa*

Strain	No. of strains with the following MIC (μg/ml):			
	1	2	4	8
A	3	17	45	35
B	5	66	26	3

Work undertaken by Sensititre substantiates this observation. The data shown in Table 1 were obtained by using plates dosed with the same inoculum of *P. aeruginosa* ATCC 27853 which had been assayed in broths from two vendors. The expected MIC is 1 to 4 μg/ml. Recently reported findings by White et al. (5) confirm this observation. White et al. observed a shift in the modal MIC of imipenem for *P. aeruginosa* ATCC 27853 from 2 to 8 μg/ml in different lots of broth. Time-kill studies showed that this was due to differences in the rate of degradation of imipenem in broth, in the time to regrow, and in the concentration of organism after 24 h of incubation. They also presented data showing that acceptable QC results can be obtained with *P. aeruginosa* ATCC 27853 on Sensititre panels stored for up to 18 months when dosed with appropriate broth.

A recent report (2) has shown that the zinc content in lots of Mueller-Hinton agar varies from vendor to vendor. Higher zinc content was associated with higher MICs of imipenem for *P. aeruginosa* but not for other gram-negative bacilli. Further studies are required to see whether this observation explains the effect observed by White et al. (5).

(iii) Organism. The condition of the *P. aeruginosa* ATCC 27853 QC strain can affect the MIC of imipenem by 1 to 2 doubling dilutions. The results shown in Table 2 were obtained from 100 determinations of a strain freshly obtained from the American Type Culture Collection (strain B) compared with a strain that had had multiple passaging (strain A). In general, the best results are obtained with strains that have had minimal subculturing.

REFERENCES

1. **Baron, E. J., and J. A. Hindler.** 1984. Bioactivity of imipenem as a function of medium, time, and temperature. Antimicrob. Agents Chemother. **25**:781–782.
2. **Cooper, G., A. Louie, R. Chu, R. P. Smith, W. Ritz, and M. Franke.** 1991. The role of zinc (Zn) on the activity of imipenem-cilastatin (IM) against *Pseudomonas aeruginosa*, abstr. C-145, p. 366. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
3. **Nadler, H., D. Pitkin, and W. Sheikh.** 1989. The effect of culture medium source on antimicrobial activity of carbapenems. Abstr. 29th Intersci. Conf. Antimicrob. Agents Chemother., abstr. 230.
4. **O'Rourke, E. J., K. G. Lambert, K. C. Parsonnet, A. B. Macone, and D. A. Goldman.** 1991. False resistance to imipenem with a microdilution susceptibility testing system. J. Clin. Microbiol. **29**:827–829.
5. **White, R. L., M. B. Kays, and L. V. Friedrich.** 1991. Effect of media and drug degradation during in vitro testing on the activity of imipenem, abstr. A-20, p. 4. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.

6. **White, R. L., M. B. Kays, L. V. Friedrich, E. W. Brown, and J. R. Koonce.** 1991. Pseudoresistance of *Pseudomonas aeruginosa* resulting from degradation of imipenem in an automated susceptibility testing system with predried panels. J. Clin. Microbiol. **29**:398–400.

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Author's Reply

We would like to acknowledge that the findings cited in our previous report may only apply under the conditions that existed in our institution during the time of the study. We suggest that our original report be read in its entirety. A number of details mentioned by Dr. Grist were addressed in the manuscript (3).

As emphasized by Dr. Grist, the complexity of this issue made it quite difficult to isolate the various factors involved. However, we and others (1, 3) have demonstrated degradation of imipenem indirectly through the use of MIC and breakpoint testing and directly through assay of panels by both microbiologic and high-performance liquid chromatographic methods.

Although the external effects suggested by Dr. Grist may have contributed to the discrepancies, degradation of approximately 50% over 1 year represents a substantial loss of potency. The "acceptable" results found by Sensititre with reserve batches may be due to the controlled conditions of their study and especially to the lack of exposure to shipping conditions.

We investigated the variables which may affect the results of microbiologic testing mentioned by Dr. Grist (with the exception of the zinc studies). As stated in our report, we only used panels with blue dessicants, suggesting that moisture was not a factor in our findings. As suggested by Sensititre, we obtained a fresh QC strain of *P. aeruginosa* ATCC 27853. However, we were able to duplicate our previous results obtained with the strain that had been subcultured.

As indicated by Dr. Grist, we reported the effects of broth on the in vitro activity of imipenem (2). It is important to note that we found that some lots of broth produced MIC results within acceptable QC limits despite the fact that panels in which only 40% of the stated potency of imipenem remained were used. However, the discrepancies due to imipenem degradation were obtained with broth supplied by Sensititre that was within its stated expiration date at the time of the study.

In summary, we tested for many of the factors that may contribute to variability during in vitro susceptibility testing and demonstrated that drug degradation substantially contributed to a decline in *P. aeruginosa* susceptibility. Our initial and interim findings were communicated to Sensititre. Although it was initially suggested that imipenem degradation was an insignificant factor, the change in the manufacturing process and the reduction in the shelf life suggest otherwise. We applaud the recent efforts by Sensititre, especially those of Dr. Grist, in addressing this problem.

REFERENCES

1. **O'Rourke, E. J., K. G. Lambert, K. C. Parsonnet, A. B. Macone, and D. A. Goldmann.** 1991. False resistance to imipenem with a microdilution susceptibility testing system. J. Clin. Microbiol. **29**:827–829.
2. **White, R. L., M. B. Kays, and L. V. Friedrich.** 1991. Effect of media and drug degradation during in vitro testing on the activity of imipenem, abstr. A-20, p. 4. Abstr. 91st Gen. Meet. Am. Soc. Microbiol. 1991. American Society for Microbiology, Washington, D.C.
3. **White, R. L., M. B. Kays, L. V. Friedrich, E. W. Brown, and J. R. Koonce.** 1991. Pseudoresistance of *Pseudomonas aeruginosa* resulting from degradation of imipenem in an automated susceptibility testing system with predried panels. J. Clin. Microbiol. **29**:398–400.

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