Failure of Quality Control Measures To Prevent Reporting of False Resistance to Imipenem, Resulting in a Pseudo-Outbreak of Imipenem-Resistant *Pseudomonas aeruginosa*

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False results showing an outbreak of *Pseudomonas aeruginosa* with resistance to imipenem were traced to a defective lot of microdilution MIC testing panels. These panels contained two- to threefold lower concentrations of imipenem than expected and resulted in artificial two- to fourfold increases in MICs of imipenem. The quality-control MIC results for *Pseudomonas aeruginosa* ATCC 27853 were 4 μg/ml, the highest value within the range recommended by the National Committee for Clinical Laboratory Standards. We recommend that this value be considered out of the quality-control range.

False determinations of resistance to imipenem by a microdilution testing system have been attributed to degradation of imipenem in the testing panels after their prolonged storage (2, 8, 9). The quality-control measures recommended by the National Committee for Clinical Laboratory Standards (NCCLS) are believed to prevent false determinations of resistance (3, 5). In our institution during the 2-year period from September 1994 to August 1996, 12% of all *Pseudomonas aeruginosa* isolates were resistant to imipenem (7). On the basis of data from a relational clinical database (6), this rate increased to 23% starting in September of 1996 and prompted an investigation. We report here results showing an outbreak of *P. aeruginosa* with false resistance to imipenem despite adherence to NCCLS quality-control guidelines.

*P. aeruginosa* was identified from clinical specimens submitted to the microbiology laboratory by using gram-negative type II identification panels (Dade International Inc., West Sacramento, Calif.). Susceptibility to imipenem was determined by microdilution broth testing (MicroScan; Dade International Inc.). Testing was performed according to the manufacturer’s instructions. Inocula were prepared by using the Prompt Inoculation System (MicroScan).

Some isolates were also tested for susceptibility to imipenem by a standard Kirby-Bauer agar disk diffusion method according to NCCLS guidelines (4). A case-control study was implemented to investigate the apparent outbreak of imipenem-resistant *P. aeruginosa*. By searching the microbiology laboratory database, every patient identified between 15 September and 31 October 1996 was considered to be a case patient if the *P. aeruginosa* isolate was resistant or intermediate to imipenem (MIC, ≥8 μg/ml) or a control patient if the isolate was susceptible to imipenem (MIC, <8 μg/ml). Variables considered in the case-control study included admission service, ward of hospitalization, whether a patient shared a room with a case patient or with a patient who shared a room with a case patient, stay in an intensive care unit, dates and types of procedures performed in the operating room, type of procedure performed, surgeon performing the procedure, transfer from another hospital or chronic care facility, and previous imipenem exposure. Isolates from four case patients were available and were studied by pulsed-field gel electrophoresis (PFGE) as previously described (1).

During the outbreak period, a specific lot of an MIC testing panel was used (dried, gram negative, urine panel type 9, lot 8/17/97, MicroScan; Dade International). To examine the possibility of false resistance, imipenem MIC test results for *P. aeruginosa* and other gram-negative rods for two periods were compared. Time B (15 September to 28 December 1996) consisted of the period during which the specific lot of the MIC testing panel described above was used. Time A (15 September 1995 to 14 September 1996) consisted of the year before the outbreak, during which 18 different MIC testing panel lots were used.

Fourteen isolates for which imipenem MICs were found to be ≥4 μg/ml when they were tested with the outbreak lot were retested with another MIC testing panel lot (dried, gram negative, combo panel type 20, lot 12/18/97, MicroScan; Dade International). Imipenem MIC weekly quality-control results for *P. aeruginosa* (ATCC 27853) and for *Escherichia coli* (ATCC 25922) for the different MIC testing panel lots used during time A and time B were compared. Fresh American Type Culture Collection strains were used during both periods.

The concentration of imipenem at each dilution in the MIC panels from five different lots was determined by high-pressure liquid chromatography (HPLC). The content of each well was reconstituted with phosphate buffer (pH 6.8), diluted to 1 ml, and assayed for imipenem by HPLC (TSPC 1000) after 1 h of incubation at room temperature. The assay results were determined against an imipenem reference standard in a pH 6.8 buffer matrix. Both the area count method and the peak-height-calculation method were used to determine the results.

All of the statistical analyses were performed with Stata Corp. (College Station, Tex.) version 5 software. Fisher’s exact test was used for discrete variables. The logarithms of the MICs during the two time periods were compared by a nonparametric test (Kruskal-Wallis). The differences between

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pairs of MIC results (the same isolate tested with two different panel lots) were compared by the paired Student t test. Analysis of variance was used to examine the variability of the MIC for the quality control strain across the different lots used during the year prior to the outbreak period. All P values calculated were two-tailed.

**Case-control study.** During the 6-week period (15 September to 31 October 1996) investigated in the case-control study, 16 cases and 49 controls were identified. Ten of the case patients were inpatients, and six were outpatients. No common source or epidemiological link was identified between the cases. Case patients were not exposed to imipenem more often than control patients. *P. aeruginosa* isolates from four case patients were studied by PFGE (Fig. 1). Each of these isolates had a distinct PFGE pattern. We examined the possibility of one of the isolates were the results concordant (within least fourfold when the isolates were retested with the second lot of the same manufacturer) and since 15 November 1996 also for *P. aeruginosa* isolates that for each imipenem MIC testing dilution examined, the MIC results for the quality-control *E. coli* strain (ATCC 25922) were ≤1 µg/ml in the two periods.

To find whether an imipenem MIC of 4 µg/ml is an unusual result for the quality-control *P. aeruginosa* strain (ATCC 27853) we examined the distribution of the quality-control results during time A. An imipenem MIC of 4 µg/ml was recorded in 6 of 55 (11%) tests during time A. Only three lots were implicated. Two of these lots were used for only one week; thus, the quality-control strain was tested only once for each. Another of these lots (dried, gram negative, urine panel type 9, lot 9/20/96, MicroScan; Dade International) was used for 4 weeks, and all quality-control imipenem MIC results were 4 µg/ml.

**Comparison of results of susceptibility tests.** In our laboratory, imipenem resistance as measured by MicroScan is routinely confirmed by Kirby-Bauer disc diffusion for Enterobacteriaceae and since 15 November 1996 also for *P. aeruginosa*. Discrepant results between the microdilution and the disc diffusion results for Entrobacteriaceae were found for 27 of 37 (73%) isolates in time A and for 42 of 46 (91%) isolates in time B (odds ratio, 3.9; \( P = 0.04 \)). Fourteen *P. aeruginosa* isolates were found to be imipenem resistant by the implicated lot after 15 November 1996; all of these isolates tested susceptible by disc diffusion.

Fourteen *P. aeruginosa* isolates for which the imipenem MICs were determined to be ≥4 µg/ml with the implicated lot were retested with another lot of the same manufacturer (dried, gram negative, combo panel type 20, lot 12/18/97). Imipenem MICs for 11 of the 14 isolates (79%) decreased at least fourfold when the isolates were retested with the second panel, and those for two isolates decreased twofold; for only one of the isolates were the results concordant (\( P < 0.001 \)).

**Quality-control results.** Weekly imipenem MIC results for the quality-control *P. aeruginosa* strain (ATCC 27853) were within the recommended range (≤1 to 4 µg/ml) both in time A and in time B, but the 25th, 50th, and 75th percentiles of MICs were 1, 2, and 2 µg/ml, respectively, in time A and 4, 4, and 4 µg/ml, respectively, in time B (\( P < 0.001 \)). The imipenem MIC results for the quality-control *E. coli* strain (ATCC 25922) were ≤1 µg/ml in the two periods.

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**Imipenem concentration.** The concentrations of imipenem as measured by HPLC are shown in Table 2. Analysis revealed that for each imipenem MIC testing dilution examined, the implicated lot had concentrations two- to threefold lower than those of the other four panels tested.

We investigated an increased incidence of imipenem-resistant *P. aeruginosa*. No epidemiological link was found between the cases, and the isolates differed in their PFGE patterns. Therefore, we studied the hypothesis of false resistance and

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**TABLE 2. Imipenem concentrations as measured by HPLC**

<table>
<thead>
<tr>
<th>Reference</th>
<th>Imipenem concn (µg/ml) at imipenem MIC testing dilution (µg/ml):</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>1</td>
</tr>
<tr>
<td>Implicated lot</td>
<td>0.3</td>
</tr>
<tr>
<td>Control lot 1</td>
<td>1.0</td>
</tr>
<tr>
<td>Control lot 2</td>
<td>0.8</td>
</tr>
<tr>
<td>Control lot 3</td>
<td>4.6</td>
</tr>
<tr>
<td>Control lot 4</td>
<td>3.8</td>
</tr>
</tbody>
</table>

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*a* All the MIC testing panels examined are products of MicroScan (Dade International). The following panel lots were tested (each lot number is similar to the expiration date): implicated lot, dried, gram negative, urine panel type 9, lot 8/17/97; control lot 1, dried, gram negative, urine panel type 8, lot 9/20/97; control lot 2, dried, gram negative, urine panel type 9, lot 11/25/97; control lot 3, dried, gram negative, urine combo panel type 1, lot 28/10/97; and control lot 4, dried, gram negative, combo panel type 13, lot 9/17/97. The implicated lot had two- to threefold-lower concentrations than the control lots.
found that the imipenem MIC results shifted towards higher values both for clinical isolates (P. aeruginosa and other gram-negative rods) and for the quality-control P. aeruginosa isolate (ATCC 27853). The increase in MIC was of a magnitude of two- to fourfold (one to two dilutions). Repeated testing of some isolates with panels from a different lot confirmed that the artifactual increase in MIC associated with the implicated lot was fourfold. These false results were related to imipenem concentrations two- to threefold lower than expected for the implicated panel. Imipenem stability in a predried susceptibility panel have been shown to be reduced after prolonged storage (2, 8). We do not think that the false resistance related to the implicated lot was secondary to this phenomenon, since the panel was used 9 to 11 months before its expiration date. It was also not related to the storage conditions, as three different shipments of this lot were used. Thus, we believe that a manufacturing error resulted in less-than-expected imipenem concentrations in the implicated lot. This error should have been detected and prevented by quality-control measures. Similarly high imipenem MIC quality-control results were retrospectively found for another lot. The quality-control criteria recommended by the NCCLS are an MIC of ≤1 to 4 μg/ml for P. aeruginosa ATCC 27853. This outbreak demonstrates that this MIC range may be too broad, and an MIC of 4 μg/ml should not be included within the acceptable range.

In conclusion, a specific MIC testing panel lot contained imipenem concentrations two- to threefold lower than stated. The quality-control measures failed to detect this faulty lot, which resulted in a shift of imipenem MIC results and a pseudo-outbreak of imipenem-resistant P. aerugi-nosa. Stricter quality-control measures are needed to prevent similar false-resistance results in the future. We suggest that an imipenem MIC of 4 μg/ml for the quality control strain P. aeruginosa ATCC 27853 should be considered unacceptable. Alternatively, strains with known resistance mechanisms and for which imipenem MICs are higher can be used as quality-control strains (8). Until such measures are implemented, we recommend that laboratories using MicroScan panels routinely confirm imipenem resistance by other susceptibility testing methods.

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REFERENCES