

## Pseudo-Outbreak of Imipenem-Resistant *Acinetobacter baumannii* Resulting from False Susceptibility Testing by a Rapid Automated System

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**Introduction of the Vitek GNS-506 susceptibility testing cards in the Hippokraton General Hospital, Thessaloniki, Greece, resulted in an apparently high prevalence of imipenem-resistant *Acinetobacter baumannii*. When 35 of these isolates were further tested by disk diffusion, broth microdilution, and agar dilution assays, 32 were imipenem sensitive by all tests and three were sensitive or intermediate, depending on the method. The pseudoresistant acinetobacters did not form a genetically homogeneous group. It is suggested that the detection of imipenem-resistant *A. baumannii* isolates by this system should be confirmed by an additional susceptibility test.**

*Acinetobacter baumannii* is increasingly implicated in hospital-acquired infections, mostly affecting debilitated patients in intensive care units, in whom such infections are associated with high mortality rates (1). The administration of appropriate antimicrobial therapy to these patients is therefore essential. The organism under appropriate selection pressure can easily become resistant to multiple antibiotics (i.e., newer  $\beta$ -lactams, aminoglycosides, and fluorinated quinolones), and currently, high proportions of acinetobacters exhibit cross-resistance to these drugs (1, 6, 9). Carbapenems usually retain good potency, with imipenem being the most active against *A. baumannii* (1). However, outbreaks due to imipenem-resistant acinetobacters are sporadically reported, and usually none of the clinically available antibiotics can be used (2, 15). In most of these cases, resistance to imipenem was related to the activity of a carbapenemase (2, 11, 12).

Previous data from our region have shown that acinetobacters are among the most frequent nosocomial pathogens, and the majority of them are multidrug resistant (6). However, imipenem-resistant acinetobacters have not been reported in Greece. As part of monitoring the antibiotic resistance trends at Hippokraton General Hospital, Thessaloniki, Greece, we noted that after the introduction of the Vitek automated system (bioMerieux, Hazelwood, Mo.) for susceptibility testing in May 1998, a considerable proportion of acinetobacters from different departments of the hospital were detected as imipenem resistant. This proportion reached 27.6% in 1998 and 22.1% in 1999, while no resistant isolates had been detected prior to 1998. After this observation, we evaluated the accuracy of susceptibility to imipenem for *A. baumannii* reported by the system.

During the study period (May 1998 to December 1999), 269 *A. baumannii* isolates were recovered from clinical infections of 185 hospitalized patients. They were tested for susceptibility

to imipenem with the Vitek system, by using GNS-506 cards that were stored at 4°C and used before the expiration date. During the study period 12 different lots of cards were used, and when each lot was in use, cards were randomly selected from the inventory present in the lab. Susceptibility testing was performed according to the instructions of the manufacturer and applying the criteria prescribed by the National Committee for Clinical Laboratory Standards (NCCLS) (7). Sixty-six (24.5%) of the isolates recovered throughout the whole study period were characterized as imipenem resistant (MIC,  $\geq 16$   $\mu\text{g/ml}$ ) and eight (3.0%) as imipenem intermediate (MIC, 8  $\mu\text{g/ml}$ ). Imipenem-resistant or -intermediate isolates were obtained by using cards from 10 lots. During the study period, two isolates were recovered from the same patient in 61 cases, while seven patients yielded three isolates and three others yielded four isolates. In all but five cases, the susceptibility results were the same for the repetitive isolates. Among these five patients, who yielded two isolates each, one isolate was characterized as resistant and the other as susceptible in four cases, and one isolate was characterized as resistant and the other as intermediate in one case. *Pseudomonas aeruginosa* (ATCC 27853) was used weekly as a control, and the MIC for this strain was consistently characterized as  $\leq 4$   $\mu\text{g/ml}$ .

Fifty isolates were selected for further testing by disk diffusion, agar dilution, and broth microdilution MIC assay methods. Thirty-five of them were chosen randomly from the isolates characterized as imipenem resistant by the system, and 15 were also obtained at random from acinetobacters characterized as imipenem intermediate (two isolates) or imipenem sensitive (13 isolates). These susceptibility methods were inoculated concurrently from the same bacterial preparation. For determination of MICs by the agar and broth dilution methods, imipenem solutions (Merck Sharp and Dohme, West Point, Pa.) and MIC plates were prepared on the day of testing. The agar dilution technique was performed according to the recommendations of the NCCLS (7), by inoculating  $10^4$  CFU/spot onto Mueller-Hinton agar plates (BBL, Cockeysville, Md.) containing antibiotic dilutions of 0.06 to 64  $\mu\text{g/ml}$ . The MIC was recorded as the lowest drug concentration at which no growth occurred. For determination of MICs by the broth

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microdilution method (7), broth cultures were diluted to yield  $5 \times 10^5$  CFU/ml in each well of the microtiter plates containing twofold serial antibiotic dilutions (0.06 to 64  $\mu\text{g/ml}$ ) in cation-supplemented Mueller-Hinton broth (BBL). The MIC was defined as the lowest concentration of the drug that resulted in no visible growth. Disk diffusion testing was performed as recommended by the NCCLS (8). Control strains of *Pseudomonas aeruginosa* (ATCC 27853) and *Escherichia coli* (ATCC 25922) were included in each assay. They were recovered from multiple cultures at  $-70^\circ\text{C}$  and were subcultured once before testing. Susceptibility categories were assigned according to NCCLS interpretative criteria (7, 8).

By the agar dilution method, 47 of the 50 isolates were susceptible to imipenem. Three isolates, characterized as resistant by the Vitek system, were imipenem intermediate. The MIC for 24 of the imipenem-resistant isolates was 2  $\mu\text{g/ml}$ , and the MIC for the remaining 8 isolates was 4  $\mu\text{g/ml}$ ; the MICs for the sensitive or intermediate isolates by the system were lower, ranging from 0.125 to 2  $\mu\text{g/ml}$ . Using broth microdilution, all isolates were imipenem sensitive except two, which were intermediate. MIC ranges were identical to those of the agar dilution assay for isolates characterized as resistant, intermediate, or sensitive by the Vitek. By disk diffusion, all isolates were defined in the susceptible category. The quality control MIC results were 2  $\mu\text{g/ml}$  for *P. aeruginosa* ATCC 27853 and 0.125  $\mu\text{g/ml}$  for *E. coli* ATCC 25922 by both the agar and broth dilution methods. Twelve of the 35 isolates classified as imipenem resistant in their initial isolation were retested using cards from five newly acquired lots. In these assays the same inoculum preparation was used with the standard MIC assay methods. Of the 60 determinations, values were within the susceptible range for 13 (21.7%), intermediate for 5 (8.3%), and resistant for 42 (70%) of them.

PCR testing of the 35 initially resistant isolates for carbapenemase genes detected in our country or neighboring countries (*bla*<sub>IMP-1</sub> and *bla*<sub>VIM-1</sub>) was done using published primers and conditions (14, 17). These genes were not detected in any isolate. Pulsed-field gel electrophoresis of *Sma*I-digested genomic DNA was performed with a CHEF-DRIII system (Bio-Rad, Hemel Hempstead, England) (13). The technique was applied to the 35 isolates, and banding patterns were compared visually by following the criteria of Tenover et al. (16). Five different genotypes were defined, each containing 3 to 15 isolates (data not shown).

Discrepancies of resistance to imipenem by automated systems have been described previously in *P. aeruginosa* (3, 4, 10, 18), but similar observations have not been reported for acinetobacters or other microorganisms for which the MIC ranges of imipenem are also relatively high. Although imipenem is stable in solution at  $-70^\circ\text{C}$  for up to 1 year, it can deteriorate over time, even in a predried format stored as recommended by the manufacturer (18). In previous reports, imipenem concentrations were determined by high-performance liquid chromatography (3, 10, 18). It has been shown that the amount of imipenem degrades over time, resulting in false increases in MICs of imipenem for *P. aeruginosa* clinical isolates. Thus, the false resistance of acinetobacters might be due to a lower-than-expected concentration of imipenem by the time the cards were used in the laboratory.

Variations of at least three dilutions were usually observed when we compared the Vitek cards with the standard dilution methods. Similar discrepancies were noticed previously with an automated system when *P. aeruginosa* isolates were tested for susceptibility to imipenem, and a loss of drug potency in customized trays was documented (10). The importance of inoculum size as a primary determinant in the accuracy of suscep-

tibility testing with the Vitek system was demonstrated previously and was more obvious when cell wall-active antimicrobials were tested (5). In our study this was possibly not the case, since care was exercised to use an inoculum equivalent to that recommended by the manufacturer, and the same inocula were used in the standard susceptibility assays as well as in the repeats of susceptibility testing by the Vitek system. However, it is possible that the invalid MIC results cannot be totally attributed to the above factors, and our results may only apply under the circumstances in our laboratory as well as the shipping conditions during the study.

The recommended quality control of the imipenem susceptibility testing using *P. aeruginosa* ATCC 27853 was unable to identify the problem. It could be noted that a previous study has shown that an imipenem MIC of 4  $\mu\text{g/ml}$  for *P. aeruginosa* ATCC 27853 should not be included within the acceptable range (3). Since the Vitek cards contain only two wells, with 4 and 8  $\mu\text{g}$  of imipenem per ml, the characterization that the MIC of imipenem for the reference strain is  $\leq 4$   $\mu\text{g/ml}$  does not allow the estimation of the precise MIC measured by the system. Thus, susceptibility cards may include a well with an imipenem concentration of 2  $\mu\text{g/ml}$  in order to estimate more sufficiently the range of MICs for the control strains as well as the susceptible bacterial population. It would also be recommended that when an acinetobacter isolate is characterized as resistant to imipenem by the Vitek system, an additional simple test, such as the disk diffusion assay, might be used.

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