

## Mixed-Morphotype Broth Microdilution Susceptibility Testing of *Pseudomonas aeruginosa* from Cystic Fibrosis Patients

KENNETH G. VAN HORN

*Clinical Microbiology, Westchester County Medical Center, Valhalla, New York 10595*

Received 11 September 1992/Accepted 13 November 1992

**Multiple morphotypes of *Pseudomonas aeruginosa* isolated from 50 respiratory specimens of cystic fibrosis patients were tested for correlation of broth microdilution susceptibility results of a mixed-morphotype inoculum with a predicted antibiogram of the individual isolates. The overall correlation was 96.0%, with only 1.6% very major or major errors.**

*Pseudomonas aeruginosa* is an important cause of pulmonary infection in cystic fibrosis patients (3, 5, 6, 8, 11). Multiple colony morphotypes of *P. aeruginosa* are often isolated (2, 11), with susceptibility tests commonly performed on each distinct morphotype since colonizing strains cannot be distinguished from infecting strains. A single susceptibility test with a mixed-morphotype inoculum of *P. aeruginosa* would be advantageous and has been reported for disk diffusion (1) and for limited antimicrobial agents with a broth microdilution method (7). This study utilized a commercially available broth microdilution MIC method to correlate susceptibility results obtained with a standardized mixed-morphotype inoculum of *P. aeruginosa* isolated from cystic fibrosis patients with a predicted antibiogram based on susceptibility results of each individual morphotype. (This work was previously presented in part [12]).

The study sample consisted of 50 sputum cultures that grew more than one *P. aeruginosa* morphotype from 29 cystic fibrosis patients. A total of 143 individual *P. aeruginosa* isolates, identified by conventional biochemical methods (4), and the 50 mixed morphotypes were tested for susceptibilities with the Microscan Neg/Urine MIC 5 Panel (Baxter Diagnostics, Inc., West Sacramento, Calif.) according to the manufacturer's instructions. A standardized organism suspension was prepared with 5 to 10 well-isolated, freshly grown (24- to 48-h) colonies of each distinct morphology. The turbidity of each suspension was adjusted to that of a 0.5 McFarland turbidity standard. A mixed-morphotype suspension was prepared for each culture by adding 100  $\mu$ l of each individual morphotype suspension to a sterile tube. All suspensions were mixed well, and the final inoculum was prepared by adding 100  $\mu$ l of each suspension into 25 ml of inoculum water. Each MIC panel was inoculated with 115  $\mu$ l per well for a final concentration of approximately  $1 \times 10^5$  to  $4 \times 10^5$  CFU/ml. Each panel was incubated at 35°C for 18 to 24 h without CO<sub>2</sub> and read manually. The antimicrobial agents evaluated and the concentration ranges tested were amikacin and ceftazidime (2 to 16  $\mu$ g/ml); gentamicin and tobramycin (0.5 to 4 and 6  $\mu$ g/ml); mezlocillin, piperacillin, and ticarcillin (8 to 64  $\mu$ g/ml); ciprofloxacin (1 to 2  $\mu$ g/ml); and imipenem (1 to 8  $\mu$ g/ml). Ticarcillin-clavulanate and aztreonam, two additional anti-pseudomonad antibiotics used to treat cystic fibrosis patients, were not evaluated. The MIC was recorded as the lowest concentration of antimicrobial agent to show no visible growth. Susceptibility interpretations were made according to National Committee for Clinical Laboratory Standards guidelines (10).

The MICs for the mixed morphotypes were compared to

the individual-morphotype MICs and considered discrepant if the observed mixed-morphotype value varied  $\pm 2$  twofold dilutions from the highest individual-morphotype MICs. The susceptibility interpretations of the mixed-morphotype antibiogram were compared with the predicted antibiogram obtained from the most resistant cumulative pattern of the individual morphotypes. Discrepancies between the predicted and observed (mixed-morphotype) interpretive results were classified as very major (VM; predicted resistant, observed susceptible), major (M; predicted susceptible, observed resistant), and minor (MN; predicted or observed intermediately or moderately susceptible, other result susceptible or resistant). A standardized disk diffusion test (9) was performed for each isolate and mixed-morphotype inoculum and was used as the reference method to resolve discrepancies.

There were a total of 450 mixed-morphotype susceptibility determinations with an overall correlation of MICs for 447 (99.3%), with only two piperacillin and one imipenem mixed-morphotype MIC discrepant by at least 2 twofold dilutions from the predicted antibiogram. Off-scale values discrepant by 1 dilution at the off-scale value were not considered as twofold dilution discrepancies. This was a limitation of the concentration ranges of the antibiotics in the panels tested. These results compare favorably to results of a previous study (7) in which only three anti-pseudomonad antibiotics were tested.

The overall correlation between observed and predicted susceptibility interpretations was 96.0% (Table 1). Seventeen cultures grew 2 morphotypes, 24 cultures grew 3 morphotypes, 8 cultures grew 4 morphotypes, and 1 culture grew 5 morphotypes. There were four VM errors (two each for piperacillin and ticarcillin). There were three M errors (two for mezlocillin and 1 for piperacillin). There were 11 MN errors (4 for ceftazidime, 2 each for tobramycin and imipenem, and 1 each for ciprofloxacin, amikacin, and gentamicin). These data correlate well with data published for the disk diffusion method for testing mixed morphotypes of *P. aeruginosa* (1), except our data had fewer aminoglycoside discrepancies. Also, the accuracy of the mixed-morphotype susceptibility determinations did not appear to be affected by the number of individual morphotypes tested as previously reported (1). There were five individual morphotypes that failed to grow in the MIC panels; however, these were not considered to affect any predicted results since all five grew on Mueller-Hinton medium used for disk diffusion and were determined by disk diffusion to be no more resistant than predicted by the antibiogram.

TABLE 1. Interpretive susceptibility results of mixed morphotypes of *P. aeruginosa*

No. of morpho- types	No. of cultures	Total no. of obser- vations <sup>a</sup>	No. of discrepancies <sup>b</sup>			Total agreement (%)
			VM	M	MN	
2	17	153	2	1	4	95.4
3	24	216	0	1	5	97.2
4 or 5	9	81	2	1	2	93.8
Total	50	450	4	3	11	96.0

<sup>a</sup> Number of cultures tested with nine antimicrobial agents.

<sup>b</sup> VM, very major; M, major; MN, minor.

Disk diffusion was used to resolve any interpretation discrepancies, and on the basis of the disk diffusion results, the two VM piperacillin errors and all three M errors were due to incorrect MICs for the individual morphotypes. The individual results that led to the three major errors may have been due to poor growth of the individual isolates in the broth microdilution system. One of the VM errors was due to a difference of less than 1 dilution (>64 µg/ml versus 64 µg/ml with interpretations of resistant and susceptible, respectively). The remaining piperacillin VM error was discrepant by at least 2 twofold dilutions (>64 µg/ml versus 16 µg/ml). Only 5 of the 11 MN discrepancies were truly considered errors on the basis of disk diffusion results. Seven of the MN discrepancies were due to off-scale values that had one result resistant with a greater-than-MIC (>*x* µg/ml) value while the intermediate or moderately susceptible value was equal to but not greater than the same off-scale MIC (*x* µg/ml). Of the overall 18 original errors, the observed mixed-morphotype MIC was within less than one MIC of the predicted MIC in 12 cases. However, further clinical trials should be performed to establish whether mixed-morphotype testing might actually lead to better in vivo therapeutic results. Also, future studies to evaluate the performance of automated systems with the mixed-morphotype method might be beneficial since panels in this study were read manually.

Susceptibility testing of multiple *P. aeruginosa* isolates from cystic fibrosis patient respiratory specimens is becoming more important because of the number of newer antibiotics available and the continued development of pseudomonal resistance to these antibiotics. Practical implementation of mixed-morphotype susceptibilities would provide for MIC testing upon initial isolation with organism identification based on typical colony morphology (mucoid, pig-

ment), a positive oxidase reaction, and no growth on *Pseudomonas cepacia* selective agar. Any definitive isolate identification would be performed after the MIC data were obtained. Early mixed-morphotype susceptibility testing of these isolates could benefit both the laboratory and the clinician and may actually correlate better with the actual predicted response of the host pseudomonal flora to antimicrobial therapy. The Microscan Neg/Urine MIC 5 Panel broth microdilution system provides accurate susceptibility results for mixed-morphotype testing of *P. aeruginosa* isolated from cystic fibrosis patients.

I thank Gary Wormser for his expert review during preparation of this manuscript and Baxter Diagnostics, Inc., for supplying the susceptibility panels.

#### REFERENCES

- Dunne, W. M., Jr., and M. J. Chusid. 1987. Mixed morphotype susceptibility testing of *Pseudomonas aeruginosa* from patients with cystic fibrosis. *Diagn. Microbiol. Infect. Dis.* 6:165-170.
- Fegan, M., P. Francis, A. C. Hayward, G. H. G. Davis, and J. A. Fuerst. 1990. Phenotypic conversion of *Pseudomonas aeruginosa* in cystic fibrosis. *J. Clin. Microbiol.* 28:1143-1146.
- Friend, P. A. 1986. Pulmonary infection in cystic fibrosis. *J. Infect.* 13:55-72.
- Gilardi, G. L. 1985. *Pseudomonas*, p. 350-372. In E. H. Lennette, A. Balows, W. J. Hausler, Jr., and H. J. Shadomy (ed.), *Manual of clinical microbiology*, 4th ed. American Society for Microbiology, Washington, D.C.
- Kulczycki, L. L., T. M. Murphy, and J. A. Bellanti. 1978. *Pseudomonas* colonization in cystic fibrosis. *JAMA* 240:30-34.
- May, J. R., N. C. Herrick, and D. Thompson. 1972. Bacterial infection in cystic fibrosis. *Arch. Dis. Child.* 47:908-913.
- Maybury, B. A., J. Blessing-Moore, S. A. deWit, N. J. Lewiston, and A. S. Yeager. 1979. Antimicrobial susceptibilities of rough, smooth, and mucoid colony types of *Pseudomonas* isolated from cystic fibrosis patients. *Antimicrob. Agents Chemother.* 15:494-496.
- Mearns, M. B., G. H. Hunt, and R. Rushworth. 1972. Bacterial flora of respiratory tract in patients with cystic fibrosis, 1950-1971. *Arch. Dis. Child.* 47:902-907.
- National Committee for Clinical Laboratory Standards. 1990. Performance standards for antimicrobial disk susceptibility tests, 4th ed. Document M2-A4. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- National Committee for Clinical Laboratory Standards. 1991. Performance standards for antimicrobial susceptibility testing, 3rd informational supplement. Document M100-S3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
- Thomassen, M. J., C. A. Demko, B. Boxerbaum, R. C. Stern, and P. J. Kuchenbrod. 1979. Multiple isolates of *Pseudomonas aeruginosa* with differing antimicrobial susceptibility patterns from patients with cystic fibrosis. *J. Infect. Dis.* 140:873-880.
- Van Horn, K. 1991. Abstr. Annu. Meet. Am. Soc. Microbiol., abstr. C-221, p. 379.