ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, July 1986, p. 35–38
0066-4804/86/070035-04$02.00/0
Copyright © 1986, American Society for Microbiology
Vol. 30, No. 1

Discrepancies between Disk Diffusion and Broth Susceptibility Studies of the Activity of Ticarcillin Plus Clavulanic Acid against Ticarcillin-Resistant *Pseudomonas aeruginosa*

FARRIN A. MANIAN AND ROBERT H. ALFORD*

Infectious Diseases Section, Veterans Administration Medical Center, and Division of Infectious Diseases, Vanderbilt University Medical School, Nashville, Tennessee 37203

Received 30 December 1985/Accepted 17 April 1986

Ticarcillin and clavulanic acid in combination were tested against 40 *Pseudomonas aeruginosa* isolates resistant to ticarcillin by disk diffusion. A total of 21 isolates (53%) were susceptible to ticarcillin-clavulanate by disk diffusion, under currently recommended criteria for ticarcillin susceptibility. Macro-broth dilution tests (ticarcillin plus clavulanic acid, 2 μg/ml) confirmed susceptibility (MIC ≤64 μg/ml) of only 8 (38%) of 21 isolates. Time-kill studies of disk diffusion susceptible isolates indicated 2 $\log_{10}$ or greater killing of most isolates at 6 h in broth containing ticarcillin (64 μg/ml) combined with clavulanic acid (1, 2, 5, or 10 μg/ml). After 6 h, regrowth was common in all concentrations of clavulanic acid except 10 μg/ml. Regrowth populations were resistant to ticarcillin-clavulanate by MIC determination. Poor bactericidal activity of ticarcillin-clavulanate against ticarcillin-resistant *P. aeruginosa* was confirmed, as most isolates did not undergo 99.9% or greater killing at 24 h in all concentrations of clavulanic acid. Serotype O-11 was our most common serotype and was associated with disk diffusion "pseudosusceptibility." Concomitant disk diffusion testing of ticarcillin-clavulanate and ticarcillin is recommended for testing the susceptibility of *P. aeruginosa* to ticarcillin-clavulanate by disk diffusion. *P. aeruginosa* isolates resistant to ticarcillin should as a rule be considered also resistant to ticarcillin-clavulanate, despite apparent susceptibility by disk diffusion.

Clavulanic acid, by virtue of its irreversible inhibition of Richmond type II, III, IV, and V β-lactamases (18, 22), has broadened the spectrum of activity of ticarcillin to include many otherwise resistant strains of *Staphylococcus aureus*, the family *Enterobacteriaceae*, and *Bacteroides* spp. (2, 6–8, 11, 15, 19). Against ticarcillin-susceptible *Pseudomonas aeruginosa*, ticarcillin-clavulanate in combination has activity in vitro like that of ticarcillin alone (2, 6, 8, 11, 15). Against ticarcillin-resistant *P. aeruginosa*, the activity of this combination has been less thoroughly studied, often with conflicting results. Some investigators have reported that most ticarcillin-resistant *P. aeruginosa* strains are resistant to ticarcillin-clavulanate by broth dilution MICs (19), while others have found that the antipseudomonal activity of the combination is superior to that of ticarcillin alone by disk diffusion (T. Fosse, E. Dupont, A. Rousset-Rouviere, D. Darmuzey, M. David, and F. Duluc, Abstr. 14th Int. Congr. Chemother. S-12-3, p. 119, 1985) or agar dilution MIC (14; R. Morisset, E. Toma, M. Poisson, D. Phaneuf, and C. Vega, Proc. Conjoint Meet. Infect. Dis., Vancouver, no. 54, 1984). Systematic correlations of disk diffusion, broth dilution, and time-kill susceptibilities of ticarcillin-resistant *P. aeruginosa* to ticarcillin-clavulanate have not been reported previously.

By disk diffusion testing, 21 (53%) of 40 ticarcillin-resistant *P. aeruginosa* isolates from our medical center were susceptible to ticarcillin-clavulanate, under currently recommended criteria for ticarcillin-susceptible isolates (12). Because of the apparently high proportion of ticarcillin-clavulanate susceptibility, we undertook to corroborate disk diffusion results by in vitro broth susceptibility tests. Additionally, we sought to determine whether ticarcillin-clavulanate susceptibility among ticarcillin-resistant isolates was serotype dependent.

* Corresponding author.

MATERIALS AND METHODS

Bacterial isolates. Initially, 65 *P. aeruginosa* clinical isolates from Nashville Veterans Administration Medical Center, either frozen or lyophilized from 1979 to 1984, were reconstituted, grown in broth, plated, and screened for ticarcillin resistance by disk diffusion testing. Of these, 21 were ticarcillin resistant (inhibition zone, <12 mm). One ticarcillin-resistant isolate from 1973 was also included. Similarly, 7 current (1985) ticarcillin-resistant isolates frozen by the Vanderbilt Hospital Microbiology Laboratory and 9 more current ticarcillin-resistant isolates from Nashville Veterans Administration Medical Center from June to August 1985 were tested, for a total of 40 isolates. All isolates, except one, were ticarcillin resistant by disk diffusion; one isolate with an intermediate inhibition zone (12.5 mm) and a ticarcillin MIC of 128 μg/ml was also included.

Disk diffusion susceptibility tests. Disk diffusion susceptibility was evaluated by the method of the National Committee for Clinical Laboratory Standards with rapid inoculum preparation modification (3). Isolates were grown on sheep erythrocyte agar for 18 to 24 h at 35°C. Five morphologically similar colonies were picked from the plates and suspended in 0.9% NaCl solution (saline) to equal a 0.5 McFarland barium sulfate turbidity standard. This bacterial suspension was repeatedly confirmed to contain approximately 10^9 CFU/ml as follows. A 0.5-ml sample of a 1:1,000 dilution in saline of this bacterial suspension was serially diluted 10-fold. A 0.01-ml sample from each dilution was cultured on sheep erythrocyte agar plates. After 18 to 24 h of incubation at 35°C, the number of colonies on plates was multiplied by the appropriate dilution to verify that bacterial suspensions did indeed contain $1 \times 10^9 \pm 0.5 \times 10^1$ CFU/ml.

Within 5 min of preparation, the inoculum was evenly spread over Mueller-Hinton agar plates by a sterile swab and

TABLE 1. Ticarcillin and ticarcillin-clavulanate MICs for ticarcillin-resistant *P. aeruginosa* determined by ticarcillin-clavulanate disk diffusion

Ticarcillin-clavulanate disk diffusion category	Antibiotic	Cumulative % inhibited at a concn (μg/ml) of:					
		32	64	128	256	512	≥512
Susceptible[a]	Ticarcillin	0	5	24	29	48	100
	Ticarcillin-clavulanate	10	38	67	71	76	100
Resistant[b]	Ticarcillin	0	25	63	75	81	100
	Ticarcillin-clavulanate	0	31	81	88	88	100

[a] $n = 21$.
[b] $n = 16$.

left undisturbed for about 10 min. Disks (supplied by Beecham Laboratories, Bristol, Tenn.) containing ticarcillin (75 μg), either alone or in combination with clavulanic acid (10 μg), were placed on a single plate, and the zones of inhibition were measured after 18 to 24 h at 35°C. Ticarcillin and ticarcillin-clavulanate disk potencies were verified with *P. aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 35218. Disk diffusion susceptibility tests were run in duplicate, and mean inhibition zones were calculated. Results were readily reproducible with all isolates. Isolates with inhibition zones of <12 mm were considered resistant; those with inhibition zones of ≥15 mm were designated susceptible.

Macro-broth dilution tests. The macro-broth dilution MIC was determined for 37 of 40 ticarcillin-resistant isolates; 3 failed to grow for retesting. Organisms were grown to log phase (4 to 6 h) at 35°C in Mueller-Hinton broth supplemented with 25 mg of magnesium and 50 mg of calcium per liter. The resultant bacterial suspension was adjusted with broth to 0.5 McFarland standard turbidity. Repeated assay of these suspensions indicated approximately 10^9 CFU/ml, as described above. This initial suspension was further diluted 1:1,000, to yield approximately 10^6 CFU/ml. Twofold serial dilutions of ticarcillin, starting with 512 μg/ml, were employed, adding clavulanic acid in a fixed final concentration of 2 μg/ml when determining ticarcillin-clavulanate MICs, as previously recommended (11). Diluted antibiotic (0.5 ml) was combined with an equal volume of the working bacterial suspension (10^6 CFU/ml) to yield a final inoculum of approximately 5×10^5 CFU/ml. Ticarcillin and ticarcillin-clavulanate broth dilution tests were run simultaneously. The MIC, which was defined as the lowest concentration of ticarcillin inhibiting visible growth of the organisms, was read after 18 to 24 h of incubation at 35°C. The MIC was redetermined for all isolates susceptible to ticarcillin-clavulanate by disk diffusion with good reproducibility of results. For six isolates in which the two MICs differed by one tube dilution, the higher MIC was arbitrarily selected. One isolate yielded greater than a twofold difference in MICs; a third MIC was determined with the final MIC based on the two closest values. An MIC of ≤64 μg/ml for ticarcillin (with or without clavulanic acid) was defined as susceptible.

Time-kill studies. To assess bactericidal activity of ticarcillin-clavulanate against ticarcillin-resistant *P. aeruginosa*, time-kill studies were performed on all 21 isolates susceptible to ticarcillin-clavulanate by disk diffusion. Inocula were prepared by the methods described above. At zero time, ticarcillin (64 μg/ml) in broth was added to glass tubes either alone or in combination with 1, 2, 5, or 10 μg of clavulanic acid per ml, which at these concentrations has no activity by itself against *P. aeruginosa* (18). Tubes containing bacterial suspension with or without 10 μg of clavulanic acid per ml served as controls. The final volume in each tube was 5 ml. Tubes were incubated at 35°C with continuous agitation by using a blood culture bottle shaker (New Brunswick Scientific Co., Inc., Edison, N.J.) to prevent regrowth of susceptible organisms caused by poor mixing with antimicrobial agents (13). At 0, 6, and 24 h, 0.5-ml samples from each tube were serially diluted 10-fold in 4.5 ml of sterile 0.9% NaCl solution (saline). The viable bacterial count was determined by culturing 0.01-ml samples from bacterial broth and saline tubes on blood agar and multiplying the resulting colony count by appropriate dilution. Also at 6 h, a duplicate ticarcillin-clavulanate (2 μg/ml) tube received an additional 2 μg of clavulanic acid per ml, and the viable cell count was determined similarly at 24 h. Regrowth was defined as 2 $\log_{10}$ or greater reduction in viable cell count at 6 h, followed by growth resulting in 24-h viable cell counts similar to those of controls.

Serotyping. A total of 17 O antisera based on the International Antigenic Typing Scheme for *P. aeruginosa* (5) were purchased from Difco Laboratories (Detroit, Mich.). Serotyping was performed on all isolates by the method of Brokopp and Farmer (5).

Statistical analysis. Statistical analysis was by the chi-square test of significance. P values of <0.05 were considered significant.

RESULTS

Of 40 ticarcillin-resistant *P. aeruginosa* isolates, 21 (53%) were susceptible to ticarcillin-clavulanate by disk diffusion. The distribution of ticarcillin and ticarcillin-clavulanate MICs in each disk diffusion susceptibility category is shown in Table 1. Of disk diffusion susceptible isolates, 38% had ticarcillin-clavulanate MICs of ≤64 μg/ml, resulting in a false-susceptibility rate of 62% for ticarcillin-clavulanate disk diffusion testing.

Time-kill results with the 21 isolates susceptible to ticarcillin-clavulanate by disk diffusion are indicated in Table 2. At 6 h, most strains underwent 2 $\log_{10}$ or greater killing by all concentrations of clavulanic acid in the presence of ticarcillin. However, at 24 h, only 10, 19, and 38% of isolates showed persistent killing in the presence of 1, 2, and 5 μg of clavulanic acid per ml, respectively. Persistent bactericidal activity at 24 h prevailed only when 10 μg of clavulanic acid per ml was combined with ticarcillin. Regrowth was evident in 19% of isolates in ticarcillin-clavulanate (10 μg/ml) but as frequently as 76% in ticarcillin-clavulanate (2 μg/ml). A decrease of ≥3 $\log_{10}$ in bacterial cell count (≥99.9% killing) at 24 h occurred in only 29% of isolates, even at higher clavulanic acid concentrations of 5 and 10 μg/ml. Clavulanic acid (2 μg/ml) added at 6 h to duplicate ticarcillin-clavulanate

(2 μg/ml) tubes conferred no significant advantage in 24-h killing over ticarcillin-clavulanate (2 μg/ml) alone. Ticarcillin (64 μg/ml) or clavulanic acid (10 μg/ml) alone was not bactericidal in time-kill studies.

To assess the emergence of more resistant subpopulations of bacteria, ticarcillin-clavulanate MIC determinations for all 21 isolates were repeated on randomly selected colonies persisting after a 24-h exposure to ticarcillin and clavulanic acid. A total of 19 isolates were retested after the 24-h exposure to ticarcillin-clavulanate (5 μg/ml), and 2 isolates were retested after 24-h exposure to ticarcillin-clavulanate (1 or 2 μg/ml) because of complete killing of these 2 isolates at 24 h with higher concentrations of clavulanic acid in the presence of ticarcillin. Of the 21 isolates retested after 24-h exposure to ticarcillin-clavulanate, 14 also demonstrated regrowth, with the remaining isolates either showing growth at 6 h (1 isolate) or ≥2 $\log_{10}$ killing at 24 h (6 isolates). All 21 isolates that recovered from 24-h time-kill studies were resistant to ticarcillin-clavulanate (MIC ≥128 μg/ml), with 16 (76%) having MICs of ≥512 μg/ml. Of the 14 regrowth populations tested, 10 (71%) had MICs of ≥512 μg/ml. All eight isolates previously susceptible to ticarcillin clavulanate by MIC before time-kill studies became resistant after 24-h exposure to ticarcillin-clavulanate, with six (75%) demonstrating a fourfold or greater increase in MIC.

Serotyping of our ticarcillin-resistant *P. aeruginosa* isolates revealed that 19 of 38 isolates (50%) belonged to serotype O-11. A total of 14 of 21 (67%) disk diffusion ticarcillin-clavulanate isolates were serotype O-11, while only 5 of 16 isolates (29%) in the disk diffusion resistant category belonged to this serotype; this difference nearly reached statistical significance ($\chi^2 = 3.83$, $P = 0.05$). The distribution of other serotypes did not differ significantly between the ticarcillin-clavulanate-susceptible and -resistant isolates.

DISCUSSION

Approximately 15 to 20% of all *P. aeruginosa* isolates from Nashville Veterans Administration Medical Center and Vanderbilt University Medical Center are resistant to ticarcillin by disk diffusion criteria, as in other centers (2, 20). More than half of these isolates appeared susceptible to ticarcillin-clavulanate by disk diffusion. Thus, we sought to further evaluate the degree of correlation of this test with macro-broth dilution MIC and time-kill studies. Knowledge of this correlation might be particularly important in centers such as ours where ticarcillin-resistant *P. aeruginosa* strains are common and ticarcillin-clavulante disk diffusion testing of *P. aeruginosa* is contemplated.

TABLE 2. Bactericidal activity of ticarcillin (64 μg/ml) plus graded concentrations of clavulanic acid in time-kill studies of 21 *P. aeruginosa* isolates resistant to ticarcillin but susceptible to ticarcillin-clavulanate by disk diffusion

Clavulanic acid concn (μg/ml)	No. of strains (%)			
	≥2 $\log_{10}$ CFU killed at 6 h	≥2 $\log_{10}$ CFU killed at 24 h	Showing regrowth[a] at 24 h	≥3 $\log_{10}$ CFU killed at 24 h
0	0	0	0	0
1	14 (67)	2 (10)	12 (57)	1 (5)
2	20 (95)	4 (19)	16 (76)	3 (14)
5	20 (95)	8 (38)	12 (57)	6 (29)
10	20 (95)	16 (76)	4 (19)	6 (29)
2 + 2[b]		7 (33)		3 (14)

[a] Isolates with ≥2 $\log_{10}$ CFU killed at 6 h, followed by growth resulting in 24-h viable cell counts similar to those of controls.

[b] Additional clavulanic acid (2 μg/ml) added to duplicate ticarcillin-clavulanate tubes (2 μg/ml) at 6 h.

Our data strongly suggested that disk diffusion testing of ticarcillin-resistant *P. aeruginosa* overestimates susceptibility of these isolates to ticarcillin-clavulanate as measured by broth MIC and time-kill studies. Although more than half of our ticarcillin-resistant isolates initially appeared susceptible to ticarcillin-clavulanate by disk diffusion, we found a very high false-susceptibility rate of 62% by MIC. Poor bactericidal activity of the combination drug against ticarcillin-resistant *P. aeruginosa* isolates was confirmed by time-kill studies in which only 14% of disk diffusion-susceptible isolates demonstrated 99.9% or greater killing at 24 h by ticarcillin (64 μg/ml) combined with clavulanic acid (2 μg/ml). Even in the presence of higher concentrations of clavulanic acid (5 and 10 μg/ml), effective killing occurred in only 29% of isolates. Despite frequent 2 $\log_{10}$ or greater killing at 6 h of ticarcillin combined with various concentrations of clavulanic acid, regrowth and rapid emergence of isolates with high-level resistance to ticarcillin-clavulanate after 24-h exposure to this drug combination were common. Although it is possible that higher clavulanic acid concentrations (>10 μg/ml) might have conferred better bactericidal activity in the presence of ticarcillin in time-kill studies, such concentrations probably have less clinical relevance since clavulanic acid levels of ≥5 μg/ml are obtained in blood only during the first 1 h of ticarcillin-clavulanate infusion (3 and 0.2 g, respectively), and not at all in blister fluid (4).

Although the mechanism(s) responsible for the discrepancies between disk diffusion and broth susceptibility testing was not systematically evaluated, one explanation may be the lower number of organisms tested in agar tests (9, 23, 25), with less opportunity for expression of resistant variants. Reports indicating superior activity of ticarcillin-clavulanate against ticarcillin-resistant *P. aeruginosa* have been based on disk diffusion (Fosse et al., 14th ICC) or agar dilution susceptibility testing (14; Morisset et al., Proc. Conjoint Meet. Infect. Dis. 1984). Lack of significant activity of this drug combination against ticarcillin-resistant *P. aeruginosa* has often been demonstrated by broth dilution (2, 11, 19).

The clinical relevance of poor correlation between disk diffusion and broth dilution susceptibility testing remains to be seen. Overestimation of susceptibility by disk diffusion testing of *P. aeruginosa*, with associated clinical failures, has been previously reported for cefoperazone (1) and moxalactam (16, 21). The clinical importance of such discrepancy also has been emphasized for cefamandole against *Enterobacter cloacae* (17, 24). Rylander et al. have demonstrated superior predictability of outcome for certain organisms in experimental infections by broth dilution MIC compared with agar dilution susceptibility testing (23).

Serotype O-11 predominated among our ticarcillin-resistant *P. aeruginosa* isolates and was associated with ticarcillin-clavulanate susceptibility by disk diffusion. This serotype accounted for 24% of all *P. aeruginosa* isolates at our institution and has been an important cause of hospital outbreaks in other centers (10).

In conclusion, disk diffusion susceptibility testing of ticarcillin-clavulanate against ticarcillin-resistant *P. aeruginosa* overestimates in vitro susceptibility to ticarcillin-clavulanate, with most isolates resistant by broth tests. We recommend concomitant disk diffusion testing with both ticarcillin and ticarcillin-clavulanate if disk diffusion susceptibility testing of ticarcillin-clavulanate against *P. aeruginosa* is per-

formed. Ticarcillin-clavulanate susceptibility by disk diffusion in the presence of ticarcillin resistance should be interpreted with caution and confirmed by broth susceptibility testing. Until the clinical efficacy of ticarcillin-clavulanate in ticarcillin-resistant *P. aeruginosa* infections is established, we concur (2, 12) that *P. aeruginosa* isolates resistant to ticarcillin should, as a rule, be considered resistant to ticarcillin-clavulanate.

ACKNOWLEDGMENTS

This work was supported by the Veterans Administration.

We thank Beverly Hosse and the Nashville Veterans Administration Medical Center Microbiology Laboratory for their invaluable technical assistance.

LITERATURE CITED

1. **Bailey, R. R., B. Peddie, E. Blake, V. Bishop, and J. Reddy.** 1981. Cefoperazone in the treatment of severe or complicated infections. Drugs **22**(Suppl. 1):76–86.
2. **Barry, A. L., L. W. Ayers, T. L. Gavan, E. H. Gerlach, and R. N. Jones.** 1984. *In vitro* activity of ticarcillin plus clavulanic acid against bacteria isolated in three centers. Eur. J. Clin. Microbiol. **3**:203–206.
3. **Barry, A. L., and C. Thornsberry.** 1985. Susceptibility tests: diffusion test procedures, p. 978–987. *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.
4. **Bennett, S., R. Wise, D. Weston, and J. Dent.** 1983. Pharmacokinetics and tissue penetration of ticarcillin combined with clavulanic acid. Antimicrob. Agents Chemother. **23**:831–834.
5. **Brokopp, C. D., and J. J. Farmer III.** 1979. Typing methods for *Pseudomonas aeruginosa*, p. 90–133. *In* R. G. Dogget (ed.), *Pseudomonas aeruginosa*. Academic Press, Inc., New York.
6. **Casey, P., and M. Glauser.** 1983. Susceptibility of gram-negative bacteria and *Staphylococcus aureus* to combinations of ticarcillin and clavulanic acid. Eur. J. Clin. Microbiol. **2**:541–547.
7. **Chattopadhyay, B., and I. Hall.** 1984. *In-vitro* activity of ticarcillin plus clavulanic acid (BRL 28500, "Timentin") against ticarcillin-resistant gram-negative rods. Curr. Med. Res. Opin. **9**:157–160.
8. **Clarke, A. M., and S. J. Zemcov.** 1984. Clavulanic acid in combination with ticarcillin: an *in-vitro* comparison with other beta-lactams. J. Antimicrob. Chemother. **13**:121–128.
9. **Ericsson, H. M., and J. C. Sherris.** 1971. Antibiotic sensitivity testing—report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sect. B Suppl. 217.
10. **Farmer, J. J., III, R. A. Weinstein, C. H. Zierdt, and C. D. Brokopp.** 1982. Hospital outbreaks caused by *Pseudomonas aeruginosa*: importance of serogroup O-11. J. Clin. Microbiol. **16**:266–270.
11. **Fuchs, P. C., A. L. Barry, C. Thornsberry, and R. N. Jones.** 1984. In vitro activity of ticarcillin plus clavulanic acid against 632 clinical isolates. Antimicrob. Agents Chemother. **25**:392–394.
12. **Fuchs, P. C., R. N. Jones, A. L. Barry, and C. Thornsberry.** 1984. Disk diffusion susceptibility testing of ticarcillin plus clavulanic acid. J. Clin. Microbiol. **19**:555–557.
13. **Gwynn, M. N., T. Webb, and G. N. Rolinson.** 1981. Regrowth of *Pseudomonas aeruginosa* and other bacteria after the bactericidal action of carbenicillin and other beta-lactam antibiotics. J. Infect. Dis. **144**:263–269.
14. **Henry, D., A. G. Skidmore, J. Ngui-Yen, A. Smith, and J. A. Smith.** 1985. In vitro activities of enoxacin, ticarcillin plus clavulanic acid, aztreonam, piperacillin and imipenem and comparison with commonly used antimicrobial agents. Antimicrob. Agents Chemother. **28**:259–264.
15. **Hunter, P. A., K. Coleman, J. Fisher, and D. Taylor.** 1980. *In vitro* synergistic properties of clavulanic acid with ampicillin, amoxycillin and ticarcillin. J. Antimicrob. Chemother. **6**:455–470.
16. **Mathisen, G. E., R. D. Meyer, J. M. Thompson, and S. M. Finegold.** 1982. Clinical evaluation of moxalactam. Antimicrob. Agents Chemother. **21**:780–786.
17. **Murray, P. R., G. G. Granich, D. J. Krogstad, and A. C. Niles.** 1983. *In-vivo* selection of resistance to multiple cephalosporins by *Enterobacter cloacae*. J. Infect. Dis. **147**:590.
18. **Neu, H. C., and K. P. Fu.** 1978. Clavulanic acid, a novel inhibitor of β-lactamases. Antimicrob. Agents Chemother. **14**:650–655.
19. **Paisley, J. W., and J. A. Washington II.** 1978. Combined activity of clavulanic acid and ticarcillin against ticarcillin-resistant, gram-negative bacilli. Antimicrob. Agents Chemother. **14**:224–227.
20. **Parry, M. F., and H. C. Neu.** 1976. Ticarcillin for treatment of serious infections with gram-negative bacteria. J. Infect. Dis. **134**:476–485.
21. **Preheim, L. C., R. G. Penn, C. C. Sanders, R. V. Goering, and D. K. Giger.** 1982. Emergence of resistance to β-lactam and aminoglycoside antibiotics during moxalactam therapy of *Pseudomonas aeruginosa* infections. Antimicrob. Agents Chemother. **22**:1037–1041.
22. **Reading, C., and M. Cole.** 1977. Clavulanic acid: a beta-lactamase-inhibiting beta-lactam from *Streptomyces clavuligerus*. Antimicrob. Agents Chemother. **11**:852–857.
23. **Rylander, M., J.-E. Brorson, J. Johnsson, and R. Norrby.** 1979. Comparison between agar and broth minimum inhibitory concentrations of cefamandole, cefoxitin, and cefuroxime. Antimicrob. Agents Chemother. **15**:572–579.
24. **Sanders, C. C., R. C. Moellering, Jr., R. R. Martin, R. L. Perkins, D. G. Strike, T. D. Gootz, and W. E. Sanders, Jr.** 1982. Resistance to cefamandole: a collaborative study of emerging clinical problems. J. Infect. Dis. **145**:118–125.
25. **Stratton, C. W.** 1983. Susceptibility testing revisited. Prog. Clin. Pathol. **9**:65–100.