

In Vitro Activity of Three Tetracycline Antibiotics Against *Acinetobacter calcoaceticus* subsp. *anitratus*

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The in vitro activity of three tetracycline antibiotics against 127 strains of *Acinetobacter calcoaceticus* (*Herellea vaginicola*) were compared. Almost all strains were susceptible to minocycline and doxycycline, whereas most strains were resistant to tetracycline.

It has been documented that *Acinetobacter calcoaceticus* subsp. *anitratus* (*Herellea vaginicola*) causes serious infections (3). Nosocomial infections due to *Acinetobacter* usually occur in seriously ill, debilitated patients, many of whom have impaired renal function (3). In our hospital the therapeutic management of such patients is often hindered by the increasing resistance of *A. calcoaceticus* to most antibiotics (6, 8; Table 1). Many of our strains remain susceptible only to kanamycin, tobramycin, carbenicillin, or trimethoprim-sulfamethoxazole or any combination of these (2, 3), but the use of adequate doses of these antibiotics is often complicated by co-existing renal insufficiency. Previous studies have shown in vitro efficacy of minocycline against *A. calcoaceticus* (5, 7, 10, 11), and, although minocycline can probably be given in adequate doses (13), its use in this fashion in patients with impaired renal function has not received official approval. Moreover, it may cause untoward effects such as nausea and dizziness (4). We, therefore, compared the in vitro efficacy of minocycline and tetracycline with that of doxycycline, an agent which is metabolized predominantly by the liver. Doxycycline has also been approved for use without dosage modification in patients with impaired renal function (12).

The 127 strains tested included 93 strains comprising all of the *A. calcoaceticus* strains isolated by the clinical microbiology laboratory of the Massachusetts General Hospital between 24 July and 18 August 1978, and 33 strains chosen from a previous study because they were known to be tetracycline resistant (3). In addition, we have previously introduced the P group plasmid, RP4, which mediates tetracycline resistance, into several clinical isolates of *A. calcoaceticus* (8). One of these, H20 (RP4), was also included in this study. Standard strains of *Staphylococcus*

aureus (ATCC 25923) and *Escherichia coli* (ATCC 25922) were utilized as controls. Species identification of all organisms was confirmed by API 20E (Analytic Profile Index, Analytic Products, Plainview, N.Y.) and in many cases by additional biochemical test (8).

Antibiotic susceptibilities were determined by the single-disk method of Bauer et al. (1) on Mueller-Hinton agar (Difco Laboratories, Detroit, Mich.). Minimal inhibitory concentrations (MIC) were determined by agar dilution in Mueller-Hinton agar by using a Steers replicator (9) to apply an inoculum of 10^5 colony-forming units. The antibiotics and antibiotic disks used were minocycline (Minocin; Lederle Laboratories, Pearl River, N.Y.), doxycycline (Vibramycin; Pfizer Inc., New York, N.Y.) and tetracycline (Pfizer, Inc.). The plates were read after 24 h of incubation at 37°C. The MIC was defined as the lowest concentration which yielded no visible growth or less than six discrete colonies.

Of the 127 strains of *A. calcoaceticus*, 123 were susceptible to $\leq 2 \mu\text{g}$ of minocycline per ml and $\leq 4 \mu\text{g}$ of doxycycline per ml (Fig. 1) and produced zones of inhibition of ≥ 19 mm by disk diffusion testing (Fig. 2). The remaining four strains were susceptible to $4 \mu\text{g}$ of minocycline per ml and $8 \mu\text{g}$ of doxycycline per ml (Fig. 1), with zones of inhibition of 17 and 18 mm for minocycline and 16 mm for doxycycline (Fig. 2). However, only 36 strains were susceptible to $\leq 4 \mu\text{g}$ of tetracycline per ml and the 4 strains with highest MIC of minocycline and doxycycline had MIC equal to $64 \mu\text{g}$ of tetracycline per ml (Fig. 1 and 2).

On the basis of these in vitro data, we believe that both minocycline and doxycycline may be of benefit in treating patients with infections due to *A. calcoaceticus* subsp. *anitratus* in which a bacteriostatic agent would be adequate. Because of its pharmacokinetic properties, doxycycline

TABLE 1. Antimicrobial susceptibility of 694 isolates of *A. calcoaceticus*^a

Antimicrobial agent	% Susceptible	Antimicrobial agent	% Susceptible
Cephalothin	0	Gentamicin	54
Tetracycline	38	Colistin	94
Chloramphenicol	2	Nitrofurantoin	0
Ampicillin	3	Tobramycin	77
Kanamycin	83	Amikacin	55

^a From Massachusetts General Hospital bacteriology laboratory, January through December 1978.

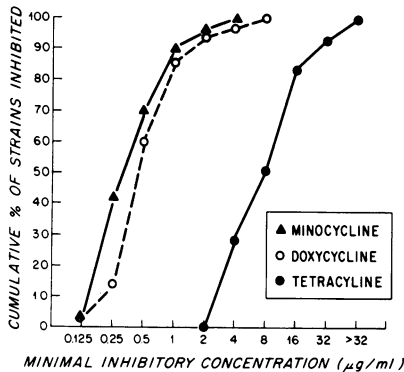


FIG. 1. Susceptibility of 127 strains of *Acinetobacter calcoaceticus* subsp. *anitratus* to doxycycline, minocycline, and tetracycline.

may be particularly useful in treating acinetobacter infections in patients with impaired renal function.

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LITERATURE CITED

- Bauer, W. W., W. M. M. Kirby, J. C. Sherris, and M. Truck. 1966. Antibiotic susceptibility testing by standardized single disc method. *Am. J. Clin. Pathol.* 45: 493-496.
- Glew, R. H., R. C. Moellering, Jr., and K. R. Buettner. 1977. In vitro synergism between carbenicillin and aminoglycosidic aminocyclitols against *Acinetobacter calcoaceticus* var. *anitratus*. *Antimicrob. Agents Chemother.* 11:1036-1041.
- Glew, R. H., R. C. Moellering, Jr., and L. J. Kunz. 1977. Infections with *Acinetobacter calcoaceticus* (*Herellea vaginicola*): clinical and laboratory studies. *Medicine* 56:79-97.
- Gump, D. W., T. Ashikaga, T. J. Fink, and A. M. Radin. 1977. Side effects of minocycline: different dosage regimens. *Antimicrob. Agents Chemother.* 12:642-646
- Maderazo, E. G., R. Quintiliani, R. C. Tilton, R. Bartlett, N. C. Joyce, and V. T. Andriole. 1975. Activity of minocycline against *Acinetobacter calcoaceticus* var. *anitratus* (syn. *Herellea vaginicola*) and *Serratia marcescens*. *Antimicrob. Agents Chemother.* 8:54-57.
- Moellering, R. C., Jr., C. Wennersten, L. Kunz, and J. Poitras. 1977. Resistance to gentamicin, tobramycin, and amikacin among clinical isolates of bacteria. *Am. J. Med.* 62:873-881.
- Montgomerie, J. Z., M. J. Pickett, R. N. Yoshimori, A. W. Chow, and L. B. Guze. 1976. Susceptibility of

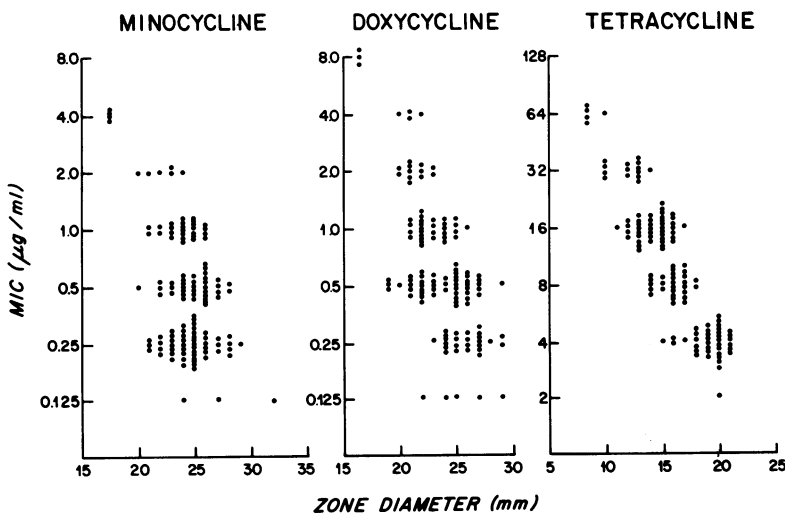


FIG. 2. Relationship of MIC (determined by agar dilution) to diameter of zone of inhibition (by disk diffusion testing) for doxycycline, minocycline, and tetracycline.

- Acinetobacter calcoaceticus* var. *anitratus* (*Herellea vaginicola*) to minocycline. *Antimicrob. Agents Chemother.* **10**:102-105.
8. Murray, B. E., and R. C. Moellering, Jr. 1979. Aminoglycoside-modifying enzymes among clinical isolates of *Acinetobacter calcoaceticus* subsp. *anitratus* (*Herellea vaginicola*): explanation for high-level aminoglycoside resistance. *Antimicrob. Agents Chemother.* **15**:190-199.
 9. Steers, E., E. L. Foltz, and B. S. Graves. 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. *Antibiot. Chemother. Washington, D.C.* **9**:307-311.
 10. Steigbigel, N. H., C. W. Reed, and M. Finland. 1968. Susceptibility of common pathogenic bacteria to seven

- tetracycline antibiotics in vitro. *Am. J. Med. Sci.* **255**: 179-195.
11. Washington, J. A., P. K. W. Yu, and W. J. Martin. 1970. In vitro antibacterial activity of minocycline and effect of agar medium utilized in its susceptibility testing. *Appl. Microbiol.* **19**:259-263.
 12. Weinstein, L. 1975. Antimicrobial agents, p. 1109. In L. S. Goodman and A. Gilman (ed.), *The pharmacological basis of therapeutics*, 5th ed. Macmillan Publishing Co., Inc., New York, N.Y.
 13. Welling, P. G., W. R. Shaw, S. J. Uman, F. L. S. Tse, and W. A. Craig. 1975. Pharmacokinetics of minocycline in renal failure. *Antimicrob. Agents Chemother.* **8**:532-537.

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