ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Mar. 1985, p. 424–426
0066-4804/85/030424-03$02.00/0

Vol. 27, No. 3

Establishment of MICs of Moxalactam for Control and Reference Anaerobic Organisms in Agar Dilution and Microdilution Techniques

VERA L. SUTTER,[1]* J. EMMERMAN,[2] EILEEN RANDALL,[3] R. J. ZABRANSKY,[4] AND R. J. BIRK[4]

Research Service, Veterans Administration Medical Center, West Los Angeles, Wadsworth Division, Los Angeles, California 90073[1]; Infectious Diseases Laboratory, Research Department,[2] and Departments of Pathology and Laboratory Medicine,[3] Evanston Hospital, Evanston, Illinois 60201; and Department of Pathology and Laboratory Medicine, Mount Sinai Medical Center, Milwaukee, Wisconsin 52301[4]

Received 16 July 1984/Accepted 7 December 1984

The MICs of moxalactam were determined for eight National Committee for Clinical Laboratory Standards control and reference strains and for *Fusobacterium nucleatum* ATCC 10953 by agar and microdilution techniques. The recommended MIC for the control strain *Bacteroides fragilis* in both agar and microdilution tests is 0.5 μg/ml. Recommended MICs for *Bacteroides thetaiotaomicron* ATCC 29741 and *Clostridium perfringens* ATCC 13124 by agar dilution are 8 and 0.063 μg/ml, respectively. These two strains gave inconsistent results in microdilution tests. Variation in results with microdilution procedures was seen, which illustrates problems in reading endpoints and with modifications of media. Recommended MICs for the reference strains are presented.

Moxalactam is the first member of a new class of β-lactam antibiotics, oxa-β-lactams, to be evaluated clinically. It has been shown to have a broad spectrum of activity against a wide variety of aerobic, facultative, and anaerobic bacteria (1, 3, 5, 6).

Reference values for quality control of in vitro susceptibility tests with moxalactam against anaerobic bacteria are unavailable. The National Committee for Clinical Laboratory Standards tentative reference agar dilution procedure for anaerobic bacteria (4) includes reference values for antimicrobial agents used in treating anaerobic infections at the time it was written, but does not include values for the newer β-lactam antibiotics.

The purpose of this study was to determine the MICs of moxalactam for the anaerobe control and reference strains by the reference agar dilution method and a microdilution method. The microdilution method was included because many laboratories use a variety of microdilution methods without comparison to a reference procedure.

Three laboratories participated: The Oral Microbiology Laboratory, Veterans Administration Medical Center, Wadsworth Division, Los Angeles, Calif.; the Infectious Disease Laboratory, Evanston Hospital, Evanston, Ill.; and the Microbiology Research Laboratory, Mount Sinai Medical Center, Milwaukee, Wis. Moxalactam was provided by Eli Lilli & Co., Indianapolis, Ind., and the same lot was used by all laboratories. The organisms used in this study were the eight control and reference strains described in the reference dilution procedure (4), *Bacteroides asaccharolyticus* ATCC 25260, and *Fusobacterium nucleatum* ATCC 10953. These organisms were added to provide a data base for additional reference strains. With the technique described in the reference dilution procedure, each organism was tested nine times in each of the three laboratories on Wilkins-Chalgren agar (Difco Laboratories) (WC) and on WC plus 5% sheep blood lysed by freezing and thawing. Each organism was also tested nine times by a microdilution technique (2) in Anaerobe Broth Experimental (Difco Laboratories) (ABE), ABE with 10% horse serum, and ABE with 5% Fildes enrichment. ABE has the same formulation as WC except that the agar is omitted. The inoculum was estimated to be 10^6 CFU/ml for microdilution methods, except where indicated. In addition, one laboratory (Mount Sinai Medical Center) tested each organism nine times in Wilkins-West broth (7), Wilkins-West broth with 10% horse serum, and Wilkins-West broth with 5% Fildes Enrichment.

TABLE 1. Modes and ranges of moxalactam MICs for anaerobic control and reference strains by agar dilution and microdilution techniques

Organism (ATCC no.)	MIC (μg/ml)[a] by:			
	Agar dilution on WC		Microdilution on ABE	
	Mode	Range	Mode	Range
B. fragilis (25285)	0.5	0.25–2	0.5	0.5–1
B. thetaiotaomicron (29741)	8	8–16	—[b]	4–32
C. perfringens (13124)	0.063	0.031–0.5	0.063	<0.016–0.125
B. vulgatus (29327)	0.125	0.125–0.5	0.125	0.125–0.25
B. thetaiotaomicron (29742)	8	8–128	—	4–128
P. variabilis (14956)	0.25 (0.5)	0.031–1	1	0.5–1
P. magnus (29328)	0.25 (0.125)	0.063–0.5	0.5	0.125–0.5
P. asaccharolyticus (29743)	0.25	0.063–0.5	0.25, 0.5	0.25–1
F. nucleatum (10953)	0.5	0.25–0.5	0.25	0.125–0.5

[a] MIC most commonly obtained. Two numbers indicate MICs of equal distribution. Numbers in parentheses indicate an MIC obtained almost as frequently as the mode.
[b] —, No mode established.

* Corresponding author.

TABLE 2. Comparison of modes of microdilution moxalactam MICs from participating laboratories

Organism (ATCC no.)	Mode MIC (μg/ml) on following agar at indicated laboratory[a]:							
	ABE			ABE + 10% horse serum			WW (MS)[b]	WW + 10% horse serum (MS)[b]
	VA	EH	MS	VA	EH	MS		
B. fragilis (25285)	1	0.5	0.5	0.5	0.5	0.5	0.5	1
B. thetaiotaomicron (29741)	4	4	32[c]	4	4	16[c]	32[c]	32[c]
C. perfringens (13124)	<0.016	0.063	0.063	<0.016	<0.016	0.063	0.063	0.063
B. vulgatus (29327)	0.125	0.125	0.125	0.25	0.125	0.063	0.25	0.125
B. thetaiotaomicron (29742)	4	8	64[c]	2	2	8[c]	64[c]	64[c]
P. variabilis (14956)	1	1	1	1	1	2	1	2
P. magnus (29328)	0.5	0.25	0.5	1	0.5	1	0.5	1
P. asaccharolyticus (29743)	0.5	0.25	0.5	0.5	0.25	0.25	0.5	0.5
F. nucleatum (10953)	0.25	0.25	0.5	0.5	0.5	0.5	0.5	1

[a] Mode MIC, MIC most commonly obtained. WW, Wilkens-West broth; VA, Oral Microbiology Laboratory, Veterans Administration Medical Center, Wadsworth Division, Los Angeles, Calif.; EH, Infectious Disease Laboratory, Evanston Hospital, Evanston, Ill.; MS, Microbiology Research Laboratory, Mount Sinai Medical Center, Milwaukee, Wis.

[b] Results of nine determinations at one laboratory are shown for WW.

[c] Higher modes reported owing to interpretation of endpoints (see text).

Table 1 lists the mode values and ranges for the agar dilution and a microdilution procedure by all three laboratories (27 determinations). The modes are the most common values obtained. When two numbers are listed, the values were obtained with almost equal frequency. A value in parentheses was obtained almost as frequently as the mode. Data from tests with ABE plus 5% Fildes enrichment are not included because it was found that some lots of Fildes enrichment inhibited growth of the *Peptococcus* strains. Also, in the tests a precipitate was formed which made interpretation of results virtually impossible. Data are not available for the *B. asaccharolyticus* strain because it usually failed to grow in the absence of blood. Longer incubation of plates (5 days total) gave somewhat better results. This strain grows much too slowly to be used as a control or reference strain. The mode values varied more than one dilution with *Bacteroides vulgatus*, *Peptococcus variabilis*, and *Peptococcus magnus* with the media tested. When 10% horse serum was added to ABE, modes could not be established for *Clostridium perfringens*, *Bacteroides thetaiotaomicron* ATCC 29741, or *B. thetaiotaomicron* ATCC 29742. When 5% lysed sheep blood was added to WC, a wide range of MICs were observed with *B. thetaiotaomicron* ATCC 29742, and modes could not be established. Similarly, both strains of *B. thetaiotaomicron* produced a range of MICs in microdilution testing, prohibiting the establishment of a mode value. The inclusion of blood in WC affected the MICs of *P. variabilis* and *P. magnus* so that MICs were twofold higher than those seen without blood. Also, broad ranges of MICs were obtained with the two strains of *B. thetaiotaomicron*.

When the individual results obtained by each laboratory for the two agar media used are examined, less variability was seen in the results with WC than with WC with 5% lysed sheep blood. The control strain, *C. perfringens*, gave the most variable results on WC, whereas all other strains gave modes within one dilution step. When blood was added, *C. perfringens* again gave variable results. In addition, there were differences in interpretation of trailing endpoints with both strains of *B. thetaiotaomicron*, with strain ATCC 29742 giving the most variable results. The remaining strains gave consistent results, although the cocci tended to yield higher modes in the presence of blood. This is probably related to the fact that their growth was enhanced on this medium.

A comparison of media and laboratories using the microdilution technique is given in Table 2. *C. perfringens* gave inconsistent results in both media. We believe the inconsistencies in both the agar dilution and microdilution techniques to be related to the growth rate of this organism, its cellular size, and the resulting inoculum size. The two strains of *B. thetaiotaomicron* gave variable results, again due to the problem of interpretation of trailing endpoints. Trailing endpoints occur when the size of the button of growth decreases appreciably from that of the control growth at a particular concentration of antibiotic but remains present for two or more concentrations above that. This made endpoint determinations questionable. Individual interpretation as to the exact endpoint could change the MIC result by two or three concentrations. Guidelines must be established for reading endpoints to prevent confusion and error. Should

TABLE 3. Recommended mode MICs for anaerobic control and reference strains for agar dilution and microdilution testing of moxalactam

Organism (ATCC no.)	Mode MIC (μg/ml) by[a]:	
	Agar dilution on WC[b]	Microdilution on ABE or WW
B. fragilis (25285)	0.5	0.5
B. thetaiotaomicron (29741)	8	NR[c]
C. perfringens (13124)	0.063	0.063
B. vulgatus (29327)	0.125	0.125
B. thetaiotaomicron (29742)	8	NR
P. variabilis (14956)	0.25 (0.5)	1
P. magnus (29328)	0.25 (0.125)	0.5
P. asaccharolyticus (29743)	0.25	0.25, 0.5
F. nucleatum (10953)	0.5	0.25

[a] Numbers in parentheses represent MICs obtained almost as frequently as the mode.

[b] Addition of blood not recommended for routine use; use only when necessary for growth.

[c] NR, No MIC recommended owing to inconsistent results (see text).

endpoints be read where there is the largest drop in button size or should they be read at the no-growth level?

One laboratory (Mount Sinai Medical Center) attempted to explain the discrepancies they obtained between agar dilution and microdilution test results and attempted to gain some insight into interpretation of trailing endpoints. Several inoculum sizes and incubation conditions were investigated by microdilution with the two strains of *B. thetaiotaomicron*. When the Medical Center used an inoculum 10-fold less than that used in the procedure used by the other laboratories, the observed MICs were the same as or lower than those obtained on WC. MICs were equal to those obtained on WC for *B. thetaiotaomicron* ATCC 29742 but were 1 to 2 dilutions higher for *B. thetaiotaomicron* ATCC 29741. Therefore, inoculum size is a factor, as shown previously (8), and appears to be strain related.

The strain variation and inoculum size effect seems to indicate β-lactamase production as the possible reason for these discrepancies and the trailing effect observed with moxalactam and these particular organisms. When the endpoint was read at the major drop-off point of the size of the button rather than at the no-growth level, the results were more in line with the agar dilution results. The main concern here is which is the more accurate reading methodology in relation to the clinical response, especially if this is due to β-lactamase production. The microdilution method might be a better indicator of resistance in these situations than the standard reference method.

Recommended MICs for the control and reference strains in the different test systems are shown in Table 3. The addition of blood or serum to the agar or broth tended to raise the MIC for some of the strains; these additives should be used only when necessary for the growth of fastidious anaerobes. Modes for the two strains of *B. thetaiotaomicron* in the microdilution system could not be established because of problems in interpretation of endpoints.

Further investigations on interpretation of endpoints with bacteria such as *B. thetaiotaomicron* in the presence of some β-lactam antibiotics are needed.

This investigation was supported by a grant from Eli Lilly & Co. and the Veterans Administration Medical Research Service.

LITERATURE CITED

1. **Gilcrist, M. J. R., and J. A. Washington II.** 1982. In vitro activity of moxalactam against anaerobic bacteria. Rev. Infect. Dis. **4**:S511–S515.
2. **Jenkins, S. G., R. J. Birk, and R. J. Zabransky.** 1982. Differences in susceptibilities of species of the *Bacteroides fragilis* group to several β-lactam antibiotics: indole production as an indicator of resistance. Antimicrob. Agents Chemother. **22**:628–634.
3. **Jones, R. N., P. C. Fuchs, H. M. Sommers, T. L. Gavan, A. L. Barry, and E. H. Gerlach.** 1980. Moxalactam (LY127835), a new semisynthetic 1-oxa-β-lactam antibiotic with remarkable antimicrobial activity: in vitro comparison with cefamandole and tobramycin. Antimicrob. Agents Chemother. **17**:750–756.
4. **National Committee for Clinical Laboratory Standards.** 1982. Tentative standard reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria, vol. 2, p. 70–101. National Committee for Clinical Laboratory Standards, Villanova, Pa.
5. **Neu, H. C., N. Aswapokee, K. P. Fu, and P. Aswapokee.** 1979. Antibacterial activity of a new 1-oxa-cephalosporin compared with that of other β-lactam compounds. Antimicrob. Agents Chemother. **16**:141–149.
6. **Rolfe, R. D., and S. M. Finegold.** 1981. Comparative in vitro activity of new beta-lactam antibiotics against anaerobic bacteria. Antimicrob. Agents Chemother. **20**:600–609.
7. **West, S. E. H., and T. D. Wilkins.** 1980. Vaspar broth-disk procedure for antibiotic susceptibility testing of anaerobic bacteria. Antimicrob. Agents Chemother. **17**:288–291.
8. **Yu, P. K. W., and J. A. Washington II.** 1983. Bactericidal activities of new β-lactam antibiotics against *Bacteroides fragilis*. Antimicrob. Agents Chemother. **24**:1–4.