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Evaluation of In Vitro Methods for Testing Susceptibility of Anaerobes to Ampicillin-Sulbactam and Amoxicillin-Clavulanic Acid

PETER C. FUCHS,[1]* ARTHUR L. BARRY,[2] AND RONALD N. JONES[2]

St. Vincent Hospital and Medical Center, Portland, Oregon 97225,[1] and The Clinical Microbiology Institute, Tualatin, Oregon 97062[2]

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A total of 97 anaerobic bacteria were tested for susceptibility to ampicillin, ampicillin-sulbactam, and amoxicillin-clavulanic acid by broth microdilution and disk elution methods, the results of which were compared with those of the reference agar dilution method. With the broth microdilution method, approximately 95% of MICs were within 1 dilution of those of the reference agar method, with a definite (0.6 to 0.7 dilution) trend toward lower MICs. The disk elution test performed satisfactorily, but additional anaerobic isolates resistant to ampicillin-sulbactam and/or amoxicillin-clavulanic acid (currently rare) are needed to assure the predictability of resistance by the disk elution test.

The 2:1 combinations of ampicillin-sulbactam and amoxicillin-clavulanic acid have been shown to have a greater spectrum of antimicrobial activity than ampicillin or amoxicillin alone (2, 7, 9). This broadened spectrum includes activity against β-lactamase-producing bacteria and is due to the β-lactamase-inhibitory activity of sulbactam and clavulanate (3, 6). Among the bacteria added to the spectrum are the β-lactamase-producing anaerobes, such as the *Bacteroides fragilis* group (1, 8, 9). Consequently, these drugs are potential therapeutic agents for anaerobic infections, and susceptibility testing may be requested of clinical laboratories. Two common methods of anaerobe susceptibility testing are broth microdilution and disk elution. This study was designed to evaluate these two methods for the testing of susceptibility of anaerobes to ampicillin-sulbactam and amoxicillin-clavulanic acid by comparing them with the reference agar dilution method.

A total of 97 strains of clinical anaerobic isolates were tested. These isolates included the following: *Bacteroides bivius* (5 strains), *B. distasonis* (6 strains), *B. fragilis* (40 strains), *B. melaninogenicus* (3 strains), *B. ovatus* (5 strains), *B. thetaiotaomicron* (6 strains), *B. uniformis* (1 strain), *B. vulgatus* (6 strains), *Clostridium bifermentans* (2 strains), *C. clostridiiforme* (1 strain), *C. difficile* (2 strains), *C. perfringens* (5 strains), *C. ramosum* (1 strain), *C. sporogenes* (2 strains), *Eubacterium lentum* (3 strains), *Peptostreptococcus anaerobius* (6 strains) and *P. asaccharolyticus* (4 strains). One strain (*C. ramosum*) did not grow in the disk elution medium. Also, one strain of *B. melaninogenicus* was tested against ampicillin-sulbactam but not against amoxicillin-clavulanic acid.

Ampicillin and sulbactam were supplied as standard powders by Roerig-Pfizer, New York, N.Y.; amoxicillin and clavulanic acid were provided by Beecham Laboratories, Bristol, Tenn. Disks of ampicillin (10 μg), ampicillin-sulbactam (10 μg/10 μg), and amoxicillin-clavulanic acid (20 μg/10 μg) were prepared by Difco Laboratories, Detroit, Mich.

The reference agar dilution susceptibility test was performed in strict accordance with the National Committee for Clinical Laboratory Standards method (4). The concentrations of ampicillin tested were serial twofold dilutions ranging from 32 to 0.5 μg/ml. The ampicillin and amoxicillin concentrations in ampicillin-sulbactam and amoxicillin-clavulanic acid, respectively, ranged from 32 to 0.25 μg/ml. The concentrations of sulbactam and clavulanate were one-half those of ampicillin and amoxicillin, respectively. Both the broth microdilution and the disk elution tests were performed as outlined by the National Committee for Clinical Laboratory Standards (5). For the broth microdilution test, Wilkins-Chalgren broth was used with drug concentrations identical to those used in the agar dilution test. With the disk elution test, four disks of ampicillin, four disks of ampicillin-sulbactam (10 μg/10 μg), or two disks of amoxicillin-clavulanic acid (20 μg/10 μg) were allowed to elute in 5 ml of thioglycolate broth, calculated to yield a concentration of 8 μg of ampicillin or amoxicillin per ml. Susceptibility as defined by the National Committee for Clinical Laboratory Standards (5) was no growth or <50% growth compared with the growth in a control tube. This endpoint was determined by visual inspection.

The comparison of the broth microdilution test results with those of the reference agar dilution test is summarized

TABLE 1. Ratios of agar dilution MIC to broth microdilution MIC for ampicillin, ampicillin-sulbactam (2:1), and amoxicillin-clavulanic acid (2:1) against 97 clinical anaerobic isolates

Antimicrobial agent and range of data[a]	No. of organisms	No. of isolates with MIC ratio[b]					Mean ratio
		0.5	1.0	2.0	4.0	≥8.0	
Ampicillin							
On scale only	55	2	24	28	1	0	1.54
All data	97	4	52	36	3	2	1.59
Ampicillin-sulbactam							
On scale only	78	1	30	44	2	1	1.72
All data	97	2	44	47	2	2	1.68
Amoxicillin-clavulanic acid							
On scale only	50	4	15	26	5	0	1.78
All data	96	4	37	48	7	0	1.70

[a] On scale only, Excludes off-scale MICs (≤0.25 μg/ml or >32 μg/ml) by one or both methods; all data, MICs of ≤0.25 μg/ml and >32 μg/ml were assumed to be 0.25 μg/ml and 64 μg/ml, respectively, for purposes of tabulation.

[b] A ratio of 1.0 indicates identical MICs; ratios of ≤0.5 indicate lower agar dilution MICs; ratios of ≥2.0 indicate lower broth microdilution MICs.

* Corresponding author.

TABLE 2. Comparison of the disk elution test for ampicillin, ampicillin-sulbactam, and amoxicillin-clavulanic acid with the reference agar dilution method

MIC (μg/ml)	No. of organisms giving indicated response in the disk elution test[a]					
	Ampicillin		Ampicillin-sulbactam		Amoxicillin-clavulanic acid	
	S	R	S	R	S	R
>32	1	21				
32	1	32				1
16	2	3	2	1		
8.0	6	5	6			2
4.0	3		8		5	
2.0	1		33		7	
1.0	4		28		22	
0.5	17		5		38	
≤0.25			13		20	

[a] Estimated final concentrations for the disk elution test were as follows: ampicillin, 8.0 μg/ml; ampicillin-sulbactam, 8.0/8.0 μg/ml; and amoxicillin-clavulanic acid, 8.0/4.0 μg/ml. Results were recorded as R (growth) or S (≤50% of growth of control).

in Table 1. For both ampicillin and ampicillin-sulbactam, 95% of microdilution endpoints were within 1 dilution of the agar dilution endpoints; this was true for 93% of amoxicillin-clavulanic acid endpoints. There was, however, a definite skewing toward slightly lower MICs with the microdilution method for all three drugs. The differences averaged 0.6 dilution for ampicillin and 0.7 dilution for ampicillin-sulbactam and amoxicillin-clavulanic acid, but these differences did not result in any susceptibility category changes and were felt to be of little practical significance.

The data comparing the disk elution test results with those of the reference agar dilution test are summarized in Table 2. For ampicillin, the disk elution test yielded ambivalent results when the agar dilution (or broth microdilution) MICs were near the break point concentrations (8 or 16 μg/ml). At each MIC, approximately half of the organisms were susceptible and half were resistant. Among the 55 isolates for which ampicillin MICs were ≥32 μg/ml, there were two false-susceptible disk elution results. All of the 93 organisms susceptible to ampicillin-sulbactam and the 94 organisms susceptible to amoxicillin-clavulanic acid (MIC, ≤8.0 μg/ml) were susceptible by the disk elution test as well. There were three organisms for which ampicillin-sulbactam MICs were 16 μg/ml (intermediate susceptibility category), two of which were susceptible and one of which was resistant by the disk elution test. There was one isolate that was resistant to amoxicillin-clavulanic acid (MIC, 32 μg/ml) by agar dilution, and it was also resistant by disk elution. Because none of the anaerobes tested were resistant to ampicillin-sulbactam and only one was resistant to amoxicillin-clavulanic acid, it was not possible to evaluate the performance of the disk elution test with resistant anaerobes. Anaerobic bacteria resistant to these β-lactamase inhibitor combinations appear to be very rare at present.

We conclude that the broth microdilution test is an acceptable alternative to the reference agar dilution test for the testing of anaerobic susceptibility to ampicillin-sulbactam and amoxicillin-clavulanic acid. The disk elution test provided reliable results for anaerobes susceptible to ampicillin-sulbactam and amoxicillin-clavulanic acid, but since resistant isolates were not available for such studies, no conclusions can be reached about the reliability of this test for anaerobes resistant to these drug combinations.

LITERATURE CITED

1. **Appelbaum, P. C., M. R. Jacobs, S. K. Spangler, and S. Yamabe.** 1986. Comparative activity of β-lactamase inhibitors YTR 830, clavulanate, and sulbactam combined with β-lactams against β-lactamase-producing anaerobes. Antimicrob. Agents Chemother. **30**:789–791.
2. **English, A. R., J. A. Retsema, A. E. Girard, J. E. Lynch, and W. E. Barth.** 1978. CP-45,899, a beta-lactamase inhibitor that extends the antibacterial spectrum of beta-lactams: initial bacteriological characterization. Antimicrob. Agents Chemother. **14:** 414–419.
3. **Fu, K. P., and H. C. Neu.** 1979. Comparative inhibition of β-lactamases by novel β-lactam compounds. Antimicrob. Agents Chemother. **15**:171–176.
4. **National Committee for Clinical Laboratory Standards.** 1985. Reference agar dilution procedure for antimicrobial susceptibility testing of anaerobic bacteria. Approved standard M11-A. National Committee for Clinical Laboratory Standards, Villanova, Pa.
5. **National Committee for Clinical Laboratory Standards.** 1985. Alternative methods for antimicrobial susceptibility testing of anaerobic bacteria. Proposed guideline M17-P. National Committee for Clinical Laboratory Standards, Villanova, Pa.
6. **Neu, H. C., and K. P. Fu.** 1978. Clavulanic acid, a novel inhibitor of β-lactamases. Antimicrob. Agents Chemother. **14**:650–655.
7. **Retsema, J. A., A. R. English, and A. E. Girard.** 1980. CP-45,899 in combination with penicillin or ampicillin against penicillin-resistant *Staphylococcus*, *Haemophilus influenzae*, and *Bacteroides*. Antimicrob. Agents Chemother. **17**:615–622.
8. **Wexler, H. M., B. Harris, W. T. Carter, and S. M. Finegold.** 1985. In vitro efficacy of sulbactam combined with ampicillin against anaerobic bacteria. Antimicrob. Agents Chemother. **27:** 876–878.
9. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1978. In vitro study of clavulanic acid in combination with penicillin, amoxycillin, and carbenicillin. Antimicrob. Agents Chemother. **13**:389–393.