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Inoculum Preparation for Anaerobic Susceptibility Tests

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The results of anaerobic susceptibility tests performed with inocula prepared directly from agar isolation media and from overnight broth cultures were compared. Altogether, 93.0% of the results with these two inoculum preparations were within one twofold dilution, including 92.5 and 95.6% of the results with *Clostridium* species and the *Bacteroides fragilis* group, respectively. Thus, inocula prepared from agar subculture plates resulted in more-timely susceptibility test results. In addition, the test organisms used in this study grew better when the inoculum was prepared directly from agar plates.

Susceptibility tests must be performed promptly if the results are to be clinically useful. Preparation of the inoculum is one step in the test procedure that has been modified to lessen the time required for performing the tests. Susceptibility tests with aerobic and anaerobic blood culture isolates can be performed accurately by using positive blood culture broths as the test inoculum, thus reducing the testing time by 1 to 2 days (2, 3). Furthermore, D'Amato and Hochstein (1) showed that disk diffusion susceptibility tests could be performed accurately with inocula prepared either from broth cultures, as recommended by the National Committee for Clinical Laboratory Standards, or directly from the primary isolation plates. Test results for aerobic and facultative organisms could be obtained earlier with this modification. In the study presented herein, we extended the observations of D'Amato and Hochstein by examining the feasibility of preparing the test inoculum for anaerobic susceptibility tests directly from agar plates.

The following laboratory standard antibiotic powders and concentrations were tested: cefazolin (0.5 to 64 μg/ml), cefamandole (0.25 to 32 μg/ml), cephalothin (0.25 to 32 μg/ml), and erythromycin (0.06 to 8 μg/ml) (Eli Lilly & Co., Indianapolis, Ind.); carbenicillin (1 to 128 μg/ml) (Roerig, New York, N.Y.); cefoxitin (0.25 to 32 μg/ml) (Merck Sharp & Dohme, West Point, Pa.); chloramphenicol (0.5 to 64 μg/ml) (Parke, Davis & Co., Detroit, Mich.); clindamycin (0.12 to 16 μg/ml) (The Upjohn Co., Kalamazoo, Mich.); doxycycline (0.06 to 8 μg/ml) (Pfizer Laboratories, New York, N.Y.); metronidazole (0.25 to 32 μg/ml) (G. D. Searle and Co., Chicago, Ill.); and penicillin G (0.5 to 64 μg/ml) (Wyeth Laboratories, Philadelphia, Pa.). The antimicrobial stock solutions were diluted serially in Wilkins-Chalgren broth and dispensed into microdilution trays. The trays were frozen at −80°C until used.

A total of 78 clinical isolates and the 8 National Committee for Clinical Laboratory Standards reference quality control organisms (4) were tested, including *Bacteroides fragilis* group (25 isolates), *Bacteroides* spp. (3 isolates), *Clostridium perfringens* (20 isolates), *Clostridium* spp. (19 isolates), *Fusobacterium* spp. (6 isolates), *Eubacterium* spp. (3 isolates), *Peptostreptococcus* spp. (4 isolates), and *Peptococcus* spp. (6 isolates).

The inocula for the susceptibility tests were prepared directly from agar plates and from overnight broth cultures. The test organisms were streaked onto Schaedler blood agar and incubated in an anaerobic chamber for 24 to 48 h. After good growth was obtained, isolated colonies were picked from the plate and suspended in Wilkins-Chalgren broth supplemented with 1% heat-inactivated horse serum. The inoculum was adjusted to a turbidity equivalent to a 0.5 McFarland standard, diluted 1:100, and inoculated into a microdilution tray. For the tests performed with broth culture inocula, isolated colonies were picked from the Schaedler blood agar plate, inoculated into thioglycolate broth supplemented with vitamin K, hemin, and sodium bicarbonate, and incubated overnight in an anaerobic chamber. The broths were then diluted in Wilkins-Chalgren broth supplemented with inactivated horse serum, adjusted to the turbidity of a 0.5 McFarland standard, diluted 1:100, and inoculated into microdilution trays. All tests were incubated at 35°C in an anaerobic chamber. After 48 h of incubation, the minimal inhibitory concentration (MIC) was determined

TABLE 1. Comparison of susceptibility test results with inocula prepared with overnight broth cultures and directly from agar plates

Antibiotic	No. of strains with MIC ratio[a] of:					% of results within one twofold dilution[b]
	≤0.25	0.5	1	2	≥4	
Carbenicillin	1	5	62	6	1	97.3
Cefamandole	5	6	52	11	1	92.0
Cefoxitin	1	9	54	8	3	94.7
Cefazolin	3	10	54	5	3	92.0
Cephalothin	3	10	51	9	2	93.3
Chloramphenicol	4	16	45	9	1	93.3
Clindamycin	4	4	60	4	3	90.7
Doxycycline	5	7	57	5	1	92.0
Erythromycin	3	17	43	11	1	94.7
Metronidazole	5	10	44	10	6	85.3
Penicillin	1	8	61	4	1	97.3

[a] MIC ratio, Ratio of MIC with broth inoculum to MIC with direct inoculum.

[b] A total of 75 comparisons was made for each antibiotic.

as the lowest concentration of an antimicrobial agent that prevented visible growth of the test organism.

Comparative quantitative susceptibility tests could not be performed with 11 isolates. Two isolates (*Clostridium sordellii, Fusobacterium necrophorum*) did not grow in the microdilution tests with inocula prepared from either agar or broth. Nine isolates (four of *Clostridium difficile* and one each of *Clostridium bifermentans, C. sordellii, Fusobacterium mortiferum, F. necrophorum*, and *Bacteroides oralis*) grew only with inocula prepared from agar. Therefore, comparative tests were performed with 75 isolates. Altogether, 93.0% of the results with inocula from agar and broth cultures were within one twofold dilution. With the exception of metronidazole, more than 90% of the results with each antibiotic were within one twofold dilution (Table 1). Furthermore, more than 90% of the results with each group of organisms were within one twofold dilution, including 92.5 and 95.6% of the results with *Clostridium* species and the *Bacteroides fragilis* group, respectively.

This study shows that inocula for anaerobic susceptibility tests can be prepared from overnight broth cultures or directly from agar media. The susceptibility test results obtained with the two methods of inoculum preparation were equivalent (Table 1). The advantages of preparing inocula directly from agar media are twofold. First, the susceptibility results should be available at least 1 day faster. At present, most laboratories initially subculture colonies from primary isolation plates to aerobic and anaerobic plates. After 1 to 2 days of incubation, the pure culture of the anaerobe is inoculated into a broth culture, incubated overnight, and then used to prepare the inoculum for the susceptibility test. However, the broth culture is unnecessary because the test inoculum can be prepared directly from the subculture agar plate. Secondly, the anaerobic test organisms used in this study grew better when the inoculum was prepared from agar plates. Altogether, 84 of 86 organisms could be tested from agar plates, compared with only 75 organisms prepared from broth cultures.

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