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Eubacterium lentum ATCC 43055, a New Reference Strain for Quality Control of Anaerobic Susceptibility Tests

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A strain of *Eubacterium lentum* (ATCC 43055) was selected for quality control of anaerobic susceptibility tests. Multilaboratory collaborative studies with 13 different antimicrobial agents were reviewed, and MIC control limits were proposed for agar dilution tests with the new control strain.

In vitro methods for determining antimicrobial susceptibility of anaerobic bacteria were reviewed recently by Finegold et al. (3). Although the clinical relevance of such in vitro data has been questioned, many diagnostic laboratories are required to test susceptibility of anaerobes on a regular basis. To obtain reproducible results, careful standardization of methods and institution of a strict quality assurance program are important.

Three quality control strains have been identified (*Bacteroides fragilis* ATCC 25285, *Bacteroides thetaiotaomicron* ATCC 29741, and *Clostridium perfringens* ATCC 13124) (4, 6). When testing many β-lactam antimicrobial agents, the *C. perfringens* control strain has proven to be unsatisfactory for two reasons: (i) the results are often too variable to be useful, and (ii) the MICs of many drugs are well below the lowest concentration that is normally tested by clinical laboratories (1, 8). Zabransky and colleagues identified a strain of *Eubacterium lentum* that was initially recovered from a postsurgical abdominal wound. That isolate was selected as a possible alternative control strain because it grew satisfactorily on most anaerobic media, it withstood freezing and lyophilization, it gave reasonably well defined endpoints with most drugs, and most MICs fell within clinically relevant ranges. This strain has subsequently been deposited in the American Type Culture Collection (ATCC 43055). Several multilaboratory studies have now been undertaken to establish acceptable MICs for the reference agar dilution procedure with this control strain. The National Committee for Clinical Laboratory Standards (NCCLS) (5) specifically requires replicate dilution tests in at least five independent laboratories using multiple lots of agar medium. A marketed control drug or other control strains with established MIC limits are normally tested at the same time for internal control purposes. The results of such five-laboratory collaborative studies are then used to define MIC control limits for each drug-microorganism combination, and these limits are published as part of the NCCLS test procedure (4). Such studies have been performed with the previously selected anaerobic control strains (1, 6–8), and ongoing efforts are being made to establish control limits for new antimicrobial agents and new control strains.

In this brief report, we describe the results of several collaborative studies that were undertaken to evaluate the *E. lentum* control strain tested against each of the 13 different antimicrobial agents listed in Table 1. The five-laboratory testing protocol was performed as previously described (1). Each of five participating laboratories generated 20 MICs with 20 separate inoculum preparations by using the NCCLS agar dilution procedure (4). Additional controls with established drugs or with established control strains gave satisfactory results and are not described further in this report. The 100 MICs that were generated in this way were then evaluated to propose an acceptable range of MICs. In most cases, the control range was the mode ± 1 doubling dilution, but when the data were skewed, the range included at least 95% of all of the datum points (2), even though the mode was not in the center. In all cases, the proposed control limits describe ranges which include three twofold dilutions; stricter limits which include only 2 doubling dilutions could have been applied to most of the study drugs (2). Two separate five-laboratory studies with cefoxitin were performed independently, and all MICs were either 4.0 or 8.0 μg/ml; only the mode differed in the two sets of data (Table 1). In both cases, MIC ranges of 4.0 to 8.0 μg/ml or 4.0 to 16 μg/ml would be appropriate for tests with cefoxitin.

Supplementary data that were collected as part of separate studies are also presented. These studies involved only three laboratories, and additional data from a fourth laboratory were added later. Those supplementary data are presented in Table 1 to confirm the initial five-laboratory study data (ceftizoxime and piperacillin). In two cases (moxalactam and cefoperazone) the supplementary data provided the only control information that has been collected systematically. Although only four laboratories contributed data for each of the latter drugs, tentative control limits could be proposed by using a mode ± 1 doubling dilution statistic.

In the initial five-laboratory study with ceftizoxime, the mode was 32 μg/ml, but the supplemental data provided a mode of 64 μg/ml. Where the true mode actually lies is a matter of conjecture. Against other microorganisms, ceftizoxime endpoints are often very difficult to read, and that can result in marked discrepancies between different laboratories or between individuals in the same laboratory. Nevertheless, the *E. lentum* control strain gives relatively well defined ceftizoxime endpoints. A range of 16 to 64 μg/ml is recommended for monitoring agar dilution tests.

When testing clindamycin against the *E. lentum* control strain, a modal value of 0.06 μg/ml was noted, and thus a control range of 0.03 to 0.125 μg/ml could have been proposed. However, MICs of 0.03 μg/ml were not reported in this series, and one of five laboratories reported all 20 of their MICs to be 0.25 μg/ml. Consequently, control limits of 0.06 to 0.25 μg/ml are tentatively proposed rather than 0.03

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TABLE 1. Reference agar dilution anaerobic susceptibility test results with *E. lentum* ATCC 43055

Antimicrobial agent	MIC (μg/ml) (no. of times each MIC was recorded)							
	Five-laboratory coordinated study[a]				Supplemental data[b]			
Cefotaxime	32 (2)	[64 (30)	128 (67)	256 (1)][c]				
Ceftizoxime	[16 (7)	32 (93)	64 (0)]		[16 (0)	32 (22)	64 (34)]	128 (4)
Moxalactam	No data				[64 (16)	128 (96)	256 (3)]	
Ceftriaxone	[128 (45)	256 (53)	512 (2)]					
Cefoperazone	No data				[32 (0)	64 (45)	128 (30)]	
Cefmetazole	[4.0 (0)	8.0 (100)	16 (0)]					
Cefoxitin	[4.0 (20)	8.0 (80)	16 (0)]		[4.0 (64)	8.0 (63)	16 (0)][d]	
Cefotetan	[32 (41)	64 (52)	128 (9)]					
Imipenem	[0.25 (47)	0.5 (53)	1.0 (0)]					
Mezlocillin	[8.0 (20)	16 (80)	32 (0)]					
Piperacillin	[8.0 (27)	16 (73)	32 (0)]		[8.0 (14)	16 (68)	32 (36)]	
Clindamycin	[0.06 (55)	0.125 (25)	0.25 (20)]					
Trospectomycin	[4.0 (43)	8.0 (48)	16 (14)]					

[a] Studies with sufficient internal controls to conform to NCCLS guidelines (5).
[b] Supplemental data collected from three or four different laboratories as part of unrelated collaborative studies.
[c] Proposed MIC control limits are enclosed within brackets.
[d] Represents a second five-laboratory coordinated study that confirms the initial study results.

to 0.125 μg/ml. These tentative standards can be changed if the need for such a change is supported by additional data that may be generated in the future.

In general, the *E. lentum* strain has proven to be capable of providing reasonably reproducible agar dilution test results. MICs of most β-lactam antibiotics are similar to those for the two *Bacteroides* species that are currently being used for quality control purposes. Because the *E. lentum* strain is less likely to give off-scale endpoints with β-lactam drugs and because it is capable of providing repeatable endpoints, we recommend that it be incorporated into quality assurance programs. For those drugs that are commonly tested in clinical laboratories, *E. lentum* ATCC 43055 can usually replace *C. perfringens* ATCC 13124. Additional studies with *E. lentum* ATCC 43055 are needed to provide control limits for drugs that were not included in the present report. All of the data presented in this report refer to agar dilution test results. Slightly different results might be expected when broth microdilution or macrodilution procedures are used or if different agar media are used. Consequently, the control limits described in this report apply only to the results of agar dilution susceptibility tests performed according to NCCLS procedure (4). Similar studies with broth microdilution tests are needed.

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