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Susceptibilities of 201 Anaerobes to Erythromycin, Azithromycin, Clarithromycin, and Roxithromycin by Oxyrase Agar Dilution and E test Methodologies

S. K. SPANGLER,[1] M. R. JACOBS,[2] AND P. C. APPELBAUM[1]*

Department of Pathology (Clinical Microbiology), Hershey Medical Center, Hershey, Pennsylvania 17033,[1] *and Department of Pathology, Case Western Reserve University, Cleveland, Ohio 44106*[2]

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The susceptibility of 201 anaerobes to erythromycin, azithromycin, clarithromycin, and roxithromycin was tested by agar dilution and E test methods by using a commercially available plate and dish system (OxyDish) to provide anaerobic conditions. Plates were incubated for 48 h. MICs for 50% of strains tested and MICs for 90% of strains tested by agar dilution and E test methods corresponded within 1 doubling dilution for all compounds. When all antibiotics were considered together, agar and E test MICs were within 1 and 2 doubling dilutions of each other in 84 to 91% and >99% of cases, respectively.

Previous studies have established the usefulness of the Oxyrase agar dilution method for susceptibility testing of anaerobes. The method allows incubation in ambient air rather than the CO_2-enriched atmosphere present in anaerobic chambers or gas-generating jar systems. This avoids the effect of CO_2 on the pH of the medium when testing the activity of macrolides and azalides against anaerobes (6–9). One problem with this method has been the time-consuming and laborious nature of preparing Oxyrase agar and performing agar dilution MIC tests (8, 9).

The E test has been established as an excellent method for MIC testing of anaerobes (1–3, 11). This study was designed to evaluate the utility of commercial Oxyrase plates and the E test to test the activity of erythromycin, azithromycin, clarithromycin, and roxithromycin against 201 clinically isolated anaerobes. Agar dilution MIC methodology, also performed in Oxyrase plates, was used as the reference system.

Organisms used in the current study were as follows (number of isolates in parentheses): *Bacteroides fragilis* (21), *Bacteroides thetaiotaomicron* (15), *Bacteroides ovatus* (10), *Bacteroides distasonis* (15), *Bacteroides vulgatus* (15), *Prevotella bivia* (3), *Prevotella disiens* (11), *Prevotella oralis* (1), *Prevotella corporis* (1), *Prevotella loeschei* (1), *Prevotella intermedia* (6), *Prevotella melaninogenica* (9), *Bacteroides ureolyticus* (5), *Bacteroides capillosus* (5), *Fusobacterium nucleatum* (6), *Fusobacterium necrophorum* (8), *Fusobacterium varium* (4), *Fusobacterium mortiferum* (10), *Fusobacterium* species (5), *Peptostreptococcus* species (1), *Peptostreptococcus magnus* (2), *Peptostreptococcus anaerobius* (2), *Peptostreptococcus asaccharolyticus* (2), *Peptostreptococcus micros* (1), *Streptococcus intermedius* (1), *Propionibacterium acnes* (2), *Bifidobacterium* species (2), *Lactobacillus* species (4), *Actinomyces meyeri* (1), *Eubacterium lentum* (1), *Clostridium perfringens* (8), *Clostridium difficile* (5), *Clostridium sporogenes* (1), *Clostridium clostridioforme* (1), *Clostridium butyricum* (2), *Clostridium tertium* (4), *Clostridium paraperfringens* (1), *Clostridium innocuum* (3), *Clostridium bifermentans* (1), *Clostridium cadaveris* (2), *Clostridium ramosum* (2), and *Clostridium* species (1). Organisms were identified by standard methods (4, 10) and stored at −70°C until use.

Antibiotic powders of known potency were obtained as follows: erythromycin and clarithromycin (Abbott Laboratories, Chicago, Ill.), azithromycin (Pfizer, Inc., New York, N.Y.), and roxithromycin (Roussel Uclaf, Paris, France). The commercially available Oxyrase plate and lid system (OxyDish; Oxyrase, Inc., Mansfield, Ohio) (Fig. 1) was used throughout the study. Agar dilution MICs were determined in these Oxyrase plates by a modification of the method recommended by the National Committee for Clinical Laboratory Standards (5) as described previously (8, 9). Media for agar dilution tests were prepared as follows. For the first 81 strains, 1 ml of an Oxyrase solution containing 5 U of Oxyrase and 45 mM lactate, 30 mM succinate, and 15 mM formate was added to 17 ml of molten Wilkins-Chalgren agar (Difco Laboratories, Detroit, Mich.)–1 ml of sterile sheep blood–1 ml of antibiotic solution. For the remaining 120 strains, a commercially available solution containing both Oxyrase and substrates (Oxyrase, Inc.) became available and was used in place of the separate Oxyrase solution and the three substrates. For E tests, plates containing medium as described above, containing 18 ml of molten Wilkins-Chalgren medium, 1 ml of blood, and 1 ml of Oxyrase solution, were inoculated with a 0.5-McFarland-standard organism suspension, and E test strips were applied (two per plate). Plates were incubated for 48 h. Standard quality control strains (*B. fragilis* ATCC 25285, *B. thetaiotaomicron* ATCC 29741, and *C. perfringens* ATCC 13124 [used only when gram-positive strains were tested]) were included in each run to check antibiotic potency and anaerobic conditions. Inocula

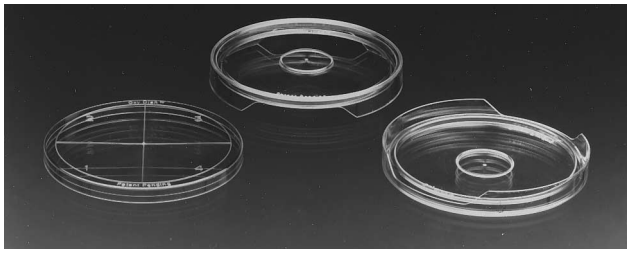

FIG. 1. Commercially available empty OxyDish plate and lid system.

* Corresponding author. Mailing address: Department of Pathology, Hershey Medical Center, P.O. Box 850, Hershey, PA 17033. Phone: (717) 531-5113. Fax: (717) 531-5021.

TABLE 1. MICs for anaerobe strains by agar dilution and E test

Antimicrobial agent and strain type	MIC_{50} (μg/ml)[a]	MIC_{90} (μg/ml)[a]
Erythromycin		
Gram negative	4.0/4.0	256.0/256.0
Gram positive	2.0/1.0	256.0/256.0
All	4.0/2.0	256.0/256.0
Azithromycin		
Gram negative	4.0/4.0	256.0/256.0
Gram positive	2.0/1.0	256.0/256.0
All	4.0/4.0	256.0/256.0
Clarithromycin		
Gram negative	2.0/2.0	256.0/256.0
Gram positive	1.0/0.5	256.0/256.0
All	1.0/1.0	256.0/256.0
Roxithromycin		
Gram negative	8.0/8.0	256.0/256.0
Gram positive	2.0/2.0	256.0/256.0
All	8.0/4.0	256.0/256.0

[a] Expressed as ratio of value by agar dilution to that by E test.

for both methods were 10^5 CFU per spot and were prepared by scraping growth off enriched blood agar plates (8, 9). Agar dilution ranges comprised 0.125 to 256.0 μg/ml. For the purposes of this study, E test and agar dilution MICs of ≤0.125 μg/ml were said to correspond.

Results of MICs for 50% of strains tested (MIC_{50}s) and MIC_{90}s by agar dilution and E test in Oxyrase plates are presented in Table 1. As can be seen, the MIC_{50}s and MIC_{90}s of all four drugs by agar dilution agreed with those obtained by E test to within 1 doubling dilution. As reported previously (9), clarithromycin was slightly more active than the other three compounds tested. Agar dilution and E test MICs corresponded to within 2 doubling dilutions in >99% of cases (Table 2). However, only 83.6 to 91.0% of E test MICs were within 1 dilution of agar MICs for all compounds tested; in most cases, E test MICs were lower than those obtained by agar dilution (Table 2).

Quality control ranges (micrograms per milliliter) by agar dilution were as follows: (i) *B. fragilis* ATCC 25285, erythromycin, azithromycin, and clarithromycin, 1.0 to 2.0; roxithromycin, 1.0 to 4.0; (ii) *B. thetaiotaomicron* ATCC 29741, erythromycin, 1.0 to 4.0; azithromycin, 2.0 to 8.0; clarithromycin, 1.0 to 4.0; roxithromycin, 2.0 to 8.0; (iii) *C. perfringens* 13124, erythromycin, azithromycin, and clarithromycin, 1.0 to 2.0; roxithromycin, 2.0. E test quality control ranges, although similar to those for agar dilution, tended to be 1 dilution lower.

Results of this study indicate that combining Oxyrase plates with the E test yields promise for testing susceptibility of anaerobes to macrolides and azalides in a routine laboratory without the need for anaerobic chambers or jars. We do not have an explanation for the E test MICs which were 2 dilutions lower than those obtained with agar dilution. This phenomenon was observed with all four agents at all MICs and for all organism groups and species tested. No differences in results obtained with commercial and in-house-prepared Oxyrase solutions were observed. Expansion of the study with additional strains from other centers may help shed light on this phenomenon. We have no data on comparison between E test and broth dilution MICs with Oxyrase.

TABLE 2. Correspondence between agar dilution and E test

Antimicrobial agent	No. of E test results agreeing with those by agar dilution								
	+3	+2	+1	0	−1	−2	−3	% ± 1 dilution	% ± 2 dilutions
Erythromycin	0	5	22	102	44	27	1	83.6	99.5
Azithromycin	0	3	38	105	40	15	0	91.0	100.0
Clarithromycin	0	0	24	101	48	28	0	86.1	100.0
Roxithromycin	0	2	26	97	52	24	0	87.1	100.0

The newly designed OxyDish is easy to use and has the advantage that the lid may be removed and replaced several times without adversely affecting anaerobiosis. Lids fit snugly, and there is no danger of loosening during handling and incubation. A solution containing Oxyrase and substrates is now commercially available, such that a flask of sterile molten Wilkins-Chalgren agar may be melted, Oxyrase solution and blood may be added, and plates may be poured whenever necessary. All strains tested grew well with this method.

Use of the Oxyrase method together with E test strips obviates the need for anaerobe jars or an anaerobic chamber for incubation of plates. A previous study using in-house-prepared Oxyrase plates (8) indicated that Oxyrase agar dilution methodology yielded clindamycin anaerobe MICs which corresponded well to those obtained in CO_2. To our knowledge, no other agents commonly used to treat anaerobic infections have been evaluated by the Oxyrase agar dilution method. Other drugs, such as cefoxitin, clindamycin, piperacillin, ceftizoxime, and imipenem, which may be used for treatment of anaerobic infections, have been tested by Oxyrase broth disk elution and macrotube broth dilution, respectively (6, 7). MICs have been shown to correspond well with those obtained by agar and macrobroth dilution MIC tests by conventional anaerobic incubation. Future studies should focus on use of Oxyrase together with E test strips for susceptibility testing of antimicrobial agents routinely used for treatment of anaerobic infections.

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