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Susceptibility Testing of *Propionibacterium acnes* Comparing Agar Dilution with E test

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***Propionibacterium acnes* has been identified as a significant agent of nosocomial infections, including endophthalmitis. Data concerning susceptibility of *P. acnes* to newer beta-lactam antibiotics and fluoroquinolones are limited. Recent reports suggest that quinolones have activity against these organisms sufficient to warrant further study. We undertook a study to select appropriate antimicrobial agents for use in a rabbit model of *P. acnes* endophthalmitis. We compared the antibiotic susceptibilities of *P. acnes* by using the National Committee for Clinical Laboratory Standards method of agar dilution with the E test. Thirteen clinical isolates obtained from eye specimens and three American Type Culture Collection control strains were tested against 14 antibiotics. All the clinical isolates were susceptible by both methods to piperacillin, piperacillin-tazobactam, ampicillin-sulbactam, ticarcillin-clavulanate, cefotaxime, cefotetan, ceftriaxone, cefoxitin, and imipenem in addition to clindamycin but were resistant to metronidazole. The clinical *P. acnes* isolates also displayed high-level susceptibility to ciprofloxacin, sparfloxacin, and ofloxacin. Almost all the *P. acnes* strains demonstrated E-test MICs within 2 dilutions of the MICs observed by the agar dilution method. Those few strains for which discrepancies were noted exhibited E-test susceptibilities three- to fivefold dilutions lower than the agar dilution method susceptibilities but only with ampicillin-sulbactam, ticarcillin-clavulanate, and/or clindamycin. On the basis of our study, all of our clinical eye isolates were susceptible to these newer antimicrobial agents and the two methods demonstrated similar susceptibility patterns.**

Propionibacterium acnes is a gram-positive, non-spore-forming, anaerobic bacillus traditionally considered nonpathogenic. More recently, it has been identified as a significant agent of nosocomial infections, including endophthalmitis (2, 13, 15). Special care is required for the isolation of this bacterium and the study of its in vitro antibiotic susceptibility profile. Although *P. acnes* has demonstrated in vitro susceptibility to many of the older-generation beta-lactam antibiotics and macrolides, treatment success in patients has been inconsistent (2). In addition, data concerning susceptibility of *P. acnes* to newer beta-lactams and fluoroquinolones are limited or unavailable (1, 14).

While quinolones are not considered the drugs of choice for anaerobic infections, recent evidence suggests that they demonstrate activity against anaerobes sufficient to warrant further study (6, 7). More importantly, the fact that endophthalmitis is a closed-compartment infection in which direct intravitreal antibiotic instillation can achieve a concentration markedly greater than the MIC for the organism justifies testing of these antimicrobial agents against *P. acnes*. Therefore, the use of these drugs against the bacterium may be indicated for our studies using an animal model of endophthalmitis (10).

We compared the antibiotic susceptibilities of *P. acnes* by using the National Committee for Clinical Laboratory Standards method of agar dilution with the E test (9, 12). The E test has been shown to provide equivalent susceptibility results for many organisms. Little information regarding the response of *P. acnes* to antimicrobial agents with the utilization of routine methods is available, and there is little information concerning the use of E-test methodology with this organism.

Two strains of *P. acnes* (ATCC 11827 and 11828) and one strain of *Bacteroides fragilis* (ATCC 25285) served as controls; 13 *P. acnes* strains isolated from eye specimens submitted to the Microbiology Division of the Long Island Jewish Medical Center, New Hyde Park, N.Y., were used in this study. Sheep blood agar (Becton Dickinson Microbiology Systems; BBL, Cockeysville, Md.) served to prepare inocula for the susceptibility studies. The bacteria were then placed in reduced brucella broth (BBL) and suspended to a density of 0.5 McFarland standard for the agar dilution and a density of 1.0 for the E test (PDM epsilometer; AB Biodisk N.A., Piscataway, N.J.). Susceptibility tests were performed on sheep blood-enriched Mueller-Hinton agar (BBL), Wilkens-Chalgren agar (BBL), or supplemented brucella agar (BBL) to select the medium which provided the best growth for the organism. Agar dilution was prepared in accordance with the National Committee for Clinical Laboratory Standards protocol (9). MICs were determined by means of both agar dilution and the E test at 48 h following incubation at 35°C in an anaerobic chamber. Standard antibiotic powders included piperacillin (Lederle Laboratories, Carolina, P.R.), piperacillin-tazobactam (Lederle Laboratories, Pearl River, N.Y.), ampicillin-sulbactam (Roerig-Pfizer, New York, N.Y.), ticarcillin-clavulanate (SmithKline Beecham, Philadelphia, Pa.), cefotaxime (Hoechst-Roussel, Somerville, N.J.), cefotetan (Stewart Pharmaceuticals, Wilmington, Del.), ceftriaxone (Roche, Nutley, N.J.), cefoxitin (Merck Sharp & Dohme, West Point, Pa.), imipenem (Merck Sharp & Dohme, Rahway, N.J.), ciprofloxacin (Miles Laboratories, West Haven, Conn.), ofloxacin (R.W. Johnson Pharmaceutical

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TABLE 1. Antimicrobial susceptibilities of anaerobic strains

Antimicrobial agent	Agar dilution or E-test MIC (μg/ml) for *P. acnes* strain[a]:															
	E418		E459		E476		E483		E485		E495		E12		E33	
	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET
Piperacillin	0.5	1	0.5	0.5	0.5	1	0.5	1	0.5	0.5	0.5	2	0.5	0.5	1	2
Piperacillin-tazobactam	0.5	0.5	0.5	0.06	0.25	0.25	0.5	1	0.5	1	0.5	1	0.03	0.06	0.5	0.5
Ampicillin-sulbactam	≤0.25	0.06	≤0.25	≤0.016	0.125	0.125	≤0.25	0.125	0.5	0.125	0.125	0.125	0.06	0.06	0.125	0.125
Ticarcillin-clavulanate	0.06	≤0.016	0.06	≤0.016	0.25	0.06	0.25	0.25	0.5	0.06	0.5	0.03	0.06	0.03	0.06	0.03
Cefotaxime	0.25	0.5	≤0.125	0.06	≤0.125	0.25	0.25	0.5	0.5	1	0.25	0.25	≤0.125	0.06	0.25	0.06
Cefotetan	0.5	1	≤0.25	0.25	≤0.25	1	1	1	1	1	1	1	≤0.25	0.25	0.5	1
Ceftriaxone	≤0.125	0.25	≤0.125	0.06	0.5	0.25	0.5	0.5	0.5	0.25	0.5	0.25	≤0.125	0.06	0.25	0.25
Cefoxitin	0.25	0.25	≤0.25	0.06	0.25	0.25	0.25	0.5	0.5	0.5	0.25	0.25	≤0.25	0.06	0.5	0.25
Imipenem	≤0.016	0.03	≤0.016	0.016	0.03	0.03	≤0.016	0.03	0.03	0.06	≤0.016	0.03	≤0.016	0.007	≤0.016	0.03
Ciprofloxacin	1	1	0.5	1	0.5	0.5	1	0.5	0.5	0.5	0.5	0.5	1	1	1	2
Ofloxacin	1	2	0.5	1	1	1	1	1	1	1	1	4	1	0.5	1	1
Sparfloxacin	0.25	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25
Metronidazole	>64	>32	>64	>32	>64	>32	>64	>32	>64	>32	>64	>32	>64	>32	>64	>32
Clindamycin	0.016	≤0.016	0.016	0.125	0.06	0.25	0.016	0.25	0.5	0.5	0.5	0.06	0.016	≤0.016	0.016	0.03

[a] AD, agar dilution; ET, E test.

TABLE 2. Antimicrobial susceptibilities of anaerobic strains

Antimicrobial agent	Agar dilution or E-test MIC (μg/ml) for[a]:															
	P. acnes E44		*P. acnes* E46		*P. acnes* E497		*P. acnes* E500		*P. acnes* E511		*P. acnes* ATCC 11827		*P. acnes* ATCC 11828		*B. fragilis* ATCC 25285	
	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET	AD	ET
Piperacillin	1	2	0.5	1	≤0.5	0.125	≤0.5	0.5	≤0.5	0.125	≤0.5	≤0.016	1	1	4	6
Piperacillin-tazobactam	0.5	1	0.25	0.25	0.5	0.5	0.5	0.5	0.25	0.125	0.06	≤0.016	1	0.25	0.125	0.03
Ampicillin-sulbactam	0.125	0.25	0.125	0.125	≤0.25	0.03	≤0.25	0.125	0.125	≤0.016	0.06	≤0.016	≤0.25	0.125	1	0.03
Ticarcillin-clavulanate	0.5	0.06	0.06	≤0.016	0.06	≤0.016	0.06	0.03	0.06	≤0.016	0.125	≤0.016	1	0.03	0.5	0.03
Cefotaxime	0.5	0.5	0.25	0.25	≤0.125	0.03	≤0.125	0.125	≤0.125	≤0.016	≤0.125	≤0.016	0.25	0.25	8	2
Cefotetan	1	1	1	0.5	≤0.25	0.06	0.5	0.5	0.5	0.06	≤0.25	≤0.016	1	0.5	1	0.5
Ceftriaxone	0.5	0.5	0.25	0.25	≤0.125	0.03	0.25	0.125	≤0.125	0.016	≤0.125	≤0.016	0.5	0.25	>32	>32
Cefoxitin	0.25	0.5	0.25	0.25	0.25	0.125	0.5	0.5	0.25	0.06	≤0.25	≤0.016	0.25	0.25	16	6
Imipenem	0.03	0.03	0.03	0.016	0.03	0.016	0.03	0.03	0.03	0.016	≤0.016	0.007	≤0.016	0.06	0.06	0.06
Ciprofloxacin	0.5	0.5	1	2	1	1	0.5	1	0.5	0.5	1	0.5	1	0.5	2	2
Ofloxacin	1	1	1	1	1	1	1	0.5	1	0.5	1	0.5	1	0.5	0.5	1
Sparfloxacin	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.125	0.25	0.06	0.25	0.25	0.5	1
Metronidazole	>64	>32	>64	>32	>64	>32	>64	>32	>64	>32	>64	>32	>64	>32	≤16	1
Clindamycin	0.5	0.016	0.016	0.03	0.016	≤0.016	0.016	≤0.016	0.016	≤0.016	0.25	0.016	0.5	≤0.016	8	0.5

[a] AD, agar dilution; ET, E test.

Research Institute, Spring House, Pa.), sparfloxacin (Rhone-Poulenc-Rorer, Collegeville, Pa.), metronidazole (McGaw, Irvine, Calif.), and clindamycin (Upjohn Co., Kalamazoo, Mich.). E strips for these drugs were kindly provided by AB Biodisk. Serial dilution of piperacillin-tazobactam was accomplished by diluting piperacillin but maintaining tazobactam at a constant of 4 μg/ml (9). The MIC for agar dilution was defined as the lowest concentration that resulted in no growth or a barely visible haze. The E-test MIC was defined as the point on the scale at which the ellipse of growth inhibition intercepts the strip. The E-test MICs that fell between two points on the scale were rounded to the next higher value (4). The National Committee for Clinical Laboratory Standards breakpoints (in micrograms per milliliter) were used for susceptibility designation (9).

Blood-supplemented Mueller-Hinton agar (BBL) provided excellent growth for the bacteria. *P. acnes* grew poorly on Wilkens-Chalgren agar. Comparison of Mueller-Hinton agar with the supplemented brucella agar (BBL) gave equivalent results and supported the choice of sheep blood-supplemented Mueller-Hinton medium for the study (3–5).

Agar dilution and E-test MIC results were read after 48 h of incubation. All tests were repeated at least twice. The results are shown in Tables 1 and 2. Almost all the *P. acnes* strains demonstrated E-test MICs within 2 dilutions of those observed by agar dilution testing. The exceptions included *P. acnes* strains E459, E485, E495, E44, and E511 and the control strains. These bacteria exhibited E-test MICs which were three- to fivefold dilutions lower than those of the agar dilution method but only with ampicillin-sulbactam, ticarcillin-clavulanate, and/or clindamycin. The agar dilution and E-test methods demonstrated equivalent susceptibility results for the organisms with all other drugs tested.

P. acnes strains were highly susceptible to the beta-lactam antimicrobial agents, including piperacillin, piperacillin-tazobactam, ampicillin-sulbactam, ticarcillin-clavulanate, cefotaxime, cefotetan, ceftriaxone, cefoxitin, and imipenem, in addition to clindamycin but were resistant to metronidazole. The clinical isolates of *P. acnes* displayed high-level susceptibility to the three fluoroquinolones included in the study.

The study was undertaken to help select suitable antimicrobial agents for evaluation in a rabbit model of *P. acnes* endophthalmitis (10). All the clinical isolates were susceptible by both methods to the beta-lactam agents, the fluoroquinolones, and clindamycin. The data obtained by agar dilution and those obtained by the E test were equivalent. The discrepancy noted with respect to some beta-lactam antibiotics and clindamycin may be explained by inoculum or antibiotic diffusion effects which could lower the MIC result for the E test (4).

Although quinolones are not generally considered drugs of choice for anaerobic infections, recent data suggest that they have activity against these organisms sufficient to warrant further study (6, 7). Our data demonstrated high-level in vitro susceptibility of *P. acnes* to sparfloxacin, ofloxacin, and ciprofloxacin.

Hecht and Lederer recently reported similar susceptibility results after comparing three different media with isolates of *B. fragilis* tested by agar dilution (8). Our selection of enriched Mueller-Hinton medium to conduct this study was based on excellent growth of *P. acnes* on this medium compared with the poor results with Wilkens-Chalgren medium. As previously stated, susceptibility studies with supplemented brucella agar performed at the same time as studies with enriched Mueller-Hinton medium produce equivalent results.

Limited information regarding susceptibility of *P. acnes* to the newer beta-lactams and quinolones had been available. We performed both agar dilution and the E test not only to generate susceptibility data for *P. acnes* but also to compare data obtained by both methods. On the basis of our study, all of our clinical eye isolates were susceptible to these newer antimicrobial agents and the two methods demonstrated similar susceptibility patterns.

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