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Comparison of Etest and Agar Dilution Method for Antimicrobial Susceptibility Testing of *Flavobacterium* Isolates

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The Etest was evaluated as a possible alternative to the standard agar dilution method for susceptibility testing of nine antimicrobial agents against *Flavobacterium* species. In studies of 100 clinical isolates, the agreement between the MICs (±1 $\log_2$ dilution) obtained by the two methods was acceptable for cefotaxime, ceftazidime, amikacin, minocycline, ofloxacin, and ciprofloxacin (>90%). Conversely, the agreement between the results obtained for piperacillin was limited (84%). The overall agreement was 92.5%.

Strains of *Flavobacterium* species, including *Flavobacterium meningosepticum*, *F. indologenes*, and *F. odoratum*, have been reported to cause a variety of infections in humans (9–12, 15, 18, 19, 21). Invasive infections caused by this organism, especially in hospitalized and immunocompromised patients, frequently necessitate effective and timely antimicrobial therapy (9–12, 15, 19, 21). Appropriate choice of effective antimicrobial agents for treatment of flavobacterial infections is difficult. First, the MIC breakpoints for resistance and susceptibility have not been established by the National Committee for Clinical Laboratory Standards (NCCLS) (14). Second, results of the disk diffusion method, the method most commonly used for routine susceptibility testing in most clinical microbiology laboratories, have been demonstrated to show considerable discrepancy from those determined by the standard agar dilution method (1, 6). Third, the performance of standard dilution susceptibility testing as a routine method to determine the MICs of the agents prescribed against an individual flavobacterial isolate of clinical significance is laborious and time-consuming.

Although broth microdilution susceptibility methods are available in automated and semiautomated test formats from various manufacturers, most of the clinical microbiology laboratories in Taiwan are not equipped with the instruments required for MIC determination. The Etest (AB Biodisk, Solna, Sweden) has been proved to be a convenient and reliable alternative to the standard dilution method for determination of MICs of antimicrobial agents against a wide array of bacteria (3–5, 13, 16). This study was undertaken to evaluate the reliability of Etest results for determining the antimicrobial susceptibility of flavobacteria.

The 100 clinical strains of flavobacteria used in this study, including 40 isolates of *F. meningosepticum*, 55 of *F. indologenes*, and 5 of *F. odoratum*, were isolated in Taiwan from January 1992 to June 1996. MICs for all isolates were determined concomitantly by the agar dilution method and the Etest. Standard powders of nine antimicrobial agents obtained from various manufacturers for agar dilution testing included piperacillin and minocycline (Lederle Laboratories, Pearl River, N.Y.), cefotaxime (Hoechst AG, Frankfurt, Germany), ceftazidime (Glaxo Operations, Ltd., Greenford, England), aztreonam (Bristol-Myers Squibb Laboratories, Princeton, N.J.), imipenem (Merck Sharp & Dohme, West Point, Pa.), amikacin (Sigma Chemical Co., St. Louis, Mo.), ofloxacin (Daiichi Pharmaceutical Co., Ltd., Tokyo, Japan), and ciprofloxacin (Bayer Co., Leverkusen, Germany). Etest strips were purchased from AB Biodisk.

MICs were determined by an agar dilution method by following NCCLS guidelines (14), with Mueller-Hinton agar (BBL, Becton Dickinson, Sparks, Md.) and a multipoint inoculator with an inoculum of 10^4 CFU per spot. Antimicrobial agent concentrations ranged from 0.03 to 128 μg/ml for all agents. The MIC of each antimicrobial agent determined by the agar dilution method was defined as the lowest concentration which inhibited visible growth of the organism.

The Etest was performed in accordance with the manufacturer's directions. The antimicrobial concentrations of nine Etest strips ranged from 0.016 to 256 μg/ml for piperacillin, cefotaxime, ceftazidime, aztreonam, amikacin, and minocycline and from 0.002 to 32 μg/ml for imipenem, ofloxacin, and ciprofloxacin. A bacterial suspension of growth from a tryptic soy agar plate (BBL) was prepared in 5 ml of Mueller-Hinton broth (BBL) and adjusted to equal the turbidity of a 0.5 McFarland standard. A cotton-tipped swab was dipped into the

TABLE 1. Antimicrobial susceptibilities of 100 *Flavobacterium* isolates determined by the agar dilution method and the Etest

Antimicrobial agent	MIC (μg/ml)[a]					
	Agar dilution			Etest		
	Range	50%	90%	Range	50%	90%
Piperacillin	1–>128	4	128	1–>256	8	>256
Cefotaxime	4–>128	64	>128	8–>256	128	>256
Ceftazidime	2–>128	128	>128	1–>256	>256	>256
Aztreonam	>128	>128	>128	>256	>256	>256
Imipenem	1–>128	>128	>128	1–>32	>32	>32
Amikacin	16–>128	128	>128	32–>256	>256	>256
Minocycline	1–16	2	8	0.25–32	4	8
Ofloxacin	0.5–64	4	64	0.5–>32	2	>32
Ciprofloxacin	0.25–128	2	64	0.25–>32	1	>32

[a] 50% and 90%, MICs at which 50 and 90% of the isolates were inhibited, respectively.

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TABLE 2. Comparison of Etest and agar dilution test results for seven antimicrobial agents tested against 100 *Flavobacterium* isolates

Antimicrobial agent	% of isolates for which Etest MICs differed from agar dilution MICs by indicated no. of $\log_2$ dilutions[a]						% Agreement[b]	No. (%) of isolates with category discrepancy[c]		
	−2	−1	0	+1	+2	≥+3		Very major error	Major error	Total error
Piperacillin	0.0	4.0	40.0	42.0	8.0	6.0	84.0	0 (0)	12 (16.7)	21 (21)
Cefotaxime	0.0	7.0	50.0	34.0	4.0	5.0	91.0	0 (0)	0 (0)	6 (6)
Ceftazidime	0.0	8.0	62.0	23.0	5.0	2.0	93.0	0 (0)	0 (0)	4 (4)
Amikacin	0.0	3.0	45.0	43.0	5.0	4.0	91.0	0 (0)	0 (0)	5 (5)
Minocycline	2.0	4.0	74.0	18.0	2.0	0.0	96.0	0 (0)	0 (0)	4 (4)
Ofloxacin	1.0	25.0	58.0	13.0	3.0	0.0	96.0	0 (0)	0 (0)	5 (5)
Ciprofloxacin	1.0	25.0	52.0	20.0	2.0	0.0	97.0	0 (0)	0 (0)	6 (6)
All agents	0.6	10.9	54.4	27.6	4.1	2.4	92.6			

[a] A difference of 0 indicates that the MICs were identical; −1 and +1 indicate a ±1 $\log_2$ dilution difference, etc.
[b] Percentage of isolates with different MICs of an indicated agent by two methods within ±1 $\log_2$ dilution.
[c] The categories of flavobacterial susceptibility to the seven agents tested were in accord with those of *Enterobacteriaceae* described by the NCCLS.

standardized suspension, excess fluid was expressed, and the suspension was streaked over the entire surface of a Mueller-Hinton agar plate (15 by 150 mm). The nine Etest strips were applied with forceps to the surfaces of two Mueller-Hinton agar plates (four and five strips in each) for each isolate. The plates were incubated at 35°C in ambient air for 16 to 18 h. The MIC was defined as the intercept of the zone of inhibition with the graded Etest strip.

Control strains for the two susceptibility test methods were *Staphylococcus aureus* ATCC 29213, *Escherichia coli* ATCC 25922, and *Pseudomonas aeruginosa* ATCC 27853. For comparison with the results of the agar dilution method, any MIC obtained by the Etest that fell between twofold dilutions was rounded up to the next twofold dilution. MICs of >128 μg/ml determined by the agar dilution method and MICs of >256 μg/ml determined by the Etest were both considered 256 μg/ml. For ofloxacin and ciprofloxacin, Etest MICs of >32 μg/ml were considered 64 μg/ml. Category discrepancies (very major, major, and minor errors) were defined on the basis of the description reported by Ferraro and Jorgensen (8). Acceptable Etest accuracy for an antimicrobial agent should provide >90% agreement with MICs determined by the agar dilution method (8).

The MICs of the antimicrobial agents obtained by the Etest and the agar dilution method were all within the recommended ranges for the three quality control strains. The differences between the MICs obtained by the two methods were all within ±1 $\log_2$ dilution.

The MICs determined by the agar dilution method and the Etest are shown in Table 1. All of the agents tested, except minocycline, had limited in vitro activities against the flavobacteria tested. The MICs of piperacillin, cefotaxime, and minocycline obtained by the Etest for 50% of the strains tested (MIC_{50}s) were 1 $\log_2$ dilution higher than those obtained by the agar dilution method. However, the MIC_{50}s of ofloxacin and ciprofloxacin were 1 $\log_2$ dilution lower than those obtained by the agar dilution method. Compared with the agar dilution method, the Etest produced higher MICs of piperacillin, cefotaxime, ceftazidime, and amikacin and lower MICs of ofloxacin and ciprofloxacin. These findings support previous observations (13, 16). The MICs of aztreonam and imipenem were not compared because the MICs obtained by both methods for most (>90%) of the isolates were at the upper end of the MIC range. For significant percentages of the isolates tested, the Etest MICs of ceftazidime (36%), cefotaxime (46%), and amikacin (48%) were greater than the highest concentration tested by the agar dilution method. As shown in Table 2, the agreement between the MICs (±1 $\log_2$ dilution) obtained by the two methods was acceptable for cefotaxime, ceftazidime, amikacin, minocycline, ofloxacin, and ciprofloxacin (>90%). Conversely, for piperacillin the agreement was the poorest (84%). The percent agreement between the MICs obtained by these two methods for all agents was 92.5%.

Regarding the MIC obtained in this study and those of previous reports, piperacillin, ofloxacin, and ciprofloxacin may be considered as potential drugs of choice for the management of infections caused by flavobacteria (2, 6, 7, 9–12, 17, 20, 22). In the present study, for 56% of the isolates tested, the Etest piperacillin MICs were higher than those obtained by the agar dilution method, and for 26% of the isolates, the Etest ofloxacin and ciprofloxacin MICs were lower than those obtained by the standard method, indicating the possibility of Etest category discrepancies. Because no NCCLS MIC breakpoints are available for *Flavobacterium* species to determine categories of susceptibility, the category discrepancies between the two methods cannot be estimated. However, when the MIC breakpoints of these seven agents for *Enterobacteriaceae* were applied to the *Flavobacterium* species tested, no very major or major category error was found for any of the agents tested, except piperacillin, for which there was a significant major category error of 16.7% (Table 2). In contrast to our findings, Baker et al. demonstrated a good agreement (92%) of Etest results for piperacillin against 140 strains of gram-negative bacteria compared with those of the agar dilution method (3). The reason for the poor correlation between the piperacillin efficacy results obtained with these two susceptibility test methods for flavobacteria is unknown and requires more thorough study.

We suggest that the Etest may be considered as an acceptable alternative for testing of the susceptibility of *Flavobacterium* species to all of the agents tested here except piperacillin. When evaluating susceptibility to piperacillin, any Etest MIC between 16 and 128 μg/ml should be reevaluated by another test procedure.

REFERENCES

1. **Aber, R. C., C. Wennersten, and R. C. Moellering, Jr.** 1978. Antimicrobial susceptibility of flavobacteria. Antimicrob. Agents Chemother. **14:**483–487.
2. **Altmann, G., and B. Bogokovsky.** 1971. In vitro sensitivity of *Flavobacterium meningosepticum* to antimicrobial agents. J. Med. Microbiol. **4:**296–299.
3. **Baker, C. N., S. A. Stocker, D. H. Culver, and C. Thornsberry.** 1991. Comparison of the E Test to agar dilution, broth microdilution, and agar diffusion susceptibility testing techniques by using a special challenge set of bacteria. J. Clin. Microbiol. **29:**533–538.

4. **Brown, D. F. J., and L. Brown.** 1991. Evaluation of the E test, a novel method of quantifying antimicrobial activity. J. Antimicrob. Chemother. **27:**185–190.
5. **Brown, D. F. J.** 1992. The E-Test challenged with selected strains. Diagn. Microbiol. Infect. Dis. **15:**465–468.
6. **Chang, J. C., P. R. Hsueh, J. J. Wu, W. C. Hsieh, and K. T. Luh.** Antimicrobial susceptibility of flavobacteria: a comparison of agar dilution MICs and disk diffusion results. Submitted for publication.
7. **Fass, R. J., and J. Barnishan.** 1980. In vitro susceptibilities of nonfermentative gram-negative bacilli other than *Pseudomonas aeruginosa* to 32 antimicrobial agents. Rev. Infect. Dis. **2:**841–853.
8. **Ferraro, M. J., and J. H. Jorgensen.** 1995. Instrument-based antimicrobial susceptibility testing, p. 1379–1384. *In* P. R. Murray, E. J. Baron, M. A. Pfaller, F. C. Tenover, and R. H. Yolken (ed.), Manual of clinical microbiology, 6th ed. American Society for Microbiology, Washington, D.C.
9. **Hsueh, P. R., J. J. Wu, T. R. Hsiue, and W. C. Hsieh.** 1995. Bacteremic necrotizing fasciitis due to *Flavobacterium odoratum*. Clin. Infect. Dis. **21:** 1337–1338.
10. **Hsueh, P. R., T. R. Hsiue, and W. C. Hsieh.** 1996. Pyomyositis in intravenous drug abusers: report of a unique case and review of the literature. Clin. Infect. Dis. **22:**858–860.
11. **Hsueh, P. R., L. J. Teng, S. W. Ho, W. C. Hsieh, and K. T. Luh.** 1996. Clinical and microbiological characteristics of *Flavobacterium indologenes* infections associated with indwelling devices. J. Clin. Microbiol. **34:**1908–1913.
12. **Hsueh, P. R., T. R. Hsiue, J. J. Wu, L. J. Teng, S. W. Ho, W. C. Hsieh, and K. T. Luh.** 1996. *Flavobacterium indologenes* bacteremia: clinical and microbiological characteristics. Clin. Infect. Dis. **23:**550–555.
13. **Huang, M. B., C. N. Baker, S. Banerjee, and F. C. Tenover.** 1992. Accuracy of the E test for determining antimicrobial susceptibilities of staphylococci, enterococci, *Campylobacter jejuni*, and gram-negative bacteria resistant to antimicrobial agents. J. Clin. Microbiol. **30:**3243–3248.
14. **National Committee for Clinical Laboratory Standards.** 1993. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Approved standard M7-A3. National Committee for Clinical Laboratory Standards, Villanova, Pa.
15. **Ratner, H.** 1984. *Flavobacterium meningosepticum*. Infect. Control **5:**237–239.
16. **Sanchez, M. L., M. S. Barrett, and R. N. Jones.** 1992. The E-Test applied to susceptibility tests of gonococci, multiply-resistant enterococci, and Enterobacteriaceae producing potent β-lactamases. Diagn. Microbiol. Infect. Dis. **15:**459–463.
17. **Scully, B. E., and H. C. Neu.** 1986. Oral ciprofloxacin therapy of infection caused by multiply resistant bacteria other than *Pseudomonas aeruginosa*. J. Antimicrob. Chemother. **18**(Suppl. D)**:**179–185.
18. **Sheridan, R. I., C. M. Ryan, M. S. Pasternack, J. M. Weber, and R. G. Tompkins.** 1993. Flavobacterial sepsis in massively burned pediatric patients. Clin. Infect. Dis. **17:**185–187.
19. **Siegman-Igra, Y., D. Schwartz, G. Soferman, and N. Konforti.** 1987. *Flavobacterium* group IIb bacteremia: report of a case and review of *Flavobacterium* infections. Med. Microbiol. Immunol. **176:**103–111.
20. **Strandberg, D. A., J. H. Jorgensen, and D. J. Drutz.** 1983. Activities of aztreonam and new cephalosporins against infrequently isolated gram-negative bacilli. Antimicrob. Agents Chemother. **24:**282–286.
21. **Tam, A. Y. C., R. W. H. Yung, and K. Fu.** 1989. Fatal pneumonia caused by *Flavobacterium meningosepticum*. Pediatr. Infect. Dis. J. **8:**252–254.
22. **von Graevenitz, A., and M. Grehn.** 1977. Susceptibility studies of *Flavobacterium* IIb. FEMS Microbiol. Lett. **2:**289–292.