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Comparison of E test and Agar Dilution for Antimicrobial Susceptibility Testing of *Stenotrophomonas* (*Xanthomonas*) *maltophilia*

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Currently recommended dilution test methods for the determination of antimicrobial susceptibility of *Stenotrophomonas* (*Xanthomonas*) *maltophilia* are labor-intensive and often impractical in many clinical laboratories. We compared the E test with the agar dilution method for susceptibility testing of 176 clinical isolates of *S. maltophilia* against 16 antimicrobial agents. The MICs obtained by E test correlated well with those determined by the agar dilution method, with an overall agreement of 94%. Very major and major errors occurred infrequently (0.6 to 2.9%) when testing β-lactam agents, tobramycin, trimethoprim-sulfamethoxazole, and fluoroquinolones. The E test was found to be accurate and easy to perform. For most antimicrobial agents tested against *S. maltophilia*, the E test is an acceptable alternative susceptibility test method.

Stenotrophomonas maltophilia is the recently proposed name for the bacterium previously referred to as *Xanthomonas maltophilia* (11). This organism has emerged as an increasingly important nosocomial pathogen capable of causing respiratory, urinary, and bacteremic infections in hospitalized patients, particularly those admitted to an intensive care unit (5, 7, 8, 16). Treatment of these infections is often difficult, because clinical isolates of *S. maltophilia* are generally resistant to multiple antibiotics (10, 12, 15). Problems in performing in vitro susceptibility testing of this organism have also been encountered. The disk diffusion method of susceptibility testing has been found to be inaccurate and not reproducible for the testing of *S. maltophilia* (4, 6, 12, 13). Agar dilution and broth microdilution are the currently recommended susceptibility test methods for this organism, but they are cumbersome to perform and impractical to implement as routine tests in many clinical laboratories. The E test (AB Biodisk, Solna, Sweden) has been reported to be a simple and accurate alternative method for determining antimicrobial susceptibility of various microorganisms, including fastidious bacteria (1, 2, 14), although experience with this method for testing *S. maltophilia* has been limited. We evaluated the accuracy of the E test, compared with that of the agar dilution method, for the susceptibility testing of *S. maltophilia*.

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A total of 176 single patient isolates of *S. maltophilia* obtained in 1992 and 1993 from three Toronto hospitals were used in this study. *S. maltophilia* was identified by Gram stain appearance, colonial morphology, motility, negative or weak cytochrome oxidase reaction, and positive reactions for gelatin hydrolysis, DNase, esculin hydrolysis, and lysine decarboxylase (3). Strains were stored at −70°C for subsequent testing.

The antimicrobial agents tested were provided by their respective manufacturers and included cefoxitin, ceftriaxone, cefotaxime, ceftazidime, cefpirome, cefepime, imipenem, piperacillin, piperacillin-tazobactam, ticarcillin-clavulanate, gentamicin, tobramycin, amikacin, trimethoprim-sulfamethoxazole, ciprofloxacin, and ofloxacin. Susceptibility testing by agar dilution with Mueller-Hinton agar plates (Becton Dickinson Microbiology Systems, Cockeysville, Md.) was done in accordance with methods recommended by the National Committee for Clinical Laboratory Standards (9). Briefly, inocula were prepared by suspending colonies from overnight growth plates in 5 ml of sterile saline to match the turbidity of a 0.5 McFarland standard. A 1:10 dilution was prepared for the inoculation of plates with the Cathra Systems replicator (MCT Medical, Inc., St. Paul, Minn.) for a final inoculum of 10^4 CFU. The MIC was defined as the lowest concentration of antimicrobial agent that prevented visible growth after incubation of plates at 35°C for 16 to 20 h. A single colony or faint haze was regarded as no growth.

The E test strips were provided by AB Biodisk and were used as recommended by the manufacturer. A suspension of each isolate in Mueller-Hinton broth, adjusted to the density of a 0.5 McFarland standard, was used to inoculate Mueller-Hinton agar plates. E test strips were placed on the plates, which were incubated at 35°C for 16 to 20 h. The MIC was read where inhibition of growth intersected the E test strip. When small colonies occurred within the zone of inhibition or a haze of growth occurred around MIC endpoints, the highest MIC intersect was recorded. *Escherichia coli* ATCC 25922 (β-lactamase negative), *E. coli* ATCC 35218 (β-lactamase positive), *Pseudomonas aeruginosa* ATCC 27853, *Enterococcus faecalis* ATCC 29212, and *S. maltophilia* ATCC 13673 were included as quality control strains for testing.

Agreement between the two test methods was defined as MICs that differed by 1 $\log_2$ dilution or less and that remained within the same susceptibility or resistance category. Discrepancies in MICs were characterized as very major, major, or minor errors. A very major error occurred if the reference method result was resistance and the E test result was susceptibility; a major error occurred if the reference method result was susceptibility and the E test result was resistance. Other

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TABLE 1. Comparison of antimicrobial susceptibility test results by the agar dilution method and E test for 176 *S. maltophilia* isolates

Antimicrobial agent	MIC_{90} (μg/ml)[a]		% Resistant[b]		% Interpretive errors		
	AD[c]	E test	AD	E test	Very major	Major	Minor
Cefoxitin[d]	>32	>32	100	100	0	0	0
Ceftriaxone	>64	>256	91	94	0.6	1.7	4.7
Cefotaxime	>64	>256	77	83	1.7	0	15.3
Ceftazidime	>64	>256	64	61	1.1	0	5.9
Cefpirome	>64	>256	90	88	2.9	0	3.1
Cefepime	64	128	55	55	1.1	0	24.9
Imipenem	>16	>32	100	100	0	0	0
Piperacillin	>128	>256	73	92	0	1.7	21.3
Piperacillin-tazobactam	>128	>256	66	78	0	0	20.6
Ticarcillin-clavulanate	128	>256	12	21	0	1.7	28.3
Gentamicin	>32	>256	88	85	0	0	9.0
Tobramycin	>32	>256	85	81	1.7	0	7.3
Amikacin	>64	>256	83	75	0	0	11.0
Trimethoprim-sulfamethoxazole	1.0	2.0	4	4	0.6	0.6	0
Ciprofloxacin	>8	>32	51	42	1.1	0	25.9
Ofloxacin	>8	32	21	24	1.7	0	17.3

[a] MIC_{90}, MIC at which 90% of the isolates are inhibited.
[b] Breakpoints used for resistance were as follows (in micrograms per milliliter): cefoxitin, ≥32; ceftriaxone, ≥64; cefotaxime, ≥64; ceftazidime, ≥32; cefpirome, ≥32; cefepime, ≥32; imipenem, ≥16; piperacillin, ≥128; piperacillin-tazobactam, ≥128:4; ticarcillin-clavulanate, ≥128:2; gentamicin, ≥16; tobramycin, ≥16; amikacin, ≥64; trimethoprim-sulfamethoxazole, ≥4:76; ciprofloxacin, ≥4; ofloxacin, ≥8.
[c] AD, agar dilution.
[d] Only 81 isolates tested.

discrepancies were interpreted as being minor errors. The E test and agar dilution susceptibility testing were repeated for strains yielding major or very major errors. The E test and agar dilution testing were also repeated with incubation of plates for 24 and 48 h, in order to determine if prolonged incubation would affect the results.

Antimicrobial susceptibility test results are shown in Table 1, and a comparison of the agreement between the E test results and the agar dilution testing results is shown in Table 2. *S. maltophilia* was found to be highly resistant to multiple antibiotics, including newer β-lactams such as cefpirome, cefepime, and piperacillin-tazobactam. The most active compounds in vitro were trimethoprim-sulfamethoxazole and ticarcillin-clavulanate. The E test resulted in few major or very major errors for the antimicrobial agents tested. There were a substantial number of minor errors, especially with cefepime, piperacillin, β-lactam–β-lactamase inhibitor combinations, and the fluoroquinolones. In most of these cases, the E test yielded MICs indicative of resistance, whereas the agar dilution testing results were in the intermediate susceptibility category. The E test yielded greater numbers of resistance results than did agar dilution testing when piperacillin ($P = 0.01$), piperacillin-tazobactam ($P < 0.001$), and ticarcillin-clavulanate ($P = 0.03$) were tested. Overall, 94% of the E test MICs were within a difference of 1 $\log_2$ dilution, compared with those obtained by agar dilution testing. However, agreement when testing some of the β-lactams, such as piperacillin (79% agreement) and ticarcillin-clavulanate (84% agreement), was not as good as that obtained with other antimicrobial agents. For isolates yielding major or very major errors, repeat testing, including prolonged incubation (48 h), did not change any of the results (data not shown).

TABLE 2. Correlation of MICs for 176 *S. maltophilia* isolates obtained by the E test and agar dilution methods

Antimicrobial agent	No. of isolates for which E test MICs were within the indicated concentration ($\log_2$ dilution) of agar dilution MICs							% Agreement within 1 $\log_2$ dilution
	<−2	−2	−1	Same	+1	+2	>+2	
Cefoxitin[a]	0	0	0	81	0	0	0	100
Ceftriaxone	1	0	2	163	1	2	7	94
Cefotaxime	7	3	1	145	11	4	5	89
Ceftazidime	3	5	6	161	1	0	0	95
Cefpirome	5	1	1	166	3	0	0	97
Cefepime	4	1	6	133	23	9	0	91
Imipenem	0	0	0	176	0	0	0	100
Piperacillin	0	0	3	135	1	3	34	79
Piperacillin-tazobactam	0	0	5	139	11	7	13	89
Ticarcillin-clavulanate	0	14	14	123	11	1	13	84
Gentamicin	2	3	9	160	2	0	0	97
Tobramycin	2	7	5	160	2	0	0	95
Amikacin	0	0	18	157	1	0	0	100
Trimethoprim-sulfamethoxazole	0	0	1	174	0	1	0	99
Ciprofloxacin	2	6	36	126	3	2	1	95
Ofloxacin	2	4	14	139	16	1	0	98

[a] Only 81 isolates tested.

The currently recommended methods for antimicrobial susceptibility testing of *S. maltophilia* are broth and agar dilution (9). Unfortunately, these methods are labor-intensive and not easily incorporated into routine laboratory procedures. Susceptibility testing of *S. maltophilia* by disk diffusion or by semiautomated methods such as Vitek (bioMerieux Inc., Hazelwood, Mo.) or MicroScan (Baxter Health Care Corp., Sacramento, Calif.) is unreliable and therefore not recommended (4, 6, 12, 13). The E test is a relatively new and innovative approach to antimicrobial susceptibility testing. This study provides added information about the use of the E test for susceptibility testing of *S. maltophilia*. The results of this study indicate that E test MICs were generally in agreement with those obtained by the reference agar dilution method. There were only small numbers of major or very major errors observed. Most of the discrepancies represented minor errors likely to be of little clinical significance. In an attempt to determine if the accuracy of the E test could be improved still further, susceptibility testing of isolates was repeated with a prolonged incubation period (48 h). Although prolonged incubation did not resolve any discrepant results, repeat testing with the E test did demonstrate excellent reproducibility.

Compared with the agar and broth dilution methods, the E test is much less labor-intensive and is easier to perform. However, it is important to note that interpretation of E test results was occasionally difficult when testing the aminoglycosides, because of the presence of tiny resistant microcolonies within the zone of inhibition. These could easily have been missed if not carefully looked for and, therefore, could have given false susceptibility results. Similar problems were encountered by Pankuch et al. (12) in their recent evaluation of test methods for the determination of *S. maltophilia* antimicrobial susceptibility. These investigators also reported the occasional presence of a haze of translucent growth within the area of inhibition. In the current study a haze was observed with both the E test and the agar dilution method, but only when testing susceptibility to trimethoprim-sulfamethoxazole.

In summary, susceptibility testing of *S. maltophilia* remains problematic for most diagnostic laboratories. However, the results of this study indicate that the E test is a reliable, easy-to-perform, and suitable alternative method for the determination of the susceptibility of *S. maltophilia* to many antimicrobial agents.

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