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Susceptibilities of 123 Strains of *Xanthomonas maltophilia* to Eight β-Lactams (Including β-Lactam–β-Lactamase Inhibitor Combinations) and Ciprofloxacin Tested by Five Methods

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This study evaluated the susceptibility of 123 *Xanthomonas maltophilia* strains to ticarcillin, ticarcillin-clavulanate, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, piperacillin-tazobactam, imipenem, and ciprofloxacin by Kirby-Bauer disk, E test, and Sensititre dehydrated microdilution MIC and conventional agar dilution MIC methodology. Intermediate susceptibility breakpoints for members of the family *Enterobacteriaceae* were used. When results were analyzed as MICs for 50 and 90% of the strains tested and percentages of strains susceptible at the breakpoint, good correlation between the methods was observed, with ticarcillin-clavulanate clearly the most active β-lactam by all four methods. However, when the various methods were compared with the agar dilution methodology by regression analysis, poor r^2 values (0.3 to 0.7) were obtained for compounds with sufficient on-scale values to permit analysis. When the number of strains with $\log_2$ ratios of reference agar dilution MICs to test MICs of +3 to −3 were analyzed, correlation was also poor, with many major and very major discrepancies for all methods tested. Results obtained with time-kill studies of nine strains with discrepant ticarcillin-clavulanate MICs appeared to correlate best when compared at 24 h with agar dilution MICs. The concentration of ticarcillin-clavulanate required to reduce the colony count by ≥2 $\log_{10}$ reduction values for eight of nine strains compared with that for growth controls was ≤16.0/2.0 μg/ml at 6 h and ranged from 16.0/2.0 μg/ml to 128.0/2.0 μg/ml at 24 h. Parallel kill curves with piperacillin-tazobactam yielded ≥2 $\log_{10}$ reduction values ranging from ≤16.0/4.0 μg/ml to 128.0/4.0 μg/ml at 6 h and >128.0/4.0 μg/ml at 24 h. The susceptibility method of choice for *X. maltophilia* has not yet been standardized, but time-kill studies correlated best with agar dilution MICs.

Infections with *Xanthomonas maltophilia* are increasingly encountered, especially in immunocompromised patients (9, 14–16, 25). This organism is inherently resistant to most β-lactam and non-β-lactam agents by virtue of permeability barriers and elaboration of at least two β-lactamases; trimethoprim-sulfamethoxazole and, perhaps, moxalactam are currently suggested as the drugs of choice for treating infections with this organism (4, 7, 9–11, 14–16, 25). β-Lactam resistance in *X. maltophilia* is mediated by production of at least two β-lactamases—a zinc-dependent metalloenzyme that breaks down carbapenems and is resistant to β-lactamase inhibitors and a cephalosporinase that is susceptible to β-lactamase inhibitors (3, 23, 24). A previous preliminary study by our group, as well as reports by other workers, have documented apparent increased susceptibility of *X. maltophilia* strains to ticarcillin-clavulanate by disk diffusion and MIC methodology compared with susceptibility to ticarcillin, ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, piperacillin, and piperacillin-tazobactam (8, 19, 21).

In order to shed more light on the phenomenon described above and to define whether some *Xanthomonas* strains are indeed susceptible to ticarcillin-clavulanate and that this finding is not a method-dependent artifact, we tested the susceptibility of 123 clinically isolated strains of *X. maltophilia* to ampicillin, amoxicillin-clavulanate, ampicillin-sulbactam, ticarcillin, ticarcillin-clavulanate, piperacillin, piperacillin-tazobactam, and imipenem by disk diffusion, microdilution, agar dilution, and E test methodologies. Ciprofloxacin was also tested by the four methods described above. Additionally, the activities of ticarcillin-clavulanate and piperacillin-tazobactam on selected strains were studied by the time-kill methodology.

MATERIALS AND METHODS

Bacterial strains. One hundred twenty-three strains (1 strain per patient) of *X. maltophilia* were isolated from clinical specimens at Hershey Medical Center and Case Western Reserve University. Identification was by conventional methodology (5), and strains were stored in double-strength skim milk (Difco Laboratories, Detroit, Mich.) at −70°C until use. Purity was checked throughout the study by Gram staining and colonial morphology. Disk diffusion testing and MIC susceptibility testing were performed at Hershey Medical Center, and time-kill studies were done at SmithKline Beecham Laboratories.

Antimicrobial agents. Susceptibility powders were obtained from the following suppliers: ampicillin, amoxicillin, ticarcillin, and clavulanate, SmithKline Beecham Laboratories, Philadelphia, Pa; sulbactam, Pfizer, Inc., New York, N.Y.; piperacillin and tazobactam, Lederle Laboratories, Pearl River, N.Y.; imipenem, Merck and Co., Rahway, N.J.; and ciprofloxacin, Miles, Inc., West Haven, Conn. Amoxicillin-clavulanate and ampicillin-sulbactam were both tested in 2:1 ratios of β-lactam

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TABLE 1. Results of susceptibility testing of β-lactams by four methods

Agent (MIC breakpoint [μg/ml])	% Susceptible by disk diffusion[a]	MIC_{50}/MIC_{90} (% susceptible) by: Agar dilution		E test	Sensititre	
		24 h	48 h		24 h	48 h
Ticarcillin (64)[b]	20	256/>256 (21)	256/>256 (12)	>256/>256 (7)	128/>256 (45)	256/>256 (24)
Ticarcillin-clavulanate at fixed 2-μg/ml clavulanate concn (64)	88	32/256 (84)	32/256 (84)	16/>256 (73)	16/128 (88)	32/>256 (71)
Ampicillin (16)	12	>256/>256 (0)	>256/>256 (0)	>256/>256 (2)	256/>256 (7)	>256/>256 (2)
Amoxicillin-clavulanate at 2:1 ratio (16)[c]	4	256/>256 (0)	256/>256 (0)	>256/>256 (1)	64/128 (4)	128/256 (1)
Ampicillin-sulbactam at 2:1 ratio (16)[c]	20	256/>256 (2)	256/>256 (0)	>256/>256 (6)	>16/>16 (6)	>16/>16 (2)
Piperacillin (64)	20	256/>256 (20)	256/>256 (15)	>256/>256 (8)	256/>256 (33)	>256/>256 (18)
Piperacillin-tazobactam at fixed 4-μg/ml tazobactam concn (64)	24	256/>256 (32)	256/>256 (20)	>256/>256 (20)	128/>256 (50)	>256/>256 (29)
Imipenem (8)	0	>32/>32 (0)	>32/>32 (0)	>32/>32 (0)	>32/>32 (0)	>32/>32 (0)

[a] Determined by Kirby-Bauer breakpoints in millimeters with intermediate values for members of the family *Enterobacteriaceae*.
[b] MIC breakpoints determined by using intermediate values for members of the family *Enterobacteriaceae*.
[c] Ampicillin breakpoint used for amoxicillin-clavulanate and ampicillin-sulbactam.

to inhibitor. Clavulanate was added to ticarcillin at a fixed concentration of 2.0 μg/ml, and tazobactam was added to piperacillin at a fixed concentration of 4.0 μg/ml. Dilution ranges with all methods were 0.016 to 256.0 μg/ml (β-lactam component for combinations) for all compounds except imipenem (0.016 to 32 μg/ml with E test and agar dilution only). Ampicillin-sulbactam dilutions in microdilution tests were 0.016 to 16.0 μg/ml (ampicillin component). Disks of all compounds were obtained from BBL Microbiology Systems (Cockeysville, Md.). E test strips were obtained from AB Biodisk (Solna, Sweden).

Susceptibility testing. (i) Disk diffusion. For disk diffusion testing, the method recommended by the National Committee for Clinical Laboratory Standards for gram-negative aerobic rods was used (18). Mueller-Hinton plates (15 cm in diameter) (Difco) were inoculated with a 0.5 McFarland standard suspension of organisms in Mueller-Hinton broth (Difco), and disks were applied. Disk contents (susceptibility breakpoints) were as follows: ampicillin, 10 μg (≥14 mm); amoxicillin-clavulanate, 20/10 μg (≥14 mm); ampicillin-sulbactam, 10/10 μg (≥12 mm); ticarcillin, 75 μg (≥15 mm); ticarcillin-clavulanate, 75/10 μg (≥15 mm); piperacillin, 100 μg (≥18 mm); piperacillin-tazobactam, 100/10 μg (≥18 mm); imipenem, 10 μg (≥14 mm); ciprofloxacin, 5 μg (≥16 mm). Zones of growth inhibition were recorded in millimeters after overnight incubation at 35°C.

(ii) Microdilution MIC determination. Specially manufactured dehydrated trays containing dilutions of all compounds were obtained from Sensititre, Inc., Westlake, Ohio. Ampicillin-sulbactam was supplied by Sensititre as "stand-alone" trays. Growth from plates was suspended in deionized water to a density of a 0.5 McFarland standard and diluted 10^{-3} in supplemented Mueller-Hinton broth (Sensititre). Each well was inoculated with a final volume of 50-μl aliquots of 10^5-CFU/ml organism suspensions by using the automated inoculator recommended by the manufacturer. Results were interpreted after overnight incubation as well as after incubation for an additional 24 h (both at 35°C).

(iii) Agar dilution MIC determination. The method recommended by the National Committee for Clinical Laboratory Standards, with Mueller-Hinton agar plates, was used (17). Growth harvested from plates was suspended in Mueller-Hinton broth (Difco) to the density of a 0.5 McFarland standard and diluted, and plates were inoculated by means of a Steers replicator with 2-μl volumes (10^4 CFU per spot). MICs were read as the lowest drug concentration that inhibited visible growth. A single colony or faint haze was regarded as no growth. MICs were interpreted after overnight incubation as well as after incubation for an additional 24 h (both at 35°C).

(iv) E test MIC determination. Growth from plates was suspended in Mueller-Hinton broth (Difco) to the density of a 0.5 McFarland standard and used to inoculate 15-cm Mueller-Hinton agar plates. E test strips were placed on plates in a radial fashion. Plates were incubated overnight at 35°C, and MICs were read where complete inhibition of growth intersected the strips according to the manufacturer's instructions. In cases in which small colonies or a haze of growth occurred around MIC end points, the higher and more conservative MIC intersect was recorded. For 15 strains, E test MICs were also interpreted after an added 24-h incubation. All E test results were interpreted with the aid of a magnifying glass.

For all MIC methods, susceptibility breakpoints were the high (or intermediate) breakpoints recommended by the National Committee for Clinical Laboratory Standards for members of the family *Enterobacteriaceae* (17): 2.0 μg/ml for ciprofloxacin; 8.0 μg/ml for imipenem; 16.0 μg/ml for ampicillin, amoxicillin-clavulanate, and ampicillin-sulbactam; and 64.0 μg/ml for ticarcillin, ticarcillin-clavulanate, piperacillin, and piperacillin-tazobactam. All results with β-lactam–β-lactamase inhibitor combinations are expressed as the concentration of the β-lactam component.

For time-kill studies, nine strains which yielded discrepant ticarcillin-clavulanate values with the four methods were cho-

TABLE 2. Correlation of ticarcillin-clavulanate results by different methods of susceptibility testing by regression analysis

Method	Correlation of ticarcillin-clavulanate results (r^2 values) by: Agar dilution (48 h)	Microdilution		E test	Disk diffusion
		24 h	48 h		
Agar dilution (24 h)	0.34	0.45	0.48	0.48	0.35
Agar dilution (48 h)		0.34	0.41	0.38	0.29
Microdilution (24 h)			0.76	0.37	0.27
Microdilution (48 h)				0.48	0.35
E test					0.68

TABLE 3. Comparison of MIC methods for ticarcillin-clavulanate by using 24-h agar dilution as the reference method

Method	No. of strains with log_2 ratio (with reference to test MIC) of:							% ± 1 log_2 dilution	No. of categorical discrepancies[a]	
	≥+3	+2	+1	0	−1	−2	−3		Major	Very major
Agar dilution (48 h)	0	1	5	70	40	6	1	93.5	2	2
Microdilution (24 h)	13	11	27	34	25	10	3	69.9	7	12
Microdilution (48 h)	6	7	15	29	31	20	15	61.0	23	8
E test	3	11	20	15	20	30	24	44.7	16	3

[a] Major errors, reference method susceptible, comparison method resistant; very major errors, reference method resistant, comparison method susceptible.

sen for analysis. Each strain was inoculated at approximately 10^5 CFU/ml into flasks containing 20 ml of Mueller-Hinton broth (BBL Microbiology Systems). Concentrations of ticarcillin-clavulanate (fixed inhibitor concentration, 2.0 μg/ml) of 16.0 and 128.0 μg/ml were added to each flask. Flasks were incubated aerobically at 35°C with constant agitation by a platform shaker. Additionally, ticarcillin-clavulanate concentrations of 4.0, 8.0, 32.0, and 64.0 μg/ml (fixed inhibitor concentration, 2.0 μg/ml) were also tested against five of the nine strains. Piperacillin-tazobactam concentrations of 16.0 and 128.0 μg/ml (fixed inhibitor concentration, 4.0 μg/ml) were tested against five of the strains. Viability counts in time-kill experiments were performed at 0, 6, and 24 h. Two *X. maltophilia* strains included in the study (2908 and 425) served as ticarcillin-clavulanate-susceptible and -resistant controls, respectively. These strains yielded similar results with all methods tested.

RESULTS

Problems of various degrees of severity in determining susceptibility of *X. maltophilia* were encountered in this study. Trailing end points were frequently encountered in agar and microdilution tests after overnight incubation; they were less frequently encountered after an additional 24-h incubation. In E test and disk diffusion tests, small resistant colonies or a haze of translucent growth inside the area of inhibition sometimes made accurate interpretation difficult.

Susceptibility testing results with the four methods are presented as the MIC for 50% of the strains tested (MIC_{50}), MIC_{90}, and percentage of strains susceptible at breakpoint in Table 1. As can be seen, reasonable correlation was found with all methods. For ampicillin, MIC_{50}s and MIC_{90}s were both ≥256 μg/ml with all methods, with ≤12% of the strains being susceptible. Ampicillin-sulbactam was also not active, with MIC_{50}s and MIC_{90}s ≥256 μg/ml, except for the microdilution system (in which the highest concentration tested was 16 μg/ml), and ≤20% of strains were susceptible. Amoxicillin-clavulanate was also not active, with MIC_{50}s ranging from 64 μg/ml to >256 μg/ml and MIC_{90}s between 128 μg/ml and >256 μg/ml with the four methods and with ≤4% of the strains being susceptible.

Ticarcillin showed little activity, with MIC_{50}s of 128 to >256 μg/ml, MIC_{90}s of >256 μg/ml, and ≤24% of the strains being susceptible (except for the 24-h microdilution reading, in which 45% of the strains were found to be susceptible). In contrast, greater activity was observed with ticarcillin-clavulanate, with MIC_{50}s ranging from 16 μg/ml to 32 μg/ml and MIC_{90}s ranging from 128 μg/ml to >256 μg/ml and with 71 to 88% of the strains being susceptible with the four methods. Piperacillin showed little activity, with MIC_{50}s and MIC_{90}s ≥256 μg/ml and 8 to 33% of the strains being susceptible. Piperacillin-tazobactam was not significantly more active than piperacillin alone, with MIC_{50}s ranging between 128 μg/ml and >256 μg/ml, MIC_{90}s of >256 μg/ml, and 20 to 50% of the strains being susceptible. All strains were resistant to imipenem, with MIC_{50}s and MIC_{90}s of >32 μg/ml (Table 1).

Because of the resistance (and therefore off-scale values) of *X. maltophilia* strains to most β-lactams tested, only ticarcillin-clavulanate, piperacillin-tazobactam, and ciprofloxacin MIC results were analyzed in greater detail (Table 2 to 8). Regression analysis of ticarcillin-clavulanate for the various methods revealed poor coefficients of determination (r^2 values of 0.27 to 0.68) (Table 2). When ticarcillin-clavulanate data were analyzed according to agreement with on-scale MICs, with 24-h agar dilution MICs as the reference method, essential agreement (± 1 doubling dilution) varied from 44.7 to 93.5%, major errors occurred in 2 to 23 strains, and very major errors occurred in 2 to 12 strains (Table 3). Similarly low rates of essential agreement and high rates of major and very major errors were seen with piperacillin-tazobactam (Table 4). Disk diffusion data also did not correlate well with other methods; there were large numbers of major and very major errors (Table 5).

When results for ciprofloxacin were analyzed, MIC_{50}s and MIC_{90}s by the different methods were found to be similar, but considerable variation in percentages of strains susceptible (30 to 56%) was found (Table 6). Regression analysis and essential agreement comparison also showed poor correlation of methods for ciprofloxacin, except for 24- and 48-h agar dilution

TABLE 4. Comparison of MIC methods for piperacillin-tazobactam by using 24-h agar dilution as the reference method

Method	No. of strains with log_2 ratio (with reference to test MIC) of:							% ± 1 log_2 dilution	No. of categorical discrepancies	
	≥+3	+2	+1	0	−1	−2	−3		Major	Very major
Agar dilution (48 h)	0	0	1	79	32	9	2	91.1	13	0
Microdilution (24 h)	19	18	21	39	16	9	1	61.8	5	28
Microdilution (48 h)	4	5	13	48	24	23	8	69.1	12	10
E test	4	1	4	34	33	29	18	57.7	19	6

[a] Major errors, reference method susceptible, comparison method resistant; very major errors, reference method resistant, comparison method susceptible.

TABLE 5. Analysis of disk diffusion data for ticarcillin-clavulanate, piperacillin-tazobactam, and ciprofloxacin by using the intermediate susceptibility zone diameter breakpoint for members of the family *Enterobacteriaceae*

Comparison method	Error rate for[a]:					
	Ticarcillin-clavulanate (20 strains resistant, 103 susceptible)		Piperacillin-tazobactam (84 strains resistant, 39 susceptible)		Ciprofloxacin (77 strains resistant, 46 susceptible)	
	Major	Very major	Major	Very major	Major	Very major
Agar dilution (24 h)	5	9	11	17	1	51
Agar dilution (48 h)	5	9	3	22	0	59
Microdilution (24 h)	8	7	23	6	2	52
Microdilution (48 h)	3	20	8	16	2	52
E test	0	17	2	21	0	50

[a] Results are expressed as error rates with 24-h agar dilution MICs as the reference method. Major errors, number of susceptible strains with resistant zones; very major errors, number of resistant strains with susceptible zones.

(Tables 7 and 8). Considerable numbers of major and very major errors also occurred by the disk diffusion method with ciprofloxacin (Table 5).

For the 15 strains whose E test MICs were read after overnight incubation as well as after an additional 24 h at 35°C, MICs were within one doubling dilution in all cases except one, for which the piperacillin MIC increased from 32 μg/ml after 24 h to 256 μg/ml after 48 h.

Analysis of time-kill curves showed that the concentration of ticarcillin-clavulanate required to reduce the colony count for eight of nine strains by ≥2 $\log_{10}$ reduction values was ≤16.0/2.0 μg/ml at 6 h and ranged from 16.0/2.0 μg/ml to 128.0/2.0 μg/ml at 24 h (Table 9). Kill curves with piperacillin-tazobactam yielded ≥2 $\log_{10}$ reduction values ranging from ≤16.0/4.0 μg/ml to 128.0/4.0 μg/ml at 6 h and >128.0/4.0 μg/ml at 24 h. These results correlated well with all susceptibility test methods (Table 10). Of the five susceptibility testing methods examined, time-kill results correlated best with agar dilution MICs (Tables 9 and 10).

DISCUSSION

Problems in interpretation of susceptibility test results with *X. maltophilia* have been encountered before, and there is currently no standardized method for susceptibility testing of this organism. Several studies attest to inconsistent results (some of them medium dependent) obtained when disk diffu-

TABLE 6. Susceptibility of *X. maltophilia* to ciprofloxacin by various methods

Method	MIC (μg/ml)[a]		% Susceptible[b]
	50%	90%	
Agar dilution (24 h)	4	16	37
Agar dilution (48 h)	4	16	30
Microdilution (24 h)	2	16	50
Microdilution (48 h)	8	>16	32
E test	4	>32	43
Disk diffusion	NA[c]	NA	56

[a] 50% and 90%, MIC_{50} and MIC_{90}, respectively.
[b] At breakpoint concentration of 2 μg/ml.
[c] NA, not applicable.

TABLE 7. Correlation of ciprofloxacin results by different susceptibility testing methods by regression analysis

Method	Correlation of ciprofloxacin results (r^2 values) by:				
	Agar dilution (48 h)	Microdilution 24 h	Microdilution 48 h	E test	Disk diffusion
Agar dilution (24 h)	0.91	0.52	0.47	0.66	0.39
Agar dilution (48 h)		0.53	0.51	0.60	0.36
Microdilution (24 h)			0.77	0.47	0.21
Microdilution (48 h)				0.43	0.26
E test					0.51

sion results were compared with agar dilution and microdilution MICs with this organism (1, 4, 7, 12, 13, 21, 22). Poulos and coworkers obtained different results for β-lactam and non-β-lactam drugs in disk diffusion susceptibility testing of *X. maltophilia* by using five media: two brands of Mueller-Hinton agar (Difco Laboratories and Oxoid Laboratories [Columbia, Md.]), Isosensitest, Columbia agar base, and Diagnostic Sensitivity Test agar (all three media from Oxoid Laboratories). In the light of these results, they have concluded that disk diffusion testing of this species cannot be performed accurately (21). The time-kill study results suggest that ticarcillin-clavulanate reduced the number of *X. maltophilia* colonies at 6 h by ≥2 $\log_{10}$ reduction values compared with that of the growth control. However, in some of the time-kill studies, there was regrowth of the organism at 24 h, which may help explain the trailing end points observed with growth on agar.

The chemical composition of the medium for *X. maltophilia* susceptibility testing may also be important (2); Hawkey and coworkers have reported that the variation in the in vitro susceptibility to imipenem seen with this species is due to differences in the medium's Zn^{2+} content and have recommended that media used for susceptibility testing of *Xanthomonas* spp. be controlled for Zn^{2+} content (6). In the current study, all strains tested were resistant to imipenem with all methods tested. The Zn^{2+} content of the media used was not controlled; additionally, the possibility that imipenem stability was poor or that degradation occurred cannot be excluded. However, *Pseudomonas aeruginosa* ATCC 27853 and *Escherichia coli* ATCC 25922, which were used as quality controls in each susceptibility run, were invariably susceptible to imipenem, with values in the control range in every case. Because a medium for *Xanthomonas* susceptibility testing has not been standardized, other as-yet-undefined components may play a role in susceptibility to β-lactams (and possibly non-β-lactams).

Preliminary evidence has suggested that some strains of *X. maltophilia* are susceptible to ticarcillin-clavulanate, but not to other β-lactams and β-lactam–β-lactamase inhibitor combinations (8, 21). A synergistic interaction between ticarcillin-clavulanate and trimethoprim-sulfamethoxazole for this species has also been reported (20). In our study, strains were more susceptible to ticarcillin-clavulanate than to other β-lactams by all methods tested. MICs were highest with the E test method, probably because of the clearly visible small colonies or growth hazes inside the inhibition ellipse. This is the first species of which we are aware for which E test MICs do not correspond well with those obtained by other methods. Although the E test is said to be medium independent, other media may yield different results with this species. If the E test is used for susceptibility testing of *X. maltophilia*, use of a

TABLE 8. Comparison of MIC methods for ciprofloxacin by using 24-h agar dilution as the reference method

Method	No. of strains with log_2 ratio (with reference to test MIC) of:							% ± 1 log_2 dilution	No. of categorical discrepancies[a]	
	≥+3	+2	+1	0	−1	−2	−3		Major	Very major
Agar dilution (48 h)	0	0	0	99	24	0	0	100	0	0
Microdilution (24 h)	4	15	34	43	22	4	1	80.5	5	23
Microdilution (48 h)	0	7	15	45	39	11	6	80.5	18	11
E test	0	0	7	37	30	13	36	59.3	17	2

[a] Major errors, reference method susceptible, comparison method resistant; very major errors, reference method resistant, comparison method susceptible.

magnifying glass is recommended and care must be taken in interpretation of results.

X. maltophilia produces at least two different β-lactamases: a clavulanate-resistant imipenemase and a clavulanate-susceptible cephalosporinase (3, 22, 24). Ticarcillin-clavulanate was the most active β-lactam in the current study. We postulate that strains susceptible to ticarcillin-clavulanate produce relatively less imipenemase, and those resistant to this combination produce relatively more imipenemase. Kazmierczak and coworkers (8) have postulated that combinations containing clavulanate (e.g., aztreonam and clavulanate) are more active against *X. maltophilia* than those containing sulbactam or tazobactam because of the greater inherent activity of clavulanate than that of sulbactam and tazobactam against these organisms. This was not tested in our study. In contrast to ticarcillin-clavulanate, amoxicillin-clavulanate was not active even though there was more clavulanate in the latter combination than in the former. In view of this, it is possible that ticarcillin-clavulanate is synergistic because of a non-β-lactamase-mediated mechanism.

Because of the different results obtained with the four methods and the difficulty in standardizing susceptibility methodology with this organism, we believe that interpretation of susceptibility testing of this organism in the clinical laboratory is problematic at the present time. Lack of correlation of ciprofloxacin results in our study points to factors other than β-lactamase production, which may play an added role in the difficult nature of susceptibility test correlation with this organism. Until further clarification is obtained, interpretation of susceptibility results with β-lactam–β-lactamase inhibitor combinations and ciprofloxacin should be regarded with caution. The results of the time-kill studies suggest that agar dilution may be the most accurate method for determining the MIC of this species. Disk diffusion, microdilution, and E test methods need to be modified in order to better correlate with the latter two methods. Other approaches to this problem that would obviate the problems mentioned above could include direct enzyme testing, to differentiate between the two β-lactamases, and susceptibility testing with different media (e.g., Diagnostic Sensitivity Test agar and Isosensitest agar).

No controlled clinical data on the therapy of *X. maltophilia* infections with ticarcillin-clavulanate are currently available. Ticarcillin-clavulanate has clinical indications for septicemia and urinary tract infections caused by *Pseudomonas* species: *X. maltophilia* would be included in the latter category, since the nomenclature was *Pseudomonas maltophilia* in 1985 when the submission to the Food and Drug Administration was made. A recent request has been made to the Food and Drug Administration to have *X. maltophilia* formally added to the therapeutic indications of ticarcillin-clavulanate. Clinical data will be necessary in order to confirm the in vitro activity of ticarcillin-clavulanate against this organism.

TABLE 9. Comparison of ticarcillin-clavulanate susceptibility testing inconsistencies for 9 strains of *X. maltophilia*

Strain	Ticarcillin-clavulanate MIC (μg/ml) by[a]:			% Susceptible by disk diffusion[b]	Kill curve at[a,c]:	
	Agar dilution (24 h)	E test	Sensititre (48 h)		6 h	24 h
3579	32	8	>256	31	16	64
2939	32	4	128	33	32	32
2969	64	4	>256	34	16	64
31	8	32	256	26	8	64
HMC-38	16	256	64	20	16	64
3218[d]	32	8	128	23	≤16	16–128[e]
423[d]	32	8	128	23	≤16	16–128[e]
12[d]	128	16	128	25	≤16	16–128[e]
84[d]	8	>256	>256	19	≤16	≤16
2908[f]	8	2	4	33	16	16
425[g]	256	>256	>256	0	64	128

[a] Results are reported in micrograms of ticarcillin per milliliter (with a fixed amount of 2.0 μg of clavulanate per ml).
[b] Ticarcillin-clavulanate zone sizes were recorded to the nearest millimeter.
[c] Concentration (micrograms per milliliter) required to reduce the number of CFU by ≥2 log_{10} reduction values compared with the growth controls.
[d] Kill curves were only determined at ticarcillin-clavulanate concentrations of 16.0/2.0 and 128.0/2.0 μg/ml.
[e] A range is given because ticarcillin-clavulanate was only tested at two concentrations.
[f] Ticarcillin-clavulanate-susceptible control strain.
[g] Ticarcillin-clavulanate-resistant control strain.

TABLE 10. Comparison of piperacillin-tazobactam susceptibility results for 5 strains of *X. maltophilia* that had ticarcillin-clavulanate testing inconsistencies

Strain	Piperacillin-tazobactam MIC (μg/ml) by[a]:			% Susceptible by disk diffusion[b]	Kill curve at[a,c]:	
	Agar dilution (24 h)	E test	Sensititre (48 h)		6 h	24 h
3579	128	>256	>256	15	<16	>128
2939	256	>256	>256	13	16–128[d]	>128
2969	>256	>256	>256	15	16–128[d]	>128
31	128	>256	>256	12	<16	>128
HMC-38	64	>256	>256	0	16–128[d]	>128

[a] Results are reported in micrograms of piperacillin per milliliter (with a fixed amount of 4.0 μg of tazobactam per ml).
[b] Piperacillin-tazobactam zone sizes were recorded to the nearest millimeter.
[c] Concentration (micrograms per milliliter) required to reduce the number of CFU by ≥2 log_{10} reduction values compared with the growth controls. Kill-curve assays were only performed at piperacillin-tazobactam concentrations of 16.0/4.0 and 128.0/4.0 μg/ml.
[d] A range is given because piperacillin-tazobactam was only tested at two concentrations.

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