JOURNAL OF CLINICAL MICROBIOLOGY, Jan. 1992, p. 214–215
0095-1137/92/010214-02$02.00/0
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Vol. 30, No. 1

Comparison of the E Test and Microdilution for Detection of β-Lactam-Resistant Mutants That Are Stably Derepressed for Type I β-Lactamase

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Received 24 July 1991/Accepted 30 September 1991

The activities of cefotaxime, ceftazidime, piperacillin, and aztreonam were compared in the E test and broth microdilution test against 30 gram-negative bacterial mutants derepressed for type I β-lactamases. The results demonstrated complete agreement between 24-h MICs of 80 to 83% and essential agreement between 24-h MICs of 90 to 97%. When sufficient growth was present for the E test to be read at 6 h, the essential agreement between 6- and 24-h E-test MICs was 100% for ceftazidime, piperacillin, and aztreonam and 85% for cefotaxime.

The E test (AB Biodisk, Solna, Sweden) is a novel test for susceptibility testing that consists of a rectangular plastic test strip that contains a predefined, continuous, and exponential gradient of antimicrobial agent and provides MICs based on the intercept of the zone of inhibition with the graded test carrier (1–3, 5). Although Baker et al. (1) compared the E test with microdilution and agar dilution tests of β-lactams against a variety of gram-negative bacilli, it is not clear whether the isolates of *Citrobacter freundii*, *Enterobacter aerogenes*, *Enterobacter cloacae*, *Serratia marcescens*, or *Pseudomonas aeruginosa* tested were wild types or mutants stably derepressed for type I β-lactamase. Nonetheless, it is of interest that Baker et al. (1) noted that MICs of piperacillin for some isolates of members of the *Enterobacteriaceae* family were higher in the microdilution test than in the E test, possibly because small numbers of resistant mutants were more readily discernible in microdilution wells because of continued growth, whereas with the E test and agar dilution 1 or 2 CFUs may not be visible or may be ignored when MICs are determined. The latter explanation, however, seems unlikely since Washington et al. (9) found that microdilution MICs were more likely to be lower than broth macrodilution or agar dilution MICs in a study of β-lactams tested against mutants stably derepressed for type I β-lactamase.

The purpose of this study was to compare microdilution and E-test MICs of cefotaxime, ceftazidime, piperacillin, and aztreonam against 30 isolates of gram-negative bacterial mutants that are stably derepressed for type I β-lactamase (kindly provided by Christine C. Sanders and including *C. freundii*, nine isolates; *E. aerogenes*, two isolates; *E. cloacae*, eight isolates; *Morganella morganii*, two isolates; *Proteus vulgaris*, one isolate; and *P. aeruginosa*, eight isolates).

Isolates were tested by microdilution according to the procedure recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (8) and with the E test according to the manufacturer's directions. Although MICs were determined after 24 h of incubation in both methods, E tests were initially examined after 6 h of incubation to assess how often growth was sufficient to obtain a MIC and, when growth was sufficient, to compare the 6- and 24-h MICs.

Discrepancies between E-test and microdilution MICs are categorized in Table 1 as being very major (E-test MIC equivalent to susceptible, microdilution MIC equivalent to resistant), major (E-test MIC equivalent to resistant, microdilution MIC equivalent to susceptible), and minor (MIC of one test equivalent to either susceptible or resistant and that of another equivalent to moderately susceptible) on the basis of NCCLS MIC interpretive criteria (8). A separate analysis of results based on NCCLS MIC breakpoints (8) is shown in Table 2 to demonstrate the numbers of very major errors (VME), major errors (ME), and minor errors (MIE) and calculations of complete agreement {CA = $[n - (\text{VME} + \text{ME} + \text{MIE})]/(n \times 100)$} and essential agreement {EA = $[n - (\text{VME} + \text{ME})]/(n \times 100)$} (6). As shown in Tables 1 and 2, complete agreement of 24-h MICs was ≥80%, primarily because of the frequency of minor errors. Essential agreement, however, of 24-h MICs was ≥90% since the effect of minor errors is eliminated in this calculation.

Only 20 isolates yielded sufficient growth in the E test after 6 h of incubation to allow determination of MICs; however, as can be seen in Table 2, essential agreement was 100% for ceftazidime, piperacillin, and aztreonam and 85% for cefotaxime. In most instances, *P. aeruginosa* represented the species yielding insufficient growth after 6 h of incubation.

As reported previously from this laboratory (9), susceptibility tests of gram-negative bacterial mutants with derepressed β-lactamases against β-lactams appear to result in a high rate of minor errors. Nonetheless, the essential agree-

TABLE 1. Classification of discrepancies between E-test MICs and reference microdilution test MICs

Antimicrobial agent	No. of isolates by error category[a]		
	Very major	Major	Minor
Cefotaxime	3	1	4
Ceftazidime	1	0	5
Piperacillin	0 (0)	0 (3)	2 (0)
Aztreonam	0	1	4

[a] Number in parentheses represents *P. aeruginosa* isolates.

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TABLE 2. Calculated complete (CA) and essential (EA) agreements of E-test MICs with microdilution MICs

Antimicrobial agent	CA (%)		EA (%)	
	6 h[a]	24 h[b]	6 h[a]	24 h[b]
Cefotaxime	55	80	85	93
Ceftazidime	95	83	100	97
Piperacillin	60	83	100	90
Aztreonam	70	80	100	97

[a] Based on only 20 of the 30 isolates since 10 isolated failed to yield sufficient growth in 6 h for MIC determination.
[b] Based on all 30 isolates.

ment rates between 24-h microdilution and E-test MICs may be acceptable for this challenging group of organisms. Problems with very major errors when testing *E. cloacae* against β-lactams within a 4- to 6-h incubation period were initially reported by Sherris and coworkers (4, 7). The frequency of such very major errors, however, is considerably decreased when incubation is increased to approximately 8 h, as was the case when gram-negative bacterial mutants with derepressed β-lactamases were tested in the Vitek system (9). Nonetheless, when growth was sufficient for MIC determination after 6 h of incubation, the essential agreement rate between 6- and 24-h E-test MICs in our study, with the possible exception of cefotaxime, was acceptable.

We thank AB Biodisk for providing E-test material required for this study.

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