

## Evaluation of Stability of Cefotaxime (30- $\mu$ g) and Ceftazidime (30- $\mu$ g) Disks Impregnated with Clavulanic Acid (10 $\mu$ g) for Detection of Extended-Spectrum $\beta$ -Lactamases

Extended-spectrum  $\beta$ -lactamase (ESBL) activity in *Escherichia coli*, *Klebsiella pneumoniae*, or *Klebsiella oxytoca* can be detected by using a standard disk diffusion susceptibility test method with cefotaxime (30- $\mu$ g), ceftriaxone (30- $\mu$ g), ceftazidime (30- $\mu$ g), cefpodoxime (10- $\mu$ g), or aztreonam (30- $\mu$ g) disks (2, 3). The presence of an ESBL can then be confirmed by a phenotypic confirmatory disk diffusion test recommended by the National Committee for Clinical Laboratory Standards (3) or by the double disk synergy test described by Jarlier et al. (1). The phenotypic confirmatory disk diffusion test is relatively simple to perform and easy to interpret. It requires testing of both cefotaxime (30- $\mu$ g) and ceftazidime (30- $\mu$ g) disks each alone and in combination with clavulanic acid (10  $\mu$ g). An increase in the zone diameter of  $\geq 5$  mm for either antimicrobial agent tested in combination with clavulanic acid over that when tested alone indicates that the isolate is an ESBL producer. However, as clavulanic acid is labile, it is recommended that the disks be used immediately after preparation and that they not be stored (3). This may be an inconvenience for many clinical laboratories. Therefore, we evaluated the stability of disks containing clavulanic acid stored at  $-20^{\circ}\text{C}$  for 14 consecutive days.

Clavulanic acid powder was obtained from Smith Kline Beecham Pharma (Oakville, Ontario, Canada). A stock solution of clavulanic acid (1,000  $\mu\text{g}/\text{ml}$ ) was freshly prepared. A 10- $\mu\text{l}$  aliquot was added to each of the cefotaxime (30- $\mu$ g) and

ceftazidime (30- $\mu$ g) disks, which were then allowed to air dry at room temperature for 30 min. The cefotaxime-clavulanic acid and ceftazidime-clavulanic acid disks were placed in separate screw-capped glass vials containing dessicators (Dricap 1.0 silica gel; Multi-Sorb Technology Inc., Buffalo, N.Y.) and placed in a  $-20^{\circ}\text{C}$  freezer. Each day for 14 consecutive days, the containers were removed from the freezer and brought to room temperature. The disks were removed, and the containers were closed and immediately returned to the freezer. The disks were used for phenotypic confirmatory disk diffusion testing (3) to detect ESBLs using *E. coli* ATCC 25922 (ESBL-negative) and a well-characterized ESBL-producing strain of *E. coli* (LPTP 9711). Linear regression analysis was used to determine if there was a significant change in the zone diameters over time.

There was no significant change in the zone diameters obtained with any of the four disks tested with the two organisms over the 14 days of evaluation (Table 1). For each of the disks tested, the maximum variability in the measured zone diameters was 3 mm. There were no statistically significant changes in the zone diameters with the  $\beta$ -lactam disks alone in comparison to those containing clavulanic acid over the 14 days. These results suggest that disks with clavulanic acid prepared for ESBL phenotypic confirmatory disk diffusion testing (3) are sufficiently stable for use up to 14 days after preparation, provided that they are stored at  $-20^{\circ}\text{C}$ .

TABLE 1. Phenotypic confirmatory disk diffusion test results using cefotaxime and ceftazidime disks, with and without clavulanic acid, that were stored frozen for up to 14 days<sup>a</sup>

Day	Zone diam (mm)											
	<i>E. coli</i> LPTP 9711 (ESBL producing)						<i>E. coli</i> 25922 (ESBL negative)					
	CTX	CTX-CL	Difference between CTX and CTX-CL	CAZ	CAZ-CL	Difference between CAZ and CAZ-CL	CTX	CTX-CL	Difference between CTX and CTX-CL	CAZ	CAZ-CL	Difference between CAZ and CAZ-CL
1	22	34	12	11	28	17	33	35	2	31	32	1
2	22	36	14	11	29	18	32	32	0	30	30	0
3	22	35	13	11	29	18	32	35	3	30	31	1
4	22	35	13	12	30	18	34	34	0	31	30	-1
5	21	34	13	11	29	18	35	36	1	31	30	-1
6	22	35	13	11	29	18	34	35	1	31	32	1
7	23	36	13	12	30	18	35	36	1	31	32	1
8	22	35	13	13	29	16	35	36	1	31	30	-1
9	24	36	12	13	30	17	35	33	-2	31	31	0
10	22	34	12	12	29	17	33	34	1	32	31	-1
11	21	35	14	12	29	17	33	35	2	30	31	1
12	22	35	13	12	30	18	34	34	0	31	32	1
13	22	36	14	12	30	18	34	34	0	30	31	1
14	22	36	14	12	30	18	34	34	0	32	30	-2
Mean diam		35.1	13.1		29.4	17.6		34.5	0.7		30.9	-0.1
SD		0.8	0.7		0.6	0.7		1.2	1.1		0.8	1.0
$r^2$		0.11	0.10		0.35	0.25		0.10	0.13		0.01	0.10
<i>P</i> value		0.25	0.27		0.02	0.85		0.81	0.20		0.97	0.27

<sup>a</sup> CTX, cefotaxime; CAZ, ceftazidime; CL, clavulanic acid.

## REFERENCES

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