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Comparative In Vitro Activities of Carbapenem L-749,345 and Other Antimicrobials against Multiresistant Gram-Negative Clinical Pathogens

GEORGE JACOBY,* PAULA HAN, AND JOHN TRAN

Lahey Hitchcock Clinic, Burlington, Massachusetts 01805, and Edith Nourse Rogers Memorial Veterans Hospital, Bedford, Massachusetts 01730

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Carbapenems L-749,345 and imipenem had the lowest MICs at which 90% of isolates were inhibited (0.5 μg/ml) of 14 antimicrobial agents tested against 76 multiresistant gram-negative clinical isolates with TEM- or SHV-type extended-spectrum β-lactamases and chromosomal or plasmid-determined AmpC β-lactamases, but the MIC of L-749,345 for one isolate of *Klebsiella pneumoniae* was 16 μg/ml.

L-749,345 is a new, long-acting, 1-β-methyl carbapenem with a broad spectrum of activity against gram-negative pathogens (4). Its potency was evaluated against strains making plasmid-mediated extended-spectrum β-lactamase (ESBL) or overproducing chromosomal group 1 enzyme (1). Seventy-six oxyimino-β-lactam-resistant clinical isolates from 32 hospitals around the United States were selected for study. As a rule, no more than one strain of a particular type (enzyme or species) was selected from each location. The ceftazidime MICs for all strains were ≥1 μg/ml. Three strains of *Citrobacter freundii*, four isolates of *Enterobacter cloacae*, and five strains of *Escherichia coli* made excess amounts of enzymes with isoelectric points (pIs) and resistance properties consistent with those of AmpC (group 1) β-lactamases. These strains did not transfer resistance to ceftazidime, but the remaining 16 strains of *E. coli* and 48 strains of *Klebsiella pneumoniae* contained transmissible plasmids encoding enzymes mediating oxyimino-β-lactam resistance (2). Twelve isolates produced an AmpC-type β-lactamase, 19 produced a TEM-type ESBL (2 made TEM-10, 4 made TEM-12, 1 made TEM-19, 2 made TEM-26, and 10 made unclassified ESBLs with pIs between 5.4 and 5.9), and 33 produced an SHV-type ESBL (as determined by pI, 3 made SHV-2, 6 made SHV-3, 11 made SHV-4, and 13 made SHV-5). Each strain transferred only one β-lactamase responsible for oxyimino-β-lactam resistance but may have possessed permeability defects or other mechanisms contributing to resistance. *E. coli* ATCC 25922 and *Pseudomonas aeruginosa* ATCC 27853 were included for quality control.

Susceptibility was determined according to National Committee for Clinical Laboratory Standards (NCCLS) criteria by agar dilution with unsupplemented Mueller-Hinton medium (Difco Laboratories, Detroit, Mich.) containing doubling concentrations of antibiotics and inocula of 10^4 or 10^6 organisms per spot applied with a replica plating device (3). For piperacillin-tazobactam, the concentration of tazobactam was fixed at 4 μg/ml. Plates were incubated 16 to 20 h at 35°C. Antibiotics were obtained from the following sources: Bayer Corp. (ciprofloxacin), Bristol-Myers Squibb (aztreonam and cefepime), Elkins-Sinn, Inc. (gentamicin), Hoffmann-La Roche Inc. (ceftriaxone), Hoechst-Roussel Pharmaceuticals Inc. (cefotaxime), Lederle Inc. (piperacillin and tazobactam), Eli Lilly & Co. (ceftazidime), Marsam Pharmaceuticals Inc. (cefazolin and cefuroxime), Merck & Co., Inc. (cefoxitin, imipenem, and L-749,345), and Zeneca Pharmaceuticals (cefotetan). Procedures for β-lactamase isoelectric focusing and resistance transfer have been described previously (2).

Table 1 shows the MIC ranges, the MICs at which 50% of isolates were inhibited (MIC_{50}s), and the MIC_{90}s of the tested agents against the 76 multiresistant isolates. MIC_{90}s were 64 μg/ml or greater for cefazolin, cefuroxime, ceftazidime, ceftriaxone, aztreonam, cefoxitin, cefotetan, piperacillin-tazobactam, and gentamicin. L-749,345 and imipenem had the lowest MIC_{90} (0.5 μg/ml) of the tested antimicrobials, with cefepime (MIC_{90}, 2 μg/ml) also conspicuously potent. Table 2 shows the number of strains of each type in each susceptibility category according to present NCCLS criteria. Problems with the current breakpoints are illustrated by the percent of strains apparently susceptible to cefazolin (22%) or cefuroxime (38%). Twenty percent or less of the strains were intermediate or resistant to cefepime, cefotetan, piperacillin-tazobactam, imipenem, or, at a cutoff of 8 μg/ml (5), L-749,345. However, raising the number of organisms in inoculum from 10^4 to 10^6 per spot increased MICs 2- to 32-fold and raised the percent intermediate or resistant to 25% or more for cefepime, ce-

TABLE 1. Comparative susceptibility of 76 isolates

Antibiotic	MIC (μg/ml)[a]		
	Range	50%	90%
Cefazolin	2–≥256	64	≥256
Cefuroxime	2–≥256	16	256
Aztreonam	1–≥256	16	128
Cefotaxime	≤0.06–256	2	32
Ceftazidime	1–≥256	32	128
Ceftriaxone	≤0.06–256	2	64
Cefepime	≤0.06–128	1	2
Cefotetan	≤0.06–256	0.5	64
Cefoxitin	<2–≥256	8	256
Piperacillin-tazobactam	0.5–≥256	4	64
Imipenem	≤0.06–2	0.25	0.5
L-749,345	≤0.015–16	0.06	0.5
Ciprofloxacin	≤0.015–64	0.03	4
Gentamicin	0.25–≥256	8	64

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

* Corresponding author. Mailing address: Lahey Hitchcock Clinic, 41 Mall Rd., Burlington, MA 01805. Phone: (617) 273-8608. Fax: (617) 744-1264. E-mail: George.A.Jacoby@Lahey.Hitchcock.Org.

TABLE 2. Number in each susceptibility category for isolates grouped by resistance mechanism

Antibiotic	Susceptibility breakpoints[a] (μg/ml)	No. of isolates in each susceptibility category[b]			
		AmpC overproducer (n = 12)	AmpC plasmid (n = 12)	TEM ESBL (n = 19)	SHV ESBL (n = 33)
Cefazolin	≤8, 16, ≥32	0, 2, 10	0, 0, 12	14, 2, 3	3, 4, 26
Cefuroxime	≤8, 16, ≥32	2, 1, 9	0, 0, 12	15, 1, 3	12, 10, 11
Aztreonam	≤8, 16, ≥32	7, 1, 4	6, 1, 5	9, 5, 5	10, 2, 21
Cefotaxime	≤8, 16–32, ≥64	5, 3, 4	7, 4, 1	19, 0, 0	29, 3, 1
Ceftazidime	≤8, 16, ≥32	4, 1, 7	2, 2, 8	7, 0, 12	9, 4, 20
Ceftriaxone	≤8, 16–32, ≥64	5, 1, 6	8, 2, 2	19, 0, 0	30, 2, 1
Cefepime	≤8, 16, ≥32	12, 0, 0	12, 0, 0	19, 0, 0	32, 0, 1
Cefotetan	≤16, 32, ≥64	5, 1, 6	4, 1, 7	19, 0, 0	33, 0, 0
Cefoxitin	≤8, 16, ≥32	1, 0, 11	0, 0, 12	16, 2, 1	28, 2, 3
Piperacillin-tazobactam	≤16, 32–64, ≥128	7, 4, 1	7, 4, 1	18, 1, 0	30, 1, 2
Imipenem	≤4, 8, ≥16	12, 0, 0	12, 0, 0	19, 0, 0	33, 0, 0
L-749,345	≤4, 8, ≥16[c]	12, 0, 0	12, 0, 0	19, 0, 0	32, 0, 1
Ciprofloxacin	≤1, 2, ≥4	12, 0, 0	10, 0, 2	16, 2, 1	23, 0, 10
Gentamicin	≤4, 8, ≥16	9, 0, 3	3, 4, 5	5, 4, 10	15, 4, 14

[a] NCCLS interpretive standards for susceptible, intermediate, and resistant isolates.
[b] Grouped as susceptible, intermediate, and resistant.
[c] Proposed (5) but not yet NCCLS approved.

fotetan, and piperacillin-tazobactam without changing the percent resistant to imipenem or L-749,345 (Table 3).

For one *K. pneumoniae* isolate the MIC of L-749,345 was 16 μg/ml. This strain was also resistant to aztreonam, ceftazidime, cefotaxime, ceftriaxone, cefepime, cefoxitin, and piperacillin-tazobactam, and the MIC of imipenem for this strain was 2 μg/ml. The isolate produced a β-lactamase with a pI of 7.6, consistent with SHV-2, that could be transferred to an *E. coli* recipient by selecting for transfer of ceftazidime resistance. Resistance to L-749,345 was not transferred (MIC ≤ 0.015 μg/ml). A ceftazidime-susceptible derivative, obtained by acridine orange treatment of the parent *K. pneumoniae* strain, could be restored to L-749,345 resistance by reintroducing the pI 7.6 β-lactamase by conjugation, indicating that both ESBL production and another property, presumably affecting L-749,345 permeability, were required for resistance.

L-749,345 and imipenem were thus the most potent of the agents tested against this group of multiresistant clinical isolates. One strain of *K. pneumoniae* among the 76 strains tested was, however, resistant to L-749,345 due to the combined presence of an ESBL and a presumed permeability limitation.

TABLE 3. Effect of inoculum

Antibiotic	Inoculum (no. of organisms per spot)	MIC_{50} (μg/ml)	MIC_{90} (μg/ml)	% Resistant[a] or intermediate
Cefepime	10^4	1	2	1
	10^6	8	64	29
Cefotetan	10^4	0.5	64	20
	10^6	1	256	26
Piperacillin-tazobactam	10^4	4	64	18
	10^6	16	>256	43
Imipenem	10^4	0.25	0.5	0
	10^6	0.5	2	0
L-749,345	10^4	0.06	0.5	1
	10^6	0.12	1	1

[a] Percent not inhibited at or above the intermediate breakpoints given in Table 2.

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