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In Vitro Antibacterial Spectrum of E1040 Compared with Those of Cefpirome and Ceftazidime and Disk Diffusion Interpretive Criteria for E1040

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E1040 is a new parenteral cephalosporin which was tested against 690 clinical isolates and compared with cefpirome and ceftazidime. E1040 had the best activity of the three drugs against *Pseudomonas aeruginosa*, inhibiting 89% of strains at 8.0 μg/ml. E1040 demonstrated good activity against members of the family *Enterobacteriaceae*, including cefpirome-resistant and ceftazidime-resistant strains. E1040 also had good activity against streptococci but much poorer activity against enterococci and staphylococci. When E1040 broth microdilution and disk diffusion susceptibility test results were compared, the 30-μg disk was recommended, with the following tentative interpretive criteria: susceptible, ≥18 mm (MIC, ≤8.0 μg/ml); intermediate, 15 to 17 mm (MIC, 16 μg/ml); and resistant, ≤14 mm (MIC, ≥32 μg/ml).

E1040 is a new semisynthetic parenteral cephalosporin characterized by a 4-carbamoylquinuclidine in position 3 and a 5-amino-1,2,4-thiadiazolyl-methoxyimino moiety in position 7 of the cephalosporin ring (3, 4). It has been shown to have a broad spectrum of activity that is bactericidal against most gram-negative bacteria (including *Pseudomonas aeruginosa*) and many gram-positive species (3, 4). It is inactive against *Bacteriodes fragilis* (3, 4). E1040 has shown marked stability when exposed to various bacterial β-lactamases and also exhibited a low affinity for these enzymes (3, 4). Its low human serum protein binding (<5%) and its apparent efficacy in the treatment of infections caused by *P. aeruginosa* and other bacteria in experimental animals suggest its potential usefulness for humans (M. Otsuki, R. Hiruma, and T. Nishino, Program Abstr. 28th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 432, 1988; H. Takeuchi, D. Yoshida, and M. Satoh, 28th ICAAC, abstr. no. 434, 1988). The purpose of this study was to determine the interpretive criteria for E1040 disk diffusion susceptibility testing, as well as to confirm its spectrum of antibacterial activity in comparison with those of cefpirome and ceftazidime.

E1040 was provided as a standardized powder by Eisai America, Inc., Teaneck, N.J. The drugs used in the comparison, ceftazidime and cefpirome, were obtained from their respective American manufacturers. Paper disks containing 10, 20, and 30 μg of E1040 and 30 μg of ceftazidime were prepared by BBL Microbiology Systems, Cockeysville, Md. The 690 clinical isolates that were tested are identified in Table 1. These included 269 *Enterobacteriaceae* isolates, 60 *Haemophilus influenzae* isolates, 56 nonenteric gram-negative bacilli, 20 enterococci, 90 streptococci, 65 staphylococci, 100 gram-negative cocci, and 30 gram-positive bacilli.

All organisms were tested for susceptibility to E1040, cefpirome, and ceftazidime by the broth microdilution method as recommended by the National Committee for Clinical Laboratory Standards (NCCLS) (1). The concentrations of antibiotics tested were serial twofold dilutions which ranged from 128 to 0.004 μg/ml for E1040 and from 16 to 0.03 μg/ml for cefpirome and ceftazidime. All organisms were tested for susceptibility to E1040 by the disk diffusion method as outlined by the NCCLS (2) by using disks impregnated with 10, 20, and 30 μg of E1040. Ceftazidime (30 μg) disks were also tested in parallel for quality assurance purposes. The quality control organisms recommended by the NCCLS for both procedures (1, 2) were also tested on each day of testing. The modal E1040 MICs (ranges are in parentheses) with 20 tests per organism were as follows: *Staphylococcus aureus* ATCC 29213, 8.0 (4.0 to 16) μg/ml; *Enterococcus faecalis* ATCC 29212, 128 (64 to >128) μg/ml; *Escherichia coli* ATCC 25922, 0.13 (0.13 to 0.25) μg/ml; and *P. aeruginosa* ATCC 27853, 0.5 (0.25 to 0.5) μg/ml. The median (ranges are in parentheses) inhibitory zone diameters around 30-μg E1040 disks with 20 tests per organism were as follows: *S. aureus* ATCC 25923, 20 (18 to 22) mm; *E. coli* ATCC 25922, 28 (25 to 31) mm; and *P. aeruginosa* ATCC 27853, 29 (26 to 32) mm. All 120 ceftazidime test results with the quality control organisms fell within the acceptable ranges published by the NCCLS (1, 2).

In this study, the antimicrobial activity of E1040 compared with those of ceftazidime and cefpirome was very similar to that reported in previously published papers (3, 4). Table 1 summarizes the broth microdilution susceptibility test results for all three drugs, expressed as the MICs for 50 and 90% of isolates (MIC_{50} and MIC_{90}, respectively). Against the *Enterobacteriaceae* as a group, cefpirome had the lowest MIC_{50} (0.06 μg/ml), but E1040 had the lowest MIC_{90} (1.0 μg/ml). With the exception of 1 *Enterobacter aerogenes* strain which was resistant to all three tested drugs, there were 5 cefpirome-resistant and 26 ceftazidime-resistant (MIC, >8.0 μg/ml) *Enterobacteriaceae* isolates, all of which were susceptible to E1040.

E1040 was the most active of the three drugs against *P. aeruginosa*, inhibiting 89% of the isolates at 8.0 μg/ml, compared with 76% for ceftazidime and 60% for cefpirome. Against other *Pseudomonas* spp., especially *Pseudomonas cepacia*, E1040 was less active than ceftazidime and more active than cefpirome.

All streptococci, *Neisseria* spp., and *Branhamella catarrhalis* strains tested were susceptible to all three cephalosporins. The relative activity of the three drugs varied

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TABLE 1. Susceptibility of 690 clinical isolates to E1040, cefpirome, and ceftazidime

Organism	No.	E1040			Cefpirome			Ceftazidime		
		MIC (μg/ml)[a]		% Susceptible[b]	MIC (μg/ml)		% Susceptible	MIC (μg/ml)		% Susceptible
		50%	90%		50%	90%		50%	90%	
Citrobacter spp.	37	0.13	2.0	100	0.06	8.0	97	0.5	>16	78
Enterobacter spp.	66	0.25	2.0	99	0.13	4.0	96	0.5	>16	76
Escherichia coli	20	0.13	0.25	100	0.06	0.25	100	0.25	0.5	100
Klebsiella spp.	21	0.13	1.0	100	0.06	2.0	95	0.25	1.0	100
Morganella morganii	10	0.13	0.25	100	0.06	0.13	100	0.25	8.0	90
Proteus mirabilis	20	0.25	0.25	100	0.06	0.06	100	0.06	0.06	100
P. vulgaris	10	0.5	2.0	100	1.0	8.0	90	0.13	0.25	100
Providencia spp.	22	0.13	0.25	100	0.06	0.5	100	0.25	1.0	100
Salmonella spp.	10	0.25	1.0	100	0.06	1.0	100	0.5	2.0	100
Serratia spp.	27	0.5	8.0	100	0.13	4.0	100	0.5	4.0	100
Shigella spp.	12	0.13	0.25	100	≤0.03	0.06	100	0.13	0.25	100
Yersinia enterocolitica	10	0.13	0.13	100	≤0.03	0.06	100	0.5	1.0	100
Other enteric organisms[c]	4	0.13_2	0.25_1, 0.5_1	100	0.06_2	0.13_1, 16_1	75	0.13_1, 0.5_1	16_1, $>16_1$	50
All *Enterobacteriaceae* isolates	269	0.13	1.0	99.6	0.06	2.0	97.4	0.25	8.0	90
Pseudomonas aeruginosa	25	1.0	16	88	8.0	>16	60	4.0	>16	76
Other *Pseudomonas* spp.	14	1.0	64	79	4.0	>16	57	2.0	16	86
Acinetobacter spp.	11	1.0	2.0	100	2.0	4.0	91	4.0	8.0	91
Other nonenteric GNBs[d]	6	16		33	>16		33	4.0		67
Enterococci	20	128	>128	10	8.0	>16	50	>16	>16	10
Streptococcus bovis	10	1.0	1.0	100	8.0	8.0	100	4.0	4.0	100
S. pyogenes	20	0.06	0.13	100	≤0.03	≤0.03	100	0.13	0.25	100
S. agalactiae	20	0.5	1.0	100	0.06	0.06	100	0.5	1.0	100
Streptococcus groups C and G	20	1.0	1.0	100	8.0	8.0	100	4.0	4.0	100
S. pneumoniae										
P-susc[e]	10	0.25	0.5	100	≤0.03	≤0.03	100	0.25	0.25	100
P-res[e]	10	1.0	2.0	100	0.13	0.25	100	4.0	4.0	100
Staphylococcus aureus										
Ox-susc[f]	20	8.0	16	85	1.0	1.0	100	8.0	16	85
Ox-res[f]	10	16	32	10	4.0	>16	60	>16	>16	0
S. epidermidis										
Ox-susc	10	4.0	8.0	90	1.0	1.0	100	8.0	16	50
Ox-res	5	8_1, 16_2 32_1, 64_1		20	4.0_1, 8.0_1, 16_1, $>16_2$		40	$>16_5$		0
S. haemolyticus	10	8.0	>128	50	1.0	>16	50	16	>16	10
Other *Staphylococcus* spp.	10	4.0	8.0	100	0.5	1.0	100	8.0	>16	70
Bacillus spp.	10	64	>128	0	16	16	40	>16	>16	0
Corynebacterium jeikeium	10	8.0	>128	80	8.0	>16	70	>16	>16	0
Listeria monocytogenes	10	>128	>128	0	>16	>16	10	>16	>16	0
Branhamella catarrhalis	30	2.0	4.0	100	0.13	2.0	100	≤0.03	0.25	100
Neisseria meningitidis	10	0.5	1.0	100	≤0.03	≤0.03	100	≤0.03	0.06	100
N. gonorrhoeae										
β-Neg[g], P-susc	21	0.5	1.0	100	NT[h]			NT		
β-Pos[g]	21	0.5	1.0	100	NT			NT		
β-Neg, P-res	18	2.0	8.0	100	NT			NT		
Haemophilus influenzae										
β-Neg	19	0.13	0.5	100	NT			NT		
β-Pos	21	0.25	0.25	100	NT			NT		
β-Neg, Amp-res[i]	20	4.0	8.0	100	NT			NT		

[a] 50% and 90%, MIC for 50 and 90% of isolates. For organisms with fewer than six isolates, individual MICs are presented with subscripts indicating number of isolates for that MIC.
[b] Percentage of isolates susceptible to 8.0 μg/ml.
[c] Includes two *Cedacea lepagai* strains and two *Hafnia alvei* strains.
[d] Includes two strains each of *Achromobacter xylosoxidans*, *Aeromonas* spp., and *Flavobacterium* spp. GNB, Gram-negative bacilli.
[e] P-susc, Penicillin susceptible; P-res, penicillin resistant.
[f] Ox-susc, Oxacillin susceptible; Ox-res, oxacillin resistant.
[g] β-Neg and β-Pos, β-Lactamase negative and positive, respectively.
[h] NT, Not tested.
[i] Amp-res, Ampicillin resistant.

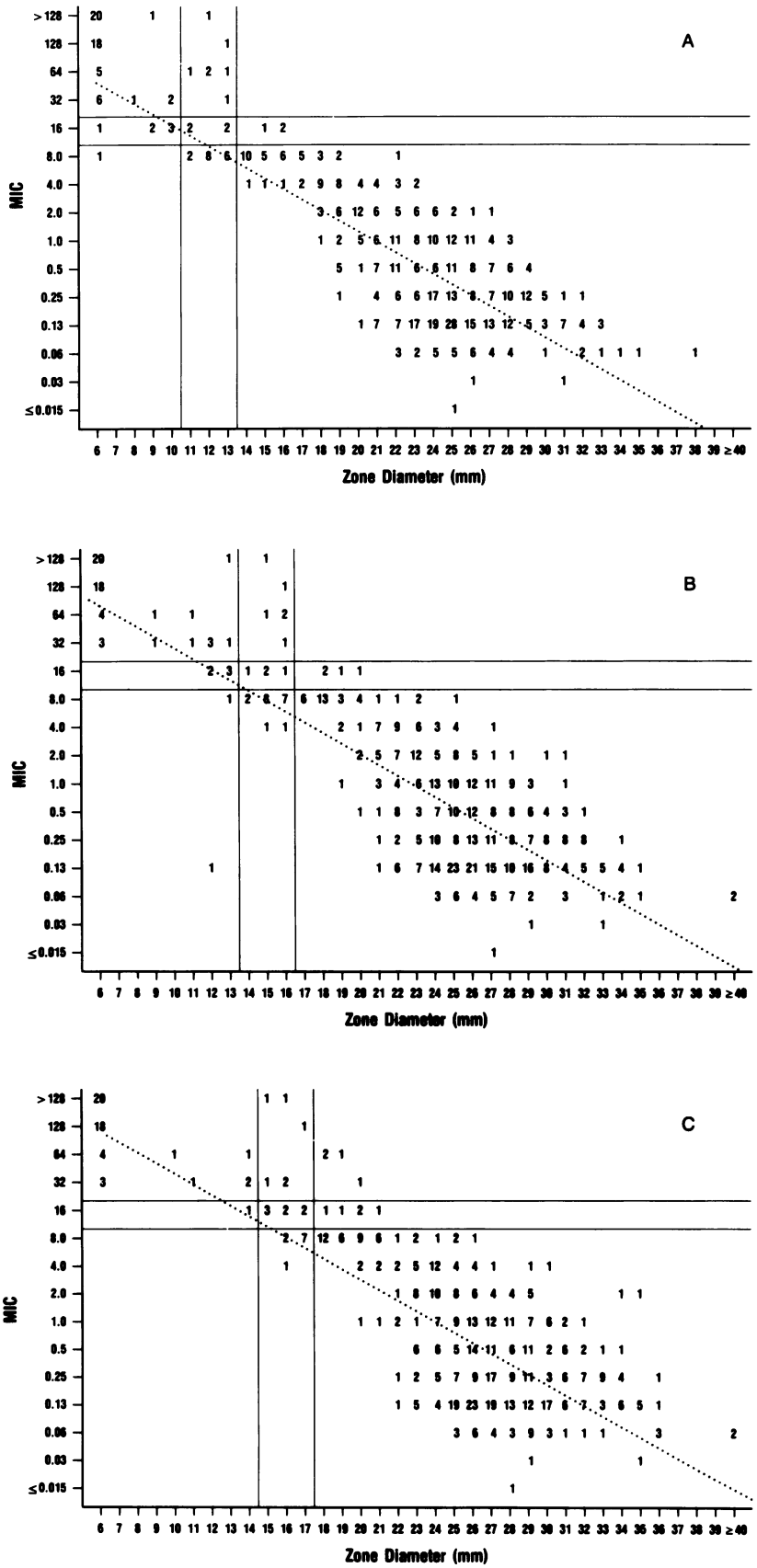

FIG. 1. Scattergrams of E1040 MICs versus zone diameters (10-μg [A], 20-μg [B], and 30-μg [C] E1040 disks). Horizontal lines represent proposed susceptible (lower line) and resistant (upper line) MIC breakpoints; vertical lines represent proposed susceptible (right line) and resistant (left line) zone diameter breakpoints.

TABLE 2. Statistical data, interpretive criteria, and error rates for E1040 disk diffusion tests

Disk (μg)	Coefficient of:		y Intercept[a]	Criteria (mm)			Error rate (%)			
	Correlation	Regression		Susceptible	Intermediate	Resistant	Very major	Major	Minor	Total
10	0.779	−0.376	16.86	≥14	11–13	≤10	0.00	0.16	5.15	5.32
20	0.732	−0.373	17.49	≥17	14–16	≤13	0.00	0.32	5.48	5.81
30	0.699	−0.376	18.10	≥18	15–17	≤14	0.65	0.00	3.55	4.20
				≥19	16–18	≤15	0.32	0.00	5.81	6.13
				≥20	17–19	≤16	0.16	0.48	6.13	6.77

[a] Log_2 + 9 scale (micrograms per milliliter).

among individual species (Table 1). By contrast, enterococci and gram-positive bacilli were resistant to these drugs.

Cefpirome showed the best activity against staphylococci; E1040 and ceftazidime were significantly less active. Many strains of *Staphylococcus haemolyticus* and oxacillin-resistant staphylococci were resistant to all three drugs. Like ceftazidime, E1040 probably should not be considered a primary antistaphylococcal drug.

Among organisms for which β-lactamase-positive and -negative strains could be compared (e.g., staphylococci, *H. influenzae* and *Neisseria gonorrhoeae*), no appreciable difference in E1040 MICs was observed, thus tending to substantiate the β-lactamase stability of E1040 (3, 4). However, significantly higher E1040 MICs did occur among strains demonstrating β-lactam resistance due to altered penicillin-binding proteins, such as oxacillin-resistant staphylococci, penicillin-resistant pneumococci, β-lactamase-negative penicillin-resistant gonococci, and β-lactamase-negative ampicillin-resistant *H. influenzae* (Table 1). With the exception of the staphylococci, the aforementioned organisms were all susceptible to E1040 at ≤8.0 μg/ml.

To date, we are unaware of published pharmacokinetic data on E1040. However, in a summary of preclinical and phase I studies for E1040 provided by the manufacturer of E1040, levels achieved in serum after intravenous administration of E1040 to healthy adult volunteers are comparable to those expected with most other parenteral cephalosporins. Consequently, we tentatively selected an MIC of ≤8.0 μg/ml as the susceptible breakpoint, since such a level is normally far exceeded and would maintain parity with susceptible breakpoints of most other parenteral cephalosporins.

Figure 1 displays scattergrams depicting E1040 MICs versus disk diffusion zone diameters for 620 organisms (excluding *Neisseria meningitidis* and *N. gonorrhoeae*). The correlation coefficient was highest with the 10-μg E1040 disks (Table 2) and lowest with the 30-μg disks. By using MIC-susceptible and -resistant breakpoints of ≤8.0 and ≥32 μg/ml, respectively, the corresponding zone diameter breakpoints for the 10-μg E1040 disk would be ≥14 mm and ≤10 mm, respectively. These breakpoints gave a very acceptable 5.3% total error rate, with no very major (false-susceptible) errors and one major (false-resistant) error.

For all cephalosporins currently available in the United States for which the susceptible MIC breakpoint is ≤8.0 μg/ml, a disk content of 30 μg is recommended, and for most of these, the susceptible and resistant zone diameter breakpoints are ≥18 mm and ≤14 mm, respectively. When the latter criteria were applied to our 30-μg disk data (Fig. 1C) (Table 2), the lowest total error rate (4.2%) was achieved, but a relatively high (0.65%) very major error rate was also observed. The four very major errors were produced by one strain each of *E. aerogenes*, *P. cepacia*, *Pseudomonas acidovorans*, and *S. haemolyticus*. Most of the false-susceptible and minor errors were accounted for by oxacillin-resistant staphylococci. The error rate could thus be lowered even more by eliminating oxacillin-resistant staphylococci from testing protocols.

Our data indicate that with the criteria proposed in Table 2, the 10- and 30-μg E1040 disks both effectively delineate susceptible bacterial isolates from resistant bacterial isolates, in accord with corresponding MIC criteria. For U.S. laboratories which utilize NCCLS methods, the 30-μg disk and proposed breakpoints would be much easier to assimilate into routine practice.

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