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In Vitro Evaluation of Ro 09-1227, a Novel Catechol-Substituted Cephalosporin

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Ro 09-1227 is a novel 7-position catechol-substituted parenteral cephalosporin that also has a 3-position radical similar to previously described cephems. The Ro 09-1227 spectrum was slightly wider than that of ceftazidime against members of the family *Enterobacteriaceae* tested, principally because of greater activity against species producing Richmond-Sykes type I β-lactamases. Ro 09-1227 was also more active than ceftazidime against some strains producing extended-spectrum plasmid-encoded β-lactamases, such as TEM-3, -4, -5, -6, -7, and -9, SHV-2 and -3, and CAZ-2. Most strains of *Pseudomonas aeruginosa, Xanthomonas maltophilia,* and *Acinetobacter* spp. were also more susceptible to Ro 09-1227 than cefotaxime, ceftriaxone, cefoperazone, and ceftazidime. *Haemophilus influenzae* (MIC for 90% of strains tested [MIC_{90}], 0.5 μg/ml), *Neisseria gonorrhoeae* (MIC_{90}, 0.015 μg/ml), and *Moraxella (Branhamella) catarrhalis* (MIC_{90}, 0.5 μg/ml) were also Ro 09-1227 susceptible. Ro 09-1227 activity against important gram-positive cocci was most comparable to that of ceftazidime. *Bacteroides fragilis* (MIC_{90}, >32 μg/ml) and the enterococci (MIC_{90}, >32 μg/ml) were resistant to Ro 09-1227. These in vitro results indicate that this catechol-substituted cephalosporin may be useful as an empiric agent, especially for some isolates resistant to currently available broad-spectrum cephalosporins.

Compound Ro 09-1227 (also known as SR-1024; Sankei) is a 7-catechol-substituted cephalosporin with the chemical formula 7-[[[(6*R*,7*R*)-7-[(*Z*)-2-(2-amino-4-thiazolyl)-2-[[1-[3-(3,4-dihydroxybenzoyl) carbazoyl]-1-methylethoxy]imino]acetamido]-2-carboxy-8-oxo-5-thia-1-azabicylo[4.2.0]oct-2-en-3-yl]methyl]thio-5-methyl-[1,2,4]triazolo[1,5-1]pyrimidine-2-carboxylic acid disodium (Fig. 1). Similar catechol cephems have been previously described by others. These compounds have acceptable activity against members of the family *Enterobacteriaceae, Pseudomonas* spp., staphylococci, *Streptococcus* spp., fastidious respiratory tract pathogens (*Haemophilus* spp. and *Moraxella (Branhamella) catarrhalis),* and most anaerobes (1, 3–5, 7, 10, 12). Because of the catechol substitution, uptake of these compounds can be enhanced by the *tonB* iron transport system (3, 7, 10, 12). However, an excess of free iron compared with the conditions that may occur in an iron-depleted infection environment may result in higher MICs (1–4, 7, 10).

In this study, we report the in vitro spectrum of Ro 09-1227 activity compared with those of currently available broad-spectrum cephalosporins, such as cefotaxime, ceftazidime, ceftriaxone, and cefoperazone. The effects of high and low medium iron content on Ro 09-1227 MICs were also assessed.

The Ro 09-1227 and ceftriaxone were obtained from Hoffman-La Roche (Nutley, N.J.). The following pharmaceutical companies kindly provided additional reagents: Hoechst-Roussel Pharmaceuticals Inc. (Sommerville, N.J.), cefotaxime and cefpirome (6); Pfizer Inc. (New York, N.Y.), cefoperazone; and Glaxo Inc. (Research Triangle Park, N.C.), ceftazidime.

The organisms tested were recent clinical isolates from patients at the University of Iowa Hospitals and Clinics. Most strains were cultured from blood or other normally sterile body fluids (not urine). An additional collection of organisms resistant to newer beta-lactams, especially cephalosporins, was tested. These 32 enteric bacilli were generally ceftazidime resistant by plasmid-mediated enzyme hydrolysis or by a stably derepressed chromosomal cephalosporinase.

MICs were determined by the broth microdilution method (8) in trays manufactured by Prepared Media Laboratory (Tualatin, Oreg.). The Mueller-Hinton medium was adjusted with divalent cations to the levels recommended by the National Committee for Clinical Laboratory Standards (8). For one series of tests, the broth was further supplemented with ferric chloride to a level of 500 μg/dl. Moreover, 2,2′-dipyridyl (100 μM/liter) was added to a limited number of

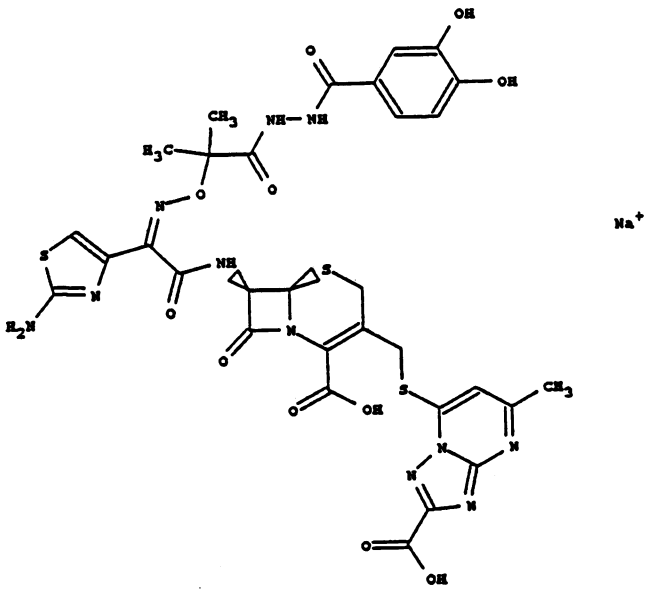

FIG. 1. Chemical structure of Ro 09-1227.

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TABLE 1. In vitro activity of Ro 09-1227 compared with other broad-spectrum cephalosporins tested

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)			% Susceptible[a]
		50%	90%	Range	
Citrobacter diversus (10)	Ro 09-1227	0.5	1	0.12–2	100
	Cefotaxime	≤0.25	0.5	≤0.25–0.5	100
	Ceftriaxone	≤0.25	1	≤0.25–1	100
	Cefoperazone	0.5	4	≤0.25–64	90
	Ceftazidime	≤0.12	0.25	≤0.12–0.5	100
Citrobacter freundii (20)	Ro 09-1227	0.25	16	≤0.06–>32	80
	Cefotaxime	≤0.25	>32	≤0.25–>32	70
	Ceftriaxone	≤0.25	>32	≤0.25–>32	65
	Cefoperazone	2	64	≤0.25–>64	65
	Ceftazidime	1	>16	≤0.12–>16	65
Enterobacter aerogenes (20)	Ro 09-1227	0.12	2	≤0.06–>32	95
	Cefotaxime	≤0.25	16	≤0.25–32	70
	Ceftriaxone	≤0.25	16	≤0.25–32	70
	Cefoperazone	≤0.25	16	≤0.25–32	95
	Ceftazidime	0.25	>16	≤0.12–>16	70
Enterobacter agglomerans (10)	Ro 09-1227	≤0.06	2	≤0.06–2	100
	Cefotaxime	≤0.25	≤0.25	≤0.25	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25	100
	Cefoperazone	≤0.25	2	≤0.25–8	100
	Ceftazidime	≤0.12	1	≤0.12–1	100
Enterobacter cloacae (20)	Ro 09-1227	1	16	≤0.06–16	85
	Cefotaxime	0.5	>32	≤0.25–>32	80
	Ceftriaxone	0.5	>32	≤0.25–>32	80
	Cefoperazone	≤0.25	32	≤0.25–64	80
	Ceftazidime	0.25	>16	≤0.12–>16	80
Escherichia coli (20)	Ro 09-1227	0.12	0.5	≤0.06–2	100
	Cefotaxime	≤0.25	≤0.25	≤0.25–2	100
	Ceftriaxone	≤0.25	≤0.25	≤0.12–1	100
	Cefoperazone	≤0.25	1	≤0.25–8	100
	Ceftazidime	≤0.12	0.25	≤0.12–8	100
Klebsiella oxytoca (10)	Ro 09-1227	≤0.06	0.12	≤0.06–0.12	100
	Cefotaxime	≤0.25	4	≤0.25–4	100
	Ceftriaxone	≤0.25	>32	≤0.25–>32	82
	Cefoperazone	0.5	>64	≤0.25–>64	80
	Ceftazidime	≤0.12	>16	≤0.12–>16	91
Klebsiella pneumoniae (20)	Ro 09-1227	≤0.06	0.5	≤0.06–1	100
	Cefotaxime	≤0.25	≤0.25	≤0.25	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25	100
	Cefoperazone	≤0.25	1	≤0.25–4	100
	Ceftazidime	≤0.12	0.25	≤0.12–0.25	100
Morganella morganii (10)	Ro 09-1227	1	2	0.25–4	100
	Cefotaxime	≤0.25	≤0.25	≤0.25–4	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25–1	100
	Cefoperazone	1	4	0.5–32	100
	Ceftazidime	≤0.12	0.25	≤0.12–8	100
Proteus mirabilis (20)	Ro 09-1227	≤0.06	≤0.06	≤0.06	100
	Cefotaxime	≤0.25	≤0.25	≤0.25	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25	100
	Cefoperazone	0.5	1	≤0.25–1	100
	Ceftazidime	≤0.12	≤0.12	≤0.12	100
Proteus vulgaris (10)	Ro 09-1227	≤0.06	0.25	≤0.06–16	90
	Cefotaxime	≤0.25	≤0.25	≤0.25–0.5	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25	100
	Cefoperazone	0.5	1	0.5–16	100
	Ceftazidime	≤0.12	≤0.12	≤0.12	100
Providencia rettgeri (10)	Ro 09-1227	≤0.06	2	≤0.06–4	100
	Cefotaxime	≤0.25	≤0.25	≤0.25–2	100

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TABLE 1—*Continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)			% Susceptible[a]
		50%	90%	Range	
	Ceftriaxone	≤0.25	≤0.25	≤0.25	100
	Cefoperazone	≤0.25	4	≤0.25–16	100
	Ceftazidime	≤0.12	2	≤0.12–2	100
Providencia stuartii (10)	Ro 09-1227	0.12	2	≤0.06–4	100
	Cefotaxime	0.5	8	≤0.25–16	90
	Ceftriaxone	≤0.25	8	≤0.25–2	100
	Cefoperazone	0.5	4	≤0.25–16	100
	Ceftazidime	1	8	≤0.12–8	100
Salmonella enteritidis (10)	Ro 09-1227	≤0.06	≤0.06	≤0.06–0.12	100
	Cefotaxime	≤0.25	≤0.25	≤0.25	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25	100
	Cefoperazone	≤0.25	0.5	≤0.25–4	100
	Ceftazidime	0.25	0.5	≤0.12–0.5	100
Serratia marcescens (20)	Ro 09-1227	0.25	1	0.12–16	95
	Cefotaxime	0.5	4	≤0.25–32	95
	Ceftriaxone	0.5	2	≤0.25–16	95
	Cefoperazone	1	16	≤0.25–32	95
	Ceftazidime	≤0.12	0.5	≤0.12–2	100
Shigella spp. (10)	Ro 09-1227	0.12	1	≤0.06–2	100
	Cefotaxime	≤0.25	≤0.25	≤0.25	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25	100
	Cefoperazone	1	1	≤0.25–1	100
	Ceftazidime	≤0.12	0.25	≤0.12–0.25	100
Yersinia enterocolitica (10)	Ro 09-1227	0.12	0.25	≤0.06–0.5	100
	Cefotaxime	≤0.25	≤0.25	≤0.25–2	100
	Ceftriaxone	≤0.25	≤0.25	≤0.25–0.5	100
	Cefoperazone	2	4	0.5–4	100
	Ceftazidime	≤0.12	0.5	≤0.12–1	100
Acinetobacter spp. (10)	Ro 09-1227	0.5	8	0.12–16	90
	Cefotaxime	8	32	≤0.25–>32	50
	Ceftriaxone	8	16	≤0.25–>32	60
	Cefoperazone	32	>64	1–>64	15
	Ceftazidime	4	8	0.5–>16	90
Pseudomonas aeruginosa (30)	Ro 09-1227	0.5	4	≤0.06–32	90
	Cefotaxime	16	>32	4–>32	33
	Ceftriaxone	16	>32	2–>32	47
	Cefoperazone	4	8	≤0.25–>64	93
	Ceftazidime	2	4	0.5–>16	93
Xanthomonas maltophilia (10)	Ro 09-1227	0.12	2	≤0.06–32	90
	Cefotaxime	>32	>32	1–>32	20
	Ceftriaxone	>32	>32	8–>32	20
	Cefoperazone	4	16	1–16	100
	Ceftazidime	4	>16	1–>16	60
Haemophilus influenzae (58)[b]	Ro 09-1227	≤0.12	0.5	≤0.12–0.5	100
	Cefotaxime	0.06	0.25	≤0.015–0.5	100
	Ampicillin	1	>32	≤0.12–>32	35
	Chloramphenicol	0.25	0.5	≤0.12–>8	97
Moraxella catarrhalis (20)[c]	Ro 09-1227	0.25	0.5	≤0.12–1	100
	Cefotaxime	0.06	0.25	≤0.015–0.5	100
Neisseria gonorrhoeae (30)[d]	Ro 09-1227	0.008	0.015	≤0.001–0.25	100
	Cefotaxime	0.004	0.015	≤0.001–0.12	100
Bacteroides fragilis (26)[e]	Ro 09-1227	32	>32	16–>32	0
Staphylococcus aureus, oxacillin-susceptible (40)	Ro 09-1227	2	4	1–8	100
	Cefotaxime	1	2	0.5–4	100
	Ceftriaxone	2	2	0.5–2	100
	Cefoperazone	2	2	1–8	100

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TABLE 1—*Continued*

Organism (no. tested)	Antimicrobial agent	MIC (μg/ml)			% Susceptible[a]
		50%	90%	Range	
	Ceftazidime	4	8	4–8	100
Staphylococcus aureus, oxacillin-resistant (10)	Ro 09-1227	>32	>32	4–>32	10
	Cefotaxime	>32	>32	8–>32	10
	Ceftriaxone	>32	>32	16–>32	0
	Cefoperazone	>64	>64	8–>64	10
	Ceftazidime	>16	>16	>16	0
Staphylococcus epidermidis (20)	Ro 09-1227	2	>32	0.5–>32	75
	Cefotaxime	1	16	≤0.25–>32	85
	Ceftriaxone	2	16	≤0.25–>32	75
	Cefoperazone	1	4	0.5–8	100
	Ceftazidime	4	>16	2–>16	60
Staphylococcus haemolyticus (10)	Ro 09-1227	>32	>32	2–>32	30
	Cefotaxime	>32	>32	0.5–>32	40
	Ceftriaxone	>32	>32	1–>32	40
	Cefoperazone	64	>64	2–>64	40
	Ceftazidime	>16	>16	4–>16	30
Coagulase-negative staphylococci (20)[f]	Ro 09-1227	4	8	1–32	90
	Cefotaxime	1	2	≤0.25–4	100
	Ceftriaxone	2	4	0.5–8	100
	Cefoperazone	1	4	≤0.25–4	100
	Ceftazidime	8	16	2–16	85
Enterococcus faecalis (21)	Ro 09-1227	>32	>32	8–>32	5
	Cefotaxime	>32	>32	2–>32	17
	Ceftriaxone	>32	>32	8–>32	13
	Cefoperazone	32	>64	8–>64	45
	Ceftazidime	>16	>16	>16	0
Enterococcus faecium (10)	Ro 09-1227	>32	>32	>32	0
	Cefotaxime	>32	>32	>32	0
	Ceftriaxone	>32	>32	>32	0
	Cefoperazone	>64	>64	64–>64	0
	Ceftazidime	>16	>16	>16	0
Streptococcus group A (20)	Ro 09-1227	0.25	0.25	≤0.12–0.5	100
	Cefotaxime	≤0.015	≤0.015	≤0.015	100
Streptococcus group B (20)	Ro 09-1227	0.5	0.5	0.25–2	100
	Cefotaxime	0.03	0.03	≤0.015–0.06	100
Streptococcus groups C, F, and G (14)	Ro 09-1227	0.5	2	0.25–2	100
	Cefotaxime	0.06	0.25	≤0.015–2	100
Streptococcus pneumoniae (30)[g]	Ro 09-1227	0.25	4	≤0.12–>16	97
	Cefotaxime	≤0.015	0.25	≤0.015–2	100
Bacillus cereus (10)	Ro 09-1227	16	32	4–>32	30
	Cefotaxime	16	32	16–32	0
	Ceftriaxone	8	16	8–16	50
	Ceftazidime	>16	>16	>16	0

[a] Susceptibility according to the National Committee for Clinical Laboratory Standards criteria, document M7-A2 (8). The Ro 09-1227 criterion was ≤8 μg/ml, e.g., comparable with that for ceftriaxone or ceftazidime.
[b] Includes β-lactamase-producing isolates (22 strains) and 16 strains that were β-lactamase negative and ampicillin resistant.
[c] Includes 16 strains producing either BRO-1 or BRO-2 enzymes.
[d] Includes 20 isolates resistant to penicillin by penicillinase production or chromosomal mechanisms.
[e] All strains were β-lactamase positive.
[f] Includes seven species other than those listed in this table.
[g] Includes 10 strains with penicillin MICs of ≥0.1 μg/ml.

TABLE 2. In vitro activity of Ro 09-1227 tested against *E. coli* strains producing plasmid-mediated β-lactamases

β-Lactamase	MIC (μg/ml) of:			
	Ro 09-1227	Ceftazidime	Cefpirome	Cefuroxime
CAZ-2	2	>16	8	16
SHV-1	0.12	1	0.5	8
SHV-2	1	>16	>16	>32
SHV-3	1	>16	>16	>32
SHV-4	32	>16	16	>32
SHV-5	>32	>16	16	>32
TEM-1	≤0.06	0.25	≤0.12	8
TEM-2	0.12	0.5	0.5	8
TEM-3	1	>16	8	>32
TEM-4	1	>16	16	>32
TEM-5	4	>16	2	>32
TEM-6	8	>16	8	16
TEM-7	1	>16	8	8
TEM-9	8	>16	8	8

trays to determine Ro 09-1227 potency changes in a low-iron state (3, 4, 7, 10–12). The haemophilus test medium was used for MIC testing for *Haemophilus influenzae, M. catarrhalis,* pneumococcus, and fastidious streptococci (8). Anaerobes were processed by the National Committee for Clinical Laboratory Standards M11-A2 procedure (9) on Wilkin-Chalgren agar (Difco Laboratories, Detroit, Mich.).

Table 1 summarizes all Ro 09-1227 MIC results and those for the four comparison cephalosporins. Ro 09-1227 was generally less active than cefotaxime, ceftriaxone, and ceftazidime against members of the family *Enterobacteriaceae* (MIC_{90} range, ≤0.06 to 16 μg/ml), but it remained more active against the ceftazidime-resistant strains of *Citrobacter freundii, Enterobacter aerogenes,* and *Enterobacter cloacae*. Ro 09-1227 was also most effective on type IVc β-lactamase-producing isolates of *Klebsiella oxytoca* that rendered nearly all other cephems inactive. Many *Acinetobacter* spp. (MIC for 50% of strains tested [MIC_{50}], 0.5 μg/ml), *Pseudomonas aeruginosa* (MIC_{50}, 0.5 μg/ml), and *Xanthomonas maltophilia* (MIC_{50}, 0.12 μg/ml) strains were more susceptible to Ro 09-1227 than to ceftazidime. Fastidious gram-negative respiratory tract pathogens (*H. influenzae* and *M. catarrhalis*) were very susceptible to Ro 09-1227, e.g., all MICs were ≤1 μg/ml. *Bacteroides fragilis* strains were not susceptible to this catechol-substituted cephalosporin (MIC_{90}, >32 μg/ml).

Ro 09-1227 activity against gram-positive species was superior to that of ceftazidime but less than that demonstrated by cefoperazone, cefotaxime, or ceftriaxone (Table 1). Oxacillin-resistant staphylococci and enterococci were Ro 09-1227 resistant. Ro 09-1227 inhibited nearly all streptococci tested (MIC_{90}, 0.25 to 4 μg/ml) but was 8- to 16-fold less active than cefotaxime. Penicillin-resistant pneumococci had the only Ro 09-1227-resistant MICs (>16 μg/ml).

A total of 212 strains were also tested in the presence of elevated medium iron content (data not shown). Ro 09-1227 MICs for the enteric bacilli (average + 4-fold), nonenteric gram-negative bacilli (average + 6-fold), and *Staphylococcus* spp. (average + 1.5-fold) were elevated. Other nonstaphylococcal gram-positive species, such as the enterococci, exhibited no significant adverse decrease in the Ro 09-1227 activity produced by medium iron. The 2,2′-dipyridyl iron-depleted-broth MIC tests showed an average twofold increase in Ro 09-1227 potency for 10 organisms from five species.

Among 18 members of the family *Enterobacteriaceae* found to be resistant to ceftazidime (MICs, ≥32 μg/ml), 83.3% had Ro 09-1227 MICs of ≤16 μg/ml. These organisms were *C. freundii* (eight strains), *E. aerogenes* (six strains) and *E. cloacae* (four strains), all having a stably derepressed type I β-lactamase. Table 2 lists the Ro 09-1227 MICs for 14 *Escherichia coli* strains having plasmid-mediated β-lactamases. Ro 09-1227 had MICs of ≤8 μg/ml against all strains except those producing SHV-4 and -5 enzymes (MICs, ≥32 μg/ml). This catechol cephalosporin appears more stable to several extended-spectrum enzymes by MIC criteria than ceftazidime, cefpirome, or cefuroxime. However, cefpirome had moderate activity (MICs, 16 μg/ml) against the two cited strains with the highest Ro 09-1227 MICs.

Compound Ro 09-1227 is a 7-catechol-substituted cephalosporin having a structure minimally modified at the C-3 pyrimidine position compared with Ro 41-1879 and Ro 09-1428 (1, 5). These compounds possess activities similar or superior to currently available cephalosporins and investigational compounds such as cefpirome (6). Because of the catechol group, the Ro 09-1227 potency is influenced by medium iron content and potential metabolic modifications by in vivo O-methylation. Although the in vitro potency of these drugs appears promising, the in vivo infection model and toxicology investigations may have more importance in the selection of a potential drug candidate for advanced clinical investigations.

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REFERENCES

1. **Arisawa, M., Y. Sekine, S. Shimizu, H. Takano, Angehrn, and R. L. Then.** 1991. In vitro and in vivo evaluation of Ro 09-1428, a new parenteral cephalosporin with high antipseudomonal activity. Antimicrob. Agents Chemother. **35**:653–659.
2. **Bullen, J. J.** 1981. The significance of iron in infection. Rev. Infect. Dis. **3**:1127–1138.
3. **Curtis, N. A. C., R. L. Eisenstadt, S. J. East, R. J. Cornford, L. A. Walker, and A. J. White.** 1988. Iron-regulated outer membrane proteins of *Escherichia coli* K-12 and mechanism of action of catechol-substituted cephalosporins. Antimicrob. Agents Chemother. **32**:1879–1886.
4. **Erwin, M. E., R. N. Jones, M. S. Barrett, B. M. Briggs, and D. J. Johnson.** 1991. In vitro evaluation of GR69153, a novel catechol-substituted cephalosporin. Antimicrob. Agents Chemother. **35**:929–937.
5. **Jones, R. N.** Antimicrobial activity of Ro 41-1879, a new parenteral cephalosporin. J. Chemother., in press.
6. **Jones, R. N., C. Thornsberry, and A. L. Barry.** 1984. In vitro evaluation of HR810, a new wide-spectrum aminothiazolyl α-methoxyimino cephalosporin. Antimicrob. Agents Chemother. **25**:710–718.
7. **Mochizuki, H., H. Yamada, Y. Oikawa, K. Murakami, J. Ishiguro, H. Kosuzume, N. Aizawa, and E. Mochida.** 1988. Bactericidal activity of M14659 enhanced in low-iron environments. Antimicrob. Agents Chemother. **32**:1648–1654.
8. **National Committee for Clinical Laboratory Standards.** 1990. Approved standard M7-A2. Standard methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. National Committee for Clinical Laboratory Standards, Villanova, Pa.
9. **National Committee for Clinical Laboratory Standards.** 1990. Approved standard M11-A2. Methods for antimicrobial suscep-

tibility testing of anaerobic bacteria. National Committee for Clinical Laboratory Standards, Villanova, Pa.
10. **Silley, P., J. W. Griffiths, D. Monsey, and A. M. Harris.** 1990. Mode of action of GR69153, a novel catechol-substituted cephalosporin, and its interaction with the *tonB*-dependent iron transport system. Antimicrob. Agents Chemother. **34**:1806–1808.
11. **Simpson, C. H., R. D. Feld, and P. K. Jaynes.** 1982. Centrifugal analysis for serum iron and iron-binding capacity using the IL Multistat III Analyzer. Clin. Biochem. **15**:313–314.
12. **Watanabe, N. A., T. Nagasu, K. Katsu, and K. Kitoh.** 1987. E-0702, a new cephalosporin, is incorporated into *Escherichia coli* cells via the *tonB*-dependent iron transport system. Antimicrob. Agents Chemother. **31**:497–504.