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In Vitro Activity of BMY-28142 Against Pediatric Pathogens, Including Isolates from Cystic Fibrosis Sputum

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The antibacterial activity of BMY-28142, a new aminothiazole cephalosporin, was measured by standardized broth microdilution and agar dilution methods against 450 gram-positive and gram-negative bacteria isolated from pediatric infections, including acute pulmonary exacerbations of cystic fibrosis. BMY-28142 activity was compared with that of aminoglycosides, β-lactams, chloramphenicol, trimethoprim-sulfamethoxazole, vancomycin, and clindamycin. The activity of BMY-28142 in combination with other antimicrobial agents against *Pseudomonas aeruginosa* was also determined. Furthermore, the effects of inoculum and pH on BMY-28142 activity were evaluated. BMY-21842 was active against most of the gram-positive and gram-negative isolates, with the exception of methicillin-resistant *Staphylococcus aureus* and *Pseudomonas cepacia*. The combination of BMY-28142 with tobramycin was often synergistic, and combinations of BMY-28142 with either polymyxin B or imipenem were usually antagonistic. BMY-28142 antibacterial activity could be adversely affected at extremes of medium pH and by high inoculum densities.

BMY-28142 (7-[alpha-(2-aminothiazol-4-yl)-alpha-(Z)-methoximinoacetamido]-3-(1-methylpyrrolidino)-methyl-3-cephem-4-carboxylate), an aminothiazole cephalosporin resulting from the 3-side chain methyl substitution with *n*-methylpyrrolidine, has demonstrated in vitro antibacterial activity against many gram-positive and gram-negative bacteria, including *Pseudomonas aeruginosa* and *Staphylococcus aureus* (J. Okumura, S. Aburaki, H. Kamachi, Y. Narita, T. Naito, and H. Kawaguchi, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 23rd, Las Vegas, Nev., abstr. no. 576, 1983; M. Bies, R. E. Buck, T. A. Pursiano, D. R. Chisholm, Y. H. Tsai, M. Misiek, K. E. Price, and F. Leitner, 23rd ICAAC, abstr. no. 577). In mice experimentally infected intraperitoneally with *Staphylococcus aureus* or *P. aeruginosa*, BMY-21842 demonstrated greater therapeutic efficacy than did cefotaxime, ceftazidime, or moxalactam, and was also more successful than these agents in treating experimental murine *Escherichia coli* and *Streptococcus pneumoniae* meningitis (Bristol-Myers Co., unpublished data). Preliminary evaluation in adult mice has revealed a close pharmacokinetic similarity between BMY-28142 and ceftazidime, with respective whole blood concentrations of 22.2 and 24.9 μg/ml 10 min after single 20-mg/kg intramuscular injections (Bristol-Myers Co., unpublished data). A peak blood concentration of 40 μg/ml, occurring 1 h after BMY-28142 administration, has been observed in suckling rats (Y. H. Tsai, M. Bies, and D. P. Henry, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 24th, Washington, D.C., abstr. no. 749).

We evaluated the antibacterial activity of BMY-28142, alone and in combination with other antimicrobial agents, against bacteria isolated from pediatric infections, including acute exacerbations of chronic cystic fibrosis pneumonitis.

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MATERIALS AND METHODS

BMY-28142 was provided by Bristol-Myers Co., Syracuse, N.Y. Stock solutions of the antibacterial agents were prepared in the recommended manner and either used immediately or stored at −70°C for no longer than 6 weeks.

A total of 450 bacterial isolates were obtained from patients at Oklahoma Children's Memorial Hospital and Oklahoma Memorial Hospital, Oklahoma City; Montreal Children's Hospital, Montreal, Quebec, Canada; Hospital for Sick Children, Toronto, Ontario, Canada (P. Fleming); Rainbow Babies' and Children's Hospital, Cleveland, Ohio (J. Klinger); St. Christopher's Hospital for Children, Philadelphia, Pa. (P. Gilligan); University of Texas Medical Branch, Galveston, Tex. (M. Kelly); University of South Alabama, Mobile, Ala. (S. Chartrand); Tulane University, New Orleans, La. (R. Daum).

Each isolate was identified by routine and standard laboratory procedures (8). Many isolates were resistant to one or more antimicrobial agents, proven by biochemical analysis (e.g., β-lactamase production, presence of chloramphenicol acetyltransferase, etc.) and by previous antibacterial susceptibility testing.

MICs for all isolates except *Haemophilus influenzae* were determined by microdilution methods with Mueller-Hinton broth, adjusted to pH 7.2 to 7.4, and supplemented with 75 mg of calcium and 25 mg of magnesium per liter for gram-negative bacteria; 2% sodium chloride for *Staphylococcus aureus* oxacillin MIC determinations; 5% defibrinated sheep erythrocytes for *Streptococcus pneumoniae*.

Plates were prepared and inoculated with the MIC-2000 (Dynatech Laboratories, Inc., Alexandria, Va.); those plates not immediately inoculated were stored at −70°C for no longer than 3 weeks.

Each well was inoculated with 2×10^5 to 6×10^5 CFU/ml. Plates were incubated for 18 to 24 h at 35°C in ambient air (except for *Streptococcus pneumoniae*, whose incubation atmosphere contained 5% CO_2).

The MIC was defined as the lowest concentration of drug preventing visible growth; for *Streptococcus pneumoniae*, the endpoint was defined as the lowest concentration of drug preventing bacterial reduction of the sheep erythrocyte hemoglobin, as determined by the method of Tarpay et al. (15).

MIC determinations for each set of isolates were controlled by inclusion of reference strains *E. coli* ATCC 25922 or *Staphylococcus aureus* ATCC 29213. Results were within one twofold dilution of established MIC values in all cases.

The MBC was defined as the lowest concentration of drug preventing growth of more than five bacterial colonies after subculture of 50-μl samples apportioned from the plate microtiter wells onto antibiotic-free Columbia sheep blood agar and overnight incubation at 35°C (>99.9% inoculum reduction). Ten percent of the isolates, selected at random, were subcultured in duplicate for this determination.

The MIC of each antibiotic against *H. influenzae* was determined by standard agar dilution methodology, using Mueller-Hinton agar plus 1% supplement C (Difco Laboratories, Detroit, Mich.). A Steers replicator was used to deliver 4×10^4 to 8×10^4 CFU of a log-phase culture to the agar surface. Each agar plate was incubated for 18 to 24 h at 35°C in 5% CO_2. The MIC was defined as the lowest concentration of drug inhibiting visible growth; a slight haze or single colony was disregarded.

Antibacterial activity of BMY-28142 in combination with each of nine antimicrobial agents was determined by the two-dimensional checkerboard broth microdilution method. Fractional inhibitory concentrations (FICs), as described by Elion et al. (5), were determined against 10 *P. aeruginosa* strains (including four tobramycin-resistant isolates) after 18 to 24 h of incubation in cation-supplemented Mueller-Hinton broth at 35°C in ambient air. Antibiotic combinations producing an FIC index ≤0.5 were defined as synergistic; >2 was considered antagonistic. Combinations were considered indifferent if the FIC index was >0.5 but ≤2.

Eight strains each of *P. aeruginosa*, *E. coli*, and *Staphylococcus aureus* were used to study the effect of inoculum density on BMY-28142 antibacterial activity. Broth microtiter plates containing serial 2-fold dilutions of BMY-28142 were inoculated with 10-fold dilutions of each isolate at inoculum densities ranging from 10^3 to 10^7 CFU/ml (verified by colony count) and were incubated for 18 to 24 h at 35°C in ambient air. For 10^7-CFU/ml densities, duplicate plates were inoculated. One plate was stationary during incubation, and the second plate was rotated at 220 ± 10 rotations per min in an Autobac-1 incubator (General Diagnostics, Morris Plains, N.J.). MICs were determined as previously described.

The same 24 strains at 10^5 CFU/ml inoculum were used to study the effect of various medium pHs on BMY-28142 activity. Bacto-Peptone broth (2%) was adjusted to pH 5, 6, 7, 8, or 9 by the addition of K_2HPO_4 or KH_2PO_4. Twofold serial dilutions of BMY-28142 were prepared in the various broths and added to microtiter plates. Inoculation and incubation were performed in the manner previously described. MICs were determined as described above.

RESULTS

BMY-28142 was the most active antimicrobial agent tested against the *Enterobacteriaceae* (Table 1). Although less active than ceftazidime against tobramycin-susceptible *P. aeruginosa*, BMY-28142 had greater antibacterial activity against tobramycin-resistant strains (MIC ≥4). BMY-28142 was generally ineffective against *Pseudomonas cepacia*.

BMY-28142 demonstrated antibacterial activity comparable to that of moxalactam against *H. influenzae* (type b and nontypable strains), although cefotaxime was more active. Neither β-lactamase nor chloramphenicol acetyltransferase production (present in four of the seven β-lactamase-positive strains tested) adversely influenced the anti-*Haemophilus* activity of BMY-28142.

The antibacterial activites of BMY-28142 and penicillin G were equivalent against penicillin-susceptible *Streptococcus pneumoniae*, although BMY-28142 was more active than penicillin G against relatively penicillin-resistant *Streptococcus pneumoniae* (MIC ≥0.12). Imipenem was most active against both susceptible and relatively resistant strains. BMY-28142 showed fair antibacterial activity against methicillin-susceptible *Staphylococcus aureus* but was not effective against methicillin-resistant strains (MIC ≥2).

The MBC was within two dilutions of the MIC for 89% of those strains tested.

BMY-28142 in combination with tobramycin demonstrated antimicrobial synergy against 5 of the 10 *P. aeruginosa* strains tested; more importantly, the combination was synergistic against three of the four tobramycin-resistant strains (Table 2). The combination of BMY-28142 and polymyxin B was antagonistic against seven isolates, and the combination of BMY-28142 with imipenem was antagonistic against nine. The anti-pseudomonal activity of combinations of BMY-28142 with the remaining antimicrobial agents tested was largely indifferent.

An inoculum density of 10^6 CFU/ml or greater often adversely affected the activity of BMY-28142; on average, there was an eightfold increase in the MIC. This increase in the MIC with higher inoculum density was observed after stationary and rotational incubation.

Loss of antibacterial activity, reflected as a marked increase in mean MIC of BMY-28142 for the specific isolate groups, was seen at extremes of medium pH. For the eight strains of *E. coli* tested, at both medium pH 5 and pH 9, mean MIC was increased nine times that of the mean MIC at medium pH 7. In a similar manner, although only observed at medium pH 5, the mean MIC of BMY-28142 for the eight *P. aeruginosa* strains increased 1.5 times that of the mean MIC at medium pH 7. Antibacterial activity against *Staphylococcus aureus*, however, was not influenced by medium pH, as the mean MICs remained constant through the range of medium pHs.

DISCUSSION

Our evaluation has shown that BMY-28142 has in vitro antibacterial activity against many gram-positive and gram-negative bacteria, including *H. influenzae*, *Streptococcus pneumoniae*, and the *Enterobacteriaceae*. Ceftazidime, an aminothiazole cephalosporin structurally related to BMY-28142, has successfully treated human infections such as pneumonia (10), pyelonephritis (6), and septicemia (9). Based on its antibacterial activities and other pharmacological properties, BMY-28142 may have similar clinical usefulness.

Bronchopulmonary infections due to *Pseudomonas* spp. in patients with cystic fibrosis are extremely difficult to treat. In view of the favorable preliminary experience with ceftazidime in this condition (4, 7), the activity of BMY-28142 was examined in vitro versus *P. aeruginosa* and *P. cepacia*. BMY-28142 demonstrated an overall anti-*P. aeruginosa* activity (against both tobramycin-susceptible and tobramycin-resistant isolates) comparable to that of ceftazidime, but it was relatively inactive against *P. cepacia*. Furthermore,

TABLE 1. In vitro activity of BMY-28142 and comparison antimicrobial agents

Strain (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
Pseudomonas aeruginosa (50)	BMY-28142	2	8	0.5–16
	Amikacin	8	16	1–32
	Tobramycin	1	2	≤0.12–2
	Ticarcillin	16	32	0.25–64
	Azlocillin	8	32	0.5–128
	Piperacillin	4	8	0.25–16
	Ceftazidime	1	2	0.5–8
	Cefotaxime	16	>32	0.5–>32
Pseudomonas aeruginosa tobramycin-resistant (20)	BMY-28142	8	16	2–32
	Amikacin	32	64	2–64
	Tobramycin	4	128	4–>128
	Ticarcillin	32	>128	0.5–>128
	Azlocillin	16	128	1–>128
	Piperacillin	8	32	0.5–128
	Ceftazidime	2	32	0.5–32
	Cefotaxime	16	>32	4–>32
Pseudomonas cepacia (21)	BMY-28142	32	>64	2–>64
	Ceftazidime	2	4	2–16
	Chloramphenicol	32	256	8–256
	Trimethoprim-sulfamethoxazole	4–80	>8–>160	0.5–>8/10–>160
Escherichia coli (29)	BMY	≤0.03	0.12	≤0.03–2
	Ampicillin	128	>128	1–>128
	Ticarcillin	2	>128	0.5–>128
	Chloramphenicol	4	8	1–128
	Tobramycin	0.5	1	0.25–128
	Trimethoprim-sulfamethoxazole	0.12–2.5	2–40	0.015–>8/0.03–>160
Shigella spp. (30)	BMY-28142	≤0.03	≤0.03	≤0.03–0.06
	Ampicillin	1	>128	0.25–>128
	Ticarcillin	1	>128	0.25–>128
	Chloramphenicol	2	4	0.25–4
	Tobramycin	1	1	0.5–4
	Trimethoprim-sulfamethoxazole	0.06–1.2	0.12–2.5	0.015–>8/0.3–>160
Salmonella spp. (21)	BMY-28142	≤0.03	0.06	≤0.03–0.06
	Ampicillin	4	>128	2–>128
	Ticarcillin	8	>128	4–>128
	Chloramphenicol	4	4	4
	Tobramycin	1	2	1–4
	Trimethoprim-sulfamethoxazole	0.25–5	0.5–10	0.12–1/2.5–20
Citrobacter spp. (11)	BMY-28142	0.06	0.12	≤0.03–0.5
	Ampicillin	64	>128	16–>128
	Ticarcillin	>128	>128	64–>128
	Chloramphenicol	4	16	1–>128
	Tobramycin	1	64	0.5–64
	Trimethoprim-sulfamethoxazole	0.5–10	>8–>160	0.12–>8/2.5–>160
Yersinia enterocolitica (30)	BMY-28142	≤0.03	≤0.03	≤0.03–4
	Ceftazidime	≤0.12	0.25	≤0.12–0.25
	Ticarcillin	128	>128	128–>128
	Chloramphenicol	2	4	1–4
	Tobramycin	0.5	0.5	0.25–0.5
	Trimethoprim-sulfamethoxazole	0.03–0.6	0.06–1.2	0.03–0.06/0.6–1.2
Klebsiella spp. (37)	BMY-28142	0.06	0.25	≤0.03–0.25
	Ampicillin	32	>128	4–>128
	Ticarcillin	128	>128	8–>128
	Chloramphenicol	2	4	0.5–>128
	Tobramycin	0.5	0.5	0.25–64
	Trimethoprim-sulfamethoxazole	0.12–2.5	0.5–10	0.06–>8/1.2–>160
Serratia marcescens (29)	BMY-28142	0.12	0.5	0.06–1
	Ampicillin	64	>128	8–>128
	Ticarcillin	4	>128	2–>128

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TABLE 1.—*Continued*

Strain (no. of isolates)	Antimicrobial agent	MIC (μg/ml)		
		50%	90%	Range
	Chloramphenicol	16	16	4–>128
	Tobramycin	2	128	0.5–>128
	Timethoprim-sulfamethoxazole	0.25–5	>8–>160	0.12–>8/2.5–>160
Enterobacter cloacae (14)	BMY-28142	≤0.03	0.25	≤0.03–8
	Ampicillin	16	>128	4–>128
	Ticarcillin	1	>128	0.5–>128
	Chloramphenicol	4	8	2–>128
	Tobramycin	0.5	1	0.25–16
	Trimethoprim-sulfamethoxazole	0.12–2.5	0.25–5	0.06–0.5/1.2–10
Enterobacter aerogenes (10)	BMY-28142	0.06	0.5	≤0.03–0.5
	Ampicillin	16	>128	2–>128
	Ticarcillin	1	64	0.5–>128
	Chloramphenicol	4	64	2–128
	Tobramycin	0.25	0.5	0.25–128
	Trimethoprim-sulfamethoxazole	0.12–2.5	>8–>160	0.015–>8–0.3–>160
Haemophilus influenzae type b, β-lactamase negative (26)	BMY-28142	0.03	0.03	0.015–0.06
	Ampicillin	<0.12	0.25	≤0.12–0.5
	Chloramphenicol	0.5	0.5	0.5
	Moxalactam	0.03	0.03	≤0.015–0.06
	Cefotaxime	≤0.008	≤0.008	≤0.008–0.03
Haemophilus influenzae type b, β-lactamase positive (7)	BMY-28142	0.03		0.015–0.03
	Ampicillin	4		4–8
	Chloramphenicol	8		0.5–32
	Moxalactam	0.03		≤0.015–0.03
	Cefotaxime	≤0.008		≤0.008
Haemophilus influenzae nontypable, β-lactamase negative (22)	BMY-28142	0.06	0.25	≤0.008–0.5
	Ampicillin	0.5	1	≤0.03–2
	Chloramphenicol	0.5	1	0.12–1
	Moxalactam	0.06	0.25	≤0.008–0.5
	Cefotaxime	0.03	0.06	≤0.004–0.06
	Trimethoprim-sulfamethoxazole	0.03–0.6	0.06–1.2	≤0.008–0.25/≤0.15–5
Haemophilus influenzae nontypable, β-lactamase positive (10)	BMY-28142	0.06	0.25	0.03–0.25
	Ampicillin	8	16	8–16
	Chloramphenicol	1	1	0.5–1
	Moxalactam	0.12	0.5	0.03–0.5
	Cefotaxime	0.015	0.06	≤0.008–0.12
	Trimethoprim-sulfamethoxazole	0.03–0.6	0.06–1.2	0.015–0.06/0.3–1.2
Staphylococcus aureus (38)	BMY-28142	4	8	1–16
	Oxacillin	0.25	1	0.12–1
	Cephalothin	0.25	0.5	0.25–1
	Vancomycin	0.5	1	0.25–1
	Clindamycin	0.06	0.06	≤0.03–0.25
	Cefotaxime	2	4	1–4
Staphylococcus aureus, methicillin-resistant (15)	BMY-28142	32	128	2–>128
	Oxacillin	64	>64	2–>64
	Vancomycin	1	2	0.25–2
Streptococcus pneumoniae (24)	BMY-28142	≤0.03	0.06	≤0.03–0.06
	Penicillin	≤0.03	0.06	≤0.03–0.06
	Cephalothin	0.12	0.12	≤0.06–0.12
	Cefotaxime	≤0.03	≤0.03	≤0.03–0.06
	Moxalactam	≤0.12	0.25	≤0.12–0.5
	Imipenem	≤0.008	≤0.008	≤0.008–0.015
Streptococcus pneumoniae, relatively penicillin resistant (6)	BMY-28142	0.25		0.06–0.5
	Penicillin	0.5		0.12–1
	Cephalothin	2		0.25–2
	Cefotaxime	0.12		≤0.03–0.5
	Moxalactam	2		0.5–4
	Imipenem	0.015		≤0.008–0.03

TABLE 2. In vitro anti-*P. aeruginosa* activity of BMY-28142 in combination with other antimicrobial agents

Antimicrobial agent combined with BMY-28142	No. of isolates (of 10) showing [a]:		
	Synergy	Indifference	Antagonism
Piperacillin	0	7	3
Aztreonam	4	5	1
Tobramycin	5[b]	4	1
Polymyxin B	0	3	7
Ciprofloxacin	2	7	1
Enoxacin	0	7	3
Rifampin	0	8	2
Ceftazidime	2	8	0
Imipenem	0	1	9

[a] Descriptions of activity are defined in the text.
[b] Includes three of four tobramycin-resistant strains.

the combination of BMY-28142 and tobramycin was synergistic against the majority of tobramycin-resistant *P. aeruginosa* isolates tested. These results suggest that BMY-28142, either alone or in combination with an aminoglycoside, may be effective in treating acute cystic fibrosis pneumonitis.

As described for other third-generation cephalosporins (11), BMY-28142 was less active against *Staphylococcus aureus* than were the penicillinase-resistant penicillins and first-generation cephalosporins, and it was inactive against methicillin-resistant *Staphylococcus aureus*.

BMY-28142 was generally bactericidal, as the MBC was within two dilutions of the MIC for 89% of those isolates tested. In no case did the MBC exceed the MIC by more than three dilutions. More important clinically, no isolate demonstrated tolerance to BMY-28142.

Although the antipseudomonal activity of BMY-28142 in combination with the other antimicrobial agents studied was usually indifferent, two specific drug interactions warrant discussion.

The synergism of BMY-28142 and tobramycin observed against three of four tobramycin-resistant isolates suggests a potential clinical application in treatment of infections caused by aminoglycoside-resistant *P. aeruginosa*, such as acute pulmonary exacerbations in older cystic fibrosis patients colonized with resistant strains.

A fourfold or greater increase in the MIC of BMY-28142 was reported for 4 of 30 *P. aeruginosa* strains incubated in subinhibitory concentrations of imipenem (F. J. De Orio, R. E. Buck, and R. E. Kessler, 24th ICAAC, abstr. no. 748). With antagonism defined as an FIC index >2 for our evaluation, we observed an even greater frequency of antagonism against *P. aeruginosa* strains tested. Although BMY-28142 has an apparently low affinity for class I β-lactamases (D. J. Phelps, D. D. Carlton, and C. A. Farrell, 24th ICAAC, abstr. no. 746), the known high β-lactamase inductive potential of imipenem (2) may explain this observed antagonism.

Because of the variable effect of combining BMY-28142 and other antimicrobial agents, combination studies should be performed against specific isolates before the use of such combinations in clinical situations.

Increases in MIC observed with higher densities of *P. aeruginosa*, *Staphylococcus aureus*, and *E. coli* reflect an inoculum-dependent effect described for other β-lactam antibiotics against *P. aeruginosa* (3), *Klebsiella aerogenes* (1), and *H. influenzae* (14, 16). This effect, postulated to be caused by the increased β-lactamase content of the larger bacterial population, may account for the observed decline in BMY-28142 antimicrobial activity.

β-Lactam antibiotics are unstable at pH extremes, due to chemical degradation of the β-lactam ring, resulting in loss of antimicrobial potency (12, 13). This adverse reaction may explain the decreased antibacterial activity of BMY-28142 against *P. aeruginosa* and *E. coli* observed at acid pHs (≤5).

BMY-28142 has considerable antibacterial activity in vitro against a wide range of gram-positive and gram-negative bacteria isolated from pediatric infections, and it has shown antimicrobial synergy against *P. aeruginosa* when combined with tobramycin and antimicrobial antagonism when combined with imipenem. BMY-28142 was not active against methicillin-resistant *Staphylococcus aureus* or against *P. cepacia*, and could be adversely influenced by higher inoculum densities and extremes of medium pH. These observations may provide useful guidelines for the clinical evaluation of this new antibiotic.

LITERATURE CITED

1. **Basker, M. J., R. A. E. Edmondson, and R. Sutherland.** 1979. Comparative antibacterial activity of azlocillin, mezlocillin, carbenicillin, and ticarcillin and relative stability to β-lactamases of *Pseudomonas aeruginosa* and *Klebsiella aerogenes*. Infection **7**:67–73.
2. **Bertram, M. A., and L. S. Young.** 1984. Imipenem antagonism of the in vitro activity of piperacillin against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **26**:272–274.
3. **Corrado, M. L., S. H. Landesman, and C. E. Cherubin.** 1980. Influence of inoculum size on activity of cefoperazone, cefotaxime, moxalactam, piperacillin, and *N*-formimidoyl thienamycin (MK0787) against *Pseudomonas aeruginosa*. Antimicrob. Agents Chemother. **18**:893–896.
4. **Dodge, J., I. Zamiri, M. Goodchild, and P. Ingram.** 1983. Experience with ceftazidime in cystic fibrosis. J. Antimicrob. Chemother. **12**(Suppl. A):325–329.
5. **Elion, G. B., S. Singer, and G. H. Hitchings.** 1954. Antagonists of nucleic acid derivatives. VIII. Synergism in combination of biochemically related antimetabolites. J. Biol. Chem. **208**:477–488.
6. **Kawamura, N.** 1983. Clinical experience with ceftazidime in urology in Japan. J. Antimicrob. Chemother. **12**(Suppl. A):65–70.
7. **Kercsmar, C. M., R. C. Stern, M. D. Reed, C. M. Myers, D. Murdell, and J. L. Blumer.** 1983. Ceftazidime in cystic fibrosis: pharmacokinetics and therapeutic response. J. Antimicrob. Chemother. **12**(Suppl. A):289–295.
8. **Lennette, E. H., A. Balows, W. J. Hausler, and J. P. Truant. (ed.).** 1980. Manual of clinical microbiology, 3rd ed. American Society for Microbiology, Washington, D.C.
9. **Lundbergh, P., C. Jarstrand, L. Morfeldt-Manson, and O. Weiland.** 1983. Ceftazidime in septicaemia. J. Antimicrob. Chemother. **12**(Suppl. A):199–205.
10. **Mandell, L. A., L. E. Nicolle, A. R. Ronald, R. Duperval, H. G. Robson, R. Feld, J. Vincelette, and I. Fong.** 1983. A multicentre prospective randomized trial comparing ceftazidime with cefazolin/tobramycin in the treatment of hospitalized patients with non-pneumococcal pneumonia. J. Antimicrob. Chemother. **12**(Suppl. A):9–20.
11. **Muytjens, H. L., and J. van der Ros-van de Repe.** 1982. Comparative activities of 13 β-lactam antibiotics. Antimicrob. Agents Chemother. **21**:925–934.
12. **Schwartz, M. A., and F. H. Buckwalter.** 1962. Pharmaceutics of penicillin. J. Pharm. Sci. **51**:1119–1128.
13. **Schwartz, M. A.** 1965. Mechanism of degradation of penicillin G in acidic solution. J. Pharm. Sci. **54**:472–473.
14. **Simard, P., and M. G. Bergeron.** 1982. Inoculum size effect on the MIC of cefoperazone, moxalactam, cefotaxime, cefoxitin

and cephalothin for 118 strains of *Haemophilus influenzae* including 'tolerant' micro-organisms. J. Antimicrob. Chemother. **10**:397–402.

15. **Tarpay, M. M., D. F. Welch, and M. I. Marks.** 1980. Antimicrobial susceptibility testing of *Streptococcus pneumoniae* by micro-broth dilution. Antimicrob. Agents Chemother. **18**:579–581.
16. **Washington, J. A.** 1983. Discrepancies between in vitro activity of and in vivo response to antimicrobial agents. Diagn. Microbiol. Infect. Dis. **1**:25–31.