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Cephamycins, a New Family of β-Lactam Antibiotics: Antibacterial Activity and Resistance to β-Lactamase Degradation

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The susceptibility to some cephalosporin antibiotics and to cephamycin C, a member of a new family of β-lactam antibiotics, was evaluated for 466 cultures representing 11 different genera or species of gram-negative clinical isolates. The susceptibility of 39 gram-negative cultures known to produce β-lactamase was also determined. The β-lactamase activity of a representative group of the clinical isolates and the 39 enzyme producers was studied with the cephalosporins (cephalothin and cephaloridine) and cephamycin C as substrates and was related to the in vitro disc susceptibility to these same antibiotics. The significant resistance to β-lactamase displayed by the cephamycins is reflected in the kinetics of enzyme activity (K_m and V_{max}) that are reported for the cephalosporins and the cephamycins. Resistance to β-lactamase is probably one of the reasons that many cephalosporin-resistant cultures are susceptible to cephamycin C.

Stapley et al. (22) described the production of cephamycins A, B, and C from fermentation broths of various species of *Streptomyces*. In spite of their resemblance to the cephalosporins (1, 19), the cephamycins demonstrate markedly greater resistance to microbial degradation by β-lactamase (EC 3.5.2.6 penicillin [cephalosporin] amido-β-lactam hydrolase) than is normally displayed by the cephalosporins. Although cephamycins A and B are endowed with broad-spectrum activity, they are limited from the standpoint of sufficient potency in many instances. Cephamycin C has been found to be particularly effective versus gram-negative bacteria of clinical importance and relatively ineffective versus gram-positive organisms.

Our efforts were concentrated on the investigation of the relative merits of cephamycin C with respect to its in vitro efficacy and stability to microbial β-lactamase degradation vis-à-vis those same properties of some cephalosporin antibiotics.

MATERIALS AND METHODS

Cultures. A group of 466 clinical isolates was obtained from several large metropolitan hospitals (Bellevue Hospital, New York, N.Y.; St. Luke's Medical Center, New York, N.Y.; D.C. General, Washington, D.C.; Presbyterian Hospital, Philadelphia, Pa; and U.S. Public Health Service Hospital, Seattle, Wash.). These cultures represent 11 different genera or species of gram-negative bacteria that were obtained randomly from various infectious disease states (urinary tract, post-surgical, wound and respiratory infections, etc.) and not selected on the basis of antibiotic resistance.

A second group consisted of 39 β-lactamase-producing organisms, obtained from M. H. Richmond (Department of Bacteriology, The Medical School, University of Bristol, Bristol, England) and used in his study, of the distinct types of β-lactamase produced by gram-negative bacteria (14).

Strains of *Proteus morganii* obtained from M. Gluck, New York University School of Medicine (*P. morganii* 251 and 356), and A. Girard, Department of Bacteriology, University of Connecticut (*P. morganii* 221), were used in the study of β-lactamase induction. The first two cultures were assigned the Merck Sharp & Dohme culture designations of MSD 3376 and 3345, respectively, and the last strain was assigned MSD 3202.

Two strains of *Aerobacter cloacae*, HSC 18410/62 and HSC 18410-M-66, were obtained from M. Goldner (The Research Institute, Hospital for Sick Children, Toronto, Ontario, Canada). The former is a cephalosporin-resistant, β-lactamase-producing clinical isolate (5, 8), whereas the latter is a mutant strain HSC 18410/62 that has lost spontaneously the capacity to produce β-lactamase (9).

Media and maintenance of cultures. Each of the cultures was transferred to slants of brain heart infusion (BHI) agar (Difco) upon receipt,

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grown overnight at 37 C, and stored in that manner at 3 to 5 C for no more than 2 weeks. Long-term preservation was accomplished by storing BHI slant cultures at −80 C in an ultra-low-temperature freezer (Revco Inc., West Columbia, S.C.). Transfers were made from these slants each time a culture was to be employed.

Antibiotic susceptibility testing. The susceptibility of the test organisms to cephalosporin antibiotics and to cephamycins A, B, and C was determined by the standard Bauer-Kirby disc method of antibiotic susceptibility testing (3). Susceptibility tests with cephalothin were done with 0.25-inch (0.64-cm) commercially prepared discs (BBL, Sensi-Disc, 30-μg potency). Discs of cephaloridine (Loridine, Eli Lilly & Co.), cephalexin (Keflex, Eli Lilly & Co.), and cephamycins A, B, and C were prepared in the laboratory such that each 0.25-inch disc would contain 30 μg of the appropriate antibiotic. A zone of inhibition equal to or greater than 18 mm in diameter was used as the criterion for susceptibility to the test antibiotics since this is the established minimal zone used for designating susceptibility to the cephalosporin family of antibiotics. A culture was judged resistant to a given antibiotic if the zone of inhibition was less than 18 mm in diameter or if five or more resistant colonies appeared in a zone of inhibition greater than 18 mm in diameter.

Assay for β-lactamase activity. β-Lactamase activity was determined by suspending whole cells from a BHI agar culture grown at 37 C overnight into 0.067 M phosphate buffer (*p*H 7.0) and adjusting the absorbance at 550 nm to 0.5 (Spectronic 20 colorimeter, Bausch & Lomb, Rochester, N.Y.). The assay for β-lactamase degradation was performed by reaction of the cell suspension for 1 hr at 37 C with 50 μg of cephaloridine, cephalothin, or cephamycin C per ml as substrate. β-Lactamase activity was determined by a microiodometric method based on the work of Novick (20), Perret (21), and Goldner et al. (8). After a 1-hr period, Novick's starch-iodine solution in 0.5 M sodium acetate buffer, *p*H 4.0, was added to stop the reaction. Iodine reduction was measured, as absorbance at a wavelength of 620 nm, exactly 10 min after addition of the complex. Appropriate controls were run for nonspecific iodine-reacting materials (13).

Kinetics of β-lactamase action. Kinetic studies were done with a crude β-lactamase preparation obtained from *A. cloacae* 18410/62. The bacterium was cultured in BHI broth (1,000 ml/2-liter flask) at 37 C, with shaking, to provide a total of 20 liters of culture fluid. The cells were collected by continuous-flow centrifugation at approximately 40,000 rev/min in a Sharples Super Centrifuge (Sharples, Westminister, Pa.), washed twice with equal volumes of cold 0.067 M phosphate buffer (*p*H 7.0), and resuspended in 200 ml of the same buffer. The cells in this suspension were disrupted by sonic treatment in a Raytheon 10-kc sonic oscillator maintained at full amplitude for 20 min and kept at 3 to 5 C by continuous recirculation of ice water through the jacket of the sonic treatment chamber. The sonic extract was centrifuged at 8,000 × *g* for 20 min in a Sorvall RC2-B automatic refrigerated centrifuge (Ivan Sorvall, Inc., Norwalk, Conn.). The pellet was discarded, and the supernatant fluid was centrifuged at 30,000 × *g* for 30 min in the same centrifuge.

The supernatant fluid was dialyzed by placing 20-ml samples in a dialysis sac (A. H. Thomas, Philadelphia, Pa; no. 4465-A2, 1⅛ inch [2.9 cm] flat) and immersing the assembly for 24 hr in a carboy containing 20 liters of cold, freshly distilled water. The dialysis fluid was agitated continuously by use of a magnetic stirrer and stirring bar. The dialysate was centrifuged at 30,000 × *g* for 30 min and the supernatant fluid was used as the source of β-lactamase.

The concentration of antibiotics tested ranged between 125 and 1,000 μM. The level of enzyme employed was adjusted for each antibiotic such that a substrate turnover of approximately 10% would be obtained in the 10 min the individual reaction rates were studied. β-Lactamase activity was measured by the microiodometric method described above. Protein concentration was determined by the method of Lowry et al. (16). The Michaelis constant (K_m) and the velocity of the reaction (V_{max}) were determined by the method described originally by Lineweaver and Burk (15) and discussed by Dixon and Webb (4).

Induction of β-lactamase. The degradation of cephalothin and of cephamycin C by three strains of *P. morganii* was studied with whole cells or cell-free extracts that were induced, or not, by the subject antibiotics. The strains of *P. morganii* were grown overnight at 37 C in BHI broth. A transfer was made to fresh BHI broth, and the culture was incubated at 37 C on a rotary shaker (220 rev/min) to a cell density equivalent to that in mid-log phase of growth. A 10-ml sample of the culture was mixed with 40 ml of BHI broth containing 12.5 to 200 μg of cephalothin/ml or 6.25 to 50 μg of cephamycin C/ml, each in twofold increments.

Induction was carried out at 37 C for 3 hr. The drug concentration in the induction mixture that yielded a cell density most closely approaching that for the control culture (no antibiotic) was used to determine the presence and extent of β-lactamase induction. This level was found to be 25 μg/ml for cephamycin C and 200 μg/ml for cephalothin.

The cells were harvested from the selected mixtures by centrifugation at 10,000 × *g* for 10 min in a Sorvall Superspeed Centrifuge equipped with an SS-34 head (Ivan Sorvall, Inc.), washed once in 10 ml of 0.067 M phosphate buffer (*p*H 7.0), and resuspeded in the buffer to an absorbance at 550 nm of 5.0. Cell-free extracts were prepared by sonic treatment of 5- to 10-ml samples in an MSE 60-w ultrasonic disintegrator operated at maximal amperage (MSE Inc., Westlake, Ohio). Sonic treat-treatment was carried out for approximately 5 min after the cell suspension and the titanium

vibrator probe (0.75 inch, 1.9 cm) had been prechilled thoroughly in iced water. All assays for β-lactamase activity were made with a substrate (cephalothin or cephamycin C) concentration of 500 μM.

RESULTS AND DISCUSSION

Susceptibility of clinical isolates. The disc susceptibility data for the 466 clinical isolates show that cephamycin C has a spectrum of activity against gram-negative bacteria very similar to that displayed by the commercial synthetic cephalosporins cephaloridine and cephalothin (Table 1). Cephamycin C is essentially inactive against gram-positive organisms (18). However, as will be demonstrated below, more gram-negative strains which are resistant to cephalosporin-like antibiotics in general or which produce β-lactamase are susceptible to cephamycin C than to cephaloridine or cephalothin.

Comparison of in vitro susceptibility and β-lactamase activity. Fifty-four cultures were selected as representative of the types of cephalosporin-resistance patterns for the various genera among the 466 gram-negative, clinical isolates whose in vitro susceptibility to cephaloridine, cephalothin, and cephamycin C had been determined (Table 1). These cultures and the 39 gram-negative, β-lactamase-producing cultures were evaluated for their capacity to degrade the subject antibiotics via β-lactamase activity. The data are listed for the individual cultues tested (Table 2) and have been organized to demonstrate more readily the relationship between disc susceptibility and the capacity to degrade by β-lactam hydrolysis (Table 3).

Cephamycin C was distinctly more resistant to degradation by β-lactamase than was cephaloridine, irrespective of the enzyme source. In comparison with cephalothin, however, the results are skewed in the favor of cephamycin C as a result of the effect of species variation. Cephamycin C was much less susceptible than cephalothin to β-lactamase degradation by the *Aerobacter-Enterobacter* group. The difference in results between cephamycin C and cephalothin was much less pronounced among the other species examined. Overall, however, cephamycin C was degraded by fewer members of these other species than was cephalothin.

No absolute correlation between β-lactamase resistance and bacterial susceptibility exists. However, a greater number of these cephalosporin-resistant and β-lactamase-producing gram-negative bacteria were susceptible to cephamycin C (40) than to either cephaloridine (31) or cephalothin (22), as shown in Table 3. In spite of the existence of species variation, the resistance of cephamycin C to hydrolysis by β-lactamase may still be an important property contributing to the advantage of this antibiotic with respect to the susceptibility of many of the strains examined.

Kinetics of β-lactamase activity. As a result of the marked resistance to β-lactamase degradation exhibited by the cephamycin family of antibiotics, a study was undertaken to compare the kinetics of inactivation of cephalothin, cephaloridine, and cephalosporin C with those of cephamycins A, B, and C. The enzyme selected with which to conduct the study was the potent cephalosporinase originally isolated from *A. cloacae* by Fleming et al. (6) and further characterized by these same authors (8).

The reaction rate of β-lactam hydrolysis for

TABLE 1. *Antibiotic susceptibility testing of gram-negative clinical isolates*

Culture	Total strains	No. susceptible		
		Cephalothin	Cephaloridine	Cephamycin C
Proteus mirabilis	112	104	102	102
P. morganii	2	0	0	0
P. vulgaris	6	1	1	5
Providencia	2	0	0	1
Paracolons	19	8	6	9
Escherichia coli	194	169	163	184
Serratia (nonpigmented)	2	0	0	0
Klebsiella spp.	66	59	56	61
Aerobacter-Enterobacter	29	10	6	8
Alcaligenes faecalis	12	2	2	2
Citrobacter sp.	1	1	1	1
Unidentified gram-negative rods	21	11	14	14
Totals	466	365	351	387

TABLE 2. *Comparison of the antibiotic susceptibility of clinical isolates or β-lactamase-producing bacteria with the capacity to degrade cephalosporins and cephamycin C by β-lactamase*

Culture	Source	Susceptibility[a]			Antibiotic degraded (%)		
		Cephalothin	Cephaloridine	Cephamycin C	Cephalothin	Cephaloridine	Cephamycin C
Aeromonas sp.							
18	M. Richmond	S	S	S	0	20	0
2/37	M. Richmond	S	S	S	0	24	0
241	M. Richmond	S	S	S	12	84	0
1390	M. Richmond	S	S	S	0	24	0
Aerobacter cloacae							
HSC18410/62	M. Goldner	R	R	R	87	97	100
HSC18410M66	M. Goldner	S	S	S	0	0	0
P99	M. Richmond	R	R	R	78	100	100
53	M. Richmond	R	S	S	0	100	0
Enterobacter cloacae							
214	M. Richmond	R	R	R	16	42	0
177	M. Richmond	R	R	R	5	92	0
533	M. Richmond	S	S	R	0	0	0
1082E	M. Richmond	R	R	S	74	100	0
1929	M. Richmond	R	R	R	5	100	0
1316	M. Richmond	R	R	R	0	54	0
Aerobacter sp. 2/46	M. Richmond	R	S	S	12	54	0
Enterobacter sp.							
6	Bellevue	R	R	R	22	49	0
15	Bellevue	R	R	R	0	0	0
42	Bellevue	R	R	R	26	45	44
349	Bellevue	R	R	R	30	100	0
369	Bellevue	R	R	R	42	94	40
68	D.C. General	S	R	R	16	36	5
231	D.C. General	R	R	R	10	32	10
242	D.C. General	R	R	S	0	0	0
244	D.C. General	R	R	R	28	50	10
301	D.C. General	R	R	R	22	44	0
Alcaligenes sp.							
97	D.C. General	R	R	R	0	0	0
249	D.C. General	R	R	R	0	0	0
251	D.C. General	R	R	R	0	0	0
257	D.C. General	S	S	S	0	0	0
385	M. Richmond	R	S	S	36	100	0
Paracolons							
53	D.C. General	R	R	R	0	18	0
111	D.C. General	R	R	R	12	50	10
237	D.C. General	R	R	R	0	50	20
256	D.C. General	R	R	R	16	38	0
260	D.C. General	R	R	R	10	36	0
309	D.C. General	R	R	R	10	32	0
Providencia							
2	Bellevue	R	R	R	0	20	0
21	St. Luke's	R	R	R	0	0	0
Serratia sp.							
187	Presbyterian	R	R	R	0	0	0
377	Bellevue	R	R	R	46	90	0
Escherichia coli							
35	Bellevue	R	S	S	0	46	44
65	Bellevue	R	R	R	26	100	52
105	St. Luke's	R	R	R	12	38	10
61	D.C. General	R	R	R	12	50	0
120	D.C. General	R	R	R	53	100	61
Fink	M. Turck	R	R	R	54	89	88
McTee	M. Turck	R	S	S	32	87	64

TABLE 2—*continued*

Culture	Source	Susceptibility[a]			Antibiotic degraded (%)		
		Cephalothin	Cephaloridine	Cephamycin C	Cephalothin	Cephaloridine	Cephamycin C
Lovell	M. Turck	R	R	R	38	87	64
8	M. Richmond	S	S	S	0	66	0
071	M. Richmond	S	S	S	0	34	0
284	M. Richmond	S	S	S	0	0	0
719	M. Richmond	R	R	R	63	100	48
1758	M. Richmond	R	R	R	0	0	5
2/40/1	M. Richmond	R	R	R	34	94	14
Denston 5003	M. Richmond	R	S	S	0	100	0
CJL	M. Richmond	R	R	R	19	100	20
R+TEM	M. Richmond	R	S	S	12	52	0
NPL 3	M. Richmond	R	R	R	26	100	48
Wickham	M. Richmond	S	S	S	12	62	26
Klebsiella sp.							
17	Bellevue	R	R	S	0	100	0
31	Bellevue	R	R	S	0	87	0
36	Bellevue	R	R	R	20	87	0
39	Bellevue	R	R	S	0	88	0
202	D.C. General	R	R	R	0	50	0
239	St. Luke's	R	R	R	12	34	20
266	St. Luke's	R	R	R	0	0	0
Burpee	M. Turck	R	R	R	0	30	0
115	M. Richmond	S	S	S	0	40	0
264	M. Richmond	S	S	S	0	26	0
311	M. Richmond	S	S	S	0	44	5
418	M. Richmond	S	S	S	0	5	5
466	M. Richmond	R	S	S	8	82	0
481	M. Richmond	S	S	S	0	30	0
D535	M. Richmond	R	R	R	82	100	95
1169	M. Richmond	R	S	S	0	58	0
9527	M. Richmond	S	S	S	0	50	0
McDonald	M. Richmond	R	S	S	46	100	0
Robinson	M. Richmond	S	S	S	0	42	0
Shine	M. Richmond	R	S	S	34	100	5
Proteus morganii							
1266	M. Richmond	S	S	S	0	0	0
Collier	M. Turck	R	R	R	0	10	0
Detenly	M. Turck	R	R	R	0	0	0
P. mirabilis							
Mauser	M. Turck	R	R	S	0	0	0
23	Bellevue	R	R	S	0	3	0
46	Bellevue	R	R	S	0	0	0
58	Bellevue	S	R	S	0	24	0
P. vulgaris 3117	M. Richmond	S	S	S	0	64	0
Proteus sp.							
11	Bellevue	R	R	R	12	41	0
16	Bellevue	R	R	R	0	15	0
20	Bellevue	R	R	S	0	5	0
26	Bellevue	R	R	R	0	3	0
63	Bellevue	R	R	R	0	0	0
92	D.C. General	R	R	R	12	40	10

[a] S, susceptible; R, resistant.

each of the six antibiotics studied, as expressed by the V_{max} (Table 4), is further evidence that the cephamycins bear an increased degree of stability to this enzymatic inactivation. Since this study was performed with a crude enzyme preparation, the absolute values stated for each parameter cannot be compared directly to data obtained with purified enzymes. However, the rela-

TABLE 3. *Composite of test cultures producing various levels of β-lactamase degradation of cephalothin, cephaloridine, and cephamycin C with respect to the disc susceptibility of these cultures to the subject antibiotics*

Antibiotic	β-Lactamase activity[a]	No. of strains	Disc susceptibility[b]	
			S	R
Cephaloridine	−	23	6	17
	+	70	25	45
	Total	93	31	62
Cephalothin	−	56	19	37
	+	37	3	34
	Total	93	22	71
Cephamycin C	−	75	37	38
	+	18	3	15
	Total	93	40	53

[a] Symbols: −, 10% or less degradation observed; +, 11% or more degradation observed.
[b] S, susceptible; R, resistant.

TABLE 4. *Kinetics of inactivation of cephem antibiotics by β-lactamase from Aerobacter cloacae 18410/62*

Substrate	V_{max}[a]	K_m (μM)
Cephamycin A.....	1.2×10^{-3}	9.5×10^{-2}
Cephamycin B.....	1.0×10^{-3}	6.2×10^{-2}
Cephamycin C...	3.3×10^{-3}	2.4×10^{-2}
Cephalothin.....	9.1×10^{-3}	0.6×10^{-2}
Cephaloridine.....	100×10^{-3}	9.2×10^{-2}
Cephalosporin C...	200×10^{-3}	14.3×10^{-2}

[a] Expressed as micromoles per minute per milligram of protein.

tionship of the antibiotic substrates with respect to this enzyme within this system are valid. To check the validity of the relationships established in Table 4, data obtained by Goldner et al. (8) and Hennessey (12), working with a purified enzyme preparation from the same organism, and by Hennessey and Richmond (13), working with a similar enzyme from *E. cloacae* 214, were compared with our data. The enzymatic hydrolysis rates of cephaloridine and cephalothin are compared relative to that for cephalosporin C in Table 5. The relationship between hydrolysis rates established with purified enzyme preparations is the same as those established within our system with a crude enzyme preparation.

Induction of β-lactamase. The indole-positive cultures *P. morganii* and *P. vulgaris* are generally found to be resistant to the cephalosporin antibiotics. The advantage in efficacy in vivo and

TABLE 5. *Comparison of the ratio of relative reaction rates of Aerobacter cloacae β-lactamase on cephalosporins and cephamycin C*

Substrate[a]	Ratio of reaction rate[b]		
	A	B	C
Cephalosporin C.....	1.0	1.0	1.0
Cephaloridine.....	0.5	1.1	0.5
Cephalothin.....	0.1	0.16	0.05
Cephamycin C.....	—	—	0.02

[a] Cephalosporin C was used as the reference substrate.
[b] (A) Data from Goldner et al. (8). (B) Calculated from data reported by Hennessey and Richmond (13) and similar to that calculated from data reported by Hennessey (12). (C) Calculated from data in Table 4.

in vitro of cephamycin C over the cephalosporins has been demonstrated with this group of *Proteus* species (17, 18). Our failure to demonstrate any significant degree of β-lactamase degradation of the cephalosporins by these resistant cultures led us to reexamine our approach to the problem and to evaluate the possible role of an inducible β-lactamase as a mechanism of resistance. Ever since Hamilton-Miller (10) demonstrated the inducibility of a β-lactamase (penicillinase) in *P. morganii*, this phenomenon in gram-negative bacteria has been the subject of considerable study and discussion (2, 7, 10, 12). The need was recognized not only to test induction itself but also to test the phenomenon in whole cells as well as ruptured cells to discount permeability or crypticity of the cultures as a factor in the interpretation of results (2, 11, 12).

Two of the strains tested, *P. morganii* 3202 and 3345, demonstrate a low-level β-lactamase degradation of cephalothin but none at all of cephamycin C by uninduced, whole cells (Table 6). Ruptured cells of these two strains display no greater activity versus cephalothin than found with whole cells; however, a very minute level of activity was detected against cephamycin C. Strain 3376 exhibited a rather high-level, constitutive β-lactamase degradation of cephalothin but not of cephamycin C. Each of these cultures apparently experiences relatively free accessibility of substrate to enzyme, as the permeability or crypticity factor is of little significance. Only strain 3345 showed any evidence of producing a higher level of inactivaion (approximately two-fold) from ruptured than from intact cells, and this occurred only when cells were induced by cephamycin C and the cell extract was reacted with cephalothin as substrate.

Induction takes on a very significant role, how-

TABLE 6. *Degradation of cephalothin and cephamycin by β-lactamase from whole cells or cell-free extracts of Proteus morganii induced, or not, by cephamycin C or cephalothin*

Cell prepn	β-Lactamase degradation[a]					
	Cephalothin			Cephamycin C		
	Uninduced	Induced by		Uninduced	Induced by	
		Cephalothin	Cephamycin C		Cephalothin	Cephamycin C
Whole cells of						
P. morganii 3202	6.2	583	367	0	35	19
P. morganii 3345	5.3	690	595	0	20	94
P. morganii 3376	204	571	571	0	28	96
Cell extract of						
P. morganii 3202	2.5	328	96	0.8	1.5	12
P. morganii 3345	4.7	345	1255	1.2	4.6	19
P. morganii 3376	120	368	835	1.3	4.6	28

[a] Activity of β-lactamase from whole cells is expressed as micromoles degraded per minute per milliliter of cell suspension. The cell suspension was adjusted to A_{550} = 5.0, which resulted in the following dry weights: strain 3202, 68 mg/ml; strain 3345, 70.5 mg/ml; and strain 3376, 69.5 mg/ml. For the cell-free extracts, the activity is expressed as micromoles degraded per minute per milligram of protein. The reaction was carried out with cell extract from a number of cells equivalent to the number of whole cells used above.

ever, and the results of our study offer some explanation for the increased efficacy of cephamycin C compared to the cephalosporins against strains of *P. morganii*. Strain 3202 was induced by cephalothin and cephamycin C to degrade the former antibiotic at a rate 97- and 61-fold, respectively, above that noted at the constitutive level. Strain 3345 was induced to degrade cephalothin at a rate 138- and 119-fold greater than recorded at the uninduced level after induction by cephalothin and cephamycin C, respectively. Strain 3376 had a high constitutive level of activity versus cephalothin and was induced by either antibiotic to degrade cephalothin at a rate three times greater than the uninduced level.

Some degradation of cephamycin C has been noted after induction by either of the subject antibiotics, but this was much lower than that noted with cephalothin. These studies demonstrate that cephamycin C is more resistant to hydrolysis by the β-lactamases from *P. morganii* than are cephalothin and cephaloridine. This difference in stability may account for the greater susceptibility of such organisms to cephamycin C.

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