HR 756, the *syn* Isomer of a New Methoxyimino Cephalosporin with Unusual Antibacterial Activity

YVES A. CHABBERT¹* AND ANDRÉ J. LUTZ²

Unité de Bactériologie Médicale, Institut Pasteur, 75724 Paris,¹ and Centre de Recherches Roussel-Uclaf, 93230 Romainville, France

Received for publication 1 September 1978

HR 756, the *syn* derivative of 7-[(2-(2-amino-4-thiazolyl)-2-methoxyimino)acetamido]cephalosporanic acid, is a new semisynthetic cephalosporin. It was 80 times more active than the *anti* derivative against β -lactamase-producing strains of gram-negative bacteria. The range of inhibitory concentrations of HR 756 against gram-negative bacteria, including *Haemophilus influenzae*, susceptible or resistant to penicillins and cephalosporins was from 0.01 to 0.1 μ g/ml. This activity was consistently higher than those observed with cephalothin, cephaloridine, cephalexin, and cefazolin. Nevertheless, some strains of *Enterobacter cloacae* were resistant. HR 756 showed very similar activity to that of ampicillin against group A streptococci and *Streptococcus pneumoniae*.

Methoxyimino derivatives of 7-aminocephalosporanic acid appear to be an interesting new category of cephalosporin antibiotics with increased stability toward β -lactamases and increased activity against *Haemophilus influenzae* (7). In these compounds the oxyimino group can be in two isomeric configurations, *syn* or *anti*. In preliminary observations by Bucourt et al. (1) on 7-[(2-(2-amino-4-thiazolyl)-2-methoxyimino)acetamido]cephalosporanic acid, it was claimed that the *syn* isomer, HR 756, is 10 to 100 times more active against gram-negative bacteria than are the cephalosporins currently available commercially. This report describes *in vitro* studies on different groups of bacteria: β -lactamase-producing strains of enteric bacteria, *Haemophilus*, *Streptococcus*, pneumococcus, and clinical isolates of various species.

MATERIALS AND METHODS

Antibiotics. The *syn* derivative HR 756 and the *anti* isomer of 7-[(2-(2-amino-4-thiazolyl)-2-methoxyimino)acetamido]cephalosporanic acid (sodium salt) were synthesized in the Research Department of Roussel-Uclaf, Romainville, France. Most of the determinations were carried out with batch 7E/0998 of HR 756, free of the *anti* isomer.

Ampicillin, amoxicillin, and carbenicillin were supplied by Beecham-Sévigé Laboratories, Paris, France, and cephalothin, cephaloridine, cephalexin, and cefazolin were supplied by Eli-Lilly France, St. Cloud.

Bacterial strains. Among the strains of enteric bacteria studied by the reference laboratory of the Unité de Bactériologie Médicale, Institut Pasteur (Paris, France), 26 were selected that produced various β -lactamases belonging to the main groups defined by Sykes and Matthew (21). These strains have been

previously characterized on the basis of the following parameters: 50% inhibitory concentrations (IC₅₀s) of available penicillins and cephalosporins, plasmid content, characterization of TEM-like penicillinase (19) and immunotype 1 and 2 β -lactamases (20), determination of β -lactamase K_m and V_{max} constants by a computerized microacidimetric method (9), and analytical isoelectric focusing by sucrose density gradient (10). The isoelectric points listed below and in Table 1 have been determined by Labia et al. (11-13; personal communication).

Escherichia coli J53 F⁻ *pro met* (from N. Datta) and *E. coli* ATCC 25922 were used as "susceptible" control strains. R-plasmid-mediated penicillinase is exemplified by *E. coli* K-12 harboring plasmid pIP111 (3), coding for TEM 1 penicillinase (pI 5.4); plasmid pIP71a (3), coding for a penicillinase different from TEM 1 and 2 belonging to the immunotype 2 (20) (pI 7.7); and plasmid pIP55 (3, 4), coding for oxacillin-hydrolyzing enzyme (pI 6.97). A penicillinase produced by *Klebsiella pneumoniae* 1103 (pI 7.1) is similar to the plasmid-coded enzyme corresponding to the immunotype 2, but that a plasmid codes for the enzyme in this strain has never been proven. Chromosomal cephalosporinases of *E. coli* similar to the enzyme of strain D31 reported by Burman et al. (2), which produces increased levels of basic β -lactamases of *E. coli* by *ampA* gene amplification (16), were observed in *E. coli* strains LA0002 (pI 9.17), O49 (pI 9.21), and SOL (pI 9.30). Other chromosomal cephalosporinases, apparently species specific, are exemplified by the following: *K. pneumoniae* H28; *Enterobacter cloacae* T45 (pI 8.34), PI116 (pI 9.02), PI46 (pI 8.34), P99 (pI 8.34), V462, G60, and J68; *Proteus morganii* F20 (pI 8.3); *Proteus rettgeri* A19; *Providencia* C22S; *Serratia marcescens* M01117 (pI 9.75), H23, and K11. Strains *K. pneumoniae* C19 and *S. marcescens* R66 each produce two β -lactamases; one is a TEM-like enzyme, and the other is a cephalosporinase. In addition, 9 strains of penicillinase-producing *H. influenzae* from

different sources have been compared with 16 ampicillin-susceptible strains. *Streptococcus pyogenes* group A (26 strains) and *Streptococcus pneumoniae* (25 strains) were randomly selected from strains collected by the Centre National de Référence, Institut Pasteur.

Clinical isolates. More than 700 recent isolates were obtained from several public and private hospitals from Paris and Strasbourg, France. Among the gram-negative strains tested were 186 *E. coli*, 25 *Salmonella*, 12 *Citrobacter*, 91 *Klebsiella* (80 *K. pneumoniae*, 11 *K. oxytoca*), 48 *E. cloacae*, 31 *Serratia*, 81 *Proteus* (40 *P. mirabilis*, 14 *P. vulgaris*, 11 *P.morganii*, 16 *P. rettgeri*), 18 *Providencia*, 6 *Neisseria gonorrhoeae*, and 40 *Pseudomonas aeruginosa*. Besides 123 staphylococci, 45 *Streptococcus faecalis*, and some strains of *Corynebacterium*, *Bacillus subtilis*, *Moraxella duplex*, *M. lacunata*, *Aeromonas hydrophila*, *Yersinia enterocolitica*, *Clostridium perfringens*, *Fusobacterium necrophorum*, and *Bacteroides fragilis* were also tested.

Determination of inhibitory concentrations. To determine inhibitory concentrations, different serial dilutions and end point readings were used, according to the degree of accuracy required.

(i) **MIC.** Minimum inhibitory concentrations (MICs) were determined according to the reference methods of the International Collaborative Study (ICS) (6), using twofold dilutions on Mueller-Hinton agar (Institut Pasteur Production) and Mueller-Hinton broth (Oxoid Ltd., London, England). For *Streptococcus* strains, brain heart infusion (Difco Laboratories, Detroit, Mich.) was used. This medium was supplemented by Fildes enrichment (Difco) for *Haemophilus*.

(ii) **IC99.** For IC99, the twofold dilution in Mueller-Hinton agar used in the ICS reference method was modified to obtain the following progression of antibiotic content (dilution factor, 1.25): 1, 1.25, 1.6, 2, 2.5, 3.2, 4, Agar plates were inoculated with a Steers replicator, using dilutions of an overnight culture to obtain 1,000 to 3,000 bacteria per spot. An additional control plate contained a 1:100 dilution of this inoculum, giving 10 to 30 bacteria per spot. The end point reading was the lowest concentration of antibiotic giving 10 to 30 isolated colonies per spot. These end points corresponded to 99% inhibition.

(iii) **IC50.** For IC50, the agar dilution method (6) in Mueller-Hinton agar was carried out, using the 1.25 dilution factor. Petri dishes were inoculated by spreading 300 to 400 bacteria per plate. After 18 h of incubation, colonies were counted with a Fisher bacterial counter model 480. The percentage of colonies growing on each antibiotic concentration was plotted on probability-log paper, and the 50% end point was determined graphically. This end point gives results similar to those obtained with the so-called single-cell end point (21) but is more quantitative.

RESULTS

Comparative activities of *syn* (HR 756) and *anti* isomers on β -lactamase-producing enteric bacteria. The IC99 of the *syn* (HR 756) and *anti* derivatives against 24 β -lactamase-pro-

ducing strains of enteric bacteria and against *E. coli* K-12 and ATCC 25922 as control strains are reported in Table 1. The IC99 of the *anti* oxymino derivative against susceptible *E. coli* K-12 was 3.12 $\mu\text{g/ml}$; this value was unaffected by the presence in this strain of plasmids pIP111, pIP71a, and pIP55, coding for different penicillinases. But the IC99s against strains producing cephalosporinases, such as *E. coli* LA0002, *K. pneumoniae* H28, *E. cloacae* P99, and *S. marcescens* R66, were consistently higher. In contrast, the activity of the *syn* derivative, HR 756, was very high against many strains, most of them being inhibited at 0.125 $\mu\text{g/ml}$. The activity of HR 756 remained unchanged by the plasmid-linked penicillinase production of *E. coli* K-12 and by the cephalosporinase of, for example, *K. pneumoniae* strain 1103 or C19, *E. cloacae* T45, or *P. morganii* F20. The ratio of IC99s obtained with the *anti* and *syn* (HR 756) derivatives was 100 for *E. coli* K-12 J53 and varied between 40 and 150 for 23 of the 26 other strains. If the IC99 against each strain of the *syn* and *anti* derivatives is plotted, the regression line shows that the ratio of these values is nearly constant for IC99s of HR 756 ranging from 0.01 to 1 $\mu\text{g/ml}$, and its value is about 80. Nevertheless, three strains of *E. cloacae*, J68, PI46, and P99, behaved quite differently, the ratios being 4, 4, and 1.5, respectively. It is noteworthy that the ratio for *E. cloacae* T45 was 70 and that for P99 was 1.5, whereas the isoelectric point was 8.34 for the β -lactamases of both.

Comparative activities of HR 756 and other penicillins and cephalosporins. A close comparison between the *syn* derivative, HR 756, and two penicillins, ampicillin and carbenicillin, and four cephalosporins, cephalothin, cephaloridine, cephalixin, and cefazolin, was carried out by determination of IC50s. The results are summarized in Table 2. With the exception of *E. cloacae* strains PI46 and P99, all IC50s of HR 756 were below 0.5 $\mu\text{g/ml}$. The activity of HR 756 against *E. coli* K-12 J53 was 30 to 150 times higher than the activities of the other antibiotics studied. The activity did not change when this strain was harboring a plasmid, such as pIP111, coding for a TEM 1 penicillinase, whereas the IC50 of ampicillin jumped from 1.42 to 880 $\mu\text{g/ml}$ and that of carbenicillin jumped from 1.64 to 6,000 $\mu\text{g/ml}$. *E. coli* cephalosporinase-producing strains LA0002 and SOL were highly resistant to cephalothin and cephalixin. The respective IC50s were 620 and 165 $\mu\text{g/ml}$ for cephalothin and 950 and 205 $\mu\text{g/ml}$ for cephalixin, versus 0.36 and 0.18 $\mu\text{g/ml}$ for HR 756. These values were similar to the IC50 observed with the *E. coli* K-12 control strain. Among *Serratia*, strain M01117 was inhibited

TABLE 1. *IC99s*

Bacterial strain	pI of enzyme	IC99 ($\mu\text{g/ml}$) of derivatives		<i>anti/syn</i> ratio
		<i>syn</i> (HR 756)	<i>anti</i>	
<i>E. coli</i> K-12 J53	9.17	0.0312	3.12	100
<i>E. coli</i> ATCC 25922		0.0625	6.25	100
<i>E. coli</i> K-12 LA0002	9.17	0.625	50	80
<i>E. coli</i> pIP111 (TEM)	5.4	0.0312	3.12	100
<i>E. coli</i> pIP71a (Im2)	7.7	0.040	3.12	80
<i>E. coli</i> pIP55 (oxa H)	6.97	0.0312	3.12	100
<i>E. coli</i> 049	9.21	0.1	8	80
<i>E. coli</i> 023		0.2	8	40
<i>E. coli</i> SOL	9.30	0.312	12.5	40
<i>Klebsiella</i> 1103	7.10	0.025	2.5	100
<i>Klebsiella</i> C19		0.025	2.5	100
<i>Klebsiella</i> H28		0.312	40	120
<i>Enterobacter</i> T45	8.34	0.0625	4	70
<i>Enterobacter</i> V462		0.08	6.25	80
<i>Enterobacter</i> PI116	9.02	0.125	8	64
<i>Enterobacter</i> G60		0.25	20	90
<i>Enterobacter</i> J68		10	40	4
<i>Enterobacter</i> PI46	8.34	10	40	4
<i>Enterobacter</i> P99	8.34	32	50	1.5
<i>P. morganii</i> F20	8.30	0.0125	0.5	40
<i>P. rettgeri</i> A19		0.25	12.5	50
<i>Providencia</i> C22S		0.062	2.5	40
<i>Serratia</i> H23		0.08	8	100
<i>Serratia</i> K11		0.08	6.25	80
<i>Serratia</i> R66		0.312	50	150
<i>Serratia</i> M01117	9.75	0.5	40	80

TABLE 2. *Comparative in vitro activities of HR 756 and reference compounds against lactamase-producing, gram-negative strains.*

Strain	IC ₅₀ ($\mu\text{g/ml}$) of:						
	Ampicillin	Carbenicillin	Cephalothin	Cephaloridine	Cephalexin	Cefazolin	HR 756
<i>E. coli</i> K-12 J53	1.42	1.65	2.15	1.10	3.6	0.67	0.023
<i>E. coli</i> K-12 pIP111 (TEM)	800	6,000	6.7	7.5	3.75	2.45	0.023
<i>E. coli</i> K-12 pIP71a	135	1,100	3.15	2.6	3.9	0.8	0.023
<i>E. coli</i> K-12 pIP55	25	98	3.7	1.4		1.08	0.023
<i>E. coli</i> LA0002	32.5	11	620	110	950	85	0.36
<i>E. coli</i> 049	3.12	2.3	7.5	1.5	8.5	1.15	0.07
<i>E. coli</i> SOL	36.5	4.3	165	7.6	205	5.5	0.18
<i>Klebsiella</i> 1103	11.7	55	1.3	1.6	2.5	0.66	0.022
<i>Klebsiella</i> C19	1,425	4,300	7.2	7.5	3.6	3.1	0.022
<i>Klebsiella</i> H28	8.5	47	45	31	80	22.5	0.28
<i>Enterobacter</i> T45	6.25	0.95	23.5	115	11	125	0.05
<i>Enterobacter</i> PI116	350	2.1	1,050	375	430	815	0.062
<i>Enterobacter</i> PI46	100	17	2,900	200	640	350	2.4
<i>Enterobacter</i> P99	320	9.5	2,500	330		1,200	27.2
<i>P. morganii</i> F20	17.5	0.32	245	280	22.5	80	0.01
<i>P. rettgeri</i> A19	370	470	900	285		250	0.1
<i>Providencia</i> C22S	10.2	0.4	19	95	15.5	2.5	0.04
<i>Serratia</i> H23	9	2.5	3,000		100	1,130	0.076
<i>Serratia</i> R66	1,400	6,300	1,100	1,125	180	1,680	0.2
<i>Serratia</i> M01117	90	5.5	11,500	7,200	5,000	12,000	0.31

only by high concentrations of cephalothin (11,500 $\mu\text{g/ml}$), cephaloridine (7,200 $\mu\text{g/ml}$), cephalexin (5,000 $\mu\text{g/ml}$), and cefazolin (12,000 $\mu\text{g/ml}$). In contrast, HR 756 inhibited this strain

at 0.31 $\mu\text{g/ml}$. A similar picture is seen with *E. cloacae* PI116 and *Serratia* H23; the cephalosporins studied were only active at concentrations ranging from 100 to 3,000 $\mu\text{g/ml}$, but the

TABLE 4. Comparative activities of HR 756 and cefazolin against clinical isolates

Isolates (no.)		MIC ($\mu\text{g/ml}$) to inhibit cumulative % of isolates					
		HR 756			Cefazolin		
		50%	75%	90%	50%	75%	90%
<i>E. coli</i>	(186)	0.07	0.12	0.23	1.60	2.60	20
<i>Salmonella</i>	(25)	0.12	0.18	0.36	1.70	2.40	3
<i>Citrobacter</i>	(12)	2.40	4.0	9.0	>40		
<i>K. pneumoniae</i>	(80)	0.08	0.13	0.24	5.50	24	>40
<i>K. oxytoca</i>	(11)	0.04	0.06	0.10	2.40	12	>40
<i>E. cloacae</i>	(48)	30	>40		>40		
<i>Serratia</i>	(31)	0.85	2.0	5.0	>40		
<i>P. mirabilis</i>	(40)	0.03	0.05	0.50	9.0	19	>40
<i>P. vulgaris</i>	(14)	0.75	1.45	1.90	>40		
<i>P. morganii</i>	(11)	0.48	1.55	3.10	>40		
<i>P. rettgeri</i>	(16)	0.50	1.40	5.0	>40		
<i>Providencia</i>	(18)	0.85	1.70	2.50	>40		

also studied on some strains belonging to different bacterial groups: *Corynebacterium*; *B. subtilis* and *Bacillus mycoides*; *M. duplex* and *M. lacunata*; *A. hydrophila*; *Y. enterocolitica*; and anaerobic bacteria, such as *C. perfringens*, *F. necrophorum*, and *B. fragilis*. Most of these strains were inhibited by 0.02 $\mu\text{g/ml}$, and all were inhibited by 0.5 $\mu\text{g/ml}$.

DISCUSSION

New cephalosporins are mainly selected to be active against β -lactamase-producing, gram-negative bacteria. It is often difficult to evaluate whether a new compound fulfills this requirement, because a classification including all β -lactamases has never been established. As pointed out by Sykes and Matthew (21), β -lactamases are likely to be gene, species, and subspecies specific, and "the diversity of β -lactamases will be as extensive as that of bacteria." In addition, β -lactamases are not the only means of bacterial resistance to cephalosporins, and so-called intrinsic factors may play a role, alone or in conjunction with these enzymes. For these reasons, the collection of 26 strains used in this report does not purport to be representative of all β -lactamases but only of the groups most commonly observed. Against such strains, the differences in activity between the two isomeric derivatives of the methoxyimino cephalosporin are clear-cut. The inhibitory concentration of the *anti* isomer against *E. coli* is 3 to 6 $\mu\text{g/ml}$. This value is not far from the geometric mean MIC reported for cefuroxime (another methoxyimino cephalosporin) by O'Callaghan et al. (18) and Eykyn et al. (7) or from the mean MICs reported for other cephalosporins, such as cephalothin, cephaloridine, cefoxitin, and cefamandole (7, 17). But, as we have seen, the *syn* derivative, HR 756, is at least 80 times more

active than the *anti* one. The range of inhibitory concentrations of HR 756 against gram-negative bacteria, susceptible or resistant to penicillins and cephalosporins, was from 0.01 to 0.1 $\mu\text{g/ml}$. This activity is regarded as unusual against enteric bacteria; such a range of inhibitory concentrations has only been observed before with penicillin G and rifamycins against cocci. In addition, the MICs observed against bacteria isolated from the upper respiratory tract, *Haemophilus*, *S. pneumoniae*, and *S. pyogenes* group A, are very low. If we compare our results on *S. pneumoniae* with those reported by Eykyn et al. (7), HR 756 appears to be the most active cephalosporin against these bacteria. Perhaps the most interesting point is the activity of HR 756 against *Haemophilus* strains, penicillinase producing or not. The MICs range between 0.004 and 0.03 $\mu\text{g/ml}$ (median, 0.01 $\mu\text{g/ml}$) for susceptible *Haemophilus* strains and for six out of nine penicillinase-producing strains (three others are inhibited by 0.1 $\mu\text{g/ml}$). In our observations, ampicillin-susceptible *Haemophilus* strains are only inhibited by 0.1 to 1 $\mu\text{g/ml}$, and ampicillin is inactive against penicillinase-producing strains. If we compare the median MIC of HR 756 (0.012 $\mu\text{g/ml}$) with the median MICs reported by Finland et al. (8) for 16 penicillin analogs, HR 756 appears to be at least 10 times more active against *Haemophilus* than the best of them. Penicillinase-producing *Haemophilus* strains harboring plasmids coding for a TEM-like penicillinase (5) are susceptible to HR 756 because the antibiotic is resistant to this enzyme.

Nevertheless, the *syn* derivative, HR 756, was not highly effective against all β -lactamase-producing enteric bacteria. Many strains of *E. cloacae*, including P99 (14, 15), were resistant, whereas strain T45 was very susceptible to HR 756. The isoelectric points of the cephalospori-

nases produced by P99 and T45 were the same, 8.34. In addition, the ratio of the activities of *anti*- and *syn*-stereoisomer derivatives was only 1.5 with P99, in contrast to 80 for the majority of strains. The resistance to HR 756 of *E. cloacae* P99 might be interpreted as a characteristic related to a modification of the permeability barrier or enzyme activity. These *syn* and *anti* derivatives might thus provide interesting tools for studying the mode of action of β -lactamase-resistant antibiotics on the cell wall.

ACKNOWLEDGMENTS

We thank Eliane Derlot and Françoise Delbos for expert technical assistance.

LITERATURE CITED

1. Bucourt, R., R. Heymes, A. Lutz, L. Penasse, and J. Perronnet. 1977. Propriétés antibiotiques inattendues dans le domaine des céphalosporines. C. R. Acad. Sci. Ser. D 284:1847-1849.
2. Burman, L. G., K. Nordström, and H. G. Boman. 1968. Resistance of *Escherichia coli* to penicillins. V. Physiological comparison of two isogenic strains, one with chromosomally and one with episomally mediated ampicillin resistance. J. Bacteriol. 96:438-446.
3. Chabbert, Y. A., M. R. Scavizzi, J. L. Witchitz, G. R. Gerbaud, and D. H. Bouanchaud. 1972. Incompatibility groups and the classification of *f_i⁻* resistance factors. J. Bacteriol. 112:666-675.
4. Dale, J. W., and J. T. Smith. 1974. R-factor mediated β -lactamases that hydrolyze oxacillin: evidence for two distinct groups. J. Bacteriol. 119:351-356.
5. Elwell, L. P., J. De Graaff, D. Seibert, and S. Falkow. 1975. Plasmid-linked ampicillin resistance in *Haemophilus influenzae* type b. Infect Immun. 12:404-410.
6. Ericsson, H. M., and J. C. Sherris. 1971. Antibiotic susceptibility testing. Report of an international collaborative study. Acta Pathol. Microbiol. Scand. Sect. B 217(Suppl.):3-90.
7. Eykyn, S., C. Jenkins, A. King, and I. Phillips. 1976. Antibacterial activity of cefuroxime, a new cephalosporin antibiotic, compared with that of cephaloridine, cephalothin, and cefamandole. Antimicrob. Agents Chemother. 9:690-695.
8. Finland, M., C. Garner, C. Wilcox, and L. D. Sabath. 1976. Susceptibility of pneumococci and *Haemophilus influenzae* to antibacterial agents. Antimicrob. Agents Chemother. 9:274-287.
9. Labia, R., J. Andrillon, and F. Le Goffic. 1973. Computerized microacidimetric determination of β -lactamase Michaelis-Menten constants. FEBS Lett. 33:42-43.
10. Labia, R., and M. Barthelemy. 1977. Problèmes de la détermination des points isoélectriques des β -lactamases. C.R. Acad. Sci. Ser. D 284:1729-1732.
11. Labia, R., G. Brunet, M. Guionie, A. Philoppon, M. Heitz, and J. S. Pitton. 1976. Céphalosporinases constitutives de *Escherichia coli*. Ann. Microbiol. 127B:453-461.
12. Labia, R., M. Guionie, and C. Fabre. 1976. Cephalosporinases inducibles de *Proteus morganii*. Biochimie 58:1083-1087.
13. Labia, R., F. Le Goffic, and J. Andrillon. 1974. Etude cinétique de deux β -lactamases responsables d'un même phénotype. Biochimie 56:1025-1030.
14. Marshall, M. J., G. W. Ross, K. V. Chanter, and A. M. Harris. 1972. Comparison of the substrate specificities of the β -lactamases from *Klebsiella aerogenes* 1082E and *Enterobacter cloacae* P99. Appl. Microbiol. 23:765-769.
15. Neu, H. C., and E. B. Winshell. 1972. Relation of β -lactamase activity and cellular location to resistance of *Enterobacter* to penicillins and cephalosporins. Antimicrob. Agents Chemother. 1:107-111.
16. Normark, S., T. Edlund, T. Grundström, S. Bergström, and H. Wolf-Watz. 1977. *Escherichia coli* K-12 mutants hyperproducing chromosomal beta-lactamase by gene repetitions. J. Bacteriol. 132:912-922.
17. Norrby, R., J.-E. Brorsson, and S. Seeberg. 1976. Comparative study of the in vitro antibacterial activity of cefoxitin, cefuroxime, and cephaloridine. Antimicrob. Agents Chemother. 9:506-510.
18. O'Callaghan, C. H., R. B. Sykes, A. Griffiths, and J. E. Thornton. 1976. Cefuroxime a new cephalosporin antibiotic: activity in vitro. Antimicrob. Agents Chemother. 9:511-519.
19. Richmond, M. H., and R. B. Sykes. 1973. β -Lactamases of Gram-negative bacteria and their possible physiological role. Adv. Microb. Physiol. 9:31-38.
20. Roupas, A., and J. S. Pitton. 1974. R factor-mediated and chromosomal resistance to ampicillin in *Escherichia coli*. Antimicrob. Agents Chemother. 5:186-191.
21. Sykes, R. B., and M. Matthew. 1976. The β -lactamases of Gram-negative bacteria and their role in resistance to β -lactam antibiotics. J. Antimicrob. Chemother. 2:115-157.