ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1986, p. 980–985
0066-4804/86/060980-06$02.00/0

Vol. 29, No. 6

Comparative Activities of the β-Lactamase Inhibitors YTR 830, Clavulanate, and Sulbactam Combined with Ampicillin and Broad-Spectrum Penicillins against Defined β-Lactamase-Producing Aerobic Gram-Negative Bacilli

MICHAEL R. JACOBS,[1]* STEPHEN C. ARONOFF,[2] SHARON JOHENNING,[1] DAVID M. SHLAES,[3] AND SHIGERU YAMABE[4]

Departments of Pathology[1] and Pediatrics,[2] School of Medicine, Case Western Reserve University, and Laboratory and Research Services, Veterans Administration Medical Center,[3] Cleveland, Ohio 44106, and Division of Chemotherapy, Kobe College Research Institute, Nishinomiya, Japan[4]

Received 23 October 1985/Accepted 11 March 1986

The in vitro synergistic activities of the β-lactamase inhibitors YTR 830, clavulanate, and sulbactam, combined with ampicillin, ticarcillin, mezlocillin, azlocillin, piperacillin, and apalcillin, were determined against 34 strains of members of the *Enterobacteriaceae* family, *Pseudomonas aeruginosa*, *Aeromonas hydrophila*, and *Haemophilus influenzae* with characterized plasmid or chromosomal β-lactamases or both. Strains were tested against fixed concentrations of β-lactamase inhibitors (8 μg/ml) combined with doubling dilutions of β-lactams. Synergy was defined as a fourfold or greater decrease in the MIC of the β-lactam. Against *Enterobacteriaceae* producing Richmond and Sykes class III and V plasmid-mediated β-lactamases, synergy was obtained against most strains with YTR 830– and clavulanate–β-lactam combinations, with sulbactam being less effective. Against *Enterobacteriaceae* producing class I chromosomal β-lactamases, combinations containing YTR 830 or sulbactam were more synergistic than combinations containing clavulanate. Against strains producing class V PSE enzymes, all three inhibitors were synergistic with piperacillin and apalcillin against strains producing PSE-1, -3, and -4 enzymes, while the PSE-2-producing strain was resistant to all inhibitors. YTR 830–β-lactam combinations were also synergistic against strains producing the novel β-lactamases OHIO-1, TLE-1, AER-1, and ROB-1. Overall, YTR 830 with piperacillin or apalcillin was the most effective combination.

Inactivation of β-lactams by β-lactamases has been known for almost 40 years, and this problem was predominantly addressed by the development of compounds resistant to the activity of β-lactamases (4). Over the last 10 years, however, attention has been paid to compounds with very little intrinsic activity, but with the ability to irreversibly inhibit β-lactamases. Such inhibitors include clavulanic acid, halopenicillanic acids, and penicillanic acid sulfones such as sulbactam and YTR 830 (6).

Clavulanate is the most extensively characterized β-lactamase inhibitor and demonstrates activity against β-lactamases of *Staphylococcus aureus*, the gram-negative plasmid-mediated enzymes (TEM, OXA, HMS, SHV, and PSE), and the chromosomal enzymes of *Klebsiella* spp., *Proteus vulgaris*, and *Bacteroides fragilis* (6). Sulbactam is a less potent but broader-spectrum β-lactamase inhibitor, with up to 100-fold less acitivity against class III enzymes and up to 100-fold better activity against class I enzymes than clavulanate (1). Sulbactam does not appear to be a potent inducer of chromosomal β-lactamases (4), whereas clavulanate is (12, 20). YTR 830 is a penicillanic acid sulfone derivative which inhibits the plasmid-encoded β-lactamases of many gram-negative bacilli (2; F. Moosden, J. D. Williams, and S. Yamabe, Proc. 14th Int. Congr. Chemother., abstr. no. S13-13, p. 123, 1985).

With the introduction of clavulanate combined with amoxicillin and ticarcillin for oral and parenteral administration, respectively, β-lactamase inhibitors have been shown to have a useful place in the therapy of many infections caused by β-lactamase-producing organisms (5, 6, 8). The spectrum of activity of these combinations includes β-lactamase-producing strains of *S. aureus*, *Haemophilus influenzae*, *Neisseria gonorrhoeae*, *Klebsiella pneumoniae* and some strains of other members of the *Enterobacteriaceae* family (6, 14).

The purpose of this study was to evaluate the activity of YTR 830 against strains of members of the *Enterobacteriaceae* and *Pseudomonas aeruginosa* with defined β-lactamases and to compare this activity with those of two other β-lactamase inhibitors, sulbactam and clavulanate.

MATERIALS AND METHODS

Study design. The study was designed to detect synergy of β-lactams with β-lactamase inhibitors, using known defined β-lactamase-producing strains of members of the *Enterobacteriaceae*, *P. aeruginosa*, *Aeromonas hydrophila*, and *H. influenzae*, and to compare the activity of the three β-lactamase inhibitors combined with six β-lactams. The β-lactamase inhibitors were tested in fixed concentrations of 8 μg/ml (2 μg/ml used for the *H. influenzae* strain) to represent achievable serum levels of clavulanate and sulbactam by intravenous administration (3, 19) and to enable optimal β-lactam–β-lactamase synergy to occur without significant antibacterial activity of the β-lactamase inhibitors alone.

Test strains. Thirty-four strains with characterized plasmid or chromosomal β-lactamases or both were used in this study. Fifteen strains were *Escherichia coli* recipients into

* Corresponding author.

which known β-lactamase-coding plasmids had been transferred by conjugation or transformation (9, 10; D. M. Shlaes, A. A. Medeiros, C. Currie-McCumber, E. Papa, and C. V. Vartian, Antimicrob. Agents Chemother., in press). Five strains were *P. aeruginosa* recipients with minimal chromosomal β-lactamase production in the absence of an inducer (A. A. Medeiros, personal communication) into which PSE and OXA-6 plasmids had been transferred. Eleven strains were wild clinical isolates of *Enterobacter*, *Citrobacter*, and *Serratia* species producing chromosomal β-lactamases and resistant to cefotaxime or moxalactam; seven of these strains also produced plasmid-mediated β-lactamases (11). Two strains were organisms with novel β-lactamases (*A. hydrophila* AER-1 and *H. influenzae* ROB-1), kindly provided by A. A. Medeiros (Miriam Hospital, Providence, R.I.) (7, 10). *E. coli* ATCC 35218 was also included as a known TEM-1 β-lactamase-producing strain. In addition, *E. coli* ATCC 25922 was included as a negative control. All strains were maintained at −70°C and subcultured in the presence of ampicillin prior to testing.

Antimicrobial agents. Laboratory reference powders of the following agents were used: ampicillin (Bristol Laboratories, Syracuse, N.Y.), ticarcillin (Beecham Laboratories, Bristol, Tenn.), mezlocillin and azlocillin (Miles Laboratories, West Haven, Conn.), piperacillin (Lederle Laboratories, Pearl River, N.Y.), and apalcillin (Sumitomo Pharmaceutical Co., Ltd., Osaka, Japan).

β-Lactamase inhibitors used were YTR 830 (Taiho Pharmaceutical Co., Tokyo, Japan), clavulanic acid (Beecham Laboratories), and sulbactam (Pfizer Inc., New York, N.Y.).

β-Lactamase production. The presence of β-lactamase was tested by the chromogenic cephalosporin method on growth in a β-lactam-containing microdilution well, using nitrocefin (Glaxo Inc., Durham, N.C.) (16). A 25-μl portion of nitrocefin solution (500 μg/ml in 0.05 M phosphate buffer, pH 7.0) was added to the test well. Development of a red color within 10 min was read as positive.

Synergy with β-lactamase inhibitors. Isolates were tested by broth microdilution in 96-well trays, using a Dynatech MIC 2000 system (Dynatech Laboratories, Inc., Alexandria, Va.) (12). Trays contained 0.1-ml volumes of doubling dilutions of β-lactams (0.5 to 512 μg/ml) alone and combined with fixed concentrations of 8 μg of the β-lactamase inhibitors per ml (2 μg/ml for *H. influenzae*). Control wells containing β-lactamase inhibitors alone at 8 μg/ml (2 μg/ml for *H. influenzae*), as well as growth and sterility wells, were included. Medium used was cation-supplemented Mueller-Hinton broth (supplemented with 1% hemin and 1% NAD for *H. influenzae*). Trays were stored at −70°C until used. Trays were inoculated with 10^5 to 10^6 organisms per ml, using a Dynatech MIC 2000 inoculator, and incubated overnight (18 to 20 h) at 35°C. The lowest β-lactam concentration showing no growth was read as the MIC. *E. coli* ATCC 25922 and 35218 were tested weekly during the study period for quality control assurance. MICs were interpreted according to current recommendations of the National Committee for Clinical Laboratory Standards (13). MICs of the β-lactamase inhibitors alone were also determined. Synergy was defined as a fourfold or greater decrease in the MIC of a β-lactam in the presence of a β-lactamase inhibitor compared with the β-lactam alone; antagonism was defined as a fourfold or greater increase in the MIC of a β-lactam alone in the presence of a β-lactamase inhibitor compared with the β-lactam alone (15; M. D. Kitzis, L. Gutman, S. Yamabe, and J. F. Acar, Proc. 14th Int. Congr. Chemother., abstr. no. P45-63, p. 412, 1985).

RESULTS

Bacterial isolates. All isolates produced β-lactamase by the chromogenic cephalosporin method. All were resistant to ampicillin (MICs > 16 μg/ml) (11), with most having MICs of >512 μg/ml (see Tables 1 to 3). Most strains were also highly resistant to ticarcillin (MICs ≥ 256 μg/ml), except for the four *Enterobacteriaceae* family strains producing chromosomal β-lactamase only, which were moderately susceptible (64 μg/ml) or just in the resistant range (128 μg/ml).

Susceptibility to the other β-lactams was variable, with susceptible strains inhibited by 4 to 16 μg/ml. *E. coli* ATCC 25922 was susceptible to all agents (MICs of 1 to 8 μg/ml), and synergy was not observed with any of the β-lactamase inhibitors. With the exception of *H. influenzae* ROB-1, no strains were inhibited by the β-lactamase inhibitors alone at the concentration tested (8 μg/ml), and MICs of the inhibitors alone were all ≥32 μg/ml. MICs of the inhibitors alone for *H. influenzae* ROB-1 were 8 to 16 μg/ml, and the concentration of the inhibitors was therefore lowered to 2 μg/ml for synergy testing of this strain.

Synergy studies. (i) Plasmid-bearing *E. coli* strains (Table 1). Synergy was demonstrated with all β-lactams combined with YTR 830 or clavulanate against all 16 strains, including OXA-producing strains susceptible to mezlocillin, piperacillin, and apalcillin alone. MICs of the β-lactams were reduced by 4- to >512-fold, with all except two MICs being reduced to <32 μg/ml. Sulbactam was synergistic with the various β-lactams against 9 to 13 of the 16 strains, with MICs reduced by 4- to >512-fold for 67 of the 97 combinations. MICs remained in the resistant range despite synergy for five strains with ampicillin-sulbactam, two with ampicillin-YTR 830, one with ticarcillin-sulbactam, and one with azlocillin-sulbactam. Strains for which no synergy with sulbactam was observed produced TEM-1, TEM-2, HMS-1, and OHIO-1 β-lactamases. For combinations showing synergy with sulbactam, the reduction in MICs of the β-lactams was equal or superior to that of clavulanate and YTR 830 for 24 and 35 combinations, respectively, and inferior for 43 and 32 combinations, respectively.

(ii) Plasmid-bearing *P. aeruginosa* strains (Table 2). No synergy between ampicillin and the inhibitors was seen. Ticarcillin and clavulanate were synergistic against strains producing PSE-1, -3, and -4 and OXA-6 enzymes; against the PSE-3-producing strain, ticarcillin-YTR 830 and ticarcillin-sulbactam also showed synergy. None of the combinations was synergistic against the PSE-2-producing strain.

Azlocillin alone was active against the PSE-1 and -3-producing strains. Azlocillin was synergistic with all three inhibitors against the PSE-4-producing strain and with sulbactam and YTR 830 against the PSE-3-producing strain. Azlocillin-clavulanate was antagonistic against the PSE-1-producing strain.

Mezlocillin was synergistic with the three inhibitors against the PSE-1-, -3-, and -4-producing strains, although the MIC of mezlocillin remained in the resistant range when combined with sulbactam and YTR 830. Piperacillin and apalcillin were synergistic with the three inhibitors against the same three strains, with reduction of MICs of the β-lactams to clinically achievable levels.

(iii) *A. hydrophilia* AER-1 (Table 2). All β-lactam combinations with YTR 830 and clavulanate were synergistic against *A. hydrophilia* AER-1, although the MIC of ampicillin-YTR 830 remained in the resistant range. Sulbactam was synergistic in combination with ticarcillin, piperacillin, and apalcillin, but not with ampicillin or azlocillin.

TABLE 1. MICs of β-lactams alone and with inhibitors against 16 plasmid-bearing *E. coli* strains[a]

Strain	β-lactamase	MIC (μg/ml)																					
		Ampicillin				Ticarcillin				Azlocillin				Mezlocillin				Piperacillin				Apalcillin			
		Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830
ATCC 35218	TEM-1	512	**2**	**2**	**2**	>512	**2**	**8**	**2**	128	**2**	**2**	**4**	64	**<1**	**<1**	**<1**	32	**<1**	**<1**	**<1**	32	**<1**	**<1**	**<1**
R6K-R	TEM-1	>512	**4**	>512	**4**	>512	**16**	>512	**8**	512	**4**	**64**	**8**	128	**1**	**16**	**2**	64	**1**	**8**	**1**	128	**<1**	**16**	**1**
C600-pBR322	TEM-1	>512	**8**	>512	**8**	>512	**64**	>512	**64**	>512	**2**	>512	**2**	512	**2**	512	**4**	128	**1**	128	**1**	>512	**1**	>512	**2**
C600-RP1	TEM-2	>512	**4**	>512	**4**	>512	**16**	>512	**16**	>512	**4**	**256**[b]	**4**	512	**1**	**64**	**2**	64	**1**	32	**2**	512	**<1**	256	**1**
1725-RP1	TEM-2	>512	**8**	>512	**8**	>512	**32**	>512	**32**	>512	**8**	>512	**8**	256	**1**	128	**1**	64	**1**	64	**1**	512	**1**	512	**1**
J53.2-R997	HMS-1	>512	**8**	>512	**4**	>512	**64**	>512	**64**	>512	**4**	>512	**8**	128	**<1**	64	**<1**	64	**<1**	32	**<1**	256	**<1**	**64**	**<1**
J53-R1010	SHV-1	>512	**1**	**256**[b]	**2**	>512	**4**	>512	**8**	512	**2**	**32**	**4**	64	**1**	**4**	**1**	32	**<1**	**4**	**<1**	64	**<1**	**8**	**<1**
1527-RGN238	OXA-1	512	**8**	**64**[b]	**64**[b]	256	**8**	**16**	**16**	256	**8**	**8**	**32**	16	**1**	**1**	**2**	16	**1**	**2**	**1**	64	**2**	**<1**	**2**
1573-R46	OXA-2	32	**<1**	**<1**	**1**	64	**<1**	**<1**	**2**	16	**2**	**1**	**2**	8	**<1**	**<1**	**<1**	4	**<1**	**<1**	**<1**	8	**<1**	**<1**	**<1**
1894E-R576	OXA-3	128	**2**	**2**	**2**	128	**4**	**1**	**1**	32	**2**	**2**	**4**	4	**<1**	**<1**	**<1**	4	**<1**	**<1**	**<1**	4	**<1**	**<1**	**<1**
7529-pMG203	OXA-4	512	**16**	**32**[b]	**64**[b]	256	**8**	**32**	**32**	256	**8**	**16**	**16**	16	**2**	**4**	**4**	16	**2**	**4**	**4**	16	**1**	**2**	**4**
C600-R388	OXA-5	256	**16**	**64**[b]	**16**	>512	**32**	**128**[b]	**32**	128	**4**	**16**	**8**	16	**<1**	**2**	**2**	16	**<1**	**2**	**1**	16	**<1**	**1**	**<1**
7181-pMG202	OXA-7	512	**4**	**64**[b]	**16**	512	**4**	**64**	**16**	256	**1**	**16**	**8**	16	**<1**	**2**	**1**	16	**<1**	**2**	**1**	16	**<1**	**2**	**<1**
C600-pD5075	OHIO-1	>512	**8**	>512	**32**[b]	>512	**16**	>512	**64**	>512	**16**	512	**32**	256	**2**	128	**2**	64	**1**	64	**1**	256	**1**	**64**	**1**
C600-pDS076	OHIO-1	512	**2**	**8**	**4**	>512	**2**	**32**	**4**	128	**4**	**4**	**4**	16	**<1**	**1**	**1**	16	**<1**	**<1**	**<1**	32	**<1**	**<1**	**<1**
7604-cilV	TLE-1	>512	**2**	**4**	**2**	>512	**2**	**8**	**2**	256	**2**	**8**	**8**	32	**<1**	**2**	**2**	32	**<1**	**1**	**1**	32	**<1**	**<1**	**1**

[a] Bold type indicates synergy (fourfold or greater reduction in MIC). *E. coli* ATCC 35218 also produces a chromosomal β-lactamase with a pI of 9.2 (S. Aronoff and P. Labrozzi, Abst. 25th Intersci. Conf. Antimicrob. Agents Chemother., abstr. no. 983, p. 274, 1985).

[b] MIC in resistant range despite synergy.

(iv) ***H. influenzae* ROB-1.** *H. influenzae* ROB-1 was tested against ampicillin, which had a MIC of 4 μg/ml, and against ampicillin combined with the three inhibitors, where MICs of ampicillin were reduced to <1 μg/ml. The ROB-1 strain was readily β-lactamase positive with nitrocefin both in solution, as used for the other study strains, and in disk form (Cefinase; BBL Microbiology Systems, Cockeysville, Md.); this contrasts with the original description of this strain, which was described as having a weak, delayed nitrocefin reaction (18).

(v) Strains of *Enterobacteriaceae* with chromosomal β-lactamases, with and without plasmid-mediated β-lactamases (Table 3). These members of the *Enterobacteriaceae* were resistant to ampicillin and resistant, or in a few instances, moderately susceptible to the broad-spectrum penicillins. No synergy was detected with the ampicillin-inhibitor combinations. The four strains with only chromosomal β-lactamase were inhibited by 32 to 128 μg of ticarcillin or mezlocillin per ml and 64 to 512 μg of piperacillin or apalcillin per ml. Synergy with the inhibitors occurred in only 4 of 20 β-lactam–clavulanate combinations. β-Lactam–sulbactam and β-lactam–YTR 830 were synergistic in 13 and 12 of the 20 combinations, respectively.

Against the four strains producing only chromosomal β-lactamase, ampicillin, ticarcillin, and azlocillin showed little useful synergy with the inhibitors. Mezlocillin-clavulanate and mezlocillin-YTR 830 were synergistic against one strain each; mezlocillin-sulbactam was synergistic against two strains. Piperacillin and apalcillin were synergistic with clavulanate against one strain, with YTR 830 against three strains, and with sulbactam against all four strains.

Comparison of the susceptibilities of strains producing only chromosomal β-lactamase and those producing both chromosomal and plasmid-mediated enzymes is shown in Table 3. Synergy occurred in both groups with many of the combinations against the *Enterobacter* and *Citrobacter* strains, but was less frequently observed with *Serratia* spp.

Overall, combined with broad-spectrum penicillins, clavulanate was synergistic with β-lactams in 27 of 55 combinations (49%); sulbactam, in 22 combinations (40%); and YTR 830, in 38 combinations (69%). Despite synergy, MICs of the β-lactams in the combinations remained in the resistant range for six ticarcillin, 12 azlocillin, 3 piperacillin, and 3 apalcillin combinations.

TABLE 2. MICs of β-lactams alone and with inhibitors against five plasmid-bearing *P. aeruginosa* strains and *A. hydrophila* AER-1[a]

Strain	β-lactamase	MIC (μg/ml)																	
		Ampicillin			Ticarcillin			Azlocillin			Mezlocillin			Piperacillin			Apalcillin		
		Alone	+ Clavulanate	+ Sulbactam	Alone	+ Clavulanate	+ Sulbactam	Alone	+ Clavulanate	+ Sulbactam	Alone	+ Clavulanate	+ Sulbactam	Alone	+ Clavulanate	+ Sulbactam	Alone	+ Clavulanate	+ Sulbactam
1937-RP11	PSE-1	>512	>512	>512	>512	**64**	512	16	**64**[b]	8	512	**64**	**128**[c]	128	**16**	**32**	32	**2**	**8**
Pu21-R151	PSE-2	>512	>512	>512	256	256	256	128	64	64	256	256	256	64	32	32	16	8	8
Pu21-RMS149	PSE-3	>512	>512	>512	**32**	**32**	**32**	64	32	**8**	256	**64**	**64**	**16**	**8**	**8**	16	2	2
Pu21-pMG19	PSE-4	>512	>512	>512	**64**	>512	256	256	**32**	**32**	512	**64**	**128**[c]	128	**32**	**32**	128	2	**8**
PAO38-pMG329	OXA-6	>512	>512	>512	256	256	256	128	64	128	128	64	128	64	**16**	64	8	4	8
A. hydrophila[d]	AER-1	>512	**16**	512	**256**[c]	256	**4**	32	**1**	16	**4**	**<1**	8	**2**	8	**<1**	**<1**	8	**2**

[a] Bold type indicates synergy (fourfold or greater reduction in MIC).
[b] Combination antagonistic.
[c] MIC in resistant range despite synergy.
[d] *A. hydrophila* VL7711. This strain produces two β-lactamases, presumably determined by chromosomal genes, in addition to the transferable AER-1 enzyme (7).

DISCUSSION

Activity of β-lactamase inhibitors has extended the spectrum of β-lactams to include strains of *Enterobacteriaceae* and *P. aeruginosa* producing class I, III, IV, and V enzymes (2, 6, 14, 17). This study has shown that β-lactams combined with YTR 830 or clavulanate have similar activity, which is superior to that of β-lactam–sulbactam combinations (Table 4). Against *E. coli* recipients of enterobacterial plasmid-mediated β-lactamases, combinations containing YTR 830 or clavulanate were essentially equivalent. Against *Enterobacteriaceae* producing chromosomal enzymes, combinations containing YTR 830 and sulbactam were equally synergistic.

Against PSE-1-producing *P. aeruginosa* strains, azlocillin and apalcillin alone were active, as was piperacillin with any of the inhibitors. Piperacillin or apalcillin combined with any of the inhibitors was most active against the PSE-3- and -4-producing strains, while none of the combinations tested was effective against the PSE-2-producing strain. Combinations containing YTR 830 and clavulanate were synergistic

TABLE 3. MICs of β-lactams alone and with inhibitors against 11 strains of *Enterobacteriaceae* with chromosomal β-lactamase, with and without plasmid-mediated β-lactamases[a]

Organism	Strain	pI of chromosomal β-lactamase[b]	Plasmid β-lactamase(s)	MIC (μg/ml)																							
				Ampicillin				Ticarcillin				Azlocillin				Mezlocillin				Piperacillin				Apalcillin			
				Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830	Alone	+ Clavulanate	+ Sulbactam	+ YTR 830
Enterobacter aerogenes	83120208	7.82	None	>512	>512	>512	>512	64	64	64	128	>512	**128**[c]	**256**[c]	512	64	**8**	**16**	32	128	**16**	**16**	**32**	64	**16**	**16**	32
	83120220	7.82	TEM-1, OHIO-1	>512	>512	>512	>512	>512	**64**	>512	**128**[c]	>512	**128**[c]	>512	**128**[c]	>512	**8**	>512	**16**	>512	**16**	>512	**16**	>512	**16**	>512	**16**
	83120210	7.9	TEM-1	>512	>512	>512	>512	>512	**64**	>512	**64**	>512	**64**	>512	**256**[c]	256	**2**	**64**	**8**	256	**8**	**64**	**32**	256	**8**	**64**	**16**
Enterobacter cloacae	83120216	7.66	None	>512	>512	>512	>512	128	256	128	128	>512	>512	512	**256**[c]	64	32	32	**16**	512	128	**32**	**16**	256	256	**64**	**32**
	83120218	8.2	OHIO-1	>512	>512	>512	>512	>512	**64**	>512	512	>512	**128**[c]	512	**128**[c]	128	**8**	**32**	**16**	128	**8**	64	**8**	128	**8**	64	**16**
	83120223	8.0	TEM-1	>512	>512	>512	>512	>512	**256**[c]	>512	>512	>512	>512	>512	512	512	**32**	512	**32**	>512	**128**[c]	512	**256**[c]	>512	**256**[c]	>512	**256**[c]
Citrobacter freundii	83120221	8.10	None	>512	>512	>512	>512	128	128	64	**32**	>512	>512	**256**[c]	**64**	32	16	**8**	**4**	64	64	**16**	**8**	64	64	**8**	**2**
	83120211	8.10	TEM-1	>512	>512	>512	>512	>512	**128**[c]	**128**[c]	**64**	>512	>512	512	**128**[c]	128	**16**	**16**	**8**	128	64	**32**	**8**	128	64	**32**	**8**
Serratia marcescens	83120230	8.5	None	256	512	256	256	64	64	**16**	32	>512	>512	**256**[c]	**256**[c]	64	128	32	64	64	128	**16**	32	128	128	**32**	**32**
	83120212	8.3	TEM-1	>512	>512	>512	>512	>512	**256**[c]	>512	**128**[c]	>512	>512	>512	512	512	256	256	**64**	256	128	128	**32**	>512	**256**[c]	512	**64**
	83120202	8.3	TEM-1, OXA-2	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	>512	512	>512	512	>512	512	>512	**256**[c]	>512	512	512	512

[a] Bold type indicates synergy.
[b] Isoelectric focusing data from Medeiros (personal communication).
[c] MIC in resistant range despite synergy.

TABLE 4. Cumulative susceptibilities of strains tested (excluding *A. hydrophila* and *H. influenzae*)

β-Lactamase class	No. of strains tested	Antibiotic	No. of strains susceptible[a]			
			β-Lactam alone	β-Lactam with[b]:		
				Clavulanate	Sulbactam	YTR 830
I (chromosomal)	4	Ampicillin	0	0	0	0
		Ticarcillin	2	0	1	1
		Azlocillin	0	0	0	1
		Mezlocillin	4	0	0	0
		Piperacillin	2	1	2	2
		Apalcillin	2	0	2	2
III (TEM, HMS, SHV, OHIO, TLE)	9	Ampicillin	0	9	2	8
		Ticarcillin	0	9	2	9
		Azlocillin	0	9	4	9
		Mezlocillin	3	6	3	6
		Piperacillin	8	1	0	1
		Apalcillin	3	6	3	6
I and III	8	Ampicillin	0	1	1	1
		Ticarcillin	0	4	1	3
		Azlocillin	0	2	1	1
		Mezlocillin	1	5	3	6
		Piperacillin	1	4	3	5
		Apalcillin	1	4	3	5
V (OXA, PSE)	11	Ampicillin	0	6	2	4
		Ticarcillin	1	9	5	6
		Azlocillin	4	7	6	6
		Mezlocillin	6	4	1	1
		Piperacillin	9	2	2	2
		Apalcillin	10	1	1	1
Totals	32	Ampicillin	0	16	5	13
		Ticarcillin	3	22	9	19
		Azlocillin	4	18	11	17
		Mezlocillin	14	15	7	13
		Piperacillin	20	8	7	10
		Apalcillin	16	11	9	14

[a] Using moderately susceptible National Committee for Clinical Laboratory Standards (≤16 μg/ml for ampicillin; ≤64 μg/ml for broad-spectrum penicillins).

[b] Number of strains only susceptible to combination.

against the novel β-lactamase-producing strains tested, OHIO-1, TLE-1, AER-1, and ROB-1.

YTR 830–β-lactam combinations were most active against strains with chromosomal (class I) enzymes with or without class III enzymes, particularly in combination with piperacillin, mezlocillin, or apalcillin. Overall, the best combinations were YTR 830 with piperacillin or apalcillin (30 of the 32 strains susceptible), followed by clavulanate with mezlocillin and apalcillin with sulbactam (29 strains susceptible). YTR 830 therefore appears to be a promising β-lactamase inhibitor, and further development and evaluation of this agent are warranted.

LITERATURE CITED

1. **Arisawa, M., and R. L. Then.** 1982. 6-Acetylmethylenepenicillanic acid: a potent beta-lactamase inhibitor. I. Inhibition of chromosomally and R-factor-mediated beta-lactamases. J. Antibiot. **35**:1578–1583.
2. **Aronoff, S. C., M. R. Jacobs, S. Johenning, and S. Yamabe.** 1984. Comparative activities of the β-lactamase inhibitors YTR 830, sodium clavulanate, and sulbactam combined with amoxicillin or ampicillin. Antimicrob. Agents Chemother. **26**:580–582.
3. **Brown, R. M., R. Wise, J. M. Andrews, and J. Hancox.** 1982. Comparative pharmacokinetics and tissue penetration of sulbactam and ampicillin after concurrent intravenous administration. Antimicrob. Agents Chemother. **21**:565–567.
4. **Bush, K., and R. B. Sykes.** 1984. Interaction of β-lactam antibiotics with β-lactamases as a cause for resistance, *In* L. E. Bryan (ed.), Antimicrobial drug resistance, p. 1–31. Academic Press, Inc., Orlando, Fla.
5. **File, T. M., J. S. Tan, S.-J. Salstrom, L. A. Johnson, and G. F. Douglas.** 1984. Timentin versus piperacillin or moxalactam in the therapy of acute bacterial infections. Antimicrob. Agents Chemother. **26**:310–313.
6. **Fisher, J.** 1984. β-Lactams resistant to hydrolysis by the β-lactamases, p. 33–79. *In* L. E. Bryan (ed.), Antimicrobial drug resistance. Academic Press, Inc., Orlando, Fla.
7. **Hedges, R. W., A. A. Medeiros, M. Cohenford, and G. A. Jacoby.** 1985. Genetic and biochemical properties of AER-1, a novel carbenicillin-hydrolyzing β-lactamase from *Aeromonas hydrophila*. Antimicrob. Agents Chemother. **27**:479–484.
8. **Holloway, W. J.** 1985. Treatment of infections in hospitalized patients with ticarcillin plus clavulanic acid. Am. J. Med. **79**:(Suppl. 5B):168–171.
9. **Medeiros, A. A.** 1984. β-Lactamases. Br. Med. Bull. **40**:18–27.
10. **Medeiros, A. A., M. Cohenford, and G. A. Jacoby.** 1985. Five novel plasmid-determined β-lactamases. Antimicrob. Agents Chemother. **27**:715–719.
11. **Medeiros, A. A., R. Hare, E. Papa, C. Adam, and G. H. Miller.** 1985. Gram-negative bacilli resistant to third-generation cephalosporins: β-lactamase characterization and susceptibility to Sch 34343. J. Antimicrob. Chemother. **15**-(Suppl. C):119–132.
12. **Minami, S., A. Yotsuji, M. Inoue, and S. Mitsuhashi.** 1980. Induction of β-lactamases by various β-lactam antibiotics in *Enterobacter cloacae*. Antimicrob. Agents Chemother. **18**: 382–385.
13. **National Committee for Clinical Laboratory Standards.** 1983. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically, M7-T. National Committee for Clinical Laboratory Standards, Villanova, Pa.
14. **Neu, H. C.** 1982. Penicillins—new insights into their mechanisms of action and clinical use. Bull. N.Y. Acad. Med. **58**:681–695.
15. **Neu, H. C.** 1983. β-Lactamase inhibitor activity of iodopenicillanate and bromopenicillanate. Antimicrob. Agents Chemother. **23**:63–66.
16. **O'Callaghan, C. H., A. Morris, S. Kirby, and A. H. Shingler.** 1972. Novel method for the detection of β-lactamases by using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:282–288.
17. **Paisley, J. W., and J. A. Washington II.** 1978. Combined activity of clavulanic acid and ticarcillin against ticarcillin-resistant, gram-negative bacilli. Antimicrob. Agents Chemother. **14**: 224–227.
18. **Rubin, L. G., R. H. Yolkin, A. A. Medeiros, and R. E. Moxon.** 1981. Ampicillin treatment failure of apparently β-lactamase negative Haemophilus influenzae type b meningitis due to novel β-lactamase. Lancet **ii**:1008–1010.
19. **Scully, B. E., N.-X. Chin, and H. C. Neu.** 1985. Pharmacology of ticarcillin combined with clavulanic acid in humans. Am. J. Med. **79**-(Suppl. 5B):39–43.
20. **Yotsuji, A., S. Minami, Y. Araki, M. Inoue, and S. Mitsuhashi.** 1982. Inducer activity of beta-lactam antibiotics for the beta-lactamases of Proteus rettgeri and Proteus vulgaris. J. Antibiot. **35**:1590–1593.