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In Vitro Activity of Furazlocillin (Bay k 4999) Compared with Those of Mezlocillin, Piperacillin, and Standard Beta-Lactam Antibiotics

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The activity of furazlocillin (Bay k 4999) was compared with those of mezlocillin, piperacillin, and standard beta-lactam antibiotics against a number of gram-positive and gram-negative organisms. These new expanded-spectrum penicillins were less active than penicillin G against most gram-positive organisms. Furazlocillin, mezlocillin, and piperacillin showed activity comparable to ampicillin and penicillin G against *Haemophilus influenzae* and penicillin-susceptible neisseriae, respectively. None of the drugs tested was effective against penicillin-resistant gonococci. The activity of furazlocillin was greater than that of mezlocillin, piperacillin, ampicillin, or carbenicillin against many *Enterobacteriaceae*. However, certain beta-lactam-resistant strains among these organisms were not highly susceptible to any of the three new penicillins. Furazlocillin was less active than piperacillin against *Pseudomonas aeruginosa* but was more active than carbenicillin or mezlocillin. Inoculum effects and discrepancies between minimal inhibitory concentrations and minimal bactericidal concentrations were observed with furazlocillin, mezlocillin, and piperacillin against several genera. The kinetics of bacterial killing by the new penicillins were often slow and incomplete over 24 h, especially in tests with *Enterobacter* and *P. aeruginosa*. Synergy was demonstrated between furazlocillin and aminoglycosides against a variety of gram-negative bacilli and *Streptococcus faecalis*.

The limited spectrum of ampicillin and the relative impotence of carbenicillin against a variety of gram-negative bacilli have stimulated development of a number of new expanded-spectrum penicillins. The major advantage of these new compounds in vitro appears to be their broad spectrum of activity which includes not only ampicillin- and carbenicillin-susceptible organisms, but also many organisms resistant to the currently available penicillins. Piperacillin, a broad-spectrum, semisynthetic aminobenzylpenicillin, has recently been shown to be more active than carbenicillin against *Pseudomonas aeruginosa* (2, 3, 8, 9). Two other new penicillins, azlocillin and mezlocillin, compare favorably with carbenicillin and ampicillin against *Escherichia coli, Klebsiella, Enterobacter, Citrobacter, Acinetobacter,* and *Serratia* (4). Furazlocillin (Bay k 4999) is the latest member of the expanded-spectrum penicillins (10). The compound appears to be more potent than azlocillin, mezlocillin, and piperacillin against many gram-negative bacilli, including *Klebsiella*, indole-positive *Proteus*, and *Enterobacter* (H. Grimm, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., Abstr. no. 455, 1978; L. Verbist, Program Abstr. Intersci. Conf. Antimicrob. Agents Chemother. 18th, Atlanta, Ga., Abstr. no. 456, 1978).

The purpose of the present study was to compare the in vitro activity of furazlocillin, mezlocillin, piperacillin, and standard beta-lactam antibiotics against a wide variety of gram-negative and gram-positive clinical isolates. The killing kinetics and relative beta-lactamase stability of these compounds were also determined. Finally, the ability of the new penicillins to act synergistically with aminoglycosides against *Streptococcus faecalis* and a number of gram-negative bacilli was investigated.

MATERIALS AND METHODS

Microorganisms. A total of 350 gram-positive and gram-negative organisms were used for susceptibility testing. All were clinical isolates obtained from the bacteriology laboratories at St. Joseph's and Veterans Administration hospitals, Omaha, Nebr.

The following criteria were used for designating a strain drug resistant: penicillin resistant—positive test for penicillinase; methicillin resistant—capable of growth on agar containing 25 μg of methicillin per ml after incubation at 32°C; ampicillin-resistant *Haemophilus influenzae*—positive test for beta-lactamase;

other ampicillin-resistant organisms— minimal inhibitory concentration (MIC), >16 μg/ml; cephalothin resistant—MIC for cephalothin, >16 μg/ml; and carbenicillin resistant—MIC for carbenicillin, >64 μg/ml.

Antibiotics. The following standard powders were used: the sodium salts of furazlocillin, mezlocillin (Delbay Pharmaceuticals Inc., Florham Park, N.J.), and piperacillin (Lederle Laboratories, Pearl River, N.Y.); ampicillin trihydrate, the sodium salt of methicillin, and the potassium salt of penicillin G (Bristol Laboratories, Syracuse, N.Y.); the sodium salt of carbenicillin (Pfizer Inc. New York, N.Y.); the sodium salt of cephalothin and streptomycin sulfate (Eli Lilly & Co., Indianapolis, Ind.); and gentamicin sulfate (Schering Laboratories, Bloomfield, N.J.). A stock solution of piperacillin was prepared in distilled water and stored at −10°C until needed. All other drugs were weighed from standard powders, the weight was corrected for potency, and the drugs were prepared in assay broth immediately before use.

Susceptibility tests. For testing of most strains, serial twofold dilutions of each drug were prepared in Mueller-Hinton broth to a final volume of 3 ml. Isolates of *Streptococcus pyogenes* and *Streptococcus pneumoniae* were tested in Todd-Hewitt broth, and *H. influenzae* isolates were tested in Levinthal broth. An overnight broth culture was used for all broth susceptibility testing to give a final inoculum of 10^5 colony-forming units (CFU) per ml in each assay tube, except for inoculum effect studies in which final inocula of 10^3, 10^5, and 10^7 CFU/ml were employed. Agar dilution procedures were used for all *Neisseria* spp. tested. GC agar base medium plus the Kellogg supplement (6) was employed for the susceptibility testing of *Neisseria gonorrhoeae;* strains of *Neisseria meningitidis* were tested on Mueller-Hinton agar. Agar plates were inoculated with a 0.01-ml calibrated loop delivering a final inoculum of 10^5 CFU/cm^2. Inoculated media were incubated for 24 h at 37°C in air, except for those containing *S. pyogenes, S. pneumoniae, H. influenzae,* and neisseriae, which were incubated at 37°C in 10% CO_2 in air. The MIC was defined as the lowest concentration of drug inhibiting macroscopic growth after 24 h of incubation. Each clear tube was subcultured (0.01-ml calibrated loop) to a drug-free agar plate, and the minimal bactericidal concentration (MBC) was defined as the lowest concentration of drug showing no growth on subculture.

Kill curves. The kinetics of bacterial killing were determined by the use of quantitative kill curves. Broth containing drug was inoculated with bacteria from overnight Mueller-Hinton broth cultures and incubated at 37°C in air. The number of CFU per milliliter was determined at 0, 2, 4, 6, 8, and 24 h by removing samples from the culture broth, treating with commercial penicillinase (BBL Microbiology Systems, Cockeysville, Md.) to inactivate any residual drug, and performing agar plate counts. Results given represent averages of duplicate counts.

Beta-lactamase studies. To elucidate the relative degree of susceptibility of the expanded-spectrum penicillins to beta-lactamase, crude cell-free preparations were made from sonic extracts of beta-lactamase-producing, gram-negative bacilli. Log-phase cultures in Mueller-Hinton broth were centrifuged at 7,000 rpm for 10 min at 4°C and then washed in 0.05 M tris(hydroxymethyl)aminomethane buffer, pH 7.2. After a second centrifugation, the washed cell pellet was suspended in 33 mM tris(hydroxymethyl)-aminomethane buffer, pH 7.3, containing 0.5 mM $MgCl_2$ and 1.0 mM dithiothreitol. The cells were then disrupted by sonication (Biosonik IV, Bronwill Scientific Inc., Rochester, N.Y.) until more than 90% of the cells were lysed, as seen by phase microscopy. Cellular debris was removed by centrifugation at 4°C for 15 min at 9,000 rpm. The crude supernatant preparation was stored at −70°C after filter sterilization (0.45-μm filter, Millipore Corp., Bedford, Mass.). Appropriate dilutions of these crude filtrates were added to 4.0 ml of each drug dissolved in 0.10 M phosphate buffer (pH 7.2) to give a final substrate concentration of 1.0 μmol/ml. After incubation at 37°C, iodine was added at a final concentration of 0.7 mM to stop further enzyme activity (7). The amount of residual drug remaining after exposure to beta-lactamase was determined by bioassay using *Bacillus subtilis* ATCC 6633 as the indicator strain, inoculated as described for the Bauer-Kirby disk diffusion procedure (1). Sterile paper disks were impregnated with drug-containing solutions, and the concentration of active drug was determined by comparing zones of inhibition to a standard curve obtained with fresh drug solutions of known concentrations.

Synergy studies. The ability of furazlocillin, mezlocillin, and piperacillin to act synergistically with aminoglycosides was determined. Quantitative kill curves were performed in Mueller-Hinton broth to determine the highest non-bactericidal concentration of each drug alone. Kill curves were then performed by using the highest non-bactericidal concentration of each penicillin in combination with the highest non-bactericidal concentration of aminoglycoside. Synergy was defined as the occurrence of at least a 2-log killing of the test organism at 24 h with the combination, with no bactericidal effect by either drug alone.

RESULTS

Susceptibility tests. The in vitro activity of furazlocillin, mezlocillin, piperacillin, and standard beta-lactam antibiotics against streptococci, neisseriae, and *H. influenzae* is shown in Table 1. All group A streptococci tested were inhibited by ≤0.015 μg of penicillin G per ml, whereas fewer than 25% of the strains were inhibited by furazlocillin, mezlocillin, and piperacillin at this level. Furazlocillin, mezlocillin, and piperacillin showed activity comparable to penicillin G against *N. meningitidis* and penicillin-susceptible *N. gonorrhoeae.* None of the penicillins tested was effective against penicillin-resistant gonococci. The four penicillins tested showed comparable activity against *H. influenzae,* with none highly active against ampicillin-resistant strains.

The activity of furazlocillin, mezlocillin, piperacillin, and penicillin G against staphylococci and enterococci is also shown in Table 1. At

TABLE 1. *Comparative activity of various penicillins[a] against gram-positive and -negative cocci and H. influenzae*

Organism	Antibiotic	MIC (μg/ml) needed to inhibit:			
		50%[b]	75%[b]	90%[b]	100%[b]
S. pneumoniae (10)[c]	PEN	0.015	0.03	0.06	0.12
	BAY	≤0.007	0.03	0.03	0.50
	MEZ	≤0.007	0.015	0.12	1
	PIP	0.03	0.12	0.25	2
Group A streptococci (20)	PEN	≤0.007	≤0.007	0.015	0.015
	BAY	0.03	0.03	0.06	0.12
	MEZ	0.03	0.06	0.06	0.06
	PIP	0.06	0.12	0.12	0.12
N. meningitidis (10)	PEN	≤0.015	0.03	0.03	0.03
	BAY	≤0.015	≤0.015	≤0.015	≤0.015
	MEZ	≤0.015	≤0.015	≤0.015	≤0.015
	PIP	0.03	0.03	0.03	0.03
N. gonorrhoeae (12) (PEN susceptible)	PEN	≤0.015	0.03	0.03	0.03
	BAY	≤0.015	≤0.015	0.03	0.03
	MEZ	≤0.015	≤0.015	0.06	0.06
	PIP	≤0.015	0.03	0.03	0.03
N. gonorrhoeae (8) (PEN resistant)	PEN	>64	>64	>64	>64
	BAY	>64	>64	>64	>64
	MEZ	>64	>64	>64	>64
	PIP	>64	>64	>64	>64
H. influenzae (11) (AMP susceptible)	AMP	0.03	0.06	0.5	0.5
	BAY	≤0.015	≤0.015	0.06	0.5
	MEZ	≤0.015	≤0.015	0.5	1
	PIP	≤0.015	0.03	0.06	2
H. influenzae (4) (AMP resistant)	AMP	4	4	4	8
	BAY	1	4	4	4
	MEZ	8	16	32	32
	PIP	2	4	4	8
Enterococci (20)	PEN	4	4	4	16
	BAY	4	8	8	64
	MEZ	2	4	16	64
	PIP	4	8	16	128
S. aureus (10) (PEN susceptible)	PEN	≤0.25	0.5	0.5	1
	BAY	2	8	16	16
	MEZ	1	4	4	32
	PIP	1	8	16	16
S. aureus (5) (PEN resistant)	PEN	0.5	4	8	8
	BAY	8	32	>256	>256
	MEZ	8	16	>256	>256
	PIP	16	64	>256	>256
S. aureus (4) (METH resistant)	BAY	>256	>256	>256	>256
	MEZ	128	128	256	256
	PIP	>256	>256	>256	>256
S. epidermidis (10)	PEN	0.5	2	8	32
	BAY	2	8	8	64
	MEZ	0.5	2	4	32
	PIP	1	4	8	64

[a] Abbreviations: PEN, penicillin; AMP, ampicillin; BAY, furazlocillin (Bay k 4999); MEZ, mezlocillin; PIP, piperacillin.

[b] Cumulative percent inhibited.

[c] Numbers within parentheses indicate the numbers of isolates.

TABLE 2. *Comparative activity of beta-lactams[a] against gram-negative bacilli*

Organism	Antibiotic	MIC (μg/ml) needed to inhibit:			
		50%[b]	75%[b]	90%[b]	100%[b]
AMP-susceptible (70)[c] *Escherichia, Citrobacter, Salmonella, Shigella,* and *P. mirabilis*	AMP	1	2	4	16
	BAY	≤0.25	≤0.25	0.5	2
	MEZ	1	2	4	8
	PIP	1	2	2	4
AMP-resistant (16) *E. coli, Salmonella,* and *Shigella*	AMP	>256	>256	>256	>256
	BAY	64	128	>256	>256
	MEZ	64	>256	>256	>256
	PIP	64	>256	>256	>256
Enterobacter spp. (20)	CEPH	>256	>256	>256	>256
	BAY	0.5	1	4	32
	MEZ	2	4	8	128
	PIP	2	4	8	256
Klebsiella spp. (13) (CEPH susceptible)	CEPH	2	4	8	16
	BAY	1	1	2	128
	MEZ	4	8	8	256
	PIP	4	4	8	>256
Klebsiella spp.(7) (CEPH resistant)	CEPH	32	>256	>256	>256
	BAY	8	16	256	256
	MEZ	8	32	256	256
	PIP	32	32	32	>256
AMP-resistant, CARB-susceptible *Enterobacteriaceae*					
Proteus morganii (28)	CARB	2	4	8	8
	BAY	1	4	16	32
	MEZ	8	16	64	64
	PIP	2	8	32	64
Proteus vulgaris (6)	CARB	16	32	32	32
	BAY	0.5	0.5	0.5	0.5
	MEZ	2	2	2	4
	PIP	0.5	1	1	1
P. rettgeri (6)	CARB	1	2	2	2
	BAY	1	2	2	2
	MEZ	2	4	4	4
	PIP	2	2	2	4
P. stuartii (7)	CARB	1	1	1	1
	BAY	0.5	1	1	1
	MEZ	2	4	4	4
	PIP	1	2	4	4
Serratia spp. (14)	CARB	8	8	16	16
	BAY	≤0.25	0.5	0.5	0.5
	MEZ	2	2	4	32
	PIP	1	1	2	2
AMP-resistant (4), CARB-resistant *Proteus, Providencia,* and *Serratia*	CARB	>256	>256	>256	>256
	BAY	64	64	>256	>256
	MEZ	256	>256	>256	>256
	PIP	128	256	>256	>256

TABLE 2. *Continued*

Organism	Antibiotic	MIC (μg/ml) needed to inhibit:			
		50%[b]	75%[b]	90%[b]	100%[b]
P. aeruginosa (30)	CARB	128	128	256	>256
	BAY	8	16	32	>256
	MEZ	64	128	128	>256
	PIP	8	16	16	32
Other nonfermenting (20), gram-negative bacilli	CARB	64	256	>256	>256
	BAY	4	8	16	64
	MEZ	32	64	64	128
	PIP	16	32	128	256

[a] Abbreviations: AMP, ampicillin; CEPH, cephalothin; CARB, carbenicillin, BAY, furazlocillin (Bay k 4999); MEZ, mezlocillin; PIP, piperacillin.
[b] Cumulative percent inhibited.
[c] Numbers within parentheses indicate the numbers of isolates.

least 90% of the enterococci tested were inhibited by 16 μg of each penicillin used per ml. The three new penicillins were less effective than penicillin G against *Staphylococcus aureus*, but were comparable to penicillin G against *Staphylococcus epidermidis*.

Table 2 shows the activity of furazlocillin, mezlocillin, piperacillin, and other beta-lactam antibiotics against a variety of gram-negative bacilli. Against 70 ampicillin-susceptible *Enterobacteriaceae*, which included *Salmonella* and *Shigella* spp., *E. coli*, *Citrobacter* spp., and *Proteus mirabilis*, furazlocillin was more active than ampicillin, mezlocillin, and piperacillin, inhibiting 99% of strains at 1.0 μg/ml. However, only one-half of the ampicillin-resistant strains of these genera/species were inhibited by the three new expanded-spectrum penicillins at concentrations of 64 μg/ml. Furazlocillin was more active than mezlocillin or piperacillin against *Enterobacter* spp. and cephalothin-susceptible *Klebsiella* spp., inhibiting 28 of the 33 strains tested at 1.0 μg/ml. Although the activity of the three new expanded-spectrum penicillins was comparable against cephalothin-resistant *Klebsiella* spp., these strains were generally less susceptible to the three drugs than the cephalothin-susceptible strains of this genus. Furazlocillin was generally more active than carbenicillin, mezlocillin, and piperacillin against indole-positive *Proteus, Providencia stuartii,* and *Serratia* spp., but somewhat less active than piperacillin against *P. aeruginosa*. Furazlocillin was the most active of the four penicillins tested against 20 other nonfermenting, gram-negative bacilli (including *Pseudomonas* spp., *Alkaligenes, Acinetobacter, Flavobacterium* spp., and *Achromobacter*).

The inhibitory activity of each antibiotic tested was affected by increasing the test inoculum from 10^3 to 10^7 CFU/ml (Table 3). An inoculum effect for furazlocillin, mezlocillin, and piperacillin of 128-fold or greater was observed for most isolates of *P. aeruginosa, E. coli,* indole-positive *Proteus, Enterobacter* spp., and *Klebsiella* spp. Two ampicillin-resistant *H. influenzae* tested demonstrated a large inoculum effect with all three penicillins, while two ampicillin-susceptible strains did not. A large inoculum effect was not observed with these drugs for the strains of streptococci tested.

To compare the degree of bacterial killing, MBCs for the various drugs were also determined. Against most genera tested, MBCs were close to MICs. However, some large discrepancies were seen. These are shown in Table 4 as the mean ratio of the MBC to the MIC. The largest discrepancies were observed with isolates of *S. faecalis, Klebsiella, Serratia,* and nonfermentative, gram-negative bacilli. Although discrepancies between MICs and MBCs for furazlocillin were as high as 64-fold, similar discrepancies occurred with piperacillin and mezlocillin.

Kinetics of bacterial killing. The kinetics of bacterial killing by furazlocillin, mezlocillin, piperacillin, and standard beta-lactams was determined against a variety of gram-negative bacilli. From this series of tests, three distinct patterns of killing were observed (Fig. 1). The first pattern, represented by *Klebsiella pneumoniae* 17 (Fig. 1C), was a slow but complete killing of the test organism by 24 h. This pattern was also observed in similar tests with an *E. coli* and *H. influenzae*, both of which were ampicillin susceptible. Each of the strains tested that gave this pattern had low MICs and MBCs for each

TABLE 3. *Effect of inoculum on in vitro activity of expanded-spectrum penicillins*

Organism	Fold increase[a] in MIC for:		
	Furazlocillin	Mezlocillin	Piperacillin
Enterococci (2)[b]	0–4	0–4	0–4
P. aeruginosa (6)	64–512	8–128	4–512
Other nonfermenters (2) *Pseudomonas maltophilia* *Pseudomonas mendocina*	128–1,024	16–128	128–256
E. coli (6)	16–512	8–512	4–512
H. influenzae (4)	0–2,048	2–1,024	2–4,096
Indole-positive *Proteus* (6)	512–2,048	256–1,024	512–2,048
Enterobacter spp. (2)	16–2,048	8–512	8–2,048
Klebsiella spp. (3)	32–1,024	8–256	8–256
Group A streptococci (2)	2–4	2	2–4

[a] Expressed as ratio of MIC with inoculum of 10^7 CFU/ml to MIC with inoculum of 10^3 CFU/ml.
[b] Numbers within parentheses indicate numbers of strains tested.

TABLE 4. *Occurrence of discrepancies between MICs and MBCs for various beta-lactam antibiotics*[a]

Organism	Mean MBC/MIC ratio			
	Standard drug	BAY	PIP	MEZ
S. faecalis (20)[b]	(PEN) 128	64	64	128
Klebsiella (20)	(CEPH) 2	16	8	8
Serratia (15)	(CARB) 4	32	32	64
P. aeruginosa (30)	(CARB) 4	64	64	8
Other nonfermenters[c] (20)	(CARB) 4	16	8	4

[a] Abbreviations: PEN, penicillin; CEPH, cephalothin; CARB, carbenicillin; BAY, furazlocillin (Bay k 4999); PIP, piperacillin, MEZ, mezlocillin.
[b] Numbers within parentheses indicate the numbers of isolates.
[c] Strains include *Pseudomonas* spp. (12), *Alkaligenes* spp. (2), *Acinetobacter* spp. (4), *Achromobacter* spp. (1), and *Flavobacterium* spp. (1).

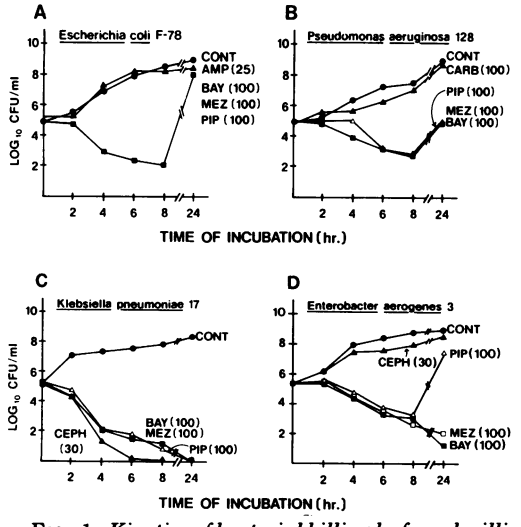

FIG. 1. *Kinetics of bacterial killing by furazlocillin (Bay k 4999 [BAY]), mezlocillin (MEZ), piperacillin (PIP), ampicillin (AMP), cephalothin (CEPH), and carbenicillin (CARB). Drug concentrations in micrograms per milliliter are shown in parentheses. CONT, drug-free control.*

of the new drugs. The second pattern observed, represented by *P. aeruginosa* 128 and *Enterobacter aerogenes* 3 (Fig. 1B and D), was a slow and incomplete killing by 24 h. This pattern was also observed in similar tests with four carbenicillin-susceptible *P. aeruginosa*, a cephalothin-resistant *K. pneumoniae*, a carbenicillin-susceptible *Proteus rettgeri*, and a second cephalothin-resistant *E. aerogenes*. Each of the strains tested that gave this pattern had low MICs and high MBCs for the new drugs. Bioassay of both *E. aerogenes* 3 and *P. aeruginosa* 128 test broths indicated that inactivation of furazlocillin, mezlocillin, and piperacillin had not occurred despite the presence of viable cells at 24 h. MICs were determined on an isolate from each of the surviving cell populations. Only the *E. aerogenes* 3 isolate from the piperacillin kill curve showed increased resistance to the drugs. The MICs of this isolate for furazlocillin, mezlocillin, and piperacillin had increased approximately 64-fold compared with an earlier determination of the parent strain.

The third pattern of bacterial killing observed, represented by *E. coli* F-78 (Fig. 1A), showed

slow killing for 8 h, after which cell regrowth near that of the drug-free controls occurred. Bioassay of each drug-containing broth at 24 h revealed no detectable activity for any of the penicillins. This pattern of regrowth with accompanying drug inactivation was also observed for a *P. rettgeri* isolate which, like *E. coli* F-78, was resistant to furazlocillin, mezlocillin, and piperacillin as reflected by high MICs and MBCs.

Susceptibility to beta-lactamase. The relative stability of furazlocillin, mezlocillin, and piperacillin to crude, cell-free preparations of beta-lactamase was compared with that of standard beta-lactam antibiotics. Table 5 shows the percentage of drug inactivated after treatment with commercial penicillinase and crude enzyme preparations from *H. influenzae* 27, *P. rettgeri* 144, and *E. coli* F-78. The data indicated that all penicillins tested were susceptible to beta-lactamase degradation.

Synergy studies. The ability of furazlocillin, mezlocillin, and piperacillin to act synergistically with aminoglycosides was determined. A number of gram-negative bacilli were chosen for synergy studies due to their resistance to various penicillins and/or aminoglycosides. Against a beta-lactamase-producing *P. rettgeri* isolate (Fig. 2), only the combination of furazlocillin and gentamicin was synergistic at the concentrations shown. Synergy could not, however, be shown with gentamicin plus furazlocillin, mezlocillin, or piperacillin against the beta-lactamase-producing *E. coli* F-78 at 24h. The combination of gentamicin plus furazlocillin or piperacillin was synergistic against a *K. pneumoniae* shown in Fig. 2. Increasing the concentration of mezlocillin to 4.0 μg/ml in the combination produced a synergistic effect at 24 h. Synergy could be demonstrated with gentamicin and furazlocillin or piperacillin against a gentamicin-susceptible *P. aeruginosa* (Fig. 3); however, it should be noted that a complete bactericidal effect was achieved only with furazlocillin in the combination. Against a *P. aeruginosa* moderately resistant to gentamicin (MIC, 12 μg/ml), gentamicin plus furazlocillin, mezlocillin, or piperacillin demonstrated synergy (Fig. 3).

The bactericidal activity of furazlocillin and penicillin alone and in combination with aminoglycosides was determined against 11 strains of enterococci (Table 6). These included five strains that were highly resistant to streptomycin (MIC, >2,000 μg/ml). Combinations of streptomycin plus furazlocillin or penicillin produced synergy with strains that were not highly resistant to streptomycin. Gentamicin plus penicillin

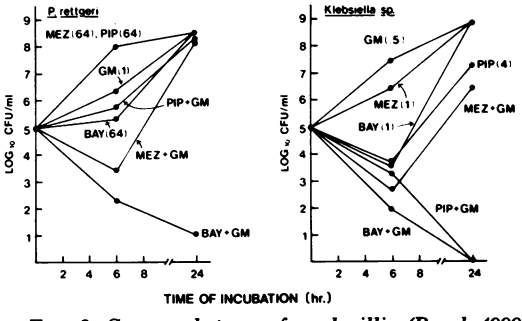

FIG. 2. *Synergy between furazlocillin (Bay k 4999 [BAY]), mezlocillin (MEZ), or piperacillin (PIP) and gentamicin (GM) against P. rettgeri and Klebsiella spp. Drug concentrations in micrograms per milliliter are shown in parentheses.*

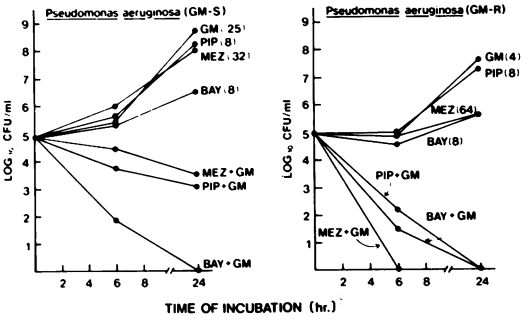

FIG. 3. *Synergy between furazlocillin (Bay k 4999 [BAY]), mezlocillin (MEZ), or piperacillin (PIP) and gentamicin (GM) against gentamicin-susceptible (GM-S) and resistant (GM-R) P. aeruginosa. Drug concentrations in micrograms per milliliter are shown in parentheses.*

TABLE 5. *Activity of cell-free beta-lactamases from various sources against some penicillins (initial drug concentration, 1.0 μmol/ml)*

Drug	*H. influenzae* 27	*P. rettgeri* 144	*E. coli* F-78	Penicillinase
Penicillin	>99[a]	81	>99	>99
Ampicillin	>99		97	>99
Carbenicillin		60		98
Furazlocillin	88	81	75	75
Mezlocillin	>99	73	>99	>99
Piperacillin	>99	81	>99	81

[a] Indicates percentage of drug inactivated.

TABLE 6. *Synergy between penicillins and aminoglycosides against enterococci*

Combination	No. of tests with six streptomycin-susceptible strains showing synergy	No. of tests with five streptomycin-resistant strains showing synergy
Streptomycin (25 μg/ml) plus:		
Penicillin (0.81 to 3.1 μg/ml)	6[a]	0
Furazlocillin (2.0 to 4.0 μg/ml)	6	0
Gentamicin (4.0 to 6.0 μg/ml) plus:		
Penicillin (0.81 to 3.1 μg/ml)	6	5
Furazlocillin (2.0 to 4.0 μg/ml)	6	4

[a] At least a 2-log killing of test organism with the combination, with no bactericidal effect by either drug alone.

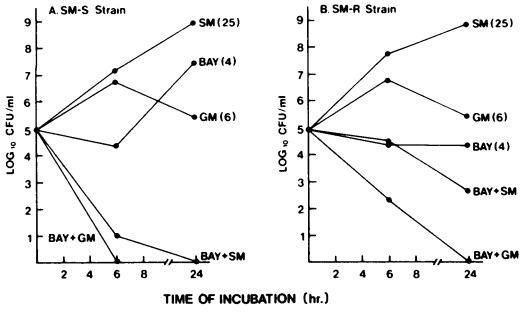

FIG. 4. *Synergy between furazlocillin (Bay k 4999 [BAY]) and gentamicin (GM) or streptomycin (SM) against streptomycin-susceptible (SM-S) and -resi ant (SM-R) S. faecalis. Drug concentrations in mi grams per milliliter are shown in parentheses.*

was synergistic against all 11 strains tested, and gentamicin plus furazlocillin was synergistic against all but 1 streptomycin-resistant strain. The killing kinetics of furazlocillin in combination with gentamicin or streptomycin are shown for streptomycin-susceptible and -resistant isolates of enterococci in Fig. 4.

DISCUSSION

This study has shown that furazlocillin is a broad-spectrum penicillin with greater activity than mezlocillin, piperacillin, and standard beta-lactam antibiotics against many gram-negative bacteria. However, furazlocillin, like mezlocillin and piperacillin, was found to be less active than penicillin G against most gram-positive organisms. The greatest advantage of furazlocillin in vitro was that its activity was greater than those of ampicillin, mezlocillin, piperacillin, and carbenicillin against a variety of *Enterobacteriaceae,* including *Klebsiella, Enterobacter,* and indole-positive *Proteus.* These findings are consistent with reports of other investigators (10) who have recently evaluated the antibacterial activity of furazlocillin (Grimm, 18th ICAAC, Abstr. no. 455; Verbist, 18th ICAAC, Abstr. no. 456). The essentially equal susceptibility of the new penicillins to beta-lactamase suggests that the greater activity of furazlocillin may involve an inherent potency of the molecule that is superior to that of other expanded-spectrum penicillins. This greater potency may be due to greater binding at lethal target sites in the susceptible organism or perhaps reflects a greater degree of penetration into the cell. The binding avidities for piperacillin to lethal and nonlethal target sites in *E. coli* have recently been studied by Iida et al. (5). These investigators observed a concentration-dependent relationship between piperacillin's antibacterial activity and its ability to induce morphological abnormalities during cell growth. Thus, it is possible that the enhanced activity of some expanded-spectrum penicillins involves selective and/or more avid attachment to the penicillin-binding proteins in the cell responsible for initiating the lethal event.

Studies on the kinetics of bacterial killing by the new penicillins revealed three distinct patterns, each of which was associated with a particular pattern obtained in dilution susceptibility tests. Strains with low MICs and MBCs generally were killed slowly but completely over 24 h. Strains with high MICs and MBCs were affected only during the interval required for their beta-lactamase to inactivate the drugs. Strains with low MICs but high MBCs were killed slowly and incompletely over 24 h. Studies with a number of such strains suggested that this incomplete killing was due to the presence of cells resistant to the penicillins by some mechanism other than beta-lactamase production. This phenomenon not only helps to explain the presence of viable cells at 24 h despite an MIC in the susceptible range, but also may be an explanation for the large inoculum effects observed with certain organisms.

Due to the nature of the infections for which the new penicillins may be used, combination chemotherapy with aminoglycosides has been an area of interest. A desirable attribute of an expanded-spectrum penicillin would be its ability to act synergistically with aminoglycosides against certain resistant organisms. In tests with gram-negative bacilli, synergy was observed most frequently when furazlocillin was used in combinations with an aminoglycoside, further demonstrating the greater inherent potency of this compound. Also, synergy with gentamicin was obtained with concentrations of furazlocillin lower than that of mezlocillin or piperacillin in most tests, including those involving strains resistant to the penicillins or gentamicin. Finally, the ability of furazlocillin to act synergistically with aminoglycosides against *S. faecalis* suggests that furazlocillin may be an alternative to penicillin in aminoglycoside-beta-lactam combinations for this organism. More extensive studies are needed to substantiate these various possibilities.

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LITERATURE CITED

1. **Bauer, A. W., W. M. M. Kirby, J. C. Sherris, and M. Turck.** 1966. Antibiotic susceptibility testing by a standardized single disk method. Am. J. Clin. Pathol. **45:** 493-496.
2. **Bodey, G. P., and B. LeBlanc.** 1978. Piperacillin: in vitro evaluation. Antimicrob. Agents Chemother. **14:**78-87.
3. **Fu, K. P., and H. C. Neu.** 1978. Piperacillin, a new penicillin active against many bacteria resistant to other penicillins. Antimicrob. Agents Chemother. **13:**358-367.
4. **Fu. K. P., and H. C. Neu.** 1978. Azlocillin and mezlocillin: new ureido penicillins. Antimicrob. Agents Chemother. **13:**930-938.
5. **Iida, K., S. Hirata, S. Nakamuta, and M. Koike.** 1978. Inhibition of cell division of *Escherichia coli* by a new synthetic penicillin, piperacillin. Antimicrob. Agents Chemother. **14:**257-266.
6. **Kellogg, D. S., Jr., W. L. Peacock, Jr., W. E. Deacon, L. Brown, and C. I. Pirkle.** 1963. *Neisseria gonorrhoeae.* I. Virulence genetically linked to clonal variation. J. Bacteriol. **85:**1274-1279.
7. **Ross, G. W., and C. H. O'Callaghan.** 1975. Beta lactamase assays. Methods Enzymol. **43:**69-85.
8. **Verbist, L.** 1978. In vitro activity of piperacillin, a new semisynthetic penicillin with an unusually broad spectrum of activity. Antimicrob. Agents Chemother. **13:** 349-357.
9. **Winston, D. J., D. Wang, L. S. Young, W. J. Martin, and W. L. Hewitt.** 1978. In vitro studies of piperacillin, a new semisynthetic penicillin. Antimicrob. Agents Chemother. **13:**944-950.
10. **Wise, R., J. M. Andrews, and K. A. Bedford.** 1978. Comparison of the in vitro activity of Bay k 4999 and piperacillin, two new antipseudomonal broad-spectrum penicillins, with other β-lactam drugs. Antimicrob. Agents Chemother. **14:**549-552.