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In Vitro Activity of BL-S640 Against Gram-Negative Bacilli and *Staphylococcus aureus* Compared with Activity of Four Other Semisynthetic Cephalosporins

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The in vitro activity of BL-S640 (cefatrizine) was determined against 674 recent clinical isolates of *Staphylococcus aureus* and *Enterobacteriaceae*. Activity against *S. aureus* was less than that of cephapirin, cephalothin, and cefazolin, but greater than that of cephalexin. Activity against gram-negative isolates was variable: BL-S640 was slightly less potent than cefazolin against *Escherichia coli* and *Klebsiella*, but more active than the other compounds. As for the more resistant gram-negative genera, BL-S640 was significantly superior to the control cephalosporins. The effect of inoculum size on the antibacterial activity was moderate for most organisms except *Enterobacter*, *Providencia stuartii*, and indole-positive *Proteus*, the median minimal inhibitory concentrations of which were 6 to 27 times lower when determined with a 10^{-4}-diluted culture compared with the undiluted one. The stability in aqueous solution at 37 C was remarkably high at the lower pH values, but low at the neutral point.

BL-S640, 7-[R-α-amino-α-(4-hydroxyphenyl)-acetamido]-3-(1,2,3-triazol-5-ylthiomethyl)-ceph-3-em-4-carboxylic acid, is a new semisynthetic cephalosporin derivative. Most cephalosporins have a broad spectrum and are used for a variety of infections caused by both gram-positive and gram-negative organisms. However, most derivatives have little or no effect against the more resistant *Enterobacteriaceae* such as members of the genera *Enterobacter*, *Serratia*, *Providencia*, and indole-positive *Proteus*. Preliminary studies with BL-S640 suggest good activity against all these isolates except *Serratia*, which is highly resistant (6, 10). The compound is well adsorbed orally and parenterally, and its therapeutic efficacy in rodents infected systemically with a variety of pathogenic bacteria appears to be significantly greater than would be predicted on the basis of its comparative activity in vitro (7). The aim of the present investigation was (i) to determine the antibacterial activity of BL-S640 against both gram-positive and gram-negative organisms; (ii) to compare the in vitro activities of cephalothin, cephalexin, cefazolin, and cephapirin; (iii) to evaluate the effect of inoculum size on antibacterial activity; and (iv) to determine the stability of the compound in aqueous solution, at various pH values, at 37, 25, and 4 C.

MATERIALS AND METHODS

BL-S640, cefazolin, and cephapirin were obtained from Bristol Laboratories; cephalothin and cephalexin were kindly supplied by Eli Lilly and Co. Bacterial strains were isolates from patients hospitalized in various Gent city hospitals. There were 215 clinical isolates of *Staphylococcus aureus*, 432 isolates of *Enterobacteriaceae*, and 27 *Pseudomonas aeruginosa*; all these strains were maintained on nutrient agar slants, stored in the refrigerator, and subcultured at monthly intervals.

Susceptibility testing methods. Susceptibility was determined by an agar dilution method on Trypticase soy agar (Difco). The inoculum consisted of a 10^{-2} dilution of an overnight culture in tryptic soy broth (Difco), containing approximately 10^9 viable cells/ml, and was applied to the surface of the agar in 1-μl quantities with the aid of the multipoint inoculator of Steers et al. (9). Swarming of *Proteus* was prevented by incorporation into the agar medium of 60 μg of 1-(4-nitrophenyl)-propantriol-(1,2,3) per ml, which was proved to influence the results of minimal inhibitory concentration (MIC) determinations in no way. Plates were incubated for 18 h at 37 C, and the MIC was recorded as the lowest concentration of antibiotic giving complete inhibition of growth, as judged by the naked eye. Solutions of all antibiotics were prepared with sterile 1% phosphate buffer, pH 6.0.

Influence of inoculum size. The influence of the inoculum size on the activity of BL-S640 was assessed by comparing the MIC values obtained with the undiluted broth culture and with a 10^{-2} and a 10^{-4} dilution as inoculum; as for cephalexin, only the undiluted culture and the 10^{-4} dilution were compared. Approximate numbers of colony-forming units deposited on the agar surface were 10^6, 10^4, and 10^2, respectively.

Stability of BL-S640 in aqueous solution. Anti-

biotic stability during storage of 0.07 M phosphate-buffered solutions (1 mg/ml) of BL-S640 was assessed at 4, 25, and 37 C and at various pH values. Phosphate-buffered solutions were used throughout for preparing stock solutions of antibiotics and had a fairly stable pH in the range 5.0 to 8.0. The acid stability at 37 C was determined by using a 1-mg/ml solution in 0.1 M hydrochloric acid, which was shown to have an approximate pH value of 1.0. Residual activities were determined turbidimetrically, according to the methods described by Arret et al. (1). The assay organism was *S. aureus* ATCC 6538P. This particular strain gave a linear calibration curve in the range 0.01 to 0.16 μg of BL-S640 activity per ml in the assay broth. The percentage of transmission versus concentration graph was found to give the best straight line over this concentration range. Antibiotic medium 3 (Difco) was used, and the test required 3 to 4 h of incubation at 35 to 37 C. Changing the pH of the assay broth from 6.0 to 8.0 (the pH range of growth of the assay organism), we found that at pH 7.0 the dose-response line was straight with the greatest slope. Decomposition of the drug at this pH during a 4-h incubation period was found not to influence the reproducibility of results.

RESULTS

Antibacterial activity. The spectrum of antimicrobial activity of BL-S640 against *S. aureus* and various *Enterobacteriaceae* is shown in Fig. 1. The antibiotic was more effective against *S. aureus* than against gram-negative bacilli. However, the latter organisms showed a rather compact susceptibility spectrum, indicating good activity of the compound against almost all *Enterobacteriaceae*. Its gram-negative spectrum included strains of *Enterobacter*, indole-positive *Proteus*, *Providencia stuartii*, and *Citrobacter*, species generally resistant to other cephalosporins (2–5). However, there was no demonstrable antibacterial activity (MIC > 100 μg/ml) against *Serratia marcescens* (13 strains) and *Pseudomonas aeruginosa* (27 strains), which are not included in the figure.

The comparative activities of the five tested cephalosporins against each individual species are illustrated in Fig. 2 to 7 and in Table 1. Cephapirin, cephalothin, and cefazolin were more active against *S. aureus* than was BL-S640 (Fig. 2). However, the latter compound in a concentration of 12.5 μg/ml inhibited almost all strains and was distinctly superior to cephalexin, the MIC values of which were about four times higher. Cefazolin was the most active compound against *Escherichia coli;* nevertheless, BL-S640 was about fourfold more active

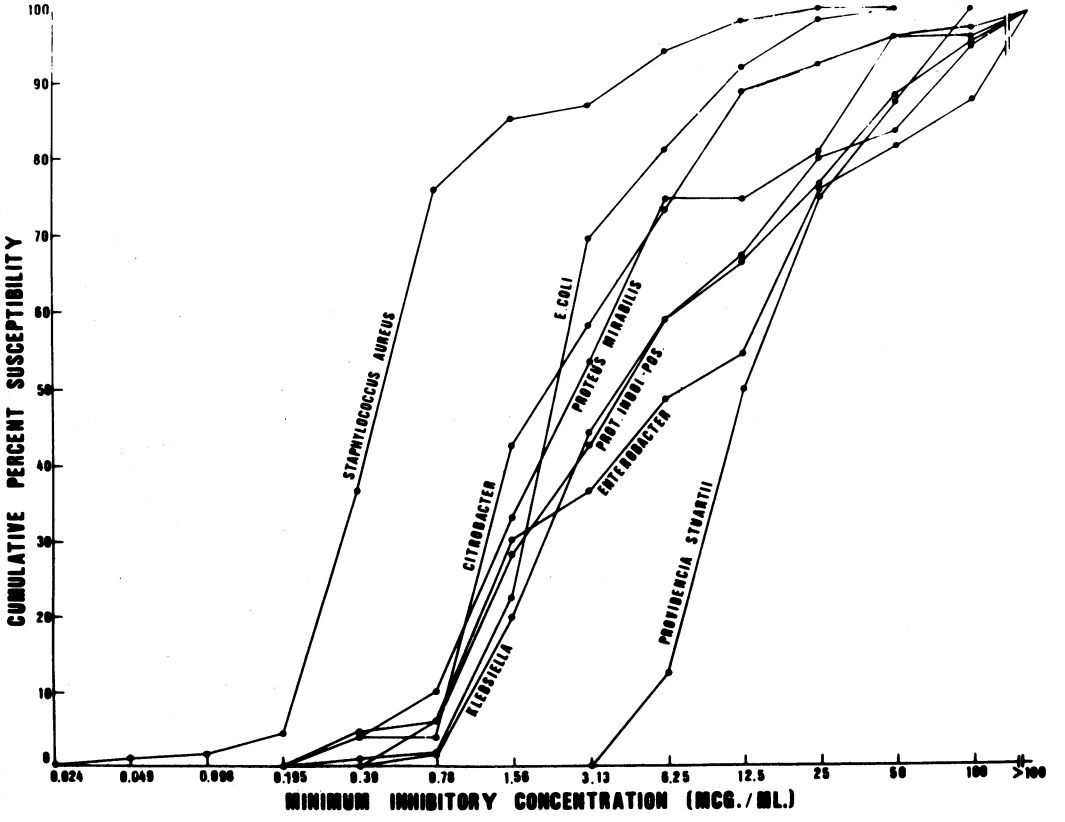

FIG. 1. *Susceptibility of various clinical isolates to BL-S640.*

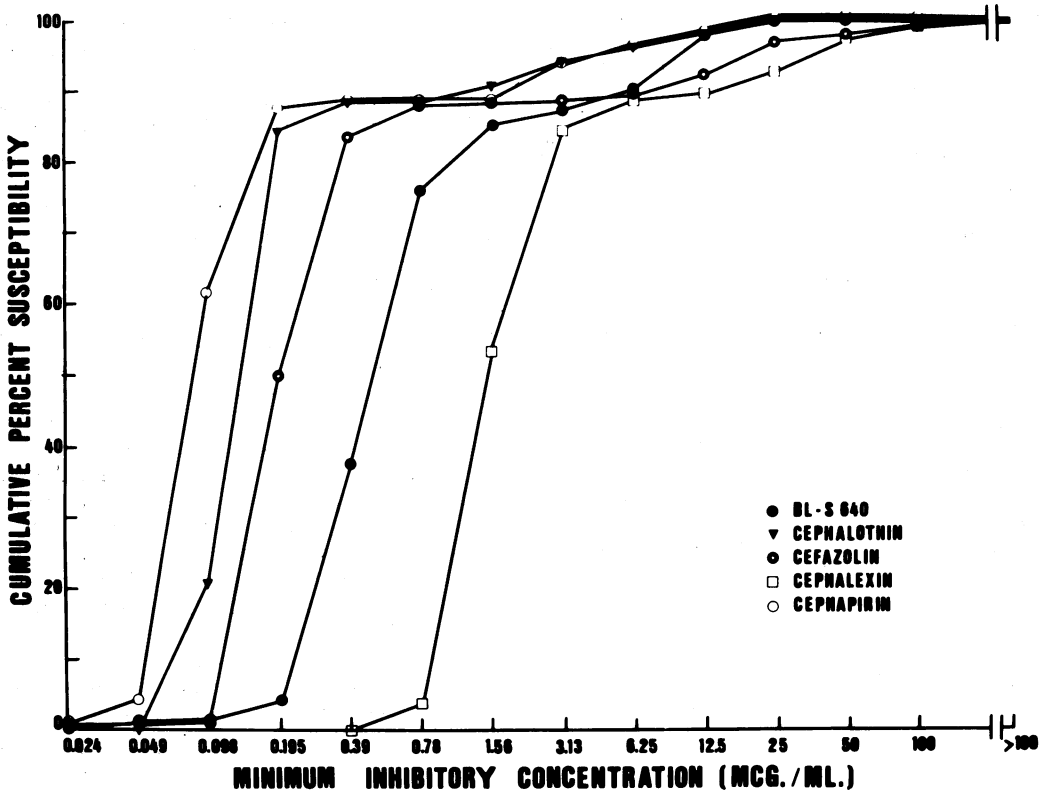

FIG. 2. *Comparative activity of five semisynthetic cephalosporins against Staphylococcus aureus (215 isolates).*

than cephalothin and cephapirin and four- to eightfold more active than cephalexin (Fig. 3). Against *Klebsiella,* cefazolin was again slightly superior to BL-S640. The latter compound had MICs equal to those of cephapirin for about half of the strains, but was twice as active for the other half, two to four times more active than cephalothin, and four to eight times more active than cephalexin (Fig. 4). However, some of the more resistant *Klebsiella* strains were as susceptible to cephalexin as to BL-S640. The *Citrobacter* strains tested showed little, if any, susceptibility to any compound except BL-S640, which was markedly active: 88% of the isolates were inhibited at 12.5 μg/ml (Fig. 5). The susceptibility pattern of *Enterobacter* strains was very similar: more than 50% were inhibited by 12.5 μg of BL-S640 per ml, whereas the activity of the control compounds was very low (Fig. 6).

To a certain degree, all derivatives showed satisfactory activity against most isolates of *Proteus mirabilis,* BL-S640 being again the most potent drug and cephalexin the least potent (Fig. 7). The MIC ratio for the two latter compounds was about 1:8. The comparative antibacterial activity of the five antibiotics against indole-positive *Proteus* and *Providencia* species is shown in Table 1. Twenty-five percent of *Proteus vulgaris* strains were inhibited by 1.56 μg of BL-S640 per ml or less, and 58% were inhibited by 6.25 μg/ml. The mean MIC values of the antibiotic against the latter species were 1.5 to 2 times higher than those against *Proteus mirabilis.* Against *Proteus morganii* and *Proteus rettgeri,* BL-S640 was more active than the control compounds by about five- and threefold, respectively. Sixty-one percent of *P. morganii* and 50% of *P. rettgeri* strains were inhibited by BL-S640 in a concentration of 6.25 μg/ml. Strains of *Providencia alcalifaciens* showed good susceptibility to BL-S640: 91% were inhibited by 12.5 μg/ml. As for *Providencia stuartii,* only 50% of the strains were inhibited by the same concentration. This activity was still markedly in excess of that of the control compounds, which had only minimal antibacterial activity against this species.

Effect of inoculum size. Varying the size of inoculum from an undiluted overnight culture

TABLE 1. *Comparative in vitro susceptibility of indole-positive Proteus and of Providenica to five antibiotics*

Organism	Antibiotic	No. of isolates	Cumulative percent susceptibility at increasing concn (μg/ml)[a]									
			0.39	0.78	1.56	3.13	6.25	12.5	25	50	100	>100
Proteus vulgaris	BL-S640	23	4		25	42	58	67		71	92	100
	Cephalothin					9	22	39	48	61	65	100
	Cephalexin						13	44	52	65	74	100
	Cefazolin			5	9	32	50	55		77	86	100
	Cephapirin				5	9	27	50	59	68	73	100
Proteus morganii	BL-S640	36	6		33	47	61	67	86	100		
	Cephalothin					11	22	25	28	31		100
	Cephalexin							25	28	31	47	100
	Cefazolin					20	26		29	43	91	100
	Cephapirin					6	23	31		34	43	100
Proteus rettgeri	BL-S640	8		13		25	50	63		88		100
	Cephalothin						13	25			38	100
	Cephalexin							13	25	50		100
	Cefazolin						13	38		50	63	100
	Cephapirin							13		25	50	100
Providencia alcalifaciens	BL-S640	11		18	27		73	91			100	
	Cephalothin		NT[b]									
	Cephalexin				18			54	73	82	100	
	Cefazolin				9	27	46	64	73	82		100
	Cephapirin					9	27	36		91		100
Providencia stuartii	BL-S640	9					13	50	75	88	100	
	Cephalothin										78	100
	Cephalexin									56	78	100
	Cefazolin								67	89		100
	Cephapirin									22		100

[a] Percentage figures are to the nearest whole number.
[b] NT, Not tested.

to a 10^{-4} dilution gave a two- to fourfold decrease of the MIC values for strains of *S. aureus, E. coli, Klebsiella,* and *Proteus mirabilis* (Table 2). As for the more resistant *Enterobacteriaceae,* inoculum effect was markedly more pronounced, with a maximum effect for *Enterobacter cloacae* and *Providencia stuartii.* The mean MIC of the two latter organisms increased respectively by a factor of up to 21 and 27 if an undiluted rather than a 10^{-4}-diluted culture was used for the inoculum. For most organisms, the effect of inoculum size was slightly to markedly greater with BL-S640 than with cephalexin.

Stability of BL-S640 in aqueous solution. Stability in 0.07 M phosphate-buffered solution at 4 C gave the following results: at pH 7.0 and 8.0 more than 50% inactivation occurred over a period of 15 days; at pH 6.0 the inactivation attained only about 15%, and at pH 5.0 activity was fully maintained after the same period of time. The stability at 37 C is shown in Fig. 8. At pH 1.0 there was a loss of activity of only 10% in 24 h, and at pH 5.0 there was a loss of about 15%. Great losses occurred at higher pH values: the half-life was 8.5 h at pH 6.0 and about 4 h at pH 7.0 and 8.0.

At 25 C, half-life times were 6 h at pH 8.0, 12 h at pH 7.0, and about 30 h at pH 6.0. At pH 5.0 no loss in activity could be detected after 24 h.

DISCUSSION

BL-S640 is known to display considerable antibacterial activity against a variety of both gram-negative and gram-positive clinical isolates (6, 10). In the present study the antibiotic was found to be less active than three of the control compounds against *S. aureus,* but nevertheless more active than cephalexin. In general, the antibacterial spectrum against gram-negative organisms was superior to that of the other cephalosporins, including cefazolin, as far as the more resistant *Enterobacteriaceae* are concerned. The overall activity of BL-S640 was well in contrast with that of cephalothin and cephapirin, which showed the highest activity

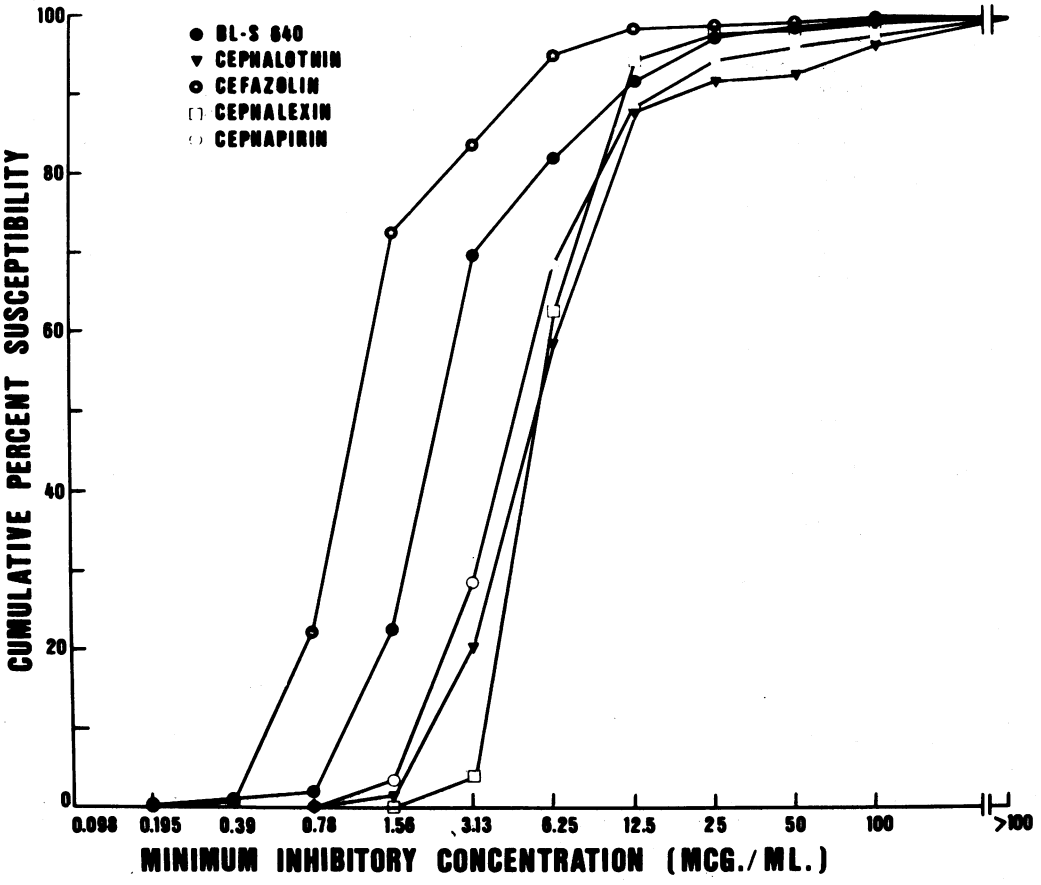

FIG. 3. *Comparative activity of five semisynthetic cephalosporins against Escherichia coli (155 isolates).*

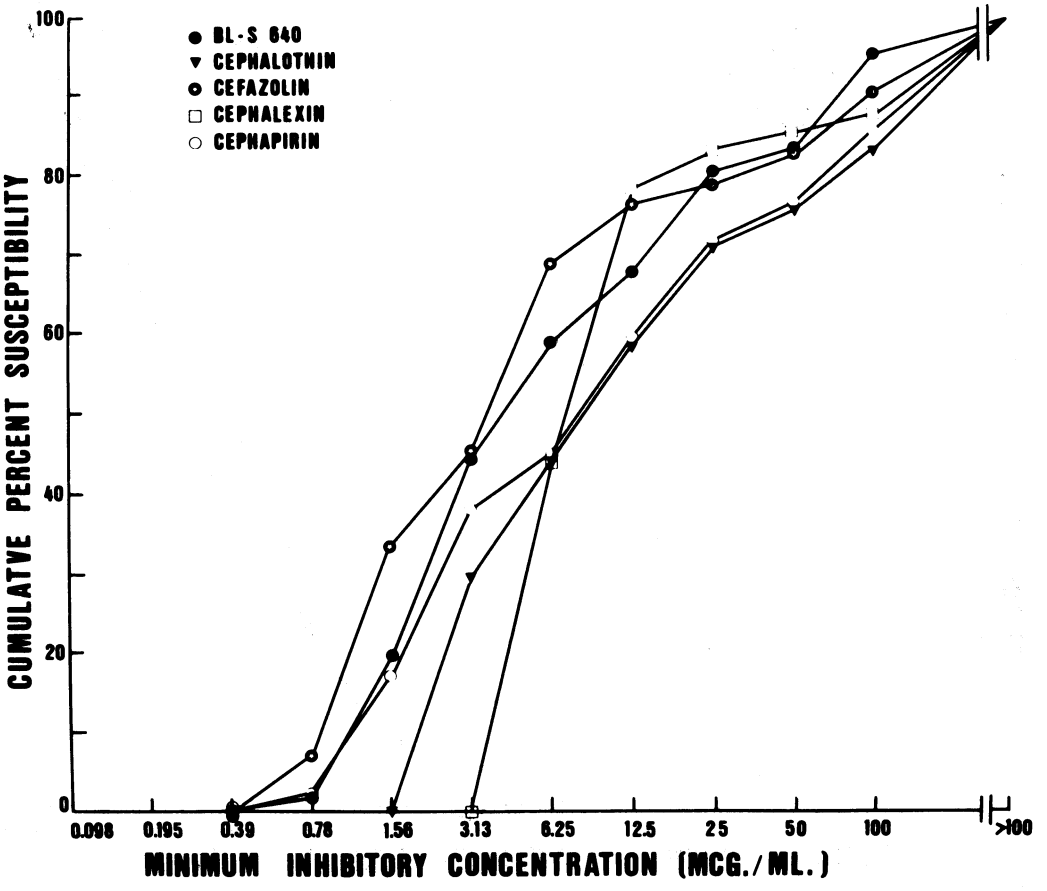

FIG. 4. *Comparative activity of five semisynthetic cephalosporins against Klebsiella species (45 isolates).*

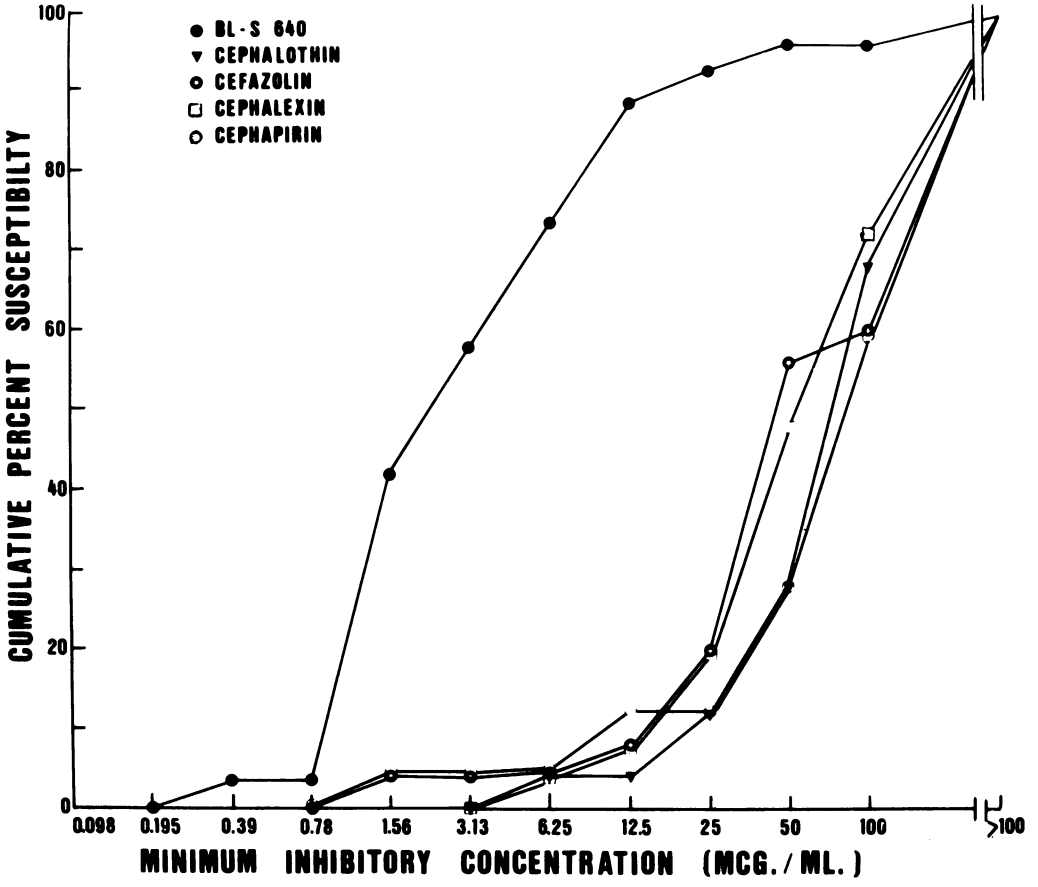

FIG. 5. *Comparative activity of five semisynthetic cephalosporins against Citrobacter species (25 isolates).*

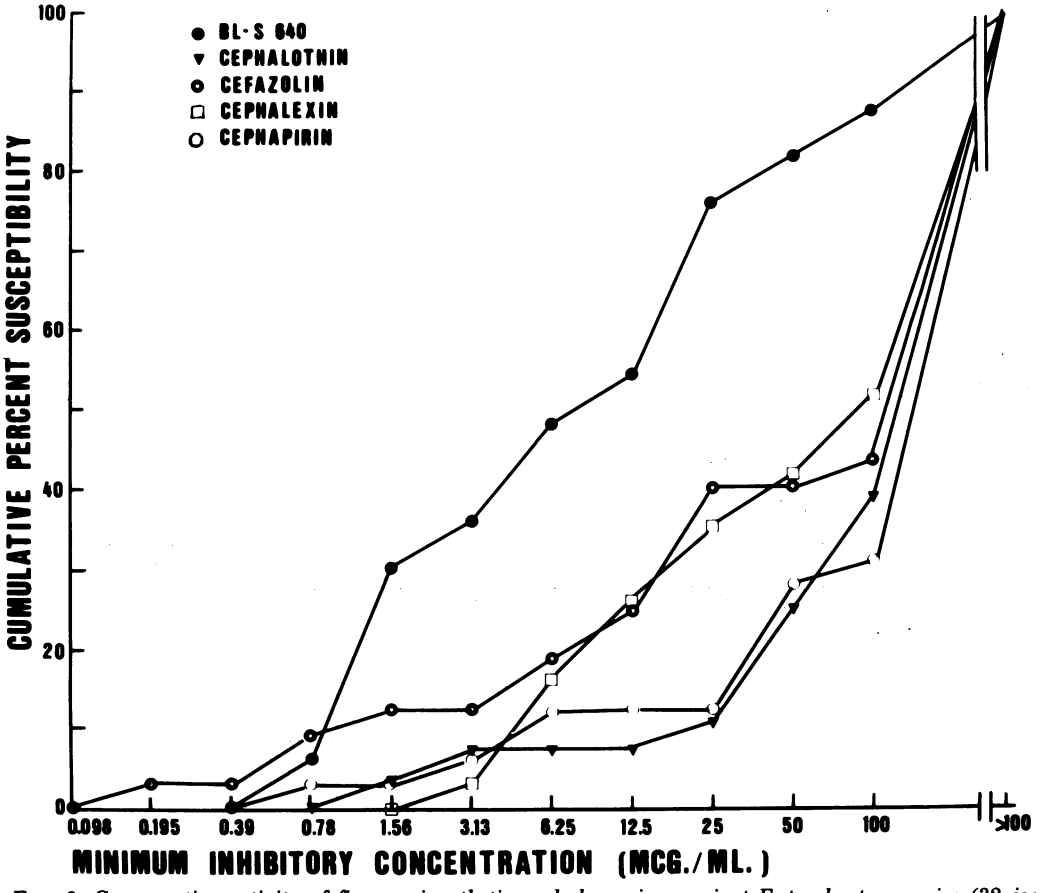

FIG. 6. *Comparative activity of five semisynthetic cephalosporins against Enterobacter species (32 isolates).*

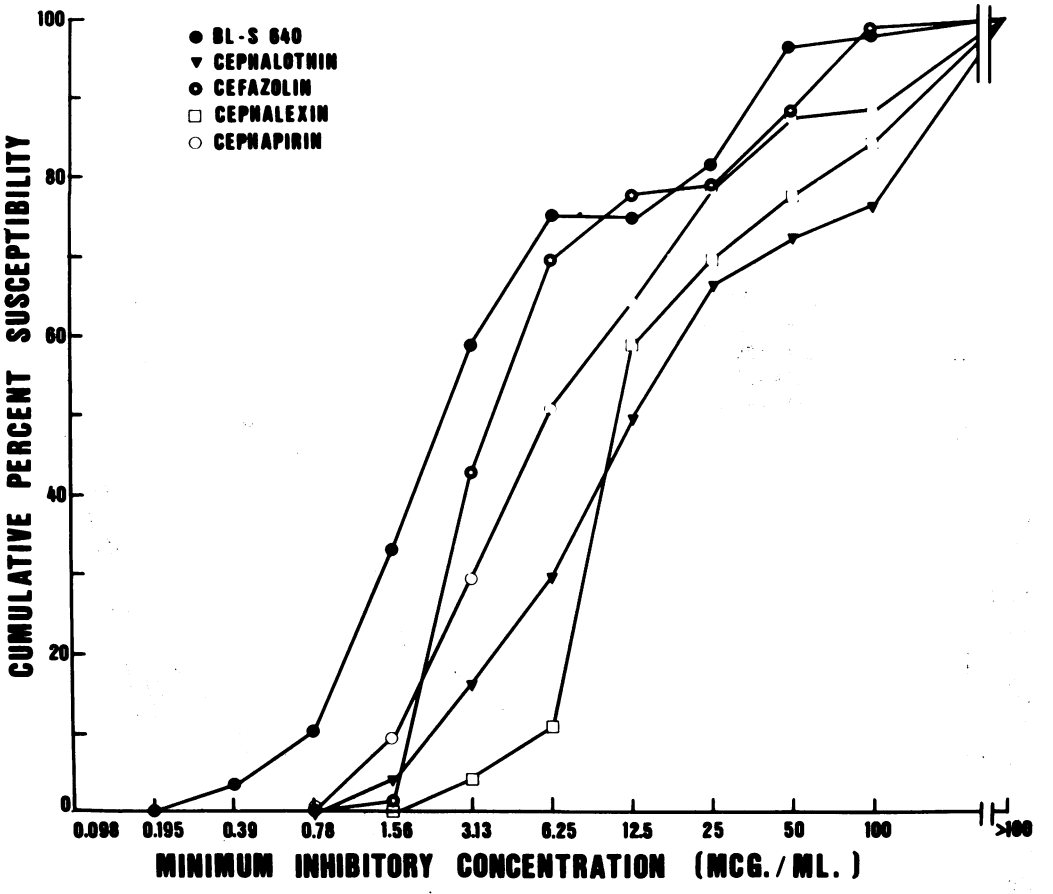

FIG. 7. *Comparative activity of five semisynthetic cephalosporins against Proteus mirabilis (75 isolates).*

TABLE 2. *Effect of inoculum size on the activity of BL-S640 and cephalexin against various organisms*

Organism	No. of strains	BL-S640			Cephalexin (MIC_a/MIC_c)
		MIC_a/MIC_b[a]	MIC_b/MIC_c	MIC_a/MIC_c	
Staphylococcus aureus	15	1.8	2.4	4.3	1.9
Escherichia coli	16	1.7	2.5	3.4	2.6
Klebsiella, sp.	5	1.6	1.2	1.8	1.8
Enterobacter cloacae	3	16.0	1.3	21.3	13.3
Proteus mirabilis	15	2.1	1.3	2.9	2.1
Proteus morganii	8	2.8	3.0	8.0	4.5
Proteus vulgaris	4	12.0	1.5	18.0	5.5
Proteus rettgeri	3	4.3	1.7	6.0	3.3
Providencia stuartii	3	8.0	3.3	26.7	16.0

[a] MIC_a: Minimal inhibitory concentration with undiluted inoculum (overnight broth culture); MIC_b: minimal inhibitory concentration with 10^{-2}-diluted inoculum; MIC_c: Minimal inhibitory concentration with 10^{-4}-diluted inoculum. Ratios are expressed as mean values for each species.

of the antibiotics tested against *S. aureus*, but among the *Enterobacteriaceae* inhibited only strains of *E. coli*, *Klebsiella*, and *Proteus mirabilis* at concentrations of drug readily achievable in man. Furthermore, BL-S640 was more active than cephalexin against all species tested. A relatively high percentage of strains belonging to the more resistant genera (*Enterobacter*, *Citrobacter*, indole-positive *Proteus*, and *Providencia*) were susceptible to BL-S640.

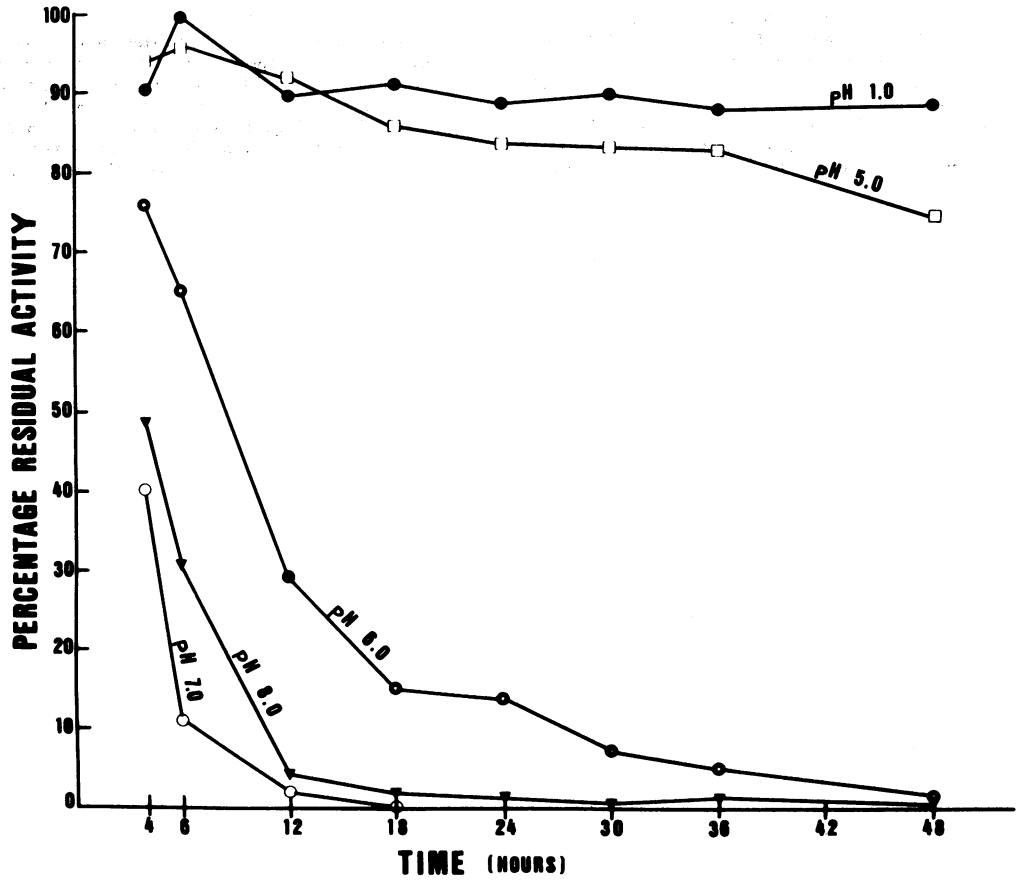

FIG. 8. *Stability of BL-S640 in aqueous solution at 37 C. Initial solutions contained 1,000-μg/ml activity. Residual activities were measured by using a turbidimetric method.*

However, similar to the other cephalosporins (2–5, 8), there was no demonstrable antibacterial activity against *Serratia marcescens* and *Pseudomonas aeruginosa*.

BL-S640 was slightly more affected in its antibacterial activity by inoculum changes than was cephalexin. This inoculum effect was moderate for strains of *S. aureus, E. coli, Klebsiella,* and *Proteus mirabilis,* but considerably higher for the more resistant organisms. In part, this may be due to enzymatic inactivation of the antibiotic by β-lactamases.

Cephalosporins have been known to undergo remarkably facile cleavage of their β-lactam bonds in aqueous solution. The acidic hydrolysis of cephalexin was found to be independent of pH, and this cephalosporin was shown to be fairly acid stable (11). In our experiments, BL-S640 also displayed remarkable stability in the pH range of 5.0 to 1.0, even at 37 C. However, above pH 6.0, stability very rapidly decreased with increasing pH.

ACKNOWLEDGMENTS

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LITERATURE CITED

1. **Arret, B., D. P. Johnson, and A. Kirshbaum.** 1971. Outline of details for microbiological assays of antibiotics: second revision. J. Pharm. Sci. 60:1689–1694.
2. **Axelrod, J., B. R. Meyers, and S. Z. Hirschman.** 1971. Cephapirin: in vitro antibacterial spectrum. Appl. Microbiol. 22:904–908.
3. **Chang, T., and L. Weinstein.** 1963. In vitro biological activity of cephalothin. J. Bacteriol. 85:1022–1027.
4. **Griffith, R. S., and H. R. Black.** 1964. Cephalothin—a new antibiotic. J. Am. Med. Assoc. 189:823–828.
5. **Griffith, R. S., and H. R. Black.** 1970. Cephalexin. Med. Clin. North Am. 54:1229–1244.
6. **Leitner, F., R. E. Buck, M. Misiek, T. A. Pursiano, and K. E. Price.** 1975. BL-S640, a cephalosporin with a broad spectrum of antibacterial activity: properties in vitro. Antimicrob. Agents Chemother. 7:298–305.
7. **Leitner, F., D. R. Chisholm, Y. H. Tsai, G. E. Wright, R. G. Deregis, and K. E. Price.** 1975. BL-S640, a cephalosporin with a broad spectrum of antibacterial activity: bioavailability and therapeutic properties in

rodents. Antimicrob. Agents Chemother. 7:306–310.
8. **Sabath, L. D., C. Wilcox, C. Garner, and M. Finland.** 1973. In vitro activity of cefazolin against recent clinical bacterial isolates. J. Infect. Dis. 128(Suppl.):S320–S326.
9. **Steers, E., E. L. Foltz, and B. S. Graves.** 1959. An inocula replicating apparatus for routine testing of bacterial susceptibility to antibiotics. Antibiot. Chemother. 9:307–311.
10. **Watanakunakorn, C., T. Bannister, and C. Glotzbecker.** 1975. Susceptibility of clinical isolates of Enterobacteriaceae to BL-S640, a new oral cephalosporin. Antimicrob. Agents Chemother. 7:381–385.
11. **Yamana, T., A. Tsuji, K. Kanayama, and O. Nakano.** 1974. Comparative stabilities of cephalosporins in aqueous solution. Jpn. J. Antibiot. 27:1000–1002.