ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, Apr. 1978, p. 669-675
0066-4804/78/0013-0669$02.00/0

Vol. 13, No. 4
Printed in U.S.A.

Use of a Heavy Inoculum in the In Vitro Evaluation of the Anti-Staphylococcal Activity of 19 Cephalosporins

MICHEL LAVERDIERE, DIANE WELTER, AND L. D. SABATH

Section on Infectious Diseases, Department of Medicine, University of Minnesota Medical School, Minneapolis, Minnesota 55455

Received for publication 14 November 1977

The in vitro activity of 19 cephalosporins against 105 clinical isolates of *Staphylococcus aureus* and *S. epidermidis* was determined by using a heavy inoculum, i.e., 10^8 to 10^9 organisms per ml, to maximally challenge the antibiotics. The anti-staphylococcal activities of cephaloridine and 87/312 were consistently decreased by the use of a heavy inoculum when compared with the activity obtained with two less-concentrated inocula. The activity of most of the other compounds was also decreased with the use of a heavy inoculum, but this was observed only with selected isolates. Cephapirin, cephalothin, and cefazaflur were the most active drugs against the methicillin-susceptible isolates. Cephaloridine, cefamandole, cefazaflur, and 87/312 had substantial activity against methicillin-resistant staphylococci even with heavy inocula. With the exception of cefaclor against *S. aureus*, the orally absorbed cephalosporins were generally one-half to one-sixteenth as active as the parenterally administered cephalosporins. The median minimal inhibitory concentrations of five of the 12 parenteral cephalosporins were lower with the methicillin-susceptible *S. aureus* than with the methicillin-susceptible *S. epidermidis* strains.

Some recently described semisynthetic cephalosporins have been reported to be more resistant to staphylococcal β-lactamase than those currently in use (4, 11). However, most of the standard in vitro studies have shown little or no difference in the anti-staphylococcal activity of those newest cephalosporins in comparison with other cephalosporins that have been available for several years or more (5, 15, 17). Previous studies (3, 14) have already established a certain correlation between the susceptibility of various cephalosporins to staphylococcal β-lactamase and the magnitude of the effect of the inoculum size when those same cephalosporins were tested against large and small inocula of staphylococci. In 1946, Luria showed that penicillinase-producing staphylococci, when diluted to the point that individual colonies were formed on agar plates, were almost as susceptible to penicillin G as were penicillinase-negative strains (9). The use of the currently suggested inoculum size for standard in vitro susceptibility tests (18) might thus fail to detect the destructive effect of staphylococcal β-lactamase on β-lactam antibiotics.

The possibility that a heavy inoculum could maximize the destructive effect of staphylococcal β-lactamase on cephalosporins and detect greater difference in the in vitro anti-staphylococcal activity of cephalosporins prompted this study.

(This paper was presented in part at the 16th Interscience Conference on Antimicrobial Agents and Chemotherapy, Chicago, Ill., 27-29 October 1976.)

MATERIALS AND METHODS

Organisms. A total of 105 isolates of staphylococci were tested, all obtained from patients at Boston City Hospitals and provided by A. Kathleen Daly and Alice McDonald. Sixty-six isolates (35 *Staphylococcus aureus* and 31 *S. epidermidis*) were susceptible to methicillin with a minimal inhibitory concentration (MIC) of 3.1 μg or less per ml. Thirty-nine isolates (17 *S. aureus* and 22 *S. epidermidis*) were resistant to methicillin, with an MIC of 50 μg or more of methicillin per ml against *S. aureus* and of 3.1 μg or greater per ml against *S. epidermidis*. The identification of each strain was based on the colonial morphology, Gram stain, coagulase reaction, and mannitol fermentation tests. All strains used in this study produced β-lactamase except for seven methicillin-susceptible strains of *S. aureus* and five methicillin-susceptible strains of *S. epidermidis*. A control strain (*S. aureus* ATCC 25923) was used in each MIC determination.

Antibiotics. Sterile standardized powders were kindly provided by their respective manufacturers: cephalothin, cephaloridine, cefazolin, cefamandole, 7-aminocephalosporanic acid, cephaloglycin, cephalexin, and cefaclor (C 99638) by Eli Lilly Laboratories for Clinical Research, Indianapolis, Ind.; cephapirin, cefatrizine (BLS 640) and BLS 786 by Bristol Research Laboratories, Syracuse, N.Y.; 87/312 Na and cefurox-

ime by Glaxo Research Ltd., Greenford, Middlesex, England; cefoxitin by Merck Sharp and Dohme Research Laboratories, West Point, Pa.; ceftezole and FR 10612 by Fujisawa Pharmaceutical Co., Ltd., Osaka, Japan; cefazaflur (SKF 59962) by Smith, Kline and French, Philadelphia, Pa.; cephacetrile (B 73-56) by Ciba-Geigy Corp., Summit, N.Y.; cephradine by Squibb and Sons, Ltd., Princeton, N.J.

The chemical composition of the three numbered compounds are as listed below, and their structural formulas are shown in Fig. 1. BL-S786 is 7-[α-(2-aminomethylphenyl)-acetamido] - 3 - [(1-carboxymethyltetrazol-5-ylthro)methyl]-3-cephem-4-carboxylic acid (8). Compound 87/312 is 3-(2,4-dinitrostyryl)-(6R,7R)-7-(2-thienylacetamido)-ceph-3-em-4-carboxylic acid, E-isomer (12). FR 10612 is 7-[D-2-(3-mesylaminophenyl)-glycinamido]-3-methyl-3-cephen-4-carboxylic acid.

Each antibiotic powder was stored in a desiccator at 4°C and removed only briefly when needed for weighing. Aqueous solutions were freshly prepared and always used immediately.

Susceptibility tests. The susceptibility testing was done by the agar dilution method using Mueller-Hinton agar and the inocula replicator of Steers et al. (16). The inoculum was an undiluted overnight culture that had been grown in Trypticase soy broth which contained 10^8 to 10^9 organisms per ml. The effect of variation in bacterial inoculum size on the MIC was determined with six methicillin-susceptible isolates (three *S. aureus* and three *S. epidermidis)* by using an undiluted overnight culture and two diluted inocula (10^{-2} and 10^{-4} dilutions) in Mueller-Hinton broth.

The MIC was defined as the minimum concentration of antibiotic that produced less than four small isolated colonies after 18 to 20 h of incubation at 30°C.

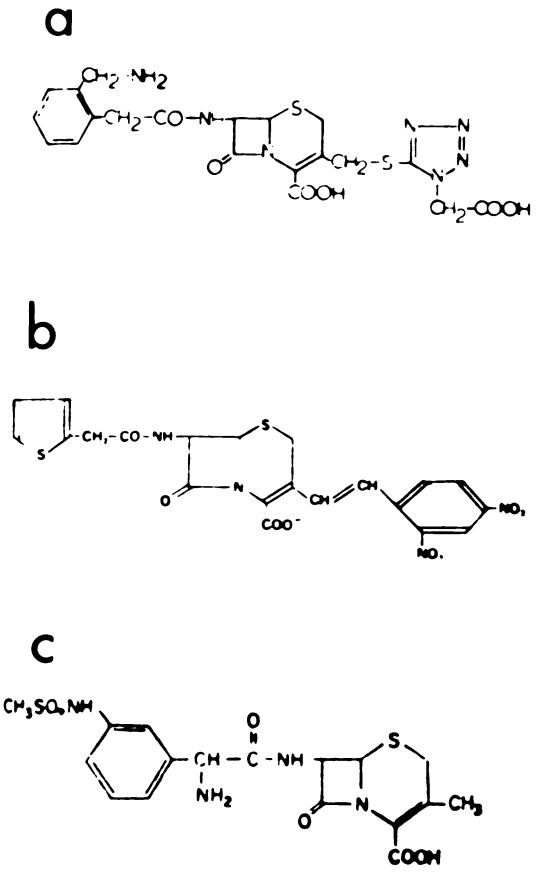

FIG. 1. *(a) BL-S786; (b) 87/312; and (c) FR 10612. See text for sources and chemical names.*

RESULTS

For the purpose of comparison, the drugs were classified in two groups according to their main route of administration: parenteral or oral. Cephradine and cefatrizine (BLS 640), the only two drugs that can be administered either way, are listed with the oral forms.

Effect of the variation in the inoculum size. Tables 1 through 4 show the effect of the size of the inoculum on the activity of the 19 cephalosporins. The greatest effect was noted with cephaloridine and 87/312: with all six isolates, a 10^{-4} dilution of the overnight culture resulted in a decrease in the MICs of those two antibiotics by as much as 64-fold when compared to the MICs obtained with the undiluted inoculum. However, most antibiotics showed marked differences (8- to 16-fold) in the inoculum effect against one or more of the strains. A significant decrease in the MICs of ceftezole and cefatrizine, for instance, was noted with *S. aureus* strain no. 2, but not with strains no. 1 and 3. Similar findings were also observed when the MICs of cefamandole and cefuroxime were determined using an undiluted overnight culture and a 10^{-4} dilution of *S. epidermidis* strain no. 2.

Activity against methicillin-susceptible organisms. The activity of the parenteral and oral cephalosporins against a heavy inoculum of methicillin-susceptible staphylococci is shown in Tables 5 and 6. The median MICs of 9 of the 19 cephalosporins against *S. aureus* and *S. epidermidis* isolates were identical. The median MICs of 8 of the remaining 10 cephalosporins were lower by two- to fourfold against *S. aureus* than against *S. epidermidis.* The only cephalosporins tested that were less active against *S. aureus* than *S. epidermidis* were cephradine and FR 10612; the median MICs were twofold lower against *S. epidermidis* than against *S. aureus.*

Thirteen of the 19 cephalosporins required 25 or more μg/ml to inhibit one or two isolates of *S. epidermidis,* even though they were susceptible to methicillin (i.e., they were methicillin

TABLE 1. *Effect of the size of the inoculum on the activity of 13 parenteral cephalosporins against S. aureus isolates (penicillinase +)*

Antibiotic	Strain no.	MIC (μg/ml) with inoculum			Ratio[a]	
		Und[b]	10^{-2} Dil[c]	10^{-4} Dil[c]	Und/10^{-2}	Und/10^{-4}
Cephalothin	1	0.78	0.78	0.39	1	2
	2	1.56	0.78	0.39	2	4
	3	0.78	0.78	0.39	1	2
Cephaloridine	1	1.56	0.09	0.02	16	64
	2	1.56	0.19	0.09	8	16
	3	1.56	0.09	0.09	16	16
Cefazolin	1	0.78	0.78	0.78	1	1
	2	3.12	0.78	0.78	4	4
	3	3.12	0.78	0.78	4	4
Cephapirin	1	0.19	0.19	0.19	1	1
	2	3.12	0.78	0.78	4	4
	3	3.12	0.39	0.39	8	8
Cefamandole	1	3.12	0.78	0.39	4	8
	2	3.12	0.78	0.39	4	8
	3	3.12	0.78	0.39	4	8
87/312	1	6.25	0.19	0.09	32	64
	2	12.5	0.78	0.39	16	32
	3	12.5	0.78	0.19	16	64
Cefuroxime	1	1.56	1.56	0.78	1	2
	2	1.56	1.56	1.56	1	1
	3	1.56	1.56	1.56	1	1
BLS 786	1	3.12	3.12	3.12	1	1
	2	6.25	3.12	3.12	2	2
	3	12.5	12.5	3.12	1	4
7-Aminocephalosporanic acid	1	>25	>25	>25	1	1
	2	>25	>25	>25	1	1
	3	>25	>25	>25	1	1
Cephacetrile	1	1.56	1.56	0.78	1	2
	2	3.12	1.56	1.56	2	2
	3	3.12	1.56	1.56	2	2
Cefazaflur	1	0.78	0.39	0.19	2	4
	2	3.12	0.78	0.39	4	8
	3	0.78	0.78	0.39	1	2
Cefoxitin	1	6.25	3.12	3.12	2	2
	2	6.25	6.25	6.25	1	1
	3	6.25	6.25	6.25	1	1
Ceftezole	1	0.39	0.39	0.39	1	1
	2	12.5	1.56	0.39	8	32
	3	3.12	0.78	0.39	4	8

[a] Ratios of the MIC with undiluted inoculum to the MIC with 10^{-2} and 10^{-4} dilutions.
[b] Und, Undiluted inoculum used in test.
[c] 10^{-2} Dil, 10^{-4} Dil, Inoculum used in test diluted 10^{-2} or 10^{-4}, respectively.

susceptible and cephalosporin resistant). Similar findings were also observed with nine cephalosporins and *S. aureus*.

The cephalosporin nucleus 7-ACA was much less active against both *S. aureus* and *S. epidermidis* (by a factor of at least 64 in comparing the median MICs) than the other semisynthetic cephalosporins.

The lowest median MICs were observed with cephapirin against both *S. aureus* and *S. epidermidis* isolates. Cephalothin showed the greater overall activity against the *S. aureus* strains inhibiting 100% of the isolates in a concentration of 0.78 μg/ml; 100% of the *S. epidermidis* strains were inhibited by 3.12 μg of cephalothin or cefazaflur (SKF 59962) per ml.

The MICs of cephaloridine and 87/312 had the widest range against both *S. aureus* and *S. epidermidis* isolates. However, ceftezole against *S. aureus* and cefuroxime against *S. epidermidis* seemed to have an equally large distribution of MICs. This wide distribution of MICs was in fact caused by a biomodal distribution in the susceptibility of the isolates for those antibiotics,

TABLE 2. *Effect of the size of the inoculum on the activity of six oral cephalosporins against S. aureus isolates (penicillinase +)*

Antibiotic	Strain no.	MIC (μg/ml) with inoculum			Ratio[a]	
		Und[b]	10^{-2} Dil[c]	10^{-4} Dil[c]	Und/10^{-2}	Und/10^{-4}
Cephaloglycin	1	3.12	3.12	1.56	1	2
	2	25	6.25	3.12	4	8
	3	12.5	6.25	3.12	2	4
Cephalexin	1	6.25	6.25	3.12	1	2
	2	25	6.25	6.25	4	4
	3	25	6.25	3.12	4	8
Cephradine	1	6.25	6.25	6.25	1	1
	2	12.5	6.25	6.25	2	2
	3	12.5	6.25	3.12	2	4
Cefatrizine	1	1.56	1.56	1.56	1	1
	2	25	6.25	1.56	4	16
	3	6.25	3.12	1.56	2	4
Cefaclor	1	6.25	3.12	1.56	2	4
	2	>25	6.25	3.12	≥4	≥8
	3	>25	6.25	3.12	≥4	≥8
FR 10612	1	3.12	3.12	1.56	1	2
	2	6.25	6.25	3.12	1	2
	3	6.25	6.25	3.12	1	2

[a] Ratios of the MIC with undiluted inoculum to the MIC with 10^{-2} and 10^{-4} dilutions.
[b] Und, Undiluted inoculum used in test.
[c] 10^{-2} Dil, 10^{-4} Dil, Inoculum used in test diluted 10^{-2} or 10^{-4}, respectively.

and the highest MICs were accounted for by only a few strains.

Cefaclor was the most active oral cephalosporin tested against the *S. aureus* isolates. In a concentration of 1.5 μg/ml, it inhibited 94% of the isolates compared with 3% with cefatrizine and 0% with the remaining four oral cephalosporins studied. This greater activity against *S. aureus* was also reflected by a median MIC one-fourth (or less) the median MICs observed with the other oral cephalosporins. With *S. epidermidis,* however, the activity of cefaclor was less striking and somewhat similar to the activity observed with FR 10612.

The parenteral drugs were more active (by 2- to 16-fold) than the oral drugs. Twenty-one of 24 median MICs obtained with the parenteral drugs (excluding 7-ACA) were equal to or less than 1.56 μg/ml, whereas 11 of 12 median MICs obtained with the oral cephalosporins were higher than 1.56 μg/ml. However, parenteral drugs cefoxitin, BLS 786, and 87/312 showed activity against *S. epidermis* isolates similar to that observed with the oral drugs, whereas cefaclor showed the same median MIC as cefazolin, cefamandole, and ceftezole against the *S. aureus* strains.

Activity against methicillin-resistant staphylococci. The activity of the parenteral and oral cephalosporins against a heavy inoculum of methicillin-resistant isolates is shown in Tables 5 and 6.

The median MIC against four of the parenteral cephalosporins for *S. epidermis* was 12.5 μg/ml. The median MIC against both *S. aureus* and *S. epidermidis* isolates for cephaloridine and cefamandole was 12.5 μg/ml, whereas that against only the *S. epidermidis* isolates for cefazaflur (SKF 59962) and 87/312 was 12 μg/ml.

DISCUSSION

This study demonstrated that even with the use of a heavy inoculum, all the drugs tested (except the cephalosporins nucleus, 7-ACA) possessed substantial anti-staphylococcal activity.

Other in vitro studies showed large inoculum effects with cephalosporins (5, 14), but in this study only cephaloridine and 87/312 consistently showed marked decreased anti-staphylococcal activity when tested against a heavy inoculum of methicillin-susceptible staphylococci. However, most of the other compounds also showed large inoculum effects with one or more strains. Some of these variations may have been due to variations in the properties of the β-lactamase produced (12). A bimodel distribution of the MICs was observed with 87/312, cephaloridine, ceftezole, and cefuroxime with the methicillin-susceptible isolates and is likely to be due to qualitative differences in the types of β-lactamase produced by the organisms and their ability or inability to hydrolyze those antibiotics. Because of these differences in the

TABLE 3. *Effect of the size of the inoculum on the activity of 13 cephalosporins against S. epidermidis isolates (penicillinase +)*

Antibiotic	Strain no.	MIC (μg/ml) with inoculum			Ratio[a]	
		Und[b]	10^{-2} Dil[c]	10^{-4} Dil[c]	Undil/10^{-2}	Undil/10^{-4}
Cephalothin	1	0.78	0.78	0.19	1	4
	2	0.39	0.19	0.19	2	2
	3	0.39	0.39	0.19	1	2
Cephaloridine	1	0.39	0.04	0.04	8	8
	2	0.78	0.04	0.01	16	64
	3	0.39	0.04	0.04	8	8
Cefazolin	1	0.39	0.39	0.39	1	1
	2	0.19	0.19	0.19	1	1
	3	0.19	0.19	0.19	1	1
Cephapirin	1	0.19	0.39	0.39	2	2
	2	0.19	0.39	0.39	2	2
	3	0.39	0.39	0.39	1	1
Cefamandole	1	1.56	0.39	0.39	4	4
	2	3.12	0.39	0.19	8	16
	3	1.56	0.78	0.39	2	8
87/312	1	0.39	0.04	0.02	8	16
	2	1.56	0.04	0.02	32	64
	3	1.56	0.09	0.04	16	32
Cefuroxime	1	1.56	1.56	0.78	1	2
	2	0.78	0.19	0.02	4	32
	3	1.56	1.56	1.56	1	1
BLS 786	1	3.12	3.12	3.12	1	1
	2	3.12	3.12	0.78	1	4
	3	3.12	3.12	3.12	1	1
7-Aminocephalosporanic acid	1	>25	>25	>25	1	1
	2	>25	>25	>25	1	1
	3	>25	>25	>25	1	1
Cephacetrile	1	1.56	1.56	0.78	1	2
	2	1.56	0.39	0.19	4	8
	3	1.56	1.56	0.78	1	2
Cefazaflur	1	0.39	0.39	0.39	1	1
	2	0.39	0.19	0.19	2	2
	3	0.39	0.39	0.39	1	1
Cefoxitin	1	6.25	6.25	3.12	1	2
	2	6.25	3.12	1.56	2	4
	3	6.25	6.25	3.12	1	2
Ceftezole	1	0.78	0.39	0.39	2	2
	2	0.78	0.19	0.09	4	8
	3	0.39	0.19	0.19	2	2

[a] Ratios of the MIC with undiluted inoculum to the MIC with 10^{-2} and 10^{-4} dilutions.
[b] Und, Undiluted inoculum used in test.
[c] 10^{-2} Dil, 10^{-4} Dil, Inoculum used in test diluted 10^{-2} or 10^{-4}, respectively.

effect of the inoculum on the MIC, it may be worthwhile, in special situations, to test the susceptibility of staphylococci to cephalosporins by using heavy inocula.

In general, *S. aureus* strains were more susceptible and were inhibited by lower MICs of cephalosporins than were *S. epidermidis* strains. A few strains of both *S. aureus* and *S. epidermidis* were resistant (e.g., were inhibited by MICs of 25, 50, or even 200 μg/ml to 7,800 μg/ml with 7-ACA) to some of the cephalosporins, even though they were methicillin susceptible.

In contrast, virtually all of the methicillin-resistant *S. aureus* strains were also resistant to these cephalosporins. The median MIC of cephapirin (0.19 μg/ml) was the lowest against both *S. aureus* and *S. epidermidis* strains, but cephalothin and cefazaflur were similar; their median MIC was 0.39 μg/ml.

Cefaclor was clearly the most active "oral" cephalosporin against *S. aureus*. The oral cephalosporins, except for cefaclor, were much less active than most of the parenteral compounds.

The situation with methicillin-resistant *S. epidermidis* strains remains unclear; although many are also resistant to the cephalosporins,

TABLE 4. *Effect of the size of the inoculum on the activity of six oral cephalosporins against S. epidermidis isolates (penicillinase +)*

Antibiotics	Strain no.	MIC (μg/ml) with inoculum			Ratio[a]	
		Und[b]	10^{-2} Dil[c]	10^{-4} Dil[c]	Undil/10^{-2}	Undil/10^{-4}
Cephaloglycin	1	12.5	3.12	3.12	4	4
	2	3.12	3.12	1.56	1	2
	3	3.12	3.12	1.56	1	2
Cephalexin	1	6.25	6.25	3.12	1	2
	2	6.25	6.25	6.25	1	1
	3	3.12	3.12	3.12	1	1
Cephradine	1	6.25	6.25	3.12	1	2
	2	6.25	6.25	1.56	1	4
	3	3.12	3.12	1.56	1	2
Cefatrizine	1	1.56	1.56	0.78	1	2
	2	1.56	0.78	0.78	2	2
	3	3.12	1.56	0.78	2	4
Cefaclor	1	3.12	1.56	1.56	2	2
	2	3.12	1.56	0.78	2	4
	3	3.12	1.56	1.56	2	2
FR 10612	1	3.12	3.12	1.56	1	2
	2	3.12	3.12	0.78	1	4
	3	3.12	3.12	3.12	1	1

[a] Ratios of the MIC with undiluted inoculum to the MIC with 10^{-2} and 10^{-4} dilutions.
[b] Und, Undiluted inoculum used in test.
[c] 10^{-2} Dil, 10^{-4} Dil, Inoculum used in test diluted 10^{-2} or 10^{-4}, respectively.

TABLE 5. *Susceptibility of methicillin-susceptible and methicillin-resistant staphylococci to parenteral cephalosporins*

Antibiotic	Methicillin-susceptible staphylococci				Methicillin-resistant staphylococci			
	S. aureus (35 strains) MIC (μg/ml)		*S. epidermidis* (31 strains) MIC (μg/ml)		*S. aureus* (17 strains) MIC (μg/ml)		*S. epidermidis* (22 strains) MIC (μg/ml)	
	Range	Median	Range	Median	Range	Median	Range	Median
Cephapirin	0.19–1.56	0.19	0.09–6.25	0.19	6.25–100	12.5	0.78–50	12.5
Cephaloridine	0.04–6.25	0.39	0.01–12.5	1.56	12.5–50	12.5	1.56–100	12.5
Cephalothin	0.09–0.78	0.39	0.04–3.12	0.39	25–400	50	0.19–200	25
Cefazaflur	0.19–3.12	0.39	0.09–3.12	0.39	25–100	50	1.56–200	25
Cefamandole	0.39–3.12	0.78	0.19–6.25	0.78	12.5–100	50	1.56–200	12.5
Cefazolin	0.39–12.5	0.78	0.19–25	0.78	12.5–200	100	3.12–200	25
Ceftezole	0.19–>25	0.78	0.19–25	1.56	100–800	200	1.56–200	200
Cefoxitin	1.56	1.56	0.78–50	3.12	50–400	200	0.09–400	200
Cefuroxime	0.78–3.12	1.56	0.39–200	1.56	3.12–>25	>25	0.04–>25	12.5
BLS 786	0.78–3.12	1.56	3.12–25	6.25	>200	>200	6.25–>200	200
87/312	0.04–>25	1.56	0.04–>25	6.25	800–>800	>800	1.56–800	200
Cephacetrile	0.78–3.12	1.56	0.78–6.25	1.56	>800	>800	0.19–>800	800
7-Aminocephalosporanic acid	200–400	400	400–>800	800	>800	>800	800–>800	>800

TABLE 6. *Susceptibility of methicillin-susceptible and methicillin-resistant staphylococci to oral cephalosporins*

Antibiotic	Methicillin-susceptible staphylococci				Methicillin-resistant staphylococci			
	S. aureus (35 strains) MIC (μg/ml)		*S. epidermidis* (31 strains) MIC (μg/ml)		*S. aureus* (17 strains) MIC (μg/ml)		*S. epidermidis* (22 strains) MIC (μg/ml)	
	Range	Median	Range	Median	Range	Median	Range	Median
Cephaloglycin	3.12–25	6.25	1.56–25	6.25	50–200	100	0.78–100	50
Cephalexin	3.12–25	6.25	3.12–50	6.25	200–400	200	6.25–400	200
Cephradine	3.12–25	6.25	1.56–100	3.12	50–400	200	6.25–50	25
Cefatrizine	1.56–50	3.12	0.78–50	6.25	200–400	400	1.56–400	400
Cefaclor	0.19–>25	0.78	0.78–>25	3.12	2.5–40	>40	20–>40	>40
FR 10612	3.12–>25	6.25	0.78–>25	3.12	>200	>200	25–>200	>200

some strains appear to be susceptible to some of these cephalosporins (note wide range, but high median in Table 5, last two columns).

ACKNOWLEDGMENTS

This study was supported by a grant from the Québec Medical Research Council and by a grant-in-aid from Glaxo Research, Ltd.

The technical assistance of Bernice Zins and Shirley Hermel in the preparation of the manuscript is deeply appreciated.

LITERATURE CITED

1. **Acar, J. F., P. Couravalin, and Y. A. Chabbert.** 1971. Methicillin-resistant staphylococcemia: bacteriological failure of treatment with cephalosporins, p. 280–285. Antimicrob. Agents Chemother. 1970.
2. **Chabbert, Y. A.** 1967. Behavior of methicillin heteroresistant staphylococci to cephaloridine. Postgrad. Med. J. **43**(Suppl):40–42.
3. **Farrar, E. W., and P. K. Gramling.** 1976. Antistaphylococcal activity and β-lactamase resistance of newer cephalosporins. J. Infect. Dis. **133**:691–695.
4. **Fong, I. W., E. R. Engelking, and W. M. M. Kirby.** 1976. Relative inactivation by *Staphylococcus aureus* of eight cephalosporin antibiotics. Antimicrob. Agents Chemother. **9**:939–944.
5. **Hall, W. H., D. Gerding, and E. A. Schierl.** 1977. Antibacterial activity of cefamandole, eight other cephalosporins, cefoxitin, and ampicillin. Curr. Ther. Res. Clin. Exp. **21**:374–389.
6. **Kiesch, A. L., and L. Bartholomew.** 1976. Comparison of the in vitro activity of several cephalosporin antibiotics against gram-negative and gram-positive bacteria resistant to cephaloridine. Antimicrob. Agents Chemother. **10**:507–510.
7. **Klimek, J. J., F. J. Marsik, R. C. Bartlett, B. Weir, P. Shea, and R. Quintiliani.** 1976. Clinical epidemiologic and bacteriologic observations of an outbreak of methicillin-resistant *Staphylococcus aureus* at a large community hospital. Am. J. Med. **61**:340–345.
8. **Leitner, F., M. Misiek, T. A. Pursiano, R. E. Buck, D. R. Chisholm, R. G. DeRegis, Y. H. Tsai, and K. E. Price.** 1976. Laboratory evaluation of BL-S786, a cephalosporin with broad-spectrum antibacterial activity. Antimicrob. Agents Chemother. **10**:426–435.
9. **Luria, S. E.** 1946. A test for penicillin sensitivity and resistance in *Staphylococcus*. Proc. Soc. Exp. Biol. Med. **61**:46–51.
10. **Nishida, M., T. Murakawa, T. Kamimura, N. Okada, H. Sakamoto, S. Fukada, S. Nakamoto, Y. Yokota, and K. Miki.** 1976. Laboratory evaluation of FR 10612, a new oral cephalosporin derivative. J. Antibiot. **29**:444–459.
11. **O'Callaghan, C. H.** 1975. Classification of cephalosporins by their antibacterial and pharmocokinetic properties. J. Antimicrob. Chemother. **1** (Suppl):1–12.
12. **O'Callaghan, C. H., A. Morris, S. M. Kirby, and A. H. Shingler.** 1972. Novel method for detection of β-lactamases using a chromogenic cephalosporin substrate. Antimicrob. Agents Chemother. **1**:283–288.
13. **Richmond, M. D.** 1965. Wild-type variants of exopenicillinase from *Staphylococcus aureus*. Biochem. J. **94**:584–593.
14. **Sabath, L. D., C. Garner, C. Wilcox, and M. Finland.** 1975. Effect of inoculum and of β-lactamase on the antistaphylococcal activity of thirteen penicillins and cephalosporins. Antimicrob. Agents Chemother. **8**:344–349.
15. **Sabath, L. D., C. Garner, C. Wilcox, and M. Finland. 1976. Susceptibility of** *Staphylococcus aureus* and *Staphylococcus epidermidis* to 65 antibiotics. Antimicrob. Agents Chemother. **9**:962–969.
16. **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.
17. **Stewart, D., and G. P. Bodey.** 1976. Comparative *in vitro* activity of cephalosporins. J. Antibiot. **29**:181–186.
18. **Washington, J. A., and A. L. Barry.** 1974. Dilution test procedures, p. 410–417. *In* E. H. Lennette, E. H. Spaulding, and J. P. Truant (ed.), Manual of clinical microbiology, 2nd ed. American Society for Microbiology, Washington, D. C.