ANTIMICROBIAL AGENTS AND CHEMOTHERAPY, June 1976, p. 962-969

Vol. 9, No. 6
Printed in U.S.A.

Susceptibility of *Staphylococcus aureus* and *Staphylococcus epidermidis* to 65 Antibiotics

L. D. SABATH,[1]* CAROL GARNER, CLARE WILCOX, AND MAXWELL FINLAND

Channing Laboratory, Boston City Hospital, Boston, Massachusetts 02118, and Departments of Medicine and Medical Microbiology, Harvard Medical School, Boston, Massachusetts 02118

Received for publication 19 January 1976

The susceptibilities of 36 recent isolates of *Staphylococcus aureus* and 35 recent isolates of *Staphylococcus epidermidis* were determined against each of 65 antimicrobial agents and against two of them in combination. Rifampin was the most active of all the agents tested against both *S. aureus* and *S. epidermidis*. Among the penicillins, cloxacillin, dicloxacillin, and nafcillin were most active, although benzylpenicillin and phenoxymethyl penicillin were more active against susceptible strains. Cephaloridine was the most active of the cephalosporins, and sisomicin was the most active aminoglycoside. Minocycline was more active than the other tetracycline analogues tested. Among the macrolide-lincomycin compounds in clinical use, clindamycin was more active, and lincomycin was less active than erythromycin. The synergy of trimethoprim-sulfamethoxazole was more striking against *S. aureus* than against *S. epidermidis*. The median minimal inhibitory concentrations of the penicillins, cephalosporins, and aminoglycosides were lower against *S. aureus*, whereas the minimal inhibitory concentrations of the tetracyclines were lower against *S. epidermidis*.

A large number of new natural and semisynthetic antibiotics with anti-staphylococcal activity have been introduced recently. This study was performed to compare their activities with those of other compounds that have been available for several or more years. The desirability of having comparative data on the same organisms from one laboratory, obtained by a uniform method, is obvious.

Tests were performed on both *Staphylococcus aureus* and *Staphylococcus epidermidis* for the purpose of observing possible differences, and also to obtain information that would be potentially useful in clinical medicine.

MATERIALS AND METHODS

Sixty-five antimicrobial preparations and two of them in combination (trimethoprim plus sulfamethoxazole in a ratio of 1:16; this was selected for convenience of making dilutions for the test—a ratio of 1:20, which has been suggested as the trimethoprim/sulfamethoxazole ratio in blood, might have been used, but no great differences in results were anticipated by using 1:16) were tested for antibacterial activity against 36 strains of *S. aureus* and 35 strains of *S. epidermidis* (in several instances, only 12 strains of *S. aureus* and 12 or 32 strains of *S. epidermidis* were tested). The antimicrobial preparations tested are listed along with their respective suppliers in Tables 1 through 3; also shown in these tables are the abbreviations for each compound, as used in the figures.

The organisms tested were all recent clinical isolates at Boston City Hospital, provided by A. Kathleen Daly and Alice McDonald, and identified on the basis of colonial morphology, Gram stain, coagulase reaction, and mannitol fermentation tests. The susceptibility testing was done by the agar dilution method using Mueller-Hinton agar and the inocula replicator of Steers et al. (8), using as inoculum 10^{-3} dilutions of overnight cultures. Plates were read for inhibition of growth after 18 h of incubation at 37 C and compared with a refrigerated, inoculated control (for no growth). All organisms were found to be susceptible to methicillin on the basis of 48 h of incubation at 30 C.

RESULTS

The range and median minimum inhibitory concentrations (MICs) of each antibiotic are shown in Tables 1 through 3, and the cumulative percentages of isolates inhibited at various concentrations of antibiotic are graphed in Fig. 1 through 10.

Among the penicillins, it is quite obvious that the penicillin nucleus, 6-APA, was much less active than any of the natural or semisynthetic penicillins against both *S. aureus* and *S.*

[1] Present address: Mayo Memorial Building, University of Minnesota Medical School, Minneapolis, Minn. 55455.

TABLE 1. *Susceptibility of staphylococci to analogues of penicillin and cephalosporin*

Antibiotic	Symbol	Supplier	*S. aureus*			*S. epidermidis*		
			No. of strains	MIC, μg/ml		No. of strains	MIC, μg/ml	
				Range	Median		Range	Median
Benzylpenicillin K[a]	PNG	Squibb	36	<0.005–12.5	0.4	35	<0.005–25	0.4
Phenoxymethyl penicillin K	PNV	Lilly	36	0.005–3.1	0.2	35	<0.005–12.5	0.1
Ampicillin sodium	AMP	Bristol	36	0.02–25	1.6	35	0.005–12.5	0.1
Epicillin	EPI	Squibb	36	0.02–12.5	1.6	35	0.01–6.3	0.2
Amoxicillin trihydrate	AMOX	Beecham	36	0.04–12.5	1.6	35	0.04–12.5	0.2
Carbenicillin disodium	CARB	Beecham	36	0.2–25	6.3	35	0.2–50	1.6
Ticarcillin disodium	TICAR	Beecham	36	0.4–25	6.3	35	0.4–50	3.1
BL-P1654 sodium	1954	Bristol	12	0.4–3.1	0.8	12	0.04–12.5	0.2
Cyclacyllin anhydrous	CYCLA	Wyeth	12	0.004–12.5	3.1	35	0.04–25	0.4
RO8-0074/003	RO8	Roche	12	0.004–3.1	0.8	12	0.04–12.5	0.4
Methicillin sodium	MC	Bristol	36	0.4–3.1	1.6	35	0.1–3.1	1.6
Oxacillin sodium	OXC	Bristol	36	0.1–0.8	0.4	35	0.1–1.6	0.2
Cloxacillin sodium	CLC	Bristol	36	0.004–0.4	0.2	35	0.1–0.8	0.2
Dicloxacillin sodium	DCC	Ayerst	36	0.004–0.2	0.2	36	0.04–0.8	0.2
Nafcillin sodium	NAF	Wyeth	36	0.1–0.4	0.4	35	0.04–0.4	0.2
6-Aminopenicillanic acid	6-APA	Bristol	36	50–200	100	35	12.5–400	50
Cephalothin sodium	CTN	Lilly	36	0.1–0.8	0.2	32	0.04–0.8	0.1
Cephaloridine	CLD	Lilly	36	0.01–0.2	0.1	32	0.005–0.4	0.02
Cephaloglycin	CGN	Lilly	36	0.8–6.3	3.1	32	0.1–12.5	0.8
Cephalexin	CLX	Lilly	36	0.8–6.3	3.1	32	0.04–12.5	1.6
Cefazolin sodium	CZ	SKF	36	0.04–0.8	0.4	32	0.04–1.6	0.2
Cephacetrile	CCT	CIBA	36	0.2–3.1	0.8	32	0.1–1.6	0.4
Cephradine	CRD	Squibb	12	1.6–6.3	3.1	12	0.8–1.6	1.6
Cefoxitin	CXT	Merck	36	1.6–6.3	3.1	32	0.04–12.5	1.6
Cephapirin sodium	CPN	Bristol	36	0.04–0.8	0.2	32	0.02–0.8	0.1
Cephanone	CNN	Lilly	36	0.04–1.6	0.4	32	0.04–1.6	0.1
Cefamandole	CMT	Lilly	36	0.04–3.1	0.8	32	0.04–1.6	0.2
87/312	87/312	Glaxo	36	0.02–6.3	0.4	32	0.005–0.8	0.02

[a] K indicates potassium salt.

TABLE 2. *Susceptibility of staphylococci to aminoglycosides, polymyxins, tetracyclines, chloramphenicol, and spectinomycin*

Antibiotic[a]	Symbol	Supplier	*S. aureus*			*S. epidermidis*		
			No. of strains	MIC, μg/ml		No. of strains	MIC, μg/ml	
				Range	Median		Range	Median
Streptomycin	SM	Lilly	36	3.1–200	6.3	35	1.6–>400	3.1
Neomycin	NM	Upjohn	36	0.4–1.6	0.8	35	0.02–12.5	0.1
Kanamycin	KM	Bristol	36	1.6–6.3	3.1	35	0.2–400	0.8
Gentamicin	GM	Schering	36	0.2–0.8	0.4	35	0.01–1.6	0.1
Betamicin	GMB	Schering	12	0.8–6.3	1.6	12	0.1–6.3	0.2
Gentamicin C_1	GMC_1	Schering	12	0.2–1.6	0.8	12	0.04–0.2	0.1
Sisomicin	SISO	Schering	12	0.2–0.8	0.2	12	0.02–0.1	0.04
Verdamicin	VERDA	Schering	12	all 0.8	0.8	12	0.02–0.2	0.04
Tobramycin	TM	Lilly	36	0.4–1.6	0.8	35	0.01–100	0.1
Amikacin	AMIK	Bristol	36	1.6–3.1	3.1	35	0.4–6.3	0.8
Butirosin	BUTI	Parke-Davis	12	50–100	50	12	0.2–>400	0.8
Polymyxin B	PMB	Burroughs-Wellcome	36	50–>100	100	35	3.1–100	50
Colistin	COLI	Warner-Lambert	36	100–>100	>100	35	6.3–>100	100
Tetracycline	TC	Lederle	36	0.04–>100	0.1	35	0.2–100	0.8
Chlortetracycline	CTC	Lederle	36	0.04–50	0.1	35	0.2–100	0.8
Oxytetracycline	OTC	Pfizer	36	0.2–>100	0.4	35	0.4–100	1.6
Demeclocycline	DMCT	Lederle	36	0.04–100	0.1	35	0.1–50	0.2
Methacycline	MTC	Pfizer	36	0.04–12.5	0.1	35	0.04–100	0.2
Doxycycline	DOXY	Pfizer	36	0.04–6.3	0.04	35	0.04–6.3	0.4
Minocycline	MINO	Lederle	36	0.02–1.6	0.04	35	0.04–0.8	0.2
Chloramphenicol	CMP	Parke-Davis	36	all 3.1	3.1	35	1.6–25	3.1
Spectinomycin	SPM	Upjohn	36	50–>400	100	35	25–>400	50

[a] Tobramycin, amikacin, chloramphenicol, and spectinomycin were supplied as the base; the other aminoglycosides and the polymyxins were all sulfates, and the tetracyclines were all hydrochlorides.

epidermidis, by a factor of 16 or more (in comparing median MIC values). The most active antibiotic against *S. aureus* was benzylpenicillin (lowest MIC in range), but the sample tested contained numerous "penicillin-resistant" (MIC, ≥0.2 μg/ml) strains with MIC values up to 12.5 μg/ml. Thus, a comparison of cloxacillin, dicloxacillin, and phenoxymethyl penicillin had the lowest median MIC (0.2 μg/ml) values for these strains, and benzylpenicillin, nafcillin, and oxacillin were close behind with median MIC values of 0.4 μg/ml. In Fig. 1, it is quite obvious that the lowest MIC values were for benzylpenicillin and phenoxymethyl penicillin, but this only obtained with about 20% of the strains. (Among penicillinase-susceptible antibiotics, the median MICs for eight penicillin-susceptible strains were 0.02 μg/ml for penicillin G and penicillin V, 0.09 μg/ml for ampicillin and epicillin, 0.19 μg/ml for amoxicillin, and 0.78 μg/ml for carbenicillin and ticarcillin.) Cloxacillin, dicloxacillin, and nafcillin inhibited all of the strains in concentrations of 0.4 μg/ml or less. Aside from 6-APA, ticarcillin and carbenicillin were the least active against *S. aureus*.

The susceptibility of *S. epidermidis* to these 16 penicillins (Fig. 2) resembled that of *S.*

TABLE 3. *Susceptibility of staphylococci to eight lincomycin analogues and to several other antibacterial agents*

Antibiotic[a]	Symbol	Supplier	*S. aureus*			*S. epidermidis*		
			No. of strains	MIC, μg/ml		No. of strains	MIC, μg/ml	
				Range	Median		Range	Median
Lincomycin hydrochloride	LM	Upjohn	36	0.4–1.6	0.8	35	0.2–>100	0.4
Clindamycin hydrochloride	CLM	Upjohn	36	0.02–0.04	0.04	35	0.02–>50	0.04
U-26, 727A	DMCL	Upjohn	36	0.02–0.1	0.04	35	0.02–>50	0.04
U-24, 729A	DPCL	Upjohn	36	0.002–0.02	0.01	35	0.002–>50	0.04
Clindamycin sulfoxide	CLSO	Upjohn	36	0.4–1.6	0.8	35	0.8–>50	0.8
U-34, 728E	HECL	Upjohn	36	0.04–0.2	0.1	35	0.04–>50	0.1
U-38, 784E	BHEL	Upjohn	36	0.04–0.2	0.1	35	0.04–>50	0.1
U-39, 745E	DMPC	Upjohn	36	0.02–0.2	0.04	35	0.04–>50	0.04
Erythromycin	EM	Lilly	36	0.1–3.1	0.2	35	0.1–>100	0.1
Rifampin	RIF	CIBA	36	0.0003–0.005	0.001	35	0.0003–0.005	0.002
Vancomycin hydrochloride	VANCO	Lilly	36	0.8–1.6	1.6	35	0.4–3.1	1.6
Bacitracin, zinc	BACI	Pfizer	36	0.8–25	12.5	35	0.4–50	25
Everninomicin	EVER	Schering	12	0.2–0.8	0.4	12	0.2–0.8	0.4
Trimethoprim lactate[b]	TMP	Burroughs-Wellcome	36	0.4–1.6	0.8	35	0.2–6.3	0.4
Sulfamethoxazole	SMZ	Roche	36	25–>100	50	35	12.5–>1,000	100
TMP in TMP 1 + SMZ 16	T/S-T		36	0.04–0.2	0.04	35	0.04–0.8	0.02

[a] The numbered antibiotics are all hydrochlorides of analogues of lincomycin (or clindamycin).
[b] The results shown for TMP and SMZ were previously reported (3).

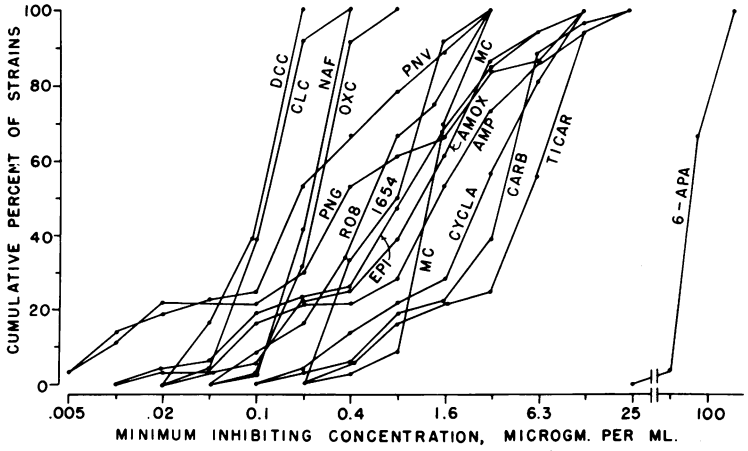

FIG. 1. *Susceptibility of strains of S. aureus to 16 penicillins. Key to symbols is given in Table 1, which also lists the numbers of strains tested with each antibiotic.*

aureus, with a few clear exceptions. The median MIC of each antibiotic was usually lower with *S. epidermidis*, by a factor as great as 16 (ampicillin), and in no instance was it higher than with *S. aureus* (Table 1). Phenoxymethyl penicillin was the most active antibiotic against *S. epidermidis*, inhibiting 50% of the organisms tested at a concentration of ≤0.05 μg/ml. Ampicillin was one of the most active against *S. epidermidis*, but it was one of the least active against *S. aureus* (Fig. 1).

The activities of 12 cephalosporins were somewhat similar to those of the penicillins in that the median MIC values for the *S. epidermidis* strains were equal to, or lower (by a factor up to 4) than, MIC results for *S. aureus* (Table 1). Cephaloridine and compound 87/312 were the most active, and cephaloglycin, cephradine, cephalexin, and cefoxitin were the least active, against both *S. aureus* and *S. epidermidis* (Fig. 3 and 4).

The 11 aminoglycosides and 2 polymyxins had similar orders of relative activity against both *S. aureus* (Fig. 5) and *S. epidermidis* (Fig. 6). Sisomicin was the most active antibiotic of this group against both *S. aureus* and *S. epidermidis*, and the polymyxins were clearly the least active. Butirosin was almost as poor in activity against *S. aureus* as polymyxin B, but against *S. epidermidis* it was slightly more

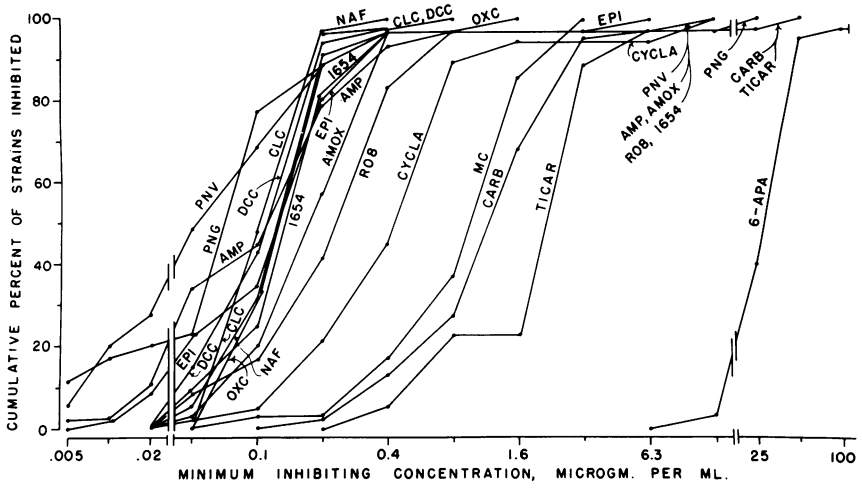

FIG. 2. *Susceptibility of strains of S. epidermidis to 16 penicillins. Key to symbols is given in Table 1, which also lists the number of strains tested with each antibiotic.*

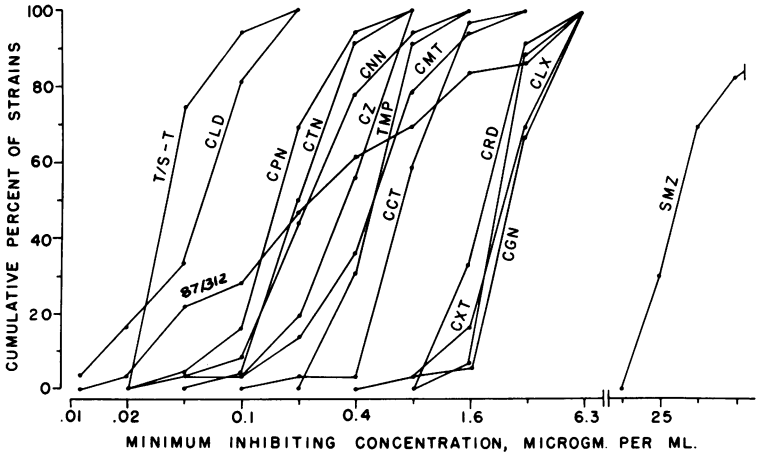

FIG. 3. *Susceptibility of strains of S. aureus to 12 cephalosporins and to trimethoprim and sulfamethoxazole, separately and combined (1:16). Key to the symbols is given in Table 1, which also lists the number of strains tested with each antibiotic.*

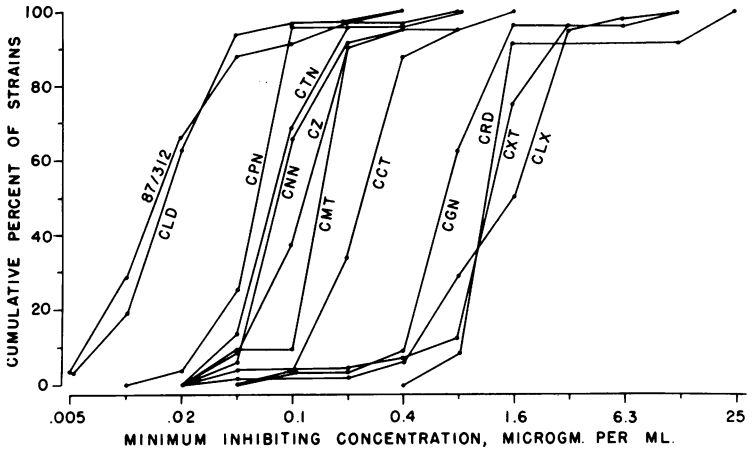

FIG. 4. *Susceptibility of strains of S. epidermidis to 12 cephalosporins. Key to the symbols is given in Table 1, which also lists the number of strains tested with each of the antibiotics.*

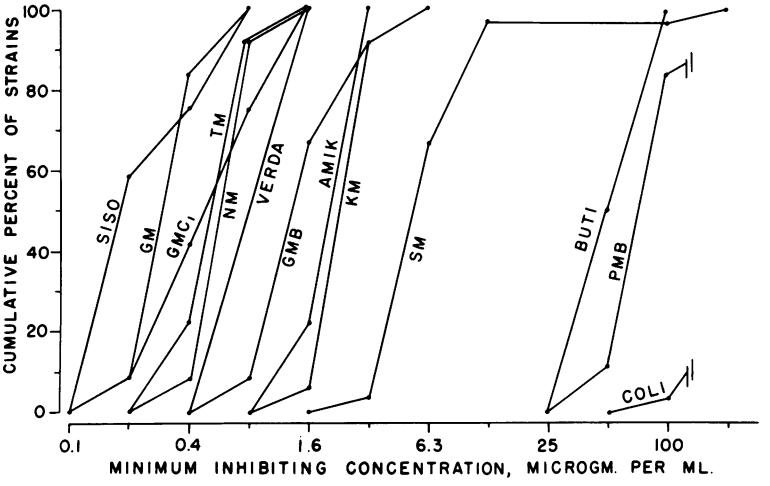

FIG. 5. *Susceptibility of strains of S. aureus to aminoglycoside antibiotics and polymyxins. Key to the symbols is given in Table 2, which also lists the number of strains tested with each agent.*

active than amikacin and kanamycin. Verdamicin, like butirosin, also showed greater activity against *S. epidermidis* (almost as good as sisomicin) than against *S. aureus*. Gentamicin was about twice as active against *S. aureus* as was tobramycin (Table 2; Fig. 5), but the two antibiotics were nearly equal in activity against *S. epidermidis*, except for about 20% of the strains, which were more susceptible to tobramycin. As with the penicillins and cephalosporins, MICs of the aminoglycosides were usually lower with *S. epidermidis* than with *S. aureus*.

The tetracyclines, unlike the penicillins, cephalosporins, and aminoglycosides, showed higher median MICs with *S. epidermidis* than with *S. aureus* (Table 2). Minocycline was clearly the most active tetracycline against both *S. aureus* and *S. epidermidis*, doxycycline was the second most active, and oxytetracycline was clearly the least active tetracycline against *S. aureus* (Fig. 7). Oxytetracycline was also least active against *S. epidermidis* (Fig. 8). However, chlortetracycline, demeclocycline, methacycline, and tetracycline were only slightly more active against *S. epidermidis* than was oxytetracycline. Spectinomycin was clearly less active than all the tetracyclines (Table 2; Fig. 7

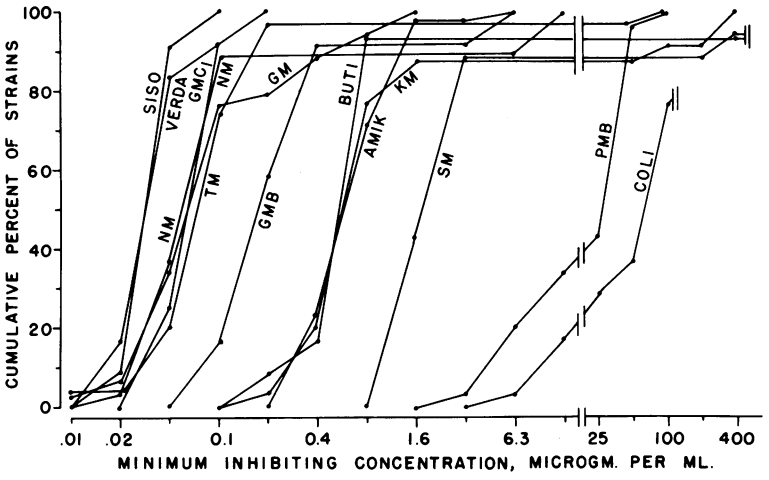

FIG. 6. *Susceptibility of strains of S. epidermidis to aminoglycoside antibiotics and polymyxins. Key to the symbols and the number of strains tested with each antibiotic are given in Table 2.*

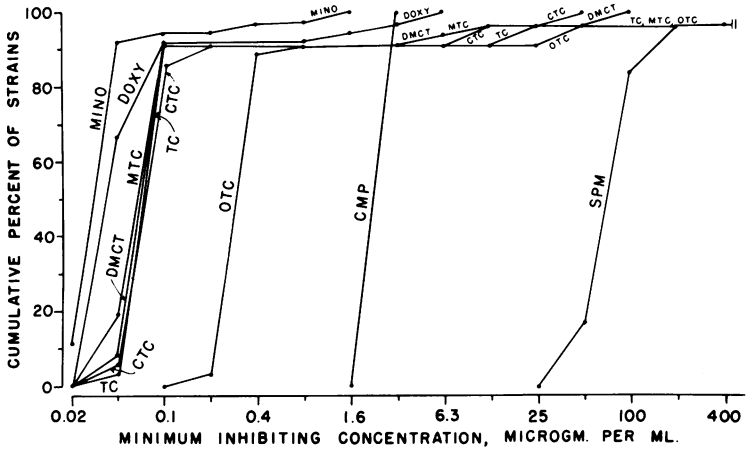

FIG. 7. *Susceptibility of strains of S. aureus to seven tetracycline analogues, chloramphenicol, and spectinomycin. Key to the symbols and the number of strains tested with each antibiotic are given in Table 2.*

and 8) and less active than chloramphenicol, which was rather uniformly active against all strains of *S. aureus* and *S. epidermidis* tested.

The results with lincomycins, erythromycin, rifampin, vancomycin, and some other antibiotics are summarized in Table 3 and Fig. 9 and 10. As with the other groups of antibiotics studied here, the relative activities of antibiotics within a group (even as heterogenous a group as this) were relatively similar against *S. aureus* and *S. epidermidis*. Rifampin and DPCL (compound U-24729A, a clindamycin derivative) were clearly the most active antibiotics in this group, and bacitracin, clindamycin sulfoxide, and vancomycin were the least active against both *S. aureus* and *S. epidermidis*. Clindamycin was more active than erythromycin and lincomycin, which was the least active of the three against strains of both *S. aureus* and *S. epidermidis*. However, with the exception of bacitracin (and all of the lincomycins tested against a few resistant strains of *S. epidermidis*), all of the MIC values were low.

The activities of trimethoprim, sulfamethoxazole, and both compounds in a 1:16 combination are summarized in Table 3. Trimethoprim was about 50 or more times as active as sulfamethoxazole, and when tested together synergy

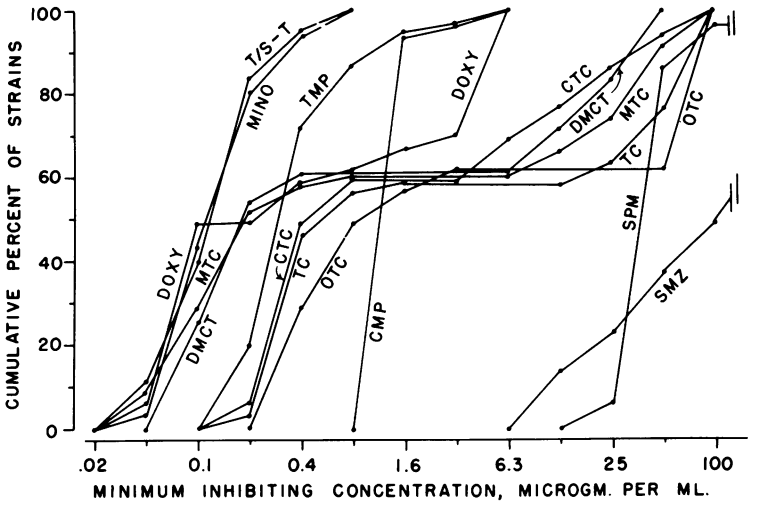

FIG. 8. *Susceptibility of strains of S. epidermidis to seven tetracycline analogues, chloramphenicol, spectinomycin and to trimethoprim and sulfamethoxazole, separately and combined (1:16). Key to symbols and the numbers of strains tested with each antibiotic are given in Table 2.*

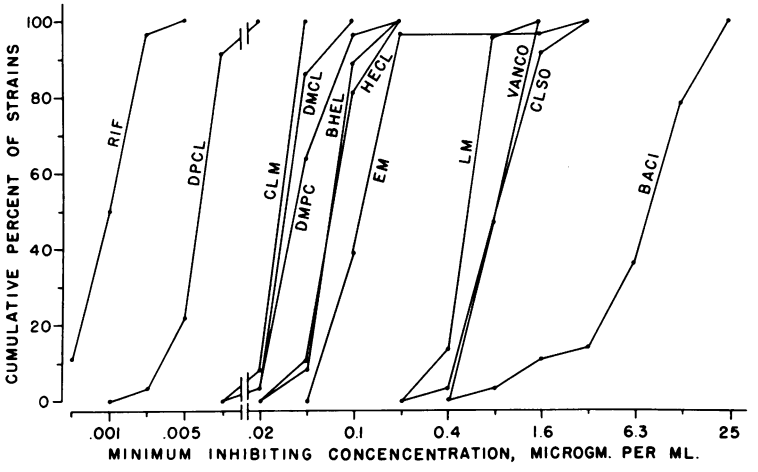

FIG. 9. *Susceptibility of strains of S. aureus to eight lincomycin analogues and to rifampin, erythromycin, vancomycin, and bacitracin. Key to the symbols is given in Table 3, which also lists the number of strains tested with each antibiotic.*

was routinely seen, but this was much more striking with *S. aureus* (Fig. 3) than with *S. epidermidis* (Fig. 8).

DISCUSSION

This study of both new and established antibiotics demonstrates the great variety of compounds with substantial anti-staphylococcal activity. However, the numerous instances in which very high MICs were required for inhibition of a few strains (even though for most of the strains, low MIC values for a given antibiotic, or group of antibiotics, were required for inhibition) emphasizes the variability in antibiotic susceptibility of staphylococci, and the necessity of determining antibiotic susceptibilities in each patient in clinical medicine, when a significant infection is present.

The numerous instances in which one, or several, antibiotics within a group showed most activity should be helpful in determining which new antibiotics should be more thoroughly studied in a clinical setting.

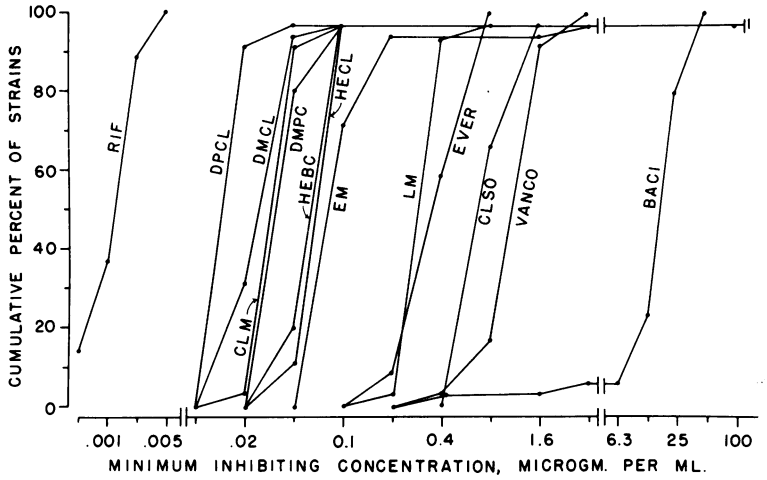

FIG. 10. *Susceptibility of strains of S. epidermidis to eight lincomycin analogues and to rifampin, erythromycin, vancomycin, and bacitracin. Key to the symbols is given in Table 3, which also lists the number of strains tested with each antibiotic.*

Obviously, in vitro activity as studied here is only one factor in determining potential clinical utility. Such problems as rapid emergence of resistance, protein binding, poor results with heavier inocula, pH effect, and toxicity clearly restrict the usefulness of many of these very active compounds; the indicated problems especially apply, respectively, to rifampin (4), isoxazolyl penicillins (5, 9), cephaloridine (6), aminoglycosides (1, 2, 6), and minocycline (10).

ACKNOWLEDGMENTS

This work was supported in part by grants 5R01-AI-23 and 5T01-AI-68 from the National Institute of Allergy and Infectious Diseases and from CIBA-Geigy, Beecham Laboratories, Lilly Laboratories for Clinical Research, the Squibb Institute for Medical Research, the Upjohn Company, and by supplementary support from the U.S. Veterans Administration.

LITERATURE CITED

1. **Abraham, E. P., and E. S. Duthie.** 1946. Effect of pH of the medium on activity of streptomycin and penicillin and other chemotherapeutic substances. Lancet 1: 455–459.
2. **Albert, A.** 1965. Ionization, p. 178–221. *In* Selective toxicity. Methuen & Co., Ltd., London.
3. **Bach, M. C., M. Finland, O. Gold, and C. Wilcox.** 1973. Susceptibility of recently isolated pathogenic bacteria to trimethoprim and sulfamethoxazole separately and combined. J. Infect. Dis. 128(Suppl.): S508–S542.
4. **McCabe, W. R., and V. Lorian.** 1968. Comparison of the antibacterial activity of rifampicin and other antibiotics. Am. J. Med. Sci. 256:255–265.
5. **Rolinson, G. N.** 1967. The significance of protein binding of antibiotics *in vitro* and *in vivo*, p. 254–283. *In* A. P. Waterson (ed.), Recent advances in medical microbiology. J. & A. Churchill Ltd., London.
6. **Sabath, L. D., C. Garner, C. Wilcox, and M. Finland.** 1975. Effect of inoculum and of beta-lactamase on the antistaphylococcal activity of thirteen penicillins and cephalosporins. Antimicrob. Agents Chemother. 8: 344–349.
7. **Sabath, L. D., D. A. Gerstein, C. D. Leaf, and M. Finland.** 1970. Increasing the usefulness of antibiotics: treatment of infections caused by gram-negative bacilli. Clin. Pharmacol. Ther. 11:161–167.
8. **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.
9. **Warren, G. H.** 1966. The prognostic significance of penicillin serum levels and protein binding in clinical medicine. A review of current studies. Chemotherapia 10:339–358.
10. **Williams, C. N., L. W. Laughlin, and Y-H. Lee.** 1974. Vestibular side-effects following minocycline therapy. Lancet 2:744–746.