Susceptibility of Pneumococci and *Haemophilus influenzae* to Antibacterial Agents

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Strains of *Diplococcus pneumoniae* and *Haemophilus influenzae* were tested for susceptibility to numerous antibiotics by a twofold agar dilution method using an inocula replicator. Undiluted, fully grown broth cultures were used as inocula for both species, and cultures of pneumococci diluted 1:1,000 were also tested. The antibiotics included most of those in common use in the United States as well as some chemical modifications recently approved and others that are under investigation. The most striking aspect of the results was the marked susceptibility of the pneumococci to all the antibiotics tested except the polymyxins and most of the aminoglycoside antibiotics, although some new aminoglycosides were active in quite low concentrations. Some of the strains of pneumococci were of decreased susceptibility to penicillin G (minimal inhibitory concentrations, 0.2 to 0.4 $\mu\text{g/ml}$), but none were tetracycline resistant, although such strains had been reported previously from this laboratory. The strains of *H. influenzae*, which were all serologically nontypable, exhibited different patterns of susceptibility to the groups of antibiotics and to the individual chemically related ones. None of these strains (isolated early in 1972) were ampicillin resistant. The most active agents against *H. influenzae* were: carbenicillin and ampicillin, analogues related to each of them, rifampin, chloramphenicol, and the polymyxins. However, the tetracycline analogues other than tetracycline, some aminoglycosides, notably tobramycin, kanamycin, gentamicin, and verdamicin, erythromycin, and some new lincomycin analogues were also active in low concentrations. Trimethoprim alone was highly active, and in combination with sulfamethoxazole it was even more active and synergistic against strains of both *D. pneumoniae* and *H. influenzae*.

The bacteria most commonly associated with acute infections of the lower respiratory tract and with acute exacerbations of chronic bronchitis are *Diplococcus* (now again classified as *Streptococcus*) *pneumoniae* and *Haemophilus influenzae*. Penicillin G (benzylpenicillin) has been the acknowledged drug of choice for the treatment of acute pneumococcal infections, and ampicillin, since it became available, has been generally considered the drug of choice for infections due to *H. influenzae*. Tetracycline is usually the second choice for either of them except in meningitis due to *H. influenzae*, when chloramphenicol may be preferred (1), but other antibiotics have also been used. The occurrence of resistance of pneumococci to tetracycline (8) and to other antibiotics has been reported (reviewed in 1972 by Finland [6]) and, more recently, ampicillin-resistant strains of *H. influenzae* have also been documented (3).

Alternative choices of drugs for therapy of infections due to *D. pneumoniae* or *H. influenzae* may be desirable or imperative not only because of resistance of the causative organisms but also because of patients who are allergic to or manifest other untoward effects from the drugs of choice or in whom colonization, mixed infections, or superinfections by other organisms with different antibiograms are present. For such patients the choice may be from among numerous antibacterial agents. The first essential in such choice is that the agents be active in killing or inhibiting the growth of the infecting organism(s). In this paper we present the results of tests for susceptibility of strains of *D. pneumoniae* and *H. influenzae* to a large number of antibiotics, including chemical analogues of several of those available and currently in common use in the United States and others that have only recently been released or are still under investigation. Included for comparison are results, already published,

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of tests carried out, mostly on the same strains, with trimethoprim and sulfamethoxazole, separately and combined in a fixed ratio (2).

MATERIALS AND METHODS

Organisms. The strains of *D. pneumoniae* (pneumococci) and *H. influenzae* used in this study were isolated, generally as the only or predominant organism, from cultures of clinical specimens obtained from separate patients with acute infections and submitted to the Microbiology Laboratory of the Boston City Hospital. They were isolated and identified by or under the direction of A. Kathleen Daly and Alice McDonald. The pneumococci were isolated and tested on two occasions, 35 in March and April 1972 and a similar number early in 1973: 10 from blood cultures, 3 from cerebrospinal fluid, 2 from conjunctival swabs, 1 from a draining ear, 36 from sputum or tracheal aspirates, and 18 from nasopharyngeal cultures. Serological typing was carried out by capsular swelling (Neufeld reaction) with 32 strains, including all those from blood, spinal fluid, eyes, and ears and 16 of those from sputum or tracheal aspirates. Thirteen serological types were represented: seven type 3, six type 8, four type 12, two each of types 6 and 19, and one each of types 7, 9, 11, 14, 16, 23, and 34. Of the strains of *H. influenzae*, one was cultured from blood, two from conjunctival swabs, and the others from sputum, tracheal aspirates, or nasopharyngeal swabs; all were serologically nontypable. Cultures of pneumococci were maintained until used by subculture every 48 h on heart infusion agar containing 8% defibrinated horse blood. The strains of *H. influenzae* were similarly maintained by subculture every 2 or 3 days on "chocolatized" Trypticase soy agar incubated in candle jars.

Antibiotics. Antibiotics were provided by the producers as sterile powders except some of the aminoglycosides, which were supplied as aqueous solutions in sterile vials. All were stored in desiccators at 4 C. Aqueous solutions containing the equivalent of 2,000 μg of the free acid or base per ml were freshly prepared for each test.

Susceptibility tests. These were done by the two-fold agar dilution method using the inocula replicator of Steers et al. (17). The inocula for the first set of tests with pneumococci were 10^{-3} dilutions of overnight cultures in brain heart infusion broth, pH 7.2, to which 2% defibrinated sheep blood had been added; similar cultures undiluted were used in the second set of tests, but these were not tested with the dilute inoculum. The organisms were inoculated on antibiotic-containing Mueller-Hinton agar, pH 7.2, containing 8% defibrinated sheep blood and incubated at 37 C. The undiluted inoculum provided approximately one million colony-forming units, and the 10^{-3} dilution provided about 1,000 colony-forming units when applied on the antibiotic-containing agar plates. With *H. influenzae* the inoculum was undiluted 24-h culture in chocolatinized Trypticase soy broth applied on plates of chocolatinized Trypticase soy agar, pH 7.2, and incubated in candle jars at 37 C. The tests with *D.*

pneumoniae were read after incubation for 20 to 24 h; those with *H. influenzae* were read after incubation for 48 h. The minimum inhibitory concentration (MIC) was taken as the maximum concentration of antibiotic that produced no growth or ≤ 5 small colonies visible with the aid of a hand lens ($\times 3$).

RESULTS

The results of the tests done with each group of related antibiotics will be presented for the strains of each species in the form of cumulative distribution curves of MICs of each antibiotic and will be summarized in tabular form.

Penicillins against pneumococci. As already noted, two groups of strains of pneumococci were tested about a year apart; for the first the inoculum was a 10^{-3} dilution of culture, whereas undiluted culture was used for the second. Sixteen penicillin analogues were tested with the latter and all but two of them, which were not available at the time, were tested with the diluted cultures. The results of both series of tests are shown in Fig. 1 and are summarized in Table 1.

The MICs were generally higher when the undiluted cultures were used (Fig. 1, lower panel) as compared with those for the same antibiotics tested with the diluted cultures (Fig. 1, upper panel). Moreover, except for the penicillin nucleus, 6-aminopenicillanic acid (6-APA), all strains were inhibited by all of the penicillin analogues tested in concentrations of $\leq 0.8 \mu\text{g/ml}$. The same was true even when the undiluted cultures were used, except for a small proportion of strains with two analogues, for each of which the MIC was $1.6 \mu\text{g/ml}$.

Most of the penicillin analogues inhibited all of the strains tested within a narrow range of concentrations (Fig. 1). However, with the diluted cultures (Fig. 1, upper panel) and each of the semisynthetic, penicillinase-resistant penicillins except nafcillin, the MICs of the strains varied over a wide range of MICs. More than one-half of the strains were inhibited by $\leq 0.02 \mu\text{g}$ of penicillin per ml, and the MIC of one-third was $0.4 \mu\text{g/ml}$; for about 60% the MICs of nafcillin were $\leq 0.02 \mu\text{g/ml}$, and for over 20% they were 0.2 or $0.4 \mu\text{g/ml}$. In the earlier tests with the diluted cultures (Fig. 1, upper panel), the MICs were lower and all were within a narrow range.

With the diluted cultures phenoxymethylpenicillin (penicillin V), nafcillin, penicillin G, and amoxicillin were about equally active and were more active than all the others. Epicillin was only slightly less active and two to four times more active than ampicillin, to which it is closely related. Ticarcillin was less active than the closely related carbenicillin, and

FIG. 1. Susceptibility of strains of *Diplococcus pneumoniae* to 16 penicillin analogues tested with undiluted cultures (below) and with cultures diluted 1:1,000 (above). Abbreviations: PNV, Phenoxymethylpenicillin K; NAF, nafcillin; AMOX, amoxicillin; PNG, penicillin G; EPI, epicillin; AMP, ampicillin; OXC, oxacillin; CYCLA, cyclacillin; CLC, cloxacillin; DCC, dicloxacillin; MC, methicillin; CARB, carbenicillin; TICAR, ticarcillin; 6-APA, 6-aminopenicillanic acid; 1654, BL-P1654; R08, R08/0074/003.

both were the least active of the 14 penicillins tested except 6-APA, which was active only in high concentrations. The penicillinase-resistant penicillins other than nafcillin were intermediate in activity against the diluted cultures.

When tested with the undiluted cultures, the new analogue BL-P1654 appeared to be the most active of the 16 penicillin analogues used. Also, amoxicillin was appreciably less active than ampicillin against the larger inoculum. The MICs of cyclacillin also were considerably greater with the larger as compared with the smaller inoculum. The new analogue designated R08/0074/03 was intermediate in activity between ampicillin and amoxicillin. With the exceptions noted, the order of activity was the same with both inoculum sizes.

Penicillins against *H. influenzae*. Only undiluted cultures of *H. influenzae* were used in the tests, the results of which are displayed in

the upper panel of Fig. 2 and also summarized in Table 1. Each of 16 penicillins tested was active within a narrow range of concentrations, which was generally higher than that required to inhibit the pneumococci. The most active of the analogues was BL-P1654, but ticarcillin, epicillin, amoxicillin, carbenicillin, ampicillin, penicillin G, and the investigational analogue R08/0074/03 were only slightly less active, within two- to fourfold, and inhibited all the strains tested in concentrations of ≤ 0.4 $\mu\text{g/ml}$. The penicillinase-resistant penicillins were appreciably less active and differed from one another over a wider range. Interestingly, penicillin V was much less active than penicillin G against *H. influenzae*, whereas both were about equally active against the pneumococci. Carbenicillin and the related ticarcillin were among the most active against *H. influenzae* and the least active of the analogues against

pneumococci. 6-APA was active against the strains of both species only in high concentrations.

Cephalosporins against *D. pneumoniae*. The strains of pneumococci were tested for susceptibility to 11 cephalosporin analogues (Fig. 3, Table 2); one of the 11, cefoxitin, was not used in the tests with undiluted cultures, and another, cephradine, was not tested with the diluted inocula.

With the diluted inocula (Fig. 2, upper panel) all of the strains tested were inhibited by 9 of the 10 analogues in concentrations of ≤ 0.4 $\mu\text{g/ml}$; the MICs of the other, cephalixin, ranged from 0.8 to 3.1 $\mu\text{g/ml}$. Most of the analogues inhibited all of the strains tested within a narrow range of concentrations, but with three of them the MIC varied over a wide range (≥ 64 -fold).

In the tests with the undiluted cultures (Fig. 3, lower panel), cephaloridine was clearly more active than the other analogues, inhibiting all of the strains tested in concentrations of ≤ 0.02 $\mu\text{g/ml}$. Cephalixin and the related analogue cephradine were clearly the least active. All or nearly all of the strains were inhibited by each of the other seven analogues in concentrations of 0.04 to 0.4 $\mu\text{g/ml}$, whereas the MICs of cephalixin and cephradine for most of the strains were 0.8 to 3.1 $\mu\text{g/ml}$. With the undiluted cultures the MICs of seven of the 10 analogues tested varied over a wide range (≥ 64 -fold).

Direct comparisons of MICs with undiluted

and diluted inocula were not carried out at the same time with the same strains. However, the MICs of control cultures of *Staphylococcus aureus* and *Streptococcus hemolyticus* included in each day's run were essentially the same (within one twofold dilution) in all tests, so that the results of the two series of tests may be compared. The MICs with undiluted cultures were generally higher than those with the diluted cultures and varied over a wider range of each of the analogues except cephaloridine (Fig. 1, Table 2).

Cephalosporins against *H. influenzae*. The results of susceptibility tests of *H. influenzae* strains to 10 cephalosporin analogues are shown in the upper panel of Fig. 4 and are summarized in the last three columns of Table 2. Each of these antibiotics inhibited all the strains tested within a relatively narrow (four- to eightfold) range of concentrations. In comparing these results with those just presented for the pneumococci, the most striking feature is that all strains of *H. influenzae* were much less susceptible to all the cephalosporins. Whereas all of the analogues, even the least active one, inhibited nearly all the strains of pneumococci in concentrations of ≤ 3.1 $\mu\text{g/ml}$, the MICs of the four most active analogues against all but a few strains of *H. influenzae* were 6.3 to 12.5 $\mu\text{g/ml}$. Cephradine, cephanone, cefoxitin, and cephapirin were about equally active and more active than all the others. Cephalothin and cephaloglycin ranked next, followed by cefazolin and cephaloridine. Cepha-

TABLE 1. Susceptibility of *D. pneumoniae* and *H. influenzae* to 16 penicillin analogues

Antibiotic	Producer	<i>D. pneumoniae</i> , undiluted			<i>D. pneumoniae</i> , 10^{-3} dilution			<i>H. influenzae</i> , undiluted		
		No. of strains	MIC ($\mu\text{g/ml}$)		No. of strains	MIC ($\mu\text{g/ml}$)		No. of strains	MIC ($\mu\text{g/ml}$)	
			Range	Median		Range	Median		Range	Median
Benzylpenicillin K	Lilly	17	0.005-0.4	0.01	33	0.005-0.02	0.01	35	0.4	0.4
Phenoxymethylpenicillin K		14	0.01-0.04	0.02	33	0.005-0.01	0.01	35	1.6-12.5	6.3
Ampicillin sodium	Bristol	15	0.01-0.04	0.02	33	0.01-0.04	0.02	35 ^a	0.04-0.8	0.8
Epicillin	Squibb	20	0.005-0.01	0.02	33	0.005-0.02	0.01	35	0.2-0.8	0.4
Amoxicillin trihydrate	Beecham	15	0.01-0.1	0.04	33	0.005-0.02	0.01	35	0.4-0.8	0.4
Carbenicillin disodium	Beecham	19	0.1-0.8	0.4	33	0.1-0.4	0.2	35 ^a	0.2-1.6	0.4
Ticarcillin disodium	Beecham	21	0.1-1.6	0.4	33	0.2-0.8	0.4	35	0.2-0.8	0.4
BL-P1654 sodium	Bristol	12	0.01-0.04	0.02	— ^b	—	—	24	0.1-0.8	0.1
Cyclacillin anhydrous	Wyeth	30	0.005-0.8	0.2	26	0.003-0.1	0.02	35 ^a	1.6-6.3	3.1
R08/0074/003	Roche	12	0.04-0.1	0.04	—	—	—	12	0.4	0.4
Methicillin sodium	Bristol	31	0.003-1.6	0.1	29	0.003-0.1	0.02	35	0.8-3.1	1.6
Oxacillin sodium	Bristol	33	0.003-0.2	0.04	29	0.001-0.1	0.01	35	6.3-50	25
Cloxacillin sodium	Bristol	32	0.005-0.4	0.2	23	0.005-0.2	0.04	35	12.5-50	25
Dicloxacillin sodium	Ayerst	32	0.02-6.3	0.1	31	0.003-0.8	0.04	35	6.3-25	12.5
Nafcillin sodium	Wyeth	30 ^a	0.005-0.4	0.02	30	0.005-0.02	0.01	35	6.3-50	25
6-Aminopenicillanic acid	Bristol	30	6.3-400	50	30	0.8-25	25	12	50-400	200

^a Twelve strains were tested on two separate occasions.

^b —, None tested.

FIG. 2. Susceptibility of strains of *Haemophilus influenzae* to 16 penicillin analogues (above), to rifampin (RIF), everninomicin (EVER), bacitracin (BACI), and vancomycin (VANCO), and to trimethoprim (TMP) and sulfamethoxazole (SMZ), singly and combined (T/S-T, T/S-S) (below). Abbreviations in top panel are as in Fig. 1.

lexin was appreciably less active and cephradine was the least active, the MIC for most of the strains being 100 $\mu\text{g}/\text{ml}$. It is interesting to note that, although cephradine was about as effective as cephalixin against all the pneumococci, the former was 4 to 16 times more active than the latter against the 12 strains of *H. influenzae* that were tested with both.

Tetracyclines, chloramphenicol, and spectinomycin against pneumococci. The results of the susceptibility tests of 33 strains of pneumococci to seven tetracycline analogues are shown in the panels on the left side of Fig. 5, and results of similar tests with chloramphenicol and spectinomycin against 34 strains are shown in the panels on the right side of the same figure. These results are all summarized in Table 3. They show the following. (i) All strains were inhibited by each antibiotic within a narrow (mostly fourfold) range of concentrations; this is evident from the nearly vertical configuration of the cumulative distribution

curves. (ii) All seven tetracycline antibiotics inhibited all the strains in concentrations of 0.02 to 0.8 $\mu\text{g}/\text{ml}$. (iii) The order of activity of the tetracycline analogues, as best delineated in the tests with the diluted inocula, was: methacycline > minocycline = doxycycline > chlorotetracycline > demeclocycline > oxytetracycline > tetracycline, which was clearly the least active. The order was essentially the same with undiluted cultures except that doxycycline was somewhat more active than minocycline and methacycline, and the differences among the others were smaller. (iv) The spread between the most active and the least active was not great and was smaller with the undiluted than with the diluted inoculum. (v) The differences between the MICs obtained with the same analogue using undiluted and diluted inocula were generally small, mostly two- to fourfold. (vi) Chloramphenicol was appreciably less active than the least active of the tetracyclines against the diluted cultures, the MICs of the

former being 3.1 to 6.3 $\mu\text{g/ml}$. Moreover, chloramphenicol showed a much greater inoculum effect, the MICs with undiluted cultures being 8- to 32-fold greater than those tested with cultures diluted 1:1,000. (vii) Spectinomycin was considerably less active than chloramphenicol against the diluted cultures; however, the former exhibited little or no inoculum effect so that it was appreciably more active than the latter in the tests with undiluted cultures.

Tetracyclines, chloramphenicol, and spectinomycin against *H. influenzae*. The results of susceptibility tests of 35 strains of *H. influenzae* against these nine antibiotics are shown in the left panel of Fig. 6 and are summarized in the last three columns of Table 3. As with the pneumococci, each antibiotic was active against all the strains within a narrow (two- to fourfold) range of concentrations, and there was a similar spread between the most and the least active of the tetracycline ana-

logues. On the other hand, chloramphenicol was as active against *H. influenzae* as the most active of the tetracyclines and spectinomycin was as active as tetracycline against these organisms. The polymyxins, also shown in the same panel, were as active as chloramphenicol and the most active of the tetracycline congeners, methacycline. Ranking next in order of activity were minocycline = demeclocycline = doxycycline > chlortetracycline > oxytetracycline, which was considerably more active than the least active analogue, tetracycline. Except for tetracycline, all the analogues and chloramphenicol inhibited nearly all the strains at $\leq 3.1 \mu\text{g/ml}$; the MICs of tetracycline and spectinomycin were 6.3 and 12.5 $\mu\text{g/ml}$.

Susceptibility of pneumococci to aminoglycoside antibiotics and polymyxins. Undiluted cultures of *D. pneumoniae* were tested for susceptibility to 11 aminoglycoside antibiotics and to polymyxin B and colistin; cultures

FIG. 3. Susceptibility of pneumococci to cephalosporin analogues using inocula of cultures undiluted (below) and diluted 1:1,000 (above). Abbreviations: CLD, cephaloridine; CMT, cefamandole; CGN, cephaloglycin; CPN, cephalpirin; CNN, cephanone; CTN, cephalothin; CZ, cefazolin; CCT, cephacetrile; CXT, cefoxitin; CLX, cephalixin; CRD, cephradine.

TABLE 2. Susceptibility of *D. pneumoniae* and *H. influenzae* to cephalosporin analogues

Antibiotic	Producer	<i>D. pneumoniae</i> , undiluted			<i>D. pneumoniae</i> , 10 ⁻³ dilution			<i>H. influenzae</i> , undiluted		
		No. of strains	MIC (μg/ml)		No. of strains	MIC (μg/ml)		No. of strains	MIC (μg/ml)	
			Range	Median		Range	Median		Range	Median
Cephalothin ^a	Lilly	31	0.01-0.8	0.2	27	0.01-0.1	0.04	35	3.1-25	12.5
Cephaloridine	Lilly	19	0.003-0.02	0.01	27	0.003-0.02	0.02	35	12.5-50	25
Cephaloglycin	Lilly	30	0.02-6.3	0.2	20	0.001-0.2	0.04	35	3.1-50	12.5
Cephalexin	Lilly	30	0.2-12.5	3.1	27	0.8-3.1	1.6	35 ^b	12.5-100	50
Cefazolin ^a	SKF	17	0.01-0.8	0.1	27	0.002-0.1	0.1	35	6.3-50	25
Cephacetrile	CIBA	18	0.01-0.8	0.2	27	0.01-0.2	0.2	35	50-200	100
Cephradine	Squibb	12	0.8-6.3	1.6	— ^c	—	—	12	6.3-12.5	6.3
Cefoxitin	Merck	—	—	—	27	0.001-0.4	0.2	35	3.1-12.5	6.3
Cephapirin ^a	Bristol	19	0.003-0.2	0.04	27	0.02-0.1	0.04	35	3.1-12.5	6.3
Cephanone	Lilly	19	0.001-0.8	0.1	27	0.005-0.1	0.04	35	3.1-12.5	6.3
Cefamandole	Lilly	18	0.01-0.2	0.04	22	0.003-0.1	0.02	—	—	—

^a Sodium salt.^b Twelve of the strains were tested on two occasions.^c —, Not tested. FIG. 4. Susceptibility of strains of *H. influenzae* to 10 cephalosporins (above) and to 8 lincomycin analogues and erythromycin (below). Abbreviations in top panel are as in Fig. 3. Abbreviations for lower panel: BHEL, U-38,784E; EM, erythromycin; DPCL, U-24,729A; HECL, U-34,728E; CLM, clindamycin hydrochloride; DMPC, U-39,745E; DMCL, U-26,727A; LM, lincomycin hydrochloride; CLSO, U-25,026A.

FIG. 5. Susceptibility of strains of *D. pneumoniae* to seven tetracycline (TC) analogues (left) and to aminoglycoside antibiotics, polymyxins, chloramphenicol (CMP), and spectinomycin (SPM) (right), using undiluted cultures (below) and cultures diluted 1:1,000 (above). Abbreviations: MTC, methacycline; MINO, minocycline; DOXY, doxycycline; CTC, chlortetracycline; DMCT, demeclocycline; OTC, oxytetracycline; GM, gentamicin, NM, neomycin; SM, streptomycin; KM, kanamycin; PMB, polymyxin B; AMIK, amikacin; BUTI, butirosin, SISO, sisomycin; VERDA, verdamicin; GMC₁, gentamicin C₁; GMB, betamycin; TM, tobramycin; COLI, colistin.

TABLE 3. Susceptibility of *D. pneumoniae* and *H. influenzae* to seven tetracyclines, chloramphenicol, and spectinomycin

Antibiotic ^a	Producer	<i>D. pneumoniae</i> , undiluted			<i>D. pneumoniae</i> , 10 ⁻³ dilution			<i>H. influenzae</i> , undiluted		
		No. of strains	MIC (μg/ml)		No. of strains	MIC (μg/ml)		No. of strains	MIC (μg/ml)	
			Range	Median		Range	Median		Range	Median
Tetracycline	Lederle	33	0.04-0.8	0.2	33	0.2-0.8	0.8	35	6.3-12.5	12.5
Chlortetracycline	Lederle	33	0.02-0.4	0.1	33	0.01-0.1	0.04	35	0.8-3.1	1.6
Oxytetracycline	Pfizer	33	0.04-0.4	0.2	33	0.04-0.4	0.2	35	1.6-6.3	3.1
Demeclocycline	Lederle	33	0.04-0.4	0.2	33	0.04-0.2	0.1	35	0.8-3.1	1.6
Methacycline	Pfizer	33	0.04-0.2	0.1	33	0.01-0.04	0.02	35	0.4-0.8	0.8
Doxycycline	Pfizer	33	0.005-0.1	0.04	33	0.02-0.1	0.04	35	0.8-3.1	1.6
Minocycline	Lederle	33	0.01-0.4	0.04	33	0.02-0.1	0.04	35	0.8-3.1	1.6
Chloramphenicol	Parke-Davis	33	12.5-100	100	34	3.1-6.3	3.1	35	0.4-0.8	0.8
Spectinomycin	Upjohn	33	6.3-25	25	34	12.5-25	25	35	3.1-12.5	6.3

^a The seven tetracycline analogues are all hydrochlorides; chloramphenicol and spectinomycin were each supplied as the base.

diluted 1:1,000 were tested with seven of the same aminoglycosides (four of the newer ones were not available to us at the time) and polymyxin B. The results are shown in the right lower and right upper panels, respectively, of Fig. 5, and all are summarized in Table 4. Each of the antibiotics inhibited all strains tested with both undiluted and diluted inocula within a two- or fourfold range of concentrations except in the tests with neomycin and the diluted cultures, for which the MICs varied over a very wide range. The basis for this deviation was not determined. Moreover, the antibiotics that were tested with both undiluted and diluted cultures

appeared to exhibit little or no inoculum effect; that is, the MICs were in about the same range in tests with each antibiotic against the undiluted and diluted cultures. Five of the new investigative aminoglycosides were more active than all the others and the polymyxins, butirosin being the most active, followed by sisomicin, verdamycin, gentamicin C₁, and betamycin. These five antibiotics inhibited all the strains with which they were tested in concentrations of 3.1 to 12.5 $\mu\text{g/ml}$; the MICs of the others were 25 to 100 $\mu\text{g/ml}$. The action of tobramycin was intermediate between that of gentamicin and streptomycin. Amikacin, kana-

FIG. 6. Susceptibility of *H. influenzae* to seven tetracyclines, two polymyxins, chloramphenicol and spectinomycin (left), and eleven aminoglycoside antibiotics (right). Abbreviations are as in Fig. 5.

TABLE 4. Susceptibility of *D. pneumoniae* and *H. influenzae* to aminoglycoside antibiotics and polymyxins

Antibiotic ^a	Producer	<i>D. pneumoniae</i> , undiluted			<i>D. pneumoniae</i> , 10 ⁻³ dilution			<i>H. influenzae</i> , undiluted		
		No. of strains	MIC ($\mu\text{g/ml}$)		No. of strains	MIC ($\mu\text{g/ml}$)		No. of strains	MIC ($\mu\text{g/ml}$)	
			Range	Median		Range	Median		Range	Median
Streptomycin	Lilly	33	50-100	50	34	50-200	100	35	1.6-6.3	3.1
Neomycin	Upjohn	33 ^b	200-400	200	20	0.4-200	50	35	1.6-6.3	3.1
Kanamycin	Bristol	33	50-200	100	34	50-200	100	35	0.4-3.1	0.8
Gentamicin	Schering	33 ^b	12.5-50	25	34	12.5-50	25	35	0.4-1.6	0.8
Betamycin	Schering	12	All 6.3	6.3	— ^c			12	3.1-6.3	6.3
Gentamicin C ₁	Schering	12	3.1-12.5	6.3	—			12	3.1-6.3	6.3
Sisomicin	Schering	12	3.1-6.3	3.1	—			12	1.6-3.1	3.1
Verdamycin	Schering	12	All 6.3	6.3	—			12	0.8-1.6	1.6
Tobramycin	Lilly	33	12.5-100	50	34	12.5-200	50	35	0.4-1.6	0.8
Amikacin	Bristol	33	50-200	100	34	50-400	200	35	1.6-6.3	3.1
Butirosin	Parke-Davis	12	1.6-3.1	3.1	—			12	6.3-35	12.5
Polymyxin B	Burroughs-Welcome	33	400->400	>400	34	50-400	200	35	all 0.8	0.8
Colistin	Warner-Lambert	33	400->400	>400	—			35	0.4-0.8	0.8

^a Tobramycin and amikacin were supplied as the base; all the others are sulfates.

^b Twelve strains were tested on two occasions.

^c —, None tested.

mycin, and neomycin were the least active of the aminoglycosides against the undiluted cultures, and the polymyxins were still less active.

Aminoglycosides and polymyxins against *H. influenzae*. The results of the susceptibility tests of undiluted cultures of *H. influenzae* to 11 aminoglycoside antibiotics are represented graphically in the right panel of Fig. 6, and for the two polymyxins they are shown in the left panel of that figure. All the results are summarized in the last three columns of Table 4. Here, too, all the strains tested were inhibited by each antibiotic within a two- to four-fold range. Colistin, polymyxin B, tobramycin, kanamycin, and gentamicin were about equally active and more active than the other aminoglycosides; they inhibited all the strains that were tested in concentrations of 0.4 to 1.6 $\mu\text{g}/\text{ml}$. The order of activity of the others was: verdamycin > sisomicin = streptomycin > amikacin = neomycin = betamycin = gentamicin C_1 > butirosin, which inhibited most of the strains tested in concentrations of 6.3 or 12.5 $\mu\text{g}/\text{ml}$.

Lincomycins and some other antibacterial agents against pneumococci. The in vitro activity of eight lincomycin analogues and of erythromycin, rifampin, vancomycin, bacitracin, and everninomicin was tested against undiluted and 10^{-3} dilutions of cultures of pneumococci. The results are displayed in Fig. 7 and summarized in Table 5. Lincomycin was clearly the least active of the eight analogues (Fig. 7); clindamycin was 8 to 16 times more active. Three of the new analogues, here designated DPCL, DMCL, and DMPC, were more active than clindamycin, and of these DPCL was the most active, especially against the undiluted cultures (Fig. 7, lower panel). The activity of one of the new analogues, clindamycin sulf-oxide, was the same as that of lincomycin, whereas two other analogues, BHEL and HECL, were about as active as clindamycin. Nevertheless, all eight analogues inhibited all the strains tested in concentrations of ≤ 0.4 $\mu\text{g}/\text{ml}$, even with the undiluted cultures, and each inhibited all the strains tested within a narrow range of concentrations. All the analogues

FIG. 7. Susceptibility of pneumococci to eight lincomycin analogues, five other antibiotics, and trimethoprim and sulfamethoxazole, separately and combined, tested with cultures undiluted (below) and diluted 1:1,000 (above). Abbreviations are as in Fig. 2 and 4.

TABLE 5. Susceptibility of *D. pneumoniae* and *H. influenzae* to eight lincomycin analogues and several other antibacterial agents

Antibiotic	Producer	<i>D. pneumoniae</i> , undiluted				<i>D. pneumoniae</i> , 10 ⁻⁴ dilution				<i>H. influenzae</i> , undiluted			
		No. of strains	MIC (µg/ml)		Median	No. of strains	MIC (µg/ml)		Median	No. of strains	MIC (µg/ml)		Median
			Range				Range				Range		
Lincomycin hydrochloride	Upjohn	33	0.05-0.4		0.2	33	0.1-0.2		0.2	35	>50		>50
Clindamycin hydrochloride	Upjohn	33	0.005-0.02		0.01	33	0.01-0.02		0.02	35	3.1-25		6.3
U-26,727A ^a	Upjohn	33	0.003-0.02		0.005	33	0.003-0.01		0.005	35	3.1-12.5		6.3
U-24,729A ^b	Upjohn	33	<0.001-0.005		0.001	33	0.001-0.01		0.005	35	0.8-12.5		3.1
U-25,026A ^c	Upjohn	33	0.01-0.4		0.2	33	0.01-0.4		0.2	35	>50		>50
U-34,728E ^d	Upjohn	33	0.005-0.04		0.02	33	0.005-0.02		0.01	35	1.6-6.3		3.1
U-38,784E ^e	Upjohn	33	0.005-0.04		0.02	33	0.003-0.02		0.01	35	0.8-3.1		1.6
U-39,745E ^f	Upjohn	33	0.001-0.02		0.005	33	0.001-0.02		0.01	35	3.1-12.5		6.3
Erythromycin	Lilly	32	0.002-0.02		0.02	34	0.002-0.02		0.02	35	1.6-6.3		3.1
Rifampin	CIBA	32	0.01-0.04		0.02	34	0.002-0.04		0.04	12	0.04-0.8		0.4
Vancomycin hydrochloride	Lilly	33	0.04-0.8		0.2	22	0.005-0.2		0.04	12	>200		>200
Bacitracin, zinc	Pfizer	32	0.04-12.5		0.8	34	0.1-12.5		0.8	24	50-400		100
Everninomicin	Schering	12	0.02-0.1		0.04	33	0.02-0.8		0.4	12	6.3-50		25
Trimethoprim lactate	Burroughs-Wellcome									35	0.2-0.8		0.4
Sulfamethoxazole	Roche												
TMP in T/S ^g						33	6.3->1000		50	35	100->1000		1000
SMZ in T/S ^h						33	0.04-0.8		0.1	35	0.04-0.4		0.1
						33	0.8-12.5		1.6	35	0.8-6.3		1.6

^a *N*-demethyl clindamycin hydrochloride.
^b *N*-demethyl-4'-depropyl-4'-pentyl clindamycin hydrochloride.
^c Clindamycin sulfoxide.
^d *N*-demethyl-*N*-(2-hydroxyethyl) clindamycin HCl·H₂O.
^e (7*S*)-7-bromo-*N*-demethyl-7-deoxy-*N*-(2-hydroxyethyl) lincomycin HCl·H₂O.
^f *N*-demethyl-*N*-(2-hydroxyethyl)-4'-depropyl-*cis-n*-pentyl-clindamycin HCL·H₂O.
^g Trimethoprim in combination with sulfamethoxazole.
^h Sulfamethoxazole in combination with trimethoprim.

except the three most active ones required somewhat greater concentrations to inhibit the undiluted as compared with diluted cultures.

Of the other antimicrobial agents shown in Fig. 7 and listed in Table 5, erythromycin appeared to be as active as clindamycin and rifampin was slightly more active, whereas vancomycin and bacitracin were much less active. Everninomicin (the product of a species of *Micromonospora* that is not active against gram-negative bacilli) was tested only with undiluted cultures, and its activity was intermediate between that of rifampin and of lincomycin. Erythromycin and rifampin appeared to be somewhat less active, and vancomycin and bacitracin were considerably less active against undiluted than against diluted cultures. Bacitracin inhibited different strains, both undiluted and diluted, over a wide range of concentration; vancomycin also showed a wide spread against diluted but not against undiluted cultures, whereas erythromycin, rifampin, and everninomicin inhibited all strains within a narrow range.

Also included in Fig. 7 and Table 5 are the previously reported results of tests with trimethoprim and sulfamethoxazole, separately and combined in a ratio of 1:16, using cultures of pneumococcus diluted 1:1,000. In those tests trimethoprim alone was about one-half to one-fourth as active as lincomycin, but when combined with sulfamethoxazole it was equally active against all strains. The MICs of trimethoprim in the combination were one-half or one-fourth those of trimethoprim alone, and sulfamethoxazole alone required eight or more times as much as in the combination. Thus the combination was considered synergistic against many but not all of the strains tested.

Lincomycin analogues and some other antimicrobial agents against *H. influenzae*. The susceptibility of undiluted cultures of *H. influenzae* to eight lincomycin analogues and erythromycin is shown in the lower panel of Fig. 4, and results of similar tests with rifampin, bacitracin, vancomycin, and everninomicin are displayed in the lower panel of Fig. 2, where the previously reported results obtained with trimethoprim and sulfamethoxazole, separately and combined, are also included. All of these results are summarized in the last three columns of Table 5.

As with the pneumococci, lincomycin and clindamycin sulfoxide were much less active than the other six analogues (MIC > 50 $\mu\text{g/ml}$). Clindamycin inhibited all the strains in concentrations of 3.1 to 25 $\mu\text{g/ml}$, but three other analogues were considerably more active, and

of these BHEL was the most active (MICs, 1.6 to 3.1 $\mu\text{g/ml}$). Erythromycin was two to four times more active than clindamycin (Fig. 4, lower panel), whereas rifampin was more active than erythromycin (MICs, 0.2 to 0.8 $\mu\text{g/ml}$) and everninomicin was about one-half as active as clindamycin. Vancomycin was essentially inactive (MICs > 400 $\mu\text{g/ml}$), and bacitracin was only slightly active (MICs, 50 to 400 $\mu\text{g/ml}$). Trimethoprim alone was about as active as rifampin against most of the strains tested and four times as active when combined with sulfamethoxazole; the sulfonamide alone inhibited only one-half of the strains in concentrations of 100 or 200 $\mu\text{g/ml}$, and the MICs of the others were $\geq 1,000$ $\mu\text{g/ml}$. In the combination, trimethoprim and sulfamethoxazole were clearly synergistic against nearly all the strains, requiring one-fourth the concentration of the former and less than 1/100 of the latter to produce inhibition when each was tested separately.

Comparison with previous results reported from this hospital. Tests for susceptibility of *D. pneumoniae* and *H. influenzae* to antimicrobial agents have been carried out in this laboratory and reported on several previous occasions, using similar materials and methods. Some of the reports dealt specifically with susceptibility of contemporary strains of either pneumococci (11-13) or *H. influenzae* (7, 10, 15) or both to all or most of the relevant antibiotics available and in use at the time. Other reports included contemporary isolates of pneumococci or *H. influenzae* among those of other common pathogenic bacterial species in studies designed to delineate the antibacterial spectrum of one or more new antimicrobial agents or analogues of previously available ones. The studies carried out through 1970 were reviewed in 1971 (6). References were made there to other reports on the occurrence of pneumococci resistant to tetracycline and to erythromycin and of decreased susceptibility of pneumococci to penicillin, but no changes in susceptibility of *H. influenzae* to any of the antibiotics in common use had been reported up to that time.

With respect to *D. pneumoniae*, the data presented here confirm the reduced susceptibility to penicillin G of a large proportion of the strains tested, and they also suggest a similar decrease in susceptibility of some strains to nafcillin, as evidenced by the bimodal distribution of MICs to these two penicillin analogues. (The median MICs for these penicillins [listed in Table 1] do not reflect the differences because more than one-half of the strains were "susceptible," hence equal to or less than the

median.) Of the 17 strains tested undiluted with penicillin G, the MICs of six were 0.2 or 0.4 $\mu\text{g/ml}$; of the six, three were type 3 and one was type 8. Of the other eleven strains, one was type 3 and three were type 8, and the MIC of penicillin G for these four strains was $<0.01 \mu\text{g/ml}$. All the type 3 and type 8 strains were from sputum except one, which was from a throat culture. It is also worth noting that nafcillin has been the only semisynthetic penicillinase-resistant penicillin reserved for parenteral use within this hospital during the past decade since its antibacterial spectrum was first reported at this hospital by Klein and Finland (14). In the present study, none of the strains of *D. pneumoniae* that were tested were resistant to any of the tetracycline analogues. The same was true of strains isolated in 1964 and 1965 and reported by Kislak et al. (13), and those isolated in 1967 and reported by Steigbigel et al. (18). However, Sabath et al. (16) found four strains, among 78 isolated in 1970, with MICs of 25 to 100 μg of tetracycline per ml; one of those strains grew for 24 h in broth containing 3.1 μg of tetracycline per ml without reducing the concentration of the antibiotic in the medium.

The strains of *H. influenzae* included in the present study, which, as previously noted, had all been isolated in May and June 1972, showed no changes in susceptibility to any of the antibiotics as compared with strains isolated in previous years and tested with those of the antibiotics that were available at the time. The large inoculum used and the 48-h incubation period should have picked up resistant strains (or clones) if there were any among the sample of strains tested.

DISCUSSION

The data presented attest to the marked susceptibility of pneumococci to all but a few of the large number and classes of antimicrobial agents that were tested. In concentrations readily achieved and maintained in the blood during treatment with doses generally recommended, all of the strains were inhibited by all the penicillin analogues (except the nucleus 6-APA), each of the cephalosporins, all the tetracycline analogues, and the analogues of lincomycin, by erythromycin, rifampin, vancomycin, everninomicin, and trimethoprim alone and particularly in combination with sulfamethoxazole; most of the strains were also similarly susceptible to bacitracin. Only the polymyxins and most of the aminoglycosides required concentrations that were too high to be achieved safely with therapeutic doses, but

three of the new aminoglycoside antibiotics currently under investigation, butirosin, sisomicin, and verdamicin, inhibited undiluted inocula or all strains in concentrations of 3.1 to 6.3 $\mu\text{g/ml}$. Chloramphenicol inhibited all strains in concentrations of 3.1 to 6.3 $\mu\text{g/ml}$ when tested with the dilute inoculum, but the MICs of those tested with undiluted cultures were 25 to 100 $\mu\text{g/ml}$, which are not readily and safely achievable with the usual doses.

Among the penicillins, carbenicillin and ticarcillin were the least active against pneumococci; penicillins G and V as well as ampicillin and the closely related ones, amoxicillin and epicillin, were the most active, and the penicillinase-resistant ones were intermediate in activity, nafcillin being the most active of that group. Cephalixin and cephadrine were clearly the least active of the cephalosporins against the pneumococci. It is of interest that some of the new lincomycin analogues were more active than clindamycin and erythromycin and equal in activity to penicillin G.

Strains of pneumococci resistant to tetracycline have been noted by others and a few were reported from this hospital (6), but none were demonstrated among the relatively small number of strains tested in the present study, and no strains resistant to erythromycin have as yet been recognized in this hospital. Decrease in susceptibility of pneumococci to penicillin G (but not to penicillin V and other penicillins) was demonstrated, however, the MIC of a large proportion of the strains being 0.2–0.4 $\mu\text{g/ml}$, whereas all the others were inhibited by $\leq 0.02 \mu\text{g/ml}$. (Strains of pneumococci with similar MICs of penicillin G were reported in 1971 by Hansman and co-workers [9] and commented on at that time by Finland [5]. However, decreased susceptibility of pneumococci or *H. influenzae* to penicillins was not noted among strains of pneumococci isolated and tested in 1970 by Sabath et al. [16], although undiluted culture was used as inoculum in that study.) Moreover, the wide range of MICs of the penicillinase-resistant penicillins and cephalosporins and the bimodal distribution of those of nafcillin suggest that the higher MICs within those ranges may become relatively more frequent as and if the use of these agents, particularly the oral forms, becomes very widespread among ambulatory patients. This was clearly demonstrated in this hospital with *S. aureus* (4), although the mechanisms of resistance with that organism are probably quite different.

There is a clearer dichotomy in the action of the penicillin analogues against *H. influenzae*. The strains of this species, which

were isolated and tested early in 1972, were all highly susceptible to ampicillin, carbenicillin, and the newer analogues related to them and somewhat less to penicillin G; they were considerably less susceptible to penicillin V, cyclacillin, and all the penicillinase-resistant penicillins including nafcillin, and still less to all the cephalosporins. The MICs of the most active of the cephalosporins were appreciably higher than those of the least active of the penicillin analogues.

There were no ampicillin-resistant strains among those tested here, but some have been encountered in this hospital since the fall of 1974. A study of such strains has been underway in this laboratory by J. O. Klein and his associates. The polymyxins chloramphenicol, rifampin, and trimethoprim, particularly in combination with sulfamethoxazole, were about as active, on a weight basis, as the more active of the penicillins. The tetracyclines were also active, but in somewhat higher concentration, and tetracycline was the least active among them (MICs, 6.3 to 12.5 $\mu\text{g/ml}$). The aminoglycosides, except butirosin, were active in concentrations of $\leq 6.3 \mu\text{g/ml}$; some of them, notably tobramycin, kanamycin, gentamicin, and verdamicin, were appreciably more active than the others (MICs, 0.4 to 1.6 $\mu\text{g/ml}$). Erythromycin, clindamycin, and several other lincosamin analogues also inhibited most or all of the strains in concentrations of $\leq 6.3 \mu\text{g/ml}$.

Only the inhibiting effects of the various antimicrobial agents on strains of pneumococci and *H. influenzae* have been considered in this paper. Other properties of such agents, not considered here, may be of equal, or of greater and sometimes even of overriding, importance from the point of view of acceptance and use. Among them are: their bactericidal action, binding to protein and pharmacokinetics, which, in turn, may affect their distribution in the body and penetration into the sites of infection to reach the offending organisms, the sensitizing potential of the agents, and the frequency and severity of any untoward effects they produce.

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