Susceptibility and Cross-Resistance of Bacteria to Four Related Antibiotics: Kanamycin, Paromomycin, Neomycin and Streptomycin. (24334)

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Kanamycin is an antibiotic derived from the fermentation products of *Streptomyces kanamyceticus;*† it has been shown to consist of 2 amino sugars linked glycosidally to 2-desoxystreptamine and it differs only slightly from that of neomycin and has much in common with that of streptomycin(2). The structure of paromomycin has not been revealed but it probably also resembles that of neomycin. It was therefore of interest to compare the activity of these 4 antibiotics against a number of strains of different organisms *in vitro* and to study the cross-resistance of strains obtained from patients and of some organisms made resistant to the individual antibiotics *in vitro.*

Materials and methods. The antibiotics were all used as sulfates and were supplied as follows: kanamycin‡ (Kantrex) by Bristol Laboratories; neomycin (Mycifradin) by Upjohn Co.; paromomycin (Humatin, formerly also called Humycin) by Parke-Davis & Co.; and streptomycin by Chas. Pfizer & Co. Aqueous solutions containing 8 μg/ml were prepared every 2 or 3 weeks, distributed in a number of tubes and kept frozen at −25°C; individual tubes were thawed and used each day. *Tests for sensitivity and for cross-resistance* were carried out on the surface of heart infusion agar, pH 7.2, containing 2-fold dilutions of antibiotic. A loopful of an overnight culture in broth was used as inoculum; incubation was carried out at 37°C, and readings made routinely at 24 hours. The minimum concentration of antibiotic on which there was no visible growth, or growth that was barely perceptible with aid of hand lens was the endpoint. Increases in resistance to individual antibiotics were accomplished by subcultures made at 2-day intervals, using suspensions of organisms from the surface of the agar containing highest concentration of that antibiotic on which growth was essentially uninhibited and transferring to another series of plates as in the test for sensitivity. At third subculture, in homologous antibiotic, suspensions were also tested for sensitivity to the other 3. To obtain fecal strains from patients receiving antibiotics, a small amount of feces was suspended and homogenized in large volume of broth, centrifuged briefly at low speed to remove gross solid particles and the supernatant centrifuged again at higher speed to sediment the bacteria; this sediment was used to inoculate antibiotic-free agar and colonies from resulting growth were picked, subcultures in broth and used in tests for sensitivity to all 4 antibiotics simultaneously, as were all strains used in comparisons. *Observations on method. Effect of inoculum size.* Two inocula were compared in simultaneous tests on agar, using all 4 antibiotics and 12 strains of Klebsiella and Aerobacter; a 2 mm loopful of undiluted cultures was compared with a similar loopful of the same cultures diluted 1:100 in broth. The endpoints were usually the same although growth just beyond the endpoint was greater from the larger inoculum. *Comparison of agar- and broth-dilution methods.* Five strains of Klebsiella and Aerobacter were tested simultaneously by the agar dilution method and by a 2-fold dilution method in brain-heart infusion broth using an inoculum of about 10^5 organisms/ml. Readings of visible growth were made of both tests at 24 hours and subcultures from broth to antibiotic-free agar were incubated 24 hours; broth cultures were reexamined at 48 hours. In tests with kanamycin, paromomy-

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† Available information about kanamycin was presented in detail at conference July 9 and 10, 1958, at N. Y. Acad. Sc.

‡ The lot of kanamycin (57K185) used was an early one and contained about 20% kanamycin B, which is said to have similar activity but more toxicity than kanamycin A which constitutes more than 95% of current lots.

TABLE I. Inhibition of Various Organisms by 4 Related Antibiotics.

Organism*	No. of strains	Anti-biotic†	Minimum inhibiting conc., μg/ml >100	100	50	25	12.5	6.3	3.1	1.6	.8	.4
			% of strains									
Staphylococcus aureus (B.C.H.)	210	S	56.2	8.6	4.3	1.9	8.6	13.8	6.2	.5		
	187	N					.5	27.8	47.6	16.6	7.0	.5
	172	P					61.6	29.7	5.8	1.2	1.2	.6
	169	K				1.8	51.5	35.5	7.7	2.4	1.2	
Escherichia coli (pathogenic types, from C.D.C.)	80	S	24	0	0	4	49	21	3			
	80	N			1	16	78	5				
	80	P			5	63	28	5				
	80	K	1	0	0	3	70	24	3			
Aerobacter (20 strains each of *A. aerogenes* & *A. cloacae*, from C.D.C.)	40	S	15‡	0	3‡	3	20	35	25			
	40	N					13	48	38	3		
	40	P				8	38	35	20			
	40	K						43	35	18	5	
Klebsiella (prototypes of all types, from C.D.C.)	73	S	12	0	0	0	3	30	47	6	3	
	73	N			1	0	0	41	44	11	3	
	73	P		1	0	4	16	41	26	10	1	
	73	K			1	1	4	15	48	22	7	1
Klebsiella-Aerobacter recent isolates, B.C.H.)	111	S	50.5	5.4	3.6	.9	4.5	15.3	9.0	8.2	2.7	
	111	N					.9	16.2	52.3	21.6	5.4	3.6
	111	P					7.2	18.0	4.05	26.1	5.4	2.7
	111	K					4.5	17.1	36.9	30.6	7.2	3.6
Proteus, species (recent isolates, B.C.H.)	32	S	31	0	13	47	9					
	32	N		28	53	16	3					
	32	P		34	25	31	9					
	32	K		22	59	13	3	3				
Pseudomonas (recent isolates, B.C.H.)	24	S	38	29	29	4						
	24	N	96	4								
	24	P	100									
	24	K	100									

* B.C.H. = Boston City Hosp., isolated from infected sources in patients; C.D.C. = Communicable Diseases Center, U.S.P.H.S., Chamblee, Ga. (obtained through courtesy of Dr. P. R. Edwards).
† S = streptomycin; N = neomycin; P = paromomycin; K = kanamycin.
‡ These were all *A. cloacae*.

cin and neomycin, endpoints of growth were nearly all identical; however, when growth was barely detectable with aid of lens on agar, there was usually no visible growth in broth containing the same concentration of antibiotic. With streptomycin, however, 2-fold greater concentrations of antibiotic were required to inhibit organisms in broth than on agar, and growth was obtained on subculture to antibiotic-free agar from 1 or 2 tubes beyond the endpoint at which there was no visible turbidity of the broth at 24 hours. The experiment was repeated with 8 strains of Proteus and subcultures to antibiotic-free agar also after 48 hours. Again, endpoints in broth and on agar at 24 hours agreed within a single 2-fold dilution in tests with kanamycin, paromomycin and neomycin; wider variations, however, were exhibited with streptomycin, some growing in 4- or 8-fold greater concentrations in broth than on agar. The endpoints of growth (or viability) in subcultures to antibiotic-free agar after 24 and 48 hours, as well as endpoints in broth at 48 hours varied: in two-thirds of the tests these endpoints were the same as in broth at 24 hours, or varied only by a single 2-fold dilution; in others, growth occurred on 2 and occasionally 3 further dilutions after the subculture or after the longer period of incubation.

Results. Susceptibility of selected groups of bacteria. Results of tests for sensitivity of various bacteria are summarized in Table I. High degrees of resistance, indicated by essentially uninhibited growth on agar containing antibiotic in concentrations of 100 μg/ml, were observed only to streptomycin; about one-half of the strains of staphylococci and the Klebsiella-Aerobacter group isolated here, and smaller proportions of Pseudomonas and of

coliform organisms were streptomycin-resistant. Nearly all strains of Pseudomonas were also resistant to neomycin, paromomycin and kanamycin, and one strain of *E. coli* was resistant to kanamycin; the possibility of previous exposure of the latter to neomycin cannot be ruled out, but it could not have been exposed to either kanamycin or paromomycin. The streptomycin-resistant strains showed no cross-resistance to the other 3 antibiotics, but the kanamycin-resistant strain of *E. coli* was also streptomycin-resistant and required 50 μg/ml of paromomycin and of neomycin to inhibit it. Susceptible strains were nearly all about equally sensitive to neomycin, paromomycin and kanamycin, although some of the staphylococci were more susceptible to neomycin, and some of the Klebsiella and Aerobacter were more sensitive to kanamycin than to the other 2 antibiotics. Some strains of Pseudomonas varied in susceptibility to streptomycin, but were resistant to the other antibiotics, most of them growing to 400 μg/ml.

Cross-resistance in vitro. This was studied in 4 strains originally sensitive to all 4 antibiotics; 2 of *Staph. aureus*, of which one (Staph. 209P, phage type 42D) was sensitive to penicillin and tetracycline and the other (Staph. A27, phage type 52,42B,80/81) resistant to them, a strain of *K. pneumoniae*, type 1(A) and the McLeod strain of *E. coli* (type 054) were used, each was subcultured serially on agar containing increasing concentrations of the 4 antibiotics. Susceptibility

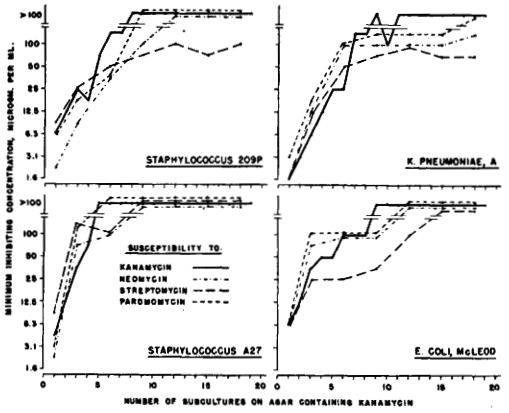

FIG. 1. Resistance and cross-resistance resulting from repeated subcultures in presence of kanamycin.

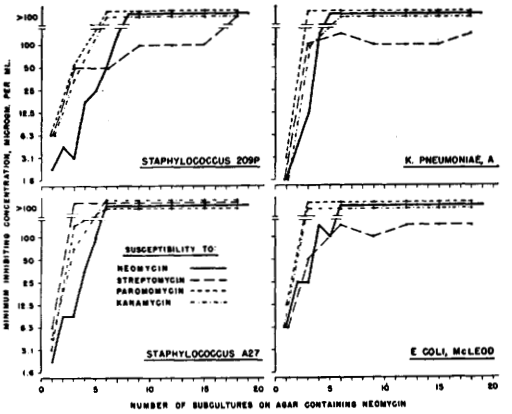

FIG. 2. Resistance and cross-resistance resulting from repeated subcultures in presence of neomycin.

of these strains to the homologous antibiotic after each subculture and to the other 3 after each third subculture is depicted in Fig. 1-3; results with paromomycin resembled those shown in Fig. 1 for kanamycin. The results indicate that resistance to the homologous antibiotic (heavy solid line) developed to a high degree within the first 5 to 10 subcultures in each instance. Cross-resistance among neomycin, paromomycin and kanamycin occurred in the early subcultures and, in most instances, was virtually complete. On the other hand, cross-resistance to streptomycin in the strains that had become resistant to the other 3 antibiotics, developed more slowly and was less complete, except in Staph. A27. Also, streptomycin-resistance resulting from exposure to that antibiotic was accompanied by lesser increases in resistance to each of the other 3 antibiotics and this was true for all 4 strains (Fig. 3).

Effect of resistance on phage types of staphylococci and on serologic specificity of coliform organisms. Phage types of parent and corresponding resistant staphylococci were identical, as were serological types of parent and corresponding resistant strains of *K. pneumoniae* and *E. coli*.§

Cross-resistance in strains from patients. Table II lists results of tests on strains iso-

§ We are indebted to Dr. Harry Shwachman, Children's Medical Center, Boston, for phage typing of staphylococci and to Dr. W. H. Ewing of the Communicable Disease Center, Chamblee, Ga., for serological typing of coliform organisms.

TABLE II. Susceptibility of Organisms Isolated from Feces of Patients during Oral Administration of Paromomycin or Kanamycin; 6 Cases.

Therapy			Minimum inhibiting conc., μg/ml			
Antibiotic	Days	Organism	S	N	P	K
P	7	*A. aerogenes*	>100	>100	>100	>100
P	7	*B. fecalis* alkaligenes	>100	>100	>100	>100
			"	"	"	"
P	7	*E. coli*	3.1	25	>100	>100
			6.3	25	100	"
			3.1	25	>100	"
K	5	*A. aerogenes*	100	12.5	6.3	100
			"	6.3	12.5	"
			"	6.3	6.3	"
K	3	"	12.5	25	50	>100
	10		3.1	50	>100	"
	10		"	"	"	"
K	3	"	6.3	12.5	25	>100

S = streptomycin; N = neomycin; P = paromomycin; K = kanamycin.

lated from feces of patients receiving oral doses of 4 g daily of either kanamycin or paromomycin. In view of the failure to demonstrate resistance to neomycin, paromomycin or kanamycin in similar organisms from patients not previously exposed to these antibiotics, these findings suggest that resistance to them may be readily acquired by organisms in the bowel during their use orally and that cross-resistance to the other 2 related ones develops at the same time or may be delayed. High degrees of cross-resistance to streptomycin, however, probably do not result from such treatment.

Discussion. Cross-resistance between kanamycin and neomycin has been noted by a number of workers in strains of *Mycobacterium tuberculosis* and of *Staph. aureus,* and a viomycin-resistant variant of *Myc. tuberculosis,* H37Rv was shown by Steenken to be resistant to kanamycin, but a variant made resistant to the latter remained sensitive to viomycin(1). Incomplete cross-resistance between streptomycin and neomycin has been demonstrated previously(3), but cross-resistance between kanamycin and streptomycin was not noted by other workers(1). It is shown here that strains of *Staph. aureus* and of a number of Enterobacteriaceae are remarkably similar in susceptibility to kanamycin, paromomycin and neomycin and that virtually complete cross-resistance results from repeated subcultures in the presence of any one of them. Similar cross-resistance probably results during treatment at least in fecal organisms when these antibiotics are given orally over a long enough time.

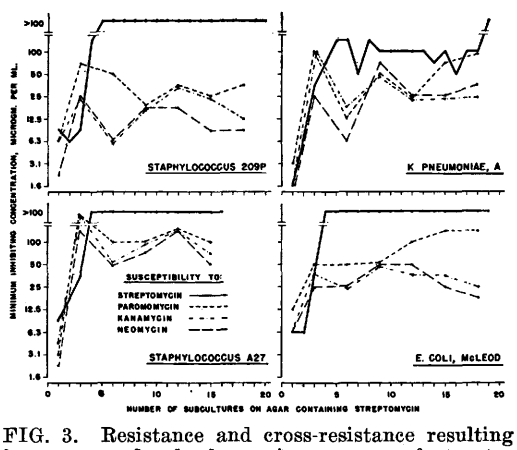

FIG. 3. Resistance and cross-resistance resulting from repeated subcultures in presence of streptomycin.

Streptomycin-resistant strains isolated from patients do not show cross-resistance to these 3 antibiotics, and results obtained with the fecal strains suggest that strains resistant to the latter, when obtained from patients under treatment, do not readily acquire cross-resistance to streptomycin. *In vitro,* however, resistance to neomycin, paromomycin or kanamycin resulting from repeated exposures to these antibiotics is accompanied by moderate cross-resistance to streptomycin, and minor increases in resistance to the other 3 antibiotics accompanies the increase in resistance

to streptomycin that occurs during subcultures in its presence. None of these 4 antibiotics show any cross-resistance with any of the other antibiotics that are now generally available.

These findings of complete cross-resistance among chemically closely related antibiotics are similar to those shown for the 3 tetracycline analogues(4) and for antibiotics of the erythromycin-group(5). In the latter, complete cross-resistance was found *in vitro* among erythromycin, carbomycin, oleandomycin and spiramycin, but cross-resistance between each of these antibiotics and streptogramin (Antibiotic 900) resembled closely that shown between streptomycin and each of the other 3 antibiotics in the present study.

Summary and conclusions. The antibiotics kanamycin, paromomycin and neomycin were shown to have essentially the same activity *in vitro* against strains of *Staphylococcus aureus* and of various Enterobacteriaceae. Bacteria made resistant to any one of these 3 antibiotics by subcultures on that antibiotic also exhibit virtually complete cross-resistance to the other two. Organisms isolated from infected sources do not show significant cross-resistance between streptomycin and any of these 3 antibiotics. However, strains made resistant *in vitro* to either kanamycin, paromomycin or neomycin show moderate increases in resistance to streptomycin, and strains made resistant to the latter exhibit only minor increases in resistance to the other 3 antibiotics. With all 4 antibiotics, corresponding parent and resistant variants of the staphylococci were of the same phage type, and resistant variants of *K. pneumoniae* and *E. coli* retained their serological specificity. Fecal organisms isolated from patients during oral treatment with paromomycin or kanamycin were resistant to the antibiotic administered and showed moderate to marked resistance also to the other one and to neomycin.

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Experimental Tick Paralysis in Laboratory Animals and Native Montana Rodents. (24335)

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Rapidly engorging females of the Rocky Mountain wood tick, *Dermacentor andersoni,* are a well-known cause of the sometimes fatal tick paralysis of man and some animals in parts of western United States and Canada. Abbott(1) has provided a good clinical and epidemiological review of this disease in various parts of the world. During the years when vaccine against spotted fever was being made from suspensions of infected adult *D. andersoni* at the Rocky Mountain Laboratory, millions of ticks in various stages, including wild-caught adults, were fed on rabbits and guinea pigs. However, so many female ticks have completed engorgement for basic vaccine

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