Development of Resistance and Cross-Resistance *in vitro* to Erythromycin, Carbomycin, Spiramycin, Oleandomycin and Streptogramin.* (22766)

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Among the major problems in the field of infectious diseases today, particularly in hospitals, is the occurrence, with increasing frequency, of strains of *Micrococcus pyogenes*, var. *aureus*, resistant to many antibiotics that are most commonly used. This has been accompanied by a serious increase in incidence and severity of infections due to such organisms and in difficulties encountered with the use of antibiotics in their management (1,2).

Among recent antibiotics, the one most active against the staphylococcus has been erythromycin, but this organism has been shown to acquire resistance quite rapidly as a result of exposure to erythromycin, both *in vitro* and during the therapy of patients(1,3, 4). Carbomycin, another antibiotic subsequently made available, was also shown to develop almost complete cross-resistance to erythromycin *in vitro*(5-7). Additional antibiotics with considerable *in vitro* activity against *M. aureus* have been developed more recently, and some of them have already received some clinical trials. The present paper presents results of studies on development of resistance and cross-resistance of *M. aureus* *in vitro* to 3 of these new agents, namely spiramycin(8-14), oleandomycin (PA-105)(15, 16) and streptogramin ("Antibiotic 900") (17), and these, in turn, were tested for cross-resistance to erythromycin and carbomycin.‡ The effect of exposures to these 5 antibiotics on susceptibility of the strains to almost all commonly used antibiotics and to some additional new ones was also tested.

Materials and methods. Four strains of *M. aureus* were used, 2 of them (strains A and B) were highly sensitive to penicillin and the tetracyclines, whereas the other 2 (strains C and D) were highly resistant to them. All 4 were originally inhibited by moderately low concentrations of each of the 5 antibiotics under study. The strains were grown on the surface of a series of plates containing heart infusion agar (Difco, pH 7.2 ±) in which graded concentrations of the antibiotics were incorporated. The original inoculum was derived from a freshly grown overnight culture of the strain in broth and this was streaked on to the agar with a 2 mm loop. The confluent growth from the surface of the agar containing the greatest concentration of antibiotic on which growth was similar to that occurring without antibiotic was used for the next passage to a similar series of plates containing the same antibiotic; this growth was scraped off with a larger loop and emulsified in 0.5 ml of broth and the resulting suspension used for the transfer. The process was repeated until full growth occurred on plates containing 400 μg/ml. the largest concentration used in this study, and was then usually transferred a number of additional times on this concentration of the agent. After each 3 subcultures on one of the antibiotics, the inoculum for the next subculture was also used for tests on similar series of plates containing the other 4 antibiotics in order to determine the extent of the cross-resistance that had developed. At the completion of the study, all of the parent cultures, which had been subcultured in parallel on antibiotic-free agar, and those that had been serially exposed to each of the 5 antibiotics were tested, in addition, for sensitivity to 10 antibiotics, including 6 that are in com-

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‡ Spiramycin and streptogramin were furnished by Sharp & Dohme Division, Merck and Co., the Oleandomycin was supplied by Parke-Davis Co. and also by Chas. Pfizer and Co., the latter also furnished the carbomycin (Magnamycin), and the erythromycin (Ilotycin) was obtained from Eli Lilly and Co.

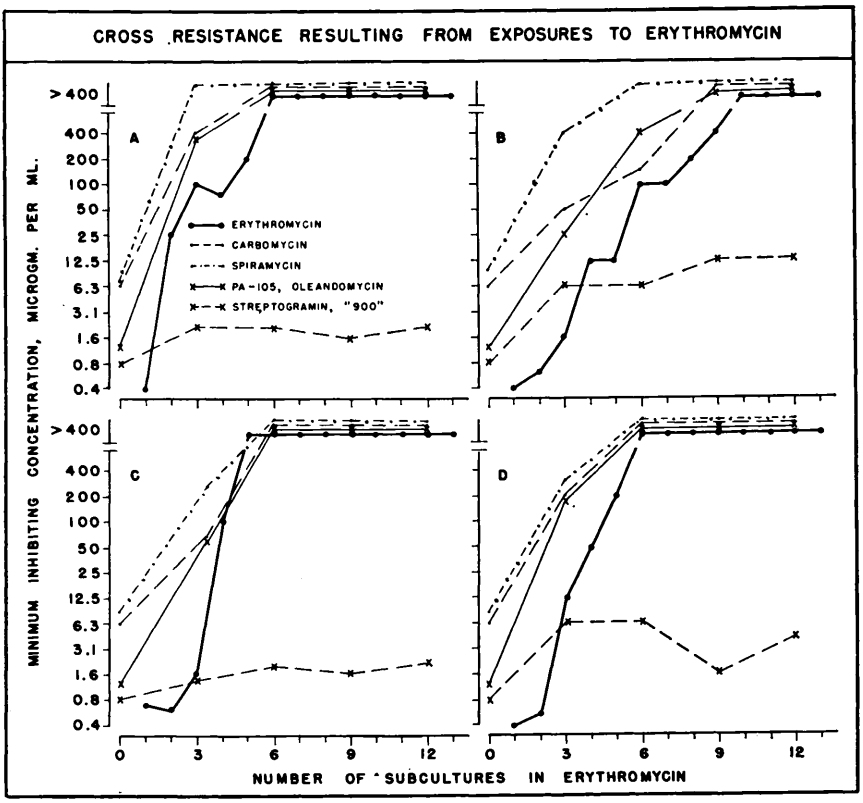

FIG. 1.

mon use and 4 that are currently under investigation.

Results. The minimum inhibiting concentration (M.I.C.) of erythromycin for each of the 4 strains§ after each successive transfer on that antibiotic, and the susceptibility to the other 4 antibiotics of these strains after each third subculture is shown in Fig. 1. All 4 strains were originally inhibited by 0.4 μg/ml of erythromycin. Each of them developed resistance to the erythromycin very rapidly in the presence of that antibiotic. Strain C grew uninhibited on agar containing 400 μg/ml of that antibiotic after 5 subcultures, Strains A and D after 6 and Strain B after 10 such exposures. Cross-resistance to 3 of the other antibiotics developed at about the same rate, as evidenced by the similar slope of the curves in the figures. The M.I.C. of each of the parent strains for these 3 antibiotics (spiramycin, carbomycin and oleandomycin) was 2 to 8 times that for erythromycin, and the number of transfers required to render the strains resistant to 400 μg/ml of these agents was somewhat greater. Streptogramin was the exception; only a slight decrease in susceptibility of the strains to this antibiotic occurred after the first few exposures to erythromycin but there was no further increase in cross-resistance after further transfers on erythromycin.

Resistance of the same strains to carbomycin resulting from the repeated exposures to that antibiotic developed at a variable rate in the different strains, most rapidly in Strain C (Fig. 2). Cross-resistance to the other antibiotics resulting from these exposures to carbomycin, varied in a similar manner, and developed either at the same rate or at somewhat slower rate. Cross-resistance to spiramycin occurred with particular rapidity, at a rate equal to or even exceeding that against the homologous antibiotic; however, the par-

§ The strains are designated A,B,C, and D corresponding to the letters shown in the panels of the text figures.

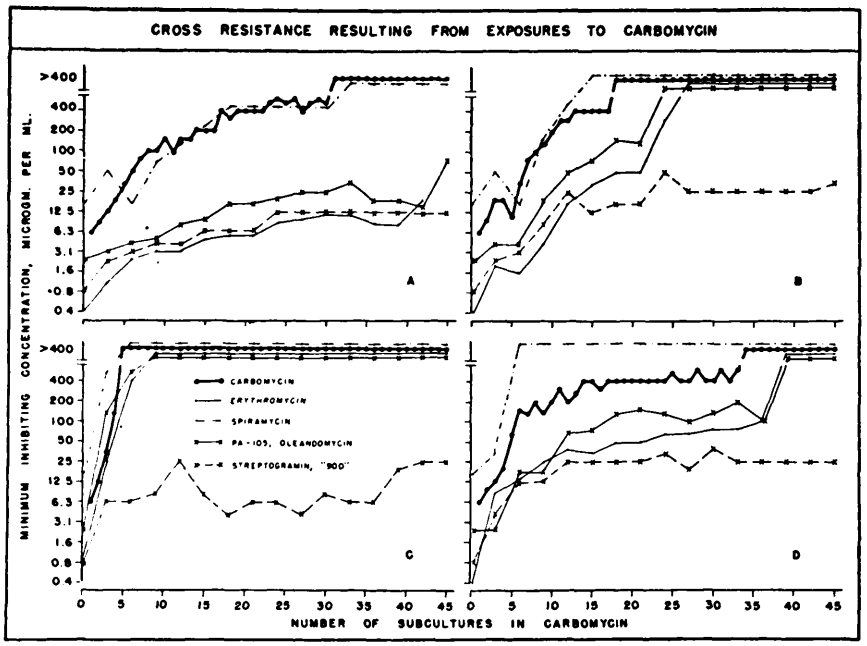

FIG. 2.

ent strains were also somewhat less susceptible to spiramycin than to carbomycin. The reverse was true with respect to the other 3 antibiotics; the parent strains were somewhat more susceptible and they developed cross-resistance at the same or somewhat slower rate, and more subcultures were required before the strains acquired cross-resistance to 400 μg/ml. For Strain A this degree of resistance was not reached after a total of 45 subcultures, or 15 transfers after this strain had acquired that degree of resistance to both carbomycin and spiramycin. Cross-resistance against streptogramin occurred to a somewhat greater extent than after exposure to erythromycin and this tended to level off after the first few exposures to carbomycin just as it did after the exposures to erythromycin.

Exposures to spiramycin (Fig. 3) resulted in the development of resistance to the homologous antibiotic and cross-resistance to the others at about the same rate as was observed with erythromycin. However, only Strain A showed any significant cross-resistance to streptogramin and this appeared to be transient.

Oleandomycin also induced resistance to the homologous agent and to all of the others, except streptogramin, quite rapidly and uniformly. There was no change whatever in the M.I.C. of the 4 strains to streptogramin during the 12 subcultures on oleandomycin (Fig. 4).

Exposures of the strains to streptogramin (Fig. 5), resulted in the development of resistance to both the homologous antibiotic and to the other 4 at a slower rate than was shown in the previous figures. From 32 to 43 subcultures on streptogramin were required to increase the resistance of the strains so that they grew in the presence of 400 μg/ml of that agent. Cross-resistance to the other agents developed at about the same rate and somewhat more slowly to erythromycin.

After these observations were completed, all of the parent strains which had been transferred in parallel on the same kind of agar without antibiotics, were again tested for their sensitivity to the 5 antibiotics used in the study and these, together with the strains that had been exposed and became resistant to the 5 antibiotics were tested for sensitivity to the following additional antibiotics: penicillin, streptomycin, neomycin, tetracycline, chloramphenicol and bacitracin, and also to 4 new antibiotics: bryamycin, cycloserine, albamy-

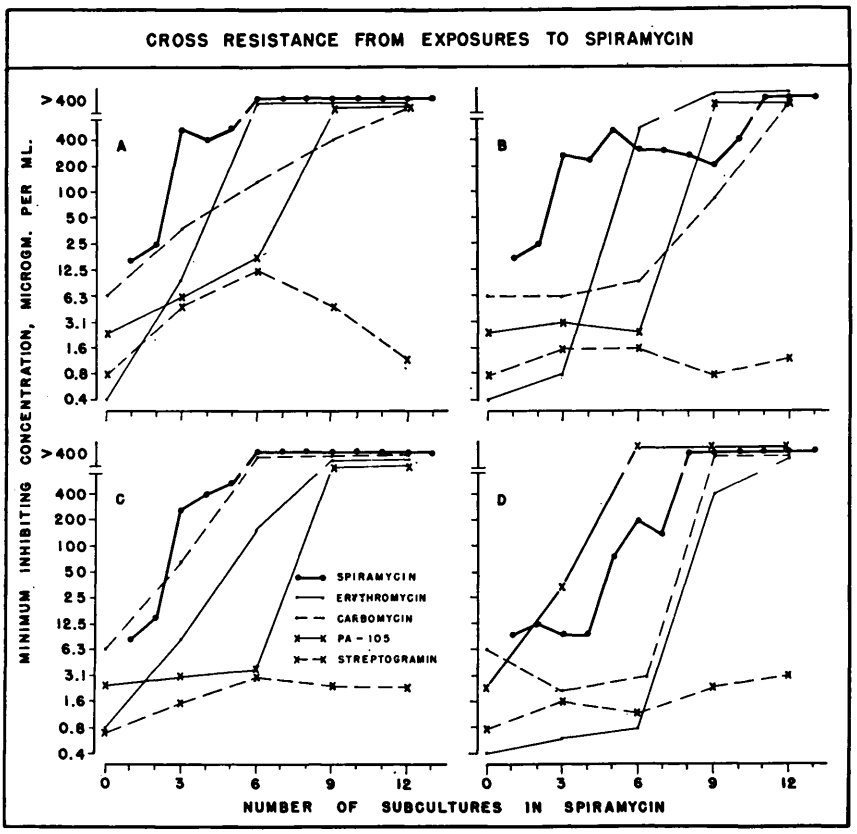

FIG. 3.

cin and vancomycin. The strains had retained their original degree of susceptibility to the 5 antibiotics during their repeated subculture on the agar without the antibiotics, and both these strains and the 5 series of resistant strains derived from them, exhibited the same susceptibility, within the limits of the method, to each of the other antibiotics. In other unpublished studies from this laboratory, strains made resistant by repeated subcultures in cycloserine, bryamin, albamycin and vancomycin showed no cross-resistance to each other, nor to any of the 5 antibiotics that were the subject of the present study, nor to the other antibiotics that are in common use. Resistance to vancomycin developed at a rate even slower than was shown here for streptogramin.

Discussion. The need for effective agents to combat the increasing number and severity of staphylococcal infections still offers a great challenge even now, with such active antibiotics as penicillin, the tetracyclines and erythromycin. These agents are still highly effective so long as the staphylococci in the lesions remain susceptible, but the nature of the staphylococcal lesion, like that in tuberculosis, is particularly favorable for the development and persistence of resistant variants. The organisms persist, often intracellularly, in necrotic and well walled-off lesions, rendering them relatively inaccessible to the full impact of systemically administered antibiotics; as a result, the staphylococci may increase in resistance and become even more difficult to eliminate. Moreover, staphylococci are readily available from the environment to contaminate superficial wounds and to spread from carriers who harbor them as saprophytes but in whom resistant variants may be selected out and then continue to be carried during antibiotic treatment or prophylaxis against other infections. This situation emphasizes the need for a highly effective

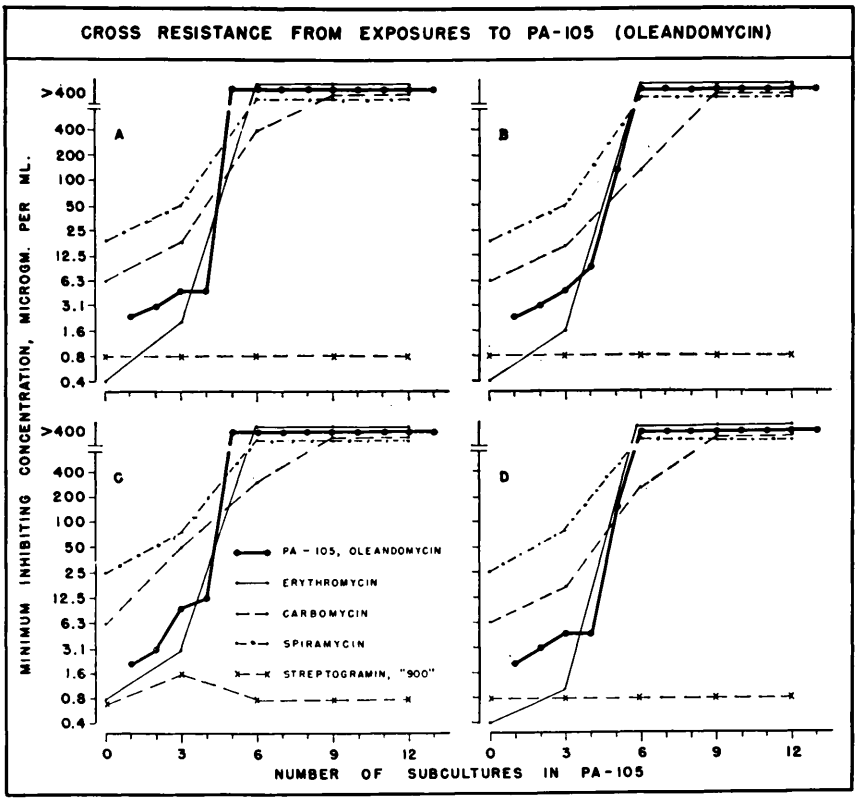
CROSS RESISTANCE FROM EXPOSURES TO PA-105 (OLEANDOMYCIN)
MINIMUM INHIBITING CONCENTRATION, MICROGM. PER ML.
>400
400
200
100
50
25
12.5
6.3
3.1
1.6
0.8
0.4
A
B
C
D
PA - 105, OLEANDOMYCIN
ERYTHROMYCIN
CARBOMYCIN
SPIRAMYCIN
STREPTOGRAMIN, "900"
0
3
6
9
12
NUMBER OF SUBCULTURES IN PA-105

FIG. 4.

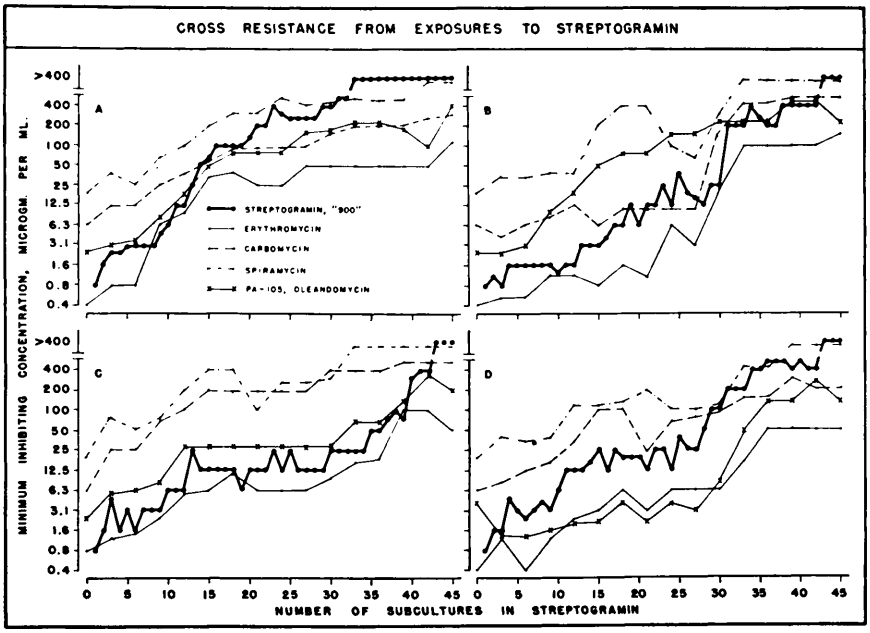
CROSS RESISTANCE FROM EXPOSURES TO STREPTOGRAMIN
MINIMUM INHIBITING CONCENTRATION, MICROGM. PER ML.
>400
400
200
100
50
25
12.5
6.3
3.1
1.6
0.8
0.4
A
B
C
D
STREPTOGRAMIN, "900"
ERYTHROMYCIN
CARBOMYCIN
SPIRAMYCIN
PA-105, OLEANDOMYCIN
0
5
10
15
20
25
30
35
40
45
NUMBER OF SUBCULTURES IN STREPTOGRAMIN

FIG. 5.

bactericidal agent, with good penetrating power, which can be used for long periods systemically, and which lacks the property of inducing resistance in staphylococci.

The present study demonstrated that at least 4 of the 5 agents that were investigated readily induced the development of resistant variants in the strains of staphylococci. There were some differences in the rate at which such resistance developed, depending on the antibiotic, and to a less extent on the strain. Moreover, cross-resistance developed at a rate comparable to that of the homologous resistance except against streptogramin; this antibiotic induced resistance more slowly than did the other 4 agents and, in turn, showed little tendency to lose its activity against the strains that had acquired resistance to those other agents. The possibility that vancomycin may be even more favorable in this respect is suggested by our own unpublished observations which corroborate those of others(18).

The data presented here were obtained *in vitro* and are valid for the conditions of the study but may not necessarily hold for all conditions of exposure of the bacteria to the antibiotics *in vitro,* and certainly may not be true *in vivo*. Ross, for example found 22 strains from patients that were resistant to erythromycin but were either sensitive, or at least moderately so to oleandomycin(19). Chabbert(10), however, found cross-resistance to erythromycin, carbomycin and spiramycin in every strain isolated from patients who had been treated with erythromycin. Observations made in this laboratory on erythromycin-resistant strains isolated from patients,‖ indicated variable degrees of cross-resistance to carbomycin and many strains of staphylococci showed none, although such cross-resistance has been observed regularly after exposures to these agents *in vitro*(5-7). However, nearly 70% of the erythromycin-resistant strains were also resistant to oleandomycin and more than one-fourth of them were resistant to spiramycin.

Summary and conclusions. Four strains of *M. aureus* were rendered resistant to erythromycin, carbomycin, oleandomycin, spiramycin and streptogramin by repeated subcultures in the presence of increasing concentrations of these antibiotics. Cross-resistance to about the same degree developed to all of these agents except to streptogramin; the latter produced homologous resistance at a slower rate than the others, but this was associated with a corresponding increase in cross-resistance to the other 4 antibiotics.

‖ Obtained from Dr. Mark H. Lepper.

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