THE ANTIBIOTIC SENSITIVITY OF PATHOGENIC STAPHYLOCOCCI

BY

W. D. LINSELL

From the Department of Bacteriology, St. Mary's Hospital Medical School, London

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Recent evidence indicates that the pathogenic bacteria are continuously changing in their susceptibility to antibiotics that have come into common clinical use. The occurrence of penicillin-resistant staphylococci in human infection has become commonplace (Anderson, Howard, and Rammelkamp, 1944; Spink, Ferris, and Vivino, 1944; Blair, Carr, and Buchman, 1946). This is thought by many to be due to the selective survival of penicillinase-producing variants during treatment (Demerec, 1945), although infection with two types of staphylococci or the occurrence of secondary infection with penicillin-resistant strains may also play a part.

The result has been a steadily increasing incidence of penicillin-resistant staphylococci in the hospital environment (Barber and Rozwadowska-Dowzenko, 1948; Forbes, 1949). Evidence has also been produced of an increased incidence of streptomycin-resistant staphylococci in hospital practice (Rountree, Barbour, and Thomson, 1951).

New antibiotics highly effective against staphylococci may bring the same change in average sensitivity, and it is the purpose of this paper to indicate the present position with regard to the newer antibiotics, in particular terramycin and aureomycin. The survey is confined to staphylococci from acute lesions in out-patients. At the same time resistance to penicillin and its relation to previous penicillin treatment was investigated. A carrier of penicillin-sensitive staphylococci might, as a result of penicillin treatment, become a carrier of a resistant strain, and if subsequent autogenous infection occurred the lesion might also yield penicillin-resistant staphylococci.

Material

Most of the strains of coagulase positive staphylococci came from finger and hand infections (60% of isolates), boils, carbuncles, and abscesses of various kinds (33%), the remainder from various skin and ear infections.

Each patient was carefully questioned in regard to previous penicillin treatment. Specimens of pus and swabs, taken from the acute pyogenic lesions of patients attending the hospital casualty department, were inoculated on blood agar, and the coagulase positive cultures were maintained on Dorset egg.

Method

One hundred and eighteen strains were isolated and tested for sensitivity to five antibiotics, using the standard serial dilution method in broth in 3 in. $\times$ $\frac{1}{2}$ in. tubes at pH 7.4.

A standard inoculum was used of 10 c.mm. of an overnight broth culture to 10 ml. of broth (approximately 10^8 organisms per ml.). In the case of aureomycin the same technique was employed, but the culture medium was adjusted to pH 6.8, as described by Anderson (1951), as this gave more consistent results than at pH 7.4, though occasionally there was little difference between the results.

The lowest concentration of antibiotic giving complete inhibition was regarded as the sensitivity level.

Results

In determining the sensitivity of 118 strains of staphylococci to penicillin, strains inhibited by concentrations of 0.25 units were arbitrarily taken as "sensitive" and those growing in concentrations of 0.5 units and above as "resistant." A resistance rate of 18.6% was obtained. Seventy-five per cent of strains were sensitive to 0.062 unit or less.

The findings in regard to resistance to penicillin and a history of previous contact with the antibiotic were analysed. Twelve out of 22 resistant strains (54.5%) came from a group of 76 cases with no previous history of contact with penicillin, whereas nine strains (40.9%) were obtained from the group of 36 cases previously treated with penicillin. Statistical analysis of this sample shows that $\chi^2 = 1.36$, which for one degree of freedom gives a probability (P) of approximately 0.25. Thus, in this sample, no statistical difference exists between the two groups; in other words, previous treatment with penicillin played no part in deciding whether a new lesion was likely to be caused by a penicillin-resistant staphylococcus.

TABLE I
SENSITIVITY OF 118 STRAINS OF COAGULASE-POSITIVE STAPHYLOCOCCI TO ANTIBIOTICS
OTHER THAN PENICILLIN

Concentration (μ g. per ml.)	Number of Strains			
	Terramycin	Aureomycin	Streptomycin	Chloramphenicol
32	2	1	2	—
16	—	—	4	1
8	—	2	4	10
4	—	—	90	101
2	—	1	17	5
1	1	72	—	1
0.5	1	42	1	—
0.25	112	—	—	—
0.125	2	—	—	—

Table I summarizes the sensitivity to the principal alternative antibiotics to penicillin. The behaviour of aureomycin and terramycin is very similar except that terramycin is inhibitory at approximately half the concentration of aureomycin weight for weight. Of two strains requiring 32 μ g./ml. of terramycin for inhibition, one required 32 μ g. and the other 8 μ g. of aureomycin for inhibition; both were penicillin-resistant. A third strain sensitive to 1 μ g. of terramycin required 8 μ g. of aureomycin. All three strains were inhibited by 4 μ g. of both chloramphenicol and streptomycin. Table II summarizes the position of individual strains classed

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TABLE II
INDIVIDUAL STRAINS RESISTANT TO ANY ONE OF THE NEWER ANTIBIOTICS

Strain Number	Terramycin	Aureomycin	Streptomycin	Chloramphenicol
11*	0.25	0.5	16	4
28*	32	32	4	4
29	32	8	4	4
34B	0.25	0.5	16	4
40*	0.5	1.0	32	4
41	0.25	0.5	32	4
49	0.25	1.0	16	4
81	1.0	8.0	2	4
110	0.25	1.0	16	4
115†	0.25	1.0	4	16

* Penicillin-resistant (10 units). † Penicillin-resistant (0.5 unit).

as resistant to one or more antibiotics. Strain 28 is of particular interest in that it was resistant to penicillin (10 units), terramycin (32 $\mu\text{g.}$), and aureomycin (32 $\mu\text{g.}$), being most sensitive to chloramphenicol (4 $\mu\text{g.}$) and streptomycin (4 $\mu\text{g.}$). There was no previous record of treatment with aureomycin or terramycin in patients yielding the strains found resistant to these antibiotics.

The two strains found resistant to terramycin were tested for an inhibitor effect. To overnight broth cultures of these and of a sensitive strain, 128 $\mu\text{g./ml.}$ of terramycin was added. All three cultures were incubated for a further 24 hours and Seitz-filtered. The three filtrates were then titrated for terramycin level. No fall in level occurred.

FIG. 1

The complete findings in this series are represented in Fig. 1. It must be emphasized that the penicillin section is displayed in units, not microgrammes, and therefore its superiority weight for weight is much greater than would appear from the figure (1 unit penicillin = 0.0006 mg. crystalline penicillin G).

Discussion

In the present series of strains of staphylococci none of those found resistant to penicillin required more than 10 units for inhibition, and detectable amounts of penicillinase were produced by strains whose resistance was greater than 0.5 unit. This is of interest in view of the highly resistant strains encountered by Spink (1951) in in-patients in whom he found cross-infection occurring with strains of staphylococci of a resistance varying between 500 and 4,000 units.

The sensitivity levels found with the newer antibiotics agree closely with the small series described by Finland, Frank, and Wilcox (1950), except that they found that higher streptomycin concentrations were required for complete inhibition. In the series of 104 strains of staphylococci described by Spink (1951) strains very highly resistant to streptomycin were also highly resistant to penicillin, though all were sensitive to aureomycin and terramycin. Spink described in his series only one strain that was resistant to aureomycin (62.5 $\mu\text{g.}$), obtained from a child after several weeks of aureomycin treatment. In his series five strains were terramycin-resistant; in no case had the patients concerned received terramycin; the most resistant strain required 125 $\mu\text{g./ml.}$ for inhibition. There were also seven chloramphenicol-resistant strains requiring 62.5 $\mu\text{g./ml.}$ or more for inhibition and unrelated to previous treatment with the drug.

From this evidence it is apparent that resistance to antibiotics of a strain of staphylococcus isolated from fresh acute lesions bears little or no relation to previous antibiotic therapy given to the patient from whom the strain is obtained. The series described gives an optimistic picture of the present value of aureomycin and terramycin in treating staphylococcal infection in this country, although it is not unlikely that strains of staphylococcus resistant to them will occur with increasing frequency, especially in infections acquired in hospital where these antibiotics are widely used. Ten strains required over 4 $\mu\text{g.}$ of streptomycin and 11 over 4 $\mu\text{g.}$ of chloramphenicol for inhibition. As 4 $\mu\text{g.}$ per ml. is a therapeutic blood level for these antibiotics, they have a place in the treatment of staphylococcal infection, though at present they appear least active, weight for weight, of the five antibiotics tested.

Summary

The sensitivity to penicillin, aureomycin, terramycin, streptomycin, and chloramphenicol of 118 strains of coagulase-positive staphylococci isolated from acute lesions in out-patients is described.

Nearly one-fifth of the strains were resistant to penicillin. Only two strains were resistant to terramycin and three to aureomycin.

In out-patients previous antibiotic therapy does not appear to influence the likelihood of encountering resistant staphylococci in acute lesions occurring later.

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