STAPHYLOCOCCUS PYOGENES: THE ANTIBIOTIC SENSITIVITY OF STRAINS ISOLATED FROM HUMAN CARRIERS

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INTRODUCTION

The treatment of staphylococcal infection is sometimes difficult because of the antibiotic resistance of the infecting organism. Since the ultimate source of these staphylococci is the human nose, it seemed important to study the sensitivity of strains, isolated from human carriers, to the antibiotics in common clinical use.

The majority of studies of the antibiotic sensitivity of the staphylococcus have been carried out on organisms isolated from hospital sources, and from these investigations it has become apparent that the relative number of penicillin-resistant strains has risen at a steady rate, until now more than 60 % are 20 times as resistant as the standard sensitive staphylococcus *in vitro* (Blair, Carr & Buchman, 1946; Barber, 1947; Nichols & Needham, 1949; Bruynoghe, 1951; Bowie, 1954), and produce penicillinase. Staphylococci exposed to streptomycin both in *in vivo* and *in vitro* rapidly develop resistance, in many instances at an even more rapid rate than to penicillin. Similarly, strains of staphylococci exposed to more recently introduced antibiotics such as aureomycin, chloromycetin, terramycin, magnamycin, and erythromycin have become resistant.

The percentage of antibiotic-resistant strains isolated from hospital out-patients has often been observed to be lower than from in-patients. A few reports of staphylococci isolated from patients and individuals unattached to hospital show an even smaller number of antibiotic-resistant strains. Thus it would seem that the antibiotic-resistant staphylococcus is essentially a hospital problem and this may be correlated with the high staphylococcal carrier rate of the staffs in hospitals and the preponderance of penicillinase producing staphylococci isolated from these carriers. Moreover, there is some evidence that staphylococcal infections arising outside hospital, and deep infections with no likelihood of surface contamination, are more often caused by sensitive staphylococci than infections contracted in hospital (Sherris & Florey, 1951).

In earlier reports of bacteriophage typing of penicillin-resistant staphylococci (Barber & Rozwadowska-Dowzenko, 1948; Rountree, Barbour & Thomson, 1951) the majority of strains were typable with group III phages and many strains in localized epidemic outbreaks of staphylococcal infection were shown to belong to the same phage group. In later reports penicillin-resistant strains have been described which react with phages belonging to other groups (Barber & Whitehead, 1949; Elwood, 1951). By means of such typing the source of many outbreaks has been traced to human carriers of the staphylococcus.

This paper records an investigation of the *in vitro* antibiotic sensitivity of *Staph. pyogenes* isolated from human carriers in the non-hospital population, with special reference to the association with bacteriophage type and previous antibiotic therapy.

MATERIALS AND METHODS

Source of the staphylococci

Staph. pyogenes aureus was isolated in nose swab cultures from pre-clinical medical students in successive years (1950–3); a group of patients from a city practice and a number of blood donors.

Nose swabs from each individual were plated on to milk-agar and individual colonies were subcultured into broth, and these broth cultures were used for all sensitivity determinations.

Sensitivity determinations

These were carried out by the disk diffusion technique (Gould & Bowie, 1952).

Penicillinase production

All penicillin resistant organisms were tested for the production of penicillinase. A modification of the method suggested by Gots (1945) was found most convenient because of the speed with which plates could be prepared and the reproducibility of the results.

Bacteriophage typing was done by the method of Williams & Rippon (1952). The majority of the results quoted have been previously reported (Gould & McKillop, 1954).

RESULTS

Sensitivity to penicillin (Table 1). Strains inhibited by less than 0.1 unit/ml. were regarded as sensitive. All those requiring a higher concentration for inhibition were penicillinase producers and regarded as resistant. Nearly all of the sensitive strains from persistent and intermittent carriers were inhibited by 0.02–0.06 unit/ml., but 15% of the strains isolated from occasional carriers were inhibited by 0.002–0.006 unit/ml., and were not typable with our phage filtrates.

Table 1. *Bacteriophage group and penicillin sensitivity of carrier strains*

Bacteriophage group	Persistent and intermittent carrier strains					Occasional carrier strains					All strains		
	Penicillin sensitivity					Penicillin sensitivity							
	No.	0.02– < 0.02	0.1 0.1	1.0 1.0	> 0.1 > 0.1	No.	0.02– < 0.02	0.1 0.1	1.0 1.0	> 1.0 > 1.0	Total no.	% sensitive to 0.1 µ/ml. penicillin	% resistant to 0.1 µ/ml. penicillin
I	68	0	67	0	1	45	6	33	6	0	113	94	6
II	44	1	42	1	0	23	2	19	0	2	67	96	4
III	52	1	30	3	18	51	0	17	4	30	103	47	53
Unclassified	16	1	13	2	0	20	3	11	3	3	36	78	22
Non-typable	23	0	21	1	1	118	30	62	22	4	141	80	20

Fourteen per cent of the strains from persistent and intermittent carriers, and 24 % from occasional carriers, were resistant to penicillin.

Bacteriophage groups of penicillin-sensitive and resistant strains. Over 90 % of group I and II strains, but less than 50 % of group III strains were penicillin sensitive (Table 1).

Sensitivity to antibiotics other than penicillin. Eleven strains were resistant to streptomycin, and one strain was resistant to chloromycetin (Table 2). Details of these strains are given in Table 3. No strains were found to be resistant to aureomycin or terramycin.

Table 2. *Antibiotic sensitivity of Staphylococcus pyogenes*

Subjects examined	Year of isolation of strains	Carrier type	Total no. of strains	Percentage of strains inhibitory concentration of penicillin				No. of strains	
				< 0.02 unit/ml. 'Sensitive'	0.1 unit/ml.	1.0 unit/ml. 'Resistant'	> 1.0 unit/ml.	Strepto- mycin resistant	Chloro- mycetin resistant
Medical students	1950	Persistent and intermittent	43	—	89	2	9	Not tested	Not tested
	1951	Persistent and intermittent	71	4	81	9	6	2	0
	1952	Persistent and intermittent	61	2	93	1	4	4	0
	1953	Persistent and intermittent	71	0	78	2	20	4	0
	1951	Occasional	79	16	53	13	18	0	0
	1952	Occasional	115	12	56	16	16	0	0
	1953	Occasional	63	5	51	12	12	0	0
	1950-3	Persistent and intermittent	246	2	84	4	10	10	0
	1951-3	Occasional	257	16	55	13	16	0	0
	1950-3	All types	503	9	67	10	14	10	0
Patients from gen. prac.	1951-2	All types	127	—	84	4	12	1	1
Children	1951	All types	17	—	87	0	13	0	0
Blood donors	1951	All types	38	—	92	4	4	0	0
All persons	1950-3	All types	785	81		19		11	1

Relationship of antibiotic-sensitive and resistant strains to previous antibiotic therapy. Fifty-seven per cent of all persons examined had had previous penicillin therapy (Table 4). Very few had had other antibiotics. More than seven times as many penicillin-resistant strains were isolated from persistent and intermittent carriers who had had previous penicillin-therapy as from those who had not. All carriers from whom streptomycin- or chloromycetin-resistant strains were isolated had had therapy with the respective antibiotic.

Table 3. *Details of streptomycin- and chloromycetin-resistant strains*

Strain no.	Carrier type from which isolated	Bacteriophage type of strain	Sensitivity to				
			P units/ml.	S	C μg./ml.	A	T*
46	Occasional	47 A/54	0.3	50	1.5	0.25	0.25
110	Occasional	51	3	50	0.75	0.25	0.25
414	Occasional	N.T.	> 3	50	1.0	0.2	2.5
417	Occasional	N.T.	0.15	10	5.0	1.0	0.5
429	Occasional	6/7/47	> 3	10	3.0	0.3	0.25
575	Persistent	3c	0.04	40	3.5	0.3	0.25
1110	Persistent	47 B	0.04	10	0.5	0.5	0.5
1237	Persistent	7/47	0.04	50	0.5	0.25	0.25
.	Persistent	7/47/76/77	> 3	> 50	1.5	0.25	0.5
.	Persistent	6/7/47/53	> 3	> 50	1.0	0.3	0.3
766	Persistent	47/53/76/77	30	> 50	1.5	0.25	0.5
785	Persistent	?	3	0.1	50	0.25	0.5

* P = penicillin; S = streptomycin; C = chloromycetin; A = aureomycin; T = terramycin.

Table 4. *Occurrence of penicillin sensitive and resistant strains in relation to previous penicillin therapy*

Source of strains	Penicillin sensitivity of strains	Number of individuals examined having had	
		Previous penicillin therapy	No previous penicillin therapy
Occasional carriers	—	265	195
	Sensitive	83	72
	Resistant	35	20
Persistent and intermittent carriers	Sensitive	73	78
	Resistant	23	3
Non-carriers	—	51	22

DISCUSSION

Five per cent of the persons examined in this survey carried penicillin-resistant *Staph. pyogenes* in their nares. This is a higher figure than those of earlier investigators of the non-hospital population (Forbes, 1949; Rountree & Thomson, 1949; Martin & Whitehead, 1949) and is in favour of some factor leading to an increase in the number of penicillin-resistant strains in the non-hospital community.

Our evidence is in favour of a connexion between the amount of antibiotic therapy and the incidence of antibiotic-resistant strains, and agrees with that of observers of the hospital population (Clarke, Dalglish & Gillespie, 1952; Summers, 1952; Needham & Nichols, 1953).

The majority of our penicillin and streptomycin-resistant strains were of phage group III. *Staphylococci* resistant to chloromycetin, aureomycin and terramycin have also been reported to belong to this group (Clarke *et al.* 1952; Lowbury, Topley & Wood, 1952). Strains of *Staph. pyogenes* of group III have been shown to mutate more readily than those of other groups and exposure to antibiotics may

induce the change from sensitive to resistant more frequently than in strains belonging to groups I and II. If this were followed by spread of the resistant strain to other persons the predominating group III strains would be explained. Such spread of antibiotic-resistant strains is well illustrated in hospital (Rountree *et al.* 1951), but the frequency of replacement of penicillin-sensitive by penicillin-resistant strains in healthy carriers, among the non-hospital population not in contact with the antibiotic, would appear to be very low.

The temporary removal of a penicillin-sensitive strain during penicillin therapy may be succeeded by replacement with a penicillin-resistant strain as conditions are then more favourable for colonization by the resistant strain. Subsequently these strains spread through the population and become dominant as has occurred in hospital. Spread among the general population has been slower and depends upon individual contact with hospital and the continued use of penicillin in general practice.

The same principles would appear to hold good for the other antibiotics.

SUMMARY

Strains of *Staphylococcus pyogenes* able to grow in 0.1 unit/ml. of penicillin produced penicillinase and were regarded as resistant. The majority of the remaining strains had the same sensitivity as the standard sensitive staphylococcus.

Five hundred and three strains were isolated from pre-clinical medical students from 1950 to 1953, and 24 % were penicillin-resistant. Two per cent were streptomycin-resistant and none were resistant to chloromycetin, aureomycin or terramycin. Two hundred and forty-six of the strains were isolated from persistent and intermittent carriers, but only 14 % were penicillin-resistant.

Another 183 strains were isolated from persons unattached to hospital, and 14 % were resistant to penicillin. Only one strain was streptomycin-resistant; one was chloromycetin-resistant and none were resistant to the other antibiotics.

Among persistent and intermittent carriers, more than 7 times as many penicillin-resistant strains were isolated from those who had had previous penicillin therapy as from those who had not.

It was concluded that the administration of antibiotic increases the number of resistant strains in the community.

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