PENICILLINASE PRODUCTION BY *STAPHYLOCOCCUS AUREUS* STRAINS FROM OUTBREAKS OF FOOD POISONING

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In the course of the routine examination of strains of *Staphylococcus aureus* isolated from outbreaks of staphylococcal food poisoning in north-west England, we were surprised to find that most of them were penicillin resistant. Nine of 11 strains, all present in large numbers in foods that had caused clinically typical outbreaks of vomiting and diarrhoea, were penicillin resistant. None of the outbreaks had occurred in a hospital. This finding was particularly interesting because, when penicillin-resistant staphylococci first became common in hospitals, the resistant strains were found to be mainly members of phage group III, the group to which most enterotoxigenic staphylococci also belong.

There are several scattered references to the incrimination of penicillin-resistant staphylococci as the cause of outbreaks of food poisoning in the years before penicillin had come into general therapeutic use. Rutherford and Crowson (1945) described an outbreak of food poisoning in Canada in 1945, in which the organism responsible was resistant to 12.5 units of penicillin in a tube test. Mason (1945) examined six "well-known" food-poisoning strains obtained from other workers and found that three of them were completely resistant when tested by the cylinder plate method. Segalove (1947) tested a collection of 15 well-authenticated enterotoxigenic strains from the collection maintained at the University of Chicago. All were known to have had no previous contact with penicillin in the laboratory, and most had been isolated before 1941. He used a tube dilution test with an inoculum of 0.1 ml. of a 1/100 dilution of a 24-hour culture in a semi-synthetic liquid medium. Seven of the strains were inhibited by 0.1 unit per ml. of penicillin or less, and eight required 10 units or more for inhibition.

Allison (1949) first noted the common association with outbreaks of food poisoning of *Staph.*

aureus strains lysed by certain phages. He examined 47 cultures, including 26 isolated in this country and 21 from the United States, Canada, Egypt, and the Sudan, and found that 38 of them were lysed by phages of the "6/47" group (including phage 42D). Others (Saint-Martin, Charest, and Desranleau, 1951; Williams, Rippon, and Dowsett, 1953; Parker, 1953) subsequently confirmed these findings. In 1953 Williams *et al.* proposed the classification of *Staph. aureus* into three broad phage groups, and included in group III strains lysed by one or more of the phages 6, 7, 47, 53, 54, 70, 73, 75, 77, 42D, and 42E. The association of strains of group III so defined with food poisoning has become even closer than that of Allison's "6/47 group" since the inclusion of further group III phages in the typing scheme and the introduction of the practice of testing apparently non-typable cultures with phage at 1,000 × the routine test dilution.

Members of phage group III form a considerable proportion of all strains isolated from human sources—between 10% and 50% according to the type of material sampled—but few of them react with phage 42D, and strains lysed only by 42D at the routine test dilution are quite rare. For example, we encountered only one "pure" 42D strain in a series of 1,494 human cultures. Such strains are, however, common in material of bovine origin and appear to be a frequent cause of mastitis of cattle (Macdonald, 1946; Smith, 1948; Price, Neave, Rippon, and Williams, 1954). It has, therefore, been proposed (see Rippon, 1956) that "type 42D" strains should be removed from phage group III to a new provisional group IV.

Materials and Methods

Strains.—The authors were fortunate in being able to secure, through the kindness of Professor G. M. Dak and Dr. M. Bergdoll, of the Food Research

Institute of the University of Chicago, a collection of 12 strains of American food-poisoning staphylococci from outbreaks before 1941. All of them had been included in the series examined by Segalove and had also been tested for enterotoxin production either in monkeys or in human volunteers. Three early American strains were also obtained from Dr. S. T. Cowan, of the National Collection of Type Cultures. The information available with two of them indicated that they should be identical with two of Professor Dack's strains, and, in fact, they proved to be culturally indistinguishable from them: the third strain appeared not to be represented in the Chicago collection. Dr. R. E. O. Williams, of the Staphylococcal Reference

Laboratory at Colindale, also provided two collections of strains from fairly recent well-investigated outbreaks, mainly in Great Britain. The origins of the 37 separate strains are shown in Table I.

Methods.—The strains were first shown to be coagulase positive by the slide method of Cadness-Graves, Williams, Harper, and Miles (1943). They were then phage typed by the method of Wilson and Atkinson (1945) as modified by Williams and Rippon (1952), using the "basic set" of 20 phages listed by Williams *et al.* (1953), omitting phage 44, but with the addition of phages 71 and 80. Strains were allotted to the three main phage groups described by Williams *et al.*, except that strains lysed only by phage 42D at routine test dilution were placed in provisional group IV. Sensitivity to five antibiotics (penicillin, streptomycin, chloramphenicol, chlortetracycline, and erythromycin) was tested on a single plate of blood agar. Plates were flooded with overnight broth cultures of staphylococci, and antibiotic tablets (Evans' "sentest") were placed on the surface after drying. Strains were considered resistant when growth occurred to within 1 mm. of a tablet after 18 hours at 37° C. The tablets contained the following quantities of antibiotic: penicillin 0.5 unit, streptomycin 20 µg., chloramphenicol 40 µg., chlortetracycline 10 µg., erythromycin 10 µg. Each culture was also tested for penicillinase production by method based on that of Bondi and Dietz (1944). Agar plates were flooded with an overnight broth culture of the Oxford staphylococcus (N.C.T.C. 6571) and, after drying, porcelain cylinders (Heatley, 1944) were placed on the surface of the medium. Equal quantities of a penicillin solution, containing 2 units/ml., and of an overnight broth culture of the staphylococcus being tested were placed in a cylinder. A control cylinder containing an equal volume of penicillin and of uninoculated broth was included on each plate. Complete absence of inhibition of growth around the test cylinder after overnight incubation at 37° C. was taken as evidence of penicillinase production.

Results

Phage-typing.—All of the 37 strains were lysed by one or more of the phages of group III as originally defined by Williams *et al.* (1953). Only 34 of them conformed to the more strict definition of group III, since three were lysed only by phage 42D at the routine test dilution and were therefore placed in provisional group IV. The fact that all three of the group IV strains were among the earliest in the series is fortuitous: a small proportion of similar strains has been noted in several other series of cultures. Allison found four among 21 British strains isolated before 1949, Williams *et al.* two out of 40 from outbreaks in 1950 and 1951, and Saint-Martin *et al.* two among 15 Canadian strains. It is unfortunate that no

TABLE I
SUMMARY OF INFORMATION ABOUT 37 STRAINS OF *STAPHYLOCOCCUS AUREUS* CONSIDERED THE CAUSE OF ENTEROTOXIC FOOD-POISONING OUTBREAKS

No.	Identification of Culture	Location of Outbreak	Year	Food Responsible	Phage Group	Penicillin
1	D 1 (also NCTC 4135)	Chicago	1929	Christmas cake	IV	S
2	D 8	Milwaukee	1930	Cake	IV	S
3	D 9 (also NCTC 4134)	Panama	1931	Chicken gravy	III	S
4	D 47	Jersey City	1931	Cake	IV	S
5	NCTC 4136	Chicago	1932	Not known	III	R
6	D 147	Tennessee	1933	"	III	R
7	D 161	Indianapolis	1935	Sandwiches	III	R
8	D 169	U.S.A.	1938	Chocolate éclair	III	R
9	D 170	"	?	Beef	III	S
10	D 171	Omaha	1938	Chipped beef	III	R
11	D 177	Baltimore	1939	Chicken	III	R
12	D 178	Cook County, Ill.	1939	Ham	III	R
13	D 196	U.S.A.	1940	"	III	R
14	C 111	Poole, Dorset	1949	Pressed meat	III	R
15	C 151	Eccles, Lancs.	1949	Black pudding	III	S
16	C 186	Boston, Lincs.	1949	Fish cake	III	R
17	C 850	Norwich	1949	Meat brawn	III	R
18	C 1363	Irlam, Lancs.	1949	Tongue	III	R
19	C 697	Brighton	1954	Raw milk	III	R
20	C 1074	Dorchester	1954	Cheese (N. Zealand)	III	R
21	C 2023	Shrewsbury	1954	Tinned ox tongue (Eire)	III	R
22	C 2072	Coventry	1954	Stew	III	R
23	C 2004	Lisbon, Portugal	1954	Fish mayonnaise	III	R
24	C 2110	Harrow, M'sx	1954	Bath chap	III	R
25	C 2431	Ipswich	1954	Potted meat	III	R
26	C 2483	Brighton	1954	Lamb tongues	III	R
27	M 1937	Manchester	1955	Brisket of beef	III	R
28	M 11406	Salford	1955	Pressed meat	III	R
29	M 27732	Stafford	1955	Meat pie	III	R
30	M 35309	Wakefield	1955	Vanilla slice	III	R
31	M 35437	Bolton	1955	" sponge	III	R
32	M 37147	Preston	1955	Fish sandwiches	III	S
33	M 37278	Shrewsbury	1955	Lamb sandwiches	III	S
34	M 41662	"	1955	Pork pie	III	R
35	M 54773	Oldham	1955	Pressed meat	III	R
36	M 55174	Prestwich, Lancs.	1955	Home canned salmon (Canada)	III	R
37	M 26569	Manchester	1956	Tinned crab (Japan)	III	R

Identification: D, Food Research Institute, University of Chicago. C, Staphylococcal Reference Laboratory, Colindale, London. NCTC, National Collection of Type Cultures. M, Manchester Public Health Laboratory.

Penicillin: S, sensitive. R, resistant.

recent group IV strains from outbreaks of food poisoning were available for testing.

Penicillin Resistance and Penicillinase Production.—Eight of the strains were sensitive to penicillin and failed to produce penicillinase: the remaining 29 were completely resistant in the plate test and all of them produced penicillinase. Eight of the 13 American strains isolated before 1941 were penicillin resistant: these included eight of the 10 members of group III but none of the group IV strains. A comparison of these results with those obtained by Segalove nine years previously revealed one discrepancy. No. 169 was sensitive to 0.1 unit of penicillin when tested by tube dilution in 1947, but when examined in this laboratory was resistant in the plate test and produced penicillinase. However, the inoculum used by Segalove was rather small, and it is possible that a weak penicillinase producer might have appeared to be sensitive to 0.1 unit under these conditions. Among the more recently isolated strains, all members of group III, 21 of 24 (88%), were penicillin resistant.

Although the number of strains available for study was small, the results suggest that at least three-quarters of all enterotoxigenic staphylococci are penicillin resistant and produce penicillinase. Whether it can be concluded that there has or has not been an increase in the proportion of penicillin-resistant strains in group III during the last 25 years will depend on whether the "type 42D" strains are separated from the "true" members of group III. Among group III strains of the sort usually found in human material, the percentage of resistant strains was of the order of 80% in the 1930s and is about the same to-day.

None of the strains was resistant to streptomycin, chloramphenicol, chlortetracycline, or erythromycin.

Penicillin Resistance Among Group III Strains from Other Sources.—Records were available of 1,494 strains, isolated from human sources between

September, 1953, and July, 1956, which had been phage typed and tested for penicillin resistance as part of another investigation. The proportion of penicillin-resistant strains and of penicillin-resistant group III strains from lesions of various sorts and from "normal" nose swabs is shown in Table II.

The material from which these strains were derived was very varied in its origin. The impetigo swabs were from cases in a number of different towns. The other specimens from patients treated at home were received from practitioners widely scattered throughout Lancashire and Cheshire. The "normal" nose swabs were from samples of children in three schools and one nursery. The material from hospital patients came from over 30 different hospitals in north-west England.

A little less than half (45%) of all the phage group III strains from sources other than hospitals were penicillin resistant, and there was no significant difference between the proportion resistant in the various types of lesion and in the nasal swabs. If the nasal swabs and the lesions other than impetigo from private cases were considered together, group III strains were more likely than other strains to be resistant (43% and 19% respectively). In the impetigo lesions, however, a smaller proportion of group III than of other strains were resistant (48% and 68% respectively) because of the preponderance of penicillin-resistant type 71 strains.

Eighty-six per cent. of group III strains from hospital patients were resistant. As is usually the case, the percentage of resistant strains from deep lesions in hospital patients was a little lower, presumably because some of the infections were acquired before admission to hospital.

It appears, therefore, that present-day enterotoxigenic group III strains, derived from sources unconnected with the hospital environment, are more likely to be penicillin resistant than strains from the noses and lesions of people outside hospital.

TABLE II

PROPORTION OF PENICILLIN-RESISTANT STRAINS AND OF PENICILLIN-RESISTANT STRAINS IN PHAGE GROUP III IN 1,494 CULTURES OF *STAPHYLOCOCCUS AUREUS* ISOLATED BETWEEN SEPTEMBER, 1953, AND JULY, 1956

	Population Not Treated in Hospital				Hospital Patients			Total
	Impetigo	Other Superficial Lesions	Deep Lesions	Nose Swabs	Infections of Newborn Infants	Older Patients		
						Superficial Lesions	Deep Lesions	
All strains	382/578 (66%)	33/97 (34%)	16/80 (20%)	33/182 (18%)	321/377 (85%)	90/110 (82%)	51/70 (73%)	926/1494 (62%)
Phage group III	30/62 (48%)	14/31 (45%)	6/10 (60%)	6/19 (32%)	83/94 (88%)	57/64 (89%)	13/19 (69%)	209/291 (72%)

Discussion

Naturally occurring strains of *Staph. aureus* are only resistant to penicillin if they produce penicillinase. Information about the prevalence of such resistant strains before the introduction of penicillin as a therapeutic agent is unfortunately scanty. There are a number of accounts of series of strains examined between 1942 and 1946, usually by a tube dilution test, but the importance of inoculum size was not generally appreciated at that time. It is difficult, therefore, to translate these results into a percentage of penicillinase-producing strains. Some undoubtedly resistant strains were encountered quite early in the period, though they were not common in suppurative lesions or wound infections in hospital. No large series of cultures from nasal swabs of normal people, from septic lesions outside hospital, or from superficial skin lesions appears to have been recorded.

The rapid increase in infections due to resistant strains in hospitals was first noted early in 1947 (Barber, 1947; Barber and Rozwadowska-Dowzenko, 1948) and was correctly attributed to the widespread therapeutic use of penicillin. Barber's opinion that penicillin acted by selecting resistant strains which were subsequently spread from patient to patient by cross-infection has been generally accepted. Most of the penicillin-resistant strains examined by Barber and her colleagues during the next two years were members of phage group III (Barber and Whitehead, 1949), but soon afterwards a large outbreak in a maternity hospital due to resistant group I strains was recorded (Barber, Hayhoe, and Whitehead, 1949). Later a considerable proportion of group II strains was found to be resistant (Williams *et al.*, 1953; Barber and Burston, 1955). These findings have been interpreted as evidence that phage group III strains yield antibiotic-resistant variants more readily than other strains of *Staph. aureus*, though the occurrence of such a mutation has never been observed. It is possible that the successive appearance of penicillin-resistant members in groups III, I, and II was only apparent. The series showing a predominance of resistant group III strains was obtained from a general hospital, and an excess of group I strains was only observed when outbreaks of infection among newborn infants were investigated. A similar preponderance of group I strains in neonatal infections and of group III strains in older hospital patients has been apparent in this area during the whole of the last three years. It is probable that similar considerations, together with certain changes in phage-typing techniques

which have been introduced since 1949, would explain the apparent emergence of penicillin-resistant group II strains in the later years.

It is becoming clear that certain classes of staphylococci prevalent in populations unconnected with hospitals are predominantly penicillin resistant. Parker, Tomlinson, and Williams (1955) reported that over 90% of all strains of *Staph. aureus* type 71, which is associated with impetigo contagiosa, are penicillin resistant, and have also produced evidence which suggests that similar strains seen in impetigo lesions in 1941 may also have been resistant (see also Tomlinson and Parker, 1956). The results reported here indicate that most group III strains isolated from foods responsible for outbreaks of enterotoxic food poisoning in recent years are penicillin resistant, and that the percentage of resistant strains from this source exceeds that among group III strains in nasal swabs and septic lesions in the population not treated in hospital. Over half of a small series of enterotoxic staphylococci isolated over 15 years ago are also resistant. It will, however, not be possible to decide whether any increase of penicillin resistance among group III strains from outbreaks of food poisoning has taken place during the last 15 years until the status of "type 42D" has been settled and a larger series of strains has been examined.

Although resistant strains were uncommon among staphylococci causing suppuration in pre-penicillin days, it is now probable that they would have been found more frequently elsewhere if they had been looked for. There may well have been foci of resistant strains in other ecological "niches" which cannot now be identified. It is clear that the occurrence of mutation need not be invoked to explain the appearance of penicillin-resistant strains in hospitals in the last 10 years.

Summary

Twenty-one of 24 strains of *Staph. aureus* from recent outbreaks of enterotoxic food poisoning were found to be penicillin resistant and to produce penicillinase. All were members of phage group III.

A greater proportion of phage group III strains from food-poisoning incidents than from other sources outside hospital were penicillin resistant.

Eight of a collection of 13 staphylococci causing food poisoning isolated before 1941 were penicillin resistant. All of these eight were members of phage group III. The five sensitive strains included two belonging to group III and three to group IV.

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