SOME CULTURAL CHARACTERISTICS OF *STAPHYLOCOCCUS AUREUS* STRAINS FROM SUPERFICIAL SKIN INFECTIONS

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(With 2 Figures in the Text)

In an investigation into the bacteriology of *impetigo contagiosa* reported by Parker, Tomlinson & Williams (1955) *Staphylococcus aureus* was isolated from 83 % of the lesions, and three-quarters of all the strains belonged to a single phage type (Type 71) which was rarely found in situations other than superficial skin lesions. Approximately 90 % of the Type 71 strains were penicillin resistant, though they had been isolated from patients few of whom had had any recent contact with hospitals; these findings recalled the result of an unpublished investigation carried out by Tomlinson in 1941, in which half of the strains of *Staph. aureus* from impetigo lesions, and a much smaller proportion from other sources, were found to inhibit the growth of corynebacteria on solid media. He also observed that when strains possessing this inhibitory property were grown on plates of serum agar made with an infusion broth base they produced zones of opacity around the developing colonies.

When these tests were applied to the impetigo strains isolated in 1953 it was found that 90 % of Type 71 staphylococci, and a minority of others, inhibited the growth of *Corynebacterium diphtheriae*. Staphylococci could give rise to one of two distinct sorts of inhibitory zone. Type 71 strains, and very few others, formed a quite narrow, sharply defined zone, but some other strains produced a wider, hazy zone of inhibition. Subsequently (Tomlinson & Parker, 1956) it was shown that 95 % of all Type 71 strains also produced opacity on serum agar plates, but that this property was not confined to Type 71; it was also seen commonly among penicillin-resistant members of phage-group III (Type 71 belongs to phage-group II).

In 1952, Gillespie & Alder had reported that many strains of *Staph. aureus* produced an opacity when grown in nutrient broth containing hen egg yolk and a little glucose, and that this reaction was inhibited by staphylococcal antitoxin. The egg-yolk reaction, as it was called, was positive in a high proportion of strains from closed suppurative lesions and from boils and abscesses seen in general practice, but most strains obtained from in-patients in a number of hospitals were egg-yolk negative. Nearly all of these 'hospital staphylococci' had been obtained from infected wounds, and had not given rise to deep suppuration. Most of the egg-yolk negative strains were penicillin resistant and belonged to phage-group III (Alder, Gillespie & Herdan, 1953; Alder, Gillespie & Thompson, 1955). It was suggested that egg-yolk negative strains, though able to survive on the skin and

to cause superficial inflammation in wounds, might be unable to give rise to lesions in deeper tissues. The fact that Type 71 strains were also egg-yolk negative, and that there was an inverse relationship between the result of the egg-yolk reaction and the serum opacity reaction (Tomlinson & Parker, 1956) lent support to this view.

It seemed desirable, therefore, to study a larger group of *Staph. aureus* cultures from a variety of human sources, and to compare the cultural characteristics of those from deep and from superficial lesions.

MATERIALS AND METHODS

A. *Origin of the cultures used in this investigation*

The origin of the 1389 strains of *Staph. aureus* used in this investigation is shown in Table 1. All were isolated between September 1953 and July 1956. The 274 strains from local impetigo cases were obtained in the years 1954–56, a period in which there was no undue prevalence of the disease; the rest of the impetigo strains were from sporadic cases in a number of other parts of England. The cultures from new-born infants included representatives of twenty-eight outbreaks of infection in twenty-five hospitals, together with four continuous series each representing the infections occurring in a ward or unit over an extended period. 330 of the 367 strains were from superficial infections, including 150 from bullous or pustular skin lesions, sixty-four from other skin infections and 116 from septic conjunctivitis ('sticky eye'); only thirty-seven were from deep lesions such as abscesses, effusions and pneumonia.

Table 1. *Origin of the strains of* Staphylococcus aureus *used in this investigation*

	Patients not in hospital		Hospital patients
Impetigo lesions			
S.E. Lancs.	274	414	
Other towns	140		
Infections of new-born infants			
Superficial	—		330
Deep	—		37
Other inflammatory lesions			
Superficial	91		104
Deep	78		60
Nasal swabs			
Hospital admissions	108	275	
School and nursery children	90		
Impetigo cases and contacts	77		
	858		531
	1389		

The 333 strains from inflammatory lesions (other than impetigo) in older persons were subdivided into those from deep and those from superficial infections. Deep lesions included closed pleural effusions, abscesses in deep tissues, pulp-space infections of the hand, and skin infections leading to pus formation beneath the

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epidermis (boils, carbuncles, *sycosis barbae*). Superficial lesions included infected wounds and burns, skin lesions confined to the epidermis (such as secondary infections of eczema, urticaria and varicella) and discharges from eyes, ears, noses and sinuses. 164 of the 333 strains were from patients in nineteen hospitals, and 169 were from non-hospital patients in widely scattered parts of Lancashire and Cheshire.

The nasal strains included 108 from patients on admission to an Infectious Diseases Hospital, ninety from normal school and nursery children and seventy-seven from impetigo cases and their family contacts.

B. *Methods*

After isolation and identification as *Staph. aureus* by methods described previously (Parker *et al.* 1955) the cultures were stored in Lemco agar 'stabs' at room temperature in the dark. They were subcultured infrequently and colony selection was avoided wherever possible. Each strain was subjected to the following tests: phage typing, penicillin resistance, inhibitory action on *C. diphtheriae*, the egg-yolk reaction and the opacity reaction on horse-serum agar. Also, 1277 strains isolated after mid-1954 were tested for resistance to streptomycin, chloramphenicol, chlortetracycline and erythromycin.

(i) *Phage typing.* Cultures were phage typed in batches by the method of Wilson & Atkinson (1945) as modified by Williams & Rippon (1952): strains showing no strong lysis with any phage at the routine test dilution were also tested at 1000 times the strength of the routine test dilution (1000 × RTD). The phages comprised the 'basic set' of Williams, Rippon & Dowsett (1953), omitting phage 44, but with the addition of phage 71. Phage 80 (Rountree & Freeman, 1955) became available in September 1955 and was included in the routine 'set' from then onwards.

Only strong reactions (greater than fifty plaques) were considered. Strains giving strong reactions with one or more phages of group I, group II or group III (Williams *et al.* 1953) at the RTD were allocated to that group. Strains failing to give a strong reaction with any dilute phage, but giving a strong reaction at 1000 × RTD with one or more phages of any one group, were similarly allocated. Strains reacting with dilute phages of more than one group, or, not being typable with dilute phage, giving reactions with phages of more than one group at 1000 × RTD were considered to be unclassifiable.

All strains lysed by phage 71 (either dilute or at 1000 × RTD), but not lysed by any other dilute phage, were included in 'Type 71', and the term *Staph. aureus* Type 71 was applied to all such strains whatever their other characteristics.

One strain reacting only with phage 42D at RTD was encountered in this series. Although such strains have now been provisionally allocated to a new phage-group IV (Rippon, 1956) it has not been shown separately, but included in group III. The single strain of Type 80 isolated between September 1955 and July 1956 has been included in phage-group I and will not be considered further.

(ii) *Antibiotic sensitivity tests.* The first 112 strains were tested for penicillin resistance only by a paper-strip method. All later strains were tested for gross

resistance to a number of antibiotics simultaneously on one plate; blotting paper disks prepared by the method of Fairbrother & Martyn (1951) were used in the early stages of this series but were replaced later by Evans 'Sentest' tablets. All strains showing apparent resistance by the disk method were retested with the tablets. Blood-agar plates were flooded with 18 hr. broth cultures and, after a brief period of drying at room temperature, the tablets were placed on the surface of the medium. Readings were made after overnight incubation at 37° C. When growth occurred up to within 1 mm. of a tablet the strain was considered to show gross resistance. The tablets used contained the following amounts of antibiotic: penicillin 0·5 unit, streptomycin 20 μg., chloramphenicol 40 μg., chlortetracycline 10 μg. and erythromycin 10 μg.

(iii) *Inhibition of* Corynebacterium diphtheriae. All strains were tested within 4 weeks of isolation. A thick suspension of a non-virulent strain of *C. diphtheriae mitis* was made by emulsification in broth of the growth from a blood-agar plate. Further blood plates were flooded with this suspension. As soon as the surface was dry small loopfuls of overnight broth cultures of staphylococci were spotted on to the plates. After overnight incubation at 37° C. the corynebacteria had grown as an even confluent sheet, with zones of inhibition around some of the circular areas of staphylococcal growth. We were concerned only with strains producing sharp, clearly defined zones of inhibition ('sharp-zone' inhibitors or DI + S strains), as the ability to form wider zones of inhibition with hazy margins (DI + H) was not correlated with any of the other characters under investigation.

(iv) *The egg-yolk reaction.* The technique of Gillespie & Alder (1952) was followed, using filtered glucose egg-yolk broth that had been matured for at least 4 weeks in the refrigerator (Alder *et al.* 1953). Final readings were made after 3 days' incubation at 37° C.

(v) *The serum opacity reaction.* After preliminary tests the following technique was adopted as a routine. The basal medium was a meat infusion broth (Wright, 1933) at pH 7·4 solidified with 1·0 % New Zealand Agar. To this 5 % of fresh horse serum was added at about 50° C. Plates were poured and dried for a brief period. The staphylococci were spot-inoculated on to the plates with a straight wire in the late afternoon, and read after not more than 16 hr. incubation at 37° C. Narrow, homogeneous zones of opacity extending continuously outward for 0·5–1·5 mm. from the edge of the growth, and visible to the naked eye, were considered as positive.

RESULTS

A. *Phage typing*

The distribution of the 1389 cultures of *Staph. aureus* among the main phage groups is summarized in Table 2, and the following general conclusions can be drawn. A little over half of all strains from impetigo lesions belonged to Type 71. There was, as before, some evidence for the association of Type 71 with infections of new-born infants: 11 % of all strains from superficial infections in this age group, and 21 % of 150 strains from bullous or pustular lesions, were members of Type 71. There were only two Type 71 strains among 175 from deep lesions

Table 2. *Phage typing of strains of* Staphylococcus aureus *with the numbers resistant to penicillin in brackets*

	Superficial lesions				Deep lesions			Nasal swabs			
			Older patients			Older patients					
Phage group	Impetigo	New-born infants in hospital	In hospital	Outside hospital	New-born infants in hospital	In hospital	Outside hospital	Hospital admissions	School and nursery children	Impetigo cases and contacts	Total
I	25	139	13	19	17	21	26	28	31	10	329
	(6)	(124)	(11)	(5)	(15)	(20)	(8)	(20)	(4)	(1)	(214)
II: Type 71	216	36	7	6	1	0	1	4	10	29	310
	(199)	(36)	(6)	(4)	(1)		(0)	(3)	(10)	(28)	(287)
Other patterns	48	15	2	12	2	3	27	17	10	5	141
	(9)	(5)	(1)	(2)	(1)	(1)	(0)	(1)	(0)	(1)	(21)
III	53	79	63	28	14	17	10	19	13	13	309
	(28)	(70)	(57)	(14)	(12)	(13)	(6)	(10)	(6)	(8)	(224)
Not typable	59	49	17	19	3	16	11	27	9	13	223
	(15)	(39)	(10)	(5)	(3)	(12)	(1)	(9)	(1)	(4)	(99)
Unclassifiable	13	12	2	7	0	3	3	13	17	7	77
	(6)	(7)	(2)	(3)		(1)	(0)	(7)	(1)	(1)	(28)
Total	414	330	104	91	37	60	78	108	90	77	1389
	(263)	(281)	(87)	(33)	(32)	(47)	(15)	(50)	(22)	(43)	(873)

and one of these, from a whitlow, possessed none of the other cultural characteristics associated with this type. Group II strains, other than members of Type 71, made up 10–20 % of the isolations from non-hospital patients but were not frequently encountered in hospitals.

Group I strains were particularly common in neonatal infections (43 %) and uncommon in impetigo lesions (6 %). Group III strains made up a considerable proportion of those isolated from all sources. They formed the majority (60 %) in superficial infections of hospital patients occurring after the neonatal period but they were less frequent (23 %) in superficial infections of new-born infants.

B. *Resistance to penicillin*

The penicillin sensitivity of the strains is shown in brackets in Table 2, and is related both to the result of phage typing and to their source (see also Figs. 1, 2). Nearly all Type 71 strains, whether from impetigo lesions or from other sources,

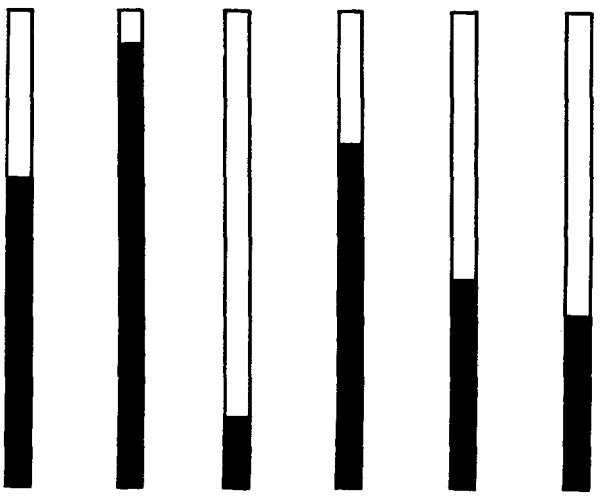

Fig. 1. Percentage of penicillin-resistant strains in Type 71 and in the other main groups of phage types.

I	II		III	Not typable	Unclassifiable
	Type 71	Other			
65	93	15	72	44	36

were penicillin resistant. In fact, the percentage of resistant strains in this type (93 %) far exceeded that in any other group. The lowest percentage (15 %) occurred among members of group II other than Type 71, and most of the small number of resistant strains seen were derived from superficial infections, particularly impetigo and neonatal infections. Group I infections acquired in hospital were nearly all (89 %) due to resistant strains, but only 27 % of group I strains from non-hospital infections were resistant. In group III, however, the difference between the percentage of resistant strains in hospital and outside infections (88 and 53 %, respectively) was not so great.

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Only 19 % of the boils, abscesses and other closed lesions of non-hospital patients were due to resistant staphylococci, but in superficial infections (other than impetigo) of non-hospital patients the percentage was somewhat higher (36 %): in impetigo 64 % of the strains were resistant. Although this figure was somewhat lower than that observed during the impetigo epidemic in Lancashire in 1953–54, it was still remarkably high. 84 % of all hospital infections were due to resistant strains, and the difference between the percentages from deep and from superficial lesions was not significant. Most of the resistant strains from impetigo lesions belonged to Type 71, but in other lesions they were mainly members of groups I and III.

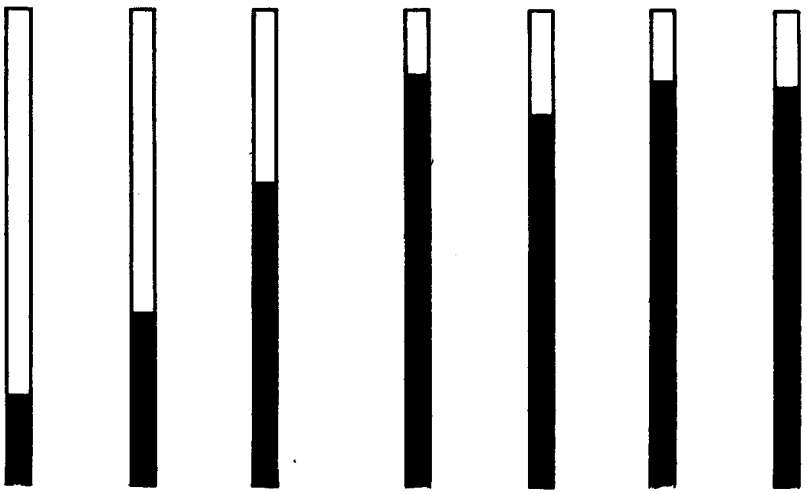

Fig. 2. Percentage of strains from various sources resistant to penicillin.

Outside hospital			In hospital			
	Superficial lesions		Deep		Superficial	
Deep lesions	(other than impetigo)	Impetigo	New-born infants	Older patients	New-born infants	Older patients
19	36	64	86	78	85	84

Neither of the two series of strains from nasal swabs of healthy persons appears to have been entirely representative of the carrier state of the general population. Nearly half of the cultures in the hospital admission series were penicillin resistant and this may possibly be attributed to the frequency of readmission and inter-hospital transfer among these patients. In the school and nursery child group 24 % of the strains were penicillin resistant; this was an unexpectedly high figure, since a similar group examined 3 years previously (Parker *et al.* 1955) had contained only 12 % of resistant strains. A comparison of the type distribution in these two series showed that the difference was almost entirely due to the presence of a nasal 'carrier epidemic' of Type 71 (unassociated with any skin infections) in one of the schools sampled in 1955. Unfortunately, no opportunity presented itself for the examination of nasal swabs from entirely unconnected persons, such as the blood donor series of Rountree and her colleagues (see Rountree & Rheuben, 1956).

C. *Resistance to other antibiotics*

Tests of sensitivity to streptomycin, chloramphenicol, chlortetracycline and erythromycin were carried out on 1277 cultures. Erythromycin-resistant strains were not encountered. The relationship between the result of the phage typing and resistance to penicillin, streptomycin, chloramphenicol and chlortetracycline is shown in Table 3. Strains sensitive to penicillin were very rarely resistant to any other antibiotic. As expected, most of those resistant to one or more antibiotic in addition to penicillin were members of phage-group III (Rountree & Thompson, 1952; Barber & Burston, 1955), and all except three of the ninety 'multiple-resistant' group III strains were from infections acquired in hospital. There was some evidence that resistance to several antibiotics was less common among strains from new-born infants than from older hospital patients. Thus, 52/58 penicillin-resistant group III strains from superficial hospital infections in older patients, but only 31/82 from infections of new-born infants, were also resistant to one or more other antibiotic.

D. *The egg-yolk reaction*

The results of the egg-yolk reaction on our series of strains confirmed and somewhat extended the observations of Gillespie and his colleagues (Table 4). Most members of phage-group I were egg-yolk positive (EY+) whether they were resistant to penicillin or not. In all the other groups there was a tendency for more of the resistant than the sensitive strains to be egg-yolk negative (EY−). In Type 71 over 99% of all penicillin-resistant strains, and over half of the small number of sensitive strains were EY−. Penicillin-sensitive members of the rest of group II and of group III were predominantly EY+, but over half of the resistant strains were EY−. Most of the EY− cultures in the series were members either of Type 71 or of group III.

The proportion of EY− strains among cultures from different sources (Table 5) is in general agreement with the results of Alder *et al.* (1953). The lowest proportion of EY− strains occurred among penicillin-sensitive cultures from deep lesions. The majority of penicillin-resistant strains from impetigo lesions and from superficial hospital infections were EY−, but the proportion EY− among strains from neonatal infections was lower. The predominance of Type 71 in impetigo lesions and of penicillin-resistant group III strains in superficial hospital infections accounted for the main excess of EY− cultures from these sources.

E. *The serum opacity reaction*

(i) *Techniques.* Preliminary experiments were made with the medium Tomlinson had used in his early work, a 5% rabbit-serum agar with an infusion broth base. On this medium most strains of *Staph. aureus* from impetigo lesions, which were members of Type 71 and inhibited corynebacteria, produced zones of opacity after overnight incubation at 37° C. Other strains, not members of Type 71 and not inhibitors of corynebacteria, also produced opacity: many of them had been obtained from superficial infections in hospitals and were members of phage-group III. The zones of opacity were in most instances dense and homogeneous and extended

Table 3. *Numbers of strains resistant to penicillin, streptomycin, chloramphenicol and chlortetracycline*

Sens, sensitive to all four antibiotics; P, resistant to penicillin; S, resistant to streptomycin; C, resistant to chloramphenicol; T, resistant to chlortetracycline.

Group	Sens	P	P+S	P+S+C	P+S+T	P+S+C+T	P+C	P+T	P+C+T	S	C	T	C+T
I	110	196	7	—	—	—	1	1	—	—	—	—	—
II: Type 71	19	259	—	—	—	—	12	3	1	—	—	—	—
Other patterns	111	20	—	—	—	—	1	—	—	—	1	—	1
III	74	117	35	11	37	4	1	—	—	1	1	—	—
Not typable	108	63	6	1	3	—	—	3	—	—	—	2	—
Unclassifiable	47	15	—	—	1	2	2	—	—	—	—	—	—
Total	469	670	48	12	41	6	17	7	1	1	2	2	1

Table 4. *Number of egg-yolk positive and egg-yolk negative cultures among penicillin-sensitive and penicillin-resistant members of the main phage groups*

	Penicillin			
	Sensitive		Resistant	
Egg-yolk reaction ...	+	−	+	−
Group				
I	100	15	196	18
II: Type 71	5	18	2	285
Other patterns	107	13	7	14
III	69	16	111	113
Not typable	101	23	70	29
Unclassifiable	43	6	17	11
Total	425	91	403	470

Table 5. *Number of egg-yolk positive and egg-yolk negative cultures among penicillin-sensitive and penicillin-resistant strains from various sources*

	Penicillin			
	Sensitive		Resistant	
Egg-yolk reaction ...	+	−	+	−
Impetigo	112	39	39	224
Other superficial lesions				
Older hospital patients	10	7	31	56
New-born infants (hospital)	39	10	184	97
Older patients (outside hospital)	47	11	20	13
Deep lesions, all sources	79	2	76	18
Nasal swabs, unconnected with impetigo	110	16	42	30

0·5–1·5 mm. outwards from the edge of the growth, but some strains gave weaker reactions, visible only with the hand-lens, which were difficult to interpret. On media containing 5 % horse or human serum, however, the zones were more marked and could nearly always be seen with the naked eye, but from time to time fainter reactions, consisting of one or more rings of haziness with intervening clear areas were also seen. These faint 'target zones' were inconstant in their appearance when the same strains were tested repeatedly, and their distribution among staphylococci of the various groups was haphazard. Target zones were rarely seen on rabbit-serum agar. Somewhat similar appearances could be induced to develop around growth that had produced no opacity in the routine test by placing the plate in the refrigerator for a few hours after incubation at 37° C. Most strains not producing opacity in the medium around the growth at 37° C. gave rise to areas of subcolonial haziness. Consistent results could be obtained on horse-serum agar if the faint target zones were ignored. Only strains giving rise to a narrow, homogeneous zone extending continuously outwards from the edge of the growth, and visible to the naked eye, were therefore considered positive. Repetition of the tests, using different batches of horse serum and of infusion base gave consistent results.

Serum became unsuitable after prolonged storage in the refrigerator: after 6 weeks it gave less than half of the expected positive results, and all the zones of opacity were faint. Heating the serum to 62° C. for $\frac{1}{2}$ hr. had no effect on the results, but reduction of the percentage of serum in the medium to 0·5 % completely abolished the reaction. No opacity was produced when the basal medium was 1 % Lemco agar, 1 % Evans peptone agar, or when the plates were incubated anaerobically. The reaction was uninfluenced by the presence of 20 % carbon dioxide in the atmosphere or by a staphylococcal antitoxic serum (Wellcome). No characteristic appearances were seen on infusion agar containing 1·5 % human γ-globulin or 2·0 % bovine albumen fraction V. Most strains produced wide, faint zones of haziness, presumably due to coagulase, on fibrinogen agar, but narrow dense zones were not seen.

A series of seventy-five cultures was inoculated in parallel on to 5 % rabbit-serum agar and 5 % rabbit-plasma agar, and the narrow dense zones of opacity were seen equally well on both. At the same time they were tested for fibrinolysin by the method of Christie & Wilson (1941) on plates of infusion agar containing rabbit plasma that had been heated to 56° C. for 20 min. All but three of the cultures produced a wide zone of clearing of the heat-precipitated fibrinogen, and within this clear area narrow zones of dense opacity were seen around the growths of most of the cultures that had been positive on rabbit-serum agar. Plates of 20 % human plasma agar were made by the method recommended by Reid & Jackson (1945) for the plate coagulase test. With suitable batches of plasma two quite distinct appearances could be seen: a wide faint zone of haziness, presumably due to coagulase, around all the cultures and a narrow dense zone only around those that had given a positive result in the serum opacity test.

Table 6. *Result of the egg-yolk reaction (EY) and the serum opacity reaction (Op) on penicillin-sensitive and penicillin-resistant members of the main phage groups. The number of strains in each category producing a sharp zone of inhibition of* Corynebacterium diphtheriae *is shown in brackets*

	Penicillin sensitive				Penicillin resistant			
	EY+		EY−		EY+		EY−	
Group	Op+	Op−	Op+	Op−	Op+	Op−	Op+	Op−
I	0	100 (3)	5	10 (1)	0	196 (1)	6	12
II: Type 71	0	5 (1)	14 (11)	4 (2)	0	2 (1)	280 (269)	5 (4)
Other patterns	0	107	11 (3)	2 (1)	0	7	12 (9)	2 (1)
III	2	67 (1)	4	12 (2)	0	111 (1)	91 (1)	22 (2)
Not typable	0	101	4 (1)	19 (3)	0	70	19 (10)	10
Unclassifiable	0	43 (3)	1	5	0	17	6	5

(ii) *The characters of* Staphylococcus aureus *strains giving a positive serum opacity reaction.* Strains forming characteristic opacity zones on 5 % horse-serum agar were considered to be positive (Op+); negative reactions were recorded as Op−. The distribution of Op+ strains among the main phage groups is essentially the inverse of the distribution of EY+ strains. The relationship between the result of the serum opacity test and the egg-yolk reaction in individual strains is shown by the fact that 59 % of the strains were EY+, Op− and 32 % were EY−, Op+. Only 8 % were EY−, Op− and were evenly distributed in all the groups; these reactions have a doubtful significance and may well be due to a lack of sensitivity of one or both of the tests. Two out of 1389 cultures were EY+, Op+. Both were

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penicillin-sensitive group III strains which gave a strongly positive egg-yolk reaction in repeated tests: numerous single-colony 'picks' from both cultures gave a weak but definite positive result in the serum opacity test.

F. *Association between certain cultural characteristics in Type* 71 *and in other groups of* Staphylococcus aureus

An attempt must now be made to summarize the information obtained about the distribution among strains of *Staph. aureus* of the cultural characteristics under consideration. Table 6 shows the result of the egg-yolk reaction and the serum opacity reaction on penicillin-sensitive and penicillin-resistant members of each phage group: the number of strains in each category having the ability to produce a sharp zone of inhibition of *C. diphtheriae* (DI + S) is also shown.

The following conclusions may be drawn.

(i) *The demarcation of Type* 71. The original definition of Type 71 was derived from the observation that strains from a particular sort of lesion had a uniform pattern of susceptibility to the typing phages. The difference in pattern between Type 71 and the rest of phage-group II is quite small, and it is therefore surprising how sharply they are demarcated by the results of the other tests, the significance of which were also established on epidemiological grounds. Of 310 Type 71 strains (defined by phage susceptibility) 269 (87 %) conformed to the typical pattern (penicillin resistant, EY −, Op +, DI + S) and 295 (95 %) differed in no more than one characteristic. Only four of the 310 strains (1 %) differed from the typical pattern in every characteristic except phage susceptibility.

Table 7. *Result of the egg-yolk reaction (EY) and the serum opacity reaction (Op) on penicillin-sensitive and penicillin-resistant members of phage-group III from various sources*

	Penicillin sensitive				Penicillin resistant			
	EY +		EY −		EY +		EY −	
	Op +	Op −	Op +	Op −	Op +	Op −	Op +	Op −
Superficial lesions								
Older patients in hospital	0	3	1	2	0	13	41	3
New-born infants in hospital	0	7	1	1	0	32	30	8
Outside hospital (including impetigo)	1	31	1	6	0	32	7	3
Deep lesions								
Older patients in hospital	0	4	0	0	0	9	3	1
New-born infants in hospital	0	2	0	0	0	8	4	0
Outside hospital	0	4	0	0	0	4	0	2

(ii) '71-*like*' *strains.* The number of DI + S strains that were not members of Type 71 was quite small. Occasional examples were found in all phage groups, but 28/43 of them were either other group II strains or were non-typable, that is to say, the difference between their phage-reaction patterns and that of Type 71 was only a quantitative one. Most Type 71 strains are lysed by other group II phages at 1000 × RTD and if such a strain were to become somewhat more susceptible to

phage 3*C* or to phage 55, for example, it would become an 'other group II' strain. If it became less susceptible to phage 71 it would be non-typable. Nineteen of these twenty-eight strains were also penicillin resistant, EY −, Op +, and were therefore '71-like' in all characters except phage susceptibility. All nineteen were isolated from impetigo lesions or from *pemphigus neonatorum*. Over half of all penicillin-resistant members of phage-group II were '71-like'.

(iii) *The general characters of strains from superficial lesions.* The largest single group of EY −, Op + strains other than Type 71 was found within phage-group III, but here we are not dealing with a characteristic of the group as a whole, since less than one-third of all group III strains, and less than one-half of the penicillin-resistant group III strains, were EY −, Op +. It will be seen from Table 7, which shows the cultural characters of group III strains from different sorts of lesions that most of the penicillin-resistant strains from superficial lesions in hospital patients were EY −, Op +, but that most group III strains from deep lesions (and from superficial lesions in non-hospital patients) are EY +, Op − whether penicillin sensitive or resistant.

DISCUSSION

In certain skin infections such as *pemphigus neonatorum* and *impetigo contagiosa* the lesion is confined to the epidermis and heals without scarring. Over 50 years ago many dermatologists (Almquist, 1891; Unna & Schwenter-Traschler, 1899; Dohi & Kurita, 1904; Clegg & Wherry, 1906) believed that these diseases were due to a special variety of *Staph. aureus* which was able to produce characteristic lesions in the skin but could not invade the deeper tissues. Although this view was supported by strong clinical and epidemiological evidence it was eventually abandoned, largely because of the failure of many attempts (see Epstein, 1934, 1935) to demonstrate that this organism differed culturally from the *Staph. aureus* of boils and abscesses.

In recent years there has been renewed interest in the possibility that *Staph. aureus* strains might differ in their pathogenicity not only quantitatively but qualitatively, and that such differences could be correlated with susceptibility to typing phages. Allison (1949) observed that enterotoxin-producing strains were almost all members of phage-group III and Williams *et al.* (1953) found a striking predominance of group I strains in cases of fulminating pneumonia during two epidemics of influenza A. In these instances the strains exhibited a variety of phage-reaction patterns within the group, but in the last 3 years two examples of staphylococcal phage types with characteristic biological properties, and defined by susceptibility to a single phage, have been reported.

Type 80 was first identified in Australia (Isbister, Durie, Rountree & Freeman, 1954; Rountree & Freeman, 1955) as a cause of severe skin lesions in new-born infants and of abscesses in older patients: in both it was characterized by a tendency to invade deeper tissues. The association of Type 71 with staphylococcal impetigo, and its presence almost exclusively in superficial situations, was reported in the following year. Both types had been identified during the investigation of a clinically recognizable infection occurring in epidemic form and had been untypable

at RTD with the routine phages. In both instances the phage specific for the type also lysed many other strains of *Staph. aureus*: the essential character of both types was that they were lysed by the one phage and by no other. Many group I strains were lysed by phage 80 in addition to one or more of the phages 29, 52, 52*A* and 79, and this justified the inclusion of Type 80 in phage-group I. Type 71 was included in phage-group II for similar reasons.

Type 71 strains possessed all the usual cultural characters of a virulent *Staph. aureus*. They produced a yellow pigment, fermented mannitol, liquefied gelatin, clotted rabbit and human plasma promptly, formed α- and δ-toxins, produced abundant hyaluronidase and formed at least five antigen-antibody flocculation lines when tested by the method of Elek & Levy (1950). It was soon apparent, however, that they had several characters which separated them sharply from other members of phage-group II. Most were penicillin resistant, inhibited the growth of *C. diphtheriae*, produced a zone of opacity on horse-serum agar, and gave a negative egg-yolk reaction, and it seemed possible that some or all of these properties might be useful as 'markers' for the detection of other sorts of *Staph. aureus* capable of similar activities in the animal body.

It became clear that the 'markers' were of two sorts. The ability to produce a sharp zone of inhibition of *C. diphtheriae* on solid medium was associated almost exclusively with Type 71. Most of the few other strains with this ability had phage patterns not very different from that of Type 71, had other '71-like' cultural characters and were associated with superficial skin lesions. The other 'markers' (penicillin resistance, EY−, Op+) were not exclusively associated with Type 71, and their distribution among members of the other phage groups was therefore of greater interest.

The finding that most Type 71 strains derived from infections acquired outside hospital were penicillin resistant was surprising. The reasons for believing that the use of penicillin in general practice was unlikely to have brought this about have already been discussed (Parker *et al.* 1955). Tomlinson found that the predominant strain of *Staph. aureus* in impetigo lesions in 1941 was both DI+S and Op+. Strains with this combination of characters occur very rarely among penicillin-sensitive staphylococci. The resistance of Type 71 to penicillin, like that of all naturally resistant *Staph. aureus*, is associated with the production of penicillinase, and it is probable that penicillinase production is a natural characteristic of Type 71. A somewhat similar situation exists among enterotoxic members of phage-group III, which were predominantly penicillinase producers even before 1941 (Parker & Lapage, 1957). It is possible that, apart from the selective influence of therapeutic penicillin, a greater proportion of the *Staph. aureus* strains from superficial than from deep lesions may be penicillinase producers.

Gillespie & Alder's observation that egg-yolk negative strains were most commonly found in superficial infections, and were usually penicillin resistant, was confirmed. Penicillin-resistant strains in all phage groups except I were more commonly EY− than penicillin-sensitive strains, but the majority of EY− cultures were members either of phage-group III or of Type 71. Further experience with the serum opacity test showed that the results were an almost perfect mirror

image of those of the egg-yolk reaction. The reason for this is not yet clear. The production of opacity in egg-yolk broth is thought to be due, not to lecithinase, but to a lipase, though the substrate has not been identified (Gillespie & Alder, 1952; Klinge, 1957). The serum opacity reaction appears to be due to the production of a substance which diffuses slowly through agar and acts on serum protein: the experiments reported here make it certain that it is not due to coagulase, and the fact that Type 71 strains produce only α- and δ-toxins makes it unlikely that the substance responsible could be β-toxin (see Christie & North, 1941). Whatever its mechanism, the serum opacity test appears to be a very valuable 'marker for superficiality' in *Staph. aureus*.

SUMMARY

1. A collection of 1389 strains of *Staphylococcus aureus* of human origin was examined by the following tests: phage typing, antibiotic resistance, ability to inhibit the growth of *Corynebacterium diphtheriae*, the egg-yolk reaction and the serum opacity reaction.

2. Nearly all strains of *Staph. aureus* Type 71 were penicillin resistant, inhibited *C. diphtheriae*, gave a negative egg-yolk reaction, and produced a zone of opacity on horse-serum agar.

3. The penicillin resistance of Type 71 strains is unlikely to have arisen as a result of the therapeutic use of penicillin, and is probably a natural characteristic of the type.

4. *Staph. aureus* strains which gave a negative egg-yolk reaction and a positive serum opacity reaction occurred almost exclusively in superficial lesions. The majority of them were members of Type 71, or were penicillin-resistant members of phage-group III. It is suggested that these organisms can cause superficial inflammation, but are usually unable to invade deeper tissues.

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