J. clin. Path. (1966), **19**, 305

Cultural characters of a newly recognized group of hospital staphylococci

M. PATRICIA JEVONS, MADELEINE JOHN, AND M. T. PARKER

From the Cross-infection Reference Laboratory, Colindale, London

SYNOPSIS Members of a newly recognized group of hospital staphylococci, which are believed to have arisen from 83A staphylococci by lysogenization, differ from them in several cultural characters. Some but not all of these characters appear to be determined by the carriage of phage.

In 1960, a new group of *Staphylococcus aureus* strains not lysed by any of the basic set of typing bacteriophages made their appearance in British hospitals. These organisms gave inhibition reactions with some of the group III phages applied at 1,000 times the routine test dilution. This suggested that they were related to each other, and possibly also to staphylococci that were lysed only by phage 83A, which gave similar inhibition reactions and had first been recognized about two years earlier as causes of endemic infection in British hospitals. Jevons and Parker (1964) produced evidence that the new untypable staphylococci had arisen from the 83A organism by lysogenization with a phage which blocked the reaction with phage 83A. The blocking phages obtained from several distinct strains of the new organisms were, however, all different in host range, and included members of several serological groups. Strains from different places also differed in their susceptibility to lysis by a set of experimental phages. It was concluded that the new organisms had arisen by a number of separate lysogenizations, and probably not all from the same 83A strain. They did not constitute a 'type', but rather a group or complex of related strains.

In 1963, several authors reported that the new untypable staphylococci had unusual cultural characters. Willis and Turner (1963) in Leeds found that many *Staph. aureus* strains that were resistant both to penicillin and to tetracycline formed a lemon-yellow pigment on glycerol monoacetate agar. According to Jacobs, Willis, Ludlam, and Goodburn (1963), most of these lemon-yellow organisms were untypable, but gave inhibition reactions with group III phages at routine test dilution $\times$ 1,000. In this they resembled the new untypable organisms prevalent in Glasgow and Liverpool (Temple and Blackburn, 1963; Turner, 1963; Willis, Jacobs, and Goodburn, 1963). Further investigations (Willis *et al.*, 1963, 1964) showed that lemon-yellow, tetracycline-resistant cultures differed from most others in that they were usually not 'proteolytic', *i.e.*, did not cause darkening of heated blood agar (99%), but were often β-lysin positive (33%) and staphylokinase (fibrinolysin) negative (68%); the incidence of these characters in other *Staph. aureus* cultures was, for absence of protease 21%, for β-lysin production 3%, and for absence of staphylokinase 24%. It was later shown that the new untypable staphylococci seldom hydrolysed Tween 80 (Smith, Willis, and O'Connor, 1965). Robertson (1963) found that most of them were resistant to neomycin (see also Jacobs and Willis, 1963, 1964; Lowbury, Babb, Brown, and Collins, 1964). It seemed, therefore, that the new organisms were characteristically lemon-yellow, non-'proteolytic', β-lysin positive, staphylokinase-negative, Tween-negative and neomycin-resistant, but that none of these characters was invariably present.

Similar organisms had been reported in Cincinnati, U.S.A., before they were seen in England (Thomas, Hill, Culbertson, and Altemeier, 1960), and were widespread in parts of Canada by mid-1961 (Comtois and Bynoe, 1963). There were also numerous accounts of neomycin-resistant strains of *Staph. aureus* in the U.S.A. at about the same time (Quie, Collin, and Cardle, 1960; Tisdale, Fenster, and Klatskin, 1960; Finegold and Gaylor, 1960; Griffith, Ostrander, Smith, and Beswick, 1961; Cohen, Fekety, and Cluff, 1962). These organisms were variously reported as being untypable, or lysed at routine test dilution $\times$1,000 by group III phages, but there is little doubt that they belonged to the same group (see Mitchell, 1964). Untypable organisms with inhibition reactions in group III recently

Received for publication January 1966

became widespread in Australia (Jacobs and Willis, 1964) and were sometimes resistant to bacitracin as well as to neomycin (Rountree and Beard, 1965). There is no evidence that 83A organisms were ever common in North America or Australia, and attempts to obtain phages which block the 83A reaction from untypable strains from these countries have so far been unsuccessful (Rountree and Beard, 1965; Comtois, 1965).

In the present investigation we compared the cultural characters of strains of the new untypable staphylococcus obtained from diverse sources with those of the 83A organism, and of other staphylococcal strains.

METHODS

COLLECTIONS OF STAPH. AUREUS CULTURES 1 A group of 34 untypable *Staph. aureus* cultures with inhibition reactions in group III was collected in 1963. Only one culture from each hospital was included, unless the reactions with five experimental phages (see Jevons and Parker, 1964) suggested that two distinct strains were present; there were 27 from Britain and seven from other countries (Switzerland two, U.S.A. two, Canada one, Norway one, Peru one). For comparison we used 22 British 83A cultures isolated in 17 different hospitals at about the same time. Two or more cultures from the same hospital were included only if they gave different reactions with the experimental phages.

2 As part of a continuous study of staphylococci responsible for sepsis we have received collections of cultures from a number of London hospitals. Each consisted of about 100 consecutively isolated cultures from staphylococcal lesions in hospital in-patients. Collections made in 1963 and 1964 were used in the present investigation.

3 Some other cultures from our routine material were used in surveys of antibiotic resistance.

EXPERIMENTAL CULTURES AND PHAGES Most of the *Staph. aureus* cultures and phages used in the experimental section of the work have already been described (Jevons and Parker, 1964). They include (*a*) 83A staphylococci and their lysogenic derivatives, which are untypable but have the characteristic inhibition pattern, and are designated in the usual way, for example 1116 (1126′) is staphylococcus 1116 carrying a phage derived from strain 1126; (*b*) four untypable organisms (1108, 1126, 9512 and 4331) and their non-lysogenic derivatives (1108DL etc.); (*c*) two untypable organisms (4331 and 14358) and Tween-positive variants of them; (*d*) strain 57 (Winkler, de Waart, and Grootsen, 1965), a sensitive indicator strain detecting phages of lytic groups I, II, and III; (*e*) blocking phages derived from new untypable organisms (Jevons and Parker, 1964).

PHAGE TYPING The method of Blair and Williams (1961) was used with the basic set of typing phages (Report, 1963), and with the five experimental phages D, 77Ad, 12100, UC18, and B5 (Jevons and Parker, 1964).

RESISTANCE TO ANTIBIOTICS Resistance to antibiotics was determined with Oxoid Multodiscs containing penicillin (1·5 unit), streptomycin (10 μg.), tetracycline (10 μg.), chloramphenicol (10 μg.), and erythromycin (10 μg.) on nutrient agar plates flooded with a six-hour broth culture and incubated at 37°C. for 18 hours. With the first four antibiotics, an organism was considered resistant if it grew to within 1 mm. of the disc, but with erythromycin a zone-radius of 4 mm. or less was taken as evidence of resistance. Neomycin (5 μg./ml.) and bacitracin (5 units/ml.) were incorporated in nutrient agar plates which were inoculated with a loopful of a six-hour broth culture of each organism. The appearance of more than 10 colonies after overnight incubation was evidence of resistance.

RESISTANCE TO MERCURY SALTS We used the disc method of Green (1962).

PIGMENTATION ON GLYCEROL MONOACETATE AGAR We used the methods of Willis and Turner (1962) and Jacobs, Willis, and Goodburn (1964). Six-hour broth cultures were spotted with a loop on to plates of Bacto brain-heart infusion agar containing 1% glycerol monoacetate. Consistent results were obtained with this basal medium, but not with several others.

PRODUCTION OF β-LYSIN, STAPHYLOKINASE, AND 'PROTEASE' To detect β-lysin, cultures were stabbed into agar plates containing 10% washed sheep erythrocytes and incubated anaerobically for 48 hours at 37°C., and kept for a further two hours at 4°C. They were examined immediately on removal from the anaerobic jar. Fibrin-agar plates, prepared as described by Jacobs and his colleagues (1964), were inoculated with 1 mm. loopfuls of six-hour cultures and examined after 24 and 48 hours at 37°C. A clearing around the inoculated area indicated the production of staphylokinase. 'Proteolysis' was detected by the surface-inoculation of chocolate agar plates containing 5% horse blood. An area of darkening around the inoculated area after 48 hours at 37°C. was a positive result.

HYDROLYSIS OF TWEEN 80 One mm. loopfuls of six-hour broth cultures were spotted on to a plate of peptone agar containing 1% Tween 80 and 0·01% $CaCl_2$ (Sierra, 1957). The appearance of an opaque halo around the bacterial growth indicated lipolysis (Tween positive).

RESULTS

CULTURAL CHARACTERS OF REPRESENTATIVE 'NEW UNTYPABLE' AND 83A STAPHYLOCOCCI The 34 cultures belonging to the new group of untypable staphylococci were chosen to include organisms from a wide geographical area, and were further diversified by the inclusion where possible of strains giving different reactions with the experimental phages. They were compared with a collection of 22 distinct British 83A staphylococci (Table I).

There were several resemblances between the two groups of cultures. All the untypable organisms, and

TABLE I

CULTURAL CHARACTERS OF REPRESENTATIVE MEMBERS OF THE NEW GROUP OF UNTYPABLE ORGANISMS AND OF 83A STAPHYLOCOCCI

	New Group	*83A*
Number examined	34	22
Resistance to penicillin	34 (100%)	20 (91%)
Resistance to streptomycin	34 (100%)	21 (96%)
Resistance to tetracycline	34 (100%)	20 (91%)
Resistance to erythromycin	29 (85%)	15 (68%)
Resistance to oleandomycin	2 (6%)	0
Resistance to chloramphenico	8 (24%)	3 (14%)
Resistance to novobiocin	6 (18%)	4 (18%)
Resistance to mercury salts	34 (100%)	21 (96%)
'Protease'	4 (12%)	2 (9%)
Tween positive	8 (24%)	22 (100%)
Lemon-yellow pigmentation	23 (69%)	3 (14%)
β-lysin positive	11 (32%)	2 (9%)
Staphylokinase positive	20 (59%)	21 (96%)
Resistance to neomycin	24 (71%)	0
Resistance to bacitracin	21 (62%)	0

all but one of the 83A organisms, were resistant to several antibiotics and to mercury salts. 'Proteolytic' activity was low in both groups, less than 10% of each causing darkening of chocolate agar.

The six remaining tests showed clear differences between the two groups of cultures. All the 83A organisms, but less than a quarter of the untypable organisms, gave a positive Tween reaction. Lemon-yellow pigmentation was common among the untypable organisms (69%) but rare among the typable organisms (14%). All but one of the 23 lemon-yellow untypable organisms were Tween negative, and seven of the 11 which did not produce a lemon-yellow pigment were Tween positive.

The production of β-lysin (β+) and of staphylokinase (K+) may be considered together. Twenty of the 22 83A cultures were β−, K+ (the combination most often seen in *Staph. aureus* from human sources), one was β+, K+ and one was β+, K−. Less than half of the untypable cultures (16 out of 34) were β−, K+; four were β+, K+, seven were β+, K−, and seven were β−, K−. In all, 32% of the untypable cultures but only 9% of the 83A cultures were β+.

All the 83A staphylococci were sensitive to neomycin and to bacitracin, but 24 of the 34 untypable staphylococci (71%) were resistant to neomycin and 21 (62%) were resistant to bacitracin.

Thus, the two collections of staphylococci were similar in their pattern of resistance to antibiotics other than neomycin and bacitracin, and both contained few 'proteolytic' cultures. The new untypable group of staphylococci as a whole differed from 83A staphylococci in that they usually produced lemon-yellow pigment but did not split Tween 80, they often formed β-lysin and failed to form staphylokinase, and they were frequently resistant to neomycin and bacitracin; but no single character was invariably present in the one nor absent from the other.

There was no clear-cut difference between the cultural characters of strains from particular geographical areas, nor any association between these characters and the pattern of sensitivity to the experimental phages. In Table II, the characters of the untypable organisms from foreign countries are shown in detail. Six of the seven cultures were Tween negative and four formed lemon-yellow pigment, but only one was β+, K−. Six were resistant to neomycin and four to bacitracin. The three strains from European countries resembled the British strains in carrying phages which lysed 83A staphylococci. No phage with this property was demonstrated in the four American strains, though they carried other phages which could be detected by lysis of strain 57. The American strains were all neomycin resistant, and three of them were Tween negative and lemon-yellow.

TABLE II

CULTURAL CHARACTERS OF UNTYPABLE CULTURES FROM FOREIGN SOURCES

Origin	*Lysis by Experimental Phages*[1]	*Carriage of Phage Lysing*		*Resistance to*[2]	*'Protease'*	*Tween Reaction*	*Lemon-yellow Pigmentation*	*β-lysin*	*Staphylokinase*
		83A Strains	*Strain 57*						
Norway	77Ad/12100/B5	+	+	P S T C N B Hg	−	−	+	−	+
Switzerland 1	None	+	−	P S T C E Hg	−	−	−	−	+
Switzerland 2	77Ad/B5	+	−	P S T E N Hg	−	−	−	−	+
U.S.A. 1	D/77Ad/12100/UC18/B5	−	+	P S T C N B Hg	−	−	+	−	+
U.S.A. 2	77Ad/UC18/B5	−	+	P S T C N B Hg	−	−	+	−	+
Canada	D/77Ad/UC18/B5	−	+	P S T N B Hg	−	−	+	+	−
Peru	12100/B5	−	+	P S T C N Hg	+	+	−	−	+

[1]see Jevons and Parker (1964).

[2]P = penicillin
S = streptomycin
T = tetracycline
C = chloramphenicol
E = erythromycin
N = neomycin
B = bacitracin
Hg = mercury salts

ASSOCIATION OF CULTURAL CHARACTERS WITH LYSOGENY There was reason to believe that the cultural characters of these organisms might be related to their state of lysogeny. Rosendal, Bülow, and Jessen (1964) had shown that the acquisition by 80/81 staphylococci of a phage which blocked the 81 reaction also changed the Tween reaction from positive to negative. This phage was obtained from a Tween-negative organism lysed only by phage 80, and every lysogenized cell became Tween-negative. The change was therefore due to lysogenic conversion. It had also been known for many years that *Staph. aureus* cultures seldom produced both β-lysin and staphylokinase (Christie and Wilson, 1941). The reason for this became apparent when Winkler and his colleagues (de Waart, Winkler, and Grootsen, 1962; Winkler *et al.*, 1965) found that the acquisition of certain serological group F phages caused in many *Staph. aureus* strains the change from β+, K− to β−, K+, and that this was also due to lysogenic conversion. Each lysogenized cell possessed both of the new characters, and the conversion appeared to be due to two separate loci on the same phage. Some cultures that had the characters β+, K+ consisted of a mixture of lysogenized and unlysogenized cells, and lysogenization with a converting phage sometimes also caused a change in the phage-typing pattern.

Tween reaction and pigmentation We examined four 83A cultures and the untypable organisms made by lysogenizing them with blocking phages originally obtained from 'new untypable' staphylococci. The cultures and phages were described in an earlier publication (Jevons and Parker, 1964). Two of the four 83A cultures (1116 and 1124) were successfully lysogenized with each of the seven phages, but the other two (443 and 36) could be lysogenized only by some of the phages because they were not sensitive to the lytic action of the others.

All four of the 83A organisms were Tween positive, and some of them became Tween negative on lysogenization (Table III). All four of the cultures lysogenized with phage $14368_2'$, and all three lysogenized with phage $1108_1'$ were Tween-negative, but none of the cultures lysogenized with the remaining five phages showed any change of Tween reaction. Both of the phages which changed the Tween reaction from positive to negative on lysogenization ($14368_2'$ and $1108_1'$) were obtained from untypable organisms that were themselves Tween-negative. Two of the five phages that were without effect on the Tween reaction were derived from Tween-positive untypable organisms (1126′ and 13564′). A third ($14368_1'$) came from a Tween-negative donor strain which carried a second phage ($14368_2'$) which converted the Tween reaction. The reason why the remaining two phages from Tween-negative donors had no influence on the Tween reaction is uncertain. Non-lysogenic variants could be obtained from neither of them. Tween-positive variants selected from strain 9514 were still untypable but were sensitive to a phage carried by the original Tween-negative

TABLE III

EFFECT OF LYSOGENIZATION WITH BLOCKING PHAGES ON THE CULTURAL CHARACTERS OF 83A STAPHYLOCOCCI

Strain	*Typing Pattern*	*Tween Reaction*	*Lemon-yellow Pigmentation*	*β-lysin*	*Staphylokinase*	*Neomycin Resistance*
1116	83A	+	−	−	+	−
1116 ($1108_1'$)	Untypable	−	−	−	+	−
1116 (1126′)	Untypable	+	−	−	+	−
1116 (14361′)	Untypable	+	−	−	+	−
1116 ($14368_1'$)	Untypable	+	−	−	+	−
1116 ($14368_2'$)	Untypable	−	−	−	+	−
1116 (9514′)	Untypable	+	−	−	+	−
1116 (13564′)	Untypable	+	−	−	+	−
1124	83A	+	−	−	+	−
1124 ($1108_1'$)	Untypable	−	+	−	+	−
1124 (1126′)	Untypable	+	+	−	+	−
1124 (14361′)	Untypable	+	−	−	+	−
1124 ($14368_1'$)	Untypable	+	−	−	+	−
1124 ($14368_2'$)	Untypable	−	+	−	+	−
1124 (9514′)	Untypable	+	−	−	+	−
1124 (13564′)	Untypable	+	+	−	+	−
443	83A	+	−	−	+	−
443 ($1108_1'$)	Untypable	−	−	−	+	−
443 ($14368_2'$)	Untypable	−	−	−	+	−
36	83A	+	−	+	−	−
36 (1126′)	Untypable	+	−	+	−	−
36 (14361′)	Untypable	+	−	+	−	−
36 ($14368_1'$)	Untypable	+	−	+	−	−
36 ($14368_2'$)	Untypable	−	−	+	−	−

strain. This suggests that 9514 carries two phages, one of which is responsible for the Tween reaction. Whether one or both block the 83A reaction could not be determined by these experiments.

Two of the four 83A cultures (443 and 36) formed a bright orange pigment on glycerol monoacetate agar and one other (1116) was a pale buff colour. None of the lysogenized derivatives of these cultures showed the characteristic lemon-yellow pigmentation. Culture no. 1124, on the other hand, gave white colonies on glycerol monoacetate agar, and four of the lysogenized derivatives were lemon-yellow. These included the two lysogenized with phages $14368_2'$ and $1108_1'$, which also converted the Tween reaction.

Non-lysogenic variants were sought in a number of naturally occurring cultures of the new untypable staphylococcus by methods described previously (Jevons and Parker, 1964) and were found in four of them (Table IV). Three of the four were Tween-negative, and their non-lysogenic variants were Tween-positive. The fourth was Tween-positive, and this reaction was unchanged on delysogenization. Unfortunately, none of the untypable organisms from which we succeeded in obtaining non-lysogenic variants formed lemon-yellow pigment.

The isolation of non-lysogenic variants by orthodox procedures was a laborious task, and it seemed that the Tween reaction might be a useful way of screening large numbers of colonies for non-lysogenic variants. This proved to be so, although mixed clones consisting of lysogenic and non-lysogenic cells sometimes gave a positive reaction. The surface of 10 plates of the Sierra medium were flooded with a log-phase broth culture, the excess fluid was removed and the plates were allowed to dry. They were then exposed to ultra-violet light for sufficient time to leave about 200 surviving colonies after overnight incubation at 37°C. Tween-positive colonies were revealed by the presence of a halo. Subcultures from them were tested for susceptibility to lysis by phage 83A, and supernatants of broth cultures were spotted on to a suitable indicator strain such as no. 1116.

TABLE V

SELECTION OF TWEEN-POSITIVE VARIANTS FROM TWO UNTYPABLE STAPHYLOCOCCI AND RESULTS OF TESTS FOR LYSOGENY AND PIGMENTATION

Strain	*Tween Reaction*	*Typing Pattern*	*Lysis of 1116 by Supernatant*	*Lemon-yellow Pigmentation*
4331	−	Untypable	+	−
5 variants	+	83A	−	−
4 variants	+	Untypable	+	−
14358	−	Untypable	+	+
12 variants	+	Untypable	+	−

The upper half of Table V shows the results of a typical experiment with strain no. 4431. About 10^6 cocci were screened, and nine Tween-positive colonies were obtained. Five of them were shown to consist of non-lysogenic organisms, in that they were susceptible to phage 83A and did not yield a phage which lysed strain no. 1116. The other four, though Tween positive, still carried some phage, and could be shown to be unstable colonies consisting of mixtures of lysogenic and non-lysogenic cocci.

The same method of selection was applied to one of the lemon-yellow Leeds cultures (no. 14358) supplied by Dr. A. T. Willis. Twelve Tween-positive colonies were found. All of them had lost the lemon-yellow colour when tested on glycerol monoacetate agar, but continued insensitivity to phage 83A and the presence of carried phage in the supernatants indicated that the colonies were unstable. The appearance of Tween-negative, lemon-yellow colonies on subculture confirmed that re-lysogenization had occurred.

β-lysin and staphylokinase production Three of the four 83A cultures used in the lysogenization experiments were β−, K+ and one was β+, K−. No change in β-lysin or staphylokinase production resulted from lysogenization with any of the blocking

TABLE IV

EFFECT OF DELYSOGENIZATION ON UNTYPABLE STAPHYLOCOCCI AND THEIR CULTURAL CHARACTERS

Strain	*Typing Pattern*	*Tween Reaction*	*Lemon-yellow Pigmentation*	*β-lysin*	*Staphylokinase*	*Neomycin Resistance*	*Bacitracin Resistance*
1108	Untypable	−	−	+	−	+	+
1108 DL	83A	+	−	+	−	+	+
4331	Untypable	−	−	−	+	+	−
4331 DL	83A	+	−	−	+	+	Not done
9512	Untypable	−	−	−	−	+	−
9512 DL	83A	+	−	−	−	+	Not done
1126	Untypable	+	−	+	−	−	−
1126 DL	83A	+	−	+	−	−	Not done

DL = non-lysogenic variant

phages (Table III). Nor was there any difference in this respect between the four naturally occurring untypable organisms and their non-lysogenic variants (Table IV). The presence or absence of blocking phage appeared, therefore, to have no influence on these two characters.

Neomycin and bacitracin resistance The sensitivity of the four 83A cultures to neomycin and to bacitracin was unchanged by the acquisition of blocking phages (Table III). Three of the four untypable organisms shown in Table IV were resistant to neomycin and one was resistant also to bacitracin. Non-lysogenic variants obtained from them were also resistant.

We compared the rate of mutation to resistance of neomycin-sensitive 83A cultures and of their lysogenized derivatives, and also of naturally occurring neomycin-sensitive 'new untypable' organisms (Table VI). Of a five-hr. broth culture 0·2 ml. was spread on each of three agar plates containing 5 μg. neomycin/ml., and the colonies appearing after 48 hours' incubation at 37°C. were counted. Viable counts were made of the broth cultures on blood agar plates by the method of Miles, Misra, and Irwin (1938). No consistent differences in the rate of mutation were found.

STAPHYLOCOCCI PREVALENT IN LONDON HOSPITALS

These experimental results suggested that at least three separate events must occur to produce a 'new untypable' staphylococcus with the characteristic pattern of cultural reactions from an 83A staphylococcus: first, lysogenization with the blocking phage, which sometimes changes the Tween reaction and, with certain 83A organisms, leads to the formation of lemon-yellow pigment; second, the change from $\beta-$, K+ to $\beta+$, K−; and lastly, the acquisition of resistance to neomycin and bacitracin. Naturally occurring strains might, therefore, be found in which the second and the third events had not taken place.

We therefore looked at the untypable organisms isolated during surveys of staphylococcal infection in a number of London hospitals. Twelve collections of *Staph. aureus*, each consisting of about 100 cultures consecutively isolated from septic lesions in in-patients, from eight hospitals (two in 1963, two in 1964 and four in both years) were phage-typed and examined culturally. Duplicate isolations from the same patient, and cultures from doubtful staphylococcal lesions, were excluded. There were 113 'new untypable' staphylococci among the remaining 1102 cultures, and most of them had the expected characters (Table VII). Indeed, the incidence of these characters (94% Tween negative, 76% lemon-yellow, 84% neomycin resistant, 60% $\beta+$, 74% K−) was somewhat higher than in the earlier series of 'new untypable' cultures (Table I) which had been obtained from a wide geographical area.

Strains of the new untypable staphylococcus were

TABLE VI

RATE OF MUTATION TO NEOMYCIN RESISTANCE

	Strain	*No. of Cocci Sampled*	*No. of Resistant Colonies*	*Mutation Rate*
Naturally occurring 83A organisms	1116	$1{\cdot}32\times10^{8}$	47	$10^{-6.4}$
	1124	$1{\cdot}02\times10^{8}$	37	$10^{-6.4}$
	443	$1{\cdot}26\times10^{8}$	13	$10^{-7.0}$
	36	$9{\cdot}9\times10^{7}$	11	$10^{-7.0}$
Lysogenized 83A organisms	1116 (1108_1)	$1{\cdot}38\times10^{8}$	9	$10^{-7.2}$
	1124 (1108_1)	$1{\cdot}02\times10^{8}$	4	$10^{-7.4}$
	443 (1108_1)	$1{\cdot}11\times10^{8}$	3	$10^{-7.6}$
	36 (1108_1)	$1{\cdot}38\times10^{8}$	5	$10^{-7.5}$
Naturally occurring 'new untypable' staphylococci	1126	$9{\cdot}95\times10^{7}$	75	$10^{-6.1}$
	1994	$1{\cdot}8\times10^{8}$	145	$10^{-6.1}$
	397	$1{\cdot}34\times10^{8}$	44	$10^{-6.5}$

TABLE VII

CULTURAL CHARACTERS OF 1102 STAPHYLOCOCCUS AUREUS CULTURES ISOLATED FROM PATIENTS IN LONDON HOSPITALS

Phage Group	*I*	*II*	*III*			*Miscellaneous*	*Other Untypable*
			83A	*'New Untypable'*	*Other*		
Tween negative	134 (27%)	25 (30%)	2 (4%)	106 (94%)	58 (29%)	18 (24%)	17 (20%)
Lemon-yellow pigmentation	13 (3%)	0	1 (2%)	86 (76%)	10 (5%)	5 (7%)	1 (1%)
Neomycin resistant	1 (0·2%)	0	1 (2%)	95 (84%)	0	0	0
β-lysin positive	19 (4%)	19 (23%)	2 (4%)	68 (60%)	25 (13%)	11 (14%)	11 (13%)
Staphylokinase positive	382 (77%)	54 (65%)	39 (83%)	29 (26%)	130 (66%)	54 (71%)	57 (66%)
Total	499	83	47	113	197	76	87

endemic in five of the hospitals. Table VIII shows the cultural characters of the organisms isolated from hospitals in which an annual collection included more than five such cultures. In three hospitals (nos. 2, 3, and 4) the predominant organism had the typical reactions (Tween negative, lemon-yellow, neomycin-resistant, $\beta+$, K$-$). The organisms from hospital no. 7 were nearly all $\beta-$, K$+$, but otherwise typical. Those from hospital 5, however, were not lemon-yellow or neomycin-resistant, and most of them were $\beta-$; they also differed from the strains prevalent in other hospitals in that they were sensitive to erythromycin.

None of the cultural characters associated with the new untypable staphylococci was unique. Lemon-yellow pigmentation was occasionally seen in other phage groups (Table VII), and sometimes in organisms with no other cultural resemblances to the new untypable staphylococci. In all there were 29 lemon-yellow organisms which did not belong to the 'new untypable' or 83A groups, and 18 of them were unrelated organisms which occurred sporadically. Two other lemon-yellow strains gave rise to small groups of associated infections: one, with the phage-typing pattern 75/77 was resistant to several antibiotics and was Tween-negative; the other was a multiple resistant, Tween-negative member of phage group I. Neomycin resistance was seen in only two other organisms, one an 83A staphylococcus and the other a member of the 52, 52A, 80, 81 complex. Producers of β-lysin, which were usually K$-$, formed a considerable minority in most phage groups. Tween-negative organisms with many different phage-typing patterns were found.

RESISTANCE TO NEOMYCIN AND BACITRACIN Resistance of many of the new untypable staphylococci to neomycin and bacitracin may have conferred upon them a selective advantage over other organisms in the hospital environment. We therefore attempted by retrospective investigation to find out when these resistances first made their appearance. In 1964 we tested all the available cultures isolated in earlier years for resistance to the two antibiotics (Table IX). Few cultures isolated before 1962 were available. Already in that year nearly half of the new untypable staphylococci were resistant both to neomycin and to bacitracin, and the proportion has not changed greatly since. Although the representative collection of 83A staphylococci examined in 1963 (Table I) did not contain any that were resistant, it is clear from Table IX that a small proportion resistant to both antibiotics has existed since at least 1962. A substantial minority of the untypable organisms were resistant to neomycin but sensitive to bacitracin, but bacitracin resistance without neomycin resistance was not seen in this or indeed in any other series of cultures. This suggests that bacitracin resistance confers a significant advantage on an organism only when it accompanies resistance to neomycin.

TABLE VIII

CULTURAL CHARACTERS OF 'NEW UNTYPABLE' STAPHYLOCOCCI ENDEMIC IN CERTAIN LONDON HOSPITALS[1]

Hospital No.	*Year*	*Number*					
		Cultures Examined	*Tween Positive*	*Lemon-yellow*	*Neomycin Resistant*	*β-Lysin Positive*	*Staphylokinase Positive*
2	1964	8	0	8	8	8	0
3	1963	11	0	11	11	8	0
3	1964	14	1	13	13	9	1
4	1963	30	0	27	26	27	3
4	1964	15	2	13	12	9	5
5	1963	8	1	0	0[2]	3	2
7	1964	21	0	21	12	2	16

[1]The hospitals included are those in which more than five of 100 consecutive cultures of *Staphylococcus aureus* from patients' lesions were 'new untypable' organisms.
[2]All cultures sensitive also to erythromycin

TABLE IX

RESISTANCE TO NEOMYCIN AND BACITRACIN

Year of Isolation	*83A Staphylococci*					*'New Untypable' Staphylococci*				
	No. Tested	*Sensitive*	*N*[1]	*B*[2]	*N+B*[3]	*No. Tested*	*Sensitive*	*N*	*B*	*N+B*
1961	2	2	0	0	0	1	1	0	0	0
1962	26	24	0	0	2	22	11	1	0	10
1963	46	44	0	0	2	92	33	14	0	45
1964	30	26	1	0	3	113	40	0	0	73
Total	104	96	1	0	7	228	85	15	0	128

[1]*N* = neomycin resistant.
[2]*B* = bacitracin resistant.
[3]*N+B* = resistant to neomycin and bacitracin.

DISCUSSION

Interest has been aroused by the recent appearance, in hospitals in many different countries, of somewhat similar untypable staphylococci. There is little doubt that these organisms have arisen independently on a number of occasions. Those prevalent in Britain and Western Europe appear to have been derived from the 83A organism that was previously widespread in the same areas.

Most of these untypable organisms have cultural characters not possessed by the 83A organism. These fall into three groups.

1 The negative Tween reaction and the production of a lemon-yellow pigment on glycerol monoacetate agar appear to be consequences of the acquisition of the phage which blocks the 83A reaction and so makes the organism untypable, but they are not invariable consequences. Two out of seven blocking phages were responsible for changing the Tween reaction whenever they were acquired; four of the seven caused the appearance of lemon-yellow pigmentation, but only in an 83A culture which did not form orange pigment. The fact that most of the untypable organisms were both Tween-negative and lemon-yellow suggests that strains with these characters survived better, or were disseminated more widely than the others.

2 Many more of the new untypable strains than of other staphylococci from human sources produce β-lysin, and those which do are usually staphylokinase negative. In these respects they resemble bovine staphylococci, a fact which led Willis and his colleagues (1964) to suggest that they are of animal origin. If, indeed, they arose from 83A staphylococci, which seldom form β-lysin, this is unlikely. The work of Winkler and his colleagues (de Waart *et al.*, 1962; Winkler *et al.*, 1965) suggests that the characters $\beta-$, K+ are determined in some strains of *Staph. aureus* by the carriage of a phage of serological group F. The loss of such a phage might explain the occurrence of the characters $\beta+$, K− in an untypable organism derived from an 83A staphylococcus which was $\beta-$, K+, but we have been unable to demonstrate this. The acquisition of the blocking phage which makes the organism untypable is without influence on the production of β-lysin or staphylokinase. Though the appearance of the characters $\beta+$, K− in some strains of the new untypable staphylococcus may be due to the loss of a phage, this does not appear to be caused by prophage substitution when the blocking phage is acquired.

3 Most of the new untypable organisms are resistant to neomycin, and many are also resistant to bacitracin. Acquisition or loss of the blocking phage was without effect on the sensitivity of the organism to either antibiotic. Nor did the lysogenized organisms differ significantly from their parents in the rate of mutation to resistance.

Resistance to neomycin and bacitracin arose early in the history of the new untypable staphylococci and probably conferred on them a considerable advantage over other staphylococcal strains in hospitals in which these antibiotics were used widely and often in combination. The fact that 83A organisms resistant to these antibiotics were isolated as early as 1962 but did not subsequently become prevalent may reflect the ease with which in the hospital environment they acquire phages which block the 83A reaction. Thus untypability may in this case be a 'symptom' of long residence in the hospital, and a consequence of the advantages associated with resistance to many antibiotics.

The neomycin-resistant staphylococci prevalent in America and Australia are also frequently untypable, Tween-negative and lemon-yellow, although they do not appear to have arisen from 83A staphylococci. This suggests some underlying similarity between staphylococci which are able to acquire resistance to neomycin.

REFERENCES

Blair, J. E., and Williams, R. E. O. (1961). *Bull. Wld. Hlth Org.*, **24**, 771.
Christie, R., and Wilson, H. (1941). *Aust. J. exp. Biol. med. Sci.*, **19**, 329.
Cohen, L. S., Fekety, F. R., and Cluff, L. E. (1962). *New Engl. J. Med.* **266**, 367.
Comtois, R. D. (1965). *Canad. J. publ. Hlth*, **56**, 199.
——, and Bynoe, E. T. (1963). *Ibid.*, **54**, 357.
Finegold, S. M., and Gaylor, D. W. (1960). *New Engl. J. Med.*, **263**, 1110.
Green, S. M. (1962). *J. clin. Path.*, **15**, 249.
Griffith, L. J., Ostrander, W. E., Smith, Z. F., and Beswick, D. E. (1961). *J. Bact.*, **81**, 157.
Jacobs, S. I., and Willis, A. T. (1963). *Lancet*, **2**, 459.
——, —— (1964). *J. clin. Path.*, **17**, 612.
——, ——, and Goodburn, G. M. (1964). *J. Path. Bact.*, **87**, 151.
——, ——, Ludlam, G. B., and Goodburn, G. M. (1963). *Lancet*, **1**, 972.
Jevons, M. P., and Parker, M. T. (1964). *J. clin. Path.*, **17**, 243.
Lowbury, E. J. L., Babb, J. R., Brown, V. I., and Collins, B. J. (1964). *J. Hyg. (Lond.)*, **62**, 221.
Miles, A. A., Misra, S. S., and Irwin, J. O. (1938). *Ibid.*, **38**, 732.
Mitchell, A. A. B. (1964). *Lancet*, **1**, 859.
Quie, P. G., Collin, M., and Cardle, J. B. (1960). *Ibid.*, **2**, 124.
Report (1963). *Int. Bull. bact. Nomencl.*, **13**, 119.
Robertson, J. J. (1963). *Lancet*, **2**, 333.
Rosendal, K., Bülow, P., and Jessen, O. (1964). *Nature (Lond.)*, **204**, 1222.
Rountree, P. M., and Beard, M. A. (1965). *Med. J. Aust.*, **1**, 498.
Sierra, G. (1957). *Antonie v. Leeuwenhoek*, **23**, 15.
Smith, J. A., Willis, A. T., and O'Connor, J. J. (1965). *Lancet*, **2**, 772.
Temple, N. E. I., and Blackburn, E. A. (1963). *Ibid.*, **1**, 581.
Thomas, D. C., Hill, E. O., Culbertson, W. R., and Altemeier, W. A. (1960). *Surg. Forum*, **10**, 334.
Tisdale, W. A., Fenster, L. F., and Klatskin, G. (1960). *New Engl. J. Med.*, **263**, 1014.
Turner, G. C. (1963). *Lancet*, **1**, 1156.
Waart, J. de, Winkler, K. C., and Grootsen, C. (1962). *Nature (Lond.)*, **195**, 407.
Willis, A. T., Jacobs, S. I., and Goodburn, G. M. (1963). *Lancet*, **2**, 67.
——, ——, —— (1964). *J. Path. Bact.*, **87**, 157.
——, and Turner, G. C. (1962). *Ibid.*, **84**, 337.
——, —— (1963). *Ibid.*, **85**, 395.
Winkler, K. C., de Waart, J. ,and Grootsen, C. (1965). *J. gen. Microbiol.*, **39** 321.