PHAGE-TYPING OF COAGULASE-NEGATIVE STAPHYLOCOCCI

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THE clinical importance of infection with coagulase-negative staphylococci (*Staphylococcus epidermidis*) is increasing (Quinn, Cox and Drake, 1966; Verhoef, 1970). Strains isolated from patients with endocarditis or colonisation of Spitz-Holter valves often belong to biotype I in Baird-Parker's scheme (Holt, 1969), and seem to be different in their metabolic activities from most of the strains at carrier sites (Ivler, 1965). Little is known about the source of the strains that cause disease or of their route of spread.

The isolation of a number of phages from coagulase-negative staphylococci (Verhoef, van Boven and Winkler, 1971*b*) made it possible to develop a phage-typing system for *Staph. epidermidis*. We typed a large number of strains from a variety of sources with the available phages, using techniques standardised in the same way as for the phage typing of *Staph. aureus* (Blair and Williams, 1961). Experience has shown that even with such standardised techniques there is a certain amount of variation in the phage-typing pattern of *Staph. aureus*. It was therefore necessary to study the variation of the typing patterns of *Staph. epidermidis* and to find out whether the rules developed for the interpretation of the results of phage typing of *Staph. aureus* (Williams and Rippon, 1952) could be applied to *Staph. epidermidis*.

MATERIALS AND METHODS

Bacterial strains. The origin of many of the strains has already been reported (Verhoef *et al.*, 1971*a*). In addition, a collection of strains from clinical material that had been isolated by Dr R. J. Holt (Queen Mary's Hospital for Children, Carshalton, Surrey) were received from Professor R. E. O. Williams (St Mary's Hospital Medical School, London).

Biochemical classification of strains. This was performed as previously described (Verhoef *et al.*, 1971*a*).

Phages. Table I shows the numbers of the phages used and of their propagating strains (see also Verhoef *et al.*, 1971*b*).

Phage-typing. This was carried out by the standard methods of Blair and Williams.

RESULTS

Typability and phage patterns

The 18 phages were tested against 240 strains from various sources. Most of the strains belonged to biotype I, but four strains of biotype 3 and five of biotype 4 were included. Table II shows that 181 strains (75.5 per cent.) were

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susceptible to one or more of the phages, 127 (53 per cent.) at routine test dilution ($\text{RTD} \times 1$) and 54 (22.5 per cent.) at $\text{RTD} \times 1000$.

The typing patterns of the strains that were typable at $1 \times \text{RTD}$ are given in table III. Though numerous patterns were observed, 100 of the 127 strains fell

TABLE I
The typing phages and their propagating strains

Serial numbers of phages and, below them, their propagating strains																	
71	275	48	51	82	130	456	130A	82A	157	71A	471	275A	275B	448	459	108	489
240	98	81	41	87	87	76	141	368	382	382	330	380	48	407	407	407	407

clearly into a relatively small number of "main patterns", which are numbered 1-7. The remaining 27 strains showed 17 different patterns, none of which was seen in more than two strains.

A strain was considered to belong to one of the seven main patterns when its spectrum of lysis did not differ in more than one strong reaction from that

TABLE II
The typability of strains of Staphylococcus epidermidis

Source of strains	Number of strains tested	Number (and percentage) of strains susceptible to the phages		
		at $\text{RTD} \times 1$	at $\text{RTD} \times 1000$	Total
Hospital staff (the Netherlands)	98	56	20	76 (78)
Normal population (the Netherlands)	113	56	30	86 (76)
Patients (14 from Britain, 15 from the Netherlands)	29	15	4	19 (66)
All sources	240	127 (53)	54 (22.5)	181 (75.5)

pattern given in table III. The strains within main patterns that involved few reactions were fairly homogeneous, but strains in the broader main patterns could be further subdivided into individual patterns. For example (table IV), pattern 5 included four recognisable individual patterns.

Table V shows the frequency of main patterns among strains from different sources. About 60 per cent. of all typable strains belonged to patterns 1, 5 and 6. Of 56 strains from hospital personnel in the Netherlands, 24 (43 per cent.) fell into patterns 1 and 6. These patterns occurred equally frequently in strains from the normal population (41 per cent.). However, these patterns were not observed in strains from patients (partly from Britain), which often belonged to pattern 5. This limited experience suggests that strains from patients might belong to different "types".

TABLE III
Main typing patterns at routine test dilution of 127 typable cultures of *Staphylococcus epidermidis*

Pattern no.*	Number (and percentage of cultures)	Degree of lysis of these cultures by phage no.																	
		157A	71A	82	130	275A	275B	456A	130A	82A	471A	448	459	108	489	275	71	48	51
1	26 (21)	+†	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—	—
2	7 (6)	—	—	+	+	—	—	—	—	—	—	—	—	—	—	—	—	—	—
3	10 (8)	—	—	+	+	+	+	—	—	—	—	—	—	—	—	—	—	—	—
4	8 (6)	—	—	—	—	—	—	+	+	+	—	—	—	—	—	—	—	—	—
5	25 (20)	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	—
6	21 (17)	—	—	—	—	—	—	+	+	+	+	+	+	+	+	+	+	+	+
7	3 (2)	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+	+

* In addition, 27 strains (21 per cent.) showed 17 different patterns, none of which occurred in more than two strains.
† — = No phage reaction; + = >20 plaques. {In most cases strong reactions (++ = >50 plaques) were observed.}

Reproducibility of phage-typing patterns

To gain some experience about reproducibility, ten strains of *Staph. epidermidis* with various phage patterns were serially subcultured ten times over a period of 14 days. Subcultures were made with a loop in a sterile cabinet.

TABLE IV
Individual typing patterns within main pattern 5

Strain no.	Main pattern no.	Related to strain no.	Degree of lysis of strain by phage no.									
			456A	130A	82A	471A	448	459	108	489	275	51
637	5	532, 535, 933	++*	++	++	++	++	++	++	++	++	—
532	5	535, 637, 933	++	++	±	++	+	+	+	+	++	—
535	5	532, 637	++	++	++	++	++	++	++	++	++	++
933	5	532, 637	++	++	++	+	++	±	++	++	—	—
456	4	933	++	++	++	—	—	—	±	+	—	—

* ++ = >50 plaques; + = 20–50 plaques; ± = <20 plaques; — = no phage reaction.

TABLE V
Phage-typing patterns of Staphylococcus epidermidis strains from different sources

Origin	Total number of strains tested	Number (and percentage) of strains in main type							
		1	2	3	4	5	6	7	8–24
Nose (hospital staff)	56	13 (23)	3 (5)	1 (2)	4 (7)	11 (20)	11 (20)	1 (2)	12 (21)
Patients	15	0	1 (7)	1 (7)	1 (7)	8 (53)	0	0	4 (27)
Nose (normal population)	56	13 (23)	3 (5)	8 (14)	3 (5)	6 (11)	10 (18)	2 (4)	11 (20)
All	127	26 (21)	7 (6)	10 (8)	8 (6)	25 (20)	21 (17)	3 (2)	27 (21)

Subcultures 1, 4, 7 and 10 were used for phage typing: the first subculture was phage-typed directly; subcultures 4 and 7 were streaked on a blood agar plate, and three colonies were picked for phage-typing; and from the tenth subculture five colonies were typed. In the event, the complete set of comparisons was made on only eight of the ten strains.

The results permitted an analysis of the variation between a number of pairs of phage-typing patterns (table VI).

TABLE VI
Reproducibility of phage-typing patterns

Relationship of staphylococci compared	Number of			Number (and percentage) of pairs showing				
	strains	typings	pairs	no variation	variation in strength of reaction only	loss or gain or both of $\pm$ or + reactions only	loss or gain of one + + reaction	loss or gain of more than one + + reaction
1. Duplicate tests on same culture	10	60	30	22 (73)	5 (17)	3 (10)	0	0
2. Different colonies from same culture	8	87*	124	74 (60)	33 (27)	16 (13)	1 (1)	0
3. Before and after three subcultures	8	95*	213	82 (39)	67 (32)	38 (18)	25 (12)	1 (<1)
4. Before and after six subcultures	8	95*	141	68 (48)	18 (13)	30 (21)	20 (14)	5 (4)
5. Before and after nine subcultures	8	47*	39	15 (39)	11 (28)	8 (21)	5 (13)	0

* From the 10th subculture of one strain, four colonies instead of five were phage typed.

(1) *Variations in duplicate tests from one culture.* From every strain three single-colony isolates were typed twice, giving 30 sets of pairs (line 1).

(2) *Variations among single-colony isolates from the same culture.* The three colonies a, b and c each yielded three pairs ab, ac and bc. The five colonies of the tenth subculture yielded ten sets (in one case only six). For the eight strains examined $(7 \times 16) + 12 = 124$ sets of pairs could be compared (line 2).

(3) *Variations in pattern after three subcultures.* The five isolates from the tenth subculture could be compared with those of the seventh subculture, and these with the isolates of the fourth subculture, yielding 213 pairs in all (line 3).

(4) *Variations in pattern after six subcultures* (line 4).

(5) *Variations in pattern after nine subcultures* (line 5).

Variations in pattern between duplicates were relatively small, and identical results were obtained in 73 per cent. of pairs. Minor variations between single-colony isolates were somewhat more frequent, but differences in strong reactions were observed only once. After three, six and nine subcultures, variations of one strong reaction were relatively frequent (12–14 per cent.), and an occasional variation exceeding one strong reaction was seen. This kind of variation after repeated subculture is more frequent in broad patterns like main patterns 5 and 6 than in patterns with few reactions. In main pattern 5, for example, 5 of 36 pairs (14 per cent.) showed variation exceeding 1 ++ reaction after six subcultures, but four of the five variations were observed in one strain.

*Phage-typing pattern of strains isolated from patients at
different times and from different sites*

Dr Holt's selection of strains came from six patients that had colonisation of Spitz-Holter valves (patients A–F); the seventh (patient G), from the Netherlands, had a wound infection after implantation of a pace-maker (see table VII). The strains in this table are not included in table II.

From patients A, B, D and F, strains with identical phage-typing patterns were isolated from the Spitz-Holter valve and from blood cultures. Two isolates from the skin of patient B had the same pattern as the strains isolated from the lesions. In the case of patient A, strains with identical patterns were isolated from the valve, from cerebrospinal fluid (CSF) and from blood cultures.

The three isolates from the wound of patient G with an implanted pace-maker had rather similar patterns, which were different from those of his skin strains. The strains from patients C and E are more difficult to evaluate because many of them were untypable. The strains from patient B showed only a weak reaction with phage 82. The phage could, however, be adapted to this strain (phage 82C). The consistency of these results seem to show that phage typing can be of value.

Phage-typing patterns of strains isolated from the nose

From nose samples from seven persons, giving n colonies on the plate, $\sqrt{n}$ colonies were phage-typed. The main patterns are indicated in table VIII in the same manner as in table III. Some people carried only one coagulase-negative

TABLE VII

Phage-typing patterns of sets of Staphylococcus epidermidis cultures isolated from patients

Patient	Source of strain	Phage-typing pattern*
A	CSF Blood: strain 1 Blood: strain 2 Valve†	N. 275B//275A, 275 NT‡ N. 275B//275A, 275 N. 275B//275A, 275
B	Blood culture no. 1 Blood culture no. 2 Blood culture no. 3 Valve† Axilla Nose	82C/ 82C/ 82C/ 82C/ 82C/ 82C/
C	Blood: strain 1 Blood: strain 2 Valve†: strain 1 Valve†: strain 2 CSF Axilla Nose	456A, 130A, 82A, 448/471A/459, 108, 489 NT NT NT NT NT NT
D	Blood (5 Apr. 1968) Valve† (11 Apr. 1968)	48// 48//
E	Blood: strain 1 Blood: strain 2 Blood: strain 3 Valve† Nose	NT N. 48// NT NT NT
F	Blood (26 Nov.) Valve† (30 Nov.) Blood (22 Dec.) Valve† (4 Jan.) Blood (12 Jan.) Valve† (18 Jan.)	N. 456A, 130A, 82A, 448/471A, 459, 108, 489 N. 456A, 130A, 82A, 448/471A, 459, 108, 489 N. 456A, 130A, 82A, 448/471A, 459, 108, 489 NT NT NT
G	Skin: strain 1 Skin: strain 2 Nose Wound swab 1 Wound swab 2 Wound swab 3	NT N. 48/130A/456A NT 275/130A, 448, 459, 108, 489/82A, 471A /130A/448, 459, 108, 489 275, 130A/471A/456A, 448, 459, 108, 489

* N = RTD × 1000; before first (/) stroke, ++ and +++ reactions; between first and second stroke, + reactions; after the second stroke, ± reactions.

† Spitz-Holter valve.

‡ NT = Not typable.

strain in the nose, but others had two or more strains with quite distinct phage patterns.

From each of a series of weekly nose swabs two colonies of coagulase-negative staphylococci were selected for phage typing. It is clear (table IX) that some persons carried the same strain for a long time (e.g., no. 1, 3, 5, 7, 18, 20 and 23); in others, however, certain strains appeared to populate the nasal area only temporarily, for coagulase-negative staphylococci from successive isolates belonged to different phage patterns.

TABLE VIII
Phage-typing patterns of multiple isolates from the same nose swab

Person no.	Number of colonies									not typable
	tested	typing in pattern no.								
		1	2	3	4	5	6	7	8-24	
1	3	—	—	—	—	—	—	—	3	—
2	8	6	—	—	2	—	—	—	—	—
3	8	5	—	—	—	—	—	—	3	—
4	6	—	—	—	—	—	2	—	—	4
5	10	—	—	—	—	10	—	—	—	—
6	6	—	—	—	—	6	—	—	—	—
7	8	6	—	—	—	1	—	—	—	1

The main pattern numbers are as in table III.

DISCUSSION

Three-quarters of the coagulase-negative staphylococci examined were lysable by means of our phages. The strains were characterised by the pattern of their lysis in the same way as are *Staph. aureus* strains (Blair and Williams, 1961). Certain combinations occur more frequently than others, and we were able to distinguish seven main patterns. It is too early yet to decide whether these main patterns can be ordered into non-overlapping lytic groups, as is possible for *Staph. aureus* (Parker, 1962). In our case the phages isolated from biotype I have many characters in common (Verhoef *et al.*, 1971*b*). This suggests that the differences between phages may be phenotypic. Indeed, some data have been obtained indicating that the phage patterns are mainly due to restriction and that the lytic pattern of the phages is due to modification induced by the propagating strains.

Among the 15 typable strains from clinical material, eight belong to main pattern 5, whilst the patterns 1 and 6, though common in the nose, did not occur among these strains. If the different patterns indeed reflect more fundamental differences, the strains from clinical material might belong to a different type (see also Ivler, 1965). To establish this for certain it would be necessary to examine more carrier and lesion strains isolated in the same geographical area.

TABLE IX

Phage-typing patterns of Staphylococcus epidermidis from weekly nasal swabs

Person no.	Swab no.	Main typing pattern of strains isolated in week											
		1	2	3	4	5	6	7	8	9	10	11	12
1	1	6	6	3	6	6	—	—	—	—	—	—	—
	2	NT*	NT	NT	6	6	—	—	—	—	—	—	—
2	1	1	1	1	—	1	—	—	—	—	—	—	—
	2	6	1	NT	—	1	—	—	—	—	—	—	—
3	1	5	5	—	—	—	—	NT	8a	1	6	5	—
	2	6	6	—	—	—	—	8a	8a	NT	NT	6	—
4	1	6	5	NT	5	1	—	—	—	NT	NT	—	—
	2	NT	5	NT	1	1	—	—	—	NT	NT	—	—
5	1	2	5	—	—	—	—	5	—	5	5	—	—
	2	2	5	—	—	—	—	5	—	5	5	—	—
6	1	5	—†	—	—	5	5	—	—	—	—	—	—
	2	5	—	—	—	5	5	—	—	—	—	—	—
9	1	5	—	8a	3	—	8a	—	—	—	—	—	—
	2	NT	—	8a	3	—	8a	—	—	—	—	—	—
10	1	NT	NT	8a	—	—	—	1	8a	8a	8a	—	—
	2	1	1	NT	—	—	—	NT	—	NT	1	—	—
11	1	NT	2	NT	2	NT	—	—	—	—	—	—	—
	2	6	2	NT	NT	NT	—	—	—	—	—	—	—
12	1	2	2	—	—	—	—	—	—	2	2	—	—
	2	5	2	—	—	—	—	—	—	5	2	—	—
13	1	5	—	8a	—	—	—	—	NT	NT	—	—	—
	2	8a†	—	8a	—	—	—	—	7	8a	—	—	—
14	1	5	8a	5	8a	—	—	—	—	—	—	5	5
	2	5	NT	8a	6	—	—	—	—	—	—	5	5
15	1	2	5	NT	2	—	—	—	NT	NT	NT	8	NT
	2	5	NT	2	2	—	—	—	NT	NT	NT	NT	NT
16	1	8†	2	8	—	NT	—	—	—	NT	2	NT	NT
	2	8	8	2	—	NT	—	—	—	NT	6	NT	NT
17	1	1	1	—	NT	1	—	—	—	1	—	6	8a
	2	1	1	—	NT	1	—	—	—	1	—	8a	8a
18	1	1	1	—	—	—	—	1	1	1	1	1	—
	2	6	6	—	—	—	—	6	6	7	6	6	—
19	1	1	—	1	1	—	—	—	—	—	—	2	5
	2	1	—	6	6	—	—	—	—	—	—	2	NT
20	1	1	—	1	6	6	—	—	—	—	—	7	7
	2	7	—	NT	7	7	—	—	—	—	—	7	7
22	1	5	5	—	NT	—	—	—	—	—	—	—	—
	2	5	5	—	5	—	—	—	—	—	—	—	—
23	1	1	1	1	1	1	—	—	—	—	—	—	—
	2	NT	1	1	1	1	—	—	—	—	—	—	—

The main patterns are as in table III.

* NT = not typable. † — = *Staph. epidermidis* not isolated.

‡ 8 = Typing patterns 8–24; 8a is included in typing patterns 8–24, and is identical with those of other 8a strains from the same patient.

Duplicate tests of one culture showed less variation in pattern than successive cultures from one strain. Patterns with many reactions like main patterns 5 and 6 showed more differences than patterns with few reactions.

The variation in phage patterns of related coagulase-negative staphylococci, however, seemed to be of the same order of magnitude as that for related coagulase-positive staphylococci.

The *Staph. epidermidis* flora in the nose can be very variable. Some people carry the same strain for months, others harbour one or more strains only temporarily. This will make epidemiological studies fairly difficult. The value of the phage-typing system in epidemiology will have to be studied extensively. Should strains from lesions indeed prove to be restricted to a small number of patterns the value of phage-typing is obvious. That similar patterns were found for strains from different sites in five out of seven patients with colonised Spitz-Holter valves is encouraging.

SUMMARY

A set of 18 bacteriophages was used to type 240 coagulase-negative staphylococci; 75.5 per cent. of the strains were lysed by one or more of the phages, 53 per cent. at RTD and a further 22.5 per cent. only at RTD $\times$ 1000. Most of the reactions at RTD could be grouped into seven main patterns. The relative frequency of occurrence in cultures from patients with lesions was somewhat different from that in cultures from nose swabs from healthy persons.

In spite of careful control of technique, variation in phage-typing patterns was observed. The amount of variation appeared to be rather similar to that encountered in phage typing of *Staph. aureus*.

Serial studies of the nasal flora indicate that some persons carry the same strain for a long time, but that others carry a strain only transitorily. Simultaneous carriage of two distinct strains occurs frequently.

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REFERENCES

- BLAIR, J. E., AND WILLIAMS, R. E. O. 1961. Phage typing of staphylococci. *Bull. Wld Hlth Org.*, **24**, 771.
- HOLT, R. J. 1969. The classification of staphylococci from colonized ventriculo-atrial shunts. *J. Clin. Path.*, **22**, 475.
- IVLER, D. 1965. Comparative metabolism of virulent and avirulent staphylococci. *Ann. N. Y. Acad. Sci.*, **128**, 62.
- PARKER, M. T. 1962. Phage-typing and the epidemiology of *Staphylococcus aureus* infection. *J. Appl. Bact.*, **25**, 389.
- QUINN, E. L., COX, F., AND DRAKE, E. H. 1966. Staphylococcal endocarditis. A disease of increasing importance. *J. Amer. Med. Assoc.*, **196**, 815.
- VERHOEF, J. 1970. Fagotypering van coagulase-negatieve stafylococci. M.D. Thesis, Univ. Utrecht.

- VERHOEF, J., VAN BOVEN, C. P. A., AND WINKLER, K. C. 1971a. Lysogeny in coagulase-negative staphylococci. *J. Med. Microbiol.*, **4**, 405.
- VERHOEF, J., VAN BOVEN, C. P. A., AND WINKLER, K. C. 1971b. Characters of phages from coagulase-negative staphylococci. *J. Med. Microbiol.*, **4**, 413.
- WILLIAMS, R. E. O., AND RIPPON, JOAN E. 1952. Bacteriophage typing of *Staphylococcus aureus*. *J. Hyg., Camb.*, **50**, 320.