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Phage typing of *Staphylococcus aureus* from dairy cattle in Australia

By A. J. FROST
Department of Veterinary Preventive Medicine, University of Queensland, Brisbane, Australia

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INTRODUCTION

Phage typing is an established technique in epidemiological studies of *Staphylococcus aureus* infection in man. The technique has also been used on animal strains, particularly those from dairy cattle, in which mastitis due to this organism is a serious problem in all the major dairying countries.

Some workers have used the 'human' phages to type bovine strains (Macdonald, 1946; Smith 1948*a*; Price, Neave, Rippon & Williams, 1954); others have isolated new phages where the human phages were unsatisfactory (Smith, 1948*b*; Seto & Wilson, 1958; Coles & Eisenstark, 1959*a*, *b*; Nakagawa, 1960). Davidson (1961) carried out an intensive study, and a set of phages suitable for typing bovine strains was suggested.

This paper records observations of phage typing of bovine strains in Australia, with the subsequent selection of a series of phages for typing such strains.

MATERIALS AND METHODS

Staph. aureus was isolated from milk samples collected mainly from herds in the Brisbane milk supply area. All strains were coagulase positive when tested by the tube method with rabbit plasma diluted 1/10.

The basic technique used in phage typing was as described by Blair & Williams (1961). The human set of phages (termed 'human phages') was obtained from Dr P. Rountree, Royal Prince Alfred Hospital, Sydney. New phages were isolated by the cross-culture method of Fisk (1942). Nine additional phages were obtained from the Central Veterinary Laboratory, Weybridge, U.K., propagated and tested (Davidson, 1961). Phages were used at routine test dilution (RTD) for typing; a machine similar to that described by Tarr (1958) was used.

Preliminary typing using the human phages (29, 52, 52A, 79, 80, 3A, 3B, 3C, 55, 71, 6, 7, 42E, 47, 53, 54, 73, 75, 77, 31B, 47D, 42D, 81, 187) was carried out by Miss Y. Battey of the Department of Health, Brisbane. This was the recommended human set (Blair & Williams, 1961) with the addition of 73, 31B, and 47D, three group III phages. These phages were all used at RTD.

RESULTS

Results of typing with human phages

The effect of the human phages was assessed from the typing of 1820 strains of *Staph. aureus*, isolated from 51 herds. Forty-four of these herds were sampled once for a survey (Frost, 1962). The remaining strains came from seven herds in which control of mastitis due to *Streptococcus agalactiae* was studied; these herds were sampled frequently and many strains of *Staph. aureus* were isolated and

Table 1. *Phage typing of bovine strains of* Staph. aureus *with the human phage set*

Origin	Phage group I	II	III	IV	Misc.	Non-typable	Total
44 survey herds*	144	8	338	9	21	329	849
7 control herds*	9	—	94	57	18	102	280
Total	153	8	432	66	39	431	1129
Percentage	13·6	0·7	38·3	5·8	3·5	38·2	—
All strains tested							
Total	172	10	603	308	155	572	1820
Percentage	9·5	0·5	33·1	16·9	8·6	31·4	—

* The typing of strains from a single herd sampling only are considered. Phages used at RTD.

Table 2. *The classification of phage patterns from* 19 *survey herds in which* 20 *or more strains were examined*

Herd no.	Phage group I	II	III	IV	Misc.	Non-typable	Total
1	3	—	18	1	—	8	30
5	4	—	19	—	—	11	34
6	6	—	1	4	—	9	20
8	7	—	3	—	—	22	32
10	24	—	37	—	—	2	63
11	2	1	17	—	—	11	31
12	—	—	6	—	—	23	29
13	8	—	—	—	—	29	37
17	1	2	7	—	2	9	21
18	18	—	2	—	—	8	28
21	6	—	18	—	—	4	28
23	—	—	3	—	—	26	29
25	4	—	17	—	1	24	46
28	2	—	12	—	1	5	20
34	9	—	15	—	—	21	45
35	—	—	9	2	1	18	30
36	—	—	17	—	2	7	26
38	—	—	31	—	—	4	35
42	1	—	8	—	—	18	27
Total	95	3	240	7	7	259	611
Percentage	15·5	0·5	39·3	1·1	1·1	42·4	—

Phages used at RTD.

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typed. Results are summarized in Table 1. For this classification, staphylococci were allotted to phage groups as defined by Parker (1962).

However, unless the proportion of strains typed is assessed in relation to the herd, these results can be misleading. Table 2 shows the classification of strains typed on a herd basis, where 42·4% of staphylococci were non-typable. The proportion of non-typable strains was too high in some herds to differentiate the strains present.

Table 3. *The lysis of* 336 *staphylococci, non-typable with the human phage set at RTD, with* 19 *additional phages*

Phage and origin		No. of strains lysed	Percentage
78		1	0·3
31B		1	0·3
101	Davidson (1961)	5	1·4
102		227	82·4
107		57	17·0
108		311	92·6
110		5	1·4
111		317	94·3
115		0	0·0
10	Brisbane	299	68·2
11		3	0·9
12		2	0·6
13		3	0·9
186		268	80·0
221		241	71·7
367		254	75·6
373		234	69·6
425		291	86·6
600		239	71·1

Phages used at RTD.

Table 4. *The number of phages by which each strain was lysed*

(From the typing of 336 strains shown in Table 3.)

No. of phages causing lysis...	11	10	9	8	7	6	5	4	3	2	1	Total
No. of strains	29	97	68	28	15	31	33	13	3	10	9	336
Percentage	8·6	28·9	20·2	8·3	4·5	9·2	9·8	3·9	0·9	3·0	2·7	100

The use of other phages

A number of new phages were isolated, propagated, and used at RTD to type 'non-typable' strains. Preliminary results with eight new phages showed that only 13 of 100 selected strains, non-typable with the human phages above, were not lysed. Then 376 such non-typable strains were typed at RTD with 19 phages; these consisted of ten new phages and nine from the bovine set of Davidson (1961). In general, these phages were either very active, lysing many of these staphylococci, or else lysed only a small number. Forty staphylococci were still not lysed by any phage, but since these were derived from a group of 1820 strains originally

examined, it was clear that a combination of the human phages and selected bovine phages would lyse a high proportion of bovine staphylococci. The results on the 336 strains which were lysed are shown in Tables 3 and 4.

In order to compare the phages under similar conditions, a series of 1404 strains of staphylococci was then typed with 42 phages, using two lawns of each strain and two sets of phages, as follows:

Human phages	Bovine phages
29, 52, 52A, 79, 80	78, 31B, 101, 102, 107
3A, 3B, 3C, 55, 71	108, 110, 111, 115, 221
6, 7, 42E, 47, 53,	373, 10, 11, 12, 13,
54, 75, 77, 42D, 81	186, 1050, 1054, 367, 425
187	600

The first nine of the bovine set of phages were those recommended by Davidson (1961). The remainder comprise the ten Brisbane phages shown in Table 3, together with two other phages of local origin (nos. 1050 and 1054).

There were 94 (6·7 %) strains which were non-typable with all 42 phages, 417 (29·7 %) were non-typable with the human phages but were lysed by one or more of the bovine phages. Twenty-seven strains which were typed by the human phages were non-typable with the bovine phages. Of these strains, nine were lysed by phage 53, one by phage 80 and seven were lysed by phages 80 and 81. The remainder were lysed by phages of group III (seven different patterns) and group II (three different patterns). Phage 187 did not lyse any of the 3224 staphylococci which were typed in these and the previous observations.

As before, many of these strains were from a limited number of control herds, and the value of the phages was assessed mainly from the typing of 397 of these strains from 328 herds; 280 of these strains were isolated from bulk milk. Not more than two strains were included from any one herd and these always belonged to different phage groups. Eighty-one strains were not lysed by the human phages, but were lysed by one or more of the bovine phages. A further 32 strains lysed by one or more bovine phages gave weak reactions with some of the human phages. Only four strains were non-typable with any phage.

The association of lysis between phages on these 397 strains is shown in Fig. 1, where only strong reactions were considered. This figure consists of a series of bars corresponding to the percentage of strains lysed by one phage (shown in the left-hand column) which were also lysed by the other phage (shown in the horizontal row at the top). Of the group I phages, 52 and 52A usually gave lysis only with that of 29 and 80. The new phage 13 appeared to belong to this group, although its lytic spectrum (see Table 5) was similar to the other bovine phages. Only phage 71 appeared useful among the group II phages. Group III phages gave rise to many different patterns of lysis.

Lysis by phage 42D was observed in staphylococci from 8 of 52 control and survey herds and from 13 of 280 isolates from bulk milk. This phage often gave lysis with phages of lytic group III, especially phages 42E and 47.

Phage 81 acted with the group III phages, except in the seven strains of phage type 80/81 mentioned above.

Phages 101, 102, 107, 108 behaved generally as group III phages and phage 102 gave lysis with phage 42D more than with any of the group III phages. Phage 110 was broader in its action and lysed in the main strains which could not be allotted

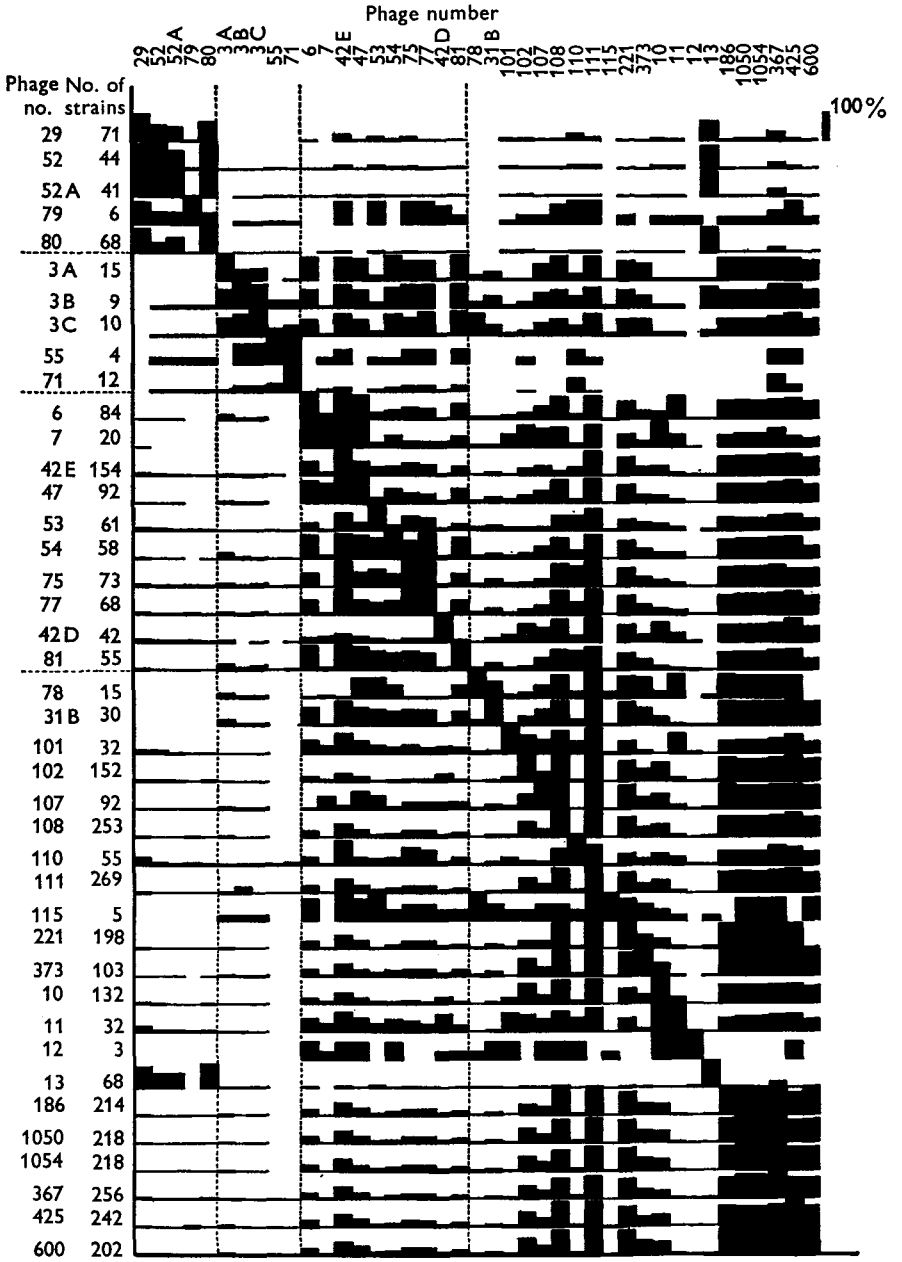

Fig. 1. Patterns of lysis of 397 strains of *Staphylococcus aureus* by 41 phages. Each horizontal line shows the pattern of lysis of those strains which gave a strong reaction with the phage shown in the column on the extreme left. The height of each bar indicates the percentage of these strains which were also lysed by the phage shown in the top line. The strains were derived from 328 herds, and included only one member of each phage group from each herd.

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to any lytic group. It was associated particularly with phage 42E, and also phage 29. Phage 111 was the most active of all, and lysed 70% of all strains typed.

Many of the phages isolated in Queensland were actively lytic, and phages 1050 and 1054 gave identical patterns of lysis. Although phages 367, 425 and 600 appear similar in Fig. 1, they each gave lysis alone on a number of strains.

Table 5 shows the lytic spectra of four of the new phages (nos. 13, 367, 425 and 600) on a set of test strains, some recommended by Blair & Williams (1961) for testing the specificity of the human typing phages, and the rest from the bovine set of Davidson (1961). The serological groups of the four phages, which were determined by Dr P. M. Rountree of the Royal Prince Alfred Hospital, Sydney, are also given in the table.

Table 5. *Lytic spectrum and serological group of new phages*

	Phage			
	13	367	425	600
Serological group	B	B	B	A
Propagating or test strain:				
52	—	—	—	—
52A/79	—	1	—	—
53	—	1	—	—
54	—	1	—	2
29	—	—	—	—
42C	—	—	—	—
42E	—	3	—	2
47	—	—	—	—
2009	—	3	2	—
8719	—	—	—	—
31B	—	—	—	—
42D	—	3	1	1
71	—	—	—	—
75	—	—	—	—
77	—	1	—	—
80	—	—	—	—
78	—	1	—	—
101	3	3	3	4
102	—	—	3	2
105/107	3	4	4	3
108	3	4	4	4
110	1	4	2	4
111	3	4	4	4
115	—	—	—	—

DISCUSSION

The human set of phages was extremely useful on its own to type bovine strains, and the lytic groups were distinguished here as recognized in the typing of human staphylococci. This is with the exception of group II, where lysis was uncommon and strains lysed by these phages were usually unable to be classified into a specific lytic group.

The position of phage 42D is rather curious. Early workers found that bovine

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staphylococci were commonly lysed by this phage (Macdonald, 1946; Smith, 1948*b*), and for this and other reasons strains of this phage type were considered of probable animal origin. The phage was separated from phages of lytic group III and is the only member of lytic group IV in the human phage set. In the present study, the endemic strains in the first two herds examined were lysed by this phage and this accounts for the large number of group IV and miscellaneous strains in Table 1. When observations were extended to other herds, staphylococci lysed by this phage were not common. The phages of bovine origin often lysed strains along with phages of group III, and phage 42D was usually associated with the bovine phages. When weak reactions with phage 42D were considered, in 39 strains this was associated with strong lysis by the bovine phages, in 53 strains with both these and group III, but never with phages of group III and without the bovine phages. Thus in some respects it was a 'link' between group III phages and those of bovine origin. However, Fig. 1 shows that phage 42D did tend to form a group of its own and in this respect it was not greatly dissimilar to phage 53, which, although usually reacting with other phages of group III, also reacted separately from others of the group. Perhaps the bovine group, along with phage 42D, could be considered a subgroup of group III.

The 42 phages were also used to type 96 strains from other animals. Only two of 78 dog strains were typed. One belonged to lytic group III, and a strain of identical pattern was isolated from the nose of the owner. The other was lysed by phage 187. This is the only strain ever lysed by this phage in this laboratory. Four of nine strains from cats were lysed, with the following patterns: 53, 53/10, 81, 6/75/77/10. The remaining strains, from horses, fowls and sheep were not lysed by any phage.

No set criteria can be laid down to define a suitable phage, and the selection of phages suitable for the typing of bovine strains is largely a matter of trial and error. However, it is important to consider the purpose of phage typing when a selection of phages is to be made. In studies on human strains its greatest use is in small epidemiological investigations, especially in hospitals. Here it is immaterial which phages are used to distinguish between cultures from related sources. It has been most useful in some epidemiological investigations (Parker & Kennedy, 1949; Sompolinsky, Hermann, Oeding & Rippon, 1957). From this type of investigation, it became clear that strains of particular importance appeared, and it was desirable to gain information of their distribution within and between countries. It is then essential that the phages used be similar. Such investigations resulted in the selection of the human set as a basis for the typing of human strains, with any hospital laboratory using additional phages if required.

In the selection of a set of phages for typing bovine staphylococci, therefore, the nature of the information required should be considered. Apart from research, at the present time there seems little likelihood of typing being of any practical value in investigating herd outbreaks where, as pointed out above, the particular phages used are unimportant.

On the broader scale, however, we need to know much more of the distribution of patterns among the dairy cattle population, and it is highly desirable that we

can compare these patterns with those in other countries. A suitable basic set for typing bovine strains should be able to satisfy the following criteria, in order of importance:

(1) It should bear as close a resemblance as practicable to the human set so that comparisons can be made.

(2) A high proportion of strains should be typed. However, provided the proportion of *herds* in which non-typable strains are predominant is low, it is not an ideal that every strain should be typed.

(3) Each phage should have a limited host range so that overlapping of phage patterns is minimized.

(4) Enough phages should be present in each lytic group to distinguish types within groups as clearly as possible. These types should be consistent and not too complex.

(5) It is not practicable to use more than 25 phages on one carpeted plate, so the set should be less than this to allow for additions.

A set of phages was selected from the 42 which was considered suitable for use on bovine strains in Queensland and probably Australia. The set consists of the following phages:

Group I	29, 80, 13
Group II	71
Group III	6, 7, 42E, 47, 53, 77, 31B
Group IV	42D
Others	81
	101, 102, 107, 110, 600, 367, 425.

Phage 80 was selected from the '52/52A/80 complex' of Rountree & Asheshov (1961). This along with phages 29 and 13 were the most useful of group I. As phage 81 is also included, the recognition of phage type 80/81, an important human pathogen, should be possible. Phage 71 was the obvious choice from group II, but more difficulty was found with group III, and perhaps one or more of 6, 7, 47 and 77 could be deleted if necessary.

This set of 20 phages, on the series of 1404 strains of staphylococci, would not have typed a further 35 strains, making a total of 129 or 9·2% of non-typable strains. Since this investigation the set has been used to type a large number of bovine staphylococci from all states of Australia, and has so far proved satisfactory.

Lysogeny among staphylococci would appear to be a universal phenomenon and it is clear that many phages thus isolated have too broad an action to be useful in phage-typing. It is of interest to note that the phages isolated here which showed the least action, were from cross-spotting of cultures from widely different sources. This approach should perhaps be followed in isolating new phages, rather than obtaining them from local strains where prophage immunity may be significant.

SUMMARY

Preliminary typing of 1820 strains of *Staph. aureus* from 51 herds with the human set of phages showed that non-typable strains were common (31·4%).

Other phages were investigated and 1404 strains from 328 herds were typed with 42 phages; these included the human phage set, phages from Weybridge and phages isolated in Brisbane. The following 20 phages were selected as suitable for typing bovine strains of *Staph. aureus* in Australia: 29, 80, 13, 71, 6, 7, 42E, 47, 53, 77, 31B, 42D, 81, 101, 102, 107, 110, 600, 367, 425. Phages 13, 367, 425, 600 were from Brisbane and 101, 102, 107 and 110 were from Weybridge.

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