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Staphylococcus aureus strains of phage-group IV

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Phage-group IV was established to include strains of *Staphylococcus aureus* which are lysed only by phage 42D (Report, 1953). This phage had been obtained by Wilson & Atkinson by adaptation of phage 42C (see Williams & Rippon, 1952). Macdonald (1946) found that a large proportion of staphylococci isolated from cows' milk in Britain were lysed only by this phage, and many subsequent authors have confirmed this observation. Strains from human sources are rarely lysed by this phage. Williams Smith (1948*a*,*b*) obtained six other phages from bovine staphylococci which appeared to have a range of specificity rather similar to that of phage 42D. With these phages, he obtained a number of pattern reactions with staphylococci lysed by phage 42D, and was also able to increase the proportion of typable strains among bovine staphylococci. It is now customary to refer to phages which resemble phage 42D in host-range as members of group IV, and to classify *Staph. aureus* cultures lysed only by these phages as group IV strains.

Phage 42D is the only group IV phage included in the international basic set of staphylococcal phages. Therefore, group IV strains are not typed with this set, but only grouped. Investigators of human staphylococcal disease have been quite satisfied with this situation, but the growing interest in animal staphylococci, particularly those of bovine origin, has created new problems. According to our own observations (Meyer, 1965) bovine *Staph. aureus* strains give reactions with the international basic set of typing phages nearly as often as do strains of human origin, but this is not necessarily identical with typing. Pulverer (1965) reported that he was able to differentiate only about 50 % of bovine staphylococci with the international basic set. As will be demonstrated later, this was owing to the inadequate recognition and differentiation of members of group IV.

Williams Smith (1948*a*, *b*) did not recommend the use of his additional phages for typing bovine staphylococci, because he thought that the differences in pattern he observed were caused mainly by resistance acquired through lysogenization of the strains with the respective phages. This pessimistic conclusion was not confirmed by the observations of Nakagawa (1960*a*). This worker (Nakagawa, 1960*b*) selected a number of new phages from *Staph. aureus* strains obtained from bovine milk. With the help of these phages he could subdivide bovine staphylococci which had been allotted to phage group IV with the international set of phages into two types within which a number of lytic patterns could be distinguished. Davidson (1961) also proposed the use of a number of new phages, in addition to some of the

phages of the international set, for the differentiation of bovine strains. Amongst them, phages 102, 107, 108 and 111 were members of group IV.

We will now discuss how the application of additional phages of group IV influences the classification of *Staph. aureus* strains from various sources.

MATERIALS AND METHODS

First we examined the results we had obtained by applying the phages of the international basic set (Blair & Williams, 1961; Report, 1963) to 8202 *Staph. aureus* strains in the years 1956–64, in order to find out what proportion of them were lysed by phage 42D either alone or in combination with other phages. This material was composed of: 2523 strains from human clinical material (pus, various secretions); 4308 strains from nasal swabs and other environmental investigations of human beings; 626 strains from foodstuffs; 745 strains from various animals (616 from cattle and 129 from other animals).

Table 1. *Phages used for investigation*

1. International basic set:*	
Gr. I	29, 52, 52A, 79, 80
Gr. II	3A, 3B, 3C, 55, 71
Gr. III	6, 7, 42E, 47, 53, 54, 75, 77, 83A
Gr. IV	42D
Gr. M ('Miscellaneous')	81, 187
2. Davidson's phages:	
Gr. I/III	101, 110
Gr. IV	102, 107, 108, 111
Gr. M. ('Miscellaneous')	115
3. Additional phages:	
Gr. III	31B
Gr. IV	42F
Gr. M. ('Miscellaneous')	44A, 78

* As constituted in 1962 (Report, 1963). Certain modifications have since been made at the 1966 meeting of the International Subcommittee on Phage-Typing of Staphylococci (Report, 1967).

We then tested 921 strains with a combined set of phages which will be described later. These included 307 isolated from bovine milk. All of them were coagulase positive when tested with human plasma, but with the aid of the bovine-plasma test (Meyer, 1966*a*, *b*) they were subdivided into 250 bovine-plasma coagulase positive and 57 bovine-plasma coagulase-negative strains. The remaining 614 strains were of human origin; 156 were isolated from nose-swabs of dairy workers, milkers and butchers, and 458 from clinical material. All were coagulase-positive with human plasma and none was positive with bovine plasma. Because there was no difference between the two groups in their reaction with phage 42D they were jointly evaluated.

The combined set of phages consisted of the international basic set (Report, 1963), together with seven of Davidson's phages (Davidson, 1961) and four others

(see Table 1). All strains were examined with this set at routine test dilution (R.T.D.). Those that were untypable at R.T.D. were tested with the same phages at R.T.D. × 1000.

RESULTS

We observed that the phages of group IV very frequently reacted together with phages of other groups, especially with phages of group III. In our epidemiological analysis we were unable to find a clear line of demarcation between strains that were lysed only by group IV phages and those which were also lysed by phages of other groups, to which we shall refer subsequently as 'phage-group IV/mixed', and we shall not make a fundamental distinction between them.

Table 2. *Reactions with phage 42D, and with other members of the international basic set of phages, of* 8202 *strains of* Staphylococcus aureus

Origin of strains	No of strains examined		No. lysed by: Phage 42D (alone or with other phages)		No. lysed by: Phage 42D alone		No. lysed by: Phage 42D at R.T.D.	
Man	6831		104 = 1·5 %		23		19	
Cow	616		214 = 34·7 %		111		93	
Pig	51		6		1		4	
Dog	32		0		0		0	
Sheep	26		3	12 = 9·3 %	3		3	7
Fowl	18	129	2		1	5	0	
Goat	1		1		0		0	
Chinchilla	1		0		0		0	
Foodstuff	626		41 = 6·5 %		14		17	
Sum	8202		371		153		136	

R.T.D. = routine test dilution.

Table 2 contains data concerning the frequency of reactions with phage 42D in *Staph. aureus* strains tested only with the basic set of phages. While this phage relatively seldom lysed strains of human origin (1·5 %), it lysed 30·3 % of strains from animal sources. Among the animal strains, however, it reacted with 34·7 % of bovine strains but only with 9·3 % from other animals. Only 23 of 104 human strains lysed by phage 42D were lysed only by this phage (22·1 %), but 111 of 214 bovine strains (51·9 %) were lysed exclusively by phage 42D. A larger proportion of the bovine strains (43·4 %) than of the human strains (18·2 %) which were lysed by phage 42D gave this reaction at R.T.D. Among the bovine strains, reactions with phage 42D occurred more often at R.T.D. among those lysed exclusively by phage 42D (72 of 111: 64·9 %) than among those belonging to phage-group IV/mixed (20 of 103: 19·4 %).

In Tables 3–5, the distribution of 921 strains of *Staph. aureus* among the various phage-groups as a result of typing with the international basic set, and with the combined set of phages, is contrasted. The difference was most impressive in the bovine-plasma coagulase-positive *Staph. aureus* strains from cows' milk (Table 3)

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but is also perceptible in the bovine-plasma coagulase-negative bovine staphylococci (Table 4) and in the strains of human origin (Table 5).

The use of the additional group IV phages in the combined set increased the number of group IV strains among 250 bovine-plasma coagulase-positive *Staph. aureus* cultures from 37 to 170, and group IV plus group IV/mixed from 72 to 244 (Table 3). In other words, 97·6 % of the cultures were lysed by one or more of the group IV phages in the combined set, either alone or together with lysis by phages

Table 3. *Grouping of the phage-patterns of* 250 *bovine-plasma coagulase-positive strains of* Staphylococcus aureus *when tested with the basic set and with the combined set of phages*

	With basic set			With combined set			
Phage-groups	R.T.D.	R.T.D. × 1000	Total	R.T.D.	R.T.D. × 1000	Total	Difference
I: 80/81 + other phages	0	0	0	0	0	0	−5
I: other phages	3	4	7	2	0	2	
II	1	0	1	0	0	0	−1
III	20	62	82	1	0	1	−81
IV	29	8	37	168	2	170	+133
I/III	6	61	67	0	1	1	−83
II/III	2	5	7	0	0	0	
I/II/III	0	10	10	0	0	0	
I/IV	0	1	1	1	1	2	+39
II/IV	0	0	0	1	0	1	
III/IV	1	14	15	15	0	15	
I/III/IV	3	12	15	49	3	52	
II/III/IV	0	1	1	0	1	1	
I/II/III/IV	0	3	3	2	1	3	
Untypable			4			2	−2

Table 4. *Grouping of the phage-patterns of* 57 *bovine-plasma coagulase-negative strains of* Staphylococcus aureus *when tested with the basic set and with the combined set of phages*

	With basic set			With combined set			
Phage-groups	R.T.D.	R.T. × 1000	Total	R.T.D.	R.T.D. × 1000	Total	Difference
I: 80/81	1	0	1	1	0	1	−4
I: 80/81 + other phages	4	1	5	3	1	4	
I: other phages	5	2	7	3	1	4	
II	6	3	9	5	3	8	−1
III	8	3	11	7	3	10	−1
IV	0	0	0	1	0	1	+1
187	2	1	3	2	1	3	−
I/III	8	6	14	4	4	8	−6
I/IV	0	0	0	1	0	1	+5
III/IV	1	0	1	3	2	5	
I/III/IV	0	0	0	6	3	9	
II/III/IV	0	0	0	1	0	1	
Untypable	—		6	—	—	2	−4

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Table 5. *Grouping of the phage-patterns of* 614 Staphylococcus aureus *strains of human origin when tested with the basic set and with the combined set of phages*

Phage groups	With basic set			With combined set			Difference
	R.T.D.	R.T.D. × 1000	Total	R.T.D.	R.T.D. × 1000	Total	
I: 80/81	112	20	132	112	17	129	
I: 80/81 + other phages	31	10	41	30	9	39	−22
I: other phages	49	45	94	45	32	77	
II	27	11	38	27	11	38	—
III	75	30	105	67	20	87	−18
IV	0	0	0	6	1	7	+7
187	9	44	53	9	44	53	—
I/II	0	1	1	0	1	1	
I/III	29	46	75	20	36	56	−19
I/II/III	1	1	2	1	1	2	
I/IV	0	2	2	6	9	15	
III/IV	0	2	2	10	13	23	+58
I/III/IV	2	6	8	11	21	32	
I/II/III/IV	0	1	1	0	1	1	
Untypable	—	—	60	—	—	54	−6

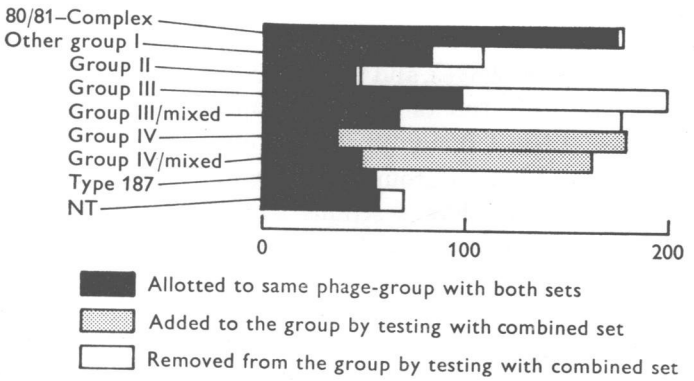

Fig. 1. Change in frequency of phage-groups when *Staphylococcus aureus* strains were tested first with the international basic set, and then with the combined set of phages.

of other groups. With the 57 bovine-plasma coagulase-negative strains from cows' milk (Table 4), there was little increase in the number of strains lysed only by the group IV phages, but the use of the combined set increased the number of group IV/mixed strains from 1 to 16 (1·8% to 28·1%). Similar results were obtained with the 614 human strains (Table 5), where the increase of group IV strains was from 0 to 7, and of group IV/mixed strains was from 13 to 71 (2·1% to 11·6%). It will be seen from Fig. 1 that the increase of group IV and group IV/mixed strains was almost exclusively at the expense of strains classified as group III and group III/mixed by the international basic set.

The results of typing at R.T.D. and at R.T.D. × 1000 with the two sets of phages may also be compared in Tables 3–5. With the basic set, a considerable minority

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(8 out of 37) of the strains lysed only by phage 42D were not lysed at R.T.D. With the combined set, however, all but three of the 178 strains from all sources that were lysed only by group IV phages gave this reaction at R.T.D. With group IV/mixed strains, the situation was different. Many of the bovine-plasma coagulase-positive strains from milk were typable only at R.T.D. × 1000 with the basic-set phages, but most were typable at R.T.D. by the combined set (Table 3). Among the human strains, however, over half of all the group IV/mixed strains (44 of 71) were untypable at R.T.D. with the combined set (Table 5).

Table 6. *Number and percentage of bovine and of human strains of* Staphylococcus aureus *reacting with each of the group IV phages*

Phage no.	Bovine strains	Human strains
102	240 (78·2)	24 (3·9)
107	231 (75·2)	11 (1·8)
108	220 (71·7)	7 (1·1)
111	242 (78·8)	46 (7·5)
42F	122 (39·7)	38 (6·2)
42D	68 (22·2)	8 (1·3)
Any phage	261 (85·0)	79 (12·9)
Total number examined	307	614

Table 6 shows the number of strains of human and of bovine origin that were lysed by any of the group IV phages, and the number lysed by each of the individual phages (42D, 42F, 102, 107, 108 and 111). It appeared that phage 42D was the least productive, since it lysed about 1/4 of the bovine and only 1/10 of the human strains that were susceptible to group IV phages. It must be added that the reactions with about half of the bovine strains, and with most of the human strains, occurred only at R.T.D. × 1000. The greatest range of activity was with phage 111, and most of the reactions were at R.T.D. The range of activity of the four Davidson phages 102, 107, 108, and 111 on our bovine plasma coagulase-positive strains was approximately the same.

Most of our bovine-plasma coagulase-negative *Staph. aureus* strains from cows' milk which reacted with group IV phages belonged to group IV/mixed. They were generally lysed only by a single group IV phage, and only at R.T.D. × 1000. Considered as a group, they behaved more like strains of human origin than like the bovine-plasma coagulase-positive strains.

In addition, we examined 58 strains of *Staph. aureus* from cases of bovine mastitis received from Dr Davidson of Weybridge. For the most part they were sensitive to group IV phages, but usually only to a minority of the six phages. We are inclined to attribute this to regional differences in type-distribution rather than to differences in pathogenicity.

DISCUSSION

It appears from our findings that *Staph. aureus* strains belonging to phage-group IV are imperfectly recognized when the international basic set of phages is used. According to reports in the literature (Pöhn, 1957; Pulverer, 1964; Brandis

& Morgenroth, 1965) not more than 0·5 % of human strains are allotted to phage-group IV when phage 42D is the only member of this group used in typing. We found a considerably greater proportion of the strains lysed by phages of group IV in our human material, but this was attributable to a single epidemic of wound infection and mastitis from which a number of group IV/mixed cultures were isolated. When these are eliminated the proportion is decreased to 0·6 %. The proportion of strains from foodstuffs which react with group IV phages is rather variable; according to our experience (Meyer & Rische, 1966) it is influenced to a large extent by the percentage of milk and dairy products in the material examined.

Most authors agree that *Staph. aureus* of phage-group IV is most frequently found in cattle. Among the bovine strains we examined, about 1/4 reacted with phage 42D, but half of these were also lysed by phages of other groups. The application of the combined phage set led to an astonishing change in the grouping of the bovine organisms, and virtually all the bovine-plasma coagulase-positive strains reacted with one or more of the group IV phages. Our earlier work with bovine staphylococci, on the coagulase reaction with various kinds of plasma (Meyer, 1965, 1966*a*, *b*, *c*) and on the crystal-violet test (Meyer, 1967*a*, *b*) suggests that bovine-plasma coagulase-positive strains form a special variety of the species *Staph. aureus* which we have called var. *bovis*. Strains from milk which are bovine-plasma coagulase-negative are rather more like human staphylococci, and for this reason we consider them to be bovine only with reservation. It now appears that nearly all *Staph. aureus* var. *bovis* belong to phage-group IV or to the IV/mixed groups, and that other strains of *Staph. aureus* react much more rarely with group IV phages.

This conclusion could not have been reached had phage 42D been the only group IV phage in the typing set. With this phage we should have recognized at most 26·1 % of the bovine and only about 10 % of the human strains which react with group IV phages. It is true that by using the combined set of phages an increased proportion of human strains appears to be related to phage-group IV, but their number is not large. Most of the human strains with group IV/mixed patterns are recognized only at R.T.D. × 1000, and generally do not seem to be of great epidemiological significance.

Often, when strains reacting with group IV phages are found in human material it is supposed that they are of bovine origin and may have been transmitted from cattle to man. This must not be taken as proven. It is interesting that, according to Markuse (1960), Vogelsang, Wormnes & Östervold (1962) and Pulverer & Fritsche (1965), human group IV strains are generally egg-yolk positive. In our experience, the great majority of bovine *Staph. aureus* strains are egg-yolk negative.

The problem of establishing standardized systems for the phage-typing of *Staph. aureus* strains in a number of different animal hosts is a difficult one. A single system for all *Staph. aureus* strains is probably impracticable. It is suggested that the international basic set should be used for typing strains of human origin, and that some such set as that proposed by Davidson (1961), which includes a

number of group IV phages, should be used for bovine staphylococci. In investigations of the possible inter-specific spread of infection, it may be necessary to use both sets of phages.

SUMMARY

Because the international basic set of staphylococcus typing phages includes only one member of group IV—phage 42D—the group is insufficiently characterized. This set of phages is therefore not suitable for typing bovine strains of *Staph. aureus*. The addition of several further group IV phages would remedy this deficiency.

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