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The basic phage set for typing bovine staphylococci

By P. J. CARROLL and P. G. FRANCIS

Ministry of Agriculture, Fisheries and Food, Central Veterinary Laboratory, New Haw, Weybridge, Surrey KT15 3NB

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SUMMARY

Two hundred and sixty-nine *Staphylococcus aureus* cultures isolated from bovine milk were subjected to phage typing using the International basic set of 16 phages at Routine Test Dilution. In the current study 73·6% of cultures were 'typable' compared with 84–89% in 1972 when the set was first recommended. The set remains capable of typing the majority of bovine staphylococci but shows a reduction in lysogenicity of most of its phages.

INTRODUCTION

In 1972 Davidson reported on a collaborative study carried out by workers in 17 countries to establish an internationally recommended set of bacteriophages for typing bovine staphylococci (Davidson, 1972). Periodic reviews of the recommended set of phages for typing human staphylococci are carried out by the International Sub-committee on Phage Typing of Staphylococci. No such review has been performed in the UK for the bovine set since 1972. The widespread use of antibiotics in bovine mastitis therapy is likely to have exerted selection pressure on bovine staphylococci and the opportunity was taken to re-examine the recommended set of 16 phages suggested by Davidson. The objective of this review was to establish if changes in the 'native' population of bovine *Staphylococcus aureus* since 1972 have altered the typing ability of Davidson's recommended set, and, if so, to establish which phages show changed lysogenicity.

MATERIALS AND METHODS

Two hundred and sixty-nine coagulase positive *S. aureus* cultures isolated from bovine milk were collected from 21 Veterinary Investigation Centres throughout England and Wales, during January to April inclusive in 1983. The International basic set of 16 phages for typing bovine staphylococci and their propagating strains were obtained from the Staphylococcal Reference Laboratory, Central Public Health Laboratory (CPHL), Colindale, London and the Biological Products and Standards Department at the Central Veterinary Laboratory (CVL), Weybridge, Surrey. The composition of the basic set is given in Table 1.

The internationally recommended methods for phage propagation, testing and typing as published by Blair & Williams (1961) detailed by Parker (1972) and in

Table 1. *The composition of the basic phage set for typing bovine staphylococci*

Group	Phage
I	29, 52A
II	3A, 116
III	6, 42E, 53, 75, 84
IV	42D, 102, 107, 117
Miscellaneous	78, 118, 119

Table 2. *Lysis patterns produced by the phage set on the propagating strains*

	Phage typing patterns at R.T.D.		
Propagating strain	Strong reactions	Weak reactions	Possible weak reactions not always present
29	29	—	—
52A	52A	—	118
3A	3A, 116	—	102, 107, 117, 118, 119
116	116	—	—
6	6, 53, 75, 84	42E(±)	107, 117, 118, 119, 116
42E	42E	—	118, 119, 116
53	53, 75, 84	—	107, 117, 119, 116
75	75, 53, 84	—	102, 116
84	84	—	—
42D	42D	—	102, 107, 117, 118, 119
102	102, 107	—	42D(+)
107	107, 102, 117	—	42D(±)
117	117, 102, 107	—	42D(±)
78	78	—	—
118	118	—	—
119	119, 102	—	84, 42D, 107

current use at the International Staphylococcal Reference Laboratory, CPHL, Colindale were used.

Nutrient broth (Oxoid CM1) and nutrient agar (Oxoid BAB CM55) plates were used for growing the cultures and the *S. aureus* typing agar plates were prepared as described by De Saxe, Coe & Wieneke, 1982.

Phage typing patterns of the propagating strains were determined for each batch of media and each batch of cultures examined (Table 2). All tests were carried out using phages at Routine Test Dilution (RTD), i.e. the highest dilution of phage filtrate that produced almost confluent lysis when a standard loopful was placed on a lawn of its propagating strain. The lysis was graded with a weak reaction of 1–19 plaques recorded as ± and the actual number of plaques noted if less than 10. Lysis showing 20–50 plaques were recorded as + but still regarded as a weak reaction. From 51 plaques to confluent lysis was recorded as + + and considered a strong reaction.

No variations from the patterns in Table 2 occurred during the experiment and these patterns are similar to those described by Davidson (1972), De Saxe, Coe & Wieneke (1982) and information supplied by CPHL, Colindale.

Duplicate examinations were carried out on the *S. aureus* strains and all results were reproducable. Very occasional variations occurred in weak reactions only.

Table 3. *Frequency of individual phage reactions*

Phage	Davidson: Cultures strongly lysed (%)	Carroll: Cultures strongly lysed (%)	Carroll: Cultures weakly lysed (%)	Change in cultures strongly lysed (%)
29	38	27	3	−11
52A	25	12	6	−13
3A	2	0·4	1	−1·6
116	2	0·4	0·7	−1·6
6	31	15	9	−16
42E	37	14	20	−23
53	7	4	10	−3
75	22	13	3	−9
84	13	7	4	−6
42D	20	2	10	−18
102	18	13	9	−5
107	14	16	7	+2
117	49	17	5	−32
78	9	12	6	+3
118	6	0·4	8	−5·6
119	9	9	1	0

Table 4. *Comparison of order of reactivity of phages (strong reactions only)*

Davidson	Carroll	Positional change
117	29	+1
29	117	−1
42E	107	+6
6	6	0
52A	42E	−2
75	75	0
42D	102	+1
102	52A	−3
107	78	+2
84	119	+2
78	84	−1
119	53	+1
53	42D	−6
118	118	0
3A	3A	0
116	116	0

Strains were considered typable if they were lysed at the + + level by one or more phages. Strains that were weakly (+ or ±) or not lysed at all were considered untypable.

RESULTS

All 269 cultures were examined and of these 198 (73·6 %) were strongly lysed by one or more phages and therefore typable. The remaining untypable cultures consisted of 58 (21·6 %) which were not lysed at all and 13 (4·8 %) weakly lysed by at least one phage.

The percentage of cultures lysed by each of the 16 phages and the comparison

of strong reactions in this study with that carried out by Davidson is shown in Table 3. Table 4 lists the phages in order of reactivity (117, 29 the most reactive, 116 the least reactive) and shows their relative positional changes compared with Davidson's results.

DISCUSSION

The number of 'typable' cultures at RTD (73·6 %) compares favourably with results (70 %) obtained in typing human strains of *S. aureus* with the International set of phages recommended for human isolates (De Saxe, 1983, personal communication). It is also consistent with the range of results (69–73 %) obtained by Davidson's collaborators when typing bovine *S. aureus* cultures from other countries using similar groups of phages to the set used in this study (Davidson, 1972). Holmberg (1975) employing at RTD the 16 phages used in this study was able to type 75·4 % of *S. aureus* strains isolated from bovine milk in Sweden. However, Davidson found that 84 % of UK strains were typable using 9 phages of this set and up to 89 % when using a wider range of 30 phages. It would appear therefore that the UK bovine *S. aureus* population has become less susceptible to lysis by this set of 16 phages.

The major change is in the number of strong reactions occurring for each culture as indicated by the percentage strongly lysed for each phage (Table 3). Only three phages (107, 78, 119) lysed the same or a greater percentage of cultures in this study with the remaining 13 showing a current reactivity less than that found by Davidson. The decrease in the average number of strong reactions per strain from 3·02 (Davidson) to 1·63 in this study reduces the ability of the set to demonstrate significant differences between strains, i.e. to differ by at least two strong reactions (Parker, 1972; Blair & Williams, 1961). The relatively small numbers of cultures examined in this study did not justify a fuller assessment of the discrimination between strains as carried out by Davidson in his much larger international trial. The apparent decrease in percentage lysogenicity may have been accentuated for the same reason however the results are directly comparable with the UK element of his trial and the reduction in lysogenicity of the set overall is marked. If more phages were added to the set it should result in a greater number of reactions occurring for each strain and therefore enhance the possibility of discrimination between strains. Holmberg (1975) suggested that the inclusion of two more phages from the International human set would increase the typing ability of the bovine set in Sweden.

The relative order of reactivity for most of the phages in the set has changed only slightly since Davidson's work (Table 4) except for phage 107 which has apparently become appreciably more lysogenic and 42D far less.

Further periodic monitoring and the evaluation of other potentially useful phages, could, in time, result in recommendations for the removal of the least lysogenic phages and/or the inclusion of other more reactive ones to the set. This would maintain it usefulness in typing the bovine *S. aureus* population in the UK.

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