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## PHAGE TYPING OF STAPHYLOCOCCUS AUREUS STRAINS ISOLATED IN SWEDEN FROM BOVINE MILK

By  
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HOLMBERG, O.: *Phage typing of Staphylococcus aureus strains isolated in Sweden from bovine milk.* Acta vet. scand. 1975, 16, 411—419. — Both the human and the bovine international sets of phages were used for typing of 372 bovine *Staphylococcus aureus* (Sa) strains, whereas the bovine set alone was used for typing of a further 1183 strains. In addition, 338 of the strains were tested for antibiotic sensitivity. Out of 372 Sa strains 85.5 % could be typed with the human and 89.8 % with the bovine phage set. Of all the 1555 Sa strains used 92.4 % were lysed by the bovine phage set. Several phage types can be present in one and the same herd and some of them can predominate. Resistance to most of the tested antibiotics was very low. The incidence of resistance to penicillin and ampicillin was 10.0 % and 4.4 % respectively.

staphylococcus aureus; mastitis; phage typing;  
antibiotic sensitivity.

Phage typing has played an important role in epidemiological investigations of infections caused by coagulase-positive staphylococci (*Staphylococcus aureus*) and many workers have engaged in the standardization of this method of typing (for references see *Koiranen* 1969). The International Committee for Staphylococcal Phage typing has recommended a standard set of 22 phages for typing of coagulase-positive staphylococci isolated from man. Since this phage set does not give satisfactory results at typing of *Staphylococcus aureus* (Sa) of bovine origin, another set has been recommended for this purpose (*Davidson* 1961, and Subcommittee on Phage Typing 1971).

In the present study, both the human and the bovine set of phages were used for typing of 372 bovine Sa strains, whereas

the bovine set alone was used for typing of a further 1183 strains. In addition, 338 of the strains were tested for antibiotic sensitivity.

## MATERIAL AND METHODS

### *Animals*

Sa strains were isolated from 1000 animals in 170 herds. Most of the herds were situated in the counties of Gotland, Uppsala, and Södermanland and belonged to the Swedish Red and White Breed (SRB).

### *Bacterial strains isolated*

The investigation comprised 1555 cultures of Sa strains isolated over the period 1967—1972 from an equal number of udder quarters at bacteriological examination of milk.

### *Identification of *Staphylococcus aureus* strains*

In those cases in which, judging by their appearance, the bacterial colonies in cultures of milk samples on nutrient agar containing about 8 % cattle blood were assessed as suspect Sa colonies, the material was kept for further study and stored in 2 % glucose broth at  $-20^{\circ}\text{C}$ . Gram-positive strains of immobile cocci which produced catalase, fermented glucose aerobically and anaerobically, and were coagulase-positive were classified as *Staphylococcus aureus*.

### *Biochemical studies*

Gram staining, catalase tests, fermentation tests of glucose aerobically and anaerobically, and coagulase tests were performed as described by *Holmberg* (1973).

### *Antibiotic tests*

Antibiotic sensitivity was determined by *Ericsson's* (1960, 1964) method. The sensitivity was graded as follows: I. "Sensitive", II. "Fairly sensitive", III. "Slightly sensitive", and IV. "Resistant".

### *Phage sets*

Two sets of phages were used:

1. The internationally used phage set for typing of Sa strains

isolated from human material (human set). It included the following phages (human phages):

Lytic group I	phages	29, 52, 52A, 79, 80
II	„	3A, 30, 55, 71
III	„	6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85
IV	phage	42D

2. Davidson's phage set for typing of bovine Sa strains (bovine set). It included the following phages:

Lytic group I	phages	29, 52A
II	„	3A, 116*
III	„	6, 42E, 53, 75, 84
IV	„	42D, 102*, 107*, 117*
Miscellaneous	„	78*, 118*, 119*

\* bovine phages.

### *Culture media*

The following media were used:

1. Nutrient broth (8 g Bacto Nutrient Broth, Difco B 3, 5 g of pure NaCl, 1000 ml of distilled water).
2. Agar for staphylococcal phages (20 g of Bacto Nutrient Broth, Difco B 3, 5 g of pure NaCl, 9 g of Bacto Agar, Difco B 140, 1000 ml of distilled water, 5 ml of 4 % CaCl<sub>2</sub> after autoclaving).
3. Sa agar (90 g of Bacto Agar, Difco, 150 g of Bacto-Trypton, Difco, 45 g of glucose, 120 g of NaCl, 15.000 ml of distilled water pH 7.6).

### *Phage typing*

Typing was done according to the method described by *Blair & Williams* (1961). The routine test dilution (RTD) was defined as the highest dilution of phages producing an almost confluent lysis. RTD and 1000 × RTD were used in the typing.

## RESULTS

### *Typing of 372 strains with the human and the bovine phage set*

It will be seen from Table 1 that out of the 372 Sa strains 85.2 % (74.4 % with RTD and 10.8 % with 1000 × RTD) could be typed with the human and 89.8 % (78.8 % with RTD and 11.0 % with 1000 × RTD) with the bovine phage set.

Fig. 1 shows the number of strains that were lysed by the respective human phage. The nine human phages that have been incorporated in the bovine set lysed four (phage 84) to 138 (phage 42D) strains. Five other phages of the human set (55,

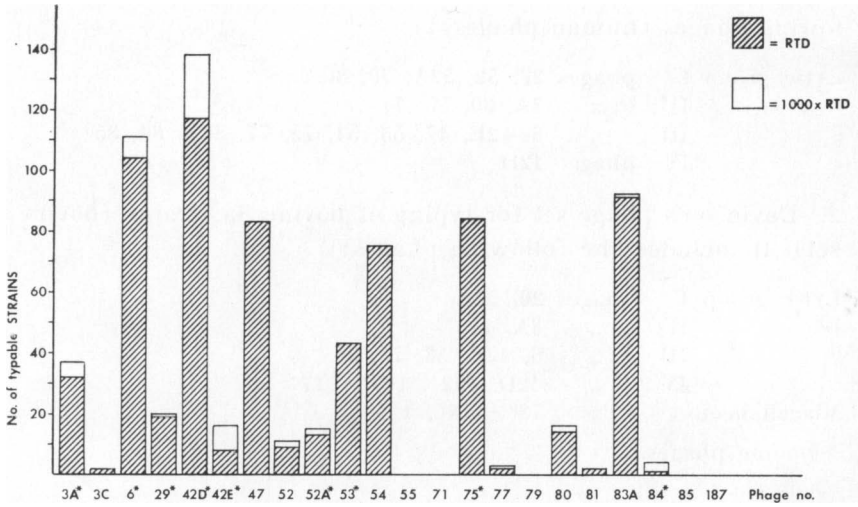


Figure 1. Numbers of strains lysed by each of the human Sa phages at typing of 372 strains.

\* Phages included both in the human and the bovine phage set.

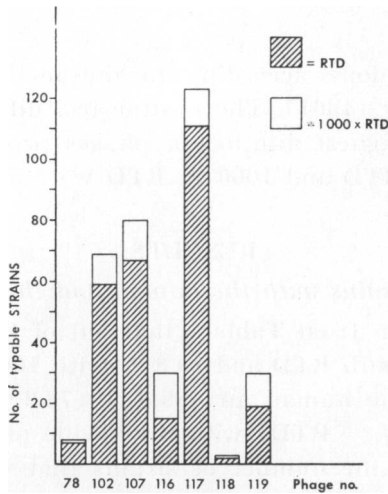


Figure 2. Numbers of strains lysed by each of the bovine Sa phages at typing of 372 strains.

Table 1. Typing of 372 *Staphylococcus aureus* strains with the human and the bovine set of phages.

Phage set	RTD	1000 × RTD	Total
human	278 (74.4 %)	40 (10.8 %)	318 (85.2 %)
bovine	293 (78.8 %)	41 (11.0 %)	334 (89.8 %)

71, 79, 85, and 187) lysed no strain. The phages 47 and 83A lysed 83 and 92 strains, respectively.

The numbers of strains that were lysed by the respective bovine phage are shown in Fig. 2. Between three (phage 118) and 123 (phage 117) strains were lysed.

#### *Typing of all the 1555 strains with the bovine phage set*

It will be seen from Table 2 that of all the 1555 Sa strains used in this study 1437 (92.4 %; 75.4 % with RTD and 17.0 % with 1000 × RTD) were lysed by the bovine phage set.

Table 2. Typing of 1555 *Staphylococcus aureus* strains with the bovine phage set.

RTD	1000 × RTD	Total
1173 (75.4 %)	264 (17.0 %)	1437 (92.4 %)

#### *Number of phage types within the herds*

In six large herds with 40 to 200 cows there were up to 12 different phage types within each herd. Table 3 shows the lysis of the strains in these herds with each phage of the bovine phage set. In herds 1 and 3 most of the strains were lysed by phage 117. In the other herds, the most useful phages were phage 116 in herd 2, phage 119 in herd 4, and phage 6 in herds 5 and 6.

#### *Antibiotic sensitivity*

Table 4 shows the result of the test for antibiotic sensitivity of 338 Sa strains. It will be seen that resistance to most of the tested antibiotics was very low. The incidence of resistance to penicillin and ampicillin was 10.0 % and 4.4 % respectively.

Table 3. Distribution of lysed *Staphylococcus aureus* strains between phages and within herds.

Phage	Herd					
	1	2	3	4	5	6
Number of investigated strains	89	120	142	17	88	332
3A	5	47	6	1	2	6
6			4		50	246
29		14		4	7	2
42D	44		55		14	3
42E	6	2	5	1	22	13
52A		15		4	10	1
53					4	211
75	1		2		17	30
78	7	1	6		3	32
84					3	45
102	58	2	110	1	10	30
107	58	3	90	2	11	36
116		75	7	1	1	1
117	79	4	126	1	26	63
118	1			2		1
119	2	24	2	12	9	1

Table 4. Antibiotic sensitivity\* of 338 *Staphylococcus aureus* strains.

	I	II	III	IV	III+IV %
Sulphonamide	295	43			0
Penicillin	300	4	34		10.0
Ampicillin	309	14	15		4.4
Streptomycin	335	2	1		0.3
Aureomycin	336			2	0.6
Terramycin	336			2	0.6
Chloramphenicol	319	19			0
Neomycin	334	4			0

\* I = sensitive.

II = fairly sensitive.

III = slightly sensitive.

IV = resistant.

## DISCUSSION

At phage typing of 372 Sa strains isolated from cow's milk 85.2 % of the strains were typable with the human and 89.8 % with the bovine phage set. Thus, a greater number of strains could be defined with the bovine than with the human phage set, and this result agrees with what has been found both in Sweden (*Thörne & Hallander 1970*) and in other countries (for references see *Koiranen 1969*).

Of the human phages not included in the bovine set, phages 47 and 83A lysed a relatively great number of bovine strains (22.3 % and 24.7 % respectively). It would thus be possible to type more bovine Sa strains in Sweden, if the human phages 47 and 83A were added to the bovine phage set.

As is evident from these investigations, several phage types of Sa can be present in one and the same herd and some of them can predominate (Table 3). In herds in which the stock is renewed by purchase of animals there is likely to be an accumulation of Sa strains belonging to different phage types. The predominating phage types might possess such genetic properties that they could spread in the herd more easily than others.

Among the strains used in this study there was a certain resistance to penicillin (10.0 %) and to ampicillin (4.4 %), which are the two antibiotics most frequently used in Sweden in "dry-period treatment". The incidence of penicillin-resistant Sa strains in Sweden is the same as in the other Nordic countries, whereas it is much lower than in non-Nordic countries. *Madsen (1968)* found 4.5 %, *Koiranen 10.3 %*, and *Nygård et al. (1968)* 7.2 % in Denmark, Finland, and Norway respectively. On the other hand, the proportions reported by *Wilson (1961)* from England were 70.6 %, by *Marshall (1964)* from New Zealand 67.0 %, and by *Jones & Bennet (1965)* from the USA 44.5 %.

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#### SAMMANFATTNING

##### *Fagtypning av Staphylococcus aureus stammar isolerade i Sverige från komjolk.*

Både det humana och bovina internationella fagseten har använts för att typa 372 bovina Staphylococcus aureus (Sa) stammar medan endast det bovina setet använts vid typning av ytterligare 1183 stammar. Därutöver har 338 av stammarna prövats avseende antibiotikakänslighet.

Av 372 Sa stammar kunde 85,5 % typas med det humana och 89,8 % med det bovina fagsetet. Av de humana fagerna som inte fanns i det bovina setet lyserade fag 47 och 83A ett relativt stort antal stammar (22,3 % respektive 24,7 %). I Sverige skulle man kunna fagtypa flera bovina stammar om det bovina fagsetet kompletterades med dessa fager.



Av samtliga 1555 Sa stammar kunde 92,4 % typas med det bovina fagsetet. I en och samma besättning förekom ett flertal fagtyper av vilka några dominerade.

Resistensen för det flesta testade antibiotika var mycket liten. Mot penicillin och ampicillin var 10,0 % respektive 4,4 % av stammarna resistent.

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