Bacteriophage Typing of Canine Staphylococci

I. Typing by Use of the International Phage Sets for Human and Bovine Staphylococci

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Abstract. Bacteriophage typing was made on 221 strains of coagulase-positive staphylococci isolated from dogs by use of the N.C.T.C. and bovine phage sets. Of these strains, eleven (5.0%) were typable by the former and ten (4.5%) by the latter. The typable strains were distributed mainly in groups I, II and III. All of them produced yellow pigment, but none of them were white-pigment producers, which were predominant in canine staphylococci and susceptible to some phages. No special relationship was observed between the source of strains and the phage pattern. Thus, the existing two phage sets were concluded to be useless for the typing of canine staphylococci.

In some countries, such as France, Japan, U.K. and U.S.A., studies have been made on the bacteriophage typing of staphylococci of canine origin by use of the International Phage Sets, which were established for the typing of human or bovine staphylococci, revealing the uselessness of these phage sets owing to their low typability [1, 2, 4-8, 13]. Such studies, however, have not been performed on canine staphylococci in Taiwan.

The author intends to establish a phage set useful for epizootiological studies on canine staphylococci. The present investigation was made as the first part of a series of studies to meet this purpose.

Materials and Methods

Staphylococcal strains examined: Phage typing was made on 221 coagulase-positive strains which had been isolated from dogs in the rural area of Taipei during a period from November, 1970 to

October, 1973. Of the 221 strains, 204 produced white pigment and were considered as strains of canine origin on the basis of their biological properties [4, 12]. Of these white strains, 100 were isolated from otitis externa, 14 from pyoderma, 30 from the ear canals of healthy dogs, 30 from the skin of healthy dogs, and 30 from the skin of dogs suffering from otitis externa. The other 17 strains produced yellow pigment and were suspected to be of human or bovine origin. Of them, four were isolated from otitis externa, four from pyoderma, eight from the skin of dogs with otitis externa, and one from the skin of a healthy dog.

Typing phages used: The National Collection of Type Cultures (N.C.T.C.) phage set and the bovine phage set which had kindly been supplied by Dr. M. Nakagawa, of the National Institute of Health, Japan, were used for typing. Details of the sets are as follows.

N.C.T.C. phage set

Group I	: 29, 52, 52A, 79 and 80
Group II	: 3A, 3C, 55 and 71
Group III	: 6, 42E, 47, 53, 54, 75, 77, 83A and 84
Group IV	: 42D
Miscellaneous	: 81 and 187

International phage set for bovine staphylococci (bovine phage set) [3].

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

Methods of typing: Phage typing was made by the methods of Nakagawa [9-11].

a) Medium—Agar medium consisting of 8 g of beef extract, 10 g of polypeptone, 2 g of sodium chloride, 12 g of agar powder, and 1,000 ml of distilled water (final pH was adjusted to 7.4-7.6) was used for propagation and titration of phages and for phage typing.

b) Propagation of phages—Overnight broth cultures of propagating strains at 37°C were inoculated on the surface of agar plates and 0.2 ml of appropriate phage dilution was spread on the top of the agar by the aid of a glass spreader after the surface was dried. In order to confirm that the culture was free from autolysis of propagating strains, a small part of the agar surface was kept free from phage as a control. After overnight incubation at 37°C, phage suspensions were obtained by pouring 5 ml of broth onto the agar plate, holding for 1 to 2 minutes, and then scrubbing the surface of the plate. After centrifugation at 3,000 rpm for 30 to 40 minutes, bacteria were removed from the resulting supernatant of the phage suspension by filtration through 0.45 μ Millipore filter or killed by addition of a piece of crystal thymol. The phage suspension was kept at 2-5°C as an original phage stock.

c) Titration of phage—Tenfold serial dilutions of phages were made with broth. One drop of each dilution was spotted on an agar plate previously seeded with 5-6-hour broth culture of a propagating strain. After overnight incubation at 37°C, the highest dilution that showed a complete lysis was regarded as a routine test dilution (RTD).

d) Typing—A six-hour broth culture of a staphylococcal strain to be examined was spread over an agar plate, the surface of which was dried. One drop of phage dilution was spotted on the inoculated agar plate by the aid of a 1/4 G needle attached to a tuberculin syringe. The whole procedure of spreading bacteria and dropping phages was completed within 90 minutes.

The plates were then incubated at 37°C overnight. The lysis was scored in 5 degrees of Williams and Rippon [15] as follows.

- CL: Complete lysis without secondary growth of resistant bacteria
 ‡‡: Complete lysis with secondary growth

‡‡: More than 50 plaques showing semi-confluent lysis

+: 20-50 plaques

±: Less than 20 plaques

For typing, both 1 and 1,000 RTD of phage were used. Strains showing ‡‡ and higher degrees of lysis were judged as typable.

Before use, the lytic spectra of all the phages were confirmed to be the same as the original ones with propagating strains as indicators.

Results

1. Results of typing

A total of 221 strains of canine staphylococci were submitted to typing with 21 phages of N.C.T.C. The results obtained are shown in Table 1. Only 6 strains (2.7%) were typable at 1 RTD of phage. Of them, three were classified into type III, two into type II, and one was into type I. Typable strains increased to only 11 (5.0%) when 1,000 RTD of phage was used. Of the 11 strains, three belonged to type I, three to type II, two to type III, two to mixed type III+IV, and one to mixed type I+III.

The results of typing by use of bovine phages are shown in Table 2. Of the 221 strains submitted, only two (0.9%) belonging to phage type III were typable at 1 RTD, and ten (4.5%) at 1,000 RTD. The 10 strains consisted of three of type II, two of type I, two of type III, two of mixed type III+IV, and one of mixed type I+III.

All the strains typable with the two phage sets were staphylococci producing yellow pigment. None of 204 white-pigment producers exhibited sensitivity to any of the phages used.

Phage patterns of the typable strains are shown in Table 3. Both N.C.T.C. and bovine phage sets lysed the same strains, except one which was susceptible to only N.C.T.C. phage 80. Strain 631Y showed the same phage pattern as strain 654Y and strain 713Y as strain 715Y. The rest showed

Table 1. Typing of canine *Staph. aureus* by using the International Phage Set for Human *Staph. aureus*

Phage group	Number of strains	
	1 RTD	1,000 RTD
I	1	3
II	2	3
III	3	2
IV	0	0
I+III	0	1
III+IV	0	2
Untypable	215 (97.3)	210 (95.0)
Total	221	221

Table 2. Typing of canine *Staph. aureus* by using the International Phage Set for Bovine *Staph. aureus*

Phage group	Number of strains	
	1 RTD	1,000 RTD
I	0	2
II	0	3
III	2	2
I+III	0	1
III+IV	0	2
Untypable	219 (99.1)	211 (95.5)
Total	221	221

Table 3. Phage patterns of yellow-pigment producing *Staph. aureus* of canine origin

Strain No.	Phage set for :	
	Human staphylococcus	Bovine staphylococcus
649Y	29 (I)	29 (I)
407Y	80 (I)	Untypable
646Y	52A/79 (I)	52A (I)
654Y	3A/3C/55/71 (II)	3A/116 (II)
631Y	3A/3C/55/71 (II)	3A/116 (II)
553Y	3A (II)	3A/116 (II)
628Y	42E/53/77/83A (III)	42E/53 (III)
630Y	53/77/83A (III)	53 (III)
717Y	52A/79/42E/53 (I+III)	52A/42E/53 (I+III)
713Y	6/47/54/75/83A/84/42D (III+IV)	6/75/84/42D (III+IV)
715Y	6/47/54/75/83A/84/42D (III+IV)	6/75/84/42D (III+IV)

Remarks.

In parentheses is shown a phage group.

different patterns from one another. N.C.T.C. phage 83A lysed four strains, and phages 3A, 53 and 116 three strains each. Thus, no strain showing a particular phage pattern was common in canine staphylococci.

2. Source of strains and phage type

Of the 11 typable strains, four were originated from otitis externa, four from pyoderma, and three from the skin of dogs with otitis externa. No special relationship, however, was found between the phage type and the source of strains.

Discussion

There are several reports which deal with phage typing of staphylococci originated from the canine nasal cavity. Blouse and Meekins [1] pointed out that only 29 (9.5%) of 348 staphylococcal strains isolated from canine respiratory infections and from normal canine nasal cavities were typable by N.C.T.C. phages. Mann et al. [7, 8] found susceptibility to N.C.T.C. phages in 15% of 107 strains. Garner et al. [6] also obtained similar results, indicating that

11.6% of 214 strains were typed by either N.C.T.C. or bovine phages and that many of the typable strains were classified into group III and a mixed group. Daigo [4] reported that six (8.8%) and four (5.8%) of 68 strains were typable by N.C.T.C. and bovine phages, respectively.

Only a few workers, however, have studied phage typing of staphylococci originated from dogs with otitis externa or pyoderma. Coles [2] reported that of 313 strains isolated from the skin and external auditory canal of diseased dogs, seven strains, including six of skin origin and one of auditory canal origin, were typable by either N.C.T.C. or bovine phages. They classified one strain into group II, two into group III, two into a miscellaneous group, one into phage S-5 group, and one into a mixed group. According to Fouace and Guilhan [5], four (13.8%) of 29 strains isolated from pyogenic dermatitis were typable by N.C.T.C. phages, consisting of one strain of group I, one of group III, and two lysed by phage 187.

In the present study 221 strains isolated from the skin and external auditory canal of diseased and healthy dogs were subjected to phage typing. Of these strains, eleven (5.0%) and ten (4.5%) were typable by N.C.T.C. and bovine phages, respectively. These percentages of typable strains were a little lower than those, 5.7–15%, reported by other investigators. Moreover, all the typable strains were staphylococci producing yellow pigment. None of the white-pigment producers were lysed by any of N.C.T.C. or bovine phages.

Such a tendency was also shown by Ochi and Shimizu [12–14] who classified staphylococcal strains into the following six types on the basis of combinations of the origin of stains and their bio-types: A_1 and A_2 types (human origin), B_1 and B_2 types

(bovine and equine origin), C type (canine origin) and D type (equine origin). Type C strains were white-pigment producers and completely non-susceptible to lysis by N.C.T.C. phages.

Taking the results obtained by the present and previous authors into consideration, it is obvious that the existing phage sets are not adequate for the typing of canine staphylococci. Moreover, it seems to be impossible to select phages useful for the typing of canine staphylococci from the existing N.C.T.C. and bovine phages.

On the other hand, there was no significant difference in the group distribution of typable strains between the present and previous studies. These strains were distributed similarly in groups I, II, and III. It should be pointed out, however, that two strains were also lysed by phage 42D of group IV in combination with group III phages. This phage is known not to be lytic on staphylococci of human origin but to be active on animal strains, especially those of bovine origin. Therefore, the results mentioned above suggest the contamination of strains not only of human origin but also of bovine origin among staphylococci isolated from dogs. This suggestion is also supported by a similar rate of strains typable by N.C.T.C. phages and by bovine phages.

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