Phage Typing of Coagulase-negative Staphylococci Isolated from Various Animals and Humans in Japan

Akira SHIMIZU, Junichi KAWANO, and Shige KIMURA

Department of Animal Hygiene, Faculty of Agriculture, Kobe University, Kobe, Hyogo 657, Japan

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ABSTRACT. The phage typing of 226 strains of coagulase-negative staphylococci (CNS) isolated from 9 different animal species and humans was performed. CNS were identified using the simplified scheme of Kloos and Schleifer. With the human *Staphylococcus epidermidis* phage set of Pulverer, it was possible to type 109 (48.2%) of 226 strains of CNS at routine test dilution (RTD). Typabilities of the human, cattle, horse, swine, dog, rabbit, mouse, chicken, pigeon and quail strains were 60.0%, 54.5%, 50.0%, 40.7%, 100%, 53.8%, 100%, 37.7%, 0% and 100%, respectively. Although 33 different phage patterns were detected, 64 of the 109 typable strains were of phage pattern Ph5/Ph9/Ph10/Ph12/Ph13/U4/U14/U16/U20/U46. There was no significant relation between phage types and the origin of the strains. The phages Ph5, Ph9, Ph10, Ph12, Ph13, U4, U14, U16, U20 and U46 showed lytic activity on *S. xylosus*, *S. simulans*, *S. epidermidis*, *S. cohnii*, *S. haemolyticus*, *S. capitis*, *S. hominis* and *S. warneri*. The reacting ability of phages Ph6, Ph14, Ph15, Ph16 and U15 seemed to be limited to *S. xylosus* and *S. epidermidis*. Attempts were made to isolate phages for typing CNS strains from animals. Seven phages were isolated from lysogenic strains of CNS and their usefulness for the typing of CNS was evaluated. Of the 226 CNS strains examined, only 27 (11.9%) were typable at RTD. The temperate phages were of limited value in typing CNS strains of animal origin, although 9 of 117 strains untypable by Pulverer's phages were lysed by the present phages. —**KEY WORDS:** lysogenicity, phage typing, staphylococci.

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The first isolation of useful phages for typing *Staphylococcus epidermidis* strains from human specimens was reported by Verhoef *et al.* [19-21] in 1971-72. In 1975, Pulverer *et al.* [11] also described the isolation and characterization of 90 different phages obtained from human *S. epidermidis* strains, sixteen of which were used in their phage typing set [4]. Since then, attempts have been made by other several workers [2, 10, 16, 18] to type strains of *S. epidermidis* from humans using the phages isolated by these workers.

There are a few reports concerning the phage typing of coagulase-negative staphylococci (CNS) strains isolated from bovine normal milk and abnormal milk samples [5, 6, 15]. However, to our knowledge, there has been no report of any attempt to phage-type strains of CNS isolated from

various other animals. In Sweden, Holmberg [5], using the 18 human phages of Verhoef *et al.* [19-21], reported that only 7 (3.2%) of 218 strains of CNS isolated from bovine milk were typable at either routine test dilution (RTD) or 1,000 × RTD, and pointed out the unsuitability of Verhoef's human phages for typing the bovine milk strains of CNS. Holmberg [5] introduced a set of 11 phages for the phage typing of bovine strains of CNS and using these 11 phages, typed 49 (22.5%) of the 218 isolates from bovine milk in Sweden. Holmberg *et al.* [6] also examined phage types of 133 strains of CNS isolated from bovine milk samples collected in southeastern Minnesota in the United States using the 11 phages of the phage set and found only one typable strain. In Japan, Fukata *et al.* [75th Annual Meeting of the Japanese Society of Veteri-

Table 1. Origin and number of coagulase-negative staphylococci strains used

Origin	No. of individuals	No. of strains	Site of isolation	Staphylococcal species (No. of strains)
Human	22	30	Skin (H) ^{a)}	<i>S. simulans</i> (1), <i>S. epidermidis</i> (23), <i>S. hominis</i> (4), UC ^{b)} (2)
Cattle	8	11	Nares (H) Normal milk	<i>S. xylosus</i> (2), <i>S. epidermidis</i> (1), <i>S. cohnii</i> (2), <i>S. haemolyticus</i> (1), <i>S. hominis</i> (2), <i>S. saprophyticus</i> (1), UC (2)
Horse	13	24	Nares (H)	<i>S. xylosus</i> (7), <i>S. epidermidis</i> (2), <i>S. cohnii</i> (2), <i>S. haemolyticus</i> (4), <i>S. warneri</i> (5), <i>S. saprophyticus</i> (2), UC (2)
Swine	42	81	Skin (D) ^{c)} Nares (H)	<i>S. xylosus</i> (23), <i>S. simulans</i> (25), <i>S. cohnii</i> (6), <i>S. haemolyticus</i> (1), <i>S. capitis</i> (4), <i>S. hominis</i> (1), <i>S. warneri</i> (1), UC (20)
Dog	1	1	Nares (H)	<i>S. xylosus</i> (1)
Rabbit	13	13	Nares (H)	<i>S. xylosus</i> (3), <i>S. cohnii</i> (10)
Mouse	2	2	Skin (H)	<i>S. cohnii</i> (2)
Chicken	35	53	Nares (H) Skin (H)	<i>S. xylosus</i> (5), <i>S. simulans</i> (4), <i>S. epidermidis</i> (11); <i>S. cohnii</i> (8), <i>S. haemolyticus</i> (13), <i>S. capitis</i> (4), <i>S. hominis</i> (1), UC (7)
Pigeon	1	1	Nares (H)	<i>S. xylosus</i> (1)
Quail	7	10	Intestinal contents Nares (H)	<i>S. xylosus</i> (1), <i>S. simulans</i> (7), <i>S. capitis</i> (1), <i>S. warneri</i> (1)

a) Healthy animals.

b) Unidentifiable.

c) Exudative epidermitis, a skin involvement with a clinical sign of erythematous skin rash and no exudation.

nary Science, 1973], using 9 phages they had isolated from the sewage of dairy farms, reported that 46 (49.5%) of 93 strains of CNS isolated from the milk of cows suffering from mastitis were typable at 100 × RTD. Sincoway and Kume [15], using 9 phages of Fukata *et al.*, found that only 35 (24.8%) of the 141 bovine milk strains of CNS consisting of 9 staphylococcal species and unidentifiable strains were typed at 100 × RTD.

One purpose of the present study was to determine whether the phages of Pulverer's *S. epidermidis* set [4, 11] could be utilized for typing strains of CNS consisting of 9 staphylococcal species and unidentifiable strains isolated from various animals and humans in Japan. In addition, this paper describes the typing results of 7 phages isolated from lysogenic strains of CNS of animal origin in the course of the present

study.

MATERIALS AND METHODS

Bacterial strains: A total of 226 strains of CNS isolated between 1970 and 1983 from 9 species of healthy or diseased animals and healthy humans in 9 prefectures in Japan were used. These CNS strains were identified by the simplified scheme of Kloos and Schleifer [8]. The number of strains and their origins are shown in Table 1. All strains had been maintained in trypto-soy agar (Nissui Seiyaku Co., Tokyo) stab cultures at ambient temperature. Before use, stock cultures of CNS were transferred to nutrient agar containing 7.5% NaCl, incubated at 37°C for 48 hours, then incubated in heart infusion broth (Nissui) for 6 to 18 hours at 37°C, and used for phage typing.

Table 2. Phage typing of 226 coagulase-negative staphylococci with the phage set of Pulverer

Staphylococcal species	Origin of strains ^{a)}										Total (%)
	Hu	Ca	Ho	Sw	Do	Rab	Mo	Ch	Pi	Qu	
<i>S. xylosus</i>	—	1/ 2 ^{b)}	4/ 7	10/23	1/1	2/ 3	—	2/ 5	0/1	1/ 1	21/43(48.8)
<i>S. simulans</i>	0/ 1	—	—	9/25	—	—	—	3/ 4	—	7/ 7	19/37(51.4)
<i>S. epidermidis</i>	14/23	1/ 1	0/ 2	—	—	—	—	4/11	—	—	19/37(51.4)
<i>S. cohnii</i>	—	1/ 2	1/ 2	4/ 6	—	5/10	2/2	3/ 8	—	—	16/30(53.3)
<i>S. haemolyticus</i>	—	1/ 1	2/ 4	0/ 1	—	—	—	1/13	—	—	4/19(21.1)
<i>S. capitis</i>	—	—	—	1/ 4	—	—	—	3/ 4	—	1/ 1	5/ 9(55.6)
<i>S. hominis</i>	4/ 4	0/ 2	—	0/ 1	—	—	—	0/ 1	—	—	4/ 8(50.0)
<i>S. warneri</i>	—	—	4/ 5	0/ 1	—	—	—	—	—	1/ 1	5/ 7(71.4)
<i>S. saprophyticus</i>	—	0/ 1	0/ 2	—	—	—	—	—	—	—	0/ 3
Unidentifiable	0/ 2	2/ 2	1/ 2	9/20	—	—	—	4/ 7	—	—	16/33(48.5)
total (%)	18/30 (60.0)	6/11 (54.5)	12/24 (50.0)	33/81 (40.7)	1/1 (100)	7/13 (53.8)	2/2 (100)	20/53 (37.7)	0/1 (0)	10/10 (100)	109/226 (48.2)

a) Abbreviations: Hu, Human; Ca, Cattle; Ho, Horse; Sw, Swine; Do, Dog; Rab, Rabbit; Mo, Mouse; Ch, Chicken; Pi, Pigeon; Qu, Quail.

b) No. of typable strains/No. of strains tested.

Phages: The 15 phages used for phage typing were derived from Pulverer's *S. epidermidis* set [4, 11]. These phages were kindly provided by Dr. S. Mitsuhashi, Gunma University, via Dr. K. Kurosaka, The Tokyo Jikeikai University School of Medicine, Tokyo, Japan. This phage set included the following phage/host combinations: U4/V 1852, U14/V 505, U15/368, U16/V 505, U20/V 505, U46/16, Ph5/53544, Ph6/Q 239, Ph9/V 542, Ph10/6497, Ph12/SK 176 SI, Ph13/23 SV, Ph14/Q 239, Ph15/SK 176 SI and Ph16/TGHGB.

Culture media: Nutrient broth, consisting of 10 g of beef extract (Mikuni Chemical Co., Tokyo), 10 g of polypeptone (Daigo Nutritive Chemical Co., Osaka), 2 g of NaCl, and 1,000 ml of distilled water (pH 7.4) and nutrient agar medium (nutrient broth added with powdered agar at 1.2%) were used for propagation and titration of phages and for phage typing.

Phage typing: Phage typing was performed at RTD only, which was the highest phage dilution to produce confluent lysis of the propagating strains. Strains showing

strong lysis (more than 50 plaques to confluent lysis) were regarded as typable.

Isolation of phages: The detection of lysogenic strains was performed for isolation of phages. Induction of phages, detection of lysogenic strains, and technical procedures used for isolation, propagation and titration of phages were the same as those described previously [13, 14].

RESULTS

1. Phage typing using Pulverer's set

Typability: The results of phage typing of 226 strains of CNS are shown in Table 2 according to origins from which they were derived and staphylococcal species. A total of 109 strains (48.2%) were typable with one or more of the phages at RTD. Typabilities of the human, cattle, horse, swine, dog, rabbit, mouse, chicken, pigeon and quail strains were 60.0%, 54.5%, 50.0%, 40.7%, 100%, 53.8%, 100%, 37.7%, 0% and 100%, respectively. The proportion of typable strains varied substantially between the different staphylococcal species; 71.4%

Table 3. Phage patterns of 109 strains typable with Pulverer's phage set

Tentative group	Phage pattern	Origin (Staphylococcal species ^{a)} and No. of strains)
I	Ph5 Ph6 Ph9 Ph10 Ph12 Ph13 Ph14 Ph15 Ph16 U4 U14 U15 U16 U20 U46 Ph5 Ph6 Ph9 Ph10 Ph12 Ph13 Ph15 Ph16 U4 U14 U15 U16 U20 U46 Ph5 Ph9 Ph10 Ph12 Ph13 Ph16 U4 U14 U15 U16 U20 U46 Ph5 Ph6 Ph9 Ph10 Ph12 Ph13 Ph14 Ph16 U4 U14 U15 U16 U20 U46 Ph5 Ph9 Ph10 Ph12 Ph13 Ph15 U4 U14 U15 U16 U20 U46	Chicken (<i>xyl</i> ,1; <i>epi</i> ,1), Horse (<i>xyl</i> ,2), Human (<i>epi</i> ,3) Swine (<i>xly</i> ,1) Quail (<i>xly</i> ,1) Human (<i>epi</i> ,1) Human (<i>epi</i> ,2)
II	Ph5 Ph9 Ph12 Ph13 U4 U14 U16 U20 U46 Ph5 Ph9 Ph10 Ph12 Ph13 U4 U14 U16 U20 U46 Ph5 Ph9 Ph10 Ph12 Ph13 U4 U14 U16 U46 Ph5 Ph10 Ph12 U4 U14 U16 Ph5 Ph9 Ph10 Ph12 Ph13 Ph15 U4 U14 U16 U20 U46 Ph5 Ph10 Ph12 Ph13 U4 U16 U20 U46 Ph5 Ph10 Ph12 Ph13 U4 U14 U16 U20 Ph5 Ph10 Ph12 Ph13 U14 U16 U46 Ph5 Ph10 Ph12 Ph13 U4 U16 Ph5 Ph10 Ph12 Ph13 U4 Ph5 Ph12 Ph13 U4 U20 Ph5 Ph12 Ph13 U4 U16 U20 U46 Ph5 Ph12 Ph13 U4 U14 Ph5 Ph12 Ph13 Ph5 U4 U14 U16 U46 Ph5 U4 U16 U20 Ph5 Ph9 Ph10 Ph12 Ph13 U4 Ph9 Ph10 Ph12 Ph13 U4 U14 U16 U20 U46 Ph12 Ph13 U4 Ph12 Ph13 Ph12 Ph13 U4 U16 U20 U46 U4 U16 U20 U46 U4 U16 U46 U4 U16 U4 U16 U46	Chicken (<i>coh</i> ,1) Swine (<i>xly</i> ,8; <i>sim</i> ,4; <i>coh</i> ,3; UC,7), Chicken (<i>cap</i> ,2; <i>coh</i> ,1; <i>epi</i> ,3; UC,3), Rabbit (<i>coh</i> ,5), Horse (UC,1; <i>hae</i> ,1; <i>war</i> ,4; <i>xly</i> ,1; <i>coh</i> ,1), Mouse (<i>coh</i> ,2), Cattle (<i>coh</i> ,1; <i>epi</i> ,1; UC,1), Quail (<i>cap</i> ,1; <i>sim</i> ,7; <i>war</i> ,1), Human (<i>epi</i> ,5; <i>hom</i> ,1) Chicken (<i>sim</i> ,1; <i>xly</i> ,1) Chicken (<i>sim</i> ,1) Chicken (<i>cap</i> ,1) Rabbit (<i>xyl</i> ,2) Cattle (<i>hae</i> ,1) Swine (<i>sim</i> ,1) Swine (UC,1) Cattle (UC,1) Swine (<i>cap</i> ,1) Swine (UC,1) Swine (<i>xyl</i> ,1) Cattle (<i>xyl</i> ,1) Chicken (UC,1) Chicken (<i>hae</i> ,1) Horse (<i>hae</i> ,1) Human (<i>epi</i> ,1) Dog (<i>xyl</i> ,1) Human (<i>epi</i> ,1) Swine (<i>sim</i> ,1), Chicken (<i>sim</i> ,1) Horse (<i>xyl</i> ,1) Swine (<i>sim</i> ,1) Swine (<i>sim</i> ,1), Chicken (<i>coh</i> ,1), Human (<i>hom</i> ,1) Swine (<i>sim</i> ,1) Human (<i>epi</i> ,1) Swine (<i>coh</i> ,1)
III	U15	Human (<i>hom</i> ,2)

a) Abbreviations: *xyl*, *S. xylosus*; *sim*, *S. simulans*; *epi*, *S. epidermidis*; *coh*, *S. cohnii*; *hae*, *S. haemolyticus*; *cap*, *S. capitis*; *hom*, *S. hominis*; *war*, *S. warneri*; UC, Unidentifiable.

of strains of *S. warneri* were sensitive compared with 21.1-55.6% of the strains in 7 other species. About half (48.5%) of the unidentifiable strains were sensitive.

Phage patterns: Phage patterns of the 109 typable strains are shown in Table 3. A total of 33 phage patterns detected were grouped arbitrarily into the following 3 major phage

Table 4. Frequency of lysis with Pulverer's phages

Species		Phage														
		Ph									U					
		5	6	9	10	12	13	14	15	16	4	14	15	16	20	46
<i>S. xylosus</i>	(43) ^{a)}	19 ^{b)}	4	15	17	20	21	3	4	5	20	16	5	18	17	18
<i>S. simulans</i>	(37)	14	0	12	14	16	13	0	0	0	16	14	0	17	12	15
<i>S. epidermidis</i>	(37)	17	5	18	18	19	19	5	6	5	19	18	7	18	18	18
<i>S. cohnii</i>	(30)	14	0	14	13	14	14	0	0	0	15	14	0	16	14	16
<i>S. haemolyticus</i>	(19)	4	0	2	3	3	3	0	0	0	4	2	0	3	3	1
<i>S. capitis</i>	(9)	5	0	4	4	5	5	0	1	0	5	4	0	4	5	4
<i>S. hominis</i>	(8)	1	0	1	1	1	1	0	0	0	2	1	2	2	1	2
<i>S. warneri</i>	(7)	5	0	5	5	5	5	0	0	0	5	5	0	5	5	5
<i>S. saprophyticus</i>	(3)	0	0	0	0	0	0	0	0	0	0	0	0	0	0	0
Unidentifiable	(33)	16	0	12	13	15	15	0	0	0	16	12	0	15	12	13
Total	(226)	95	9	83	88	98	96	8	11	10	102	86	14	98	87	92

a) No. of strains tested.

b) No. of strains showing lysis of more than one plaque at 1×RTD.

Table 5. Occurrence of lysogenic strains

Species	No. of strains	lysogenic strains	
		No.	%
<i>S. xylosus</i>	18	0	0
<i>S. cohnii</i>	15	1	6.7
<i>S. simulans</i>	12	6	50.0
<i>S. haemolyticus</i>	3	0	0
<i>S. capitis</i>	2	0	0
<i>S. hominis</i>	1	0	0
<i>S. warneri</i>	1	0	0
Unidentifiable	9	7	77.8
Total	61	14	23.0

groups; group I- phage patterns of strains lysed by 12 or more of the 15 phages; group II- phage patterns of strains lysed by all or any of the phages Ph5, Ph9, Ph10, Ph12, Ph13, U4, U14, U16, U20 and U46; and group III- phage patterns of strains lysed by any of the phages Ph6, Ph14, Ph15, Ph16 and U15. Of the 109 typable strains, 95 (87.2%) belonged to group II, 12 (11.0%) to group I and 2 (1.8%) to group III. Among those of group II strains, the phage pattern Ph5/Ph9/Ph10/Ph12/Ph13/U4/U14/U16/U20/U46 was the most common, appearing in 64 strains. There was no significant relation between phage types and

Table 6. Experimental phages and their propagating strains

phage			Propagating strain		
No.	Species	Origin	No.	Species	Origin
1	Unidentifiable	swine	123	<i>S. simulans</i>	swine
4	<i>S. cohnii</i>	mouse	57	<i>S. cohnii</i>	mouse
6	<i>S. simulans</i>	chicken	88	<i>S. simulans</i>	swine
8	<i>S. simulans</i>	chicken	95	<i>S. haemolyticus</i>	cattle
15	Unidentifiable	swine	74	<i>S. xylosus</i>	horse
17	<i>S. simulans</i>	swine	120	<i>S. simulans</i>	swine
18	<i>S. simulans</i>	swine	198	<i>S. simulans</i>	quail

the origin of the strains.

Lytic range of *Pulverer* phages: In order to analyse the lytic ranges of the 15 phages, the phages of $1 \times \text{RTD}$ were applied to a total of 226 strains of CNS. The number of strains showing lysis of more than one plaque by each phage is presented in Table 4. It was evident that the phages Ph5, Ph9, Ph10, Ph12, and Ph13 of the Ph series and the phages U4, U14, U16, U20, and U46 of the U series were highly lytic active against all 8 species of CNS except for *S. saprophyticus*. The reacting ability of the phages Ph6, Ph14, Ph15, Ph16 and U15 seemed to be limited largely to both *S. xylosus* and *S. epidermidis*.

2. Phage typing using phages isolated in this study

Occurrence of lysogenic strains: A total of 61 strains consisting of 7 staphylococcal species and unidentifiable strains were ex-

amined for lysogenicity. Lysogenicity was detected using the mitomycin C induction technique. Each strain tested for lysogenicity was used, in turn, as an indicator strain. In this way, a total of 3660 combinations (61×60) were made. Of the 61 strains, 14 (23.0%) were proved to be phage carriers (Table 5), and 33 (54.1%) were shown to be susceptible to any of the phages released from the carriers. The lysogenic strains were detected only in *S. cohnii* (1/15), *S. simulans* (6/12) and unidentifiable strains (7/9). As a result, 7 phages were selected from the 14 phages for the typing of CNS strains by judging their activity ranges, propagation abilities and degrees of lysis (Table 6).

Typability: The present phages were tested on the 226 strains of CNS (109 typable and 117 non-typable with the *Pulverer*'s phage set). Of the 109 typable strains, 18 were also lysed by the present phages at RTD (Table 7). Only 9 of 117 non-typable strains could be typed with the present phages. This rate was far lower than the 48.2% obtained by use of the phages of the *Pulverer* set. Phage patterns of the 27 typable strains are shown in Table 8 according to staphylococcal species. The phages released from strains of *S. simulans* (phages 6, 8, 17, 18) and *S. cohnii* (phage 4) were largely active against strains of the corre-

Table 7. Relationship between *Pulverer*'s phages and our experimental phages

Experimental phages	<i>Pulverer</i> 's phages	
	Typable (n=109)	Untypable (n=117)
Typable (n=27)	18	9
Untypable (n=199)	91	108
Total	109	117

Table 8. Phage patterns of 27 strains typable with experimental phages

Phage pattern	Species (origin and no. of strains typable)
1/15/17/18	<i>S. xylosus</i> (ho,1) ^a , <i>S. warneri</i> (ho,1)
1/15/17	<i>S. xylosus</i> (ho,1)
1/15	<i>S. xylosus</i> (ho,1)
1	<i>S. xylosus</i> (ho,1), <i>S. simulans</i> (sw,1)
4	<i>S. cohnii</i> (rab,1, mo,1)
6/8	<i>S. simulans</i> (sw,2), <i>S. haemolyticus</i> (ch,1, ca,1)
15	<i>S. simulans</i> (sw,1), <i>S. xylosus</i> (ch,1)
17/18	<i>S. simulans</i> (qu,7), <i>S. capitis</i> (qu,1)
17	<i>S. simulans</i> (sw,3), <i>S. epidermidis</i> (hu,1)
18	<i>S. simulans</i> (sw,1)

a) Abbreviations: ho, horse; sw, swine; rab, rabbit; mo, mouse; ch, chicken; ca, cattle; qu, quail; hu, human.

sponding species.

DISCUSSION

Attempts have been made by several workers [4, 9, 17] to type strains of CNS from humans using the phages isolated by Pulverer *et al.* [11]. In West Germany, Heczko *et al.* [4] found that 595 (72.5%) of 821 strains of *S. epidermidis* isolated from normal human skin and from acne lesions were typable by one or more of the 16 phages including phage U33 and that phage patterns of Ph10/Ph12/U14/U16 and Ph5/Ph6/Ph9/Ph10/Ph12/Ph13/Ph14/Ph15/Ph16/U4/U14/U15/U16/U20/U33/U46 were the most common among the strains. Kurosaka [9] in Japan found that phage patterns of Ph5/Ph9/Ph10/Ph12/Ph13/U4/U14/U16/U46 and Ph5/Ph6/Ph9/Ph10/Ph12/Ph13/Ph14/Ph15/Ph16/U4/U14/U15/U16/U20/U46 were the predominant types among strains of CNS isolated from the nostrils, throats or skins of healthy humans and from clinical materials (pus and urine). Staal *et al.* [17] observed that strains which were sensitive to phages 5, 9, 10, 12, 13 of the Ph series and phages 4, 14, 15, 16, 20, 46 of the U series were common in CNS of human origin. In the present study, the typable strains were differentiated into 33 phage patterns indicating that predominant pattern of strains from 7 kinds of animals and humans was Ph5/Ph9/Ph10/Ph12/Ph13/U4/U14/U16/U20/U46. Also, the present study found no significant relation between phage patterns and origin of the strains. Previous investigators [4, 9, 17] described a high proportion of strains susceptible to phages Ph6, Ph14, Ph15, Ph16 or U15 in human CNS. On the other hand, the data obtained in the present study indicated low incidence of phage patterns including these phages in animal CNS. These facts seem to strongly suggest that phage susceptibilities of animal CNS are somewhat different from those of hu-

man strains. Further experiments will be carried out to determine this point.

In the present study, some of the phages, such as Ph5, Ph9, Ph10, Ph12, Ph13, U4, U14, U16, U20 and U46, were proved to be extraordinarily active against several other different staphylococcal species as well as against *S. epidermidis*, the phages generally having a broader generic specificity. On the other hand, phages Ph6, Ph14, Ph15, Ph16 and U15 were largely active with *S. xylosum* and *S. epidermidis*.

Most of the phages used for typing *S. aureus* had been isolated from lysogenic *S. aureus* strains. Lysogenicity in *S. epidermidis* strains was thought to be rare [1], although some phages had been isolated [12]. According to Verhoef *et al.* [19], 45 (26.0%) of 173 strains of *S. epidermidis* from human sources were found to be lysogenic, as a result of mitomycin C induction experiment. In order to obtain new useful phages in the present study, we examined strains of CNS from various animals and found lysogenicity in 14 (23.0%) of 61 strains of CNS consisting of 7 staphylococcal species and unidentifiable strains tested, although this frequency was lower than that in *S. aureus* [13] or *S. hyicus* subsp. *hyicus* [3, 7]. The 14 lysogenic strains were detected in *S. cohnii* and *S. simulans* out of 7 staphylococcal species and in unidentifiable strains. The detection rate of lysogenic strains depends not only on the method of induction, such as by the use of mitomycin C or ultraviolet irradiation, but also on the number and variety of indicator strains used.

In the present study, 7 phages could be detected in lysogenic strains of *S. cohnii* (n=1), *S. simulans* (n=4) and of unidentifiable strains (n=2) of animal origin and applied to the typing of 226 strains of CNS originating from various animals and humans. As a result, only 27 (11.9%) of the 226 strains tested were typable at 1 × RTD.

None of the 7 phages isolated in the present study appeared capable of increasing significantly the percentage of typable strains, although 9 of 117 strains untypable by the phages of the Pulverer set could be typed with any of the 7 phages. However, it was particularly worthy of notice that the 7 phages isolated from lysogenic strains of animal origin were much more highly active with animal strains than with human strains.

In conclusion, the phages isolated by Pulverer *et al.* [11] appeared to be useful for typing strains of CNS from various animals, but not at satisfactory percentages. Accordingly, additional phages will be needed in order to increase the number of typable strains.

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要 約

動物およびヒト由来コアグラーゼ陰性ブドウ球菌のファージ型別：清水 晃・河野潤一・木村 重（神戸大学農学部家畜衛生学教室）——9種類の健康・病例動物およびヒトから分離されたコアグラーゼ陰性ブドウ球菌（CNS）226株について、Kloos & Schleiferの簡易同定法によって菌種分類し、ついでPulvererらのヒト由来*Staphylococcus epidermidis* ファージセットを用いて、型別を試みた。226株中109株（48.2）%が1 xRTDで型別でき、宿主由来別の型別率はヒト60.0%，ウシ54.5%，ウマ50.0%，ブタ40.7%，イヌ100%，ウサギ53.8%，マウス100%，ニワトリ37.7%，ハト0%，ウズラ100%であった。型別された菌株のファージパターンは合計33であり、最も多いパターンはPh5/Ph9/Ph10/Ph12/Ph13/U4/U14/U16/U20/U46（64株）で、ついで15種すべてのファージに溶菌される株（7株）であった。菌種との関連で見ると、Phシリーズの5，9，10，12，13およびUシリーズの4，14，16，20，46は*S. xylosus*, *S. simulans*, *S. epidermidis*, *S. cohnii*, *S. haemolyticus*, *S. capitis*, *S. warneri*と多くの菌種に溶菌活性を示した。ファージPh6，Ph14，Ph15，Ph16，U15の溶菌域は*S. xylosus*と*S. epidermidis*の2菌種に限定されていた。また、動物由来CNSの溶原株から分離した7ファージを、226株に応用したところ、27株（11.9%）が型別されたにすぎなかった。しかし型別できた菌株は1株を除いてすべて動物由来のものであった。*S. simulans*および*S. cohnii*の溶原株由来ファージはそれぞれの菌種の菌株を溶菌する傾向を示した。