Bacteriophage Typing of *Staphylococcus aureus* Isolated from Laboratory and Wild Rodents

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ABSTRACT. Phage typing was performed on 267 strains of *Staphylococcus aureus* isolated from laboratory (mice, rats, nude mice, rabbits) and wild (*Rattus norvegicus*, *Rattus rattus*, mice, a ferret) rodents, using the phages of international basic sets for typing human and bovine *S. aureus*. Of the 139 laboratory rodent strains tested, 105 (75.5%) could be typed with the human set and 130 (93.5%) with the bovine set, whereas of the 128 wild rodent strains, 42 (32.8%) could be typed with the human set and 80 (62.5%) with the bovine set. When the human phage set was used, the predominant phage groups of typable strains in laboratory rodents were group III in rabbits, II in mice, I and mixed groups in rats and mixed groups in nude mice. Among the wild rodent strains, group III was the most common. There was a significant difference in phage group distribution of typable strains between mice and rats which were obtained from the same laboratory animal facility. When the bovine phage set was applied to strains isolated from *Rattus norvegicus*, *Rattus rattus* and wild mice, predominant phage groups of typable strains were significantly different among the three species of animals; group IV in *Rattus norvegicus*, group III in *Rattus rattus* and group II in wild mice.—**KEY WORDS—** phage typing, rodents, *Staphylococcus aureus*.

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Numerous studies have been published on the phage typing of *Staphylococcus aureus* isolated from various animals in Japan. The majority of these studies have been conducted with strains isolated from bovine [13], equine [15], porcine [9], canine [14] and poultry [12, 17] sources. *S. aureus* has also been isolated from a variety of common laboratory rodents including mice, rats, guinea pigs and rabbits [1, 3, 5, 7, 11, 16], although the resident status of this organism and that of other coagulase-negative staphylococci in these animals are uncertain. Wild rodents have been shown to carry *S. aureus* in their nares, skin or intestinal contents [8, 9, 16]. This paper reports the results of the phage typing of strains of *S. aureus* isolated from laboratory and wild rodents in Japan using the phages of international basic sets for typing human and bovine *S. aureus*.

MATERIALS AND METHODS

Bacterial strains: A total of 267 strains of *S. aureus* isolated between 1970 and 1986 from laboratory (n=139) and wild (n=128) rodents in Japan were used. The numbers of *S. aureus* strains obtained from each host or source are shown in Tables 1, 2 and 3. Most (n=227) of them had been used in the previous biotyping study [16], but 40 strains were additionally included in the present study. These strains were isolated by Dr. H. Tamura of the Laboratory Animal Center, Teikyo University School of Medicine, Tokyo, Japan from 20 nude mice with subcutaneous abscesses in the period 1985–1986.

Bacteriophages: The following two phage sets were used; 21 phages of the international set for typing human *S. aureus* (group

Table 1. Phage typing of *S. aureus* strains from laboratory and wild rodents with the human and the bovine sets of phages

Origin	No. of strains	No. (%) of strains typable with					
		Human set			Bovine set		
		RTD	100×RTD ^{a)}	Subtotal	RTD	100×RTD ^{a)}	Subtotal
Laboratory							
Rabbit	9	8	1	9 (100 %)	8	1	9 (100 %)
Mouse	25	0	22	22 (88.0)	0	22	22 (88.0)
Rat	64	7	26	33 (51.6)	48	10	58 (90.6)
Nude mouse	41	0	41	41 (100 %)	0	41	41 (100 %)
Subtotal	139	15	90	105 (75.5)	56	74	130 (93.5)
Wild							
<i>Rattus norvegicus</i> ^{b)}	61	9	3	12 (19.7)	7	18	25 (41.0)
<i>Rattus rattus</i>	27	24	0	24 (88.9)	24	0	24 (88.9)
Mouse	31	4	2	6 (19.4)	25	6	31 (100 %)
Ferret	9	0	0	0	0	0	0
Subtotal	128	37	5	42 (32.8)	56	24	80 (62.5)
Total	267	52	95	147 (55.1)	112	98	210 (78.7)

a) Strains untypable at RTD were tested at 100×RTD.

b) All strains were kindly supplied by Dr. M. Mori [9].

I-29, 52, 52A, 79, 80, group II-3A, 3C, 55, 71, group III-6, 42E, 47, 53, 54, 75, 77, 83A, 84, 85, miscellaneous-81, 187) and 16 phages of the international set for typing bovine *S. aureus* (group I-29, 52A, group II-3A, 116, group III-6, 42E, 53, 75, 84, group IV-42D, 102, 107, 117, miscellaneous-118, 119, 78).

Phage typing: Phage typing was performed first at routine test dilution (RTD) and strains untypable at RTD were retested at 100×RTD. The culture media used for propagation and titration of the phages, and the phage typing method, were the same as described previously [12]. Strains showing strong lysis (more than 50 plaques to confluent lysis) were recorded as typable.

RESULTS

Typabilities of 267 strains of *S. aureus* isolated from laboratory and wild rodents with the 21 phages of the human set and the

16 phages of the bovine set are shown in Table 1.

Human set: A total of 147 (55.1%) strains were typable, 52 strains at RTD and additional 95 at 100×RTD. Typabilities of the strains from rabbits, mice, rats, nude mice, *Rattus norvegicus*, *Rattus rattus*, wild mice and a ferret were 100%, 88.0%, 51.6%, 100%, 19.7%, 88.9%, 19.4% and 0%, respectively.

Bovine set: A total of 210 (78.7%) strains were typable, 112 strains at RTD and 98 at 100×RTD. Typabilities of strains from rabbits, mice, rats, nude mice, *Rattus norvegicus*, *Rattus rattus*, wild mice and a ferret were 100%, 88.0%, 90.6%, 100%, 41.0%, 88.9%, 100% and 0%, respectively. In total, the percentage typable with the bovine set was far higher than that with the human set.

Table 2 shows the results of typing by use of the phages of human set. Details of the results according to animal species were as

Table 2. Phage typing of 267 *S. aureus* strains from laboratory and wild rodents with the human phage set

Origin	Source	No. of strains	Phage group and pattern ^{a)}				
			I	II	III	Mixed	UT ^{b)}
Laboratory							
Rabbit (H) ^{c)}	nares	9	—	—	8	1	—
Mouse (D)	abscesses	2	—	—	—	—	2
Mouse (H)	intestinal contents, nares, skin	23	—	22	—	—	1
Rat (H)	intestinal contents, nares, skin	64	19	—	2	12	31 ^{d)}
Nude mouse (D)	abscesses	40	—	—	—	40	—
Nude mouse (H)	intestinal contents	1	—	—	—	1	—
Wild							
<i>Rattus norvegicus</i> (H)	skin	61	—	—	12	—	49
<i>Rattus rattus</i> (H)	intestinal contents, nares	27	1	—	20	3	3
Mouse (H)	intestinal contents	31	2	—	4	—	25
Ferret (H)	intestinal contents, nares, skin	9	—	—	—	—	9

a) Phage patterns of typable strains were as follows. Rabbit strains: 53/77 (7 strains), 53/75/77/85+ (1), 29/52/79/54+ (1). Mouse strains: 3A/3C/71 (21), 71+ (1). Rat strains: 29+ (1), 52+ (17), 29/52+ (1), 53/77 (1), 6/53/77 (1), 29/52/42E+ (10), 29/52/81+ (1), 52/85 (1). Nude mouse strains: 52A/79/47/53/77/83A/85 (41). *Rattus norvegicus* strains: 6/42E/47/54/75/77 (1), 6/53/54/77/85/84/83A (1), 6/54/75/84+ (2), 53/54/77/85/84/83A (1), 53/77+ (1), 53/77/85/84/83A (1), 75+ (3), 85/84 (1), 85 (1). *Rattus rattus* strains: 29 (1), 53/77+ (20), 29/53 (3). Wild mouse strains: 52+ (2), 75+ (2), 85 (2).

b) Untypable.

c) (H): Healthy, (D): Diseased.

d) All the 31 strains were typable with bovine phage 116 at RTD.

follows.

Rabbit strains: Nine typable strains were distributed into 3 phage patterns of 53/77 (n=7), 53/75/77/85+ (n=1) and 29/52/79/54+ (n=1).

Mouse strains: All of 22 typable strains isolated from the nares, skin and intestinal contents of healthy mice belonged to group II, showing the main phage pattern of 3A/3C/71.

Rat strains: Thirty-three of 64 strains were typable. Of the 33 typable strains, 19 belonged to group I, 2 to group III and 12 to mixed groups. The distribution of phage groups in rat strains was significantly different from those in mouse strains, in spite of the fact that these 2 species of animals were

obtained from the same laboratory animal facility. It was noteworthy that all of 31 strains untypable by the phages of human set were typed with group II bovine phage 116 at RTD.

Nude mouse strains: All of 40 typable strains isolated from nude mice with subcutaneous abscesses showed an identical phage pattern of 52A/79/47/53/77/83A/85. One strain obtained from the intestinal contents of a healthy nude mouse also showed the same phage pattern.

***Rattus norvegicus* strains:** All of 12 typable strains belonged to group III, showing 9 phage patterns.

***Rattus rattus* strains:** Twenty-four typable strains were distributed into 3 phage pat-

Table 3. Phage typing of 267 *S. aureus* strains from laboratory and wild rodents with the bovine phage set

Origin	Source	No. of strains	Phage group and pattern ^{a)}						
			I	II	III	IV	Mis	Mixed	UT ^{b)}
Laboratory									
Rabbit (H) ^{c)}	nares	9	—	—	8	—	—	1	—
Mouse (D)	abscesses	2	—	—	—	—	—	—	2
Mouse (H)	intestinal contents, nares, skin	23	—	21	—	1	—	—	1
Rat (H)	intestinal contents, nares, skin	64	—	31	2	—	11	14	6
Nude mouse (D)	abscesses	40	—	—	—	—	—	40	—
Nude mouse (H)	intestinal contents	1	—	—	—	—	—	1	—
Wild									
<i>Rattus norvegicus</i> (H)	skin	61	—	—	7	15	2	1	36
<i>Rattus rattus</i> (H)	intestinal contents, nares	27	1	—	15	—	—	8	3
Mouse (H)	intestinal contents	31	4	23	—	—	2	2	—
Ferret (H)	intestinal contents, nares, skin	9	—	—	—	—	—	—	9

a) Phage patterns of typable strains were as follows. Rabbit strains: 53 (7 strains), 53/75 (1), 29/102/78 (1). Mouse strains: 3A (19), 3A/116 (2), 102 (1). Rat strains: 116 (31), 53 (1), 6/53 (1), 118 (11), 29/42E/42D/102/118 (10), 29/42D/102/118+ (2), 29/102/118 (1), 42D/118 (1). Nude mouse strains: 52A/53/102/107/118 (41). *Rattus norvegicus* strains: 6/75+ (2), 53+ (3), 6/53+ (1), 75+ (1), 117 (15), 118 (2), 6/42E/75/42D (1). *Rattus rattus* strains: 29 (1), 53+ (15), 29/53 (3), 53/118+ (5). Wild mouse strains: 52A+ (4), 116 (23), 118+ (2), 75/42D/118 (2).

b) Untypable.

c) (H): Healthy, (D): Diseased.

terns of 29 (n=1), 53/77+ (n=20) and 29/53 (n=3).

Wild mouse strains: Six typable strains were distributed into 3 phage patterns of 52+ (n=2), 75+ (n=2) and 85 (n=2).

Ferret strains: None of 9 strains was sensitive to any of the 21 human phages at RTD and 100×RTD used in this study.

Table 3 shows the results of typing by use of the phages of bovine set. Details of the results according to animal species were as follows.

Rabbit strains: Nine typable strains were distributed into 3 phage patterns of 53 (n=7), 53/75 (n=1) and 29/102/78 (n=1).

Mouse strains: Twenty-one of 22 typable strains belonged to group II, showing the main phage pattern of 3A.

Rat strains: Fifty-eight of 64 strains were

typable. Of the 58 typable strains, 31 belonged to group II, 2 to group III, 11 to miscellaneous and 14 to mixed groups. All of the 31 strains belonging to group II showed an identical phage pattern of 116.

Nude mouse strains: All of 41 typable strains isolated from nude mice with or without subcutaneous abscesses showed an identical phage pattern of 52A/53/102/107/118.

***Rattus norvegicus* strains:** Fifteen of 25 typable strains showed a phage pattern of 117, and the remaining 10 were distributed into 6 different patterns.

***Rattus rattus* strains:** Twenty-four typable strains showed four phage patterns but 23 of them were lysed with phage 53.

Wild mouse strains: Twenty-three of 31 typable strains showed a phage pattern of

116.

Ferret strains: None of 9 strains was sensitive to any of the 16 bovine phages at RTD and 100×RTD used in this study.

DISCUSSION

In foreign countries, several studies [1–3, 6, 10, 11] have been done on the phage typing of *S. aureus* of laboratory rodent origin by use of the international phage set, which was established for the typing of human *S. aureus*, resulting in a high frequency of typable strains. Such studies, however, have rarely been performed on *S. aureus* from laboratory rodents in Japan [4, 18].

In the present study, strains of *S. aureus* isolated from laboratory rodents such as rabbits, rats, mice and nude mice, were subjected to typing with both the phage sets for human and bovine *S. aureus*. As a result, out of 139 strains tested, 75.5% were typable by the human set and 93.5% by the bovine set. The two phage sets, especially the latter, were thus considered to be useful for typing of *S. aureus* of these laboratory rodent origins.

On the other hand, Ishihara [4] reported that 18 (90.0%) of 20 strains isolated from guinea pigs affected with dermatitis were typable at 100×RTD of phages of the human set with addition of bovine phage 42D and the typable strains were distributed into 2 phage patterns of 54 (n=16) and 52/80/42E/54/42D+ (n=2). In the present study, the same strains were subjected to phage typing with the bovine phages, but only 4 (20.0%) of the 20 strains were typable at 100×RTD, distributing into 2 phage patterns of 118 (n=2) and 42E/42D/102/118+ (n=2) (data not shown).

When the human set of phages was used, all of the typable strains isolated from the nares, skin, and intestinal contents of heal-

thy mice belonged to human phage group II, whereas most of the typable strains isolated from the nares, skin, and intestinal contents of healthy rats belonged to group I and mixed groups. Human group II strains were never found among rat strains. These healthy mice and rats used for isolation of *Staphylococcus* in this study were obtained from the same laboratory animal facility. Therefore, it seemed to be that different staphylococcal strains were natural inhabitants in these two species of animals.

There have been a few reports concerning the phage typing of *S. aureus* strains isolated from wild rodents. Mori *et al.* [9] and Mori [8] subjected 65 strains of *S. aureus* isolated from *Rattus norvegicus* living in an urban area and 112 strains from *Rattus norvegicus* captured in a small solitary island in Tokyo Bay, Japan, to phage typing by use of phages of the human set, and found that 16.9% (11/65) of the former and 27.7% (31/112) of the latter were typable, the majority belonging to group III. Similarly in the present study, it was apparent that the human phage set was of limited use in the typing of wild rodent strains, and much better results were obtained when the bovine phage set was used. Out of 128 wild rodent strains tested using the bovine phages, 80 (62.5%) strains were typable. This is far higher than 32.8% obtained by use of the human phages, but not a satisfactory percentage. Typabilities of the *Rattus norvegicus*, *Rattus rattus* and wild mouse strains were 41.0%, 88.9% and 100%, respectively. It was interesting to note that predominant phage groups of typable strains were significantly different among the three species of animals; group IV in *Rattus norvegicus*, group III in *Rattus rattus* and group II in wild mice. The use of phages of the bovine set was thus shown to be a great help in the typing of *S. aureus* from wild rodents.

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要 約

げっ歯類由来黄色ブドウ球菌のファージ型別：清水 晃・河野潤一・葉杖真二・木村 重・田村 弘¹⁾（神戸大学農学部家畜衛生学教室，¹⁾帝京大学医学部動物実験施設）——実験用のマウス（ヌードマウスを含む），ラット，ウサギおよび捕獲されたドブネズミ，クマネズミ，ハツカネズミ，フェレットから分離された *Staphylococcus aureus* 267株について，ヒト系（Hセット）およびウシ系（Bセット）*S. aureus* 型別用ファージセットを用いて，型別を試みた。実験用小動物由来139株の型別率はHセットで75.5%，Bセットで93.5%であり，捕獲げっ歯類由来128株の型別率はHセットで32.8%，Bセットで62.5%であった。Hセットを用いて，同一飼育場から購入したマウス・ラットの分離株をしらべると，マウス株のすべてがⅡ群に，ラット株の大多数がⅠ群および混合群に属した。Bセットを用いると，捕獲されたドブネズミ株の多くはⅣ群に，クマネズミ株の多くはⅢ群に，ハツカネズミ株の多くはⅡ群に属した。捕獲げっ歯類由来株の型別には，Bセットが型別率，識別能力の点でHセットより有用と思われた。