Distribution of *mecA*-harboring Staphylococci in Healthy Mares

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ABSTRACT. The prevalence of staphylococci that harbor the *mecA* gene responsible for methicillin resistance was examined in healthy breeding mares. Staphylococci often cause diseases of horses such as metritis, keratitis, and abscess. Methicillin-resistant staphylococci would make antibiotic treatments ineffective, so it may be significant to know the distribution of *mecA*-harboring staphylococci in mares. Isolation of *mecA*-harboring staphylococci was achieved from nares and pasterns of 100 mares in Hokkaido, Japan. From 13% of the mares, *mecA*-harboring staphylococci, including 15 isolates of *Staphylococcus sciuri* and 3 of *Staphylococcus lentus*, were isolated. Isolates of *S. sciuri* were found to be genetically polyclonal by pulsed-field gel electrophoresis. These isolates produced no PCase and showed low or no resistance to β -lactam and other classes of antibiotics. Distribution of staphylococcal species and levels of antibiotic resistance were found to be different between isolates from the present mares and those previously reported from riding-horses. Antibiotic pressure may lead to these differences. In addition, it appears that *mecA*-harboring *S. sciuri* may be native to horses.

KEY WORDS: equine, *mecA* gene, methicillin-resistant staphylococci, *Staphylococcus lentus*, *Staphylococcus sciuri*.

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Since methicillin-resistant *Staphylococcus aureus* (MRSA) was first recognized in England in 1961 [4], it has become an etiologic agent of serious hospital infections throughout the world. MRSA is resistant to all β -lactam antibiotics. This resistance is mediated by an extra penicillin-binding protein, PBP 2' or PBP 2a, which has a low affinity for β -lactam antibiotics [12, 19, 27]. PBP 2' is encoded by the *mecA* gene, located on the bacterial chromosome [23, 26]. The *mecA* gene is widely distributed among staphylococcal species, including coagulase-negative staphylococci (CNS), and it has been speculated that the gene may be horizontally transferred among staphylococci [2, 3].

MRSA has sometimes been isolated from animals treated surgically in veterinary hospitals [6, 8, 15]. In contrast, *mecA*-harboring CNS has been isolated from several domesticated and healthy animals [7, 17, 28]. It has been hypothesized that *mecA*-harboring staphylococci such as vancomycin-resistant enterococci [5] are prevalent in these animal species as potential reservoirs of human infection. Nowadays, various antibiotics are frequently used for animal disease treatment, thus it may be significant to clarify the distribution of *mecA*-harboring staphylococci in animals.

Staphylococcal diseases of horses are usually caused by *S. aureus*, but in rare cases by CNS species such as *S. epidermidis* from lesions [15, 16, 21, 22]. MRSA was isolated from mares with metritis between 1989 and 1991 in Japan [1] and a horse with postoperative wounds in the U.S.A. [13]. We have previously reported that Japanese healthy riding-horses do not carry MRSA, but frequently carry

mecA-harboring CNS with high resistance to various antibiotics [28]. However, it has been unclear whether isolates of *mecA*-harboring staphylococci are native to horses, since riding-horses have a great deal of contact with humans and antibiotics. In the present study, breeding mares having less contact with humans and antibiotics than riding-horses were investigated, and we discuss the characteristics of *mecA*-harboring staphylococci with reference to the differences in the circumstances of the horses.

The aim of the present investigation was to report on the following points: distribution of *mecA*-harboring staphylococci in healthy mares; characteristics of the isolates; comparison of the characteristics of *mecA*-harboring staphylococci that were isolated from mares and riding-horses. In addition, we evaluated the presence of *mec* regulator genes, *mecI* and *mecRI*, which negatively regulate the expression of the *mecA* gene [14].

MATERIALS AND METHODS

Isolation of *mecA*-harboring staphylococci: The animal subjects were a total of 100 mares (68 Thoroughbreds, 32 Anglo-Arabs) between 3 and 23 years of age kept for breeding on 25 farms in Hidaka District, Hokkaido, Japan. Isolation of *mecA*-harboring staphylococci was carried out according to the method described previously [28]. Namely, cotton swabs taken from the nares and pastern were applied onto a medium selective for MRSA (MS medium), which consisted of nutrient agar supplemented with 25 μ g/ml of ceftizoxime, 3.0% NaCl, 1.0% mannitol, and 0.004% bromocresol purple, pH 7.2. The plate was incubated for 48 hr at 30°C. Yellow or white colonies were selected and tested for catalase. Catalase-positive colonies were gram-

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stained and tested for susceptibility to lysostaphin (Sigma Chemical Co., St. Louis, Missouri, U.S.A.). Gram-positive cocci with irregular clusters that were susceptible to lysostaphin were tentatively identified as *mecA*-harboring staphylococci.

Isolates were tested for coagulation of rabbit plasma. Identification of isolates was conducted by use of API-Staph system (Bio Merieux SA, Marcy l'Etoile, France) and confirmed on the basis of additional characteristics of resistance to novobiocin (1.6 µg/ml), and detection of oxidase.

Species-specific PCR for 16S rRNA: Identification of *Staphylococcus sciuri* and *Staphylococcus lentus* was determined by two steps of species-specific PCR for the 16S rRNA gene. First, the *S. sciuri*-specific primers (5'-CAATAGTGAAAGACGGTTTCGG-3' and 5'-GGAAGACTCTATCTCTAGAGC-3') described by Gribaldo *et al.* [11] were used. Although we recognize that this primer set amplifies both *S. sciuri* and *S. lentus*, it can identify both species from other staphylococcal species. Then, additional *S. sciuri*-specific primers (5'-GAACCGCATGGT-TCAATAG-3' and 5'-GACTCTATCTCTAGAGCGG-3') were used to amplify a fragment of nucleotide position 188-1032 (DDBJ accession No. S83569), and *S. lentus*-specific primers (5'-GAACCGCATGGTTCAATGT-3' and 5'-AACTCTATCTCTAGAGCGA-3') were used to amplify the corresponding fragment of nucleotide position 163-1007 (DDBJ No. D83370) [20]. As controls, the following type strains were used: *S. sciuri* subsp. *sciuri* GIFU 9126 (=ATCC 29062^T, supplied by the Department of Microbiology, Gifu University School of Medicine); *S. sciuri* subsp. *carnaticus* ATCC 700058^T (purchased from the American Type Culture Collection); *S. sciuri* subsp. *rodentium* ATCC 700061^T (purchased from the American Type Culture Collection); and *S. lentus* ATCC 29070^T (kindly supplied by Dr. Shizunobu Igimi of the National Institute of Infectious Disease).

PCR for *mecA*, *mecR1*, and *mecI* genes: The PCR technique was used to detect the *mecA*, *mecR1*, and *mecI* genes. The primers used have been described elsewhere [28]. Briefly, primers (5'-AGATTGGGATCATAGCGTCA-3' and 5'-GAAGGTATCATCTTGTACCC-3') for detecting the *mecA* gene were designed to amplify a 258 bp DNA fragment (nucleotides 375 to 632, DDBJ No. Y00688), primers (5'-TTAGTGCTCGTCTCCACGTT-3' and 5'-CCTACGAACCATCCATTTGC-3') for detecting the *mecR1* gene were designed to amplify a 1511 bp DNA fragment (nucleotides 348 to 1858 of the *mecR1* gene, DDBJ No. X63598), and primers (5'-CATCTGCAGAATGGGAAG-3' and 5'-ACAAGTGAATTGAAACCGCC-3') for detecting *mecI* gene were designed to amplify a 271 bp DNA fragment (nucleotides 2007 to 2277, DDBJ No. X63598). PCR was performed in a thermal cycler (Perkin Elmer Cetus, Emeryville, California, U.S.A.) using a *Taq* reagent kit (Takara Shuzo Co., Ltd., Shiga, Japan). For DNA amplification, the following thermal program was used: denaturation at 94°C for 1 min, annealing at 55°C according to the primer for 1 min, and extension at 72°C for

2 min for 25 cycles to completion; the final cycle consisted of extension at 72°C for 5 min. Detection of amplified DNA was accomplished by use of 1% agarose gel electrophoresis in 1X Tris-acetate-EDTA buffer at 100 V for 20 min. After electrophoresis, gels were stained with 1 µg/ml of ethidium bromide.

Pulsed-field gel electrophoresis (PFGE): The procedure for PFGE analysis of *SmaI*-digested chromosomal DNA followed the method of George and Kloos [10]. The DNA fragments were separated in a 1% agarose gel slab using the CHEF-DR II (Bio-Rad, Hercules, California, U.S.A.) system. Electrophoresis was performed in 0.5X Tris-borate-EDTA buffer with a ramped pulse time of 5 to 40 sec at 6 V/cm for 22 hr. The gel was stained with ethidium bromide (2 µg/ml) for 20 min.

Hybridization probe and condition: The Southern hybridization technique was used for detection of the *mecA* gene in the bands revealed by PFGE. *SmaI* DNA fragments of PFGE gel were transferred to a nylon membrane by vacuum blotting. The hybridization probe used was the 1.8 kbp PCR product of the *mecA* gene that was made with MRSA strain NCTC 10442 using primers (5'-GTTGTAGT-TGTCGGGTTTGG-3' and 5'-CCACCCAATTTGTCT-GCCAGTTTCTCC-3'). The probe was labeled with a nonradioactive labeling kit, AlkPhos direct system (Amersham Pharmacia Biotech UK Ltd., Buckinghamshire, England). Hybridization was carried out under normal-stringency conditions at 55°C. Strain NCTC 10442 was supplied by the National Collection of Type Cultures.

Determination of minimum inhibitory concentration (MIC): The method of MIC and criteria for resistance followed those described in the NCCLS standard (M7-A5, 2000). Antibiotics were serially twofold diluted in a modified Mueller-Hinton agar, sensitivity disk agar-N (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan). A bacterial isolate was incubated with a sensitivity test broth for 18 hr at 35°C, and the broth was diluted 1:10 with fresh broth to make a suspension of approximately 10⁷ CFU/ml for use as an inoculum. Then 1 to 2 µl of inoculum suspension (c.a. 10⁴ CFU) was spotted with an inoculum replicating device onto the test agar plates by an inoculum replicator (Microplanter, Muto Machine Works, Tokyo) and the plates were incubated for 18 hr at 35°C. The following antibiotics were tested: benzylpenicillin-G (PCG), methicillin (DMPPC), oxacillin (MPIPC), ampicillin (ABPC), cefazolin (CEZ), cefmetazole (CMZ), ceftizoxime (CZX), imipenem (IPM), clindamycin (CLM), tetracycline (TC), doxycycline (DOXY), erythromycin (EM), gentamicin (GM), kanamycin (KM), tobramycin (TOB), and vancomycin (VCM).

Detection of penicillinase (PCase): Production of PCase was detected by the iodine-starch method [24]. Namely, the isolates were grown on trypticase soy agar containing 6.25 µg/ml of CZX for 18 hr 35°C, then the agar was overlaid with 4 ml of iodine-starch agar. Colonies surrounded by transparent zones in the violet background in 2 to 10 min were regarded as positive. The iodine-starch agar was made by adding 1 ml of a 2% I₂ and 53.2% KI solution and 1 ml of

a solution of 20 mg of benzylpenicillin per ml to 10 ml of a preheated 0.75% agar solution containing 1% starch.

RESULTS

Staphylococci that harbored the *mecA* gene were isolated from 13 of the 100 mares examined (Table 1). The 13 mares were kept on 7 farms that are not focused geographically. Twenty-five colonies were isolated from these mares by the selective medium for *mecA*-harboring staphylococci. Each colony isolated from one horse was tested by PFGE analysis, and colonies showing the same PFGE patterns were then regarded as one isolate. Consequently, 18 *mecA*-harboring staphylococci were isolated from the 13 mares (Fig. 1). Nine of the 13 mares yielded only one isolate each. One of the remaining 4 mares yielded 2 isolates of 2 species, and the other 3 mares yielded 2 or 3 isolates of *S. sciuri* differentiable by PFGE patterns.

Ten isolates were first identified as *S. sciuri*, and the other 8 isolates were identified as *S. lentus* by the API-Staph system. However, some data obtained from the PFGE analysis conflict with the identification results obtained by the API-Staph system. The PFGE pattern of the 10B isolate that was first identified as *S. lentus* was similar to those of other isolates of *S. sciuri* such as 19A and 90A (Fig. 1). *S. sciuri* and *S. lentus* are closely related with regard to characteristics used in the API-staph system, with only one difference in acid production from raffinose, i.e., negative for *S. sciuri* and positive for *S. lentus*. The 10B isolate was positive for the production of acid from raffinose. We then used species-specific 16S rRNA PCR for confirmation of species identification (Fig. 2), and five isolates (10B, 18A1, 18A2, 66A1, and 65A) were corrected to the species of *S. sciuri*. Thus, 15 isolates were identified as *S. sciuri* and the other 3 as *S. lentus* (Table 2).

The *mecA* gene was detected in all of the 18 isolates, while *mecR1* and *mecI* genes, which are the regulatory genes of *mecA*, were found to be present in 17 of the 18 isolates by PCR (Fig. 3). The latter 2 genes were not detected with an isolate of *S. sciuri* (81B).

With regard to PFGE analysis (Fig. 1), 3 *S. lentus* isolates (7B, 8A and 9A1) from 3 mares bred on the same farm were found to be identical in their banding patterns, while all 15 *S. sciuri* isolates showed different PFGE patterns. Subsequently, the PFGE bands were tested for hybridization with a *mecA* probe prepared from an MRSA strain of NCTC 10442 (Fig. 4). In all isolates, the *mecA* probe hybridized with a single band. In each of the 3 *S. lentus* isolates, the *mecA* probe hybridized with the same band, whereas in *S. sciuri* it hybridized with different bands.

The MICs measured are shown in Table 3. Some isolates of *S. sciuri* were resistant to PCG, ABPC, and CZX, but all were susceptible to DMPPC, MIPIC, CEZ and CMZ. In contrast, 3 isolates of *S. lentus* were resistant to β -lactams, except for CMZ. However, the resistances of penicillin-derivatives in all isolates were not so high. As for the other antibiotics, all isolates were found to be susceptible to KM,

Table 1. Isolation of *mecA*-harboring staphylococci from mares reared in Hokkaido, Japan

Farm	No. of tested horses	No. of positive horses	No. of isolates
A	9	4	5
B	6	2	4
C	5	2	3
D	8	2	3
E	10	1	1
F	8	1	1
G	6	1	1
H	5	—	—
I	4	—	—
J	5	—	—
K	7	—	—
L	6	—	—
M	6	—	—
N	10	—	—
O	5	—	—
Total	100	13	18

Fig. 1. PFGE patterns of isolates after *SmaI* digestion. Lane L, bacteriophage lambda DNA standard ladder. Lanes 7B–65A, isolates. The 10B isolate was first identified by API-Staph system as *S. lentus*, but its PFGE pattern was similar to those of 19A and 90A of *S. sciuri*. Several mares yielded 2 or 3 isolates showing different PFGE patterns: mares c (9A1, 9A5), f (66A, 66A1, 66A2), g (33A1, 33A2) and i (18A1, 18A2). For visual comparison, the lanes were reordered via computer imaging with Adobe Photoshop.

GM, TOB and VCM. *S. sciuri* isolates were susceptible to TC, DOXY, CLM and EM, but *S. lentus* isolates were resistant or intermediately resistant to these antibiotics. PCase was not detected in any of the isolates.

DISCUSSION

MRSA has been isolated from diseased horses, but rarely from healthy ones [9]. In a previous study, 6 species (*S. epidermidis*, *S. saprophyticus*, *S. lentus*, *S. xylosus*, *S. sciuri*, and *S. haemolyticus*), including potentially pathogenic staphylococci such as *S. epidermidis* and *S. haemolyticus*,

Fig. 2. Agarose gel electrophoresis patterns of the species-specific PCR products of 16S rRNA gene for *S. sciuri* (A) and *S. lentus* (B). Lanes are as follows: M, Φ X174/*Hinc*II digest size markers; S.s.s, *S. Sciuri* subsp. *sciuri* GIFU 9126; S.l, *S. lentus* ATCC 29070^T; S.s.r, *S. sciuri* subsp. *rodentium* ATCC 700061^T; S.s.c, *S. sciuri* subsp. *caraticus* ATCC 700058^T; 9A5-9A1, isolates. Each isolate reacted with one of the 2 primer sets (data not shown). Five isolates (10B, 18A1, 18A2, 66A1, and 65A), which were first identified as *S. lentus* by API-Staph system because of their acid production from raffinose, were subsequently identified as *S. sciuri* by PCR. The remaining 3 isolates (7B, 8A, and 9A1) that produced acid from raffinose were identified as *S. lentus*, and all isolates that did not produce acid from raffinose were identified *S. sciuri*.

Table 2. Origins of *mecA*-harboring staphylococci

Isolate	Source			Species ^{a)}
	Farm	Mare	Site	
7B	A	a	pastern	<i>S. lentus</i>
8A	A	b	nares	<i>S. lentus</i>
9A1	A	c	nares	<i>S. lentus</i>
9A5	A	c	nares	<i>S. sciuri</i>
10B	A	d	pastern	<i>S. sciuri</i> *
65A	B	e	nares	<i>S. sciuri</i> *
66A	B	f	nares	<i>S. sciuri</i>
66A1	B	f	nares	<i>S. sciuri</i> *
66A2	B	f	nares	<i>S. sciuri</i>
33A1	C	g	nares	<i>S. sciuri</i>
33A2	C	g	nares	<i>S. sciuri</i>
36A	C	h	nares	<i>S. sciuri</i>
18A1	D	i	nares	<i>S. sciuri</i> *
18A2	D	i	nares	<i>S. sciuri</i> *
19A	D	j	nares	<i>S. sciuri</i>
90A	E	k	nares	<i>S. sciuri</i>
38A	F	l	nares	<i>S. sciuri</i>
81B	G	m	pastern	<i>S. sciuri</i>

a) Asterisks denote that isolates were first identified as *S. lentus* by API-Staph system by reason of acid production from raffinose. However, results from PFGE analysis and PCR for 16S rRNA confirmed their attribution to *S. sciuri*. All of the unmarked isolates were identified unequivocally by all methods.

Fig. 3. Agarose gel electrophoresis patterns of the PCR products of the *mecA*, *mecR1* and *mecI* genes of isolates. Lane M, Φ X174/*Hinc*II digest size markers (*mecA* and *mecI*), λ /*Hind*III digest and Φ X174/*Hinc*II digest size markers (*mecR1*). Lanes 7B-65A, isolates. The *mecA* gene was detected in all of the 18 isolates. The 81B isolate lacks *mecR1* and *mecI* genes, while the remaining 17 isolates harbor both genes as shown.

Fig. 4. Hybridization of *Sma*I digests of the isolates with a *mecA* probe. Lanes 7B-65A, isolates. In each of the 3 *S. lentus* isolates (7B, 8A and 9A1), the *mecA* probe hybridized with the same band, whereas in *S. sciuri* it hybridized with different bands of 4 or more clusters.

which harbored the *mecA* gene responsible for methicillin resistance, were isolated from approximately 30% of riding-horses in the suburbs of cities in Japan [28]. We found, in this study, that *mecA*-harboring staphylococci were present on 13 of 100 healthy mares on farms of the Hidaka District,

Table 3. MICs ($\mu\text{g/ml}$) of antibiotics for *mecA*-harboring staphylococcal isolates

Antibiotic	<i>S. lentus</i>			<i>S. sciuri</i>														
	7B	8A	9A1	9A5	33A2	38A	10B	19A	90A	36A	66A2	33A1	18A1	18A2	66A	81B	66A1	65A
PCG	1	1	0.25	a)								0.25	0.25	0.25				
DMPPC	16	8	8	4	2	4	4	2	4	2	2	2	4	4	1	4	2	2
MPIPC	8	8	4	1	1	2	1	1	1	1	0.5	1	2	2	0.5	0.5	1	1
ABPC	4	8	8	1	2	4	1	1	1	1	1	2	4	2		0.5	0.5	2
CEZ	>128	>128	>128	2	2	2	2	1	2	1	1	1	2	2	0.5	1	1	1
CMZ	1	4	2	2	2	2	1	2	4	4	1	2	3	3	0.25	1	1	2
CZX	>128	64	64	64	32	4	8	16	16	16	1	64	64	64	8	2	2	2
IPM																		
EM	>128	>128	>128												1			
CLM	4	2	2	0.5	0.25	0.25	0.25	0.25	0.25	0.25	0.25	0.5				0.25	0.25	0.25
TC	64	64	128	0.25			0.25		0.25						0.5	0.25	0.25	0.25
DOXY	8	8	16												1			
GM																		
KM	2	1	1	0.5	0.25	1	0.5	1	1	0.5	0.5	0.5	0.5	0.5		1	0.5	0.5
TOB		0.25																
VCM	1	1	1	1	1	1	1	0.5	1	1	0.5	1	0.5	0.5	0.5	1	0.5	1

a) Blanks denote <0.125 ($\mu\text{g/ml}$).

Hokkaido, the center of horse breeding in Japan. However, the identified staphylococcal species were restricted to 2 species of *S. sciuri* and *S. lentus*, which are known to be less pathogenic. Species distribution of *mecA*-harboring staphylococci may vary according to the circumstances of the horses.

In addition to the change in species distribution, differences in antibiotic resistance were also noted between the isolates from riding-horses and breeding mares. Specifically, the previous study [28] showed that, isolates from riding-horses had high resistance to several antibiotics, and two-thirds of them produced PCase. In this study, however, no isolates from any mare produced PCase, and almost all had low or no resistance to β -lactams and other classes of antibiotics. Therefore, there were obvious differences in characteristics between *mecA*-harboring staphylococci from breeding mares and riding-horses. We speculate that the differences are due to the amounts of antibiotics used for horses. In Hidaka, CEZ, PCG, and ABPC are the primary β -lactam antibiotics used for treating horses, and approximately 0.1 g, 1.0 g, and 0.35 g, respectively, per horse is used per year (data is based on records of 2200 mares in Hidaka made by the Mitsuishi office of the Hidaka Agriculture Mutual Aid Association in 1996). The amount of antibiotics used for the mares is approximately 200 times less than that used for racing-horses (data on 2000 racing-horses recorded by the Miho Training Center of Japan Racing Association in 1999), and would have little effect on survival of *mecA*-harboring staphylococci. Although it was difficult to collect data on riding-horses, antibiotic amounts

comparable to those used for racing-horses may be used for riding-horses. Furthermore, in Japan, riding-horses are commonly retired racing-horses. Thus, selective pressure by antibiotics may lead to changes in drug resistance of staphylococci.

PFGE analysis revealed that 15 isolates of *S. sciuri* from 11 mares were all different clones, and each of the isolates had a different band in hybridization with a *mecA* probe. This polyclonal configuration of isolates was shown even in cases in which mares were bred on the same farm. Additionally, 3 mares yielded several isolates of *S. sciuri* showing different PFGE patterns. These data suggest that isolates of *S. sciuri* were not spread recently among the mares by a certain clone. Although it is not known whether or not each *mecA*-harboring *S. sciuri* is conserved by individual mares, those possessing diverse PFGE patterns would have been inherited at a rate of approximately 10% or more among the horse population. In contrast, 3 isolates of *S. lentus* from the 3 mares that were bred on the same farm were found to possess the same PFGE pattern and the same hybridized band with the *mecA* probe. Therefore, these *mecA*-harboring *S. lentus* had the same source and would have been spread recently among the 3 mares through mutual contact or transmission by humans.

In horses, Devriese *et al.* [9] reported that *S. xylosus* and *S. sciuri* might be normal staphylococci, and we also found that *S. xylosus* (81%) and *S. sciuri* (12.3%) were predominant, and that *S. haemolyticus* (0.9%), *S. lentus* (0.6%), and *S. epidermidis* (0.1%) are a minority based on the mares examined in the present study [20]. Therefore, *S. sciuri*

forms normal staphylococcal flora. Although it has been speculated that the *mecA* gene may be horizontally transmissible among staphylococcal species [2, 3], it would not spread easily among other species of equine staphylococcal normal flora such as *S. xylosus* as shown by the present result that no *S. xylosus* harbored the *mecA* gene.

An atypical characteristic was noted in the present study with some isolates of *S. sciuri*. Specifically, acid production from raffinose was positive, which is common to *S. lentus* but not to *S. sciuri*. Specific PCR for 16S rRNA allowed for species confirmation. Further study is necessary to determine if the raffinose-positive isolates form a new group in *S. sciuri* at the subspecies level.

In conclusion, the *mecA*-harboring *S. sciuri* is an indigenous feature of horses, but their drug resistances were found to be altered according to the career of the horse. After a horse is born, it may get normal flora, including *mecA*-harboring *S. sciuri*, from the mother mare. When it becomes a riding-horse via a racing-horse, the flora of *mecA*-harboring staphylococci is changed, as indicated by the rate of positive horses increasing from 13% to 30%, the antibiotic resistances increase, and the number of staphylococcal species increases. The *mecA*-harboring *S. sciuri* and *S. lentus* have an advantage for survival under antibiotic pressure. With regard to the other species of *S. epidermidis*, they are in the minority in mares but the majority in humans [18]. Racing-horses and riding-horses have frequent human contact. It may be that highly resistant staphylococci are transferred from humans rather than that the *mecA* gene is horizontally transferred to *S. epidermidis* and *S. haemolyticus* from *S. sciuri* in horses. Consequently, when females return to farms as breeding mares, the number of *mecA*-harboring staphylococci with high resistance may decrease with decreasing antibiotic pressure, as the resistant *S. sciuri* or human *S. epidermidis* and *S. haemolyticus* are not part of the natural flora of horses.

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