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Original Communication

Isolation of methicillin-resistant *Staphylococcus aureus* from laboratory animals

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ABSTRACT

The aim of this study was to investigate whether methicillin-resistant *Staphylococcus aureus* (MRSA) infections have spread among laboratory animals housed in our animal facility which is located close to the university hospital. Several epidemics of MRSA infection have occurred in the hospital, and we attempted to isolate these bacterial strains from the animals housed between 1994 and 2011. A total of 6,425 strains of *S. aureus* were isolated, of which 5,120 (79.7%) were isolated from rat feces. Only one MRSA isolate was subsequently detected in rats which were not treated with any antibiotics. The isolated MRSA harbored type II staphylococcal cassette chromosome *mec* (SCC*mec*), which was identical to that of a strain isolated at our university hospital. However, the coagulase type of the hospital isolates was II, which is the most endemic type in Japan, whereas our isolate was not identified to any coagulase type. These results indicate that MRSA is very rare in our animal facility, despite frequent contact between laboratory animals and medical school and hospital faculty.

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KEYWORDS: animal facility, laboratory animals, MRSA, rat, *Staphylococcus aureus*

INTRODUCTION

Staphylococcus aureus (*S. aureus*) is one of the most important pathogens in humans and in other animals as it causes a variety of potentially serious conditions such as dermatitis, mastitis, septicemia, abscesses, and other infectious diseases [1-4]. The prevalence of methicillin-resistant *S. aureus* (MRSA) nosocomial infections has increased dramatically worldwide [5, 6]. MRSA infection has become widespread in hospitals, particularly in intensive care units [7, 8]. MRSA isolates are often resistant to treatment with multiple antibiotics [9-11] and can result in potentially serious therapeutic problems.

Although the prevalence of MRSA in humans, livestock and companion animals has been reported [12, 13], a survey of MRSA infection and colonization in laboratory animals is lacking. Furthermore, because many of the researchers who have access to the animal laboratories at our university are medical school and hospital faculty, it is possible that these staff may transmit MRSA to the laboratory animals and vice versa. Therefore, we initiated a series of studies to investigate MRSA infection and colonization in laboratory animals of our animal facility. In this study, we describe the isolation of *S. aureus* and MRSA

from laboratory animals housed in our animal facility between 1994 and 2011.

MATERIALS AND METHODS

Animals and housing

A total of 644 mice were used in this study. These animals were originally obtained from commercial breeders and maintained on a laminar flow bench (Tokiwa Kagaku Co. Ltd., Tokyo, Japan; Tokiwa) under specific pathogen-free (SPF) conditions. They were subsequently housed in sterilized TPX cages (170 x 235 x 125 mm; CLEA Japan, Inc., Tokyo, Japan; CLEA) with stainless steel lids containing sterilized wooden chips, with a maximum of three mice per cage. They were fed a commercial diet (CE-2; CLEA) and received filtered drinking water *ad libitum* from an automatic dispenser and a cage change every week. A total of 312 rats, originally obtained from commercial breeders, were also incorporated into this study and maintained in sterilized TPX cages (270 x 430 x 200 mm; CLEA) and under the same rearing conditions as the mice. A total of 104 rabbits from commercial breeders were used and housed individually in autoclaved fiber reinforced plastic cages (360 x 520 x 350 mm; Tokiwa), and were maintained on an automatic-washing system rack. They were fed a commercial diet (RM-4; Funabashi Farm Co., Ltd, Chiba, Japan; Funabashi) and received filtered drinking water *ad libitum* from the automatic dispenser. Rabbit cages were changed every two weeks. 6 Japanese monkeys were tested and housed individually in stainless steel cages (600 x 600 x 650 mm; Tokiwa) and fed a commercial diet (PS; Oriental Yeast Co., Ltd, Tokyo, Japan; Oriental) and received filtered drinking water *ad libitum* also from the automatic watering system. A total of 78 mongrel dogs and 4 beagle dogs from commercial breeders were also examined. These animals were housed individually in stainless steel cages (800 x 800 x 700 mm; Tokiwa) and also fed a commercial diet (ED-1; Sanwa Kagaku Kenkyusho Co., Ltd., Nagoya, Japan) and provided with filtered drinking water *ad libitum*. The 2 goats and 8 pigs that were used were maintained in individual pens. The goats were fed both hay and a commercial diet (ZF; Oriental) and the pigs were fed a commercial diet (Super Milko; Funabashi) and provided with fresh water *ad libitum*.

All animals were kept under standard lighting conditions with white light illumination from 07:00 to 19:00 hr (local time). The ambient temperature of each of the rooms was maintained at 22 ± 2 °C and the relative humidity kept at 50 ± 20%. The animals received humane care in compliance with both institutional and Japanese government guidelines for animal experimentation.

Sample collection

Feces samples were collected from each of the animals that had been housed for at least two months after rearing began. Fresh feces were weighed and suspended in sterile saline (0.1 g of feces per ml) and 25 μl was then plated onto Mannitol salt agar plates (Nissui Pharmaceutical Co., Ltd., Tokyo, Japan; Nissui) containing 5% egg yolk (EY-MSA) [14]. Samples of nasal-swabs and swab-samples of hands and gloves of animal caretakers and researchers were collected as follows: sterilized swabs were moistened with sterile saline and placed into the nostril of each individual subject with a gentle twirling motion or applied to the hands or laboratory gloves of each individual. These swabs were then plated onto EY-MSA. Samples from the equipments in the laboratory animal rooms were also collected from sixteen inanimate sites. Sterilized swabs moistened with sterile saline were used to swab areas of about 10 cm^2 and then plated onto EY-MSA.

Identification of *S. aureus*

EY-MSA plates inoculated with each of the collected samples were incubated at 37 °C for 48 hr. A maximum of 30 lecithovitellin reaction positive colonies, which were suspected to be cases of *S. aureus* infections present in individual samples, were selected at random and streaked onto Heart Infusion agar plates (HI agar; Eiken Chemical Co., Ltd., Tokyo, Japan). *S. aureus* was identified according to standard characteristics and this was confirmed using an N-ID-Test SP-18 (Nissui) according to the manufacturer's instructions. Strains identified as *S. aureus* were reinoculated in casitone semisolid medium and stored at room temperature until required for susceptibility testing. A methicillin-sensitive *S. aureus* strain (MSSA; ATCC 25923) was used as a control.

Detection of methicillin resistance

Methicillin resistance was confirmed by agar screening tests. Isolated strains of *S. aureus* were inoculated onto Muller-Hinton agar (Difco Laboratories, Detroit, USA; Difco) containing 4% NaCl and 6 mg/ml oxacillin (methicillin screening agar) at 35 °C for 24 hr. The susceptibility of these isolates was determined according to the National Committee for Clinical Laboratory Standards (NCCLS) broth microdilution guidelines [15, 16]. Briefly, two-fold serial dilutions (final concentrations of 128 to 0.25 μg/ml) of oxacillin were prepared in each well of a sterile microtiter plate. 100μl of prepared bacterial inoculum (5×10^4 cells/ml), using cation-adjusted Muller-Hinton broth (Difco) supplemented with 2% NaCl, was inoculated in each well containing the test drug and into control wells, followed by incubation at 35 °C for 24 hr.

Multiplex polymerase chain reaction (M-PCR)

Staphylococcal cassette chromosome *mec* (SCC*mec*) typing was performed using an M-PCR strategy as described by Kondo *et al.* [17]. Briefly, M-PCR 1 for *ccr* type assignment, M-PCR 2 for *mec* class assignment and M-PCR 4 for identifying specific ORFs in J1 regions of four subtypes of type II SCC*mec* elements were used. For M-PCR 1, the reaction mixtures contained 10 ng chromosomal DNA, oligonucleotide primers (0.1 μM), 200 μM each of deoxynucleotide triphosphates, Ex *Taq* buffer, and 2.5 U Ex *Taq* polymerase (Takara Bio Inc., Kyoto, Japan) in a final volume of 50 μl. The concentration of $MgCl_2$ was 3.2 mM. A Takara PCR thermal cycler was used for amplification with an initial denaturation step (94 °C, 2 min) followed by 30 cycles of denaturation (94 °C, 2 min), annealing (57 °C, 1 min), extension (72 °C, 2 min), and a final elongation step at 72 °C for 2 min. For M-PCRs 2 and 4, the reaction mixtures were the same as those for M-PCR 1 except that the concentration of $MgCl_2$ was 2 mM and the annealing temperature was raised to 60 °C for 1 min to avoid the generation of nonspecific DNA fragments. PCR products were visualized by agarose gel electrophoresis.

Coagulase typing

The *S. aureus* isolated from rats, rat associated equipments and caretakers were examined for their coagulase types by using a staphylococcal coagulase antiserum kit (Denka Seiken, Tokyo, Japan), in accordance with the manufacturer's instructions.

RESULTS AND DISCUSSION

Isolation of *S. aureus* from various laboratory animals

The anterior nares are the most frequent site of *S. aureus* carriage; however, only about 20% of individuals in the population are persistent nasal carriers of *S. aureus*, while 30% are intermittent carriers, and about 50% are never colonized [18]. Furthermore, transient contamination has been identified where MRSA can be isolated from a nasal swab in humans [19]. Our preliminary experiments showed that *S. aureus* could be isolated from the nasal cavity of a dog, but swabs collected from the same dog 1 month later were negative, although *S. aureus* could be isolated from the feces in both tests. Thus, we selected the feces as a sample for detection of *S. aureus*, as it is easy to get samples and there is no need to kill or anesthetize the animals.

S. aureus was isolated from feces of 81/468 mice (17%), 198/312 rats (63%), 3/102 rabbits (3%), 2/6 monkeys (33%), and 14/82 dogs (17%), but could not be detected in either the goats (n = 2) or pigs (n = 4) not treated with antibiotic (Fig. 1A, left panel). In antibiotics treated experiments (cefotiam for mice; ampicillin for rabbits and pigs), *S. aureus* was isolated from feces of 56/176 mice (31.8%), but could not be detected in either the rabbits (n = 2) or pigs (n = 4) (Fig. 1A, right panel). Thus, *S. aureus* was isolated from 354/1158 (30.6%) laboratory animals and 6,425 strains were obtained. Rats were the major carrier of *S. aureus* among laboratory animals housed at our facility, accounting for 5,120 isolates (Fig. 1B). The other isolates were obtained from mice (651 isolates), dogs (386 isolates), rabbits (60 isolates), and monkeys (60 isolates) with no antibiotic treatment, and mice (148 isolates) treated with antibiotics. After inoculation onto methicillin screening agar plates, only one strain (0.3%, 1/312 rats), which had been isolated from a rat, survived under these growth conditions (Fig. 1 A and B). The oxacillin minimum inhibitory concentration (MIC) was then determined for this strain using a broth microdilution test and was 12 μg/ml, confirming this isolate to be methicillin-resistant.

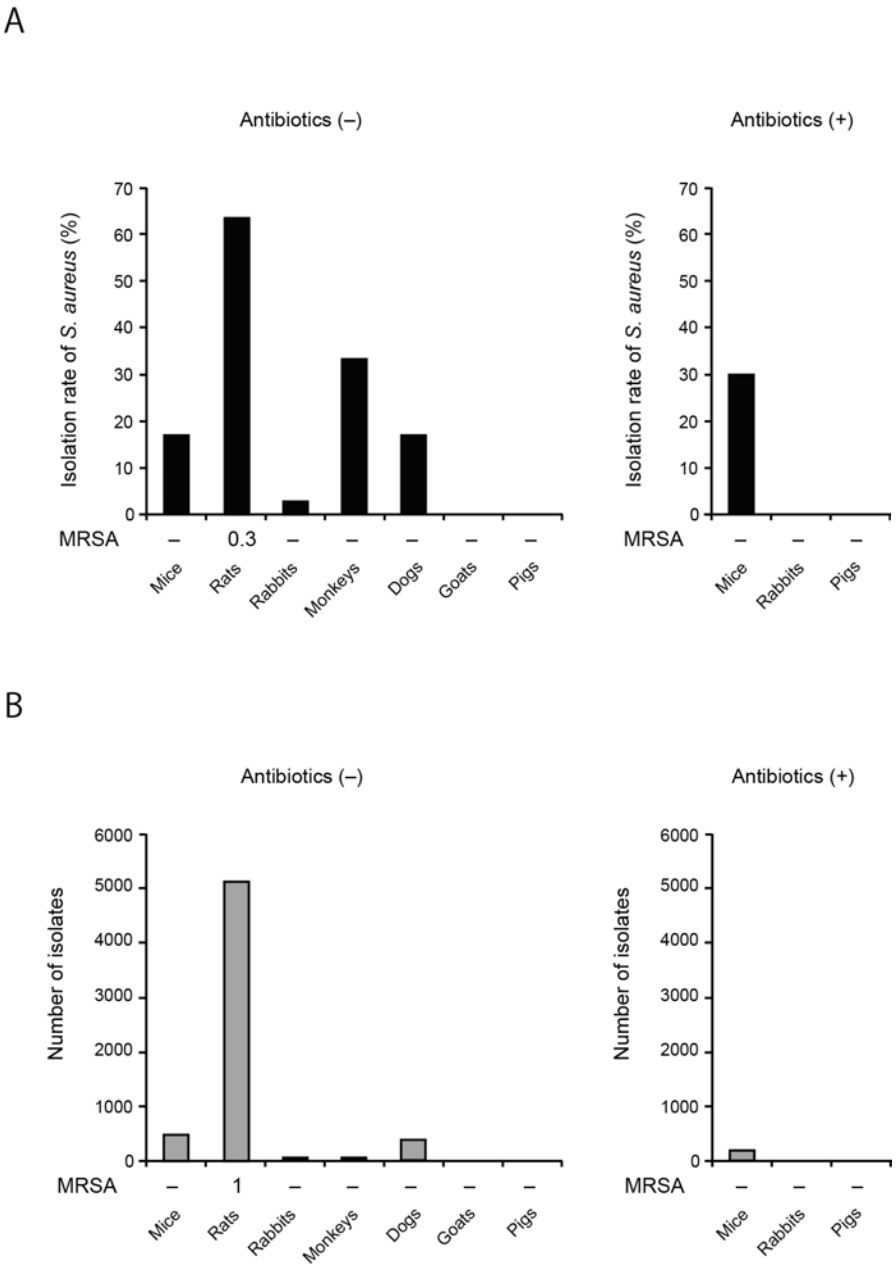


Fig. 1. Isolation of *S. aureus* from laboratory animals. (A) Rates of *S. aureus* isolation in each species without antibiotic treatment (left panel) and with antibiotic treatment (right panel). Methicillin- resistant *Staphylococcus aureus* (MRSA) was detected in 0.3% (1/312 rats) only. –; not detected. (B) Total number of *S. aureus* isolates in each species without antibiotic treatment (left panel) and with antibiotic treatment (right panel). Only a strain of MRSA was detected in rat. –; not detected.

Isolation of *S. aureus* from equipments in the animal rooms

S. aureus was successfully isolated from a variety of locations in the rooms used for laboratory animal housing and experimentation; however no MRSA was detected in any of the equipments in the animal rooms (Fig. 2A and B). A high frequency of detection was observed in animal cages (53%), beddings (52%), diets (52%), laboratory benches (33%), floors (24%), weighing machines (14%), and racks

from a laminar flow bench (10%) (Fig. 2A). A total of 2,123 strains of *S. aureus* were isolated and all the strains were found to be MSSA (Fig. 2B). *S. aureus* was mainly detected in the equipments which the animals always had contact with. These results suggested that *S. aureus* might get transferred to the equipments from the animals, and that *S. aureus* does not survive for long periods on equipment that is not in constant contact with the animals, likely because of efficient cleaning methods (e.g. ethanol spray).

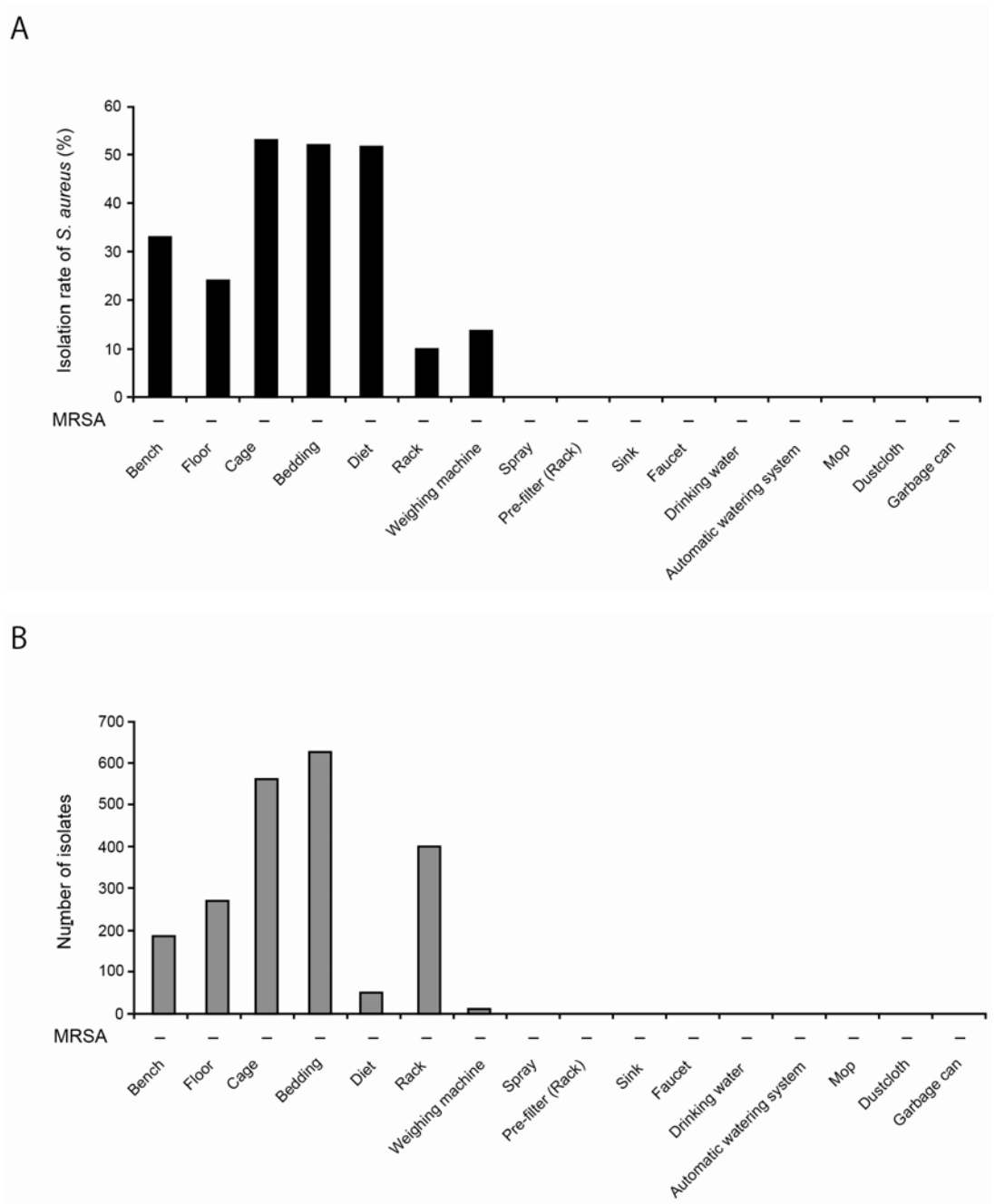
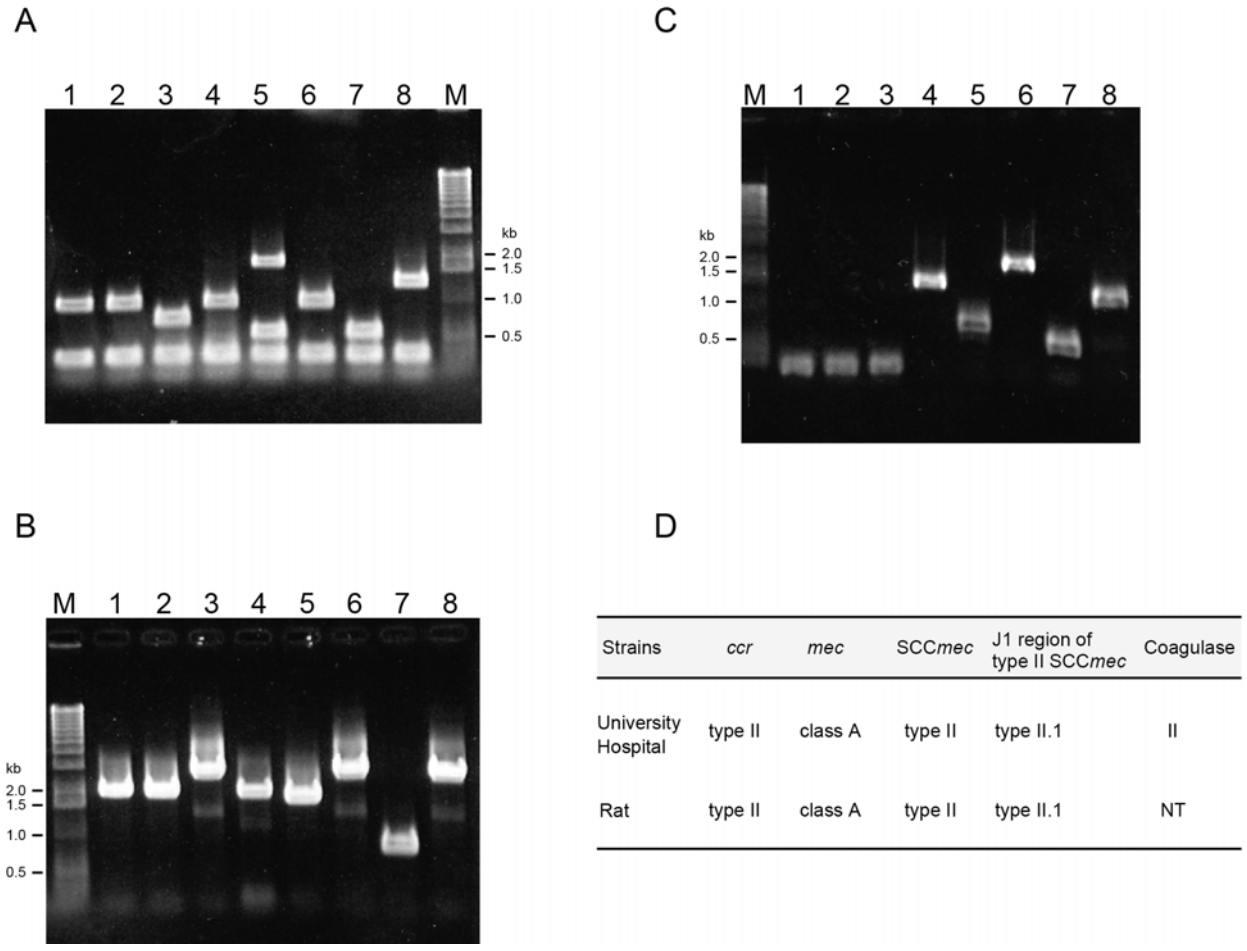


Fig. 2. Isolation of *S. aureus* from equipments in the animal facility. (A) Rates of *S. aureus* isolation in each equipment in the animal facility. No MRSA was detected in any of the equipments in the animal facility. (B) Total number of *S. aureus* isolates in each equipment in the animal facility. –; not detected.

SCC*mec* types identified in MRSA isolated from rats by multiplex PCR

Next, we compared the SCC*mec* type of the rat isolate with that of the strain isolated from Kumamoto University Hospital (Fig. 3). A summary of the reference strains included in each multiplex PCR is shown in Table 1. Multiplex PCR 1 was used to amplify and determine the *ccr* type (Fig 3A). The amplified DNA fragments from the rat isolate (lane 1) and the hospital isolate (lane 2) matched with N315, type II (lane 4). Multiplex PCR 2 (Fig. 3B) revealed that the *mec* gene complexes of the rat isolate (lane 1) and the hospital isolate (lane 2) matched with N315, class A *mec* (lane 4). Multiplex PCR 4 (Fig. 3C) showed that the rat isolate (lane 1) and the hospital isolate (lane 2) were of the SCC*mec* type II.1 subgroup, which includes the strain N315 (lane 3). These results



Strains	*ccr*	*mec*	SCC*mec*	J1 region of type II SCC*mec*	Coagulase
University Hospital	type II	class A	type II	type II.1	II
Rat	type II	class A	type II	type II.1	NT

Fig. 3. Results of multiplex PCRs used to assess SCC*mec* type. (A) Multiplex PCR 1 for identification and typing of the *ccr* gene complex. Lane 1, rat; lane 2, Kumamoto University Hospital; lane 3, 10442 type I; lane 4, N315 type II; lane 5, 85/2082 type III; lane 6, JCSC1968 type IV; lane 7, JCSC3642 type V; lane 8, HDE288 type VI; M, molecular marker. (B) Multiplex PCR 2 for identification of three gene alleles belonging to the *mec* gene complex. Lane 1, rat; lane 2, Kumamoto University Hospital; lane 3, 10442 class B; lane 4, N315 class A; lane 5, 85/2082 class A; lane 6, JCSC1968 class B; lane 7, JCSC3642 class C; lane 8, HDE288 class B; M, molecular marker. (C) Multiplex PCR 4 for J1 region-based subtyping of type II and type III SCC*mec* elements. Lane 1, rat; lane 2, Kumamoto University Hospital; lane 3, N315 type II.1; lane 4, 36-1 type II.1; lane 5, BK351 type II.3; lane 6, RN7170 type II.4; lane 7, 85/2082 type III.1; lane 8, WIS type V; M, molecular marker. (D) Summary of characterization of MRSA isolated from Hospital and rat. The coagulase type of the rat MRSA isolate could not be identified based on known coagulase types. NT, non-typeable.

Table 1. Summary of reference strains of each multiplex PCR.

		M-PCR* 1	M-PCR 2	M-PCR 4	
Lane	Strains	*ccr*	*mec*	Strains	subtype
3	10422	I	B	N315	II.1
4	N315	II	A	36-1	II.2
5	85/2082	III	A	BK351	II.3
6	JCSC1968	IV	B	RN7170	II.4
7	JCSC3642	V	C	85/2082	III.1
8	HDE288	VI	B	WIS	V

*M-PCR: multiplex PCR.

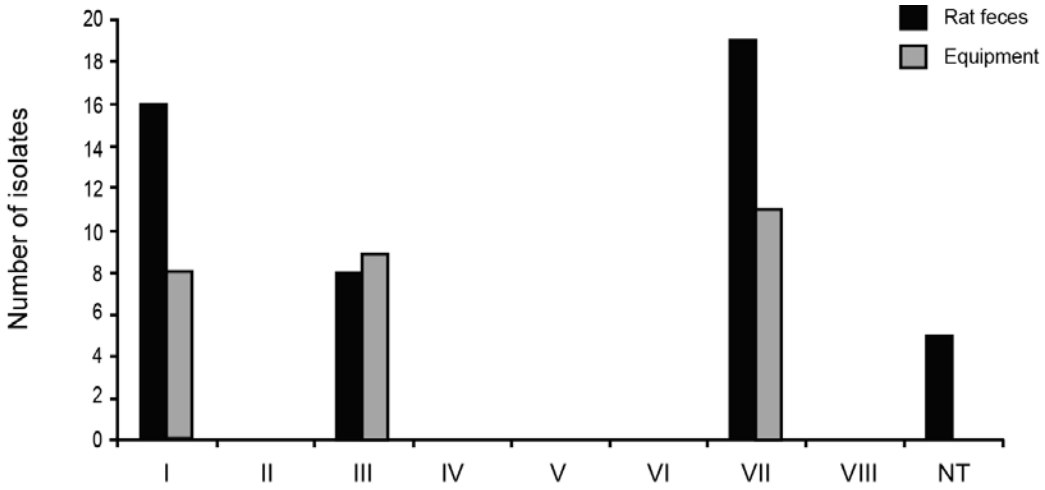


Fig. 4. Coagulase type of *S. aureus* isolated from feces of rats and rat associated equipment. The equipments included laboratory bench, floor, cages and racks. NT, non-typeable.

suggest that the rat and hospital isolates carried the same SCC*mec*, which is also carried by the prevalent strain of MRSA in Japan [20, 21]. Thus, we speculated that the epidemic strain of MRSA in the hospital was carried by the researchers into the animal facility and then, infected and proliferated in the rat itself or transferred the SCC*mec* element into MSSA colonized in rats. The coagulase type of the hospital isolate and rat isolate was tested to verify these possibilities. The coagulase type of the hospital isolate and the most prevalent strain in Japan has been previously confirmed to be type II [20]. The coagulase type of the rat MRSA isolate could not be identified based on known coagulase types (Fig. 3D). These results indicated that the hospital epidemic strain of MRSA itself did not grow in the rat reared in our animal facility.

Coagulase types in *S. aureus* isolated from rat feces and equipments associated with rat rearing

The coagulase type of the MRSA isolated from rat feces was non-typeable. Thus we investigated what coagulase types of *S. aureus* were spread in rats. A total of 48 and 28 strains of *S. aureus* isolated from rat feces and the equipments associated with rat rearing (laboratory bench, floor, cages and racks), respectively, were examined (Fig. 4). In rat feces,

the 48 strains were classified into 4 groups, 19 type VII (39.6%), 16 type I (33.3%), 8 type III (16.7%) and 5 non-typeable (10.4%). In the equipments, the 28 strains were classified, same as rat feces except of non typeable, into 11 type VII (39.3%), 9 type III (32.1%) and 8 type I (28.6%). There are only a few papers on the coagulase typing of *S. aureus* isolated from rats [22, 23]. Coagulase types V and VII were the most predominant in urban-living rats [23]. In this study, we could not detect the type V and the most predominant types were I and VII in laboratory rats. However, it may be noted that about 10% of *S. aureus* strains isolated from both urban-living and laboratory rats were non-typeable. Thus, there is no denying completely the fact that MSSA possessing non-typeable coagulase in the rats became MRSA through the acquisition of the SCC*mec* element from the epidemic strain of MRSA in the hospital, although only a single MRSA was detected in this study.

Isolation of *S. aureus* from laboratory animal caretakers and researchers

Next, we investigated whether animal caretakers or researchers might transfer *S. aureus* to laboratory animals and vice versa. Animal caretakers in our animal facility always use a cap, coverall, disposable mask and gloves during their animal rearing work. *S. aureus* was detected in the nasal cavities of six out of 24 tested caretakers (25%) before or after their work days (Fig. 5A, left panel), and 130 and 106 strains of *S. aureus* were isolated from the same caretakers before and after their work, respectively. No *S. aureus* was detected from the hands of any caretakers (data not shown). Also, *S. aureus* was detected in the wearing gloves of 8 out of 24 tested caretakers (33.3%) after work, although no *S. aureus* was detected in the gloves before work (Fig. 5A, left panel). 86 strains of *S. aureus* were isolated from these caretakers after work (Fig. 5A, right panel). No *S. aureus* was detected in the nasal cavity, hand or gloves of 5 researchers performing rat experiments before or after their animal studies (data not shown). They worked in animal rooms and had a contact with the animals for a short time (within 30 min) compared to animal caretakers (at least 6 hours a day). Furthermore, they usually used a disinfectant (e.g., ethanol) for their experiments and often disinfected their gloves during the experiments.

Before rearing work, coagulase type of *S. aureus* detected in the nasal cavity of caretakers was type I only. After their work, *S. aureus* detected from disposable gloves of *S. aureus* positive caretakers were 5 type I and 27 type VII (Fig. 5B, left panel), whereas *S. aureus* detected from gloves of *S. aureus* negative caretakers were 8 type I and 46 type VII (Fig. 5B, right panel). These results suggest that animal-to-human transfer of *S. aureus* would often occur in our animal facility; however the use of disposable masks and gloves could be effective to protect the *S. aureus* infection. In this study, we

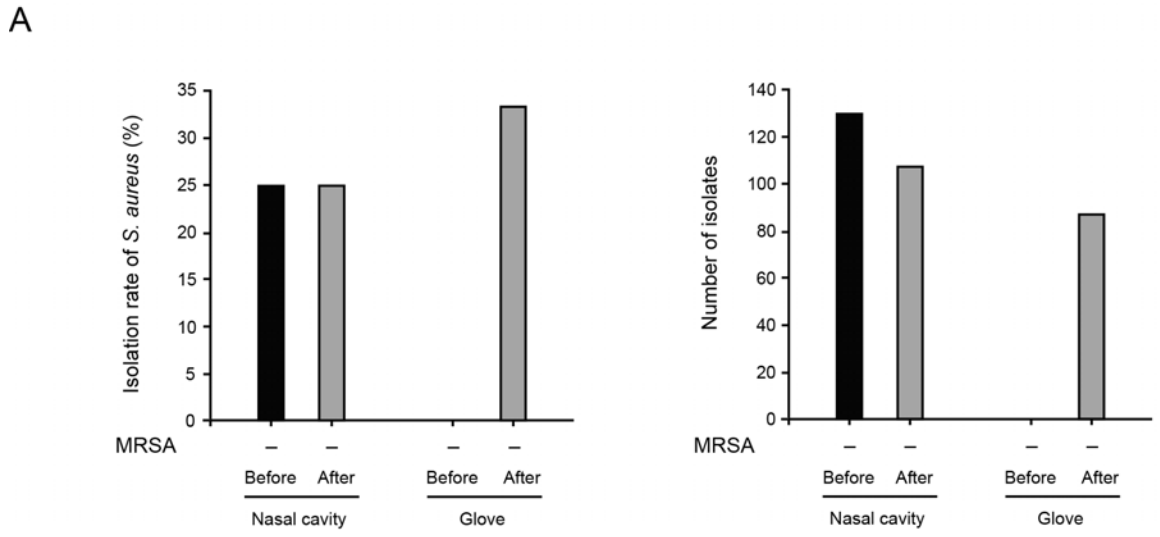


Fig. 5

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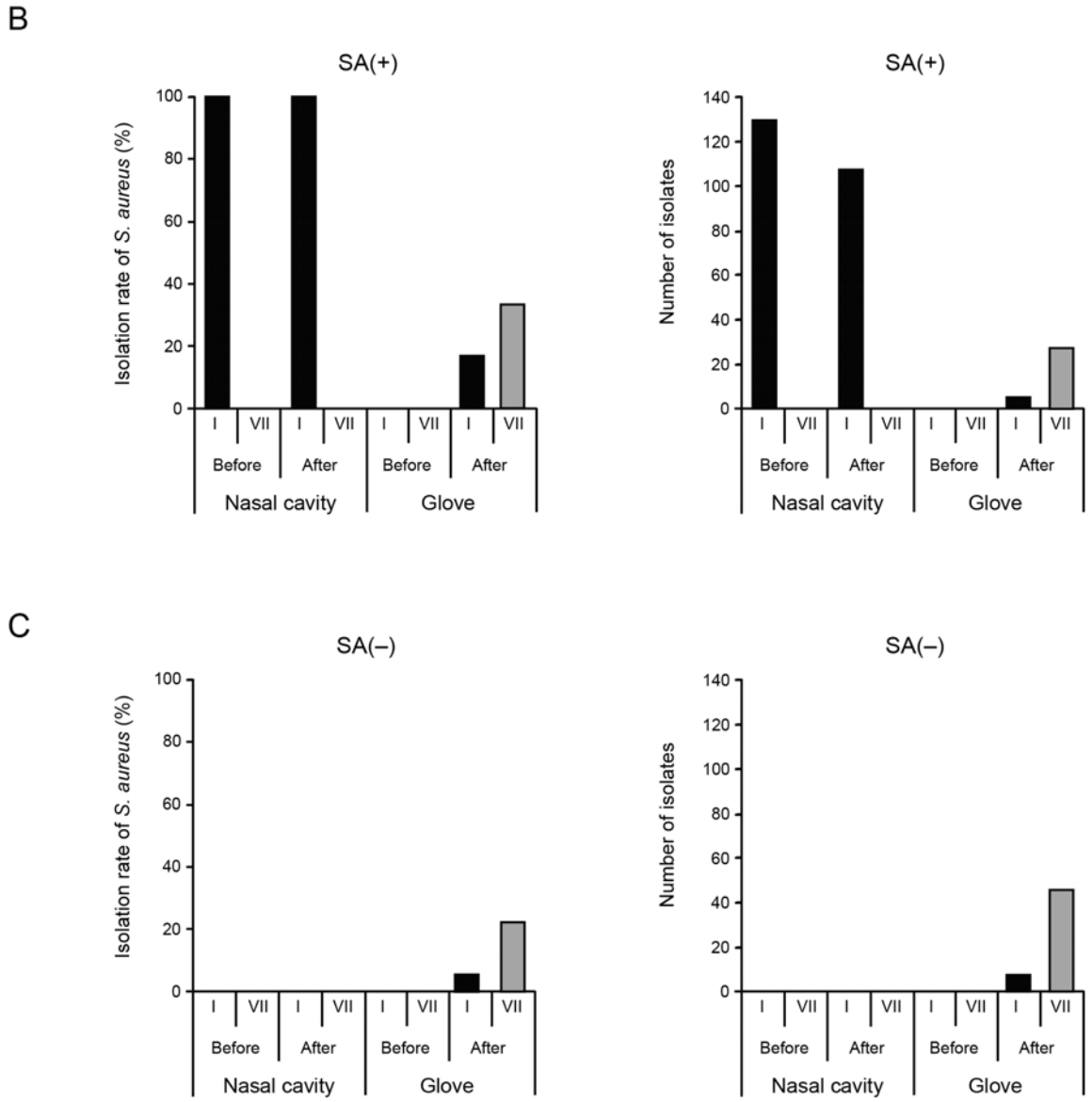

Fig. 5. Isolation of *S. aureus* from laboratory animal caretakers before and after rearing works. (A) Rates (left panel) and number (right panel) of *S. aureus* isolated from the nasal cavity and disposal gloves of animal caretakers. No MRSA was detected in any caretakers. (B) Rates (left panel) and number (right panel) of coagulase types of *S. aureus* isolated from the nasal cavity and disposable gloves of six *S. aureus* positive caretakers (SA(+)) before and after animal rearing work. (C) Rates (left panel) and number (right panel) of coagulase types of *S. aureus* isolated from the nasal cavity and disposable gloves of 18 caretakers without *S. aureus* (SA(–)) before and after animal rearing work.

could not find any evidence for animal caretakers or researchers, who worked at the university hospital during an epidemic of MRSA infection in the hospital, carrying MRSA to laboratory animals housed in our animal facility.

CONCLUSION

In conclusion, we attempted to isolate MRSA from laboratory animals housed at our facilities over a period of 17 years. Notably, we isolated a single MRSA strain from a rat colony, even though

several epidemics of MRSA infection have occurred in our hospital during this time. These results indicate that MRSA is not prevalent in our animal facility, despite frequent contact between laboratory animals and medical school and hospital faculty. Nevertheless, we must consider the risk of MRSA emergence in rats, particularly if they are treated with antibiotics, because most of the *S. aureus* isolated were detected in rats (79.7%) in this study.

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CONFLICT OF INTEREST STATEMENT

The authors declare that they have no conflict of interest.

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