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Note

Prevalence of methicillin-resistant *Staphylococcus aureus* among veterinary staff in small animal hospitals in Sapporo, Japan, between 2008 and 2016: A follow up study

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ABSTRACT

The aim of the present study was to determine and compare the prevalence of methicillin-resistant *Staphylococcus aureus* (MRSA) and their molecular characteristics among veterinary staff in Sapporo in 2008 and 2016. We isolated MRSA from veterinarians (Vet; n = 91), veterinary technicians (VT; n = 113), and other staff members (n = 24) from 45 small animal hospitals (animal hospitals), as well as from surface swabs (n = 123) obtained from 37 animal hospitals, in 2016. MRSA was observed in 14 Vets (15%), 7 VTs (6%), 2 other staff members (8%), and 6 environmental samples (5%). The prevalence of MRSA among veterinary staff tended to decrease, in comparison to 2008. All the MRSA isolates were classified as CC5/SCC*mec*II, which is commonly observed in medical settings in Japan. Upon performing pulse-field gel electrophoresis, with *Sma*I and *Eag*I, and *clfB* sequence typing, it was observed that 16 of the MRSA isolates from 2016 were highly similar to those obtained in 2008. This suggests that some MRSA isolates persisted throughout 8 years, although their origins remain unclear. The continuation of education and monitoring of MRSA is necessary for the prevention and control of infection in these settings.

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Methicillin-resistant *Staphylococcus aureus* (MRSA) is a major cause of nosocomial infection. MRSA infection leads to increased healthcare costs, primarily due to the associated high morbidity and mortality, and is an emerging concern in veterinary settings [1,2]. MRSA carriage has been frequently noted among veterinary staff worldwide [1]. In many cases, the prevalence rates of MRSA in dogs and cats were lower than in veterinary staff, and their genotypes were associated with MRSA isolates from humans [3]. MRSA is usually assumed to originate from an in-contact human [2,4]. As such, veterinary staff must pay attention to hygiene management in order to minimize cross-contamination.

In 2008, our group reported the prevalence rates of MRSA in veterinarians (Vets: 22.9%) and veterinary technicians (VTs: 10%), who worked at 71 small animal hospitals (animal hospitals) in Sapporo, Hokkaido [3]. The MRSA infection control committee of Sapporo Veterinary Medical Association (SVMA) established the '*Manual for preventing a nosocomial infection of MRSA for animal hospital*' (the MRSA manual) in 2009. The SVMA distributed the manual to animal hospitals in Sapporo, and conducted seminars. However, the contents of the manual have not been re-evaluated according to current prevalence rates of MRSA.

In this study, we investigated the occurrence and characteristics of MRSA isolates in animal hospitals in Sapporo in comparison to those observed in 2008.

Nasal swabs for the isolation of MRSA were collected using Seedswab γNo. 2 'Eiken' (Eiken Chemical, Tokyo, Japan) from 91 Vets, 113 VTs, and 24 other staff (including 13 desk workers and 11 dog groomers) who were working in 45 primary animal hospitals in Sapporo, Hokkaido, Japan, between July and September in 2016. On a single day, a total of 123 environmental surface samples (e.g. from floors, top of the medical examination stands, cages, doorknob) from 37 of 45 primary animal hospitals were collected.

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Environmental surface samples were taken by wiping 10 × 10 cm of each region of environment.

To compare the current and prior characteristics of MRSA in Sapporo's veterinary setting, we used the data for a total of 35 MRSA isolates collected in our previous study in 2008 [3]. In the 2008 study, nasal swabs were collected from 96 Vets and 70 VTs, from 71 primary animal hospitals, and 72 environmental surface samples were collected from 6 hospitals. The prevalence rates of MRSA in 2008 were 22.9% in Vets (22/96), 10% in VTs (7/70) [3], 8% in the environmental samples (6/72; the data has not published), and 31% in hospitals (22/71). In 2008, we did not collect samples from other staff members; therefore, the corresponding data are absent.

Swabs were incubated in 3 mL of tryptic soy broth (Becton Dickinson Japan, Tokyo, Japan) containing 6.5% NaCl, 5 μg/mL ceftizoxime (Tokyo Chemical Industry CO., LTD., Tokyo, Japan), and 75 μg/mL aztreonam (Tokyo Chemical Industry CO., LTD) at 37 °C for 24 h. A loopful of enrichment broth was then plated onto an oxacillin-resistant screening agar base (Oxoid, Basingstoke, England), and incubated at 37 °C for 24–48 h. In the case of suspected MRSA, 1 to 3 colonies per sample were transferred to trypticase soy agar (Becton Dickinson Japan). *S. aureus* isolates were identified through matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the Bruker MALDI Biotyper system (Bruker Daltonics, Bremen, Germany) with the ethanol-formic acid extraction method and PCR for the *femA* gene [5], specific to *S. aureus*. For the detection of MRSA, the *mecA* gene [5], which encodes the penicillin-binding protein 2′, was investigated by PCR. The *mecA*-positive *S. aureus* isolates were identified as MRSA.

SCC*mec* typing [6], determination of the clonal complex (CC) by phage open reading frame (ORF) typing [7], pulsed-field gel electrophoresis (PFGE) using 30 U *Sma*I (TaKaRa, Otsu, Japan) or 20 U *Eag*I (New England Biolabs Japan, Tokyo, Japan) [8] were performed as previously described. Since MRSA strains isolated in 2008 and 2016 displayed 100% similarity, as estimated through *Sma*I, the clumping factor B gene (*clfB*) typing was performed as previously described [9]. The *clfB* gene, containing a highly variable serine-aspartate repeat region, was capable of discriminating within identical PFGE clusters duo to nucleotide mutations and ease of repeat duplication/deletion via slipped-strand mispairing during replication [9].

Antimicrobial susceptibility was tested by the agar dilution method using the Mueller-Hinton Agar (Oxoid), following Clinical and Laboratory Standards Institute (CLSI) recommendations [10] for: ampicillin (AMP; Sigma–Aldrich, St. Louis, MO, USA), oxacillin (OXA; Sigma–Aldrich), kanamycin (KAN; Sigma–Aldrich), gentamicin (GEN; Sigma–Aldrich), erythromycin (ERY; Sigma–Aldrich), clindamycin (CLI; Sigma–Aldrich), vancomycin (VAN; Sigma–Aldrich), enrofloxacin (ERFX; Sigma–Aldrich), and tetracycline (TET; Wako Pure Chemical Industries, Osaka, Japan). *S. aureus* ATCC 29213 and *Enterococcus faecalis* ATCC 29212 were used as quality control strains. The breakpoints of these antimicrobial agents were determined according to CLSI interpretation criteria [10].

This study was approved by the Ethics Committee of the Graduate School of Dairy Science, Rakuno Gakuen University (RGU), Japan (No. 16-1).

In 2016, MRSA isolates were obtained from 13 animal hospitals (29%), 14 Vets (15%), 7 VTs (6%), and 2 other staff members (8%; 1 dog groomer and 1 desk worker). Six environmental surface samples (5%) from 3 hospitals were MRSA-positive, and the corresponding areas were: the floor of the medical examination room (n = 2), the cage for inpatient animals (n = 1), the top of the medical examination table (n = 1), the top of the X-ray table (n = 1), and forceps for medical use (n = 1). The rate of antimicrobial resistance is shown in Fig. 1. All the MRSA isolates in 2016 were resistant by 100% to ampicillin, oxacillin, kanamycin, erythromycin and clindamycin, 93% to gentamicin and enrofloxacin, and 86% to tetracycline. The prevalence of MRSA in Vets, VTs, and the environment were lower in 2016 than in 2008. This may be due to the effect of the MRSA manual which insisted on thorough hygiene management. However, the 2016 prevalence rate was still higher than that of veterinary staff in other countries [11], and of medical staff in Japan [12].

The genetic relationship among the MRSA isolates, based on PFGE, is shown in Fig. 1. The 29 MRSA isolates from 2016 were classified into CC5/SCC*mec*II, a major strain in hospital and veterinary settings in Japan [3]. Upon PFGE, the MRSA isolates from different individuals or origins (dog or environment) in the same hospital displayed 100% similarity in both 2008 and 2016. Furthermore, some MRSA clones with 100% similarity were found across animal hospitals. Through the investigation regarding the relationship among animal hospitals by SVMA, a relationship was found among animal hospitals which showed 100% similarity in the MRSA clone as follows: MRSA-positive animals visited both 16-21 (year-hospital code) and 16-25, and animal patients and owners sometimes moved between 16-45 and 16-48. These findings could be due to the insufficient control of nosocomial MRSA infections in Japanese veterinary settings. Education programs focusing on nosocomial infection could improve infection rates [13]. Therefore, continued education for hospital staff is essential for nosocomial pathogen infection control.

In addition, 16 MRSA strains isolated in 2008 (n = 13) and 2016 (n = 3) displayed 100% similarity through *Sma*I PFGE. The similarity was estimated using different restriction enzyme *Eag*I and *clfB* sequence typing (Fig. 2). After the *Eag*I PFGE analysis, the isolates were categorized into 2 groups, which showed close band patterns and remarkably high similarity (≥98%). For the *clfB* sequence, the repeat profile of MRSA was different between 2008 and 2016, although the *clfB* repeat profiles were similar. The *clfB* repeat profile of MRSA in 2016 had a defective repeat number from 9 to 6 (illustrated through underlined text) compared with the MRSA profile in 2008 (5′-1-2-3-4-5-6-4-7-8-8-4-5-6-7-17-34-3′ and 5′-1-2-3-4-5-6-4-7-8-8-4-5-6-7-9-10-11-12-12-18-30-6-33-13-14-8-14-33-12-14-8-15-6-16-6-17-34-3′, respectively). This shows that certain MRSA clones could persist in veterinary settings for at least 8 years. According to the investigation by SVMA, the animal hospitals and individuals studied in 2008 and 2016 were nonidentical. The hospital corresponding to 16-33 had opened recently, and had not participated in the 2008 investigation. The employees of 16–33 did not work at the hospitals in which clonal MRSA was isolated. The hospitals in which clonal MRSA was isolated in 2008 (08-18, 24, 37, 52, 57, and 70) did not participate or produced negative results in 2016. Although, a relationship between staff and the movement of animals was noted: the staff of 08-18 and 16-33 were friends and may have met directly, and an MRSA-positive animal patient from 08-24 had moved to 16-33 in 2015. Humans could carry certain MRSA strains for several years [14], and humans, animals, and environments may act as reservoirs of MRSA in veterinary settings [2,15], although the origin of the MRSA remains unclear.

In conclusion, the prevalence of MRSA among veterinary staff is still high, and MRSA strains could persist for long periods in veterinary settings in Japan. Regular monitoring would be useful for MRSA prevention through providing information for discussing the route of transmission, local MRSA epidemiology, and education regarding hygiene management.

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