Outbreak of methicillin-resistant *Staphylococcus aureus* sequence type 1, *spa* type t1784, in an equine hospital in Japan

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ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) has often been isolated from livestock and companion animals, including horses. Seven cases of MRSA infection in Thoroughbred racehorses were observed in an equine hospital in Japan in 2020. In this study, MRSA isolates from these seven horses and nine veterinarians in the equine hospital were studied to examine their genetic relatedness and evaluate the possibility of MRSA transmission. The MRSA isolates were subjected to whole-genome sequencing for multi-locus sequence typing, *S. aureus* protein A (*spa*) typing, staphylococcal cassette chromosome typing, and antimicrobial resistance gene detection. Minimum inhibitory concentrations of antibiotics were assessed to determine the antimicrobial susceptibility phenotype of the isolates. Phylogenetic trees based on single nucleotide polymorphisms were constructed to identify genetically close isolates. All isolates from horses and veterinarians belonged to sequence type (ST) 1, spa type t1784, with a point mutation in *gyrA* and double point mutations in *grlA*, which is known to cause fluoroquinolone resistance. All ST1-t1784 isolates were genetically closely related based on the phylogenetic tree. Our results suggested an outbreak and horse–veterinarian transmission of ST1-t1784 strains in an equine hospital.

Abbreviations

MRSA methicillin-resistant *Staphylococcus aureus*
ST sequence type
SCC*me*c Staphylococcal cassette chromosome
MIC minimal inhibitory concentration
SNP single nucleotide polymorphism

1. Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has often been isolated from livestock and companion animals (Cuny et al., 2015; Leonard & Markey, 2008). From horses, MRSA sequence type (ST) 1, ST5, and ST8 have been isolated, and transmission of MRSA between humans and horses has been suspected (Carfora et al., 2016; Cuny et al., 2008; Loncaric et al., 2014). In Japan, ST5 infection in Thoroughbred racehorses, ST1 isolation from a wound in a racehorse, and ST5 colonization in veterinarians in equine hospitals have been reported (Kuroda et al., 2016; Sekizuka et al., 2020).

In 2020, we encountered seven cases of MRSA infection in Thoroughbred racehorses in an equine hospital in Japan. To evaluate the possibility of MRSA transmission among cases and from horses to veterinarians, we examined MRSA colonization in 28 veterinarians in the equine hospital. Furthermore, MRSA isolates from the horses and veterinarians were subjected to whole-genome sequencing to examine their genetic relatedness and characteristics.

2. Material and methods

2.1. Ethical approval

This study was approved by the Research Planning Committee of Japan Racing Association (study approval number 2018–3263–05). Informed consent was obtained from the horse owners.
Veterinarians were informed about the aims of the investigation, and their consent was obtained.

2.2. Cases

Seven horses were treated for a surgical site infection after orthopedic surgery (case No. 1) or ophthalmic infections (cases No. 2–7) in an equine hospital from December 2019 to December 2020 (Fig. 1). The equine hospital is located in a training facility with an average of 2000 Thoroughbred racehorses, with 19 hospitalization stalls. Case No. 1 (female, 4-year-old) was hospitalized in June 2019 for bone fractures of the front leg and underwent surgery in December 2019; she was treated with benzylpenicillin and streptomycin until draining from the surgical area that began two weeks after the surgery. Case No. 2 (male, 6-year-old) had a tumor on the third eyelid with associated corneal ulceration and was hospitalized. He was treated with lomefloxacin before the isolation of MRSA. Case No. 3 (male, 2-year-old), No. 4 (male, 3-year-old), No. 5 (gelding, 6-year-old), No. 6 (male, 2-year-old), and No. 7 (female, 6-year-old) had corneal wounds and hospitalized after one to 24 days of treatment in the stables (Fig. 1). They were treated with antimicrobial eye drops (No. 3: tobramycin and levofloxacin; No. 4: levofloxacin; No. 5: tobramycin and ofloxacin; No. 6: tobramycin, levofloxacin, and cefmenoxime; and No. 7: tobramycin and cefmenoxime) before the isolation of MRSA.

2.3. MRSA isolation from cases

Samples from the surgical site infection (case No. 1) and swab samples of the cornea (cases No. 2–7) were subjected to bacterial culture (Fig. 1) using Columbia agar with 5% horse blood. Isolates were identified as *S. aureus* using MALDI Biotyper (Bruker Japan, Kanagawa, Japan) and showed the presence of mecA using Cica Geneus Staph POT KIT (Kanto-Kagaku, Tokyo, Japan), which were determined to be MRSA according to the Clinical and Laboratory Standards Institute (CLSI) criteria (M100Ed29E) (Clinical & Laboratory Standards Institute, 2017).

2.4. MRSA isolation from veterinarians and the environment in the hospital

To investigate the colonization of MRSA in veterinarians in the equine hospital, swab samples from nasal cavities were collected from 28 veterinarians in January 2021. Twenty-five environmental samples were obtained from 15 sampling points including equipment commonly used by veterinarians and instruments used to treat horses in January 2021 (Supplementary Table 1). The samples were cultured in mannitol salt agar with egg yolk and 4 μg/mL cefoxitin to selectively obtain MRSA. Colonies were identified as *S. aureus* with the MALDI Biotyper (Bruker Japan), and the presence of mecA was used to identify MRSA.

2.5. Genetic characterization of MRSA isolates

DNA samples were extracted from each horse and veterinarian isolate using the UltraClean Microbial Kit (QIAGEN, Hilden, Germany). Libraries were prepared using the DNA Prep Tagmentation kit (Illumina, San Diego, USA), and whole-genome sequencing was performed using the iSeq 100 system (Illumina). Obtained reads (accession numbers in DNA Data Bank of Japan: DRR347960–DRR347975) were trimmed with Trimmomatic v.0.39 (http://www.usadellab.org/cms/?page=trimmomatic) and assembled with SPAdes genome assembler v.3.15.2 (https://usegalaxy.org). Contig reads were subjected to multi-locus sequence typing with PubMLST (https://pubmlst.org/) using mist software (https://github.com/tseemann/mist). Furthermore, *S. aureus* protein A (spa) typing was performed with SpaServer (https://spaserver.rindom.de/) using SpaTyper v.0.3.3 (https://github.com/HGB-IGTP/spatyper). Identification of staphylococcal cassette chromosome (SCCmec) type was done using SCCmecFinder 1.2 (https://cge.cbs.dtu.dk/services/SCCmecFinder/), and the antimicrobial resistance genes were detected using ResFinder 4.1 (https://cge.cbs.dtu.dk/services/ResFinder/). Minimal inhibitory concentrations (MICs) for oxacillin, gentamicin, and levofloxacin were determined using the dry plate “Eiken” (Eiken Kagaku, Tokyo, Japan), and susceptibility was determined based on CLSI criteria (M100Ed29E).

2.6. Phylogenetic analysis of MRSA isolates

To evaluate strain relatedness and genomic characteristics of genetically close isolates from the horses and veterinarians, single nucleotide polymorphism (SNP) calling and phylogenetic tree analysis were performed. Sequences of the isolates after trimming and 24 published *S. aureus* genome sequences obtained from NCBI GenBank (https://www.ncbi.nlm.nih.gov/genbank/) were submitted for read mapping, variant calling, and core genome mapping against the *S. aureus* WBG8287 reference genome (accession no. CP070986) using Snippy v.4.4.0 (https://github.com/tseemann/snippy) and phylogenetically analyzed using Gubbins v.3.1.4 (https://github.com/sanger-pathogens/gubbins). A phylogenetic tree was constructed using MEGA v.11 (https://www.megasoftware.net/), and pairwise SNP counts were obtained using BioEdit v.7.1 (http://www.mbio.ncsu.edu/BioEdit/bioedit.html). The 24 *S. aureus* isolates were selected for comparison with a phylogenetic analysis because the 24 isolates belonged to the same clade as the isolates identified in this study, as shown in a phylogenetic tree generated using complete or chromosome sequences of 848 *S. aureus* sequences obtained from the NCBI GenBank (data not shown).

3. Results

3.1. MRSA isolates from horses, veterinarians, and the environment in the hospital

MRSA was isolated from cases as shown in Fig. 1 as well as from nine veterinarians (vets A–I), and the first MRSA isolate from each case and one MRSA isolate per veterinarian was used for subsequent studies.
MRSA was not isolated from environmental samples. Table 1 shows the ST, spa type, SCCmec type, detected antimicrobial resistance gene, detected point mutation causing fluoroquinolone resistance, and antimicrobial resistance phenotype of MRSA isolates from infected horses and veterinarians. The seven horse and two veterinarian isolates (vets B and F) were classified as ST1-spa type t1784-SCCmec type IVa. The remaining seven veterinarian isolates were classified as shown in Table 1. All nine ST1-t1784 isolates from horses and veterinarians had a point mutation in gyrA (S84L, TCA > TTA) and double point mutations in grlA (S80F, TCC > TTC; and E84G, GAA > GGA), which caused fluoroquinolone resistance. All nine isolates were resistant to oxacillin and levofloxacin based on their MICs (>4 μg/mL). Analysis of other antimicrobials revealed the presence of qacA and aac(6′)-aph(2′) that encode for disinfectant resistance and aminoglycoside resistance, respectively, in six of the seven isolates from horses. Gentamycin showed MICs >8 μg/mL for these isolates, and they were not susceptible to it (Table 1).

### Table 1

| Isolated from | ST   | spa type | SCCmec | AMR gene          | AMR point mutation | AMR phenotype |
|---------------|------|----------|--------|-------------------|--------------------|---------------|
| Case No. 1    | 1    | t1784    | IVa    | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| Case No. 2    | 1    | t1784    | IVa    | anr(9)-la, blaz, mecA, qacA | grlA S84L, grlA S80F, grlA E84G | OXA, LVX |
| Case No. 3    | 1    | t1784    | IVa    | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| Case No. 4    | 1    | t1784    | IVa    | anr(9)-la, aac(6)-aph(2′) | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| Case No. 5    | 1    | t1784    | IVa    | anr(9)-la, aac(6)-aph(2′) | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| Case No. 6    | 1    | t1784    | IVa    | anr(9)-la, aac(6)-aph(2′) | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| Case No. 7    | 1    | t1784    | IVa    | anr(9)-la, aac(6)-aph(2′) | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| vet A         | 8    | t1767    | IV     | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| vet B         | 1    | t1784    | IVa    | blaz, mecA        | grlA S84L, grlA S80F, grlA E84G | OXA, LVX |
| vet C         | 8    | t273     | IV     | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN |
| vet D         | 5    | 1442     | II     | anr(9)-la, aac(6)-aph(2′) | grlA S84L, grlA S80F, grlA E84G | OXA, GEN, LVX |
| vet E         | 8    | t1767    | IV     | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN |
| vet F         | 1    | t1784    | IVa    | blaz, mecA, ermC  | grlA S84L, grlA S80F, grlA E84G | OXA, LVX |
| vet G         | 8    | t1767    | IV     | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN |
| vet H         | 8    | t1767    | IV     | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN |
| vet I         | 8    | t1767    | IV     | aac(6)-aph(2′)    | grlA S84L, grlA S80F, grlA E84G | OXA, GEN |

*ST, sequence type; AMR, antimicrobial resistance; OXA, oxacillin; GEN, gentamicin; LVX, levofloxacin.

No known mutations were identified.

3.2. Phylogenetic analysis of MRSA isolates

The phylogenetic tree based on 4611 core-genome SNPs showed that the ST1-t1784 isolates from horses and veterinarians obtained in this study were arranged in one clade and differed by 0–45 SNPs with pairwise SNP counts (Fig. 2).

4. Discussion

MRSA ST1-t1784 isolates were isolated from seven horses in an equine hospital in 2020. The duration of hospitalization for some horses overlapped (Fig. 1), and all MRSA isolates from the seven cases were ST1-t1784. For five cases (No. 3–7), MRSA was isolated during hospitalization, although the cases were MRSA-negative before or at the time of hospitalization. These results suggest an outbreak of the ST1-t1784 strain in the hospital and transmission of MRSA to the five cases in the hospital. Measures to control MRSA transmission, including early detection of MRSA outbreaks and sampling to clear the transmission route, are needed to prevent MRSA infection in hospitalized horses. In addition, the five cases had been treated with antimicrobials including fluoroquinolones, tobramycin, and cefoxime prior to isolation of MRSA, even though MRSA isolates were suggested to be resistant to these antimicrobials according to their antimicrobial genes. The administered antimicrobials might have acted as a selection pressure in our cases.

Two hospital veterinarians, who were treating the cases, were found to harbor MRSA ST1-t1784 isolates, suggesting MRSA transmission between horses and veterinarians. One limitation of this study is that the veterinarians were sampled only once, and thus it was not clear whether the veterinarians were carrying MRSA before and during treatment of the cases; therefore, we were unable to determine the direction of the transmission route between horses and veterinarians.

ST1-t1784 MRSA isolates from human blood samples have been previously reported in Japan (Aung et al., 2021). In the phylogenetic tree based on SNPs, the ST1-t1784 isolates obtained in this study shared the origin with a strain isolated from a human in Japan (accession no. AP018923) (Cui et al., 2019). Because of the information on these isolates from humans, it was thought that the ST1-t1784 strain might have entered the equine hospital from humans, even though the route of entry was not clear in this study.

Isolates from cases Nos. 3–7, which were negative for MRSA at the beginning of treatment, differed by only 0–4 SNPs in the phylogenetic tree. Isolates from veterinarians B and F differed from those in the horses by 9–45 SNPs. Veterinarian isolates were isolated 2–12 months after isolation from the horses, and thus the core genome was expected to have changed at a constant rate (1 SNP per ~6 weeks) during that period (Harris et al., 2010). While one limitation was that the sampling periods of horses and veterinarians were different, our results suggest that the ST1-t1784 isolates in the present study are genetically close to each other, and that MRSA was transmitted between horses and veterinarians in the hospital.

5. Conclusion

MRSA ST1-t1784 was isolated from horses and veterinarians in an equine hospital. The results suggest that all isolates were genetically closely related and transmission of ST1-t1784 isolates between horses and veterinarians had occurred. This is the first report of an outbreak of
the ST1-t1784 strains in an equine hospital and the first to suggest transmission between horses and veterinarians. Our study highlights the need to control MRSA transmission through veterinarians in equine hospitals.

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Ethical statement

This manuscript has not been published or presented elsewhere in part or in entirety and is not under consideration by another journal. An informed consent was obtained from the horse owners, and the study design was approved by the appropriate ethics review board. We have read and understood your journal’s policies, and we believe that neither the manuscript nor the study violates any of these. Details about competing interests are provided separately.

Declaration of Competing Interest

The authors declare that they have no known competing financial interests or personal relationships that could have appeared to influence the work reported in this paper.

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Supplementary materials

Supplementary material associated with this article can be found, in the online version, at doi:10.1016/j.vas.2022.100259.

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