Evidence of Multiple Virulence Subtypes in Nosocomial and Community-Associated MRSA Genotypes in Companion Animals from the Upper Midwestern and Northeastern United States

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Objective: Not much is known about the zoonotic transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) in companion animals in the United States. We report the rate of prevalence of *S. aureus* and MRSA recovered from clinical samples of animals requiring treatment at veterinary clinics throughout the upper midwestern and northeastern United States.

Design: We compared phenotypes, genotypes, and virulence profiles of the MRSA isolates identified in companion animals, such as cats, dogs, horses, and pigs, with typical human nosocomial and community-associated MRSA (CA-MRSA) genotypes to assess implied zoonotic transmission or zoonanthroponosis. Five hundred thirty-three coagulase-positive staphylococci (CPS) isolates recovered between 2006 and 2008 from a variety of animal-source samples were screened for *S. aureus* by *S. aureus*-specific 16S rDNA primers and were screened for methicillin-resistance. All MRSA isolates were genotyped by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and *spa* typing. They were also screened for common staphylococcal enterotoxin and adhesion genes by multiplex and singleplex PCR.

Results: Among the 533 CPS isolates recovered, 66 (12.4%) were determined to be *S. aureus* and 24 (4.5%) were MRSA. The percent of animals that were positive for *S. aureus* were as follows: 6.6% (32 of 487) dogs, 39.6% (19 of 48) cats, 83.3% (10 of 12) horses, and 100% of pigs, rabbits, hamsters and rats. Notably, 36.4% of all *S. aureus* identified were MRSA. Methicillin-resistant *S. aureus* was present in clinical samples from 12 of 487 dogs (2.5%), 6 of 48 cats (12.5%), 5 of 12 horses (42%), and 1 of 2 pigs (50%). The 24 MRSA isolates resolved into 4 PFGE clones: USA100 (50%), USA300 (16.7%), USA500 (20.8%) and USA800 (12.5%) and 6 sequence types (ST5, ST8, ST105, ST830, and ST986) or 2 clonal complexes, CC5 and CC8. Five major virulence profiles (clusters A to E) were observed in these MRSA isolates. Genotypic and virulence profiles of cats and dogs were more similar to each other than to those of horses. A Panton-Valentine leukocidin positive isolate with ST8:USA300 background was identified in a pig causing skin and soft infection.

Conclusion: The presence of human MRSA clones in these animals suggests possible reverse zoonotic transmission. This study reports the first case of a USA300 genotype in a pig. Presence of multiple virulence profiles within a MRSA genotype in these animals suggests the potential of emergence of new MRSA clones by gaining or losing additional virulence genes.

Keywords: CA-MRSA; MRSA; Nosocomial; *Staphylococcus aureus*; Virulence; Zoonotic

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Staphylococcus aureus is a common human pathogen capable of producing a wide range of diseases from skin and soft tissue infections to life-threatening endocarditis, bacteremia, and necrotizing pneumonia.¹ Infections due to *S. aureus* have assumed new public health importance due to emerging multiple antibiotic resistant strains, particularly methicillin-resistant *S. aureus* (MRSA) and its epidemic clones increasingly being found in communities and hospitals.^{2,3} Typically, community-associated MRSA genotypes belong to USA300, USA400, USA1000, and USA1100, whereas healthcare-associated MRSA genotypes belong to USA100, USA200, and USA500.^{4,5}

While MRSA has long been recognized in human healthcare settings and in people in the community without known risk factors, its ability to produce disease in domesticated companion and farm animals (eg, dogs, cats, horses, and pigs) has gained attention in recent years. The first case of MRSA reported in an animal setting was in 1972 from Belgian dairy cows with mastitis.⁶ Since that time, reports have documented cases of MRSA in dogs, cats, horses, pigs, and other animal species.⁷⁻¹⁵ Except for ST398, the majority of MRSA isolated from these animals have been human MRSA clones known to circulate in hospital settings,^{11,14-20} leading to the speculation that MRSA could be transmitted from humans to animals. Most of these reports of zoonotic MRSA have come from Europe, Asia, and Canada.^{15,21-25} There are few reports describing the prevalence of MRSA recovered from animals in the United States.²⁶⁻²⁸ The aim of this study was to determine the frequency, genotypic characteristics, and virulence attributes of MRSA recovered from animal samples submitted to a regional veterinary diagnostic reference laboratory. These clinical samples were submitted from animals requiring treatment located in the midwestern and northeastern regions of the United States.

Methods

Collection of Study Isolates

Five-hundred thirty-three isolates of coagulase positive staphylococci (CPS) were recovered from companion animals that were treated at numerous veterinary clinics located throughout the midwestern and northeastern United States (Michigan, Minnesota, New Hampshire, Ohio, Pennsylvania, Wisconsin). Clinical specimens were submitted to Marshfield Labs, a diagnostic laboratory of Marshfield Clinic (Marshfield, Wisconsin) from March 2006 to October 2008 for routine culture and sensitivity testing. All CPS isolates collected during that time period were included in this study.

Microbial Susceptibility Testing

Microbial susceptibilities were performed using the disk-diffusion method according to the Clinical Laboratory Standards Institute guidelines.²⁹ Antimicrobials tested included ampicillin, enrofloxacin, cephalothin, neomycin, amoxicillin/clavulanic acid, erythromycin, gentamicin,

marbofloxacin, orbifloxacin, oxacillin, trimethoprim/sulfamethoxazole, and tetracycline.

Identification of *S. aureus*

All study isolates were identified as being *S. aureus* using a *S. aureus*-specific 16S rDNA polymerase chain reaction (PCR) assay developed in our laboratory. Forward and reverse primers were StaA-F 5'-GACGAGAAGCTTGCTTCTCTGATG-3' and StaA-R2 5'-TAATGCAGCGCGGATCCA-3', respectively. Polymerase chain reaction assay conditions have been described previously.³⁰

mecA Screening

Isolates that were identified as *S. aureus* and were oxacillin resistant based on antimicrobial susceptibility testing were further confirmed using *mecA* PCR as previously described.³¹

Molecular Typing

All MRSA isolates were genotyped by pulsed-field gel electrophoresis (PFGE), multilocus sequence typing (MLST), and staphylococcal protein A (*spa*) typing using protocols previously described.³²⁻³⁴ The PFGE-based dendrogram was created using the Dice coefficient and the unweighted-pair group method using arithmetic averages. Clones were defined using 80% genetic similarity and 1.25% tolerance by PFGE in combination with the staphylococcal cassette chromosome *mec* (SCC*mec*) and MLST typing data. Staphylococcal cassette chromosome *mec* types were determined using the method of Oliveira and de Lencastre.³⁵ Multilocus sequence typing-based sequence types (ST) were clustered into clonal complexes (CCs) using eBURST analysis (<http://eburst.mlst.net/>). A CC was defined as having six out of seven identical loci of the MLST allelic profile. Using the Based Upon Repeat Pattern (BURP) algorithm within Ridom StaphType software (<http://www3.ridom.de/spa-server/>; version 1.5.17; Ridom GmbH, Wurzburg, Germany), *spa* types were also grouped into *spa* clonal complexes (*spa*CC). Default parameters ($x=5$; $y=4$) were applied.³⁶ BURP analysis allows determination of clonal relatedness based on *spa* types of *S. aureus*.

Virulence Gene Profile PCR Assay

Methicillin-resistant *S. aureus* isolates were screened by a combination of multiplex and singleplex PCR for several virulence genes which included staphylococcal enterotoxins (*sea*, *seb*, *sec*, *sed*, *see*, *seg*, *seh*, *sei*, *sej*, *sek*, *sel*, *sem*, *sen*, *seo*), Panton-Valentine Leukocidin (*lukSF-PV*), and staphylococcal adhesions (*fnbA*, *fnbB*, *clfA*, and *clfB*). The list of primers and PCR methods has been described previously.³⁷ The PCR results were analyzed as binary data using Dice coefficients and maximum parsimony tree analysis (BioNumerics, Applied-Maths, Kortrijk, Belgium).

Results

The distribution of clinical specimens from animals found to be positive for CPS ($n=533$) included dogs ($n=487$), cats

Table 1. Number of animals screened along with the rate of positivity for *S. aureus* and MRSA.

Animal	CPS-positive (n)	<i>S. aureus</i> n (%)	MRSA-positive <i>S. aureus</i> n (%)	MRSA-positive CPS (%)
Dog	487	32 (6.6)	12 (37)	2.5
Cat	48	19 (39.6)	6 (32)	12.5
Horse	12	10 (83.3)	5 (50)	42
Pig	2	2 (100)	1 (50)	50
Rabbit	1	1 (100)	0 (0)	0
Hamster	1	1 (100)	0 (0)	0
Rat	1	1 (100)	0 (0)	0
Total	533	66 (12)	24 (36.4)	5

MRSA, methicillin-resistant *Staphylococcus aureus*; CPS, coagulase-positive staphylococci

(n=48), horses (n=12), pigs (n=2), hamster (n=1), rabbit (n=1), mink (n=1), and rat (n=1) (table 1). Sixty-six of the CPS isolates (12.4%) were identified as *S. aureus* by the *S. aureus*-specific PCR. The number and percent of animals that were positive for *S. aureus* were as follows: 32 of 487 (6.6%) dogs, 19 of 48 (40%) cats, 10 of 12 (83%) horses, and 100% of pigs, rabbits, hamsters and rats. Twenty-four of these 66 (36.4%) *S. aureus* isolates were confirmed to be *mecA* PCR positive. These MRSA isolates originated from samples obtained from dogs (n=12), cats (n=6), horses (n=5), and pig (n=1). The percent of *S. aureus* in each animal that were MRSA were dogs 37%, cats 32%, horses 50%, and pigs 50%. However, the percent of CPS that were found to be MRSA in horses, cats, and dogs were 42%, 12%, and 2% respectively. The month and year of isolation of the MRSA, state from

which samples were received, and source of the clinical samples was documented (table 2).

Most of the samples represented skin/soft tissue infections or abscesses. As expected, all *mecA* positive isolates were phenotypically resistant to oxacillin (≥ 4 μ g/ml). Most isolates were susceptible to trimethoprim/sulfamethoxazole (83%), tetracycline (75%), and gentamicin (71%), and were less susceptible to erythromycin (29%), marbofloxacin (29%), enrofloxacin (30%), orbifloxacin (31%), and neomycin (37%) (table 3). Interestingly, unlike MRSA isolates from dogs and cats, most MRSA isolates from horses were resistant to trimethoprim/sulfamethoxazole and tetracycline but were susceptible to enrofloxacin and erythromycin.

Table 2. List of MRSA isolates, their animal sources, date of isolation, origin of state and clinical source for each specimen.

Isolate #	Species	Isolate Date	Origin	Source
V-385	Canine	11/2006	OH	Skin
V-394	Canine	12/2006	PA	Skin
V-164	Canine	5/2006	WI	Wound
V-536	Canine	2/2008	PA	Catheter
V-563	Feline	3/2008	OH	Leg
V-607	Canine	4/2008	PA	Implant
V-379	Feline	2/2007	OH	Arm
V-031	Canine	3/2006	MN	Bone
V-411	Canine	1/2007	NH	Biopsy
V-223	Canine	9/2006	MN	Other
V-381	Canine	11/2006	MN	Thigh
V-501	Feline	6/2007	MI	Tissue
V-516	Feline	2/2008	MI	Tissue
V-272	Canine	12/2006	PA	Skin
V-450	Feline	1/2008	MN	Lesion
V-522	Pig	2/2008	WI	Abscess
V-569	Canine	3/2008	MN	Paw
V-453	Feline	6/2007	MN	Lesion
V-046	Equine	4/2006	PA	Heel
V-446	Equine	5/2007	WI	Discharge from incision
V-519	Equine	2/2008	WI	Draining tract
V-526	Equine	2/2008	WI	Draining tract
V-708	Equine	8/2008	MN	Draining tract
V-744	Canine	10/2008	OH	Abscess

Table3. Antimicrobial susceptibility profile of the MRSA isolates.

Isolate #	Source	MLST	AMP	BAY	CEP	NEO	CLA	ERY	GEN	MAR	ORB	OXA	SXT	TET
V-031	Canine	ST5	R	R	R	R	R	R	S	R	R	R	S	S
V-046	Equine	ST8	R	S	R	S	R	R	S	S	S	R	R	R
V-164	Canine	ST5	R	R	R	R	R	R	S	R	R	R	S	S
V-223	Canine	ST5	R	R	R	R	R	R	S	R	R	R	S	S
V-272	Canine	ST986	R	S	R	S	R	S	R	S	S	R	S	S
V-379	Feline	ST5	R	R	R	R	R	R	S	R	R	R	S	S
V-381	Canine	ST5	R	R	R	R	R	R	S	R	R	R	S	S
V-385	Canine	ST105	R	R	R	R	R	R	S	R	R	R	S	S
V-394	Canine	ST105	R	R	R	R	R	R	R	R	R	R	S	S
V-411	Canine	ST5	R	R	R	R	R	R	R	R	R	R	S	S
V-446	Equine	ST8	R	S	-	-	-	S	R	-	-	R	R	R
V-450	Feline	ST8	R	R	R	-	R	R	S	-	-	R	S	S
V-453	Feline	ST8	R	R	R	-	R	R	S	-	-	R	S	S
V-501	Feline	ST5	R	S	R	S	R	S	S	S	S	R	S	S
V-516	Feline	ST5	R	S	R	S	R	S	S	S	S	R	S	S
V-519	Equine	ST8	R	S	-	-	S	S	R	-	-	R	R	R
V-522	Pig	ST8	R	R	R	-	R	R	S	-	-	R	S	S
V-526	Equine	ST8	R	S	-	-	S	S	R	-	-	R	R	R
V-536	Canine	ST5	R	R	R	S	R	R	S	R	R	R	S	S
V-563	Feline	ST5	R	I	R	S	R	R	S	S	S	R	S	S
V-569	Canine	ST8	R	-	R	-	R	R	S	R	-	R	S	R
V-607	Canine	ST5	R	R	R	I	R	R	S	R	R	R	S	S
V-708	Equine	ST830	R	I	R	-	R	S	R	-	-	R	-	R
V-744	Canine	ST5	R	R	R	R	R	R	S	R	R	R	S	S

MLST, multilocus sequence typing; AMP, Ampicillin; BAY, enrofloxacin; CEP, Cephalothin; NEO, Neomycin; CLA, Amoxicillin/Clavulanic acid; ERY, Erythromycin; GEN, Gentamicin; MAR, Marbofloxacin; ORB, Orbifloxacin; OXA, Oxacillin; SXT, Trimethoprim/Sulfamethoxazole; TET, Tetracycline; - = not determined

Genotypes of MRSA Identified

Genotypic data were analyzed in the context of CDC- (Center for Disease Control and Prevention, USA) defined USA-genotypes, MLST-based STs and CCs, and *spa* types and *spa*CCs. Four PFGE types, USA100, USA300, USA500, and USA800, were identified from the 24 MRSA isolates (figure 1). USA100 was the predominant clone, represented by 12 isolates, 10 of which originated from dogs and 2 from cats. All USA100 isolates belonged to CC5: ST5 or ST105 and SCCmec type II. Four *spa* types were observed with t002 being predominant (78%) and one representation of t242, t442, and t045 each in CC5 (figure 1). The three USA800 strains represented by ST5 and ST986 and SCCmec type IVa came from two cats and one dog. The two cat isolates were of *spa* type t688, whereas the dog isolate belonged to *spa* t002. Four USA300 isolates belonging to CC8 (ST8, SCCmec type IV) with *spa* type t008 were identified. Two of these isolates came from cats and one each from a dog and a pig. All five USA500 isolates also belonged to CC8 and came from horses. Four of the five horse isolates were of *spa* type t064.

Overall, 62.5% of the MRSA isolates belonged to CC5, whereas 37.5% belonged to CC8. Staphylococcal cassette chromosome *mec* types II and IV were primarily associated with ST5 and ST8 respectively, with some exceptions. Of the nine *spa* types identified (figure 1), type t002 was the most common (n=8) and was mainly associated with dog isolates

(7 of 8). BURP analysis of the nine *spa* types revealed two *spa*CC and two singletons. The *spa*CC t002 consisted of *spa* types t002, t045, t242, and t548, with t002 as its founder (figure 2). The other *spa*CC consisted of types t008 and t064 with no identified founder. The two *spa* types identified as singletons were t190 and t688.

Distribution of Virulence Genes

Five virulence genes clusters (A to E) were seen in the 24 MRSA isolates tested (figure 3A). All MRSA isolates were positive for genes encoding clumping factors A and B (*clfA* and *clfB*), and all but one were positive for a fibrinogen binding protein gene, *fnbA*. Isolates in the CC5 showed two major virulence genes clusters, A and B, whereas CC8 displayed three clusters, C, D, and E. Only isolates in cluster D (CC8, ST8:SCCmec IVa) harbored Pantone-Valentine leukocidin (PVL), a known tissue necrosis factor and one of the major virulence genes in CA-MRSA. Isolates belonging to CC5 were predominantly positive for an enterotoxin gene cluster (*egc*), *seg*, *sei*, *sem*, *sen*, *seo*, and *sej*, but were negative for *seb*, *see*, *seh*, *sek*, and *sel*. Enterotoxin genes *sea*, *seb*, *sec*, and *sed*, and *fnbB* were inconsistently present in CC5 isolates. Of the three clusters in CC8, cluster C was represented by a single equine isolate, V046, and harbored enterotoxin genes *seb*, *sek* and *egc* in addition to *clfA*, *clfB*, *fnbA* and *fnbB*. The cluster D isolates differed from cluster C due to absence of *fnbB*, *seb*, and *egc* but the presence of *lukSF-PV*. One

Figure 1. Pulsed-field gel electrophoresis-based dendrogram of the 24 methicillin-resistant *Staphylococcus aureus* isolates identified in this study. The dendrogram was created using the Dice coefficient and the unweighted-pair group method using arithmetic averages. Clonal relatedness was determined by 80% genetic similarity and 1.25% tolerance.

Panton-Valentine leukocidin positive isolate causing skin and soft tissue infection (ST8:USA300) was identified in a pig. The cluster E isolates, on the other hand, harbored *seb* but not *lukSF-PV* in conjunction with *clfA*, *clfB*, *fnbA*, and *sek*, with the exception of isolate V-078 which had gained the sea gene. All CC8 isolates contained *sek*, unlike CC5 isolates. Conversely, all CC5 isolates had *sem* and *sen*, while none of the CC8 isolates did (figure 3A). The virulence profile of isolate V-046, a CC8 isolate, was unique in that it had three of the *egc* (*seg*, *sei*, *sej*) found only in CC5, but lacked two of the other *egc*, *sem* and *sen*. This dichotomy was very evident following maximum parsimony (MP) analysis shown in figure 3B, showing V-046 as an island connecting the CC5 and CC8 isolates. It appeared to be a transitional genotype between the two groups, because it shared the virulence genes of CC5 and CC8, including *sek*. The MP analysis (figure 3B) showed the genetic distance as measured by additive gain or loss of single virulence genes. Based on MP analysis, USA300 and USA500 isolates were closer relative to each other, but separated from the USA100 and USA800 clones.

The ST8:USA300 isolates were branched off from the ST8:USA500 isolates due the presence of the *lukSF-PV* genes. There was extensive overlap in the virulence profiles of the USA100 and USA800-like strains associated with CC5.

Discussion

Human to human transmission of MRSA occurs commonly in hospitals and in people who live in crowded settings.³⁸⁻⁴⁰ Methicillin-resistant *S. aureus* has also been identified in many small and large animals.^{6,8,11,26,27} However, the identification of human MRSA genotypes in either healthy or diseased animals raises the intriguing possibility of zoonotic and reverse zoonotic transmission of MRSA. Indeed, several reports from Canada and Europe documented the presence of identical PFGE types in animals and their handlers or owners.^{12,15,19,23,24,41-45}

In our study, the proportion of *S. aureus* and MRSA in all CPS was 12% and 5%, respectively. However, the proportion

Figure 2. A snapshot of the *spa* type-based clonal relatedness of the 24 MRSA isolates based on BURP analysis. Each dot represents a unique *spa* type, and the blue dot represents the group founder with the highest founder score which is a *spa*CC. Shown here is the *spa* clonal complex 002 with its founder and two *spa* types t008 and t064 with no founder. The lines joining the *spa* types do not represent the genetic distance between them.

of MRSA among *S. aureus* isolates recovered was rather high (36.4%). In cats, dogs, horses, and pigs, the four frequently studied species for MRSA prevalence, we found the rate of MRSA in CPS was highest for pigs (50%), followed by horses (42%), cats (12%), and dogs (2%). Interestingly, however, the proportion of MRSA in *S. aureus* in dogs (37%) was higher than cats (32%). Pigs had a higher proportion of MRSA compared to dogs and cats (table 1). Two recent studies have provided surveillance data for the rate of prevalence of *S. aureus* and MRSA in healthy and inflammatory skin diseased (ISD) cats and dogs.^{26,27} The cat study showed that 20% of healthy and 29% of the diseased cats were culture-positive for *S. aureus*, whereas only 2% and 4%, respectively, were positive for MRSA.²⁶ A similar study showed that nearly the same percentages of healthy (12%) and the ISD (10%) dogs carried *S. aureus*. Within this population, 1.7% of ISD dogs had MRSA compared to 0% in the healthy group.²⁷ Our study, which looked only into CPS isolates of the animals with wounds or infections, found MRSA at the rate of 2% in dogs and 4% in cats. Also, the percent of CPS that were *S. aureus* in our study was higher for dogs but smaller in cats compared to the findings of Abraham et al²⁶ and Griffith et al.²⁷ In a study that focused on the rate of prevalence of *S. aureus* and MRSA carriage in non-healthcare workers, healthcare workers, and veterinary healthcare workers and their symptom-free healthy household pets, Kottler et al²⁸ showed that ~8% of dogs carried methicillin-sensitive-SA (MSSA) compared to ~6% of cats. The percent of MRSA were 3.3% for dogs and ~4% for cats. One of the conclusions from that study was that “animals were less likely to be colonized with MSSA than people, and there was no statistical difference in the proportion of MRSA among *S. aureus* isolated from people or pets.”²⁸ This study also did not find statistical difference in the proportion of dogs compared to cats colonized with MSSA or MRSA. These differences in the rates of colonization or infections from *S. aureus* and MRSA are likely to be influenced by geographical region, patient population, source

of the clinical samples (nasal vs. rectal in case of colonization), and degree of exposure to healthcare environments.

In our study, different USA genotypes were found to be associated with dogs and cats as compared to horses. The dog and cat isolates predominantly belonged to the USA100 type (71%; *spa* type:t002, 60% and t548, 17%) whereas 100% of the horse isolates⁴⁶ were in USA500 type (t064, 80% and t190, 20%). Indeed, USA100 and USA800 were the two most common PFGE types reported in humans colonized with MRSA during the study period of 2001 to 2004 in the United States.^{47,48} A higher proportion of USA100 PFGE types in cats and dogs in our study is notable because this clone was the most frequently identified PFGE type in invasive MRSA infections in a population-based surveillance study in the United States.⁴⁹ The identification of four cases of ST8:USA300 genotypes in cats, dogs, and pigs was also significant. To the best of our knowledge this is the first report of a skin and soft tissue infection in a pig due to a USA300 genotype.

Weese et al^{20,46} have also reported from studies done in Canada that MRSA clones similar to USA100 were found to colonize and/or infect small animals, and whereas MRSA clones similar to USA500 were prevalent in equine isolates. This pattern of two different MRSA lineages affecting two different animal populations has also been observed in veterinary personnel who worked either with small or large animals in the United Kingdom and Ireland.¹⁷ MRSA clones isolated from veterinary personnel who worked with small animals were similar to USA100 (ST5, SCCmec type II), whereas MRSA isolated from veterinary personnel who worked with large animals were represented by clones similar to USA500 (ST8, SCCmec type IV).^{22,46} It would be interesting to identify which staphylococcal genetic factors favor such host specificity for infection in small versus large animals. Most of the horse MRSA isolates in our study were resistant to tetracycline and sulfamethoxazole (SXT). A higher percent of resistance to these antimicrobials has also been reported in MRSA isolates recovered from horses in Canada.^{15,46} Indeed, SXT and tetracycline are commonly used antibiotics in horses while enrofloxacin and erythromycin are not. Our virulence profile data of equine isolates suggests the presence of three clusters. Cluster C included a single isolate which harbored *seb*, *sek* and three genes of the *egc* in addition to the four adhesion genes. Cluster D which was mainly distinguished from the cluster C and E due to the presence of PVL genes and lack of *seb* and *egc*. One of the isolates in this cluster harbored *sea*. The observation of multiple virulence profiles within a clonal complex in equine isolates suggests either the gain or loss of those genes in USA500 PFGE types.

The recovery of both nosocomial (USA100 and USA500) and CA-MRSA (USA300) associated genotypes in animal isolates in our study suggests a high likelihood of interspecies transmission. Indeed, other studies have shown indistinguishable MRSA strains isolated from humans and animals.^{17,19,20,45}

Figure 3. Results and analysis of the virulence gene profile PCR assay. (A) The dendrogram was generated based on the presence/absence of virulence genes. The royal blue boxes indicate the presence and yellow boxes the absence of the corresponding virulence genes. Four virulence groups (clusters A-E) were identified among the strains based on >85% similarity. The colored boxes to the right of the virulence gene data correspond to the color scheme in figure 1, where the isolates were grouped based on their USA types. The far right column (CC) refers to the clonal complex of the particular strain. (B) Maximum parsimony analysis of virulence gene profile results. The size of the circle and colored fractions within are proportional to the number of isolates of a particular USA type that have the specific virulence genotype. The length of the lines (and corresponding numbers) separating genotype circles represent the number of virulence gene differences between the adjacent groups.

One could speculate that owners and/or caretakers were the source of the particular MRSA strain(s) which was subsequently transmitted to animals. This would not be too surprising since humans and companion animals are often in close contact with each other (eg, licking, petting, grooming, lap sitting). This study was not designed to determine whether infected animals could serve as reservoirs for colonization or infection to humans and other animals; however, it is reasonable to assume that a MRSA-positive human or animal could be the source of a new infection both in animals and humans. It is also possible that such animals may have acquired MRSA strains from environmental or other non-human sources, since *S. aureus* is known to be a hardy pathogen that can survive in hospital fomites for extended periods.⁵⁰ However, more definitive studies need to be done to determine the persistence of MRSA in dogs and cats and their role as potential short- or long-term reservoirs. This is especially relevant for animals that may be carriers of CA-MRSA through contact with MRSA carriers or contaminated inanimate objects.³⁹ Panton-Valentine leukocidin-positive CA-MRSA has been a major cause of skin and soft tissue infections in humans lacking traditional risk factors.^{1,2,3} In addition, CA-MRSA has been reported from hospitals in the United States.² Four isolates in this study had the genotypes of CA-MRSA identified in humans (CC8, SCCmec Type IVa, *lukSF-PV*+, and *spa* type t008), suggesting transmission from humans to those animal species. Notably the infection of the pig was due to a PVL-positive USA300 isolate which has not been reported to date. Our study also identified six MLST types belonging to two CCs: CC5 and CC8. In order to better understand the dynamics of transmission of MRSA between humans and animals, a simultaneous screening for MRSA colonization and/or contamination should be performed in owners of the animals, immediate environments of animals where they are housed, and veterinary healthcare providers for the affected animals.

The potential for MRSA to become a problematic zoonotic pathogen could dramatically affect the epidemiology of MRSA in humans. Increased awareness of animals serving as a reservoir for MRSA is important for understanding the changing epidemiology of this pathogen. As the prevalence of MRSA in animals continues to rise, there is an inherent risk for new MRSA clones to evolve secondary to horizontal gene transfer and host selection pressure and then spread to human hosts. This is evident from the different virulence gene profiles of USA100 and USA800 strains identified in this study (figure 3A). Thus, the presence of MRSA in animals is a concern not limited only to veterinarians and animal health care workers, but to public health at large. Identification of MRSA carriers in both humans and animals will be important if recurrent MRSA colonization and/or infection is identified in animals. The possibility of an increase in zoonotic MRSA and its implications for public health warrants further investigation. Furthermore, because the direction of transmission of MRSA between animals and humans is poorly understood, long-term studies tracking carriage status

of animals and their human contacts should be conducted. Collaboration between veterinary and human healthcare workers will be necessary to help control the spread of this pathogen between humans and our companion and food-production animals.

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