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Characterization of MRSA Isolated from Companion Animals, Healthcare Providers and Environmental Surfaces of a Veterinary Teaching Hospital

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Abstract

Purpose: Hospital-acquired (HA)-MRSA has been reported to be associated with small animal personnel, as well as companion animals. The objective of this study was to explore the characteristics of MRSA isolated from the patients, healthcare providers, and environmental surfaces within the "small animal" area of a veterinary teaching hospital (VTH).

Methods: Isolates of *S. aureus* were tested for antimicrobial susceptibility, as well as the presence of the *mecA* and PVL genes; PFGE and MLST were performed.

Results: A total of 30 MRSA isolates were identified; 6 from humans, 23 from animals, and 1 from an environmental surface. The most prevalent clone of HA-MRSA (USA100: ST 5) was identified among the faculty and patients within the VTH. Additionally, community-acquired (CA) MRSA (USA 300:ST8: PVL+) was identified from multiple samples over the course of approximately four months from a single veterinary student.

Conclusion: Despite low sample size, the presence of both HA- and CA-MRSA within this VTH is notable. Additionally, evidence of transmission of MRSA among patients and health care providers was observed. The implication of this is massive and more work to identify the routes and association of transmission is needed.

Keywords: HA-MRSA: Hospital-Acquired Methicillin-Resistant *Staphylococcus aureus;* CA-MRSA: Community-Acquired Methicillin-Resistant *Staphylococcus aureus;* VTH: Veterinary Teaching Hospital; PVL: Panton-Valentine Leukocidin; ST: Sequence-Type; ECC: Emergency Critical Care Ward; OS: Orthopedic Surgery Ward; STS: Soft Tissue Surgery Ward; IM: Internal Medicine Ward

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Introduction

The presence of antimicrobial resistance among the human population is a major public health threat and has been compounded by our interaction with animals. Companion animals, such as cats and dogs, represent realistic reservoirs of antimicrobial resistance due to their close contact with humans and the extensive use of antimicrobials in veterinary medicine. This role has increased within veterinary hospitals, where antimicrobial exposure to both patients and veterinary personnel is more direct, and contact between the patient and healthcare provider is more intimate than in human hospitals. There have been conflicting opinions on whether the patients or veterinary personnel are at a higher risk of exposure to antimicrobial resistance from each other [1,2]. One qualitative risk assessment for methicillin-resistant *S. aureus* (MRSA) acquisition by veterinary teaching hospital (VTH) patients found that veterinary personnel pose the greatest risk, followed by environmental surfaces [3]. Studies in human hospitals have documented medical equipment and healthcare providers as vehicles for the transfer of bacteria, such as vancomycin-resistant enterococci (VRE) [4,5]. Comparable research in veterinary hospitals is sparse, although there is evidence that exam tables, cages, and surgical tables can harbor pathogenic bacteria [6,7].

Lin et al. [2] has reported differentiation of MRSA association with large animal compared with small animals. MRSA isolates grouped with the national MRSA strain USA100 made up the majority of MRSA isolated from dogs and cats, while isolates grouping with national strain USA500 were from horses [2]. Additionally, it has been reported that MRSA USA100/ST5 are more likely to be isolated from veterinary personnel who work with small animals, and that MRSA USA500/ST8 are more likely to be isolated from veterinary personnel working with large animals [2]. Multiple studies

have reported that hospital- and community-based fall into different genotypic groups [2,8-10]. Those identified from hospital-acquired (HA) isolates are predominantly USA100/ST5, USA200/ST36, and USA500/ST8; while those identified from community-acquired (CA) isolates are predominantly USA800/ST5 & USA 300/ST8 [2,9,10]. Additionally, it has been reported that CA-MRSA strains are more likely to carry toxin genes, including the virulence factor, Panton-Valentine Leukocidin (PVL) – which has been reported to be carried by USA300/ST8. [8,9,11].

Much work needs to be done to characterize the overlap of HA-MRSA being associated with small animal personnel, as well as companion animals, and of CA-MRSA being associated with large animal personnel, as well as horses. This study describes the characteristics of MRSA isolated from the patients, health care providers, and environmental surfaces within the "small animal" area of a veterinary teaching hospital (VTH). Although this will not answer the larger question, this paper hopes to shed more light on the connection of CA-MRSA and companion animals.

Materials and Methods

Study design

A longitudinal study was conducted in four wards in the Michigan State University (MSU) VTH, from February, 2007 through December, 2009, to describe the prevalence and patterns of resistance in companion animals, environmental surfaces, and veterinary students and staff. Methods and results describing the prevalence and antimicrobial resistance of *Enterococcus spp* and *Staphylococcus spp* isolated from repeated sampling of surfaces within the VTH [12] and the acquisition and persistence of antimicrobial resistant bacteria isolated from companion animals admitted to the VTH [13] have already been published. Methods and results describing the role of veterinary students and staff are described below.

The study focused on the Emergency Critical Care (ECC), Orthopedic Surgery (Ortho), Soft Tissue Surgery (STS), or Internal Medicine (IM) wards within the MSU VTH. Sample collection from environmental samples and veterinary students rotating through the four wards during their 3-week clinical rotations and VTH faculty/staff who worked in these areas were invited to enroll in the study, which was approved by the Institutional Review Board for Research on Human Subjects, and written informed consent was required. Students were invited to enroll during their orientation in the study wards and were asked to provide samples and complete questionnaires at the beginning and end of their rotations in these clinics. Faculty and staff who worked in the study wards were invited to enroll at any point during the three-year study and were asked to provide samples and complete questionnaires at designated sample collection dates (within five days of the start of every fourth clinical rotation).

Students and faculty/staff were asked to complete a questionnaire about the three weeks prior to every sample collection. Information collected focused on antibiotics taken, clinical procedures they performed while at the VTH, small and large animals in their home and antibiotic usage of those animals, exposure to human hospital, physician's office & nursing home, and the same information as it pertained to their roommate or household contact.

Identification of MRSA isolates

Isolates of MRSA obtained from animal and environmental samples were processed as described previously [12,13] and stored in a freezer. Frozen samples were thawed and plated on "TSA" with 5% SBA for use in molecular and genetic testing. Isolates of MRSA obtained from human samples were processed as follows:

Biological sample collection

Stool samples were collected in specimen collection tubes with Cary Blair Medium, while nasal samples were collected via a sterile swab eSwab Transport System. Collected samples were then sent to the Michigan Department of Community Health's (MDCH) ureau of Laboratories for bacterial isolation. Presumptive isolates of staphylococci and enterococci were transported via courier back to the MSU Center for Comparative Epidemiology (CCE)-Microbial Epidemiology Laboratory for further processing.

Bacterial isolation and identification

Presumptive isolates of staphylococci were streaked onto a Columbia CNA plate and incubated for 48 hours at 37°C. Up to five isolates demonstrating typical *Staphyloccocus spp* morphology were chosen for identification, which was completed using previously described, standard methods [12].

Antimicrobial susceptibility testing

Confirmed (from environmental surfaces and companion animals) and suspected MRSA isolates were submitted to MSU Diagnostic Center for Population and Animal Health (DCPAH) for processing. The Staphylococcus isolates submitted were first identified to the species level by matrix-assisted laser desorption ionization-time of flight (MALDI-TOF) mass spectrometry using the Bruker Biotyper Microflex LT operating with RUO library 4110. Isolates were run in duplicate and final identifications were generally determined by MALDI score values of >2.0. Susceptibility testing was then performed following CLSI standard MIC testing methods for bacteria of animal origin (CLSI document M31-A3*) using the Companion 2F Trek Panel [14].

Molecular characterization of MRSA isolates

All unique isolates of MRSA collected from any source throughout the study were submitted for identification of the mec and PVL genes. Additionally, PFGE was performed and compared to the national clones, USA100, USA200, USA300, USA400, USA500, USA600, USA700, USA800, USA1000, and USA1100. Finally, the MRSA isolates underwent MLST procedures.

Gene identification

Presence of the mecA and PVL genes were performed at DCPAH using standard PCR procedures, and confirmed by nucleic acid sequencing of the PCR amplicons.

Pulsed-Field Gel Electrophoresis

In order to identify instances of common sources of MRSA, pulsed field gel electrophoresis (PFGE) using the restriction enzyme *SmaI* was performed. Electrophoresis was performed using the CHEF system (CHEF-DRIII), ramping the switch times from 4 to 35 seconds, with an overall run time of 20 hours, using standard procedures for PFGE by the MSU DCPAH.

Sample band patterns were compared to the patterns of national MRSA strains using Bionumerics software, version 3.5. All patterns were normalized for comparison and a dendogram was produced. Clones were identified as those having 85% or higher Dice Coefficient [15].

Multilocus Sequence Typing

To determine clonal relatedness among MRSA isolated from

Table 1: Summary of MRSA characteristics (N=30).

Study ID	Subject ID	Source	Location	Date	MecA	PVL	PFGE Grouping*	Sequence Type (ST)**
SA-115	H2	HUM	Dermatology	4/3/2009	Y	N	USA100	5
SA-118	H2	HUM	Dermatology	5/6/2009	Y	N	USA100	5
SA-104	H3	HUM	General	10/23/2007	Y	N	USA100	5
SA-109	A9	ANI	NCU/ICU	12/17/2008	Y	N	USA100	5
SA-112	A11	ANI	ECC	3/24/2009	Y	N	USA100	5
SA-114	A12	ANI	ECC	3/27/2009	Y	N	USA100-Pr	5
SA-196	A3	ANI	ST/IM	4/18/2008	Y	N	A	5
SA-202	A3	ANI	ECC	4/16/2008	Y	N	A	5
SA-101	A2	ANI	ST/IM	2/20/2008	Y	N	B	5
SA-102	A2	ANI	ST/IM	2/20/2008	Y	N	B	5
SA-122	A3	ANI	ST/IM	4/16/2008	Y	N	B	5
SA-123	A3	ANI	ST/IM	4/18/2008	Y	N	B	5
SA-189	A4	ANI	ECC	3/21/2009	Y	N	C	5
SA-190	A4	ANI	ECC	3/21/2009	Y	N	C	5
SA-199	A4	ANI	ECC	3/21/2009	Y	N	C	5
SA-203	A14	ANI	NCU/ICU	1/22/2009	Y	N	C	5
SA-103	A7	ANI	ECC	3/30/2008	Y	N	S	5
SA-111	H1	HUM	ECC	1/30/2009	Y	Y	USA300	8
SA-113	H1	HUM	ECC	2/20/2009	Y	Y	USA300	8
SA-119	H1	HUM	Ortho	6/9/2009	Y	Y	USA300	8
SA-120	E1	ENV	ECC	9/14/2009	Y	N	S	8
SA-105	A8	ANI	ST/IM	10/9/2008	Y	N	S	72
SA-106	A1	ANI	ECC	10/10/2008	Y	N	E	p
SA-107	A1	ANI	ECC	10/10/2008	Y	N	E	p
SA-108	A1	ANI	ECC	10/10/2008	Y	N	E	p
SA-117	A5	ANI	NCU/ICU	4/22/2009	Y	N	D	p
SA-200	A5	ANI	NCU/ICU	4/22/2009	Y	N	D	p
SA-100	A6	ANI	ECC	3/17/2008	Y	N	S	p
SA-110	A10	ANI	ECC	1/22/2009	Y	N	S	p
SA-121	A13	ANI	ST/IM	9/28/2009	Y	N	P	p

* "s" indicates a singleton group

** "p" indicates the test did not produce viable results

humans, animals, and environmental surfaces, multilocus sequence typing (MLST) was performed at the MSU Genomic Research Support Technical Facility according to previously described methods [16].

Results and Discussion

Results

From the three-year study, a total of 852 samples were collected, yielding 317 isolates of *S. aureus* and 30 isolates of MRSA (6 from humans, 23 from animals and 1 from an environmental surface). All 30 MRSA isolates tested positive for the presence of the *mecA* gene. Three isolates tested positive for the PVL genes, all collected from a single human subject.

(Table 1) shows the molecular characteristics of the 30 MRSA isolates, and Figure 1 shows the PFGE dendrogram analysis. Three isolates were USA300:ST8:PVL+, five isolates were USA100:ST5:PVL- and an additional isolate were probable USA 100 and also ST5:PVL-. Eleven isolates were ST5:PVL-, but did not match a nationally recognized PFGE clone, with an additional ST8:PVL- isolate that did not match a national clone. The three PVL+ isolates were collected from the same person at three different dates. The five USA100:ST5:PVL- isolates were collected from two people and two animals. Eight isolates only produced results for one of the seven primers used for MSLT, after two attempts. Being that the same primer sets were used for all isolates, and that at least one primer did produce results, we are uncertain as to why we were unable to obtain a complete ST.

Discussion

Over the course of three years, 30 isolates of MRSA were collected from animals, their caregivers, and environmental surfaces of MSU's VTH, yielding a three-year prevalence of 9.5%. Similar human [17] and veterinary [2] studies reported MRSA prevalence of 4.2% (0%-22.1%) during 2010 and 4.5% between 2006 and 2009, respectively [17]. Assessing the prevalence of MRSA, regardless of infection status, gives insight into the potential for hospital acquired infections. The overall prevalence of MRSA in human subjects in this study was 3.1%, which agrees with other studies reporting commensal MRSA prevalence from 1-1.5% of the general population [18,19], but was

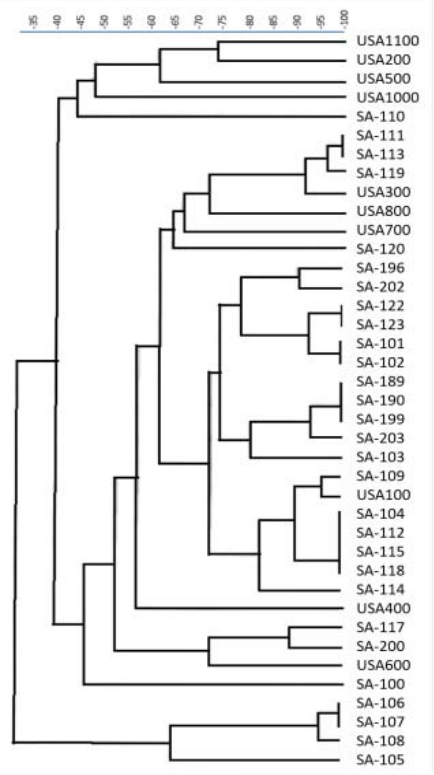

Figure 1: Dendrogram of PFGE cluster analysis. 30 MRSA isolates were compared to 10 national strains.

lower than other studies of MRSA in veterinary health professionals, which ranged from 6.5% to 12.5% [20-23]. Studies of MRSA in VTH staff in contact with MRSA patients were much higher, ranging from 16% to 18% [6,24]. However, given the low prevalence of MRSA identified in VTH patients in this study (17/622 dogs), these findings are not unexpected.

There were two distinct clones of MRSA observed: USA300:ST8: PVL+, which has been referred to as CA-MRSA and USA100: ST5: PVL-, which has been reported as the most prevalent clone of HA-MRSA [9,10,25]. The USA100:ST5 MRSA clone detected in humans and animals in the VTH has been reported as healthcare-associated MRSA [25], and has been isolated from faculty/staff working with small animals [2,20,21,26]. Isolates belonging to the USA100:ST5 clone in this study were obtained from different faculty members (18 months apart) and from seven dogs in three different wards. This distribution over multiple areas in the VTH suggests that multiple sources available to both humans and dogs may be the cause for the spread of this strain. The current study was unable to identify possible routes or direction of transmission, which would be necessary to determine whether patients or VTH personnel were serving as sources of USA100:ST5.More research is needed to identify sources of MRSA, for development of protocols to reduce MRSA spread.

USA300:ST8, commonly identified as CA-MRSA, was found only in the ECC (environmental sample) and a student who rotated through the ECC. Hudson et al concluded that CA-MRSA is becoming established in hospitals, especially where there is a community influx (ex: pediatrics and OB/GYN) of patients without prior healthcare exposure [9]. This supports the conclusion that the isolation patterns observed could be evidence of a resident strain of CA-MRSA in the VTH ECC. The USA300:ST8 isolated from a human was also PVL+, providing more evidence for it be considered CA-MRSA. The ST8 isolate obtained from an environmental surface in the ECC was PVL- and did not cluster with the USA300 PFGE MRSA clone. These stark differences lead us to believe that this isolate cannot be directly linked with the CA-MRSA isolated from the human subject. The proximity of samples (both human and environmental surface being from the ECC) may be inconsequential.

Although we could not link CA-MRSA found in a veterinary student with the environmental surface, there is much concern regarding CA-MRSA persistently being isolated from a healthcare provider. Reports that MRSA USA300 clones have selective characteristics, which may make it a better competitor in a healthcare setting, have been reported [9]. Tenover et al. [8] noted a substantial increase in USA300:ST8, from 8% in 2001 to 17.2% in 2004 among healthy adults participating in the National Health and Nutrition Examination Survey (NHANES) in the United States [8]. Additionally, one isolate of MRSA, ST72, was obtained from an animal subject admitted to the IM/ST ward. This clone has been identified from both hospital- and community-acquired isolates [10], and has been increasing in prevalence in other parts of the world as there is growing concern over MRSAS T72:PVL- in Korea, which is the main cause of CA-MRSA in that country [27].

Conclusion

The results of this study confirm what others have reported with regard to USA100:ST5 being commonly found among small animal patients and health care personnel. Additionally, despite the single source, our finding of a veterinary student carrying CA-MRSA, which has been reported to contain increased toxin and virulence genes, is notable. CA-MRSA has been reported as becoming established in clinical settings where there is increased exposure from community members, making the need for vigilant infection control practices paramount. A large limitation of our study is the low sample size, and we believe that a multi-site study which focuses on the three arms of this study (environmental samples, patient, and healthcare providers) would offer more conclusive findings into the transmission of MRSA within a VTH.

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