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Methicillin-resistant *Staphylococcus aureus* in Central Iowa Wildlife

Shylo E. Wardyn,[1,2] **Lin K. Kauffman,**[3] **and Tara C. Smith**[1,2,4] [1] Center for Emerging Infectious Diseases, University of Iowa, College of Public Health, 2501 Crosspark Road, MTF B170, Coralville, Iowa 52241, USA; [2] Department of Epidemiology, University of Iowa, College of Public Health, 105 River Street, Iowa City, Iowa 52242, USA; [3] Department of Veterinary Clinical Science, Iowa State University, College of Veterinary Medicine, 1514 Vet Med Bldg., Ames, Iowa 50011, USA; [4] Corresponding author (email: tara-smith@uiowa.edu)

ABSTRACT: Livestock and pets have been identified as carriers of *Staphylococcus aureus*; however, the role of wild animals as a reservoir of *S. aureus* strains has not yet been examined. We conducted a pilot study to determine the prevalence of methicillin-sensitive *S. aureus* (MSSA) and methicillin-resistant *S. aureus* (MRSA) in 37 species of wild animals rehabilitated at a university clinic. Nasal, wing, wound, and cloacal swabs were collected. Of 114 animals, seven (6.1%) were MSSA-positive and three (2.6%) were MRSA-positive. The MRSA isolates were obtained from two eastern cottontail rabbits (*Sylvilagus floridanus*) and a Lesser Yellowlegs (*Tringa flavipes*), a migratory shorebird. Antibiotic resistance testing of the MRSA isolates revealed that two were additionally resistant to tetracycline and erythromycin, and the third isolate was also resistant to erythromycin, clindamycin, and levofloxacin. All three isolates were positive for the Panton-Valentine leukocidin (PVL) gene. Sequence typing of the staphylococcal protein A (*spa*) region revealed one MRSA isolate to be t002, whereas the other two MRSA isolates were found to be t008. Our results suggest that *S. aureus*, including MRSA, is being carried by wild animals, although at a low prevalence with the limited number of animals tested. Additional studies are needed to determine how this may impact human health.

Key words: Antibiotic resistance, MRSA, *Staphylococcus aureus*, wildlife, zoonosis.

A complete understanding of infectious disease in humans requires an ecological approach. It is not sufficient to investigate microbes solely in the context of human infection; we also must gather knowledge about infection of other species with which we share our spaces and our natural resources. Almost 75% of emerging human pathogens originated in animals (Woolhouse and Gowtage-Sequeria, 2005), leading to the concept of "One World, One Health" (One Health, 2010). This concept is the idea that the same microorganisms can have effects on both human and animal health, necessitating communication between individuals working in both of these areas.

Staphylococcus aureus is a common bacterium that causes a variety of infections. Although 30% of Americans are colonized with *S. aureus* (Graham et al., 2006), colonization by strains of *S. aureus* that are resistant to the antibiotic methicillin is less common. Methicillin-resistant *Staphylococcus aureus* (MRSA) also infects animals, including livestock and pets; however, examination of infections and carriage in wildlife is lacking. The bacterium has been isolated from horses (*Equus ferus caballus*; Weese et al., 2006), cattle (*Bos primigenius*; Juhasz-Kaszanyitzky et al., 2007), dogs (*Canus lupus familiaris*) and cats (*Felis catus*) (Baptiste et al., 2005), and swine (*Sus scrofa domesticus*; Huijsdens et al., 2006; Khanna et al., 2008; Smith et al., 2009). Isolates from swine and their human caretakers frequently are indistinguishable, suggesting transmission between these species (Huijsdens et al., 2006; Khanna et al., 2008). Limiting investigation to human isolates of potential pathogens provides us with a narrow snapshot of the overall ecology of these pathogens, and might cause us to miss potential routes of transmission between humans and animals.

Using sterile swabs, we collected 158 samples from the nasal mucosa, wings, wounds, and cloaca of 114 orphaned or injured wildlife patients of 37 species presenting to the Wildlife Care Clinic at Iowa State University. Ames, Iowa, USA, from July 2009 to May 2010. Animals were swabbed immediately upon entry to the facility to minimize opportunities to acquire *S. aureus* within the facility. Swabs were placed on ice and mailed to the

TABLE 1. Molecular characteristics of methicillin-sensitive *Staphylococcus aureus* isolates from wildlife species in central Iowa.

Isolate	Species	Antibiotic resistance[a]	Panton-Valentine leukocidin gene presence	Staphylococcal protein A type	Multilocus sequence type
WCC5001	Great Horned Owl (*Bubo virginianus*)	None	No	t4735	
WCC7801	European Beaver (*Castor fiber*)	None	Yes	t4368	ST1959
WCC8501	Great Blue Heron (*Ardea herodias*)	None	No	t2603	
WCC10001	Rock Pigeon (*Columba livia*)	T, hGISA	No	t4634	ST2018
WCC10101	Rock Pigeon	None	No	t1059	
WCC10901	Fox squirrel (*Sciurus niger*)	None	No	t1166	
WCC13301	Screech Owl (*Megascops* sp.)	T	No	t094	

[a] T = Tetracycline, hGISA = heterogeneous-glycopeptide intermediate *S. aureus*.

University of Iowa, Iowa City, Iowa, USA, for bacterial culture, as previously described (Smith et al., 2009), with the addition of a mannitol salt agar plate for detection of methicillin-susceptible *S. aureus*. The presence of the Panton-Valentine leukocidin (PVL) gene and determination of staphylococcal protein A (*spa)* type and multilocus sequence type (MLST) (selected isolates only) were carried out as previously described (O'Brien et al., 2012).

All isolates were tested for antimicrobial susceptibility by the broth dilution method (CLSI, 2009). Isolates were tested for susceptibility to an array of antimicrobials, including: penicillin, oxacillin, tetracycline, erythromycin, clindamycin, trimetrexate, quinupristin/dalfopristin, gentamicin, levofloxacin, moxifloxacin, linezolid, daptomycin, vancomycin, and rifampin. Isolates also were tested using the Etest GRD (glycopeptide resistance detection) test (AB Biodisk, Solna, Sweden) for the detection of GISA (glycopeptide-intermediate *Staphylococcus aureus)* or hGISA (heterogeneous-GISA).

Seven of 114 animals (6.1%) were nasal carriers of methicillin-sensitive *Staphylococcus aureus* (MSSA). MSSA isolates were obtained from one Great Horned Owl (*Bubo virginianus*), one European beaver (*Castor fiber*), one Great Blue Heron (*Ardea herodias*), two Rock Pigeons (*Columba livia*), one fox squirrel (*Sciurus niger*), and one Screech Owl (*Megascops* sp.). The MSSA isolate from the European beaver was PVL-positive and identified as *spa* type t4386; however, it exhibited no resistance to any of the antibiotics tested. This isolate was determined by MLST to be the recently identified type ST1959. The MSSA isolates from one rock pigeon and the screech owl were resistant to tetracycline; the Rock Pigeon isolate also was found to express intermediate resistance to glycopeptide antibiotics (hGISA), and was *spa* type t4634. The MLST revealed it to be a relatively new type, ST2018. A variety of *spa* types were observed (Table 1).

Three of the 114 animals (2.6%) were positive for MRSA, including nasal samples from two eastern cottontail rabbits (*Sylvilagus floridanus*) that were littermates, as well as nasal and wound samples from a Lesser Yellowlegs (*Tringa flavipes*) shorebird. The rabbit MRSA isolates were PVL-positive. Additionally, these isolates were resistant to tetracycline and erythromycin and were identified as *spa* type t008. The isolates from the Lesser Yellowlegs were resistant to oxacillin, erythromycin, clindamycin, and levofloxacin and were identified as *spa* type t002 (Table 2).

This study shows that *S. aureus* is being carried by various wild animals commonly found in Iowa, and one migratory shorebird. Numerous investigators have found that *S. aureus* carriage occurs in livestock and pets (Baptiste et al., 2005; Weese et al., 2006; Juhasz-Kaszanyitzky et al., 2007; Khanna et al., 2008; Smith et al.,

TABLE 2. Molecular characteristics of methicillin-resistant *Staphylococcus aureus* isolates in central Iowa.

Isolate	Species	Antibiotic resistance[a]	Panton-Valentine leukocidin gene presence	Staphylococcal protein A type
WCC4301	Lesser Yellowlegs (*Tringa flavipes*)	O, E, C, L	No	t002
WCC5201	Eastern cottontail (*Sylvilagus floridanus*)	O, T, E	Yes	t008
WCC5301	Eastern cottontail	O, T, E	Yes	t008

[a] C = Clindamycin, E = Erythromycin, L = Levofloxacin, O = Oxacillin, T = Tetracycline.

2009). Wildlife also is an important source of zoonotic infections (Woolhouse and Gowtage-Sequeria, 2005), as well as a potential risk factor for spreading pathogens throughout the environment (Meerburg, 2010). *Staphylococcus* sp., including *aureus* have been found in insectivores and rodents (Hauschild et al., 2010) and white-eared opossums (*Didelphis albiventris*; Siqueira et al., 2010). Methicillin-resistant *S. aureus* has been found in other animals such as hamsters (*Mesocricetus* sp.; Ferreira et al., 2011), birds (Class Aves), rabbits (Family Leporidae), chinchillas (*Chinchilla* sp.), guinea pigs (*Cavia porcellus)*, turtles (Order Testudines), squirrels (Family Sciuridae), seals (Superfamily Pinnipedia), and bats (Order Chiroptera) (Rich and Roberts, 2004; O'Mahony et al., 2005; Rankin et al., 2005; Walther et al., 2008). Ours is the first study to look for carriage of *S. aureus* by a variety of wild animals found in the midwestern United States. Because transmission is so poorly understood, more studies addressing carriage in animals, including wildlife, are necessary.

Due to our opportunistic sampling method and the time required to ship isolates, we might be underestimating the prevalence *S. aureus* infection in wild animals in Iowa. We did not sample workers in the Wildlife Care Clinic, so we cannot rule out the possibility of transmission from human carriers; however, caretakers were trained staff members and took proper hygiene measures, including wearing gloves when handling animals; thus, human transmission is unlikely. The molecular typing also supports our conclusion that nosocomial transmission was unlikely to have been involved in bacterial acquisition. The two MRSA-positive rabbits were littermates, so it is understandable that they would be colonized with the same type; the Lesser Yellowlegs had the same type isolated from both the nasal and wound swab.

Risk of transmission of MRSA from wild animals is not restricted to wildlife caretakers. Companion animals and livestock can be in contact with wildlife, and transmission between animal species is possible. Small animals, such as newborn rabbits and squirrels, can be found in residential areas and are handled by people. Migratory birds can carry pathogens over long distances, thus facilitating pathogen dissemination among human and animal populations. Although not linked to any outbreaks, MRSA has been identified in rats (*Rattus rattus)* on swine farms (van de Giessen et al., 2009). Thus, wildlife might spread and transmit these pathogens throughout the farm.

Our finding of an hGISA isolate from wild animals was unexpected. Glycopeptides are among the first choice of drug therapy for *S. aureus* infections due to the high levels of resistance of β-lactams. With the increased use of vancomycin in cases of MRSA, strains of *S. aureus* are increasingly resistance to glycopeptides such as vancomycin (Appelbaum, 2007). While still relatively rare, the global prevalence of hGISA is increasing (Rybak et al., 2008). It is unknown how the rock pigeon became colonized with this strain, but additional studies examining the prevalence of hGISA should be conducted.

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LITERATURE CITED

APPELBAUM, P. C. 2007. Reduced glycopeptide susceptibility in methicillin-resistant *Staphylococcus aureus* (MRSA). International Journal of Antimicrobial Agents 30: 398–408.

BAPTISTE, K. E., K. WILLIAMS, N. J. WILLAMS, A. WATTRET, P. D. CLEGG, S. DAWSON, J. E. CORKILL, T. O'NEILL, AND C. A. HART. 2005. Methicillin-resistant staphylococci in companion animals. Emerging Infectious Diseases 11: 1942–1944.

CLINICAL AND LABORATORY STANDARDS INSTITUTE (CLSI). 2009. Methods for dilution antimicrobial susceptibility tests for bacteria that grow aerobically. Report No.: M07-A7 Clinical and Laboratory Standards Institute, Villanova, Pennsylvania. 65 pp.

FERREIRA, J. P., V. G. FOWLER Jr, M. T. CORREA, R. LYMAN, F. RUFFIN, AND K. L. ANDERSON. 2011. Transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) between human and hamster. Journal of Clinical Microbiology 49: 1679–1680.

GRAHAM, P. L., III, S. X. LIN, AND E. L. LARSON. 2006. A U.S. population-based survey of *Staphylococcus aureus* colonization. Annals of Internal Medicine 144: 318–325.

HAUSCHILD, T., P. SLIZEWSKI, AND P. MASIEWICZ. 2010. Species distribution of staphylococci from small wild mammals. Systematic and Applied Microbiology 33: 457–460.

HUIJSDENS, X. W., B. J. VAN DIJKE, E. SPALBURG, M. G. VAN SANTEN-VERHEUVEL, M. E. HECK, G. N. PLUISTER, A. VOSS, W. J. WANNET, AND A. J. DE NEELING. 2006. Community-acquired MRSA and pig-farming. Annals of Clinical Microbiology and Antimicrobials 5: 26.

JUHASZ-KASZANYITZKY, E., S. JANOSI, P. SOMOGYI, A. DAN, L. VAN DER GRAAF-VAN BLOOIS, E. VAN DUIJKEREN, AND J. A. WAGENAAR. 2007. MRSA transmission between cows and humans. Emerging Infectious Diseases 13: 630–632.

KHANNA, T., R. FRIENDSHIP, C. DEWEY, AND J. S. WEESE. 2008. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. Veterinary Microbiology 128: 298–303.

MEERBURG, B. G. 2010. Rodents are a risk factor for the spreading of pathogens on farms. Veterinary Microbiology 142: 464–465; author reply 466.

O'BRIEN, A. M., B. M. HANSON, S. A. FARINA, J. Y. WU, J. E. SIMMERING, S. E. WARDYN, B. M. FORSHEY, M. E. KULICK, D. B. WALLINGA, AND T. C. SMITH. 2012. MRSA in conventional and alternative retail pork products. PLoS One 7: e30092. doi: 10.1371/journal.pone.0030092.

O'MAHONY, R., Y. ABBOTT, F. C. LEONARD, B. K. MARKEY, P. J. QUINN, P. J. POLLOCK, S. FANNING, AND A. S. ROSSNEY. 2005. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from animals and veterinary personnel in Ireland. Veterinary Microbiology 109: 285–296.

ONE HEALTH. 2010. One Health Initiative will unite human and veterinary medicine. http://www.onehealthinitiative.com/index.php. Accessed 26 August 2010.

RANKIN, S., S. ROBERTS, K. O'SHEA, D. MALONEY, M. LORENZO, AND C. E. BENSON. 2005. Panton valentine leukocidin (PVL) toxin positive MRSA strains isolated from companion animals. Veterinary Microbiology 108: 145–148.

RICH, M., AND L. ROBERTS. 2004. Methicillin-resistant *Staphylococcus aureus* isolates from companion animals. Veterinary Record 154: 310.

RYBAK, M. J., S. N. LEONARD, K. L. ROSSI, C. M. CHEUNG, H. S. SADER, AND R. N. JONES. 2008. Characterization of vancomycin-heteroresistant *Staphylococcus aureus* from the metropolitan area of Detroit, Michigan, over a 22-year period (1986 to 2007). Journal of Clinical Microbiology 46: 2950–2954.

SIQUEIRA, D. B., F. M. AESSIO, R. A. MOTA, M. F. MARVULO, J. F. MAUFFREY, S. R. MONTEIRO, R. C. FARIAS, R. C. CUNHA, R. L. OLIVEIRA, T. C. SOUZA, E. S. MEDEIROS, AND J. C. SILVA. 2010. *Staphylococcus aureus* mastitis in a white-eared opossum (*Didelphis albiventris*) in the Atlantic Forest of northeast Brazil. Journal of Zoo and Wildlife Medicine 41: 526–529.

SMITH, T. C., M. J. MALE, A. L. HARPER, J. S. KROEGER, G. P. TINKLER, E. D. MORITZ, A. W. CAPUANO, L. A. HERWALDT, AND D. J. DIEKEMA. 2009. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. PLoS One 4: e4258. doi: 10.1371/journal.pone.0004258.

VAN DE GIESSEN, A. W., M. G. VAN SANTEN-VERHEUVEL, P. D. HENGEVELD, T. BOSCH, E. M. BROENS, AND C. B. REUSKEN. 2009. Occurrence of methicillin-resistant *Staphylococcus aureus* in rats living on pig farms. Preventive Veterinary Medicine 91: 270–273.

WALTHER, B., L. H. WIELER, A. W. FRIEDRICH, A. M. HANSSEN, B. KOHN, L. BRUNNBERG, AND A. LUBKE-BECKER. 2008. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolated from small and exotic animals at a university hospital during routine microbiological examinations. Veterinary Microbiology 127: 171–178.

WEESE, J. S., J. ROUSSEAU, B. M. WILLEY, M. ARCHAMBAULT, A. MCGEER, AND D. E. LOW. 2006. Methicillin-resistant *Staphylococcus aureus* in horses at a veterinary teaching hospital:

Frequency, characterization, and association with clinical disease. Journal of Veterinary Internal Medicine 20: 182–186.
WOOLHOUSE, M. E., AND S. GOWTAGE-SEQUERIA. 2005. Host range and emerging and reemerging pathogens. Emerging Infectious Diseases 11: 1842–1847.

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