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Prospective multicenter surveillance identifies *Staphylococcus aureus* infections caused by livestock-associated strains in an agricultural state

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ABSTRACT

We conducted a surveillance study to investigate the epidemiology of *Staphylococcus aureus* infections in Iowa, using a convenience sample. Diagnostic laboratories submitted 20 *S. aureus* isolates per month for a 20-month period between 2011 and 2013. Of the 2226 isolates analyzed, 73.6% were methicillin-resistant *S. aureus* (MRSA) and 26.4% were methicillin-susceptible *S. aureus* (MSSA). *S. aureus* infections in 25 patients (1%) were caused by ST398- and ST9-associated strain types, and appeared to be a common occurrence in areas of the state with the highest numbers of hogs and hog farms. Twenty nine (5.1%) of MSSA isolates and 10 (40.0%) livestock-associated strains were multi-drug resistant.

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1. Introduction

Staphylococcus aureus (*S. aureus*) is a common commensal and highly adaptable organism of humans and animals that causes a wide range of infections, from superficial skin and soft tissue infections (SSTIs) to life-threatening invasive diseases (Fitzgerald, 2012). In the 1990s, new strains of community-associated (CA-) methicillin-resistant *S. aureus* (MRSA) were identified as a cause of infections among previously healthy people and were occasionally fatal (David and Daum, 2010; Herold et al., 1998). A 6-month nationwide surveillance study conducted in 2011 observed a predominance of the USA300/t008 strain type as a healthcare associated pathogen in all tested regions and infection sites, particularly wounds and skin infections (Diekema et al., 2014). Although invasive CA-MRSA infections are tracked by the Centers for Disease Control and Prevention's Active Bacterial Core Surveillance, a great majority of CA-MRSA infections are not invasive such as SSTIs and would not be captured by the surveillance program (Dukic et al., 2013; Klevens et al., 2007). A recent meta-analysis of studies conducted in the US noted an increase in CA-MRSA infections between 1990 and 2012 and, speculate that these infections could be endemic and at unprecedented levels in many regions (Dukic et al., 2013). However, this meta-analysis was modeled largely on studies from urban populations and may not accurately reflect the trajectory of CA- infections in a rural region.

More recently, a novel *S. aureus* sequence type (ST) 398 was reported to colonize livestock. Human carriage and infection caused by ST398 were first reported in Europe (Fitzgerald, 2012; Smith and Pearson, 2011). It was suggested that these *S. aureus* strain types associated with livestock production were responsible for the increase in incidence of CA-MRSA since the MRSA strains were isolated from farmers and their livestock (Harrison et al., 2013; Hasman et al., 2010). Swine are observed to be the most common reservoir for the ST398; however, it has also been found to colonize other livestock. Typical human strains belonging to ST5, ST8, ST22, ST97, and ST1 have also been isolated from livestock (Fitzgerald, 2012; Hasman et al., 2010; Osadebe et al., 2013). Severe infections due to ST398 strains have emerged globally (Monaco et al., 2013; Verkade and Kluytmans, 2013). Increased rates of colonization with livestock-associated (LA-) MRSA in areas with high density of livestock such as the Netherlands and Germany, could potentially lead to increased transmission and infection by these strains (Kock et al., 2013; Wulf et al., 2012). A population-based study conducted in Iowa observed that swine farmers are at an increased risk of colonization with not only *S. aureus* but also livestock strains with varying antibiotic

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resistance, such as multidrug-resistant *S. aureus* and tetracycline resistant strains (Wardyn et al., 2015). In addition, studies conducted in USA and Europe have identified ST398 as an increasing cause of disease among people with potentially no livestock contact (Larsen et al., 2015; Mediavilla et al., 2012; Uhlemann et al., 2013).

Little is known regarding the epidemiology of *S. aureus* infections in Iowa—a rural population with exposure to hospitals, nursing homes, and livestock, all of which are implicated in *S. aureus* acquisition. The aim of our surveillance study is to characterize pathogenic *S. aureus* strains, and determine the prevalence of resistance to tested antibiotics. We included both methicillin-susceptible *S. aureus* (MSSA) and MRSA strains to be able to capture a regional perspective on *S. aureus* evolution. We anticipated ST398 infections in our population, as there is evidence of circulation of these strains in Iowa (Leedom Larson et al., 2010; O'Brien et al., 2012; Wardyn et al., 2015).

2. Materials and methods

A prospective, multi-laboratory *S. aureus* infection surveillance study was conducted between June 1, 2011 and February 28, 2013. A waiver of consent was obtained from The University of Iowa Human Subjects Office (HSO) since isolates were obtained for surveillance and personal identifiers were not collected.

2.1. Participating laboratories

The Iowa State Hygienic Laboratory (SHL), Coralville coordinated recruitment of laboratories that participated in a MRSA statewide surveillance system, as reported in a previous study (Van De Griend et al., 2009). Of these, 13 diagnostic laboratories serving 23 hospitals in and around Iowa volunteered participation in our study. One laboratory did not submit any isolates during the study period. Eleven laboratories were hospital-affiliated and one was an independent laboratory, affiliated with healthcare centers in Iowa and Illinois. As per the 2012 US Census data and report available from the Iowa Hospital Association, we estimate that the participating laboratories cumulatively served roughly one-third of Iowa's population (Table A.1).

2.2. Surveillance data and S. aureus isolate collection

All isolates were de-identified and assigned study-defined identifiers. A short data collection form was developed to obtain patient information not designated as Personal Health Information. Data was obtained on exposures such as hospitalization including admission to an intensive care unit (ICU), antimicrobial exposure, dialysis, occupational exposure to healthcare-settings, and correctional facility in the past one year. Data on patients past and/or current comorbidities, presence of indwelling catheter and/or other medical devices, type of infection and source of the isolate was also acquired. The Infection Control Preventionist for each participating lab filled the data collection form using information available with isolates; patient medical records were not accessed for data collection.

Laboratories were asked to submit twenty clinically significant MRSA and/or MSSA infection isolates (only 1 isolate per patient) per month, collected at any time in a month (Table A.2). Laboratories were instructed not to send isolates representing colonization. Isolates from nasal, throat or oral swabs, and nasopharyngeal aspirate or drainage were presumed to be colonizers and excluded from final analysis (n=29). Isolates were classified as invasive *S. aureus* infections based on previously published and validated definitions (Klevens et al., 2007). We developed this protocol for feasibility of the study but anticipate the potential for selection bias due to the convenience sampling methodology.

2.3. Molecular analysis

All *S. aureus* isolates were cultured and re-confirmed to be *S. aureus* at the Center for Emerging Infectious Diseases (CEID), as described previously (O'Brien et al., 2012). Presence of methicillin-resistance (*mec*A) and Panton-Valentine leukocidin (PVL) genes, and determination of *spa* type was performed using published methods and primers (Bosgelmez-Tinaz et al., 2006; Lina et al., 1999; Shopsin et al., 1999). Isolates were classified as MRSA or MSSA based on presence of the *mec*A gene. The Based-Upon Repeat Pattern (BURP) analysis to identify *spa* cluster complexes (*spa*CC) was conducted using the Ridom StaphType software using default parameters (version 2.2.1; Ridom GmbH, Würzburg, Germany) (Mellmann et al., 2008). Positive and negative controls were used in all molecular assays.

2.4. Antimicrobial susceptibility testing

The antibiogram for *S. aureus* isolates were obtained from corresponding diagnostic laboratories tested in accordance with the Clinical Laboratory Standards Institute (CLSI) standards (Clinical Laboratory and Standards Institute, 2012). Multidrug resistance (MDR) was defined as isolates determined to be MRSA or observed to be resistant to at least three discrete non beta-lactam antimicrobial classes (Magiorakos et al., 2012). Antimicrobial susceptibility percentages were analyzed for individual antibiotics using antibiogram classification of isolates as resistant or susceptible as determined by laboratories. Isolates with intermediate or inducible resistance were analyzed as a separate category.

2.5. Data analysis

Analyses were performed using the SAS software (Version 9.3, SAS Institute Inc., Cary, NC). Prevalence of *S. aureus* infections during the study period was calculated using the 2012 mid-year population for Iowa. The chi-square test for equal proportions or Fisher's exact test was used to analyze categorical variables. P values <0.05 were considered statistically significant.

2.6. Spatial analysis

Lab locations were geocoded and isolates were mapped according to the submitting lab in ArcMap10.3 (ESRI, Redlands, CA). Isolates were assigned to the three-digit leading prefix of the home address ZIP code in order to understand how the lab samples are representative of Iowa *S. aureus* isolates. The locations and numbers of swine animal units (swine AU) in concentrated animal feeding operations (CAFOs) were accessed from the Iowa Department of Natural Resources (DNR) and the number of swine AU per square kilometer in each three-digit ZIP code was calculated. Animal Units (AU) are a measure developed by the Iowa DNR to compare manure production across species and ages of animals. One fully grown hog equals 0.4 AU, immature hogs are smaller portions of AU.

3. Results

Of the 2226 *S. aureus* infection isolates analyzed, 73.6% were MRSA and 26.4% were MSSA by *mec*A presence. Interestingly, 1.1% (n = 25) of the isolates were noted to be *spa* types with previously known livestock association (Ballhausen et al., 2014; Cuny et al., 2013; David et al., 2013; Hasman et al., 2010; Kock et al., 2013; Normanno et al., 2015; Silva et al., 2014). Patient characteristics were significantly different by hospitalization at the time of infection culture, prior antibiotic use, prior surgery, and exposure to long-term care facility between MRSA, MSSA, and LA infections (Table 1). Population demographics in participating three-digit ZIP code areas were noted to be homogeneous in the proportion of people over 65 years of age and white race. Four ZIP

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