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Table of Contents

Abstract **page 2**

Section 1 **page 4**

Section 2 **page 6**

List of Terms and Abbreviations

CFU: colony-forming units

MRSA: methicillin-resistant *Staphylococcus aureus*

LA-MRSA: livestock-associated MRSA

PVL: Panton-Valentine leukocidin

Abstract

A recent report has documented the clinical impact of methicillin-resistant *Staphylococcus aureus* (MRSA), suggesting that MRSA caused over 94,000 invasive infections and more than 18,000 deaths in the United States in 2005, eclipsing HIV as a leading infectious disease killer. In 2005, the majority of these infections were associated with healthcare exposure; however, this trend is changing, and new groups at risk of acquiring MRSA have emerged. Initially individuals with health care contact were the main risk group for MRSA carriage and infection; however, in the past decade, athletes, injection drug users, and prisoners have also emerged as groups at risk of MRSA infection. The most recent occupational group identified as at-risk for MRSA infection are individuals in contact with live swine and other animals. As such, we examined the presence and molecular types of *S. aureus* on swine farms in Iowa using environmental sampling and before/after self-swabs of veterinary students on swine rotations through these farms; examined airborne transmission within swine barns using Andersen sampling, and the potential for a biofilter system to mitigate risk of transmission of this bacterium; and examined MRSA infections in patients in a livestock-dense area of the state.

MRSA was detected in 30% of the pork farms and in 22% of the students following an exposure to a MRSA-positive pork farm. All students found to be MRSA-positive initially following farm visit were negative for MRSA within 24 hours post visit, suggesting short duration of carriage via farm exposure or contamination rather than

biological contamination. Most common spa types recovered were t002 (79%), t034 (16%) and t548 (4%).

MRSA was also detected within a swine facility, mostly large particles (>5µm), which tend to be associated with feed. This was also detected in small particles (<5µm) 215 meters downwind of a swine facility. These smaller particles tend to be associated with dried feces and shed skin cells. Feed itself was also tested and was positive for MRSA even prior to entering the facility, suggesting that feed imported onto farms may itself be a source of MRSA in the facility.

Mitigation of MRSA with a wood chip-based biofilter was also examined. Both hardwood chips and western red cedar chips were effective at preventing the emission of viable MRSA particles in the exhaust air from swine feeding facilities. Efficiency ranged from 77 percent of particles with mean particle size of 1.6 µm to 100 percent using western red cedar media with particles with a mean size of 5.85 µm. As such, these inexpensive filters may provide a way to reduce transmission of MRSA into the external air, but additional means are needed to reduce the burden of MRSA within barns.

Finally, an examination was made of cases of MRSA reporting to a physician's clinic in western Iowa, in a swine-dense area. Fifteen case patients were enrolled with 10 total samples received from the clinic as possible MRSA infections. *S. aureus* was positively identified in 4 samples. None were identified as common livestock-associated (LA) strains.

Section 1

Key Findings

1) Determine the prevalence and molecular types of *S. aureus* on swine farms in Iowa

    a. Subaim 1: examine airborne *S. aureus* on swine farms and use of a biofilter to reduce transmission
    b. Subaim 2: determine the effectiveness of an N95 respirator at reducing potential colonization with *S. aureus*

2) Establish duration of colonization with *S. aureus* associated with farming

3) Capture symptomatic cases of MRSA infections in livestock-dense areas

For Aim 1, we examined 40 swine farms in central Iowa. Veterinary students carrying out swine rotations obtained samples from animals and the environment as a course of these rotations. MRSA was found on 30% of farms (12/40). Molecular types were found to be largely *spa* types t002 (sequence type 5) or t034 (sequence type 398, "classic" livestock-associated MRSA).

As a subaim, we carried out air sampling at one confirmed MRSA-positive farm in eastern Iowa. MRSA were found in the air both within the swine facility and downwind of the barns, suggesting a potential exposure route both for workers and for individuals living in close proximity to swine confinement facilities. We also investigated the use of a biofilter employing wood chips to reduce the amount of MRSA ventilated to the external environment, which did show a reduction in colony-forming units (CFU) of MRSA released from the barns. We further examined the effectiveness of an N95 respirator mask on reducing colonization with MRSA in this setting, using a model system. This respirator was found to be effective at eliminating MRSA using this model system.

For aim 2, we examined veterinary students on swine farm rotations in association with Aim 1. All students were sampled (nasal swabs) prior to farm visits and then followed up after visiting a farm. In students who visited MRSA-positive swine farms, no MRSA was detected in the nasal cavities of students for longer than 24 hours, suggesting that colonization with livestock-origin MRSA is transient in these students, or that positive MRSA samples may be a result of contamination rather than biological contamination.

For aim 3, we partnered with a physician's practice in Northwestern Iowa (an area with a high density of swine farms) in order to enroll patients with potential MRSA

infections. Unfortunately we were unable to enroll a significant number of patients for this. The final enrollment was only 15 individuals, and data on occupation or livestock exposure was only available for 12 individuals, and only 4 of those submitted samples which were positive for *S. aureus*. As such, we were unable to draw any conclusions for this portion of the study.

Translation of findings

Our findings suggest that MRSA and other antibiotic-resistant strains of *S. aureus* are indeed present on Iowa swine farms, and may pose an occupational risk to farmers and others working on and near such facilities. Due to the apparently short duration of carriage/colonization seen via Aim 2, it is unlikely from these studies that the community at large is at risk from MRSA originating on farms via human-to-human transmission. However, larger studies of longer duration should be performed in order to confirm this. The use of an inexpensive wood chip-based biofilter was able to reduce the amount of MRSA vented from barns to the exterior, but this needs further validation, as does the use of an N95 respirator to protect workers from colonization. The latter needs additional research within an active farming setting, in order to determine effectiveness in a real-life setting and adherence to use.

Outcomes/impact

As this was largely an exploratory study, the impact remains to be seen. We did find, as noted above, that MRSA is present on a large number of tested swine farms in Iowa. At present, the impact regarding the likelihood of developing an infection when exposed to such MRSA strains is not known, though it is thought to be relatively low in comparison to other more common "human" MRSA strains. While the biofilter may assist in reducing burden of transmission outside of swine farms, we still do not have an effective intervention to reduce the levels of MRSA within the farm building. While respirators may protect workers, previous studies by other groups have suggested that workers have low adherence to regular use of such masks in the farm setting.

Section 2: Scientific report

Aim 1

Introduction

Staphylococcus aureus is a common bacterium found on the skin and nasal passages of healthy people. Approximately 25–40% of the population is colonized with *S. aureus*. It is also a common cause of skin and soft tissue infections and sometimes causes severe disease such as pneumonia, bacteremia, meningitis, sepsis, and pericarditis. *S. aureus* bacteria harboring the *mecA* gene are resistant to methicillin and other β-lactam antimicrobials and are referred to as methicillin-resistant*S. aureus* (MRSA). In the United States it is estimated that 1.5% of the population (~4.1 million persons) is colonized with MRSA [1] leading to at least 94,000 invasive infections and over 18,000 deaths annually [2]. Various categories of MRSA based on epidemiologic characteristics are commonly used and include healthcare-associated MRSA (HA-MRSA), community-associated MRSA (CA-MRSA) and livestock-associated MRSA (LA-MRSA). HA-MRSA infections are most commonly found in immunocompromised people who have spent time in hospitals or healthcare centers, while CA-MRSA infections occur among otherwise healthy adults and children in the wider community. Livestock-associated MRSA (LA-MRSA) refers to strains of MRSA in which animals, particularly production animals, serve as the main reservoir of infection to humans.

LA-MRSA emerged as a public health concern in 2005 with reports of a specific multilocus sequence type (ST398) being found in higher than expected numbers in swine workers in France and the Netherlands [3]–[5]. Since ST398 was found at high levels in both pigs and pig farmers and very low levels in the general population, it was initially referred to as the "swine-associated" MRSA. Several studies attempting to determine the prevalence of ST398 in pigs have been conducted including a large multi-national study conducted by the European Food Safety Authority (EFSA) which found the prevalence of MRSA ST398 in swine farms to be 25.5% but varied from 0% to 50.2% among European Union Member States [6]. In Ontario, Canada a study found that 25% of the pigs from 20 farms were colonized with MRSA and that ST398 was the predominant sequence type[7]. A study in the U. S. examined 299 animals from two swine production systems in Iowa and Illinois and 45% were found to carry MRSA. All isolates typed were ST398 [8].

It is apparent that those workers who spend considerable time in production animal farms are more likely to carry MRSA than those who don't. One study in The Netherlands demonstrated a 26% carriage rate among pig farmers [4]. The Canadian and U. S. studies previously mentioned found MRSA is 20% and 45%, respectively, in the swine workers tested. Isolates obtained from swine and their human caretakers are frequently indistinguishable, suggesting transmission between the two animal species [7]. Several studies have indicated that veterinarians working with swine are more likely to carry MRSA, primarily ST398, than non-swine focused colleagues [9]–[12]. While there are concerns that ST398 may establish itself in people, it appears that human to human spread of ST398 is limited [13], [14] and transmissibility within

hospitals is less likely than non-ST398 MRSA strains [15], [16]. Additionally, colonization in persons exposed to livestock appears to be dependent on intensity of animal contact [17]. Studies indicate that short-term exposure to MRSA-positive pig farms does not lead to long-term colonization [17], [18]. Similar studies assessing the risk of short but intense exposure to MRSA-positive pork farms in the U. S. have not been done. Therefore the objectives of this study were to: i) assess the rate of MRSA acquisition and longevity of carriage in uncolonized students exposed to pork farms during the two week course, ii) characterize recovered MRSA isolates by *spa* typing and antimicrobial susceptibility testing to assess the relatedness between pork farms and veterinary student isolates.

Methods

Ethics Statement

The ISU Institutional Review Board (IRB) approved the protocols. Animal samples tested were obtained from samples submitted as part of the diagnostic workup for field case investigations and did not require institutional animal care committee (IACUC) approval. All animals sampled were under a valid veterinary-client-patient relationship (VCPR).

Enrollment

Veterinary students were provided written informed consent and voluntarily enrolled during participation in swine courses at Iowa State University (ISU) from May to November, 2010. Students answered a short questionnaire related to potential risk factors for MRSA such as recent respiratory illness with fever and sore throat, skin or soft tissue infections (SSTI), antibiotic use, hospitalization, visitation to pork production or prior diagnosis of MRSA. Age and gender information was also collected. Students participated in diagnostic investigations at pork farms as would normally occur during the two-week clinical swine medicine fourth year elective course. Diagnostic investigations at pork farms were based on requests to ISU Veterinary Diagnostic and Production Animal Medicine (VDPAM) department by swine veterinarians and producers seeking assistance with animal health-related problems. Students were randomly assigned to an investigation and were generally at the pork farms for 3 to 4 hours. No prior knowledge of MRSA status or MRSA-related disease in pigs or humans at the pork farms was available. The type of farm and approximate age of animals were recorded at the time of visit, but no further farm data was made available for this study.

Sample collection

Student.

Students were sampled at the following intervals: 1) the beginning of the course before any visits to pork farms, 2) before entry into a pork farm, 3) immediately after leaving a pork farm, 4) weekends or non-visit weekdays during the course, 5) daily for 4 consecutive days after the end of the clinical swine medicine course. Sample collection

was accomplished by using sterile swabs (BBL CultureSwab, Sparks, MD) containing Stuart's medium inserted approximately 2 cm into one naris, rotated against the anterior nasal mucosa and repeated with same swab in second naris. The swabs were transported on ice to the ISU Veterinary Diagnostic Laboratory (VDL) within 6 hours. All samples were submitted using an assigned student study ID and date.

Animal.

As part of the routine diagnostic investigation, when nasal samples were collected from manually restrained pigs for other diagnostic purposes, 3–5 of these nasal samples where then also submitted for MRSA testing. All samples were obtained as part of normal diagnostic investigation during student visit using materials and techniques described above for students. Samples were identified using a sample kit ID and date. Pigs were selected from pens with and without illness. Health status of the pig was not included when the sample was forwarded for MRSA testing.

Environmental.

The environmental samples were collected from the same farms visited by participating students during the time of the visit. The sampling sites included, but were not limited to, treatment carts, fences and gates. Typically swab samples were collected from 3–5 areas in each farm. Samples were acquired by swabbing an approximate three square inch area with a sterile Speci-Sponge (Nasco, Fort Atkinson, WI) in 5 ml of enrichment broth, placed in Whirlpak bag, and transported on ice to the ISU VDL within 6 hours. Samples were identified using the date and same sample kit ID used for animal samples.

To maintain client confidentiality, each farm was assigned a farm study ID by an individual not involved in the study. A master spreadsheet was created that included the farm ID, sample kit ID, student IDs that visited the farm, sampling date, farm type, and approximate pig age.

Isolation and identification of bacteria

Student and pig nasal swabs were inoculated directly into 2 ml of enrichment broth containing 10 g tryptone/L, 75 g NaCl/L, 10 g mannitol/L and 2.5 g yeast extract/L. Bags containing environmental sponges received an additional 10 ml of enrichment broth. Samples were incubated for 24 h at 35°C, then inoculated onto selective MRSA agar plates (MRSASelect, Bio-Rad, Hercules, CA), which were then incubated for 24–48 hours at 35°C. All plates were examined for MRSA and*Staphylococcus* species. Up to 3 suspect colonies from each sample were further identified by biochemical tests (coagulase, maltose, lactose, trehalose, and Voges-Proskauer). All *S. aueus*isolates were screened for methicillin resistance by disc diffusion (6 µg/ml oxacillin) on Mueller Hinton agar with 2% NaCL. Oxacillin-resistant isolates were tested for the presence of penicillin binding protein 2′ (PBP 2a) using latex agglutination kit (MRSA latex agglutination test, Oxoid Ltd., Hants, UK). At least one *S. aureus* isolate which was also PBP 2a positive from given sample was forwarded for molecular testing.

Molecular testing

Genomic DNA was extracted using the Wizard Genomic DNA preparation kit (Promega, Madison, WI). Polymerase Chain Reaction (PCR) was performed on all isolates. A multiplex PCR assay was used to determine the presence of the *mecA* gene, and the *nuc* gene (present only in *S. aureus*)[19]). Amplification of the *Staphylococcus* protein A (*spa*) gene was performed through PCR as previously described [20], using primers validated for use with Ridom-StaphType software [21]. The presence of PVL toxin genes (*lukS, lukF*) was determined by an additional PCR [22]. All molecular procedures utilized known positive and negative controls.

Antimicrobial Susceptibility Testing

Isolates were selected for antimicrobial susceptibility testing by broth dilution using minimum inhibitory concentration (MIC) method as described by the Clinical and Laboratory Standards Institute [23] using TREK Veterinary Sensititre equipment (Thermo Fisher Scientific, Cleveland, OH). Isolates were tested for susceptibility to chlortetracycline (CHL), clindamycin (CLI), enrofloxacin (ENR), florfenicol (FLO), gentamicin (GEN), neomycin (NEO), oxytetracycline (OXY), spectinomycin (SPE), sulfadimethoxine (SUL), tiamulin (TIA), tilmicosin, (TIL) and trimethoprim/sulfamethoxazole (TMP/SMZ). Beta-lactam antimicrobials were not considered. Breakpoints used for interpretation of resistance were based on information provided by TREK Diagnostic Systems and were as follows: CHL (≥8 μg/ml), CLI (≥2 μg/ml), ENR (≥1 μg/ml), FLO (≥4 μg/ml), GEN (≥8 μg/ml), NEO (≥8 μg/ml), OXY (≥8 μg/ml), SPE (≥32 μg/ml), TIA (≥32 μg/ml), TIL (≥16 μg/ml), TMP/SMZ (≥2 μg/ml). Multidrug resistance was defined as resistance to ≥4 antimicrobials. The reference strain *S. aureus* ATCC 29213 served as a quality control strain in the MIC determinations.

Data Analysis

Descriptive analyses were initially performed. Factor associations were investigated using χ^2analysis and assessed with Fisher's exact test. Associations were deemed significant at $p<0.05$ level and subsequently odd ratios (OR) determined as appropriate. No allowance was made for multiple comparisons. Statistical analysis of data sets was performed using SAS software, version 9.1 (SAS Institute, Inc., Cary, NC).

Results

Pork farms samples

Forty (40) pork farms of various types and animal age groups were visited during the study period. No farm was visited more than once. MRSA was detected in 30% (12/40) of the pork farms tested by either pig or environmental sampling. Two sites did not have pig samples collected, but were positive for MRSA from the environmental samples. A total of 362 samples were collected from these sites including 194 from pigs and 168 from the environment. Overall MRSA was detected in 17.4% (63/362) of the samples

tested including 17.5% (34/194) of the pig samples and 17.3% (29/168) of the environmental samples. In MRSA-positive farms, either animal or environmental samples were positive 60.1% (63/104) of the time. Of these, 69.4% (34/49) of pig samples and 52.7% (29/55) of environmental samples were MRSA-positive. There was no significant differences in MRSA detection between pig and environmental samples (p = 0.08). Pig and environmental sample results at the farm level matched 97.4% (37/38) of the time. The type of farm and age of animals was recorded for 82.5% (33/40) farms visits. In MRSA-positive farms, pigs less than 10 weeks of age were nearly 6 times (OR 5.95; 95% CI 1.22–28.95) more likely to also be present than not. Pork farm sample testing results are summarized in Table 1.

Student samples

Thirty (30) veterinary students were enrolled in a study. Only one student elected not to participate as she was taking the clinical swine course for a second time. Complete questionnaires were available for 29 students. The mean student age was 26.4 with a range of 24–35. Twenty females and 10 males participated in the study. Seven students reported using antibiotics in the previous 3 months. Also in previous 3 months, 0, 3, 1, 17 students reported hospitalization, respiratory disease with fever, SSTI, and pork farm visit, respectively. One student reported diagnosis of MRSA occurring 7 years prior. All students were negative for MRSA by nasal swab on the initial sampling. Six hundred and four (604) student samples were collected during the study period and MRSA was detected in 8 samples (1.3%, 8/604). Twenty-one (70%, 21/30) students visited MRSA-positive pork farms at least once and 6 students visited MRSA-positive farms on two separate occasions. Therefore, there were 27 student exposure events and MRSA was detected 6 times in separate students (22.2%, 6/27). MRSA was detected in 5 of these 6 students from the first nasal sample following the visit to a MRSA-positive farm. In one student MRSA was not detected until 5 days after a visit to a MRSA-positive farm. MRSA was not detected in any student for more than 24 hours, and no student subsequently became MRSA-positive again during the study period. MRSA was not detected in any student following visits to pork farms which were negative for MRSA. There was no significant association between detection of MRSA and recent respiratory disease with fever (p = 0.53), recent antimicrobial use (p = 0.29), SSTI (p = 0.29), or recent swine farm visit (p = 0.15). Additionally MRSA detection was not associated with gender (p = 1.00) or multiple exposures to MRSA-positive farms (p = 0.62). Age range in the exposed group was 24–35 years old. However, all except one student were between 24 and 28 years old. Therefore, age was not analyzed for risk. No students reported symptoms compatible with staphylococcal infections during the study period.

Molecular testing

One hundred and six isolates from 69 separate samples were positive for both *mecA* and *nuc*genes and negative for PVL genes. All 106 MRSA isolates were *spa*-typed and results are shown inTable 2. In summary, six *spa* types were found including: t002 (78.3%; n = 83), t034 (14.2%; n = 15), t548 (4.7%; n = 5), t10065 (0.9%, n = 1), t126 (0.9%; n = 1), and t1107 (0.9%; n = 1). The *spa* types found in pork farms from either pig or environmental samples included: t002, t034, t548 and t10065.

The *spa* types found in students included: t002, t034, t548, t1107, and t126. The sequence types (MLST) that have been associated with these *spa* types includes: ST398 (t034, t10065) [24], [25], ST5 (t002, t548, t1107) [21], [25], [26], and ST72 (t126) [21].

Pig and environmental *spa* types matched in all MRSA-positive farms with two exceptions. In one site, t034 was recovered from pig samples and one environmental sample. However, a second environmental sample from the same site was positive for MRSA with *spa* type t10065, which appears be a derivative of t034. In another site, t548 was recovered from all pig samples and t002 recovered from all environment samples. Both of these *spa* types (t548, t002) are associated with ST5 [25]. The *spa* type recovered from students and the pork farms closely matched those recovered from students with two exceptions; i) three *spa* types (t1107, t002, t548) were recovered from a student within 24 hours following exposure to a MRSA-positive farm where only t002 and t548 was detected. However, t1107 is also considered to be associated with ST5. ii) *spa* type t126, ST72-associated, was isolated from a student 5 days following exposure to a MRSA-positive farm with only *spa* type t002 detected. This isolate may represent exposure to a MRSA source not associated with pork farms. The combined results from pork farms and veterinary students are shown in Table 3.

Antimicrobial Susceptibility

Antimicrobial susceptibility panel testing (AST) was performed on 67 MRSA isolates from separate samples. Sources of MRSA isolates for AST included: pigs (n = 31), environment (n = 28) and students (n = 8). The *spa* types for AST included: t002 (n = 51), t034 (n = 12) and t548 (n = 4). Resistant levels to antimicrobials for all isolates included: CHL (n = 58, 86.6%), CLI (n = 31, 46.3%), ENR (n = 11, 16.4%), FLO (n = 26, 38.8%), GEN (n = 15, 22.4%), NEO (n = 49, 73.1%), OXY (n = 58, 86.6%), SPE (n = 67, 100%), SUL (n = 2, 3.0%), TIA (n = 15, 22.4%), TIL (n = 23, 34.3%), TMP-SMZ (n = 0, 0.0%) Percentage of all isolates that were resistant to a given antimicrobial is shown inFigure 1. Significant differences in level of resistance by source were seen only with enrofloxacin (p = 0.024) and florfenicol (p = 0.0006). The student isolates were more resistant than farm isolates for both antimicrobials. Significant differences in level of antimicrobial resistance among *spa* types were seen for: FLO (p = 0.0002), NEO (p = <0.0001), and TIL (p = 0.01) as shown in Figure 2. When related *spa* types (t002, t548) were combined, significant differences compared to t034 were found for only FLO (p = 0.002) and NEO (p = <0.0001) (Figure 3). In the case of NEO, if resistance was found the odds that the isolate was either t002 or t548 was very high (OR = 75.4, 95% CI = 8.4–677.6). There was 23 different resistant profiles in the isolates tested. The most common resistant phenotypes are shown in Table 4. Sixty -four (95.5%, 64/67) isolates were resistant to 3 or more antimicrobials. One isolate was resistant to 10 antimicrobials (t002; CHL-CLI-FLO-GEN-NEO-OXY-SPE-SUL-TIA-TIL).Combined resistance to tetracyclines (CHL, OXY), neomycin, and spectinomycin was seen in 67.2% (45/67) of the isolates overall but only in 8.3% (1/12) of the ST398 isolates. The proportion of multidrug-resistant isolates (≥4 antimicrobials) was higher in non-ST398 MRSA (94.5%, 52/55) versus ST398 (58.3%, 7/12) isolates (p = 0.0005).

Discussion

MRSA transmission to students

In this study we investigated the transmission dynamics associated with MRSA found in pork farms. We found that following short-term exposure (3–4 hr) to MRSA-positive pork farms, MRSA could be detected in students approximately 22% of the time. However, MRSA was not detected in any students for more than one day post-farm visit and did not reappear later on in the study. This suggests that the strains of MRSA from the pork farms did not become established in the students. These findings are consistent with other studies investigating LA-MRSA that have shown that short-term exposure to production animal farms does not lead to colonization [18], [27] or that carriage rapidly decreases when exposure is removed [17]. Studies have investigated the prevalence of MRSA in occupationally exposed people such as veterinarians with varying results. Some studies have used convenience sampling conducted at meetings or conferences and found detectable MRSA in swine veterinarians at levels such as 3% [28], 3.9% [11], and 12.5% [29]. A cross-sectional study found the prevalence of MRSA in livestock veterinarians to be 1.4% and 9.5% in Denmark and Belgium, respectively [30], while an epidemiological study in Germany found 23% of meat inspectors, laboratory personnel, and veterinarians tested were positive for MRSA ST398 [12]. Differences in prevalence can be expected based on geographic location, frequency of exposure, time since exposure, veterinary practices and study design. However, the level of MRSA detection in students enrolled in this study is rather consistent with other veterinarian prevalence studies indicating that this study may accurately represent the occupational exposure encountered by swine veterinarians. Additionally this study might provide insight into possible transmission risk to other sectors of the population with limited animal contact, such as agricultural fairgoers or petting zoo visitors. An advantage of this study over point-in-time prevalence studies is that participants were sampled frequently over time and therefore represents true incidence and temporal association to exposure. Although certain risk factors were investigated in this study (i.e. recent respiratory illness, SSTI, antibiotic use, hospitalization, pork farm visit), sample size limits the extent to which any conclusions can be drawn regarding these risk factors. Future studies targeting known MRSA-positive pork farms would increase the level of exposure and allow better assessment of human risk factors and MRSA colonization, but this would require a different approach than what could be achieved with the limitations associated with this study.

MRSA prevalence in pork farms

This study provides an estimate of the prevalence of MRSA on pork farms in the Midwestern U. S. While there have been a large number of studies examining prevalence of MRSA is pork farms in Europe [5], [6], [31]–[43], there have been rather few similar studies in the North America [7], [8]. However, finding MRSA in 30% of the pork farms in this study is consistent with these studies (Smith 50%, Khanna 45%). If MRSA was detectable in a farm it was generally easily detectable by either pig or environmental samples. MRSA was detected in approximately 60% of the samples collected at MRSA-positive farms. A higher level of detection was seen in pigs from MRSA-positive farms,

but the results were not conclusive. In fact, in one farm all pigs were negative while MRSA was detectable in the environment. In all farms with both pig and environmental testing MRSA status matched 97.4% (37/38) of the time indicating either method is equally likely to detect MRSA from a positive farm. Environmental dust samples have been used for surveillance purposes in other studies [6], [44] and in practice environmental samples are a more convenient method of collection versus live animals. Although this study was not designed to assess risk factors for MRSA on pork farms, there was a strong relationship between presence of young pigs (<10 weeks of age) and detection of MRSA (OR = 5.95). Other studies have reported an age-related association with MRSA status with highest prevalence reported in piglets between 6–12 weeks of age [8], [45].

spa types

The findings of many studies investigating MRSA in pork farms have indicated that ST398 is the predominant MLST present. In fact, discovery of an untypeable strain of MRSA in the Netherlands and subsequent investigations linking this strain to ST398 and pork farms initiated the process leading to the term "livestock-associated" MRSA [4], [5], [9], [31], [46], [47]. There were 6 *spa* types observed in this study (t002, t034, t126, t548, t1107, t10065) associated with 3 sequence types (ST5, ST398, ST72). However, non-ST398 *spa* types (t002, t548, t1107) predominated and accounted for 84% of the *spa* types observed and were found on 75% MRSA-positive farms. On the other hand, ST398-associated *spa* types (t034, t10065) accounted for 15% of *spa* types observed and were found on only 3 of 12 MRSA-positive farms. MRSA ST5 has been isolated from backyard-raised pigs in Michigan [48] and MRSA t002 was found in Canadian pigs [7], pigs at agricultural fairs [49], U. S. pork products [50], [51], and recently from Ohio pork farms [52]. This study also documents MRSA ST5 subtypes (t002 or t548) directly from pork farms in the U.S. Other studies indicate that non-ST398 (ST9) MRSA strains can be found in pigs and pig carcasses in Asia [44],[53]–[55]. Thus is appears that LA-MRSA is more diverse than ST398-associated strains and geographic differences exist.

Studies using whole-genome sequence typing have examined differences between livestock- origin and human- origin ST398 isolates [56], [57]. The first study reported that human-associated isolates carried phages that were largely missing from livestock-associated isolates. These phages were associated with innate immunomodulatory genes and considered virulence factors in humans. The authors theorized that during the jump to livestock these genes were lost, antibiotic resistance genes gained, and the resulting strains became less capable of re-infecting humans. The Uhleman study similarly reported differences in mobile genetic elements between human- and livestock-associated ST398 strains, but also reported enhanced adhesion of human isolates to human skin keratinocytes and keratin. Both studies found that genes responsible for PVL toxin production were missing in all livestock-associated ST398 strains. Similarly, in our study all ST398 and non-ST398 isolates lack*lukS-lukF*. Taken together, a picture that appears to be emerging is one of initial transmission of human-associated *S. aureus* strains or subtypes to livestock facilitated by loss of human virulence factors. However once established in livestock, the ability to re-infect humans appears reduced,

albeit not totally eliminated. MRSA ST398 is perhaps only one example of this process that may have occurred in other sequence types. A similar scenario was reported to be associated with the introduction of human *S. aureus* ST5 into chickens and broilers and subsequent global dissemination [58]. In that study, Lowder provided evidence that subtypes of ST5 found in poultry had undergone genetic diversification leading to acquisition of avian-specific accessory genes and inactivation of human virulence genes. This study suggests a similar process may have occurred with subtypes of ST5 leading to host-adaptation in swine with as yet only local distribution.

Antimicrobial resistance patterns

All isolates were resistant to spectinomycin, an aminocyclitol. Spectinomycin resistance in ST398 has been reported [59]–[61], however at lower levels than found here. Resistance to tetracycline derivatives (chlortetracycline, oxytetracycline) overall was quite high (87%). Tetracycline resistance is a common feature of ST398 [24], [62], but was also found here with high frequency in non-ST398 isolates (84%). Aminoglycoside resistance (gentamicin, neomycin) averaged approximately 48% with neomycin resistance much higher than gentamicin. A striking difference in neomycin resistance between non-ST398 (87%) and ST398 (8%) isolates was observed. Macrolide resistance (tilmicosin) was 34% while lincosamide (clindamycin) resistance was just over 46%. As a class, the least resistance was seen with sulfonamides (sulfadimethoxine, trimethoprim/sulfamethoxazole). Fluoroquinolone (enrofloxacin) resistance was 16% and resistance to florfenicol, a phenicol derivative, was nearly 39%. A Belgian study [42] which tested 643 pig MRSA ST398 isolates reported similar resistant rates in comparable drug classes for tetracycline (100%), aminoglycosides (48%), macrolides (56%), and sulfonamides (2%). However, that study found higher resistance with lincosamides (73%), and fluroroquinolones (32%), and lower resistance to the phenicol derivative, chloramphenicol (5%). In this study pleuromutilin resistance (tiamulin) was 22%. Additionally, tiamulin resistance appeared to be associated with clindamycin resistance (12/15), which may indicate presence of *vga*(A) as recently reported in ST398 [63]. There was a wide diversity of resistance phenotypes found in the isolates tested in this study with combined resistant to tetracyclines, neomycin, and spectinomycin seen most commonly particularly in ST5 subtypes. These subtypes were also more likely to be multidrug resistant.

Resistance patterns can be expected to vary based on location, drug approval, and farm level management. Due to study constraints, site-specific antimicrobial use was not recorded. Other limitations in this study include non-random selection of production sites and clustering of sites within production systems. Since the selection of pork production sites that were sampled was based on a request for assistance to the ISU Swine Production Group, presumably health-related problems existed at the farm. Management practices and farm conditions which contribute to health problems may also contribute to the presence of MRSA. Additionally, it is not uncommon for swine course diagnostic investigations to involve multiple pork farms within a common production system. Therefore, use of common practices, equipment, and breeding stock could lead to MRSA contamination of multiple farms and significantly affect the

prevalence of particular MRSA strains. Detailed information on the pork farms was withheld in this study.

Conclusions

The findings from this study support some of the findings from other studies. We found that following short-term exposure to MRSA-positive pork farms MRSA could be detected in students 22% of the time, but this level of exposure did not lead to stable colonization in participants. The prevalence of MRSA in pork farms was 30%, which is lower than results from many prevalence studies in Europe, but similar to results from other studies in North America. One of the surprising findings was the predominance of ST5 subtypes on farms and in students. ST398 subtypes were not detected in any exposed student. It was interesting that some the characteristics of the these non-ST398 isolates resembled ST398 in that none contained the PVL toxin gene but were likely to be tetracycline resistant. However, non-ST398 isolates differed in their resistance profile particularly in regard to a high level of resistance to neomycin and association with multidrug phenotype. Further investigation of these isolates by molecular analysis is needed to determine if these isolates fit the pattern associated with LA-MRSA, but it seems likely that MRSA subtypes from multiple lineages have made the human-to-livestock leap. Whether the impediments to human re-adaptation remain in place is still unknown.

Subaim 1

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) has emerged as a pathogen of public health concern (1-6). Recent evidence has demonstrated MRSA carriage among both livestock and the workers caring for these animals, suggesting that MRSA is a zoonotic agent of potential occupational and environmental health significance (7). In a recent study in the Netherlands, swine and cattle farmers had increased odds of being carriers of MRSA relative to unexposed comparison groups. Further, MRSA of livestock origin ("livestock-associated MRSA," or LA-MRSA) has been recognized as causing more than 20% of the cases of MRSA in the Netherlands (8). Of special note, MRSA occurrences in the study population clustered predominantly around areas of dense pig farms. In another Dutch study, a high MRSA carriage rate was found among workers on farms positive for MRSA in pigs or environmental dust samples, suggesting this organism may also be transmitted via the environment (9). As some health care workers in the Netherlands have been found to carry LA- MRSA, (10) they are now screened to prevent possible transmission to patients.

Recent research has shown that, in addition to the Netherlands, LA-MRSA also exists in North America. Khanna et al. revealed in a study in Canada that LA-MRSA in pigs correlated with human MRSA carriers (20% prevalence) on the swine farms and the *spa* type t034/sequence type (ST) 398 was the predominant stain in both swine and

swine workers (11). Smith et al. was the first group in the U.S. to identify and study human associations with LA-MRSA. In a pilot study, Smith et al. reported that almost half of the swine workers and pigs studied on two farming systems in Iowa and Illinois were colonized with MRSA (12), also found to be ST398.

The detection of MRSA in the nasopharynx of livestock, farmers, veterinarians and other occupationally exposed persons suggests airborne spread as another possible route of transmission (13). In Switzerland, total airborne MRSA was found on pig farms with pigs colonized with *Staphylococcus aureus*(*14*). A study conducted in animal feeding operations detected *S. aureus* as the predominant viable bacterial species present in air samples (15). A later study found that aerosolized *S. aureus* accounted for 76% of viable bacteria detected 150 meters downwind from an animal feeding operation (16). Antibiotic-resistant strains of *S. aureus* were also isolated from air samples taken downwind at dairy cattle feeding operations (17).

A study in Texas suggested that residential houses in the vicinity of livestock facilities may also be contaminated with antibiotic-resistant bacteria (18). Gandara et al. was able to recover antibiotic resistant bacteria in concentrations higher inside the residential homes than outside the homes. This finding, along with the Gibbs et al. detection of antibiotic resistant bacteria being recovered 150 m downwind of swine feeding operations, illustrates the potential of transmission of antibiotic resistant bacteria from swine feed operations to nearby communities (19).

To our knowledge there has not been a study evaluating the viable airborne concentration and size of MRSA ,using the Andersen Sampler and an Optical Particle Counter , associated particles inside a swine facility and downwind of a swine facility over 150 meters. The main objective of this study was to determine the viable airborne MRSA concentration and particle size both inside and downwind over 200 meters from a swine feeding operation. As prior studies have shown that airborne particles inside swine buildings come from two major sources large particles (> 5 µm, feed origin) and small particles (< 5µm, pig origin) (20). The findings of this study can help determine the major source of MRSA. Furthermore, data from this study may assist in determining if the present separation distances of swine facilities from residential areas are adequate to prevent the spread of MRSA.

Finally, biofilters, a system which generally uses compost and wood chips to biologically degrade odors, have been used to reduce odor emissions from swine CAFOs[28]. Evaluation of these biofilters have shown them to be efficient at mitigating odor emissions from swine CAFOs[28-30]. Biofilters have also been shown to reduce the concentration levels of dust, endotoxins and bacteria from CAFOs[31,32].

The objective of this study was to determine the efficiency of biofilters to mitigate airborne MRSA emitted from a swine CAFO. The efficiency of biofilters to reduce the

concentration of airborne MRSA particles was tested using a mobile biofilter unit. A working swine facility in which pigs, workers and air were culture positive for MRSA was affixed with duct work which connected the mechanically vented exhaust fans to the mobile biofilter unit. The duct work allowed the exhausted air from the swine feeding facility to be pulled through biofilters which contain one of two different types of media for a comparison of effectiveness (Hardwood chips and Western Red Cedar). Air inside the building and air pulled through the biofilter media was compared to air pulled through the two negative control biofilter units (lacking biofilter media).

Materials and Methods

Sample site

The study site was selected as it was representative of modern swine production facilities, and we had previously documented that the workers and swine at the facility were culture positive for MRSA (12). The producers were willing to cooperate for this study; informed consent was obtained and all IRB requirements were followed. The veterinarian for the facility helped facilitate the study, providing consultation in the conduct of sampling at the facility. The study site consisted of two buildings and produced approximately 48,000 feeder pigs/yr. Pigs entered the buildings at 14 days of age and left at the age of 60 days and weighing approximately 50 lbs. The stocking density of the two buildings was one pig per 4 ft^2.

Ventilation for the facility was provided by sixteen 24 inches and eight 14 inches wall fans (thermostat controlled) and eight 9 inches continuous pit fans. The facility had double-sided curtain for increased ventilation during warm seasons. The volume of the study room was 12,847 ft^3. The sampled facility was power washed with detergent and a biocide, Keno X5 (active ingredients hydrogen peroxide 26.5% and peroxyacetic acid 4/9%, CID Lines, Belgium, Europe), between cycling of hogs (46 days) due to the all-in, all-out, nature of the site. Topography of the area surrounding the facility was generally flat with one row of tree wind barriers.

Air sampling

The concentration of viable MRSA and total particulates inside the swine feeding facility and 215 meters downwind of the facility was determined by collecting air samples using stages one (> 7μm), two (4.7- 7μm) and five (1.1-2.1μm) of a six stage viable Andersen Cascade Impactor (Andersen Sampler In., Atlanta, GA, USA) and an Optical Particle Counter (GRIMM Technologies Inc., Douglas, GA, USA) with fifteen channels. Particle size was categorized as non-respirable (>5 μm) and respirable (<5 μm). The same instruments were used in the study for quality assurance and quality control.

The basic principles of bio-security (standard practices to keep disease out) of the facility management was practiced throughout the study (21). Prior to entry into the buildings, all of the sampling instruments were disinfected either by autoclave, or by spraying with 70% ethanol. All sampling instruments were calibrated before field sampling. The Q-Trak and VelociCalc were sent to the manufacturer for calibration prior to the study using the methods outlined in Edimansyah et al 2009 (22). The Optical Particle Counter (OPC) was calibrated at a flow rate of 1.2 liters per minute (LPM) each day prior to sampling (23) and the vacuum pump for the six stage Andersen Cascade Impactor was calibrated at a flow rate of 28.3LPM according to the methods of Middendorf with the Sensidyne Gilibrator ™ (Clearwater, Fla.) (24).

Air samplings were collected on three days: November 9th, 10th, and 11th of 2010. The sampling dates were chosen based on having access to the facility. Based on preliminary studies, sampling times inside the facility were set at 30 seconds, 1 minute and 3 minutes. Sampling was conducted in triplicate, at each sampling time to increase data reliability. Air sampling instruments were set at a height of 1.3 m on a table inside the animal feeding operation. Sampling locations inside the swine facility were conducted inside an empty pen in the middle of the building. Selective media used for MRSA isolation were CHROMagar plates (Becton, Dickinson and Company, Sparks, MD, USA). The plates were placed on stage one, two, and five of the Andersen Cascade Impactor to collect aerosolized MRSA.

Outside environmental conditions were measured including temperature, relative humidity, carbon monoxide (CO) concentration, carbon dioxide (CO_2) concentration using the Q-trak instrument (TSI, Inc., Minneapolis, MN, USA), air velocity using the Velocicalc Air Velocity Meter (TSI, Inc., Minneapolis, MN, USA), and solar intensity (RadioShack Auto Range Multimeter, RadioShack Corp., Fort Worth, TX, USA). Solar intensity and wind speed were used to determine atmospheric stability classes (categories of the stability of the atmosphere which can influence plume dispersion). Outside the animal feeding operation, sampling was conducted at a distance of 215 meters downwind from the animal feeding operations on a table at a height of 1.3 m with sampling times set at 3 minutes, 5 minutes and 10 minutes. The distance of 215 meters was chosen due to limitation of energy source beyond 215 meters. Additionally, wind direction downwind of the swine facility and boundary limitations was used to determine sample collection location.

After each sampling period the culture plates were sealed with tape, labeled, placed in a Ziploc bag and finally placed (agar face-down) into a cooler with ice packs for transport to the laboratory. Sampling time and volume of air collected by the Andersen Cascade Impactor N-6 ACI were used to determine the concentration (colony forming units, cfu/m^3) of airborne microorganisms.

Animal feed was collected on the sampling days directly off the feed truck when it arrived at the swine facility and from the feeding trough inside the swine facility. At the laboratory sampling was performed as previously described (25) with the modification of using animal feed instead of meat. Briefly, 25 g of animal feed was placed in Staph Enrichment Broth and incubated overnight at 35°C. The broth was then plated onto CHROMagar and CNA plates and incubated another 24-48 hours. Potential MRSA isolates were subcultured and diagnostics were performed.

Biofilter unit

We used a modified version of Hoff (200 9) biofilter design in collaboration with the Air Dispersion Laboratory (under the direction of Dr. Steve Hoff) at Iowa State University [33]. The modified biofilter design was tested and refined at the Air Dispersion Laboratory before field testing was performed to verify that constant air flow and pressure was being maintained. For the field test, the six stage Andersen Sampler (Andersen Sampler Inc., Atlanta, GA, USA) and an Optical Particle Counter(GRIMM Technologies Inc., Douglasville, GA, USA) were used to assess the particulate and viable MRSA content of the air, inside the building, the filtered air, and a negative control filter [34-36]. A plenum (duct) was connected to the exhaust fan of the CAFO and the biofilters. The mobile biofilter unit was composed of eight 50 gallon barrels with one of two biofilter media treatments (see figure 16) consisting of Hardwood chips (HWC) of 5 cm and Western Red Cedar (WRC) less than 5 cm[37]. The media dept was 25 cm for both media treatments. Prior to biofilter use, media chips were evaluated for MRSA and were found to be negative.

We assessed the air for presence of viable MRSA inside the CAFO in comparison to exhausted air. The air exhausted from the CAFO was assessed for viable MRSA, comparing the efficiency of the two different media (HWC, and WRC). The retention time of the air within the biofilters was adjusted to 4 seconds as determined by Chen et al 2009. N-6 ACI (using only 3 stages for collection) was used to sample air at three locations: 1) the center inside the CAFO in an empty pen, 2) the exhaust of the biofilters (figure 17), and 3) the control biofilter (which contained no filter media). Air sampling times of 30 seconds and 1 minute were conducted inside the CAFO. Sampling of filtered air and the negative control unit were 15 and 20 minutes. The air sampling times were selected based on preliminary trials. Environmental conditions inside the CAFO and outside the CAFO atmospheric conditions such as temperature, relative humidity, CO concentration, and CO_2 concentration were measured[36,38,39]. Each trial was conducted in triplicate for data reliability.

After each sampling period the culture plates were sealed with tape, labeled, placed in a Ziploc bag and finally placed (upside down) into a cooler with ice packs for transport to the laboratory. Air was sampled using CHROMagar plates as the collection media on

stages one, two and five. Concentration (colony forming units, cfu/m^3) was determined by multiplying sampling time and volume of air collected.

Bacterial diagnostics

Diagnostics were performed at the Center for Emerging Infectious Diseases Laboratory, CHROMagar MRSA plates were incubated at 35°C for 48 hrs. Potential MRSA colonies from the CHROMagar plates were subcultured onto Columbia CNA plates (Remel, Lenexa, KS, USA) for diagnostic testing. Identification tests for S. *aureus* isolates included the catalase test, the coagulase test and the *S. aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad). Methicillin resistance was confirmed by testing for the presence of penicillin binding protein (PBP2') (MRSA latex agglutination test, Oxoid Ltd). *S. aureus* and MRSA isolates were stored at -80°C.

Molecular typing

Additional molecular diagnostics were performed on a random selection of isolates. Molecular tests conducted included antibiotic susceptibility testing (CLSI 2006 and CLSI 2009), *mec*A PCR (26), *spa* typing (27), and PVL PCR (28). Positive and negative controls were used for all tests.

Results

Non-respirable particles and respirable particles were measured to determine the concentration of particles inside, relative to the concentration of particles downwind of the facility. It was noted that other potential sources of outdoor particles such as harvesting, nearby unpaved road or paved road and other large sources of particle emissions were not close to the outdoor sampling area. The highest counts for non-respirable and respirable particles inside and outside the swine facility were on sampling day three. The median particle count for non-respirable particles inside the facility was 1,000 particles/liter (p/L) (range =106-3,095 p/L) and for outside, downwind of the facility we measured a median of 6 p/L (range=0-6,238 p/L). The mean measure for respirable particles inside the facility was 2,470 p/L (range=1,170-1,119,093 p/L) and for downwind the facility we measured a median of 363 p/L (range=0-42,738 p/L).

The sampling time of 30 seconds resulted in the highest mean concentration of total viable particles in the non-respirable range (particles > 5µm) at 23,191 cfu/m^3 on sampling day three inside the swine facility (Table 4). The sampling time of 30 seconds on sampling day two resulted in the highest mean concentration of total viable particles in the non-respirable range at 47cfu/m^3 downwind from the swine facility. The lowest mean concentration of 1cfu/m^3 for total viable particles in the non-respirable size range with a sampling time of 600 seconds occurred on sampling day three. The respirable

particles (<5um) inside the swine facility ranged from 11.6 x 10^3 cfu/m^3 to 15.9 x10^3 cfu/m^3 with the highest mean of 13.8 cfu/m. Downwind of the facility the concentration of total viable respirable particles (<5 um) ranged from 15 cfu/m^3 to 111 cfu/m^3, with the highest mean of 63 cfu/m^3. The non-respirable MRSA particles (> 5um) inside the swine facility ranged from 547 cfu/m^3 to 1,103 cfu/m^3, with the highest mean of 825 cfu/m^3. Respirable MRSA particles inside the swine facility ranged from74 cfu/m^3 to 302 cfu/m^3; the highest mean concentration 188 cfu/m^3. Downwind of the facility, respirable MRSA particles were detected with the highest mean concentration of 5 cfu/m^3. A general trend with the MRSA particles recovered showed that as time increased, the concentration of MRSA particles recovered decreased.

The percentage of MRSA in the respirable and non-respirable size range inside the swine facility is presented in figure 1. MRSA in the non-respirable size range had the highest percentage detected in 2 of the 3 days of sampling.

The effect of emptying and disinfecting a swine building on airborne MRSA concentration was also tested. Table 5 demonstrates that after the swine building was emptied, power washed, and disinfected, the concentration of total viable and MRSA colony-forming units decreased compared to when the swine building was occupied.

Table 6 shows the results for antibiotic susceptibility testing and molecular typing. Twelve isolates (100%) were resistant to methicillin; 8/12 (67%) were resistant to tetracycline and clindamycin, and 4/12 (33%) were resistant to erythromycin. All of the isolates were *mec*A positive. One isolate was PVL positive and *spa* type t008. The other *spa* types identified were t034 and t5706. Animal feed from both the truck and inside the swine facility tested were resistant to methicillin (4/4) and erythromycin (4/4). The isolates collected from feed were also *mec*A positive and spa type t034.

Efficiency of the biofilters was defined as the ratio of the difference of the concentration of MRSA colony forming units and particle counts of negative control air inside the building and the concentration of the same contaminants in the air which passed through the biofilters divided by the unfiltered particles. The efficiency is reported as percentage.

Efficiency = (negative control particles – filtered particles)/ negative control particles) * 100

Figure 10 shows the results for non-viable particles using the OPC for the HWC biofilter. The OPC measured the size of dust particles through fifteen channels with size ranges from 0.4 μm to above 20 μm. The HWC biofilter was 89 percent efficient at filtering dust particles with the mean particle size of 1.8 μm, 88 percent efficient with the mean particle size of 4.5 μm, and 97 percent efficient with the mean particle size above 10 μm.

The results for the efficiency of WRC for non-viable particles using the OPC are shown in figure 11. The WRC biofilter was 83 percent efficient at filtering dust particles with the mean particle size of 0.9 µm, 59 percent efficient with the mean particle size of 1.8 µm, and 86 percent efficient with the mean particle size of above 8.75 µm.

The results for the efficiency of HWC for viable MRSA particles using the N-6 ACI are shown in figure 12. For the N-6 ACI, stage one collected MRSA particles the size range of 7µm and above, stage two collected MRSA particles in the size range of 4.7 µm to 7 µm, and stage five collected MRSA particles in the size range of 1.1 µm to 2.1 µm . The results show that the HWC biofilter was 92 percent efficient at filtering viable MRSA particles with mean particle size of 5.85 µm. The filtering efficiency of WRC for viable MRSA (figure 13) shows that the WRC media was 100 percent efficient at filtering viable MRSA particles with mean size of 5.85 µm.

Discussion

Our results show that viable MRSA can be detected inside and outside downwind of intensive livestock housing at both the non-respirable size range (> 5um) and respirable size range (< 5um) (figure 1). This suggests that the MRSA source can be both aerosolized feed dust, as well as aerosolized particles from the pigs themselves (dried manure, skin cells, etc). We have also shown that MRSA can be isolated from the bulk swine feed coming into the building. This study utilized a swine facility where the swine workers and swine have been previously confirmed to be MRSA-positive (12).

In this study, higher wind speeds corresponded to lower concentrations of viable particles. We speculate that wind speed affected the dispersion and dilution of particles in the air. We further speculate that increased wind speed led to the particles being mixed and dispersed further from the facility, albeit in lower concentrations (29). In a previous study, solar intensity appeared to be directly related to reduced isolation of viable bacteria, speculatively due to inactivation of the microbe by the sun and increased travel time in the air resulting in desiccation (30). We speculate that in addition to desiccation, solar intensity may decrease the size of the particle which allows it to travel further in the air stream before settling out. Thus, we surmise that wind speed and solar intensity have a dynamic relationship with MRSA plume dispersion. Wind speed and solar intensity were used to determine the atmospheric stability class. High wind speeds, and increased solar intensity create unstable atmospheric conditions resulting in increased dispersion of respirable sized particles. We speculate this was associated with being able to detect MRSA 215 meters downwind of the swine facility (the furthest isolations reported to date).

Our study showed results similar to Gibbs et al. (15) and Green et al. (16). Gibbs et al. also showed that antibiotic resistant bacteria in the respirable and non-respirable size ranges were recovered inside swine buildings and downwind of swine facilities. Gibbs et

al. used a control facility (without pigs) to determine the source of microorganisms detected were the pigs. We were able to detect MRSA on various stages of the Andersen sampler which indicated that MRSA possibly originated from multiple sources inside the swine feeding facility. Donham et al. showed that smaller particulates in swine buildings were from animal sources, such as dried fecal matter, and the larger particulates were from feed material (20). Therefore, t MRSA detected in the respirable size range likely originated from the pigs (dander, dried fecal matter and epithelial cells) and MRSA in the non-respirable size range originated from feed and dusts (figure 1). The sizes of MRSA detected inside the swine building were similar to the size of particles in other studies causing respiratory symptoms.

Green et al. showed that antibiotic-resistant *S. aureus* can be recovered from the air exhausted from swine CAFOs at distances of 150 m (16) Schulz et al detected airborne MRSA 150 m downwind from swine barns (31). In our study we recovered viable airborne MRSA at 215 meters downwind of a swine CAFO, a distance farther than recommended by Gibbs et al. (16, 19). According to Iowa Code Law for Confined Animal Feeding Operations (CAFOs) (32), there is no current separation distance requirement for the type of swine facility tested. The swine facility tested had an animal unit (AU) of 400; the Iowa Code Law does not have a separation distance for any swine facility with less than 500 AU. With the detection of MRSA at least 215 m downwind of the swine facility, we observed a distance is only 14 m less than what is required for CAFOs built prior to 1999. The separation distances for swine CAFOs built between 1999 and 2003 is less than 90 m from observed distance of MRSA detection. MRSA was detected 215 m downwind of the swine facility which was within the present Iowa Code Law for separation distances albeit very close to the required Iowa Code Law. It is worthwhile to note that the separation distances for swine CAFOs vary from state to state. The detection of airborne viable MRSA downwind of the swine facility suggests that a comprehensive MRSA surveillance program should include both air sampling and collecting nasal swabs from workers on swine farms.

Our study also found that when the swine CAFO was emptied and disinfected, no MRSA were detected in the air. This finding suggested pigs and other factors associated with their activities can influence the detection of airborne MRSA in swine feeding facilities and that the all-in, all-out method and disinfecting the swine buildings could reduce airborne MRSA.

We found that all isolates identified were resistant to methicillin. Isolates from animal feed were the only isolates that showed susceptibility to tetracycline and clindamycin. Furthermore, all of the isolates from animal feed were resistant to erythromycin. Both the animal feed tested inside the swine facility and animal feed directly from the truck prior to entering the swine facility. This finding is significant since MRSA detected inside the swine facility may have been of animal feed origin. Although Friese et al

detected MRSA in pooled samples of feces and feed from turkey barns (33), to our knowledge our study is the first to detect MRSA in feed from non-pooled samples in a swine facility. Additionally, we sampled feed from the feed delivery truck prior to the animal feed entering the facility. Cavaco et al. showed that metal supplements, such as zinc oxide and copper sulfate in animal feed, may promote selective pressure for MRSA emergence (34). We also found *spa* types t008 and t034, which have been found in other studies to be associated with MRSA in veterinarians and pig farming (35-38). ST389-associated/ *spa* type, t5706, was also identified in air samples inside the swine facility and exhausted from the swine facility. These findings suggest that airborne MRSA in these facilities may have either a human or livestock origin.

Our results showed that HWC and WRC media were highly efficient biofilters to prevent the emission of viable MRSA particles in the exhaust air from swine feeding facilities. The HWC media had an efficiency of 77 percent for particles with mean particle size of 1.6 µm. The efficiency of the HWC media increased as the bioaerosol particle size increased. Western Red Cedar was highly effective for particles with mean diameters of 1.6 µm to 5.85 µm. We speculate that the difference in efficiency shown by the two different biofilter media may have been due to the size of media mesh which may have affected the biofilter porosity [40]. The HWC (> 5 cm) media were larger than the WRC (< 5 cm) media which may have prevented the HWC from intertwining and forming a mesh with smaller pores. We also speculate that the larger size mesh for the HWC media allowed the various sized dust and MRSA particles to pass through the HWC mesh. However, as the size of the particles increased, the larger particles were apparently impacted on the HWC and were prevented from passing through the outer layer of the HWC media. On the other hand, the WRC biofilter media were shredded chips intertwined closely forming a smaller mesh thus making the WRC biofilter less porous than the HWC biofilter. As a result of the WRC biofilter being less porous than the HWC biofilter, the WRC biofilter had higher filtering efficiencies for the smaller size particles compared to the HWC biofilter. The results of the two sample t test showed that the efficiencies of the two different biofilter media were not due to the difference in the concentration of the dust particles filtered by HWC and WC. Instead we postulate that the difference in the efficiencies may have been due to the biofilter media used [28]. These findings showed that HWC and WRC were highly efficient biofilter media for reducing emissions of MRSA from swine feeding facilities.

Although this is the first study of effectiveness of biofilters on emission of MRSA, other studies have reported on the effectiveness of filtering other contaminants. Tymczyna (2007) found that biofilter media were efficient at retaining dust, gram-negative bacteria and endotoxins exhausted from a chicken hatchery room [31]. Martens (2001) found that biofilters were efficient at reducing bioaerosols from pig facilities [41]. In addition to the first study of biofilter effectiveness regards to MRSA, our study has advanced the field by evaluating the efficiency of different media (HWC and WRC) as

shown in figure 13 and 14. Both HWC and WRC were efficient at mitigating emissions of total dust particles. WRC was the more efficient media at mitigation respirable MRSA. Prior research findings[30-32], along with the results from our study, indicated that biofilters can be efficient at reducing emissions of airborne MRSA, gram-negative bacteria, endotoxins and various gases from ventilation exhaust systems of swine feeding facilities.

This study had several strengths. It was the first study to evaluate respirable and non-respirable dust particles with the OPC and the Andersen Cascade Impactor. The OPC allowed real time dust counts to be performed and the Andersen Cascade Impactor allowed viable dust particles to be recovered and cultured. The Andersen sampler was able to indicate the different potential dust particle sources and location within the respiratory tract where viable MRSA can be deposited. This information helps to define potential health risks and location of respiratory illness. This study also detected viable MRSA further downwind of a swine facility than any prior studies. We showed that when a swine CAFO is power washed and disinfected with biocides, airborne viable MRSA and total viable particles can be reduced to prevent potential transmission of antibiotic-resistant bacterial infections.

The study also had limitations. Our study was based on sampling one swine farm. This prevents generalization of results to other farms, especially in different geographical locations dissimilar from the settings in our study. Additionally, we only tested over a few days during the fall season.

Further studies need to be done to identify other potential sources of airborne MRSA inside buildings other than the pigs. As the current study resulted in MRSA isolation from the feed, a more complex ecology of MRSA in swine facilities is emerging. To help further define the ecology of this organism, a future study is needed to further evaluate animal feed as an additional source of MRSA in swine barns. A more compote understanding of the source and ecology of this organism in this environment will assist swine producers and swine veterinarians in decision making relative to mitigation and control of this agent.

Our finding suggests airborne transmission as a route of MRSA dissemination in farm settings. Our findings show that swine workers and people living near swine facilities are at risk for inhaling airborne MRSA particles.

Subaim 2

Introduction

Numerous research publications have documented a variety of aerosol exposures which resulted in respiratory symptoms in producers and workers in swine buildings. Aerosolized particulate is one of those hazardous exposures. Animal feeding operations have been found to have high concentrations of dust particles. Dust particles in swine feeding facilities consist of predominantly organic material such as pig dander, animal feed, feces, fungi, bacteria and gases (Chien et al., 2011; Clark et al., 1983; Crook et al., 1991; Donham et al., 1986)(Chien et al., 2011; Clark et al., 1983; Crook et al., 1991; Donham et al., 1986). High levels of bioaerosols in swine feeding facilities have been linked to animal and human activities. The activity of feeding pigs has been identified to increase exposure to airborne dust to swine workers (Kim et al., 2008; O'Shaughnessy et al., 2010)(Kim et al., 2008; O'Shaughnessy et al., 2010). Bioaerosols inside swine feeding facilities can lead to potential respiratory health hazards (Chang et al., 2001; Clark et al., 1983)(Chang et al., 2001; Clark et al., 1983). Respiratory symptoms or conditions such as non-allergic asthma, organic toxic dust syndrome and bronchitis have been identified in swine workers (Andersen et al., 2004; Crook et al., 1991; Donham et al., 1989)(Andersen et al., 2004; Crook et al., 1991; Donham et al., 1989).

An additional potentially hazardous exposure is antibiotics that are added to the feed or water. They are often added in growing animals at sub-therapeutic levels for the economic advantage of increased rate of gains feed efficiency and possibly disease prevention. However, using antibiotics in feed can present a risk of unintended environmental consequences. Antibiotics have been detected in the air in swine facilities (Chapin et al., 2005; Murphy et al., 2007)(Chapin et al., 2005; Murphy et al., 2007). This suggests the possibility of the development of antibiotic resistant organisms in this environment and the potential adverse health effects of resistant infections on swine and swine workers (Hong et al., 2012)(Hong et al., 2012). Antibiotic resistant bacteria have been detected in the nasal passages of swine workers (Létourneau et al., 2010; Smith et al., 2009)(Létourneau et al., 2010; Smith et al., 2009). Methicillin resistant *Staphylococcus aureus* (MRSA) has been identified as a zoonotic pathogen occurring in swine feeding facilities in swine workers, veterinarians and swine (Leedom Larson et al., 2010; Price et al., 2012)(Leedom Larson et al., 2010; Price et al., 2012). Although the clinical picture of livestock associated MRSA is unclear in the U.S., infections caused by hospital or community acquired MRSA include upper respiratory infections, pneumonia, skin lesions and nosocomial infections (Lozano et al., 2011; Ramirez et al., 2012; Smith et al., 2011)(Lozano et al., 2011; Ramirez et al., 2012; Smith et al., 2011). MRSA can spread through the environment by direct contact between swine workers and swine, contact with fomites, and through airborne transmission (Smith et al., 2010)(Smith et al., 2010). The spread of MRSA via airborne transmission in swine facilities presents a respiratory hazard to swine workers and veterinarians (Leedom Larson et al., 2010; Smith et al., 2009; Smith et al., 2010)(Leedom Larson et al., 2010; Smith et al., 2009; Smith et al., 2010).

To help protect workers in swine feeding facilities from airborne transmission of MRSA, a mitigation program protecting workers from aerosolized substances needs to be implemented. Although source control is the best approach, a respirator or personal respiratory protective device (RPD) may need to be used as an adjunct to source control, and may be the only protection perceived as possible and affordable by swine producers. Harnish et al. 2013demonstrated that the N95 was effectively at filtering H1N1(Harnish et al., 2013)(Harnish et al., 2013) . When recommending and selecting a respirator for this purpose, one needs to choose an efficient respiratory protection device (RPD). Two strapped N95 dust filtering respirators have been identified as an effective RPD to help prevent exposure to airborne contaminants including infectious agents (Cho et al., 2010; Qian et al., 1998; Rengasamy et al., 2008)(Cho et al., 2010; Qian et al., 1998; Rengasamy et al., 2008). Although respirators for use in swine production have been evaluated for effectiveness in protection from dusts, there has not been an evaluation of respirator efficiency for protection against infectious agents in swine buildings (Popendorf et al., 1995)(Popendorf et al., 1995).

The purpose of this study was to evaluate the efficiency of the N95 filtering face respirator to protect against airborne MRSA exposure in a swine feeding facility. It was hypothesized that the N95 filtering face respirator would have an efficiency of at least ninety five percent against airborne MRSA.

Materials and methods

The efficiency of the N95 respirator was determined first by developing a test exposure chamber in the laboratory. After the test chamber was refined in the laboratory it was taken to the swine facility where air within the building was sampled gravimetrically and by a photometer particle counter before and after flowing through the N95 respirator. The respirator was sealed at the intake on the testing device to assure 100% of the air went through the respirator filter .

Sampling site

The study site was selected as it was representative of modern swine production facilities, and we had previously documented the workers and swine at the facility were culture positive for MRSA (Smith et al., 2009) (Smith et al., 2009) (13). The producers were willing to cooperate for this study, and informed consent was obtained and all requirements of IRB were followed. The veterinarian for the facility helped facilitate the study, providing consultation in the conduct of sampling at the facility tested. The study site facility consisted of two buildings and produced approximately 48,000 feeder hogs/yr. Pigs entered the site at 14 days of age and left at the age of 60 days and weighing 50 lbs. The stocking density of the two buildings was one pig per 4 ft^2.

Ventilation for the facility was provided by sixteen 24" and eight 14" wall fans (both thermostat controlled) and eight 9" continuous pit fans. The facility had double-sided curtains which could be raised for increased ventilation during warm seasons. The volume of the study room was 12847 ft^3. The sampled facility was power washed with detergent and biocide between each group of pigs that cycled through the building. Topography of the area surrounding the facility was flat without any wind buffers.

Exposure test chamber

We refined the N95 respirator exposure test chamber at the Environmental Modeling and Exposure Assessment Facility at the Institute for Rural and Environmental Health, the University of Iowa. The respirator exposure test chamber was a modified version of a test chamber which had been used in a previous pilot study (Newnum, 2010)(Newnum, 2010).The following describes the laboratory set up and is depicted in figure 1. N95 respirators (Model 7130N95, North by Honeywell, Cranston, RI) were placed between two polymethyl methacrylate covers and placed inside a dust chamber with a metal tube (1/4" outer diameter) inserted at the back of the chamber. The N95 respirator was attached to this metal tube. An inlet tube was placed inside this metal tube to sample airborne particles which passed through the N95 respirator (filtered air). A second inlet tube was inserted through the back of the test chamber and was positioned next to and in front of the N95 respirator to sample unfiltered air in the test chamber. Tygon tubes were attached to both sampling air inlets (filtered and unfiltered) which were connected to a six stage viable Andersen Cascade Impactor (N-6 ACI) (Andersen Sampler Inc., Atlanta, GA, USA) at a flow rate of 28.3 liters per minute (LPM) which sampled unfiltered air and a second N-6 ACI sampled filtered air. The Tygon tube was also ported to an optical particle counter (GRIMM Technologies Inc., Douglasville, GA, USA) which sampled unfiltered air and to a second OPC which sampled filtered air (PETERS et al., 2006)(PETERS et al., 2006). Air was pulled through the sample inlets in the test chamber at 85 LPM flow rate via a stationary air mover (a work shop Vacuum) which was monitored by an inclined-vertical manometer and a Venturi flowmeter (Dwyer Instruments Inc., Michigan City, IN, USA). The OPC and the N-6 ACI were set at a sampling rate of 1.2 LPM and 28.3 LPM respectively and monitored with the digital manometer (Dwyer Instruments Inc., Michigan City, IN, USA). Viable airborne particles were collected with the N-6 ACI and deposited on CHROMagar plates (stages one, two and five) in triplicate runs. At the end of the preliminary test the test chamber was taken into the field for sampling. All instruments were calibrated according to manufacturer instructions.

The test chamber was then placed in the center of swine facility sampled (in an empty pen to prevent damage from the hogs) at a height of 1.3 m from the ground to simulate the "breathing zone" of workers. Samples downstream (filtered) the respirator were taken at 15 and 20 minutes, whereas samples from upstream (unfiltered) the respirator

were taken at 30 and 60 seconds to account for the expected high concentration (cfu/m3) of particles. A commercial air mover (vacuum cleaner designed for use in workshop) provided a constant flow rate of 85 LPM of pulled air through the N95 respirator and was monitored by a manometer. Flow rate for the viable N-6 ACI was set to 28.3LPM and the OPC was set at 1.2 LPM. Each sampling time was conducted in triplicate for data reliability. The count per liter of particles determined by the OPC was stored on the instrument and was analyzed at the laboratory for efficiency percent. The CHROMagar culture plates were sealed with tape, labeled, placed in a Ziploc bag and finally placed (upside down) into a cooler with ice packs for transport to the laboratory. Environmental conditions such as of temperature, relative humidity, CO concentration, CO2 concentration were measured using the Q-trak instrument (TSI, Inc., Minneapolis, MN, USA), air velocity were measured using Velocicalc Air Velocity Meter (TSI, Inc., Minneapolis, MN, USA).

The field results for the efficiency of the N95 respirator for particulates using the OPC are shown in figure 4. These measurements were done while the barn was populated with pigs. Particulates above the mean particle size of 2.5 μm had N95 respirator efficiency greater than 93.52 percent. The mean particulate size of ≤1.8 μm had a penetration of 27.42 percent and 72.57 percent efficiency. Particulates with a mean size of ≤ 0.45 μm had a penetration of 50.66 percent and 49.34 percent efficiency.

Discussion

The study results showed that the N95 respirator had efficiency greater than 99 percent with the six stage Andersen Cascade Impactor for viable MRSA particles. Both respirable and non-respirable viable MRSA particles were reduced from being inhaled. With an effective seal, the N95 respirator is capable of providing a high degree of protection. This finding is important to help provide assurance that a respiratory protection program in swine facilities may be effective in reducing the risk of transmission of airborne MRSA to workers. In addition to the results from the Andersen Cascade Impactor (detecting CFUs), the OPC was used to determine the efficiency of the N95 respirator for all particulates. The efficiency of the N95 respirator decreased as the size of the particles decreased. For particles smaller than 4 μm (respirable size range), the efficiency was less than 95percent. Lee et al. 2005 found that the assigned protection factor of 10 for N95 respirators is insufficient for particles smaller than <5 μm (Lee et al., 2005)(Lee et al., 2005).These findings indicated that the size of the particles affected the efficiency of the N95 respirator (Harnish et al., 2013).

The results from the present study suggest that the N95 filtering facepiece respirator can be used as an effective RPD for infection control of MRSA in hospital settings.

This study had several strengths. The respirators were evaluated both in the laboratory and an actual swine barn. Furthermore the swine barn had been previously identified as

positive for MRSA in the air and in the nasopharynx of swine workers and pigs in the building (Smith et al., 2009). In addition to using a direct reading instrument to sample particle concentration, i.e. OPC, we simultaneously used the Andersen Cascade Impactor which is a standard for viable air sampling techniques. The design of the plate covers and the how the plate covers were sealed to the N95 respirators prevented leakage.

There were also limitations with this study. The sample size of the study was small. We only performed three time trials. This study did not address the effect of relative humidity on the effectiveness of the N95 respirator and was not tested on human workers. Leakage of total dust particles may have led the respirable dust particles having efficiencies less than 95 percent. A leak was found in the cheek area of the plate covers which may have allowed the penetration of particles due to the lack of a perfect seal. With the inadequate protection provided by the N95 respirator for total dust particles of the respirable size range using an OPC, further studies are needed to assess N95 respirator efficiencies for total dust the respirable size range of less than 5 um using an OPC.

Conclusion

Our findings can be used by swine producers to help justify a N95 respiratory program in their facilities to help reduce the possibility of transmission of airborne viable MRSA particles to workers inside swine CAFOs. A compliant N95 respiratory program can be used to help protect workers in swine facilities from potential respiratory illnesses. As found in our study MRSA can be identified in a swine feeding facility and the N95 filtering respirator does provide protection against MRSA detected on larger size particles but is not as effective against the smaller size particles for MRSA in the respirable size range. We tested with a perfect seal which means for a N95 respiratory program, each person needs to be fit tested to offer the protection provided by the N95 respirator. With MRSA particles in swine building likely being associated with swine epithelial cells, dried fecal matter and feed (larger particles), the N95 respirator will provide the required protection against MRSA particles inside swine facilities. Our results are surprising and do suggest further studies are warranted to evaluate the efficiency of N95 respirators against MRSA particles.

Aim 2

This was addressed in materials for Aim 1.

Aim 3

As noted above, we were unable to complete this aim due to low participation on part of the clinics. Herein we include details of the samples we were able to obtain.

March 2011: We (principal investigator Dr. Tara Smith and Graduate Research Assistant Raj Nair) traveled to the Hawarden Community Clinic on March 15, 2011 to meet with Dr. Dale Nystrom and his team. A brief session was organized where we discussed sample collection and sample shipping protocols, and also answered questions or concerns that the Hawarden team had at that point. We were joined by Dr. Elizabeth Truesdell and students from the Northwestern College to discuss their activities in this research project. We carried with us study packets (informed consent documents, enrollment questionnaires, and brochure); study flow chart for the clinic and, swabs and shipping materials required for patient sample collection.

March 2011 to July 2012: We received samples from the clinic. These samples were analyzed in Dr. Smith's lab at the University of Iowa Centers for Emerging Infectious Diseases (CEID). Informed consent signed by patients before submitting their sample have been checked and stored in locked cabinets, as per the study protocol. We also collected relevant medical information on enrolled patients using a medical record abstraction form as well as had patients fill-out a questionnaire developed for this study. Data collected using the questionnaire and abstraction form is entered in a password protected excel database for analysis.

May 2012: Sioux Center Medical Clinic expressed their interest in participating in our study. Participating physician completed IRB training and the certificate was approved by Siouxland IRB. All Northwestern College students interested in helping with our study have completed IRB training and are approved by the Siouxland IRB.

August 1, 2012: Scheduled to meet with Sioux Center Medical Clinic personnel to provide materials and instructions on the study. In addition, refresher meeting scheduled with personnel from Hawarden Community Clinic to provide extra supplies and respond to questions.

Number of people enrolled in the study from Hawarden Community Clinic: 15

Number of people enrolled and signed informed consent available – 12

Enrollment questionnaires filled - 9

Number of samples submitted by the clinic: 10

Number of samples identified as *Staphylococcus aureus*: 4

Complete information (signed consent, enrollment questionnaire, abstracted medical record and infection sample) is available only for 8 patients. All participants enrolled in this study were from the Hawarden clinic. Sioux Center clinic did not actively participate in patient enrollment.

Final results:

Patients enrolled in the study were between ages 14 years (youngest) and 92 years (oldest). All enrolled participants reported being Caucasians/White. Five male and 4 female participants were enrolled, as per information available from the enrollment questionnaire. Most of the participants (3) reported having "some college" as their level of education. Seven of these participants reported residing in a small town (not a suburb). At least one participant reported living in a rural area (on farm or acreage in the country). All eight participants reported handling or eating at least one form of meat (turkey, chicken, pork, beef) in their lifetime.

The most common morbidity reported by participants was asthma and diabetes. Seven of the 15 participants reported presenting with a skin or soft tissue infection at the time of enrollment in the study. Of these at least two participants were previously diagnosed with methicillin-resistant *S. aureus* infection (MRSA).

Samples from four participants tested positive for *S. aureus* in our research lab. Of the 4, one was identified as MRSA by gene-based molecular analysis. This patient reported a history of previous MRSA infection. Two patients with *S. aureus* infection had a previous exposure to hospital and antibiotic use. Two patients with methicillin-susceptible *S. aureus* infection (MSSA) reported having exposure to chickens, swine and cattle as part of their occupation. The patient with chicken exposure worked for a duration of 19 years with around 50 chickens daily. This patient reported not using any protective measures during work (gloves, footwear, mask, protective clothing). The other patient who reported working with swine and cattle worked for a period of 30 years and 55 years, respectively. This patient was exposed to 350 swine and 300 cattle daily for at least 1 hour. This patient did report using mask, footwear and eye protection.

Results for molecular analysis of the four confirmed *S. aureus* infection isolates were as follows:

S. aureus type	*spa*-type	*mecA*	PVL	Risk exposures
MRSA	t008	1	1	Antibiotic use, history of

				MRSA infection
MSSA	t4303	0	0	Hospitalization, exposure to chickens
MSSA	t10494	0	0	Antibiotic use, exposure to swine and cattle
MSSA	t346	0	0	None noted

Spa-type information obtained genetic sequencing of the *spa* gene aids in identifying the strain type of the *S. aureus* isolate. *mecA* gene was tested to observe for the presence of the methicillin resistance gene. Only one isolated was observed to be positive for the Panton-Valentine leukocidin (PVL) gene, which is considered to potentially increase virulence of the bacteria.

To date, we have not had any report of adverse events on patients enrolled at the Hawarden Community Clinic. Enrollment rates at the participating clinics were very low. Clinics were contacted to discuss any potential barriers to patient participation. There was no information available on this issue. Hence, we hypothesize that patients may not be regularly visiting clinics for regular skin infections that could potentially be a *S. aureus* infection. This may result in under-diagnosis of the condition and further propagation of the bacteria, particularly in rural areas. Moreover, clinics did not enroll any non-English speaking participant. This may have resulted in loss of information on a population that could potentially be at a higher risk for such infections. Further studies are warranted in this regard to identify *S. aureus* infections in primary care clinics and rural areas.

Publications

Journal Article

Frana TS, Beahm AR, Hanson BM, Kinyon JM, Layman LL, Karriker LA, Ramirez A, Smith TC: [2013] Isolation and Characterization of Methicillin-Resistant *Staphylococcus aureus* from Pork Farms and Visiting Veterinary Students. PLoS ONE 8(1): e53738.

This article examined the prevalence and molecular types of S. aureus on 40 swine farms in Iowa, and the estimated duration of carriage of these organisms when obtained from a livestock environment.

Dissertation/Thesis

Ferguson DD: [2013] Assessment and Mitigation of Airborne Transmission of Methicillin-resistant *Staphylococcus aureus* in Animal Feeding Operations and the Outdoor Environment, Ph.D. Thesis, University of Iowa.

This thesis examined airborne MRSA in and downwind from swine barns, and the potential for biofilters as an inexpensive intervention to mitigate transmission within this environment.