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METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PORK PRODUCTION FACILITIES: OCCUPATIONAL EXPOSURES AND INFECTIONS

by

Kerry Reah Leedom Larson

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Occupational and Environmental Health
in the Graduate College of
The University of Iowa

May 2010

Thesis Supervisor: Professor Kelley J. Donham

ABSTRACT

This research focuses on occupational exposures associated with Methicillin-resistant *Staphylococcus aureus* (MRSA) in modern pork production facilities. This dissertation is composed of three related parts.

In Chapter II, "Methicillin-resistant *Staphylococcus aureus* in pork production shower facilities" we documented the presence of MRSA in shower facilities of conventional swine production systems where pigs were colonized with MRSA. We tested farms involved in different production phases (sow, nursery, and finisher) and geographical locations. In the two swine production systems studied, 3% and 26% of shower samples were positive for MRSA. Overall, the prevalence in showers was 19%.

In Chapter III, "Methicillin-resistant *Staphylococcus aureus* in pork production shower facilities: Adapting interventions from athletic facilities," we searched the literature for interventions designed to decrease MRSA infections in athletes. We then evaluated these interventions for adaptability to the pork production environment and composed swine-specific guidelines for MRSA prevention. We implemented our intervention in a pilot study to reduce MRSA in showers and locker rooms, and results were mixed. We recommend repeating this study with a larger sample, and better intervention management and oversight.

In Chapter IV, "Methicillin-resistant *Staphylococcus aureus* infection in pork production workers," we sought to determine if pork producers report veterinarian-diagnosed antibiotic-resistant skin infections in pigs, and physician-diagnosed antibiotic-resistant skin infections in workers (including MRSA). We then examined potential risk factors for infection associated with biosecurity, including shower and laundry procedures, farm-specific clothing use (clothing worn only while working on the farm), and personal hygiene. No significant risk factors were identified for either skin infections in pigs or skin infections in workers.

These studies provide evidence that MRSA can be found in pork production shower facilities, and that occupational exposures occur due to components of the biosecurity protocol. Our pilot intervention suggested that the impact of showers as environmental reservoirs can be reduced. We also reported the first prevalence estimate of MRSA infection in pork production workers in the United States. Livestock-associated MRSA remains an emerging issue and requires further study to determine the true occupational and public health risks.

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Graduate College
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CERTIFICATE OF APPROVAL

PH.D. THESIS

This is to certify that the Ph.D. thesis of

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has been approved by the Examining Committee
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LIST OF ACRONYMS

Acronyms (in order of appearance)

1. MRSA, Methicillin-resistant *Staphylococcus aureus*
2. HA-MRSA, Hospital-associated MRSA
3. CA-MRSA, Community-associated MRSA
4. U.S., United States
5. PBP, Penicillin binding protein
6. MSSA, Methicillin-susceptible *Staphylococcus aureus*
7. SCC, Staphylococcal cassette chromosome
8. *ccr*, Cassette chromosome recombinase
9. SSTI, skin and soft tissue infection
10. LA-MRSA, Livestock-associated MRSA
11. ST398, multi locus sequence type 398
12. *pvl*, Panton Valentine leukocidin
13. *spa*, Staphylococcal protein A
14. MLST, Multi locus sequence typing
15. PSA, Production system A
16. PSB, Production system B
17. CDC, Centers for Disease Control and Prevention
18. EPA, United States Environmental Protection Agency
19. PQA™/PQA Plus™, Pork Quality Assurance/Pork Quality Assurance Plus
20. USDA, United States Department of Agriculture
21. OHS, Occupational health and safety
22. NAICS, North American Industry Classification System

CHAPTER I

INTRODUCTION AND LITERATURE REVIEW

Zoonoses, or pathogens communicable between animals and humans, are an understudied aspect of global health (1). Over 61% of all infectious organisms and 75% of emerging pathogens are known to be zoonotic (2). In fact, zoonotic pathogens are twice as likely to be categorized as emerging (or reemerging) compared to non-zoonotic pathogens (3). More specifically, some pathogens with primary animal hosts can be transferred to man (anthropozoonoses). Pathogens that are found primarily in humans can also be occasionally transmitted to animals (zooanthroponoses) (4).

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a zoonosis that has garnered the attention of scientists and the public in recent years. Trends in the prevalence of two common MRSA forms have demonstrated that the epidemiology of MRSA is changing. Reports of MRSA related infections first came from the hospital environment, but increasingly MRSA is the cause of infections in persons with no hospital exposure (5). Furthermore, advances in molecular typing methods have led to the identification of different MRSA forms in different environments (6). Both humans and the environment are known reservoirs for MRSA, but animals are being implicated as a new source of human exposure (7). Animal-to-human transmission of MRSA is not well understood. Human agricultural populations have been studied for evidence of colonization with MRSA, which appears to be relatively common in certain populations. However, how colonization relates to the risk of infection for those in agricultural occupations is yet to be seen.

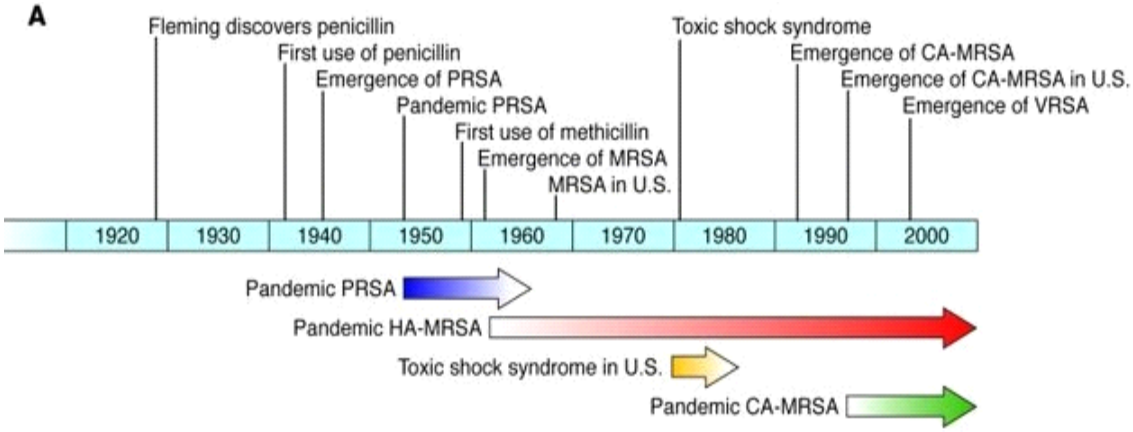
History

Staphylococcus aureus is without question the most virulent of the staphylococci due to its wide spectrum of disease, ranging from localized and systemic infections to

toxin-mediated illness (8). In fact, prior to the introduction of penicillin for treatment of *S. aureus* infections, the mortality rate from *S. aureus* infections was over 80% (9). Penicillin became available for medical use around 1940, but the first penicillin-resistant *S. aureus* isolate was identified only two years later; by 1960, about 80% of all *S. aureus* isolates were resistant to this antibiotic (10). Similarly, after the introduction of methicillin (a synthetic antibiotic related to penicillin) in the early 1960's, a methicillin-resistant form soon appeared (11).

Since then, two different types of MRSA have become established in the population, known as hospital-associated (HA-MRSA) and community-associated (CA-MRSA) based on their different microbiological, ecologic, and epidemiological characteristics (12). The distinction between these groups has recently been decreasing somewhat due to the appearance of CA-MRSA in hospital settings (10). Figure 1 summarizes the emergence of MRSA in the U.S.

Figure 1. Emergence of antibiotic-resistant *S. aureus* *



*Timeline indicates the year in which an event occurred or was reported, and arrows indicate the approximate length of time for each pandemic/epidemic.

(Reprinted with permission from DeLeo FR and Chambers HF. Reemergence of antibiotic-resistant *Staphylococcus aureus* in the genomics era. J Clin Invest. 2009;119(9):2464-2474. © 2009 American Society for Clinical Investigation.)

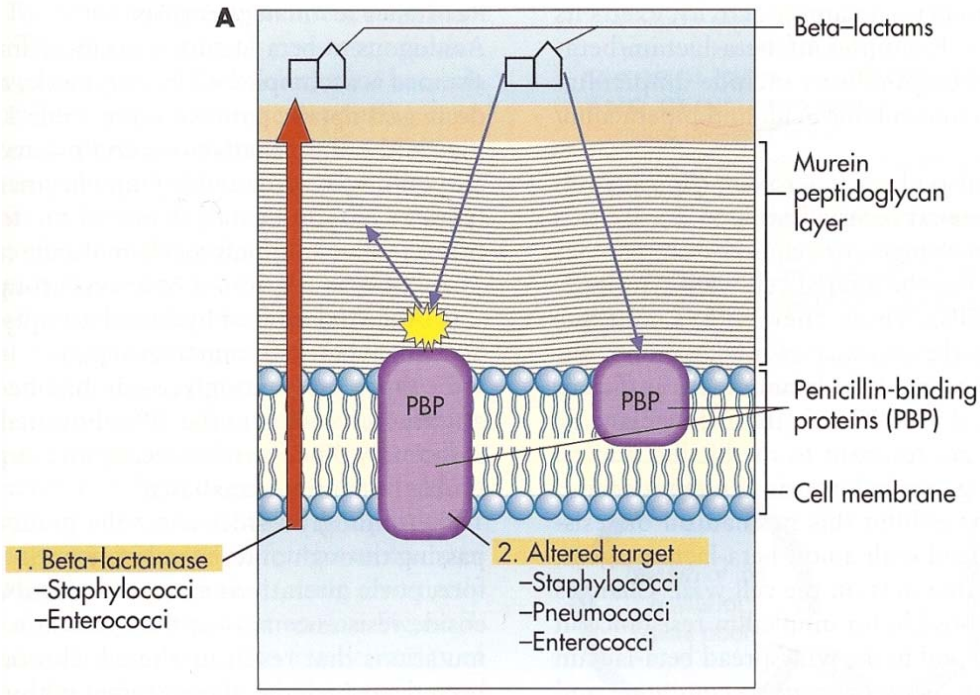
Antimicrobial resistance associated with MRSA has contributed to a substantial international burden of disease. In the United States (U.S.), it is estimated that MRSA caused 94,000 infections and over 18,000 deaths in 2005 alone (13). In fact, the number of MRSA-related hospitalizations more than doubled during the preceding six years (14). Approximately 1.5% of the U.S. population is currently thought to be nasally colonized by MRSA (15).

The organism

Microbiology *Staphylococcus aureus* are gram-positive, catalase-positive cocci (8). They have cell walls made of peptidoglycan (linked polysaccharide subunits) and may have activity that is similar to endotoxin. Other important structural components of staphylococci include polysaccharide capsules and surface proteins (for example, Protein A) (16). Staphylococci also produce enterotoxins that can cause food poisoning and shock (17). Staphylococci are commensal on the skin and mucous membranes of humans and animals, and over 30% of the U.S. population is thought to be colonized (18).

Mechanisms of resistance Resistance to methicillin and other β-lactam antibiotics among staphylococci can be bimodal. Often, resistance is due to the presence of the *mecA* gene. This gene encodes for penicillin-binding protein (PBP) 2′, an important cell membrane component (19). Methicillin-sensitive *Staphylococcus aureus* (MSSA) are susceptible to β-lactam antibiotics that bind their PBP and disrupt the cell wall; however, the altered PBP2′ that are present in MRSA cannot be bound by β-lactam antibiotics. This enables MRSA to grow in the presence of the drug (10). Most MRSA also produce enzymes that can destroy β-lactams, known as β-lactamases (20). Figure 2 demonstrates the two mechanisms of antibiotic resistance present in MRSA.

Figure 2. Diagrammatic summary of beta-lactam resistance mechanisms in gram positive bacteria



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The *mecA* gene (which confers methicillin-resistance) and the cassette chromosome recombinase (*ccr*) genes (which facilitate inclusion and excision into the *S. aureus* genome) are located on a mobile genomic island known as the staphylococcal cassette chromosome (SCC*mec*) (21). To date, eight types have been identified (traditionally designated as I-VIII); however, a naming system that includes both the *mec* class and the *ccr* type is becoming more accepted (22). Common SCC*mec* types and their associated *ccr* complexes are shown in table 1. SCC*mec* types can further be broken

down into subtypes based on regions not belonging to the *mec* or *ccr* genes, known as J regions (23).

Table 1. Summary of SCC*mec types currently described in methicillin-resistant *S. aureus***

Class of *mec* complex	Type of *ccr* complex	SCC*mec* type	Approx. size (kpb)
B	A1/B1	I	34
A	A2/B2	II	53
A	A3/B3	III	67
B	A2/B2	IV	21-24
C2	C	V	28
B	A4/B4	VI	24
C1	C	VII	27
A	A4/B4	VIII	32

*SCC*mec* = Staphylococcal cassette chromosome *mec*

(Adapted with permission from Vanderhaegen W, Hermans K, Haesebrouck F, and Butaye P. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. Epidemiol Infect. 2010; 1(1):1-20. © Cambridge University Press.)

Although the origin of SCC*mec* is not definitively known, it is generally thought that the *mecA* gene arose from transfer between two staphylococcal species (24). Two theories exist regarding the evolution of MRSA. In 1993, Kreiswirth et al. suggested that all MSRA clones have a common MSSA ancestor which acquired SCC*mec* only once (25). The later introduced multi-clone theory suggests that the SCC*mec* element was introduced into different *S. aureus* lineages multiple times (26). Currently, the multi-clone theory seems to be gaining support.

MRSA can be resistant to antimicrobials other than β-lactams. Additional drug resistance genes can become integrated into SCC*mec* as plasmids or transposons; examples include plasmid-mediated resistance to some aminoglycosides, tetracyclines, and heavy metals, and transposon-mediated resistance to macrolides, lincosamides, and streptogramin as well as cadmium (10). Some MRSA are also resistant to fluoroquinolones because of a mutation in the DNA gyrase gene (27). In addition, resistance to the powerful antibiotic vancomycin has recently been observed (28). In the U.S., the Centers for Disease Control and Prevention (CDC) conducts MRSA surveillance through the National Healthcare Safety Network (hospital-based) and the Active Bacterial Core Surveillance system (population-based) (29).

The causes of antimicrobial resistance are complex. Antimicrobial resistance in human forms of MRSA has been linked to the use of antimicrobials by man (30). MRSA strains of human origin can occasionally colonize animals, including companion animals (dogs and cats) and pigs (31-33). Similarly, resistant bacteria in livestock are likely associated with selective pressures that result from the therapeutic and sub-therapeutic use of antimicrobials in livestock (34). Specifically, the use of tetracycline has been linked to livestock related forms of MRSA in pigs (35).

The methods by which antimicrobial resistant organisms spread outside the farm have also been investigated. Evidence suggests that antimicrobial resistant bacteria, including *S. aureus*, can be transmitted downwind of pork production facilities and may be a potential human health hazard to those in the surrounding community (36, 37). There are likely multiple causes of antimicrobial resistance in the U.S. Recent legislative

efforts in the U.S. have focused media attention on the issue, and revealed it to be a highly charged issue for people in public health and the livestock industry.

Human colonization

MRSA colonization studies have been conducted both in research and clinical settings. Screening for nasal carriers, with follow-up treatment of positive persons, has been associated with a reduction in the number of MRSA infections in some healthcare facilities (38-40). In hospitalized patients, history of recent antibiotic usage or hospitalization and age over 75 years are known risk factors for MRSA carriage (41).

Despite the potential benefits of identifying patients with risk factors for MRSA carriage, or actual nasal carriers, an "active-detection and isolation" strategy for control of multidrug-resistant organisms is not currently recommended by major U.S. infection prevention professional organizations or the CDC (42). In other words, there is no consensus as to whether screening of all patients admitted to U.S. hospitals should occur. In the Netherlands and Denmark, search and destroy policies (including active hospital surveillance, preemptive isolation for patients at risk and known carriers, and treatment for nasal carriage) have been implemented to prevent MRSA transmission and have generally been considered successful (43, 44).

A variety of anatomical sites can be sampled for MRSA screening, including the nares, throat, skin, axilla, perineum, and rectum. In hospital settings, MRSA screening of the nares may detect 70% to 93% of those who are nasally colonized with MRSA (45-49). However, among patients with CA-MRSA skin and soft tissue infections (SSTI), only 25% of nasally colonized patients may be detected by nasal screening, indicating

that reservoirs other than the nares may be important in the transmission of CA-MRSA (50). When additional anatomical sites are sampled (axilla, inguinal region, and rectum) the overall detection of colonization increases to 37% (50). Study of MRSA SSTI in professional athletes indicates that, in contrast to the hospital setting, surveillance by nasal screening is ineffective in preventing infections in the community setting. (51).

Human infection

HA-MRSA This MRSA type developed soon after the introduction of the methicillin in the late 1950's (11) but took more than 20 years to become the epidemic that is known today (27). Generally, HA-MRSA infections are associated with healthcare exposures within the past year. HA-MRSA strains typically 1) cause invasive infections, 2) are resistant to clindamycin and fluoroquinolones, 3) are SCC*mec* type I, II, or III, and 4) lack the Panton-Valentine leukocidin (*pvl)* gene (10, 52). Today, MRSA causes the majority of nosocomial infections worldwide (53).

The costs of HA-MRSA infections are difficult to quantify. In a recent regional U.S. study, surgical site infections caused by MRSA led to 23 additional days of hospitalization and over $61,000 in additional charges (per admission) in 659 surgical patients (54). A literature review of MRSA in Canadian settings showed that MRSA infections prolonged hospital stays by 26 days and resulted in over $12,000 in costs (per MRSA case) in a 2007 analysis (55). Borg (2010) argued that healthcare expenditures and utilization also play a role in the epidemiology of MRSA in Europe (56) after examining the statistical link between MRSA and infant mortality rate, a known marker of healthcare expenditures.

CA-MRSA Unlike HA-MRSA, which spread throughout the world slowly in the past 20-30 years, CA-MRSA emerged only in the 1990's and has rapidly moved throughout the world (27). Generally, CA-MRSA infections are not associated with health care exposures in the past year. CA-MRSA strains typically 1) cause SSTI or pneumonia, 2) are variably resistant to clindamycin and fluoroquinolones, 3) are SCC*mec* type IV, V, or VI, and 4) contain the *pvl* gene (10, 52).

The traditional definitions of HA- and CA-MRSA are now being challenged; CA-MRSA types have increasingly been reported as causing disease in healthcare facilities in the U.S. and other countries (57-60). In fact, CA-MRSA infections in U.S. outpatients increased seven-fold from 1999-2006. CA-MRSA may be adding to the overall number of infections in hospital populations rather than replacing HA-MRSA (14, 61); alternatively, CA-MRSA may eventually become the dominant MRSA strain in hospitals (62).

LA-MRSA Contact with cows, horses, veal calves, and poultry has been associated with human LA-MRSA colonization (63-69). Contact with pigs has been most heavily studied, and swine contact now appears to be a significant risk factor for colonization with LA-MRSA. Nasal carriage of LA-MRSA has been linked to occupational swine exposure in the U.S. (70), Canada (33), the Netherlands (66, 71, 72), Denmark (73), Belgium (74), France (75), Germany (76), and China (77). Veterinarians are also a group commonly associated with LA-MRSA colonization (64, 78-80). However, some studies have failed to document LA-MRSA carriage in veterinarians expected to be at high risk due to large animal contact (81-83). Little is known about the relationship between colonization and risk of clinical infection with LA-MRSA. Denis (2009) recently

documented concurrent colonization and skin infection in one Belgian swine worker (74). The LA-MRSA form most associated with swine contact in North America is multi locus sequence type 398 (ST398).

As with other MRSA types, direct contact with colonized individuals (in this case, animals) is considered to be an important risk factor for infection with LA-MRSA based on studies of swine contact (66). The potential role of environment in LA-MRSA spread is unclear, although MRSA strains have been recovered from livestock environments (floor surfaces and feed at poultry farms) (84). Generally, information is lacking on the transmission routes of LA-MRSA and the role of environment, and more research is needed so that appropriate control measures can be implemented (85).

Recently, MRSA strains consistent with the predominant LA-MRSA type have caused outbreaks in healthcare settings. In both cases described below, animals were either present at the facility or health care workers had potential animal contact at home, although tested animals were negative for MRSA. Specifically, a hospital outbreak of ST398 in the Netherlands caused infection in five patients (86), while in a long term care facility, one resident had a clinical infection and several others became colonized with ST398 (87). Interestingly, animals living in long term care facilities can be colonized with human forms of MRSA (88, 89) and potentially serve as a new source of infection for patients.

In 14 countries, ST398 has been detected by healthcare-related surveillance (90). Very low levels of human disease caused by ST398 have been reported in Canada (91). In the U.S., no clinical infections with ST398 have been reported to date in humans.

MRSA in animals

MRSA was discovered in cattle in the 1970's as a cause of mastitis (92) and continues to be occasionally identified as such (93). Since then, MRSA has been isolated from many animal species, including companion animals (dogs and cats) (94, 95). LA-MRSA have been increasingly reported in swine, and swine associated LA-MRSA are the focus of this review.

ST398, the most common LA-MRSA strain associated with swine contact, was identified in France and the Netherlands in the early 2000's (71, 75, 96). Later studies documented colonized pigs in at least six other countries throughout Europe (90). Swine are also known to be colonized with ST398 in Canada and the U.S. (33, 70). Multiple studies in Asia have shown that ST398 does not appear to be a common strain in pigs, although another type, known as ST9, has been identified (77, 97-100). There are sporadic reports of ST398 causing disease in pigs (101-103), but generally ST398 does not seem to be an important cause of swine disease.

Human MRSA types (HA- and CA-MRSA) have also been occasionally associated with pork production. In Canada, both swine and swine workers were colonized with USA 100, a common hospital-associated strain (33). A community-associated strain, identified as ST8, has been identified in Norwegian swine (104).

MRSA in meat

Both animal and human MRSA have been detected on meat products. In the Netherlands, two studies have identified ST398 on retail meat (beef, veal, lamb, pork, chicken, turkey, fowl and game). The prevalence of positive samples ranged from 2.5%

to 11.9% (105, 106). In Asia, MRSA have similarly been isolated from pork and chicken carcasses (107). Although initial studies of ST398 on U.S. retail meat products were negative (108), a recent Iowa study found a single ST398 isolate on ground pork (prevalence=0.55% of total meat samples). However, MSSA were present in turkey, pork, chicken, and beef at higher levels (109).

Human MRSA has also been detected on chicken meat (110). It has been found in coleslaw linked to a MRSA colonized food handler as well (111). Although *S. aureus* are common food contaminants, generally MRSA (particularly ST398) do not produce enterotoxins which cause food poisoning in humans; Kluytmans (2009) has suggested that MRSA is no more dangerous than MSSA in regard to risk of food poisoning (112). However, occasional reports of enterotoxigenic MRSA do occur (113).

Occupational exposure to MRSA in pork production

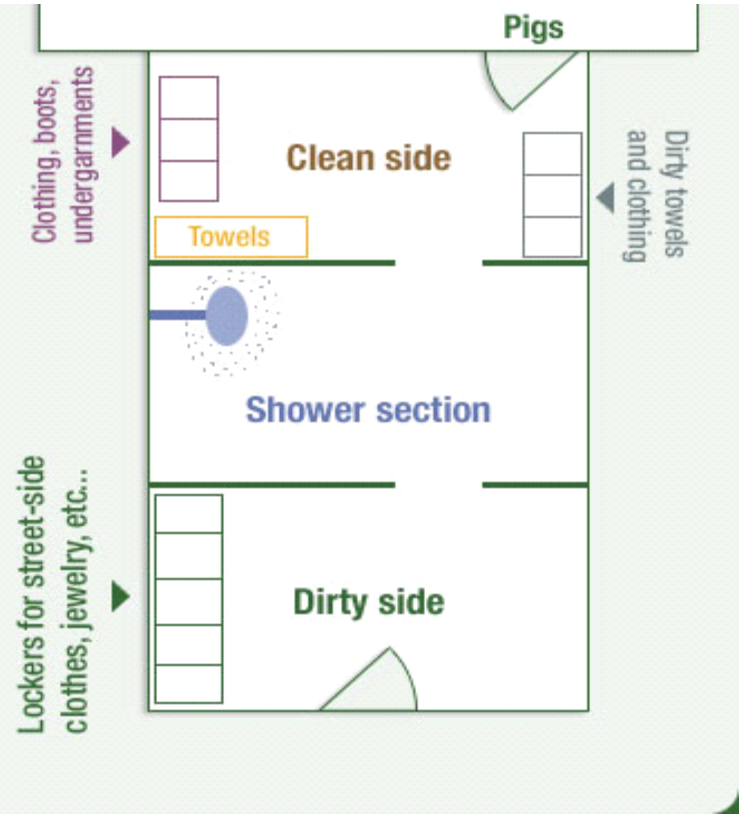
Management of modern pork production systems includes infectious disease control. Biosecurity, or the prevention of pathogens from entering and spreading throughout the production system, is an essential part of good farm management. Although the methods employed are typically aimed at decreasing swine pathogens, biosecurity plans can be adapted to include infectious disease prevention strategies for workers as well as pigs. According to the National Pork Board guidelines, which are not evidence-based, biosecurity plans typically consist of two parts: isolation biosecurity and prevention of indirect pathogen spread (114). Isolation biosecurity includes isolation and health monitoring of new breeding stock to prevent introduction of pathogens into the

swine herd. Many swine diseases can spread in this manner. In fact, MRSA has been shown to spread between pork production facilities via purchase of colonized pigs (115).

Prevention of indirect pathogen spread involves managing incidental carriers of infectious disease. This includes controlling site proximity, contact between swine herds, and contact with the public. It also includes pest and wildlife control programs, feed and transportation procedures, protocol for purchased or delivered semen, and employee behavior [including little or no contact with swine farms or pigs outside of work and required "down time" (meaning no pig contact) before re-entering the farm]. Pathogens such as MRSA can spread indirectly. Rats living on swine farms are known to carry MRSA type ST398 and could potentially contribute to the maintenance and transmission of MRSA on the farm (116).

Biosecurity plans usually require employees and visitors to shower-in and shower-out of the facility (figure 3). Generally, showering protocol includes the following steps: leave shoes outside of shower area; leave all clothing and jewelry on the "dirty" side; enter shower and wash (includes shampooing hair and washing eyeglasses if wearing inside facility); exit shower and dress in "clean" side clothing provided by the facility; if the "dirty" side is re-entered, another shower is required before re-entering the facility (114). A more detailed shower protocol issued by the National Pork Board is shown in Appendix A (shower protocol not evidence-based, and not developed for any particular pathogen). Many facilities also require 24-48 hours free from other swine exposure prior to entry (117, 118).

Figure 3. General shower room layout



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Some components of the biosecurity plan provide conditions in which MRSA transmission may occur. For example, shower facilities are required for biosecurity; however, they provide an opportunity for workers to share personal items such as towels, clothing, and soap, and they provide contact with environmental surfaces that may harbor MRSA. In the home, MRSA has been detected in bathroom sinks, on faucet handles, and in bath tubs (119, 120). Additional conditions of the pork production environment could contribute to the likelihood of MRSA infection in workers. Contact with swine and work

in barns may increase a worker's risk of skin abrasions, a known risk factor for MRSA infection.

Currently, there is no evidence to show that biosecurity practices (shower and laundry procedures, personal hygiene, or environmental exposures) are related to risk of MRSA infection in workers. The research presented in Chapters II, III, and IV seeks to further investigate this question.

Goals and aims of the study

This dissertation addresses the occupational risks associated with environmental MRSA exposure in pork production facilities. Our research questions were as follows:

- Is MRSA present in pork production shower facilities where swine are known to be colonized?
- If it is present, does a simple hygiene intervention, modeled after those used following MRSA outbreaks in athletes/athletic facilities, reduce the prevalence of MRSA in showers?
- Do pork producers report clinical MRSA infections in themselves, their workers, or their pigs, and what are the associated risk factors for infection?

The methodology to address these research questions is briefly described here.

Environmental sampling For the shower prevalence study (chapter II), samples were collected using sterile swabs moistened with sterile phosphate buffered solution. We chose sample sites based on areas that are frequently touched by workers (door handles, locker room handles, shower floors, etc.), since areas in hospitals with frequent touch contact are known to harbor pathogens (121). On flat surfaces, an approximate

10cm x 10cm area was sampled. To improve quality control, all before-and-after samples were collected by the same researcher. Some temporal variability was present in sample collection due to operational circumstances. Samples were collected in the same condition (wet vs. dry) if possible.

Laboratory techniques There are a variety of tests available to identify MRSA. Unfortunately, there is no universal standard for MRSA testing, and the cost effectiveness of most methods is unknown (6). Most simply, staphylococci can be directly detected using the Gram stain. Staphylococci, including MRSA, appear as purple colonies that are typically clustered (8). Using the gram stain alone, however, MRSA cannot be differentiated from other staphylococci. Staphylococci grow on a variety of media; for our studies, we used media selective for gram-positive bacteria (Columbia CNA, Remel, Lenexa, KS, U.S.) or media selective for MRSA (BBL CHROMagar MRSA, Becton, Dickinson and Company, Sparks, MD, U.S.). On the CHROMagar plate, MRSA appeared as distinctly round, mauve-colored colonies. Previously, CHROMagar has been shown to be a highly sensitive and specific media for the detection of MRSA (122).

Isolates suspected to be MRSA were subjected to several tests to confirm their identity. This strategy was used to prevent isolate misclassification. The catalase test, the coagulase test, and the *Staphylococcus aureus* latex agglutination assay (Pastorex Staph-plus, Bio-Rad, France) were used to definitively identify samples as *S. aureus*. Samples were confirmed as MRSA by the testing for the presence of PBP2′ with a MRSA latex agglutination test (Oxoid Ltd., Hants, UK). See Appendix B for detailed methods.

Molecular methods used in these studies were based on similar studies that have been conducted recently at the University of Iowa (70, 109). The gold-standard method for detection of human MRSA forms (123), pulsed field gel electrophoresis, is typically not appropriate for typing LA-MRSA due to the presence of a unique methylase (124). Although a number of good approaches exist, we relied upon *pvl* and *spa* sequencing to identify each isolate (see Appendix B for detailed methods). The *pvl* gene is often associated with CA-MRSA forms and severe disease, although its pathogenic role remains unclear (125). Most LA-MRSA currently lack the *pvl* gene (93). *Spa* typing is a common first line typing tool and utilizes the variable repeat region of staphylococcal protein A (126). The following *spa* types have been associated with LA-MRSA type ST398: t011, t034, t108, t567, t571, t779, t898, t943, t1197, t1250, t1254, t1255, t1451, t1456, t1457, t2346, t2383, t2970, t3015, t3119, t4208, t4872, t337, t899, and t1939 (93). Finally, we performed antimicrobial susceptibility testing using the broth dilution method (127).

Survey For the occupational infection study (chapter IV), data was collected by self-administered questionnaire (see Appendix C). Self-reported exposure assessments are commonly used due to their low cost and ease of use. The reliability of self-reported information in agricultural populations has been previously studied (128, 129). In chapter IV, we asked study participants to report on shower practices, laundry procedures, farm-specific clothing (clothing worn only while working on the farm), personal hygiene, and environmental factors, as well as antibiotic-resistant infections in both their pigs and workers.

Summary

LA-MRSA, which is microbiologically distinct from more common human forms, is known to colonize those with occupational livestock exposure. However, a complete understanding of the reservoirs and thus transmission of LA-MRSA is lacking. Agricultural environmental exposure to MRSA has been suggested by some (84), but initial studies of the environment (specifically communal showers) in pork production systems were negative (130).

Although MRSA associated with livestock has recently been a source of media scrutiny in the U.S., more research is needed to quantify the risks associated with this organism for both occupational and general (public) exposures. Within any discussion of MRSA, care should be taken to distinguish the two human forms of MRSA (HA-MRSA and CA-MRSA) from LA-MRSA. In the U.S., there is no documented evidence that LA-MRSA is causing disease in humans and the threats to public health (via meat consumption, etc) are likely to be minimal. The research presented hereafter seeks to further understand environmental reservoirs of LA-MRSA and to better define the occupational risk of MRSA infection associated with pork production work.

CHAPTER II

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PORK PRODUCTION SHOWER FACILITIES

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal carriage has recently been documented in United States (U.S.) pigs and pork production workers, and has previously been described in Canada and Europe. Of the known livestock-associated MRSA (LA-MRSA) types, multi locus sequence type 398 (ST398) has been most commonly associated with swine contact. Currently, no clinical infections with ST398 have been documented in the U.S., although anecdotal reports occur. Direct contact with colonized animals is a likely mode of LA-MRSA transmission for workers; however, other modes may occur. The role of the environment as a reservoir and in LA-MRSA transmission is poorly understood. We sought to determine if shower facilities for workers in pork production facilities can be a source of MRSA. Such showers are communal and employees often share personal items and clothing. These factors, along with poor personal and environmental hygiene, have been associated with increased risk of MRSA infection in other populations.

We sampled two conventional swine production systems in Iowa and Illinois. A variety of production phases, at different locations, were included in the study. We sampled locations in the shower and surrounding locker room that workers commonly touch, including the floor, locker handles, shower curtains, shower walls, light switches, chairs, and soap or shampoo bottles. In Production System A (PSA), a large, corporate

farm system (dominated by contract farming), 3% (1/30) of shower samples were positive for MRSA; the single positive sample was identified as *spa* type t034. In Production System B (PSB), a smaller, family-owned corporation, 26% (18/70) of shower samples were positive for MRSA. *Spa* types identified included t034, t189, t753, and t1746. Overall, we documented MRSA in 19% of samples (19/100). In both systems we also documented MRSA colonized pigs. We found 14% (7/50) of pigs were colonized in PSA, compared to 46% (41/90) in PSB. Two swine nasal swab samples from PSA were selected for *spa* analysis and were identified as t1746. All swine samples from PSB had been identified as ST398 in a previous study (70) . There were significantly more colonized pigs and showers in PSB compared to PSA (p=0.0001 and 0.01 respectively). We suspect that workers may transfer MRSA to the shower room on their clothing or footwear, or that airborne transmission of MRSA may occur. Further studies are needed to determine whether environmental sources of LA-MSRA are associated with increased risk of infection in pork production workers.

Introduction

Staphylococcus aureus is a common pathogen that is commensal on the mucous membranes of both humans and animals (16). In fact, about 30% of the United States (U.S.) population is colonized with this organism (18). Although many strains are non-pathogens, host factors such as immunosuppression may increase the risk of clinical infection. Both antibiotic-susceptible and antibiotic-resistant forms of *S. aureus* occur.

Methicillin-resistant *Staphylococcus aureus* (MRSA) was first described in the 1960s (10) and has since become a major cause of nosocomial infection (known as

hospital-associated, or HA-MRSA) (53). Since the 1990s, MRSA forms that are not associated with hospital exposure, known as community-associated (CA-MRSA), have emerged (27). About 1.5% of the general U.S. population is thought to be colonized with MRSA (15), though certain sub-populations have a higher prevalence of colonization (52, 131).

Beginning in 2004, forms of MRSA associated with livestock contact, known as LA-MRSA, have been described. Contact with many animal species has been associated with LA-MRSA, including beef, veal calves, horses, pigs, and poultry (33, 63, 64, 67-71, 132, 133). LA-MRSA types are genetically distinct from human forms. The most completely described LA-MRSA type is found in pigs, known as multi locus sequence type 398 (ST398). MRSA can also be identified by small molecular differences in the protein A region (93) detected by *spa* typing. The *spa* type most commonly associated with ST398 is t034, although up to 24 other associated *spa* types have been identified (85).

ST398 nasal carriage has been identified in people with occupational exposure to swine in Europe and North America (33, 70, 74, 133). However, the relationship between colonization and infection is not well defined in this population; while colonization appears to be relatively common, clinical infections are rarely reported. Recently, Denis (2009) documented concurrent nasal colonization and skin infection in one Belgian swine worker (74). At least 14 countries in Europe and Asia have identified ST398, or their associated *spa* types, from hospital or healthcare related surveillance (90). In the U.S., no clinical ST398 infections have been identified in the general population or in swine workers.

Direct contact with colonized or infected animals is suspected to be the major route of transmission to humans for ST398 (66). The role of agricultural environments as reservoirs in LA-MRSA transmission has not been examined closely, although preliminary evidence shows that floor surfaces and feed from poultry facilities can be a source for MRSA (84). More research is needed regarding environmental sources of LA-MRSA so that appropriate control measures can be implemented (93).

We hypothesized that shower facilities, which are an important component of modern pork production systems, can harbor MRSA. In most cases, workers and visitors are required to utilize communal showers prior to entering the facility and before exiting (130). In addition to the common environment, these showers expose workers to other shared items (including soap, towels, clothing, etc). In studies of athletes, a group at increased risk for MRSA infection, these factors have been associated disease, along with poor hygiene, failure to properly clean the environment, and inappropriate wound treatment/care. These same conditions and behaviors may be found in pork production facilities and their workers. Previous research has shown that pork production shower facilities do not harbor MRSA (130), however, the presence of MRSA on these farms or in pigs was not documented. We sought to determine if MRSA can be cultured from showers within production systems known to harbor MRSA positive pigs.

Methods

Sites Two conventional swine production systems were selected for this study based on a convenience sample located in the states ranked 1st (Iowa) and 4th (Illinois) in hog production (134, 135). Production system A (PSA) consisted of multiple sites and

production phases throughout Iowa and Illinois. We sampled two wean-to-finish sites with 6,500 pigs each (Figure 4). Production system B (PSB) also consisted of multiple sites and production phases throughout Iowa and Illinois. We sampled two nursery sites (approximately 15,000 pigs at two sites), one finisher site (8,000 pigs), and one sow site (5,200 sows) (Figure 5). At each site up to three showers were sampled.

Figure 4. Sampling scheme for Production System A (PSA)

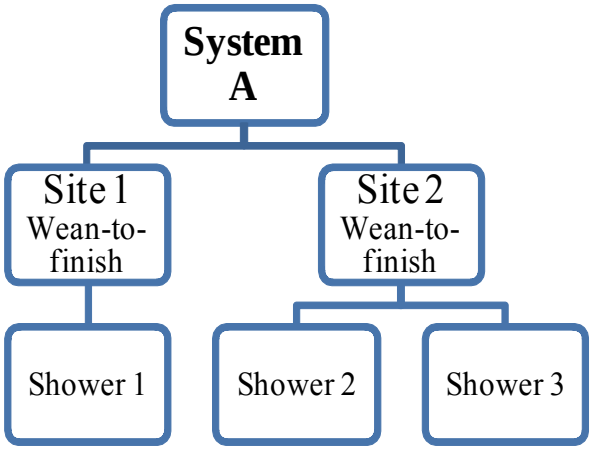
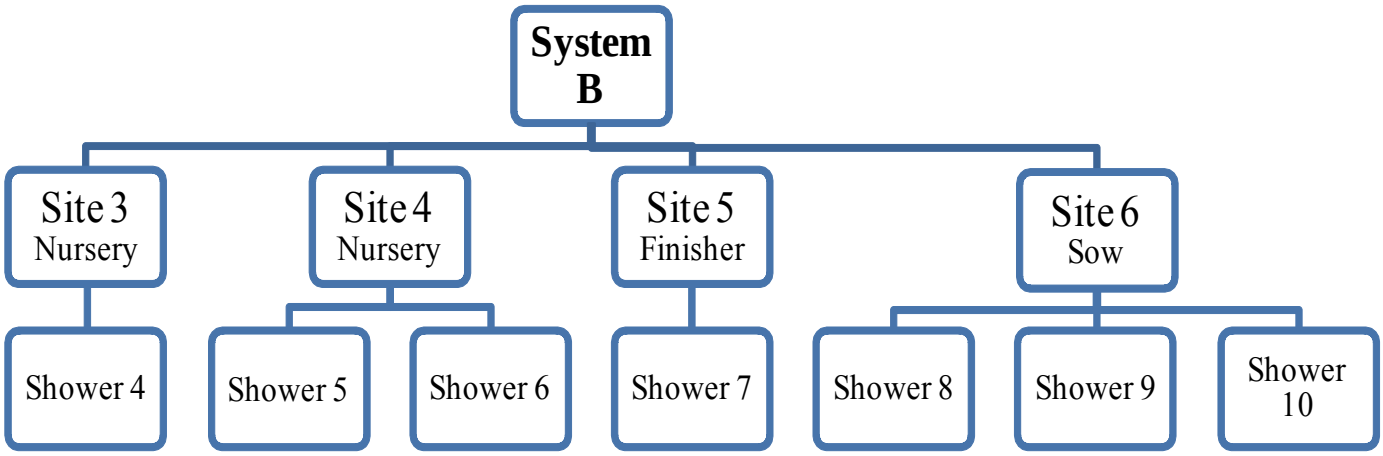


Figure 5. Sampling scheme for Production System B (PSB)



Swine nasal samples In PSA, prior to shower sampling, pigs at both wean-to-finish sites were sampled (n=50). In PSB, swine nasal swabs had been previously collected for another study (n=250) (70). In both systems, one naris was sampled with sterile swabs (BD BBL Culture Swabs with Stuart Liquid Media, Becton, Dickinson and Company, Sparks, MD, U.S.) by a local veterinarian. Swabs were transported back to the lab on ice within eight hours of collection (see bacterial isolation protocol below).

Shower samples In each shower, we collected ten samples using sterile swabs (BD BBL Culture Swabs with Stuart Liquid Media, Becton, Dickinson and Company, Sparks, MD, U.S.) moistened with sterile phosphate buffered saline. We focused on areas in the showers and changing room that workers commonly come into contact with, because so-called "hand-touch" sites have been shown to be frequently contaminated with pathogens in hospitals (121). These included: shower floors and walls, shower curtains, locker handles, light switches, locker shelves, chairs in the changing area, and shampoo or soap bottles. On flat surfaces, an approximate 10cm x 10cm area was sampled. We attempted to sample similar locations in each facility, although some differences in facility design and layout occurred. Swabs were transported back to the lab on ice within eight hours of collection.

Bacterial isolation As previously described (33, 70), swabs were inoculated into 2ml enrichment broth (containing 10g tryptone/L, 75g NaCl/L, 10g mannitol/L and 2.5g yeast extract/L) immediately upon returning to the lab. Samples were then aerobically incubated for approximately 24 hours at 35°C. Next, a loopful of broth was streaked onto gram-positive selective (Columbia CNA, Remel, Lenexa, KS, U.S.) or MRSA selective plates (BBL CHROMagar MRSA, Becton, Dickinson and Company, Sparks, MD, U.S.).

Samples were also streaked onto general media (blood agar plates). After 24 – 48 hours of aerobic incubation at 35°C, plates were examined for mauve-colored colonies likely to be MRSA. Colonies suspected to be MRSA were subjected to three confirmatory tests; the catalase test, the coagulase test, and the *Staphylococcus aureus* latex agglutination test (Pastorex Staph-plus, Bio-Rad, France). Samples were confirmed as MRSA by testing for the presence of penicillin binding protein 2' (PBP2′) with the MRSA latex agglutination test (Oxoid Ltd., Hants, UK). A subset of MRSA positive samples were re-cultured on CHROMagar for quality assurance. These samples were also re-tested with the MRSA latex agglutination test. All re-tested samples were confirmed as MRSA. See Appendix B for detailed methods.

Molecular testing Genomic DNA was extracted using the Wizard Genomic DNA purification kit (Promega, Madison, WI). All shower samples and two swine nasal samples from PSA were evaluated by molecular typing, which included *pvl* and *spa* sequencing. See Appendix B for detailed methods.

Antimicrobial susceptibility testing Selected isolates were analyzed for antimicrobial susceptibility using the broth dilution method according to the Clinical Laboratory Standards Institute guidelines (127). Isolates were tested for susceptibility to oxacillin, tetracycline, erythromycin, clindamycin, trimethoprim-sulfamethoxazole, levofloxacin, linezolid, daptomycin, vancomycin, and quinupristin/dalfopristin.

Data analysis Differences between PSA and PSB were investigated using the Fisher's exact test. A significance level of 0.05 was used in the analyses. Statistical analyses were performed using SAS software version 9.2 (SAS Institute, Inc., Cary, NC).

Spa types were identified using the Ridom SpaServer, an online database that houses known *spa* types and repeat sequences (136).

Results

Swine nasal samples In PSA, nasal swabs were collected from 50 swine (25 samples at two different sites; swine were aged 10 and 20 weeks respectively at the time of sampling). No MRSA was detected in samples from the first site, and 7/25 (28%) of samples were positive for MRSA from the second site. Overall, the prevalence for PSA was 7/50 (14%). In PSB, nasal swabs were collected from 209 swine as previously reported (70) in seven different age groups (range 9 weeks to adult). Overall prevalence was 147/209 (70%) (70). For this study, we used nasal swab data previously collected from nursery, finishing, and sow farm sites. At the nursery, 30 samples were MRSA positive (100%), at the finishing site 15 samples were MRSA positive (50%), and at the sow site 11 samples were MRSA positive (36%).

From PSA, two swine nasal samples were chosen for molecular testing. Both were *pvl* negative and *spa* type t1746. From PSB, swine nasal swab samples were previously confirmed as ST398 by multi locus sequence typing (70).

Shower samples MRSA prevalence estimates by shower are shown in table 2.

Table 2. Prevalence of MRSA in swine and showers

System		*Swine*		*Showers*		
		N	*%*		*N*	*%*
PSA						
	Site 1	0	0	Shower 1	0	0
	Site 2	7	28	Shower 2	0	0
				Shower 3	1	10
	Total	7	14		1	3
PSB						
	Site 3	15	100	Shower 4	2	20
	Site 4	15	100	Shower 5	2	20
				Shower 6	1	10
	Site 5	15	50	Shower 7	0	0
	Site 6	11	36	Shower 8	4	40
				Shower 9	3	30
				Shower 10	6	60
	Total	41	46		18	26

In PSA, we found only one positive sample from three showers, for an overall prevalence of 1/30 (3%). This sample was taken from clothing hooks on the "dirty side" of the shower changing area, where employees enter the shower area from the front of the building. The isolate was *pvl* negative, and identified as *spa* type t034.

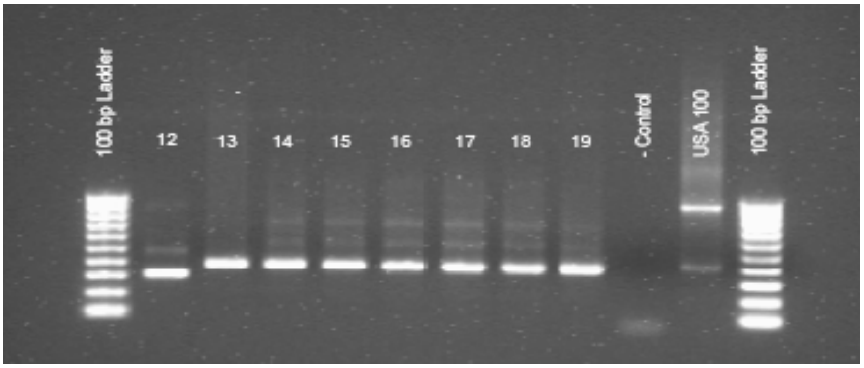
In PSB, we found 18 positive samples from seven showers, for an overall prevalence of 18/70 (26%). MRSA positive samples were collected from the following sites: shower floor (n=5), shower drain (n=3), clean side floor (n=2), locker handle (n=2), and one each from a shower curtain, shower wall, dirty side floor, light switch, chair, and soap bottle. All isolates were *pvl* negative and *spa* types t034, t1746, t189, t753 were identified. A summary of all non-t034 *spa* types identified is shown in table 3. A sample 1.5% agarose gel for *spa* testing is shown in figure 6.

Table 3. Non-t034 *spa* types* identified from swine and showers

Isolate	*Spa type*	*Repeat sequences*	*Sample source*
1	t1746	07-120-12-23-02-12-23	Swine nasal swab, PSA
2	t1746	07-120-12-23-02-12-23	Swine nasal swab, PSA
3	t1746	07-120-12-23-02-12-23	Locker room chair, sow farm, PSB
4	t189	07-23-12-21-17-34	Shower floor, sow farm, PSB
5	t753	08-16-52-25-02-25-34-24-25	Shower drain, sow farm, PSB

*All other samples identified as t034, repeat sequences 08-16-02-25-02-25-34-24-25

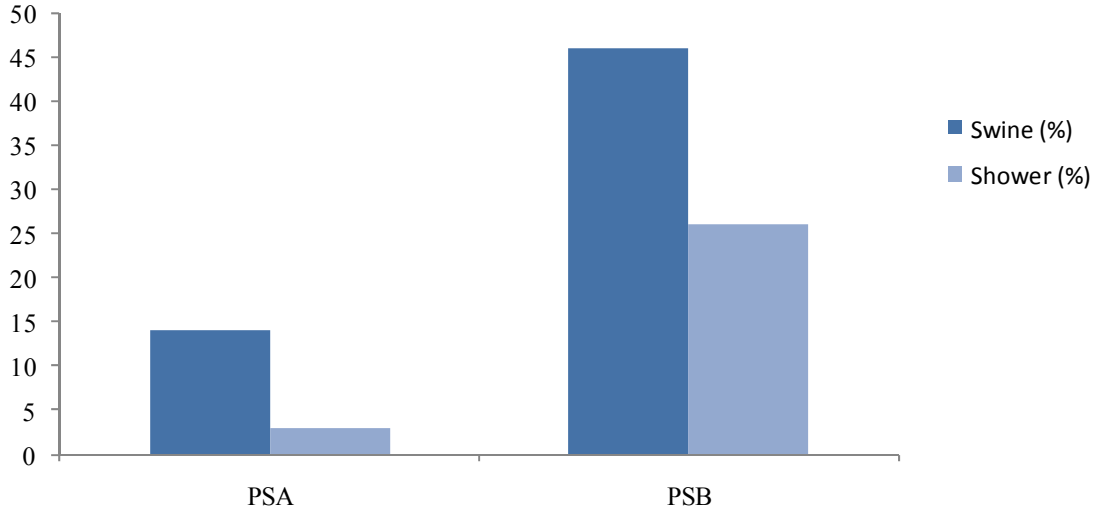
Figure 6. Sample gel from *spa* testing



Lane 1: molecular weight ladder, Lanes 2-9: shower samples
Lane 10: negative control, Lane 11: positive control
Lane 12: molecular weight ladder

There were also significantly more MRSA positive swine and shower samples collected from PSB compared to PSA (p=0.01 and 0.0001 respectively) (figure 7).

Figure 7. Prevalence of MRSA in swine nasal swabs and showers by production system



Antimicrobial susceptibility testing Nine samples (two swine and seven shower) were analyzed for antimicrobial susceptibility using the broth dilution method according to the Clinical Laboratory Standards Institute guidelines (127). Chosen samples included four t034 isolates, along with all t1746, t189, and t753 isolates. Results are shown in table 4. Six of nine (67%) isolates were resistant to oxacillin, 8 (89%) were resistant to tetracycline, and 2 (22%) were resistant to clindamycin. One (11%) isolate was intermediate for erythromycin. No inducible clindamycin resistance was observed.

Table 4. Summary of antimicrobial-resistance profiles for selected MRSA positive isolates

	PSA			*PSB*					
Antimicrobial	*1*	*2**	*3**	*1**	*2**	*3**	*4*	*5*	*6*
Oxacillin	**R**	S	S	**R**	S	**R**	**R**	**R**	**R**
Tetracycline	**R**	**R**	**R**	**R**	S	**R**	**R**	**R**	**R**
Erythromycin	S	S	S	S	S	S	S	**I**	S
Clindamycin	**R**	S	S	S	S	S	S	**R**	S
Trimethoprim-sulfamethoxazole	S	S	S	S	S	S	S	S	S
Levofloxacin	S	S	S	S	S	S	S	S	S
Linezolid	S	S	S	S	S	S	S	S	S
Daptomycin	S	S	S	S	S	S	S	S	S
Quinupristin/dalfopristin	S	S	S	S	S	S	S	S	S
Vancomycin	S	S	S	S	S	S	S	S	S

*Denotes non-t034 *spa* types

R = Resistant, I= Intermediate, S = Susceptible

Discussion

Our results show that viable MRSA organisms are present in pork production shower facilities. We tested shower facilities where MRSA positive pigs were found. Our methods, particularly the utilization of *spa* sequencing, led to the identification of several MRSA types that have not been previously reported in the U.S.

We identified *spa* type t1746 in swine nasal swab samples from PSA, and from a locker room chair in PSB. Previously, t1746 has been identified only once (2006, the Netherlands) (136). *Spa* type t189 was isolated from a shower floor at our sow site. This isolate represents 0.32% of all samples in the Ridom SpaServer database (136) and has been associated with ST188, a community-associated MRSA strain previously reported in Malaysia (137). We also identified *spa* type t753 from a shower drain at our sow site. This *spa* type has been found in the Netherlands and represents only 0.01% of the

samples in the Ridom SpaServer database (136). The most frequently identified *spa* type was t034, which has been associated with multi locus sequence type ST398, the most common form of LA-MRSA reported in the U.S. MLST was not performed for our samples and no associated multi locus sequence types have been identified for *spa* types t1746 or t753 in the literature.

We found multiple *spa* types in the same shower. Previous studies of dust in pork production (breeding and fattening facilities) have also detected multiple *spa* types in one environment, although pooled samples were analyzed (138). In the U.S., few studies of MRSA in livestock environments exist. However, previously only one *spa* type, t034, has been identified in U.S. swine workers and pigs (70). A recent report from Spain showed that humans can be colonized by multiple *spa* types, and multiple *spa* types (both livestock and human origin, or ST398 and ST1) can be recovered from human skin infections (139). As more *spa* types are identified, the ecology of MRSA in livestock can be better described.

We found higher levels of MRSA in both the pigs and showers in PSB compared to PSA. These two production systems are different in several ways. They not only have different animal sources, but are owned by two different companies, one of which is a smaller, family-owned corporation (PSB) while the other is very large corporate farm system dominated by contract farming (PSA). There may be different policies regarding environmental hygiene and infectious diseases in place at each production system that could influence the prevalence of MRSA in showers, although both systems require employees to shower-in and shower-out, and neither dictates the frequency of shower

cleaning. In both systems, there is little company control over the health and safety policies adapted by contract growers.

At site five, a finishing location in PSB, we observed that although 50% of swine sampled were colonized with ST398, no shower samples were positive. This may have been because the shower was separated from the swine barns, located in a separate office building several hundred feet away. If workers carried MRSA on their clothing or boots, it could be eliminated by the time they reached the showers. Separate shower/barn arrangements, such as those described here, are not uncommon in finishing operations. Workers in this facility were required to shower-in and shower-out, and worked only in the finishing facility. The lack of MRSA in showers separated from swine barns could also be related to a lack of airborne spread. However, the role of airborne MRSA transmission is poorly understood in pork production facilities.

We observed a high level (89%) of tetracycline and oxacillin (67%) resistance in our samples, with little resistance to other tested antimicrobials. Both production systems tested utilize antimicrobials for prophylactic and therapeutic reasons. We did not test production systems that abstain from antimicrobial use, therefore, we cannot speculate on the relationship between antimicrobial use and the finding of MRSA in showers.

Limitations This study had a small sample size. Overall, a total of six sites and ten showers were sampled. We collected ten samples per shower, for a total of 100 samples. Our samples were collected from two production systems in Iowa and Illinois, which limited the geographical scope of the study. In addition, our production systems may be different from other U.S. swine production systems which may limit

generalizability. However, the sites sampled for this study were representative of typical Midwest, modern swine production facilities.

Finally, our laboratory methods present several limitations. We did not perform MLST testing on our isolates. Although *spa* sequencing is an effective and appropriate tool for identification of MRSA, we were not able to directly compare our isolates to those from other studies that utilized MLST. *Spa* sequencing is more economically feasible for pilot studies such as this compared to MLST, and is also less labor intensive. These characteristics influenced our choice of molecular identification techniques.

Classification of *spa* types was done by comparing our results with the Ridom SpaServer database, which contains information on over 6000 *spa* types and over 115,000 strains (136). However, all MRSA researchers may not utilize the database. We also performed a literature search for each *spa* type identified to ensure that our recovered *spa* types are in fact novel in the U.S.

Conclusions

Showers can be a source of MRSA for workers in pork production facilities. We studied farms in which colonized pigs were documented. In the only previous study of pork production shower facilities tested for MRSA, the history of MRSA colonization in pigs was unknown and the study failed to document MRSA in showers (130). We suspect that workers may transfer MRSA to the shower room on their clothing or footwear, or that airborne spread may occur. Further studies are needed to determine whether environmental MRSA reservoirs are associated with increased risk of infection

in pork production workers and to determine the most effective methods of MRSA control in the shower environment.

CHAPTER III

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN PORK PRODUCTION SHOWER FACILITIES: ADAPTING INTERVENTIONS FROM ATHLETIC FACILITIES

Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) nasal colonization has been documented in swine and swine workers as well as their household contacts. MRSA has also been found in the shower facilities of conventional swine production farms (Chapter II). Known risk factors for MRSA skin and soft tissue infection (SSTI) have been identified primarily from outbreaks in athletes; they include physical contact, presence of open wounds, sharing of contaminated personal items or environments, and lack of good personal hygiene. Pork production workers are potentially at risk for MRSA infection because they are occupationally exposed to many of these same risk factors. Hygiene-based interventions have been implemented in athletic facilities to prevent MRSA outbreaks in athletes. We hypothesized that similar preventive measures could be used in swine production facilities to reduce environmental MRSA reservoirs and the risk of MRSA transmission among workers.

A review of the literature was conducted to identify measures that have previously been used to combat MRSA outbreaks in athletes and athletic facilities. These preventive measures were then evaluated for adaptability to pork production facilities as a component of a standard biosecurity plan. A best practices plan and protocol was developed to eliminate MRSA reservoirs in the shower and locker rooms of pork

production facilities. The protocol was then implemented at two conventional swine production farms. Although the overall trend was reduction in MRSA prevalence post-intervention, small sample size and lack of intervention oversight prohibit us from recommending our intervention measures for all pork producers at this time. However, the MRSA prevention guidelines developed here can be easily implemented by pork producers with little to no cost and provide a common sense approach to hygiene and infection control in the modern pork production environment.

Introduction

MRSA background MRSA is a multidrug resistant bacterium first discovered in the mid-1960s as a hospital-associated infection (HA-MRSA). Different strains of MRSA were then recognized in community environments (CA-MRSA) in the mid-1990s. MRSA associated with various livestock species (LA- MRSA) has been described since 2004. Healthy people may carry any of these strains on their mucous membranes or certain areas of their skin without causing disease (5). However, MRSA may cause a variety of infectious conditions in some people, causing this organism to be recognized as an important public health issue.

MRSA in livestock LA-MRSA is increasingly becoming a concern as a zoonotic infection, particularly for people with occupational or environmental exposure to pigs, cattle, veal calves, poultry and horses (63-66, 71, 133). Biosecurity procedures have been developed by the pork production industry to prevent the spread of communicable diseases between and within pork production facilities, and these practices have been commonplace since the 1980s. In recent times, the pork industry has also become aware

of procedures needed to prevent transmission of zoonotic infectious between workers and pigs.

Nasal colonization of swine and swine farmers with LA-MRSA was first documented in the Netherlands in 2004 (71). Canadian and U.S. swine and swine workers have since been shown to harbor MRSA (33, 70). Pork products are known to occasionally be contaminated with MRSA in Europe, Asia, and the U.S. (105, 105, 107, 108), although LA-MRSA (known as ST398) is infrequently identified on meat products (109).

Transmission of MRSA is generally thought to occur via direct contact. However, contact with inanimate environmental reservoirs may also be a mode of transmission (93). Because showers in athletic facilities have been recognized as environmental sources of CA-MRSA, we hypothesize that showers in pork production facilities may be an environmental source of LA-MRSA. In 2005, a study of ten Indiana pork production facility showers was negative for MRSA (130). However, our 2009 study sampling showers in Iowa and Illinois (at farms known to harbor MRSA positive pigs) found that 3% and 26% of shower samples were MRSA positive (see Chapter II). This finding suggests that there is potential for transmission of LA-MRSA to workers within pork production facilities from inanimate reservoirs (i.e. shower stalls), as well as direct contact with LA-MRSA colonized swine.

MRSA in athletics Reports of MRSA in athletes may describe conditions for MRSA transmission that are shared by the pork industry. The first documented MRSA outbreak in an athletic team was in 1993; 6 high school wrestlers in Vermont were diagnosed with MRSA abscesses (140). Since then, many additional MRSA outbreaks

have been recorded. Skin and soft tissue infections (SSTI) are the most commonly reported type of MRSA infection in athletes (131, 141). Among individuals presenting to university clinics with SSTI in 2008, 76% of infections were diagnosed as *S. aureus* and of those, 59% were diagnosed as MRSA (142).

Contact sports are known to predispose an athlete to MRSA infection (142). In the U.S., more MRSA case reports have been documented in football than any other sport. While high school players have been affected, outbreaks among collegiate players have occurred most commonly (143-150). MRSA has also been documented in professional football players (151). Members of an opposing team experienced MRSA infection after playing a team with recurrent MRSA infections, and genetic identification showed the strains to be identical (151).

Other contact and team sports, including wrestling, rugby, basketball, soccer, and volleyball, have been linked to MRSA outbreaks (152). However, athletes in sports that require no physical contact between participants have also been affected. Specifically, MRSA has been documented in non-contact sports such as fencing, canoeing, and weight lifting (152, 153). In these instances, sharing contaminated fomites or a common, contaminated environment may be risk factors for infection. MRSA outbreak characteristics differ slightly in contact and non-contact sports, as shown in table 5. Each outbreak reflects a single instance for a single team/group. The differences in median age may be reflective of the different populations likely to participate in the indicated sport.

Table 5. Characteristics of a MRSA outbreak in contact[a] vs. non-contact sports [b]

	College Football outbreak	*Fencing outbreak*
Median age	20 years	31 years
Players affected	11/107 (10.3%)	5/70 (7.1%)
Players hospitalized	4/11 (36.4%)	3/5 (60%)
Clinical signs	Skin abscess-elbow	Skin abscesses-legs, abdomen
Risk factors for infection	Locker near teammate with MRSA soft tissue or skin infection, sharing towels, living on campus	Sharing wire sensors under clothes that were not cleaned or disinfected
Controls implemented	Hygiene education, increased cleaning of facilities and gear, daily antibacterial showers, restriction from play, decolonization of nasal carriers, evaluation of laundry procedures	Hygiene education, cleaning of shared equipment, routine cleaning of sensor wire, treating wounds properly, showering after practice, covering cuts/abrasions, and evaluating laundry procedures

[a] Nguyen DM, Mascola L, Brancoft E. Recurring methicillin-resistant S*taphylococcus aureus* infections in a football team. Emerg Infect Dis. 2005 Apr;11(4):526-32.

[b] CDC. Methicillin-resistant S*taphylococcus aureus* infections among competitive sports participants--Colorado, Indiana, Pennsylvania, and Los Angeles county, 2000 - 2003. MMWR Morb Mortal Wkly Rep. 2003 Aug 22;52(33):793-5.

The true role of environment in MRSA infection remains poorly understood (141). However, two recent studies have shown that surfaces in high school athletic settings (locker rooms, training facilities, and wrestling mats, etc.) are commonly contaminated with MRSA (154, 155). In the hospital setting, proper environmental cleaning is thought to be a simple, cost-effective method for controlling hospital-related infections including MRSA (121). The effectiveness of a similar approach in the community is unknown.

Recent data indicate that 1.5% of the general population in the U.S. may be colonized with MRSA (15). In comparison, up to 5.4% of sports team members or day care contacts may be colonized (156). Within an individual sports team, up to 27% of athletes have been identified as carriers (140). However, the true prevalence of MRSA colonization in humans remains difficult to determine since at least some individuals may be transient carriers (157).

In general, the known association between colonization and infection with CA-MRSA is weak (158). Diagnosed MRSA infections in athletes and epidemiologic case studies remain uncommon, leading to a lack of knowledge regarding possible risk factors and the effectiveness of interventions in athletic facilities (143). Despite this, increased MRSA awareness has led many athletic organizations, such as the National Athletic Trainers' Association, to develop guidelines for prevention of MRSA transmission (159). These guidelines are often based on public health sources regarding MRSA transmission and prevention, including the Centers for Disease Control and Prevention (CDC) (153).

Recently, surveillance efforts for MRSA infections in athletes have increased. In Nebraska, 87% of state high schools have reported MRSA infection in at least one athlete, and despite prevention measures, the incidence of infection in football players and wrestlers continues to increase (160). In Texas, 32% of licensed athletic trainers in high schools have reported MRSA in their athletic department (161). Because of limited active surveillance for MRSA infection in athletes, it is difficult to determine the degree to which preventive measures decrease the incidence of disease. It is even less clear how preventive measures can affect costs associated with MRSA infection. In a study of costs associated with a hypothetical MRSA infection in a high school wrestler (starting as a

skin infection and progressing to septic shock), preventive hygiene measures for the school were estimated to cost $41, while cumulative costs for the required medical care were estimated to be over $208,000 (162).

The focus of this article is to review methods that have been used to prevent MRSA infection among athletes, and to consider the application of these methods in pork production facilities to prevent MRSA transmission among workers. We then implemented the MRSA prevention guidelines developed by literature review in a pilot intervention study to determine their efficacy in pork production operations.

Methods

We searched relevant health sciences databases at the University of Iowa (including PubMed, BioMed Central, ERIC, SPORTDiscus, and Web of Science) for articles with a combination of the following keywords: "Methicillin-resistant *Staphylococcus aureus,*" "sports," and "athletics." The search was restricted to articles written in English. One hundred and eighty three papers were initially identified. Papers that were not peer reviewed or not pertaining to MRSA primarily in sports were excluded, as were papers documenting sports outbreaks outside the U.S., since community-associated MRSA types circulating in Europe differ from those in the U.S. References for the identified papers were also checked for other relevant sources. The results yielded 39 unique papers which we examined for methods that were implemented to stop MRSA outbreaks on an athletic team, or methods that were suggested for MRSA prevention in a review (51, 131, 140-153, 155, 157, 163-184). Only three of the identified interventions were evaluated for their effectiveness. In most cases when the

MRSA outbreak ended, it is not known which, if any, infection control practices contributed to a decrease in MRSA infections.

Results

We recorded the frequency of each recommendation, and grouped them into the categories shown in table 6.

Table 6. Literature suggested methods to prevent MRSA infections among athletes

Frequency		*Methods of MRSA Prevention in Athletes and Athletic Facilities*
N	*%*	
35	89.7	Good personal hygiene/hygiene education
31	79.5	Cleaning equipment and environmental surfaces with disinfectant
24	61.5	Covering skin wounds to avoid contact with wound or wound drainage
21	53.8	No sharing of personal items including towels, clothing, or soap
19	48.7	Appropriate topical treatment of cutaneous lesions
16	41.0	Early detection and diagnosis of cutaneous lesions
15	38.5	Use of antibacterial soaps (includes use in the shower)
15	38.5	Evaluation of laundry procedures including: separating laundry and proper washing and drying
14	35.9	Exclusion from athletic participation until wounds are healed or properly covered
7	17.9	Identification and treatment of nasal carriers
7	17.9	Regular inspection of athletes for cutaneous lesions by coaches/trainers
6	15.4	Use of alcohol based hand sanitizers

Good personal hygiene and hygiene education were the most commonly cited methods for prevention of MRSA infection among athletes. The definition of "hygiene" varied somewhat among our reviewed papers, but generally referred to hand hygiene, with a focus on hand washing. Disinfection of athletic equipment and environmental

surfaces with a U.S. Environmental Protection Agency (EPA) approved disinfectant was the second most cited intervention (see Appendix D for list of appropriate disinfectants). Policies on wounds were also common; emphasis was placed on covering wounds, appropriate topical treatment of wounds, and early detection and proper diagnosis of MRSA SSTI. The emphasis on wound care was not unexpected, since contact with MRSA infected wounds is often associated with new MRSA infection in epidemiological studies of athletes. Interestingly, some prevention strategies were used less often than expected. For example, despite CDC reports that alcohol-based hand sanitizers are more effective and less irritating than soap (185), their use was one of the least frequently made recommendations.

As mentioned above, only three papers documented the effectiveness of a described intervention. Rihn et al (2005) documented nasal colonization rates of high school football players before and after administration of mupirocin nasal ointment (2%) in response to a team MRSA outbreak. After questioning, 36% of players were found to have used the mupirocin as directed. Noncompliance issues identified included: cost, inability or unwillingness to fill the prescription, or discomfort associated with use. Post-intervention, additional cases of MRSA occurred among the football players. The authors concluded that mupirocin administration was ineffective in controlling the MRSA outbreak (143). In other studies mupirocin has been shown to reduce *S. aureus* and MRSA infections among nasal carriers (186, 187), although mupirocin resistance has also been documented, particularly in hospital settings (188).

Garza et al (2009) sought to determine the effectiveness of nasal screening cultures in reducing MRSA SSTI in a professional football team. All players were

screened at the beginning of the season, and any subsequent wounds likely to be MRSA were also tested. Although no players were positive on initial screening, five culture-confirmed MRSA infections occurred during the season. The authors concluded that surveillance nasal screening was not effective in preventing MRSA infection in athletic populations (51). Overall, identification and treatment of nasal carriers was recommended by 7/39 (18%) of the papers in our literature review.

Sanders (2009) developed a non-invasive, non-pharmacological prevention and education program designed to reduce CA-MRSA infections in college football players. A 75% reduction was observed in the players in 2008 and players demonstrated increased knowledge and knowledge retention regarding MRSA (173). This type of comprehensive, educational intervention was unique among the papers in our review.

Discussion

The results from our literature review show that most suggested methods to prevent MRSA infection and transmission in athletes are similar. However, few interventions have been tested. Many of these interventions are derived from infectious disease control policies in hospitals, where MRSA is a significant nosocomial pathogen. This is problematic, because even in hospitals, guidelines for MRSA prevention may not be evidence-based (189). For example, hand hygiene is often identified as the most important measure to address hospital associated infections (190, 191), although educational efforts to increase hand hygiene are generally ineffective (192). More comprehensive hand hygiene interventions have been successful in decreasing hospital associated infections (193) as have those involving direct observation of hand washing

(194). Increased use of alcohol based hand sanitizer has also been associated with reduced MRSA infection rates (195). The effectiveness of environmental interventions are infrequently described, although there are several proposed methods of evaluating hospital cleaning practices in the literature (196, 197).

Biosecurity in swine operations Biosecurity is the prevention of pathogen introduction into a swine production system and between farms in that system. Biosecurity includes plans for isolation of new animals and prevention of indirect pathogen spread (198). Animals may be introduced into swine herds regularly, particularly breeding stock. Swine pathogens can be transmitted from introduced swine to the herd, and MRSA is known to be among them (115). Prevention of indirect pathogen spread (from sources outside of closed swine herds) includes controlling site proximity, access to other swine herds and the public, pest and wildlife control programs, and assurance of quality feed and hygienic transportation procedures. Further preventive measures include protocol for purchased or delivered semen, monitoring of employee behavior [including little or no contact with swine farms or pigs outside of work and required "down time" (meaning no pig contact) before re-entering the farm], and visitor control. Specific to MRSA, rats are known to harbor the pathogen on swine farms and could play a role in MRSA transmission and maintenance on the farm (116).

Biosecurity plans usually require employees and visitors to shower-in and shower-out, and many facilities require 24-48 hours free from swine exposure prior to entry (117, 118). However, some components of the biosecurity plan provide conditions in which MRSA transmission may occur. For example, shower facilities provide an opportunity for workers to share personal items such as towels, clothing, and soap, and

they provide contact with environmental surfaces that may harbor MRSA. Additional conditions of the pork production environment could contribute to the likelihood of MRSA infection in workers. Like athletes in contact sports, contact with swine and work in barns may increase a worker's risk of skin abrasions. Combining components of the standard biosecurity plan with lessons learned from MRSA in athletic facilities, recommendations can be made to protect workers and prevent MRSA transmission in pork production facilities, as shown in table 7. Little information exists on proven interventions for MRSA prevention in athletes, however, we considered prevention measures that were likely to be effective based on scientific principle for use in swine production systems (for example, the use of an EPA MRSA-approved disinfectant for cleaning is likely to reduce the number of MRSA present in a shower).

Table 7. Adapted methods to prevent MRSA infection in pork production facilities

Category	*Methods*
Hygiene	Educate employees about good hand hygiene Encourage use of alcohol based hand sanitizers when hands not visibly dirty Educate employees about general infection control Educate employees about MRSA transmission
Wounds	Report cuts or wounds to supervisor immediately Clean wounds or abrasions immediately and cover with a clean, dry bandage Restrict worker to a single shower stall and disinfect after each use if wound cannot be covered Encourage proper treatment of non-healing wounds by a health care professional
Showers	Advise workers to use warm water and soap Use liquid soap dispensers instead of bar soap Provide separate, clean towels for each employee Discourage sharing of personal items including soap or razors
Clothing/Laundry	Provide separate boots for each worker Provide separate, clean coveralls for each worker Wash soiled coveralls separately from other clothing and towels Wash all coveralls, clothing (including underwear and socks) and towels with hot water and soap after each use Machine dry every article of clothing and towel after washing
Environment	Develop a protocol for cleaning and disinfection of showers Include removal of visible dirt, proper dilution of disinfectant, and contact time Use dilute bleach (1/4c. Bleach: 1 gallon water) or an EPA approved disinfectant Develop a routine schedule for cleaning and disinfection of showers Provide a separate kitchen space for eating

General categories developed for the MRSA prevention guidelines included hygiene, wounds, showers, clothing and laundry, and environment. Education was a key component of the suggested guidelines, especially in the areas of hand hygiene and infection control. Most of the suggested guidelines require development of specific policies by producers in the categories above; however, the implementation of suggested guidelines (i.e. do not share personal items) falls to employees.

Preventing MRSA in pork production facilities In a pilot effort, we recruited two conventional swine production systems in Iowa and Illinois to participate in an intervention study evaluating our MRSA prevention guidelines. In both systems, swine colonization with MRSA had previously been documented. Samples were collected as previously described (see Chapter II); we swabbed ten locations each in five different showers (at two wean to finish sites and one sow farm site), focusing on areas with which workers would commonly be in contact. Although each shower and locker room varied in design, we aimed to sample the shower floor, shower walls, shampoo or soap bottles, door handles, locker handles, and chairs; in the hospital setting, areas with frequent human touch are known to harbor pathogens (121). After the initial samples were collected, pork producers were asked to implement the MRSA prevention guidelines shown in table 7. Producers were provided with information on hand washing, EPA approved disinfectants for MRSA, and general information on MRSA from the Iowa Department of Public Health (see Appendix E). A local veterinarian served as the study coordinator in each location, and was responsible for ensuring that the intervention was implemented properly. Token compensation was also offered to the swine production workers who participated in the study.

Approximately four weeks after the initial samples were collected and the intervention was implemented, investigators returned to repeat the shower sampling. The same investigator took both the before and after intervention samples, and the same locations were used for sampling in each shower. As much as possible, showers were sampled in the same condition each time (wet or dry). However, this was not always possible due to operational circumstances.

Laboratory procedures were identical to our previous studies (see Chapter II). The catalase test, coagulase test, and *S. aureus* latex agglutination test were used to confirm samples as *S. aureus*; next, the MRSA latex agglutination test was used to confirm the samples as MRSA. All samples were tested for the presence of the *pvl* gene and for *spa* type. Selected isolates were also tested for antimicrobial susceptibility as described previously (Chapter II).

Overall, results from the intervention were mixed (table 8). Results for molecular testing (*spa* sequencing) are shown in table 9. All samples were *pvl* negative. Antimicrobial susceptibility profiles for non-t034 isolates are shown in table 10.

Table 8. MRSA positive shower samples pre- and post-intervention

	System A		*System B*		
	Farm 1	Farm 2	Farm 3		
	Shower A	Shower B	Shower C	Shower D	Shower E
	N (%)	N (%)	N (%)	N (%)	N (%)
Pre-intervention	0 (0%)	1 (10%)	1 (10%)	7 (70%)	2 (20%)
Post-intervention	0 (0%)	0 (0%)	3 (30%)[a]	4 (40%)[b]	0 (0%)

[a] Three negative samples became positive post-intervention

[b] One negative sample became positive post-intervention

Table 9. *Spa* types identified pre- and post-intervention

	System A		*System B*		
	Farm 1	Farm 2	Farm 3		
	Shower A	Shower B	Shower C	Shower D	Shower E
Pre-intervention	--	t034	t189	t034	t034
Post-intervention	--	--	t374 t571 t3446	t034	--

Table 10. Antimicrobial-resistance profiles for non-t034 *spa* types

	PSB			
Antimicrobial	*1*	*2*	*3*	*4*
Oxacillin	S	S	S	**R**
Tetracycline	S	**R**	**R**	**R**
Erythromycin	S	S	S	**R**
Clindamycin	S	S	S	**R**
Trimethoprim-sulfamethoxazole	S	**R**	**R**	S
Levofloxacin	S	**R**	**R**	S
Linezolid	S	S	S	S
Daptomycin	S	S	S	S
Quinupristin/dalfopristin	S	S	S	S
Vancomycin	S	S	S	S

R = Resistant, S = Susceptible

Spa type t034, which has been associated with LA-MRSA, was the most commonly isolated *spa* type pre-intervention. We also identified t189, which has been previously associated with human CA-MRSA strains in Malaysia (137). Post-intervention, we observed reduced MRSA prevalence in 3/4 (75%) of showers. In one shower (25%), MRSA prevalence increased post-intervention. Interestingly, we isolated different *spa* types in that shower (Farm 3, Shower C) post-intervention compared to the

pre-intervention samples. None of the non-t034 *spa* types (t189, t374, t571, and t3446) isolated have been previously reported in the U.S. Type t571 has previously been associated with LA-MRSA type ST398 in Europe (138). In addition, we observed an uncommon pattern of antimicrobial resistance to both trimethoprim-sulfamethoxazole and levofloxacin that has been previously described in association with the human CA-MRSA form ST1 in Europe (139).

Given our preliminary results, there is insufficient evidence to show that MRSA levels definitively decreased during the intervention. However, many pork producers remain concerned about MRSA in the workplace. Most of the intervention components are inexpensive and could be implemented by pork producers with minimal effort. This intervention could be considered as a first step towards improved hygiene in pork production shower facilities, which likely harbor bacteria in addition to MRSA. We suggest this study be repeated with a larger sample size and more thorough oversight of the intervention by investigators.

Limitations The ecology and possible modes of transmission of MRSA in athletes and pork production workers may have some important differences. First, a major route of MRSA transmission among athletes is direct contact with contaminated wounds. While such contact is common for athletes during play, it may be less likely that pork production workers should come into direct contact with infected wounds. Second, although MRSA contamination of environmental surfaces has been documented in athletic facilities (as well as hospital and other healthcare facilities), the role of environment in MRSA transmission remains unclear. However, our evidence shows that showers in pork production facilities can be a source of MRSA. This review is also

limited in that some outbreaks of MRSA in sports go undiagnosed, and therefore a limited number of cases were available for review. Finally, few of the interventions used for MRSA outbreaks in athletes were proven to be effective. In most cases where the outbreak was ended or contained, it was unclear whether human intervention succeeded or whether the natural course of disease resulted in outbreak cessation. In general, athlete-specific guidelines were developed using information suggested by public health and sports agencies.

Our intervention pilot effort also had several potential problems. Our sample size was small, and investigators did not directly observe implementation of the intervention. In addition, the intervention instructions could have been worded more clearly in some cases. For example, we ask employers to "advise" employees to use warm water and soap in the shower. Using the statement "require" may have increased the likelihood of follow-through for employees. Also, we may not have identified all possible ways to prevent MRSA transmission with this intervention. For example, we did not ask employees to launder clothing and towels with bleach, although this has been suggested by some (160). In addition, some variation in sampling conditions was present because of operational circumstances. We suggest that this intervention study be repeated on a larger scale, with better intervention oversight and more clear instructions.

Conclusions

MRSA colonization has been documented in swine and humans with swine contact in Europe, Canada, and the U.S. MRSA has also been documented in conventional swine production system shower facilities. It is plausible that MRSA could

be transmitted among workers in pork production facilities, originating either in the community or in the barn. For that reason, pork producers should consider implementing procedures to prevent the transmission of MRSA in their facilities. We developed methods for prevention of MRSA transmission by combining recommendations from athletic teams and currently practiced swine production biosecurity measures. However, our pilot intervention study provided mixed results.

Producers should consider enhancing their existing biosecurity plans to include measures that address MRSA. For example, shower policies could easily be expanded to exclude sharing of towels, soap, and clothing within swine production facilities. Cleaning policies should also be developed to ensure regular disinfection of showers and other shared surfaces. Many of the guidelines suggested here can be implemented by pork producers with little effort and little to no cost, and can improve the conditions of the working environment for employees.

CHAPTER IV

METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS INFECTION IN PORK PRODUCTION WORKERS

Abstract

Studies in Europe and North America (including the United States, U.S.) have shown that pork production workers can be colonized with Methicillin-resistant *Staphylococcus aureus* (MRSA). How colonization is related to risk of infection is unclear. We sought to determine if U.S. pork producers report veterinarian-diagnosed antibiotic-resistant skin infections in pigs, and physician-diagnosed antibiotic-resistant skin infections in workers, and to examine associated risk factors related to biosecurity protocols (showering procedures, laundry procedures, personal hygiene, and farm clothing use).

Pork producers were selected from the National Pork Board's producer database and surveyed by paper and email questionnaires. Of all respondents, most were principal operators and farmed multiple production phases (sow, nursery, and finisher). Email respondents were more likely to operate mid-size or very large farms, and to employ an occupational health and safety professional compared to paper survey respondents.

About half of the producers owned <500 sows or finished <10,000 pigs per year. Although 71% of respondents had shower facilities, only 46% had a shower-in and shower-out policy. Soap was provided to workers by 65% of respondents, with liquid soap being most commonly used. Overall, shower cleaning was performed daily in only 4% of operations. Bleach, which is known to be effective against MRSA, was used in

about 24% of operations. Sharing of personal items, including clothing (36%), footwear (42%), and towels (48%) was frequent. Sixty percent had laundry facilities on site, although only 35% required laundry at specific time intervals. About 36% reported daily laundering. Hand hygiene was not emphasized in most operations, with hand washing policies in only 41% and alcohol based hand sanitizer use reported in 37%.

Three percent of pork producers raised pigs that had been diagnosed with antibiotic-resistant infections by a veterinarian; 4% reported workers diagnosed with antibiotic-resistant infections by a physician. Of worker infections, 83% were diagnosed as MRSA by local physicians. None of the risk factors tested were statistically significant predictors of MRSA infection in workers, or of antibiotic-resistant infections in pigs.

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly being found in the community, though it once was known only as a hospital-acquired infection. Overall, 1.5% of the United States (U.S.) general population is now thought to be colonized with MRSA (15). Livestock-associated MRSA (LA-MRSA) colonization (and infection) has recently emerged as a new concern. LA-MRSA colonization has now been documented in both Europe and North America (33, 70, 71, 133).

Specifically, multi locus sequence type 398 (ST398) has been recognized as a potential pathogen in both pigs and pork production workers. Studies from the Netherlands have shown that pig farmers have a prevalence of MRSA nasal colonization 760 times greater than patients admitted to Dutch hospitals with no pig exposure (71) and

that ST398 accounts for more than 20% of all MRSA infections detected in that country (66). In Canada, both ST398 and a human MRSA strain (USA 100) have been found in pigs and workers (33), while in the U.S., ST398 has been the solely documented strain to date in pigs (70). Although these studies indicate that MRSA colonization with swine-associated ST398 is occurring in both Europe and North America, the actual number of related symptomatic MRSA infections that have occurred is unclear. We conducted a survey to determine if U.S. pork producers report MRSA skin infection or soft tissue infection (SSTI) in their pigs and workers, and to examine potential risk factors for infection, including shower, clothing, laundry, and hygiene-related practices.

Methods

Survey Pork producers were identified from the National Pork Board's producer database and surveyed by paper and email questionnaires. The database contains over 43,000 pork producers that receive the Pork Checkoff's seasonal report and have a current hog count, or are Pork Quality Assurance (PQA®)/Pork Quality Assurance Plus (PQA Plus®) certified (PQA Plus® is a pork industry continuous improvement program designed to enhance food safety and to ensure that U.S. pork producers can measure, track, and improve animal wellbeing) (199). A random sample of 800 pork producers was selected for this study using Microsoft Excel (with a Random Sampler add-in). We based our samples size on the guideline set by Krejcie and Morgan (1970), which estimate the required study size (95% confidence, α=0.05). Given the study population base (n=43,000), 380 survey responses were required. Because we expected a low response rate, a larger number of pork producers were invited to participate. We also

considered the following calculations when determining sample size (based on 95% confidence, 80% power) (table 11).

Table 11. Sample size calculations to detect reported MRSA infection in pork production workers

Variable	*Prevalence to be detected*	*Sample size*
Shower available on premise for workers	40	67
Assume 75% have shower on premises	20	143
	5	352
Soap provided to employees	40	40
Assume 50% provide soap	20	100
	5	664
Showers cleaned with bleach solution	40	44
Assume 25% clean with bleach	20	108
	5	768
Workers have individual, farm-specific clothing	40	43
Assume 30% share clothing	20	103
	5	710

A seven-question survey (developed by infectious disease faculty at the University of Iowa's College of Public Health, and Science and Technology staff at the National Pork Board) was approved by the United States Department of Agriculture (USDA) (E. DeBord, personal communication, 14 November 2008). Institutional Review Board approval was not required since no identifying information was collected (M. Jones, personal communication, 4 June 2008). The survey included questions on job type, farm type (production phase), hog count, showering procedures, farm-specific

clothing (clothing worn only while working on the farm), laundry procedures, personal hygiene, and diagnosed antibiotic-resistant skin infections (including MRSA) in pigs and workers (see Appendix C). All survey responses were reported by pork producers themselves without validation by a physician or veterinarian (known as "self-reporting").

The survey was mailed in November 2009. Twenty-one surveys (2.6%) were returned for failure to deliver. Seventeen (2.1%) respondents indicated that they were no longer involved in hog production. Of the remaining 762 surveys, 85 (11.1%) were completed and returned via mail. In July 2008, an email version of the survey was mailed to all 800 selected producers. Because the survey was conducted anonymously, investigators could not determine which producers had previously filled out the paper survey; however, producers were instructed not to submit the email version if they had previously submitted a paper version. Two email reminders were sent at two-week intervals, with the last being in August 2009. Overall, 688 (86%) producers had an email address listed in the National Pork Board database, however, 152 (22%) were not valid. Of the 536 email surveys sent to valid email addresses, 50 (9.3%) responses were received. Combined, 135 (17.2%) surveys were received from 783 pork producers who indicated they were actively farming hogs. Investigators could not be sure that producers did not fill out multiple surveys because surveys were returned anonymously. The true response rate could range from 10.9% to 17.2% depending on the number of repeat responses that were received via email.

Data analysis Pork producer characteristics for email and paper respondents were compared, and potential risk factors for MRSA infection were investigated using the Fisher's exact test. Multivariate modeling of risk factors was performed by exact logistic

regression. A significance level of 0.05 was used in the analyses. Statistical analyses were performed using SAS software version 9.2 (SAS Institute, Inc., Cary, NC).

Results

Pork producer characteristics The characteristics of pork producers by job type, farm type (production phase) and farm size are listed in table 12.

Table 12. Characteristics of pork producers

Category		*Respondent-types*					
		Email		*Paper*		*Total*	
		N	*%*	*N*	*%*	*N*	*%*
Job type [a]							
	Owner/operator	23	46.0	--	--	23	17.0
	Company officer	0	0.0	--	--	0	0.0
	Production manager	9	18.0	--	--	9	6.7
	Site/farm owner	1	2.0	--	--	1	0.7
	Contract grower	2	4.0	--	--	2	1.5
	Production worker	0	0.0	--	--	0	0.0
	Veterinarian	1	2.0	--	--	1	0.7
	Nutrition/Pharmaceutical Rep	0	0.0	--	--	0	0.0
	Consultant	3	6.0	--	--	3	2.2
	Other	9	18.0	--	--	9	6.7
	No answer	2	4.0	85	100	87	64.4
	Total	50	100	85	100	135	99.9
Farm type [b]							
	Sow farm	30	60.0	48	56.5	78	57.8
	Nursery	31	62.0	48	56.5	79	59.0
	Finisher	38	76.0	62	73.0	100	74.0
	Other	2	4.0	2	2.4	4	3.0
	No answer	2	4.0	1	1.2	3	2.2
Farm size [c]							
	<50 or <1000	9	18.0	9	10.6	18	13.3
	51-250 or 1001-5000	9	18.0	24	28.0	33	24.4
	251-500 or 5001-10,000	2	4.0	10	12.0	12	8.9
	501-2500 or 10,001-50,000*	4	8.0	21	25.0	25	18.5
	2501-10,000 or 50,001-200,000	4	8.0	12	14.0	16	11.9
	10,001-25,000 or 200,001-500,000	4	8.0	1	1.2	5	3.7
	25,000+ or 500,000*	13	26.0	4	4.7	17	12.6
	No answer	5	10.0	4	4.7	9	6.7
	Total	50	100	85	100.2	135	100
OHS professional [d]							
	Yes*	16	32.0	8	9.4	24	17.8
	No	33	46.0	75	88.2	108	80.0
	No answer	1	2.0	2	2.4	3	2.2
	Total	50	100	85	100	135	100

* Significant difference between email and paper respondents using Fisher exact (α=0.05)

[a] Job type for email respondents only

[b] Farm type not exclusive

[c] Size in number sows or finishers marketed per year

[d] OHS = Occupational health and safety

Job types were based on pre-defined National Pork Board categories and included the following: owner/operator, company officer, production manager, site/farm owner, contract grower, production worker, veterinarian, nutrition/pharmaceutical representative, consultant, and other. Farm owner/operator was the most common job type among survey participants; however, this information was collected from email respondents only. No production workers participated in the study.

Farm type was defined by production phase (type/age of pigs raised) and categories were also based on pre-defined National Pork Board categories (using number of sows owned, or number of finishers marketed per year, see table 12). Most respondents were involved in multiple production phases

Email respondents were more likely to have mid-size farms (501-2500 sows or 10,001-50,000 finishers marketed per year) or very large farms (more than 25,000 sows or more than 500,000 finishers marketed per year) compared to paper survey respondents. Email respondents were also more likely to employ an occupational health and safety (OHS) professional compared to paper survey respondents. Overall, only 18% of producers employed an OHS professional.

We hypothesized that pork producers involved in finishing operations may be less likely to have showers available, or to have shower-in and shower-out policies in place. However, our results showed that finishing operations reported having access to showers (74%) and having shower-in and shower-out policies (75%) at higher levels compared to nursery and sow farming operations (table 13). Operating a finisher or a sow farm was associated with having showers available on the premises; only sow farming was associated with having a shower-in and shower-out policy in place.

Table 13. Showering practices by production phase

Variable	*Production-phase*					
	Sow		*Nursery*		*Finisher*	
	N	*%*	*N*	*%*	*N*	*%*
Total (*N, %*) producers reporting production phase	78	57.8	79	58.5	100	74.1
Shower available on premises for workers	77*	58.3	78	59.5	98	74.2
Company has shower in-shower out policy	68*	60.2	68	60.2	85*	75.2

* Significant using Fisher exact (α=0.05)

Shower, clothing, laundry, and hygiene practices Survey questions were divided into the following categories: showers, clothing, laundry, hygiene, and infection history. Responses for the first four categories are shown in table 14.

Table 14. Selected biosecurity-related practices among pork producers

Category	*Variable*	*Respondent-types*					
		Email		*Paper*		*Total*	
		N	*%*	*N*	*%*	*N*	*%*
Showers							
	Shower available on premises for workers	32	64.0	64	75.3	96	71.1
	Company has shower in-shower out policy	21	42.0	41	48.2	62	45.9
	Company gives instruction on how to shower properly	17	34.0	28	32.9	45	33.3
	Showering is emphasized for biosecurity	26	52.0	55	64.7	81	60.0
	Showering is emphasized for worker health	12	24.0	44	51.8	56	41.5
	Soap is provided to employees	28	56.0	60	70.6	88	65.2
	Liquid soap is used primarily	22	44.0	43	50.6	65	48.1
	Bar soap is used primarily	7	14.0	20	23.5	27	20.0
	Soap is shared among employees	28	56.0	45	52.9	73	54.1
	Shampoo is provided to employees	26	52.0	56	65.9	82	60.7
	Shampoo is shared among employees	21	42.0	51	60.0	72	53.3
	Company has cleaning schedule for shower facilities	18	36.0	29	34.1	47	34.8
	Showers are cleaned daily	4	8.0	1	1.2	5	3.7
	Showers are cleaned weekly	11	22.0	21	24.7	32	23.7
	Showers cleaned with bleach solution	13	26.0	19	22.4	32	23.7
	Shower cleaning is documented by workers	6	12.0	7	8.2	13	9.6
Clothing							
	Workers have individual, farm-specific clothing	25	50.0	54	63.5	79	58.5
	Workers share farm clothing	19	38.0	29	34.1	48	35.6
	Workers have individual, farm-specific boots and socks	25	50.0	56	65.9	81	60.0
	Workers share farm boots and socks	21	42.0	35	41.2	56	41.5
Laundry							
	Workers share towels	31	62.0	34	40.0	65	48.1
	Laundry facilities located on site	21	42.0	60	70.6	81	60.0
	Company has policy on how to launder properly	17	34.0	26	30.6	43	31.9
	Company has policy on laundry frequency	17	34.0	30	35.3	47	34.8
	Laundry is done daily	21	42.0	27	31.8	48	35.6
	Laundry is done weekly	4	8.0	2	2.4	6	4.4
	Work clothing is separated from other laundry	27	54.0	48	56.5	75	55.6
Hygiene							
	Company has policy on hand-washing	24	48.0	31	36.5	55	40.7
	Company has policy on wound/abrasion care	16	32.0	30	35.3	46	34.1
	Cuts/scrapes/wounds reported to supervisor	32	64.0	46	54.1	78	57.8
	Workers advised to cover wounds	36	72.0	61	71.8	97	71.9
	Alcohol-based hand sanitizer provided	21	42.0	29	34.1	50	37.0
Other							
	Company has infectious disease prevention policy*	16	32.0	13	15.3	29	21.5
	Kitchen space separate from shower area	31	62.0	48	56.5	79	58.5
	Kitchen space separate from barn area	30	60.0	54	63.5	84	62.2
	Barns tested for antibiotic-resistant bacteria	2	4.0	4	4.7	6	4.4

* Significant difference between email and paper respondents using Fisher exact (α=0.05)

Only one significant difference was identified between paper and email respondents; email respondents were significantly more likely to have an infectious disease policy compared to paper respondents. Overall, most premises (71%) had showers available for workers, but less than half of respondents required workers to shower-in and shower-out. Instructions for proper showering technique were reported by one-third of producers. Soap was provided by only two-third of producers, and more than half of the respondents shared soap among workers. Liquid soap was used more commonly than bar soap (48% vs. 20%). About one-third of producers had a cleaning schedule for shower facilities; showers were cleaned daily in about 4% of operations and weekly in about 24%. Only 24% of respondents used bleach solutions to clean their showers and very few indicated that shower cleaning is documented.

Nearly two-thirds of workers wore farm-specific, individual clothing, but another one-third shared these items. Similar results were found for footwear. Towels were also reportedly shared by 48% of respondents.

Laundry facilities were reportedly on-site in 60% of locations, but few respondents had policies on laundering frequency (35%) or technique (32%). Laundry was done daily at 36% of farms, and weekly at about 4%. Over half of the producers indicated that work clothing was separated from other laundry.

Hand washing policies were reported by less than half of producers. About 58% of respondents indicated that workers were encouraged to report cuts, scrapes, and wounds to their supervisors and 72% said that workers were advised to cover wounds. Alcohol-based hand sanitizer use was reported by less than half of respondents. Most

operations had kitchen facilities that were separate from the barn (62%) or shower (59%) area. Infectious disease prevention policies were present in only 22% of operations.

Bivariate modeling showed that several factors were related to having an OHS professional of staff. These included operating a very large farm (more than 25,000 sows or more than 500,000 finishers marketed per year), having a shower-in and shower-out policy, cleaning shower facilities daily, and expressed concern by workers about MRSA in the workplace.

Antibiotic resistant infections Antibiotic resistant infections, including MRSA, in pigs and pork production workers were assessed by a series of questions that focused on veterinarian-diagnosed skin infections in pigs and physician-diagnosed skin infections in workers (table 15).

Table 15. Self-reported antibiotic resistant infections in pigs and pork production workers

Variable	*N*	*(%)*
Veterinarian diagnosed antibiotic-resistant skin infections in pigs	4	3.0
Physician diagnosed antibiotic-resistant skin infections in workers	6	4.4
Physician diagnosed MRSA infections in workers	5	3.7
Workers expressed concern regarding MRSA in the workplace	10	7.4

There were no significant differences between email and paper respondents for these outcomes, so all respondents were considered as a single group in the final analysis. Only 4 (3%) respondents indicated that they had pigs diagnosed with antibiotic-resistant skin infections. Six (4.4%) respondents reported antibiotic-resistant skin infection in

workers; of these, five were MRSA soft tissue or skin infections. In addition, 10 (7%) respondents indicated that employees had expressed concern regarding MRSA or other human pathogens in the workplace.

Bivariate modeling revealed several potential risk factors for MRSA infection in workers, including working as a veterinarian (p=0.08), large farm operations (p=0.19 for farms with at least 10,001 sows or 200,001 finisher marketed per year; p=0.10 for farms with more than 25,000 sows or 500,000 finishers marketed per year), separating work laundry from other types (p=0.11), and having workers who express concern regarding MRSA (p=0.02). However, in the final model none of these risk factors were found to be significant (α=0.05). Similarly, no significant risk factors were identified for veterinarian-diagnosed antibiotic-resistant infections in pigs.

Discussion

We based our selection of potential risk factors for this study (shower, clothing, laundry, and hygiene-associated practices) on analogous factors previously identified in epidemiological studies of athletes. These included direct contact with infected wounds and sharing of contaminated personal items, clothing, and equipment (131, 142).

Our results show that most pork producers have shower facilities available for workers (71%), although only 46% require workers to shower-in and shower-out. This practice, which is an essential component of biosecurity, is recommended by the National Pork Board for all pork producers (114). Also of concern, only two-third of producers provided soap to employees, and soap was shared by over half. Other shared items included farm clothing (36%), footwear (42%), towels (48%), and shampoo (53%).

Sharing of personal items is associated with MRSA infection in athletes in shower and locker room areas, and should be discouraged in pork production facilities as well.

Covering wounds and reporting them to supervisors was common among pork producers in our study (72%), however, policies on wound care were infrequent (34%). Hand hygiene, which is likely the most important measure to prevent MRSA infection, should be improved (193). Of the producers surveyed, only 41% had policies on hand washing, and alcohol based hand sanitizer was provided by only 37%.

This study documents self-reported physician-diagnosed MRSA SSTI in U.S. pork producers, and reported veterinarian-diagnosed MRSA SSTI in pigs. Although swine contact may be a presumed cause of infection in workers, we cannot determine whether these reported infections are due to human (HA-MRSA, CA-MRSA) or LA-MRSA types. Previous work has shown that U.S. pork producers can be colonized with MRSA (70). Using a random sample selected from the National Pork Board's producer database, we found that 3% of respondents reported veterinarian-diagnosed antibiotic-resistant skin infections in pigs, and 4% reported physician-diagnosed antibiotic-resistant skin infections in workers.

Of reported worker infections, 5/6 (83%) were reportedly diagnosed as MRSA by a local physician. Because soft tissue or skin infections in workers caused by LA-MRSA are often undiagnosed or misdiagnosed (as CA-MRSA or other infections), these numbers likely represent under-reporting of MRSA infections. In a recent focus group session with rural Iowa physicians, it was indicated that anecdotally, the incidence of community-acquired MRSA seems to be increasing in rural populations (T. Smith, unpublished data, 2009. Iowa City, IA: University of Iowa).

In 2003, the Centers for Disease Control and Prevention (CDC) began recommending specific measures to prevent MRSA infections among athletes (153). These include: covering wounds, good shower hygiene (including use of adequate soap and hot water), no sharing of towels and personal items, establishing routine cleaning schedules for shared equipment, training athletes/coaches in wound recognition, and reporting of skin lesions to coaches by athletes. Practices to reduce MRSA transmission in athletic locker rooms might also reduce MRSA transmission in pork production facilities, since they share many similar features (shared showers, clothing, towels, and personal items). However, our study indicates that most pork producers do not currently follow procedures similar to the CDC recommendations.

Limitations Our results suggest that MRSA SSTI in pork producers occur, although we cannot determine if these infections are caused by swine-origin (ST398) or human-origin MRSA. This study relied on self-reports, however, we specified that reported MRSA infections in pigs and workers should be veterinarian- and physician-diagnosed. If clinical samples had been available from local veterinarians and physicians, molecular typing would have been possible in our research laboratory. At this time, diagnostics such as multi locus sequence testing are not commonly used by practicing physicians, but rather as a research tool or for national surveillance.

In total, only 135 survey responses were received. Investigators could not determine if producers filled out more than one survey, because both email and paper survey options were offered and responses were collected anonymously. The true response rate could range from 10.9% to 17.2% depending on the number of repeat responses. Although we failed to achieve the recommended sample size (200), this study

exceeded the average response rate for National Pork Board paper surveys (about 10%) (E. Risa, personal communication, 27 August 2009). Similar published surveys of U.S. agricultural populations have also achieved low response rates (201).

There are several potential reasons for our low response rate. First, pork producers may not have identified MRSA infections in their employees due to failure to seek medical treatment or misdiagnosis by rural physicians. Second, some producers may not have wanted to disclose MRSA infections in workers or pigs due to fear of identification. We conducted the survey anonymously to reduce the likelihood of this. Third, the paper version of this survey was mailed prior to the finding of MRSA colonized swine and swine workers in the U.S. (70). Therefore, there was less media attention and general knowledge regarding the issue of LA-MRSA at the time of the study. Finally, the email phase of our survey was conducted during the novel H1N1 influenza outbreak that occurred in 2009. This, along with economic pressures facing the pork industry and fear of negative publicity, may be related to a lack of response.

The National Pork Board database does not collect information on specific job type of pork producers (owner/operator, production phase manager, site manager, production worker, etc.). However, a high percentage of the producers contained in the selected database are expected to be "farm operators" as defined by the USDA. The farm operator is the person who runs the farm, making the day-to-day management decisions and the operator could be an owner, hired manager, cash tenant, share tenant, and/or a partner (202). According to the most recent Census of Agriculture, there were 30,546 hog/pig operations (as classified by North American Industry Classification System [NAICS] code 1122) in 2007.

In U.S. hog operations, principal operators are overwhelming male (91%) and white (98%) (203). Only about 1.5% of principal operators for hog/pig operations had a Hispanic or Latino background in the recent Census of Agriculture (203). For workers other than principal operators, demographic information is not available from the Census of Agriculture. Although we lack specific information on demographics in the National Pork Board database, the responding producers are likely to be from a relatively homogeneous population.

Although the National Pork Board's producer database was extremely useful for this study, it contains many smaller to mid-size pork producers which may not accurately reflect the state of the pork industry. Currently, 27 hog producing operations in the U.S. have 43% of the market share; these operations all raise more than 500,000 market hogs per year (J. Lummus, personal communication, 25 February 2010). In our study, only 12.6% of respondents indicated that they raise more than 500,000 market hogs per year. Similarly, 46.6% of our respondents raised fewer than 10,000 market hogs per year, yet they only represent 15% of the market share. Therefore, caution should be used in interpreting these results for the pork industry at large.

Finally, our survey was conducted primarily among farm owners/operators. No production workers participated in the study. Production workers may be most at risk for MRSA infection, and may not report all infections to their supervisors. Therefore, we may not have obtained accurate information on the prevalence of infection in that population.

Conclusions

This study shows that U.S. pork producers are self-reporting low levels of MRSA SSTI; however, we cannot determine whether livestock-associated or human-associated strains are the cause. Future collaboration with rural physicians could provide clinical samples from pork production workers and enable molecular typing to occur.

We also conclude that currently, U.S. pork producers are deficient in following practices similar to those designated by the CDC as preventive measures for MRSA infection in athletes. However, many U.S. pork producers currently have biosecurity practices in place that could easily be upgraded to address MRSA prevention measures at little to no cost. Worker training and education could also be easily improved. We recommend that U.S. pork producers consider implementation of these measures in their operations, although the true relationship between swine farming and MRSA infection in workers is still unclear.

CHAPTER V

CONCLUSIONS

This research focused on occupational exposures associated with Methicillin-resistant *Staphylococcus aureus* (MRSA) in modern pork production facilities. This dissertation is composed of three related parts. We sought to determine: 1) if occupational exposure to MRSA occurs in pork production facilities through the use of communal showers, 2) if a hygiene-based intervention can be designed and implemented to reduce occupational exposure to MRSA in communal showers, and 3) if pork producers self-report antibiotic-resistant skin infections in workers or in pigs.

In Chapter II, "Methicillin-resistant *Staphylococcus aureus* in pork production shower facilities," we documented the presence of MRSA in shower facilities of conventional swine production systems, where pigs are known to be colonized with MRSA. We tested farms involved in different production phases (sow, nursery, and finisher) and geographical locations. In the two swine production systems studied, 3% and 26% of shower samples were positive for MRSA. Overall, the prevalence in showers was 19%. All samples were *pvl* negative. Detected *spa* types included t034, t189, t753, and t1746. We also found significant differences in the number of positive swine and positive shower samples among our two study systems. In Production System A, a large, corporate owned system, there were significant fewer positive samples compared to Production System B, a smaller, family owned corporation.

This study shows that communal showers can be a source of exposure to MRSA for pork production workers. However, the importance of environmental exposure in MRSA transmission remains unclear in this occupational group. For human MRSA

forms, direct contact with wounds and colonized individuals are known to be the major routes of MRSA exposure. Although MRSA colonization in U.S. pigs may be common, clinical infections in U.S. pigs are not frequently reported. Therefore, direct contact may play a lesser role in swine worker colonization and infection. Exposure via showers, or other contaminated environments, could be a relevant exposure source. In addition, the importance of airborne MRSA transmission in pork production facilities deserves future study.

Although we have documented a potential source of MRSA exposure for pork production workers, more research is needed to determine if workers using showers that harbor MRSA have increased rates of clinical infection with MRSA. Workers in our tested production systems could be surveyed about their history of skin and soft tissue infections (SSTI). However, workers may not know their infection history; they may not seek treatment for SSTI, and many MRSA SSTI are likely misdiagnosed without culture and antimicrobial sensitivity testing. If clinical samples from workers in our production systems were obtained, laboratory testing could be performed, including more detailed methods of molecular identification. Because MRSA have been identified in communal showers, more study is also needed to determine the best way to reduce MRSA prevalence. The study described in chapter III sought to identify effective interventions to eliminate MRSA from communal showers.

In Chapter III, "Methicillin-resistant *Staphylococcus aureus* in pork production shower facilities: Adapting interventions from athletic facilities," we searched the literature for interventions designed to decrease MRSA infections in athletes. We hypothesized that swine production workers are similar to athletes in that they share a

shower room/locker room environment and personal items (towels, clothing, etc). We evaluated these interventions for adaptability to the pork production environment, and composed swine-specific guidelines for MRSA prevention. We focused our guidelines on the following areas: showers, clothing, laundry, wound care, and environment. In a pilot effort, we implemented our intervention to reduce MRSA in two swine production systems. Overall, results were mixed. Although the general trend showed reduction in MRSA prevalence post-intervention, in two showers we observed samples that were negative pre-intervention become positive post-intervention. We identified *spa* types t034, t189, t571, t374, and t3446 in this study.

We recommend repeating this study with a larger sample and better intervention oversight. Specifically, additional production systems should be enrolled in a follow-up study to increase the sample size. Study sites with different production phases should be included. In particular, more study sites with separate barn/shower arrangements should be sought to further investigate whether increased distance from the barn results in lower MRSA prevalence in showers. Our intervention guidelines should also be reassessed. First, the wording of some statements should be strengthened (for example, using "require" vs. "advise"). The literature should also be searched for additional measures that may be considered for the intervention, as measures relevant to MRSA control and prevention are being frequently updated in the literature.

Most importantly, implementation of the intervention should be improved in future studies. For the study outlined in Chapter III, we relied on local veterinarians to oversee the intervention process in each production system. No compliance checks were in place, and therefore investigators were not able to assess how accurately the

intervention was implemented at each site. If possible, investigators should directly observe the implementation of future interventions. Although our pilot intervention did not reduce MRSA levels in all showers tested, many of the guidelines suggested here can be implemented by pork producers with little effort and little to no cost, and can potentially improve the conditions of the working environment for employees.

In Chapter IV, "Methicillin-resistant *Staphylococcus aureus* infection in pork production workers," we sought to determine if pork producers report veterinarian-diagnosed antibiotic-resistant skin infections in pigs, and physician-diagnosed antibiotic-resistant skin infections in workers (including MRSA). Previously, reports of MRSA colonization in pork production workers have been published, but little information exists regarding actual MRSA infection in this population. We examined potential risk factors for MRSA infection in pork production workers associated with biosecurity, including shower and laundry procedures, farm clothing use, and personal hygiene. We found that 3% of respondents reported veterinarian-diagnosed antibiotic-resistant skin infections in pigs, and 4% reported physician-diagnosed antibiotic-resistant skin infections in workers. Of known worker infections, 5/6 (83%) were reportedly diagnosed as MRSA by a local physician. No significant risk factors were identified for either skin infections in pigs or skin infections in workers.

Our study is important, because it shows that U.S. pork production workers are reporting low levels of MRSA infection. However, we relied upon self-reports. In future studies, efforts should be made to obtain clinical samples from pork production workers reporting MRSA SSTI. Infections can then be definitively diagnosed, and advanced molecular testing methods can be used to help determine if MRSA are of human or swine

origin. This information would enhance the current knowledge base regarding the epidemiology and ecology of MRSA.

Furthermore, future studies should make a concerted effort to include production workers in a survey of MRSA SSTI in pork production. In our study, use of the National Pork Board's producer database for sample selection made it difficult to survey workers. Our study sample consisted mostly of farm owner/operators. This group may be less likely to work daily with pigs, and therefore less likely to contract MRSA SSTI compared to production workers. Efforts should also be made to collect demographic information in future studies. Although farm owner/operators are expected to be a relatively homogeneous group according to the 2007 Census of Agriculture, including production workers would likely increase diversity of age, sex, and race in the working population. Demographic information would help us determine the similarity of our study population, which could be very important when sample sizes are small and response rates are low.

Combined, these studies provide evidence that MRSA can be found in pork production shower facilities, and that occupational exposures occur due to components of the biosecurity protocol. We designed and implemented an intervention to decrease the level of MRSA in showers, however, our small-scale intervention was not clearly successful. We also reported the first prevalence estimate of MRSA infection in pork production workers in the United States. Livestock-associated (LA-MRSA) remains an emerging issue and requires further study to determine the true occupational risks.

In additional to occupational concerns, the larger significance of MRSA in livestock production is unclear. The prevalence of MRSA colonization in swine herds, and its impact on production costs/losses should be investigated. To date, little

information is available in the U.S. regarding MRSA colonization rates in pigs; initial studies show that up to 48% of U.S. swine are colonized (70). Prevalence trends in U.S. swine herds should also be examined to determine if colonization rates are changing. However, eliminating MRSA carriage in U.S. swine herds could be challenging. First, pork producers could consider treatment of colonized swine, either as individuals or on a herd-level. This would likely be expensive, and could be an ineffective long term strategy if pigs are easily re-colonized. Second, antibiotic use policies and practices should be evaluated in the pork industry. Industry programs support responsible antibiotic use, but more education of pork producers regarding the potential link between antimicrobial use on the farm and antimicrobial resistant bacteria in swine herds is needed. Third, the practice of shower-in and shower-out for biosecurity (and prevention of swine disease) could be evaluated. Although this practice is highly recommended in the swine industry, the effectiveness of a shower-in and shower-out policy has not been evaluated in the literature. Altogether, there is much to learn about the microbiological, ecologic, and epidemiological characteristics of LA-MRSA.

APPENDIX A

INDUSTRY RECOMMENDED SHOWER PROTOCOL (114)

ENTRANCE	
1	All staff are to enter the staff side (male and female respectively).
2	Prior to entering the shower facility, all shoes must be removed and remain outside the showering facility.
3	All items of clothing including watches must be removed and must remain off-farm. Other items such as glasses must be washed.
4	All body surfaces including hair should be washed using the supplied soap. Shower for at least 3 minutes.
5	All visitors must sign the visitor's book detailing their organization, last pig contact, and dates.
6	A selection of clean clothing must be provided on-farm, together with clean towelling.
7	Disposable socks and underwear are to be provided, or washed on-farm.
8	A dirty clothing bin is to be provided for wet towelling/used clothing.
9	A selection of clean boots are to be provided.
10	Staff boots should be named/labeled.
EXIT	
1	All boots must be cleaned thoroughly with the boot cleaner. This includes soles and heels.
2	All on-farm clothing is to be deposited in the provided laundry bin.
3	All damaged clothing is to be repaired before re-use.
IF VISITORS SHOWER OFF THE FARM	
1	Specific colored towels should be provided. These must not be moved back onto the farm and are washed separately. A different color for on-farm use should be used.
2	All shoes must be put on outside the shower block.

SHOWER ROOM HYGIENE

DAILY CHECKS		
1	Shower block	The floor of the shower should be wiped with disinfectant
		Quantity of body and hair shampoo to be checked
2	Toilet area	Toilet seats should be wiped clean
		Toilet bowl should be disinfected
		All toilets should have sterilizing tablets
		Small bins in toilet to be wiped clean with disinfectant and emptied each day
		Check quantity of toilet paper
3	Wash sinks in toilet area	Check quantity of hand disinfectant
		Check quantity of hand towels
4	Floor of toilet area	Clean with disinfectant
WEEKLY CHECKS		
1	Clothing supplies - quantity suitable	
2	All damaged clothing disposed off-farm or mended	
3	Hygiene of boots	
4	Shower curtains clean	
5	Shower works well with appropriate body soaps	
6	Bell or signal device works and gets a reply	
7	Locks on doors adequate	

APPENDIX B

LABORATORY METHODS

Bacterial isolation protocol

1. Using swab, inoculate sample into 7.5% Staphylococcus Enrichment Broth (SEB) [SEB contains 10g tryptone, 75g sodium chloride, 10g mannitol, 2.5g yeast, and 1L distilled water]
2. Incubate at 35C (no CO_2) for 24h
3. Inoculate 5µL broth onto Colistin Naladixic Acid agar (CNA agar) and CHROMagar plates
4. Check at 24h (CNA) and 48h (CHROMagar)
5. If pink colonies on CHROMagar or yellowish, beta-hemolytic colonies on CNA, subculture onto new CNA
6. Proceed with catalase test, coagulase test, staph latex test, and MRSA latex test

Catalase test

1. Remove small amount of hydrogen peroxide from bottle with plastic, disposable pipette
2. Place one droplet onto plate colony near edge of plate
3. Foaming = catalase positive

Coagulase test

Slide method:

1. Place 20 µl distilled water on a clean slide
2. Place 20 µl rabbit serum on slide (stored at -20F)
3. Emulsify sample into distilled water, then serum
4. Coagulation = positive

Tube method:

1. Place 0.5mL rabbit serum into test tube
2. Emulsify sample, mix with serum
3. Incubate at -35C for 4-24 hours
4. Clotted plasma (pellet) = positive

Staph latex

1. Remove cards from kit
2. Place a loop of bacteria and smear/mix onto control droplet
3. Go directly into test droplet and mix
4. Agglutination appears = positive

MRSA latex agglutination

1. Place about 2 drops of extraction reagent into a microfuge tube
2. Inoculate with 5µL sample; vortex
3. Place into heat block (95-100C) for 3 minutes
4. Cool to room temperature
5. Add 1 drop extraction reageant2; mix
6. Centrifuge at 3000rpm for 5 minutes
7. On testing card, label one circle with sample and one with control
8. Place 1 drop testing or control solution on card in respective location
9. Add 45-50µL sample to each circle
10. Mix thoroughly, agitate card
11. Agglutination = positive

DNA extraction (Promega: Wizard Genomic DNA Purification Kit)

1. Take an overnight culture (in glass screw-cap tube) and spin down at 2000rpm for 5 min
2. Remove supernatant and discard in bleach
3. Resuspend cells thoroughly in 480µL of 50mM EDTA
4. Pour cell and EDTA mixture in labeled microfuge tube
5. Add 15µL lysostaphin, 30µL mutanolysin, and 30µL lysozyme and pipet gently to mix
6. Incubate at 37C for 30-60 minutes
7. Centrifuge for 2 min at 13,500g and remove the supernatant
8. Add 600µ: Nuclei Lysis Solution, gently pipet until cells are resuspended
9. Incubate at 80C for 5 minutes to lyse the cells, then cool to room temperature
10. Add 2µL RNAse Solution to the cell lysate; invert the tube 2-5 times to mix
11. Incubate at 37C for 15-60 minutes then cool to room temperature
12. Add 200µL Protein Precipitate Solution to the RNAse-treated cell lysate; vortex vigorously at high speed for 20 seconds to mix
13. Incubate sample on ice for 5 minutes or later (holding period)
14. Centrifuge at 13,500g for 3 minutes
15. Transfer supernatant (about 800µL) containing DNA to clean 1.5mL microfuge tube containing 600µL room temperature isopropanol (2-propanol)
16. Gently mix by inversion until the thread-like strands of DNA form a visible mass
17. Centrifuge at 13,500g for 2 minutes
18. Carefully pour off supernatant and drain tube on clean absorbent paper; add 600µL room temperature 70% ethanol and gently invert tube several times to wash DNA pellet
19. Centrifuge at 13,500g for 2 minutes and carefully aspirate ethanol
20. Drain tube on clean absorbent paper and allow pellet to air dry for 10-15 minutes, then blot out any remaining ethanol

21. Add 100µL DNA Rehydration Solution to the tube and rehydrate DNA by incubating 65C for 1 hour (or longer if necessary) heat block on low; periodically mix the solution by tapping the tube (alternatively, rehydrate the DNA by incubating the solution overnight at room temperature or 4C)
22. Store in -20F freezer

<u>*Spa* PCR</u>

1. Make *spa* PCR master mix
    - 37uL H_2O
    - 2uL dNTP
    - 5uL 10X PCR Buffer
    - 2uL F Primer [Forward primer sequence (001-F.41070004X) 5' – GAA CAA CGT AAC GGC TTC ATC C – 3']
    - 2uL R Primer [Reverse primer sequence (001-R.41070003X) 5' – GCT TTT GCA ATG TCA TT ACT G – 3']
    - 1.25uL Taq
2. Add 48µL master mix into each tube
3. Add 2uL DNA to corresponding tube
4. Amplify on PCR instrument (PCR running conditions):
    - 1-95.0˚ for 10:00 minutes
    - 2-95.0˚ for 0:30 seconds
    - 3-60.0˚ for 0:45 seconds
    - 4-72.0˚ for 1:30 minutes
    - 5-Repeat step 2, 34 times
    - 6-72.0˚ for 10:00
    - 7-4.0˚ for ever

5. Keep in 4°C refrigerator until needed for gel
6. Run product on 1.1% agarose gel
7. Purify PCR product and submit to DNA Core for sequencing

Pvl PCR

1. Make *pvl* PCR master mix
    - 39µL d. H_2O
    - 1µL dNTP mix (dNTP: add equal parts of DNA nucleotides = final concentration of 200uM/nucleotide)
    - 2µL F primer
    - 2µL R primer
    - 5µL 10X PCR Buffer
    - 0.5µL Taq
2. Add 48µL master mix into each tube
3. Add 2uL DNA to corresponding tube
4. Amplify on PCR instrument (PCR running conditions):
    - 1-94.0˚ for 5:00 minutes
    - 2-94.0˚ for 0:30 seconds
    - 3-55.0˚ for 0:30 seconds
    - 4-72.0˚ for 1:00 minutes
    - 5-Repeat step 2, 30 times
    - 6-72.0˚ for 5:00
    - 7-4.0˚ for ever
5. Keep in 4°C refrigerator until needed for gel
6. Run product on 1.1% agarose gel
7. Purify PCR product and submit to DNA Core for sequencing

APPENDIX C

QUESTIONNAIRE

Thank you for participating in this study. Answers to all questions are anonymous. Please contact Kerry Larson with questions at (319) 470-9279 or kleedom-larson@pork.org. Surveys should be returned using the enclosed self-addressed stamped envelope. Thank you!

1. **Please circle the type(s) of productions system you operate and give the approximate number of pigs that are in each system.**

1	Farrow to wean	Number of sows:
2	Farrow to finish	Number of sows: Number pigs in nursery: Number pigs grow to finish:
3	Nursery	Number pigs in nursery:
4	Wean to finish	Number pigs in nursery: Number pigs growing: Number pigs finishing:
5	Finisher (grow – finish)	Number pigs in finisher:
6	Other	Number other:
Additional comments:		

2. **Do you have an occupational health and safety professional on staff?**

   (Circle one) Yes No

3. **Questions regarding shower facilities: Please circle yes or no**

Do you have a shower facility available for workers?	Yes	No
Do you have a shower in-shower out policy for workers?	Yes	No
Do you have a company policy on how to shower properly?	Yes	No
Do you emphasize the importance of showering for: • Biosecurity	Yes	No

• Health of animals • Health of workers	Yes Yes	No No
Do you provide soap? • Is bar soap used primarily? • Is liquid soap used primarily?	Yes Yes Yes	No No No
Do workers share soap?	Yes	No
Do you provide shampoo?	Yes	No
Do workers share shampoo?	Yes	No
Additional comments:		

4. **Questions regarding farm-specific clothing and laundry facilities: Please circle yes or no**

Do workers have their own individual clothing for farm-specific use (clothing that stays within the facility and is worn by only one individual)?	Yes	No
Is clothing ever shared among workers?	Yes	No
Do workers have their own individual socks and boots for farm-specific use (socks and boots that stay within the facility and are worn by only one individual)?	Yes	No
Are socks or boots ever shared among workers?	Yes	No
Are towels shared among workers?	Yes	No
Do you have laundry facilities on site?	Yes	No
Do you have a company policy on how to properly launder clothing and towels?	Yes	No
Do you have a company policy on how frequently laundry should be done? • If yes, how often is laundry done?	Yes	No
Are workers responsible for laundering their own clothing?	Yes	No
Is work clothing separated from other types of laundry?	Yes	No
Is laundry soap provided to workers? • If yes, what type of soap is used?	Yes	No

Additional comments:

5. **Questions regarding personal hygiene: Please circle yes or no**

Do you have a company policy on personal hygiene including: • Hand washing • Nail care • Caring for wounds/abrasions	 Yes Yes Yes	 No No No
Do workers report cuts/scrapes/wounds to their supervisor?	Yes	No
Are workers advised to cover cuts/scrapes/wounds?	Yes	No
Do you provide alcohol-based hand sanitizer for workers?	Yes	No
Additional comments:		

6. **Questions regarding environment and cleaning: Please circle yes/no or write in your answer as appropriate**

Do you have a cleaning schedule for shower facilities? • If yes, how often is cleaning performed? • What types of cleaning products are used?	Yes	No
Do you have a cleaning schedule for laundry facilities? • If yes, how often is cleaning performed? • What types of cleaning products are used?	Yes	No
Is cleaning documented by workers?	Yes	No
Do workers have a kitchen space for eating that is		

separate from : • Shower areas • Animal areas/barn	 Yes No Yes No
Additional comments:	

7. **Questions regarding Methicillin-resistant *Staphylococcus aureus* (MRSA): Please circle yes/no or write in your answer as appropriate**

Do you have a company policy on prevention of infectious diseases among employees?	Yes	No
Have you ever tested any facility or barn environment for bacteria? If yes, what were the results?	Yes	No
Have you had any known (veterinarian diagnosed) antibiotic resistant skin infections in pigs? If yes, please describe:	Yes	No
Have you had any known (physician diagnosed) antibiotic resistant skin infections in workers? If yes, please describe:	Yes	No
Have you had any known (physician diagnosed) MRSA infections in workers? If yes, please describe:	Yes	No
Have workers expressed concern regarding MRSA or other human pathogens in the workplace?	Yes	No

8. Please list any additional comments here:

THANK YOU!

APPENDIX D

EPA APPROVED DISINFECTANTS FOR METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS

EPA Reg. No.	Primary Product Name
106-72	MAXIMA 128
106-73	MAXIMA 256
106-81	MAXIMA RTU
303-223	BEAUCOUP GERMICIDAL DETERGENT
303-225	MATAR GERMICIDAL DETERGENT
498-134	SPRAYPAK SPRAY DISINFECTANT FORMULA 2
675-43	AMPHYL
675-55	LYSOL DISINFECTANT S.A. CLEANER
706-69	CLAIRE DISINFECTANT SPRAY
777-66	LYSOL BRAND DISINFECTANT DIRECT MULTIPURPOSE CLEANER
777-71	LYSOL BRAND FOAMING DISINFECTANT BASIN TUB&TILE CLEANER II
777-72	BIOSOL
777-91	CITRUS SCENT LYSOL BRAND ANTIBACTERIAL KITCHEN CLEANER II
777-96	BIOSOL or LYSOL BRAND DISINFECTANT SPRAY FOR KITCHEN
777-98	BRACE KITCHEN
777-99	BRACE
777-101	ADP 10106
777-105	ARC

1130-15	BURNISHINE GERMICIDAL SOLUTION
1043-116	TBQ RTU
1677-21	MIKRO-QUAT
1677-193	ADVACARE 120 SANITIZER/SOUR
1677-199	QUANTUM TB DISINFECTANT
1677-202	OASIS PRO 66 HEAVY DUTY ALKALINE BATHROOM CLEANER & DISINFECTANT
1839-78	NP 3.2 DETERGENT/DISINFECTANT
1839-79	NP 4.5 DETERGENT/DISINFECTANT
1839-81	NP 9.0 DETERGENT/DISINFECTANT
1839-83	DETERGENT DISINFECTANT PUMP SPRAY
1839-86	BTC 2125 M 10% SOLUTION
1839-94	NP 3.2 (D&F) DETERGENT/DISINFECTANT
1839-95	NP 4.5 (D&F) DETERGENT/DISINFECTANT
1839-96	NP 9.0 (D&F) DETERGENT/DISINFECTANT
1839-166	BTC 885 NEUTRAL DISINFECTANT CLEANER 128
1839-167	BTC 885 NEUTRAL DISINFECTANT CLEANER 256
1839-168	BTC 885 NEUTRAL DISINFECTANT CLEANER 32
1839-169	BTC 885 NEUTRAL DISINFECTANT CLEANER 64
1839-174	STEPAN TOWELETTE
1839-211	HD-64 (transferred from EPA Reg# 3377-66)
1839-212	HD-256 (transferred from EPA Reg# 3377-67)
1839-213	HD-128 (transferred from EPA Reg# 3377-68)
1839-214	ACLD-256 (transferred from EPA Reg# 3377-57)
1839-215	ACLD-128 (transferred from EPA Reg# 3377-58)
1839-216	ACLD-64 (transferred from EPA Reg# 3377-59)
2915-55	FULLSAN

3377-57	ACLD-256 (transferred to EPA Reg# 1839-214)
3377-58	ACLD-128 (transferred to EPA Reg# 1839-215)
3377-59	ACLD-64 (transferred to EPA Reg# 1839-216)
3377-66	HD-64 (transferred to EPA Reg# 1839-211)
3377-67	HD-256 (transferred to EPA Reg# 1839-212)
3377-68	HD-128 (transferred to EPA Reg# 1839-213)
3862-177	TEK-TROL DISINFECTANT CLEANER CONCENTRATE (transferred from EPA Reg# 11725-7)
3862-178	TEK-PHENE
3862-179	OPTI-PHENE CLEANER DISINFECTANT DEODORANT (transferred from EPA Reg#11725-9)
3862-181	FOAMING DISINFECTANT CLEANER
4822-359	VIREX II/64 (transferred to EPA Reg#70627-23)
5736-102	SUPER BOL
5736-104	HOSPITAL DISINFECTANT CLEANER
5736-105	LIQUID DISINFECTANT CLEANER
5736-106	FOAMING AEROSOL DISINFECTANT CLEANER
5741-18	NABC
5741-20	DMQ
5813-1	CLOROX_BLEACH
5813-21	TACKLE
5813-50	ULTRA CLOROX BRAND REGULAR BLEACH
5813-67	CLOROX 409-R
6659-3	SPRAY NINE
6836-48	BARDAC 2250-7.5
6836-57	BARQUAT 42Z-10
6836-73	LONZA FORMULATION S-38

6836-75	LONZA FORMULATION S-21
6836-77	LONZA FORMULATION S-18
6836-78	LONZA FORMULATION R-82
6836-136	LONZA FORMULATION S-18F
6836-138	LONZA FORMULATION S-38F
6836-139	LONZA FORMULATION R-82F
6836-140	LONZA FORMULATION S-21F
6836-152	LONZA FORMULATION DC-103
6836-165	LONZA FORMULATION L-7
6836-204	LONZA FORMULATION DC-110N
6836-205	LONZA FORMULATION DC-108N
6836-206	LONZA FORMULATION DC-109N
6836-244	CSP-46 CONCENTRATE
6836-245	CSP-46
6836-252	PHENOCIDE 258
6836-253	PHENOCIDE 128
6836-267	LONZA FORMULATION DCN 400-256
6836-268	LONZA FORMULATION DCN 400-128
6836-269	LONZA FORMULATION DCN 400-64
6836-309	M3 FORMULATION #4
6836-313	LONZA DISINFECTANT WIPES
7546-27	HOSPITAL DISINFECTANT CLEANER
8155-5	GENERAL PURPOSE/NON-ACID HUSKY G/P DISINFECTANT CLEANER
8155-22	HUSKY 805 C/D
8383-3	SPORICIDIN BRAND DISINFECTANT SOLUTION
8383-7	SPORICIDIN BRAND DISINFECTANT TOWELETTE

8654-9	SOLUCIDE O2
9480-4	SANI-CLOTH GERMICIDAL DISPOSABLE WIPES
9480-6	SANI-CLOTH PLUS GERMICIDAL DISPOSABLE CLOTH
9804-1	OXINE
10324-56	MAQUAT 256
10324-63	MAQUAT 10
10324-72	MAQUAT 615-HD
10324-80	MAQUAT 5.5M
10324-81	MAQUAT 7.5M
10324-85	MAQUAT 86-M
10324-93	MAQUAT 64 PD
10324-94	MAQUAT 20-M
10324-96	MAQUAT 50DS
10324-99	MAQUAT 10PD
10324-105	MAQUAT 128PD
10324-108	MAQUAT 256 MN
10324-112	MAQUAT 128 MN
10324-113	MAQUAT 64 MN
10324-114	MAQUAT 32 MN
10324-115	MAQUAT 750-M
10324-117	MAQUAT 710-M
10324-118	MAQUAT 256 EBC
10324-119	MAQUAT 128 EBC
10324-120	MAQUAT 64 EBC
10324-131	MAQUAT A
10324-134	MAQUAT 256-1010N

10324-140	MAQUAT MQ2525-M-CPV
10324-141	MAQUAT 256-NHQ
10324-142	MAQUAT MQ2525M-14
10324-143	MAQUAT 10-B
10324-144	MAQUAT 256 MN-FCS
10324-145	MAQUAT FP
10324-146	MAQUAT 128-1010N
10324-147	MAQUAT 64-1010N
10324-154	MAQUAT 64-NHQ
10324-155	MAQUAT 128-NHQ
10324-156	MAQUAT 512NHQ
10324-157	MAQUAT 32-NHQ
10324-158	MAQUAT 2420 TBD-9
10324-163	MAQUAT 12 MN
10324-164	MAQUAT 256 PD
10324-170	MAQUAT 64-PD-X
10324-171	MAQUAT 128 PD-X
10324-172	MAQUAT 128-X
10324-173	MAQUAT 64-X
10324-176	MAQUAT 2420 TBD-20
10324-177	MAQUAT 705-M
10324-179	MAQUAT 32 MN-FCS
10324-180	MAQUAT 64 MN-FCS
10324-181	MAQUAT 128 MN-FCS
10492-4	DISCIDE ULTRA DISINFECTING TOWLETTES
10492-5	DISCIDE ULTRA DISINFECTING SPRAY

11725-7	TEK-TROL DISINFECTANT CLEANER CONCENTRATE (transferred to EPA Reg#3862-177)
11725-8	TEK-PHENE CLEANER-DISINFECTANT-DEODORANT (transferred to EPA Reg#3862-178)
11725-9	OPTI-PHENE CLEANER DISINFECTANT DEODORANT (transferred to EPA Reg#3862-179)
34810-18	THYMO-CIDE
34810-19	TOPPS
34810-21	READY TO USE WEX-CIDE
34810-22	READY TO USE TOPPS
34810-25	READY TO USE THYMO-CIDE
34810-31	WEX-CIDE 128
34810-35	CLEAN-CIDE READY TO USE GERMICIDAL DETERGENT
34810-36	CLEAN-CIDE WIPES
42964-5	A-33
42964-14	OMEGA
42964-25	A-33 DRY
44446-67	CONCEPT HOSPITAL DISINFECTANT DEODORANT
46781-6	CAVICIDE
46781-8	CAVIWIPE
47371-36	HS-867Q ONE-STEP GERMICIDAL CLEANER ANS DEODORANT
47371-37	HS-267Q ONE-STEP GERMICIDAL CLEANER ANS DEODORANT
47371-129	FORMULATION HWS-258
47371-130	FORMULATION HWS-128

47371-131	FORMULATION HWS-64
47371-191	FORMULATION HWS-512
47371-192	FORMULATION HWS-32
56392-7	DISPATCH HOSPITAL CLEANER DISINFECTANT WITH BLEACH
56392-8	DISPATCH HOSPITAL CLEANER DISINFECTANT TOWELWITH BLEACH
59894-10	KWIKKILL DISINFECYANT DEODORIZING CLEANING WIPES
60142-1	VIRAHOL HOSPITAL DISINFECTANT/CLEANER & INSTRUMENT PRESOAK
60142-3	VIRAHOL HOSPITAL SURFACE DISINFECTANT TOWELETTE
61178-1	D-125
61178-2	PUBLIC PLACES
61178-4	PUBLIC PLACES TOWELETTE
61178-5	CCX 151
62472-2	KENNELSOL HC
66243-1	ODO-BAN READY-TO-USE
66243-2	ODO-BAN
67619-3	CPPC SPRAY 1
67619-8	CPPC ULTRA BLEACH 2
67519-11	CPPC SHOWER
67619-12	CPPC TSUNAMI
67619-13	CPPC STORM
67619-17	SHIELD
69687-1	SUPER-CHLOR

70144-1	OPTI-CIDE 3
70144-2	OPTI-CIDE 3 WIPES
70060-19	ASEPTROL S10-TAB
70263-6	MICROBAN QGC (transferred to EPA Reg# 70385-6)
70263-8	MICROBAN PROFESSIONAL STRENGTH MULTI-PURPOSE ANTIBACTERIAL CLEANER (transferred to EPA Reg# 70385-8)
70385-6	MICROBAN QGC transferred from EPA Reg# 70263-6)
70385-8	MICROBAN PROFESSIONAL STRENGTH MULTI-PURPOSE ANTIBACTERIAL CLEANER (transferred from EPA Reg# 70263-8)
70590-1	HYPE-WIPE DISINFECTING TOWEL WITH BLEACH
70590-2	BLEACH-RITE DISINFECTING SPRAY WITH BLEACH
70627-2	DISINFECTANT DC 100
70627-3	NADBC 101
70627-5	SCJPTABC 801
70627-6	PHENOLIC DISINFECTANT HG
70627-10	JOHNSON'S FORWARD CLEANER
70627-15	JOHNSON BLUE CHIP GERMICIDAL CLEANER FOR HOSPITALS
70627-21	VIREX II 128
70627-23	VIREX II 64 (transferred from EPA Reg#4822-359)
70627-24	VIREX II 256
70627-33	ENVY LIQUID DISINFECTANT CLEANER
70627-35	ENVY FOAMING DISINFECTANT CLEANER
70627-56	OXIVIR TB

70627-58	OXY-TEAM DISINFECTANT CLEANER
70627-60	OXIVIR WIPES
70791-2	ENVIROTRU
71654-7	VIRKON
71847-2	KLOR-KLEEN
72977-3	AXEN (R) 30
73232-1	ALPET D2
74559-1	ACCEL TB
74986-4	SELECTROCIDE 2L500
74986-5	SELECTROCIDE 5G
75512-1	EBIOX TRUKLEEN WIPES
75512-2	EBIOX TRUKLEEN SPRAY
75512-3	EBIOX TRUKLEEN CONCENTRATE
75848-1	AMERI-KLEEN WHIRLPOOL PEDICURE SPA ONE STEP DISINFECTANT
80346-1	MDF-200 MODEC DECON FORMULATION Part A
80346-2	MDF-200 MODEC DECON FORMULATION Part B
82075-1	PS DISINFECTING SURFACE WIPE
82972-1	VITAL OXIDE
83303-1	JYMRSA SPRAY SOLUTION Part A
83303-2	JYMRSA SPRAY SOLUTION Part B

Adapted from List H: EPA's Registered Products Effective Against Methicillin-resistant *Staphylococcus aureus* and Vancomycin-resistant *Enterococcus faecalis* or *faecium* – last updated January 9, 2009.

APPENDIX E
ADDITIONAL HANDOUTS FOR SHOWER PREVALENCE STUDY (CHAPTER II)

Methicillin-Resistant Staphylococcus aureus (MRSA)

What is Staphylococcus aureus? Germs called Staphylococcus aureus are bacteria. They are often just called "staph." Many healthy people carry staph in their noses or on their skin. Sometimes staph bacteria can cause infections. Usually these infections are skin infections like pimples and boils. Sometimes they are more serious infections like lung or blood infections.

What is Methicillin-Resistant Staphylococcus aureus (MRSA)? MRSA is a type of staph that has changed (become resistant) due to overuse and abuse of antibiotics. Antibiotics are drugs that kill bacteria. This resistant staph can't be killed by the usual antibiotics, like penicillin. Certain other antibiotics will still kill MRSA.

What is Community-Associated MRSA (CA-MRSA)? In the past, most infections caused by MRSA were in hospitals or nursing homes. Now, healthy people who have not recently been in the hospital are getting infections caused by MRSA. These are called community-associated MRSA infections. Community-associated MRSA infections are usually skin infections, like pimples or boils. These infections may need to be treated with carefully chosen antibiotics. It is also possible for CA-MRSA to cause blood, bone, and lung infections.

Who is at risk for MRSA infections? MRSA infections are most common in hospitals and nursing homes. Conditions that help MRSA spread are skin touching skin, cuts or scrapes, and crowded living conditions. If a person not in the hospital has a

MRSA infection, it is more likely to spread if this person is a member of certain groups. These groups include athletes, military recruits, children, prisoners, and men who have sex with men.

How is MRSA spread? The bacteria enter the body through open cuts and scrapes on the skin. The bacteria usually spread when a person with MRSA on their skin comes into contact with another person's skin. Hand washing and keeping wounds covered is important in stopping a possible spread of the infection. A less common way to spread MRSA is to share towels and sports equipment.

What does a MRSA infection look like? MRSA may cause a skin infection that looks like a pimple or boil. The infection often looks like a spider bite. It can be red, swollen, and painful. It may drain pus. If you think you may have a skin infection, see your healthcare provider. Lab tests may be run to see if your infection is caused by MRSA.

If I or someone I know has a MRSA infection, how can I keep it from spreading?

- Keep wounds that are draining covered with clean, dry, bandages.
- Clean hands regularly with soap and water or alcohol-based hand gel (if hands are not visibly soiled). Always clean hands immediately after touching infected skin or any item that has come in direct contact with a draining wound.
- Maintain good general hygiene with regular bathing.
- Do not share items that may become contaminated with wound drainage, such as towels, clothing, bedding, bar soap, razors, and athletic equipment that touches the skin.

- Wash clothing that has come in contact with wound drainage after each use and dry thoroughly.
- If you are not able to keep your wound covered with a clean, dry bandage at all times, do not join in activities where you have skin to skin contact with other persons (such as sports or in child care centers) until your wound is healed.
- Clean equipment and other environmental surfaces with which multiple individuals have bare skin contact with an over the counter detergent/disinfectant that specifies Staphylococcus aureus on the product label and is suitable for the type of surface being cleaned.

How is MRSA treated? Your healthcare provider will decide the best way to treat your infection. Some infections may need to be drained. Only a healthcare provider should drain sores. Some infections may need antibiotics. Tell your healthcare provider if you are not getting better in a few days. You may need to go to the hospital to receive antibiotics directly into your veins. Be sure to tell any healthcare provider you see if you have had an MRSA infection in the past. If anyone you know gets a similar skin infection, have them see their healthcare provider.

Adapted and used with permission from the Iowa Department of Public Health, Center for Acute Disease Epidemiology, factsheet on MRSA.

Hand washing

Wash Your Hands! How you wash and dry your hands makes a difference:

- Use soap and warm or hot running water.
- Wash for at least 15 seconds.
- Wash all surfaces, including wrists, palms, backs of hands, between fingers, and as much as possible under fingernails, by rubbing vigorously.
- Rinse hands under running water.
- Away from home, dry hands with disposable paper towels or the hot air blower. At home, provide a separate towel for each member of the household, and wash towels regularly in hot water and detergent.

When should I wash my hands?

Before you:

- Eat
- Prepare food for yourself or others
- Treat a break or cut in the skin
- Care for an ill or injured person or animal
- Insert or remove contact lenses

Immediately after you:

- Use the restroom
- Handle uncooked foods (especially raw meat, poultry, or fish)
- Change a diaper
- Blow your nose, sneeze, or cough
- Touch an animal (especially a reptile), including animals in petting zoos and fairs

Why is hand washing important? Your skin constantly makes oil that stays on its surface. Germs that get on your skin are trapped in the oil. Skin does not have to look dirty to be loaded with tiny germs that can cause big problems – like the common cold, diarrhea, and more serious diseases. Washing your hands with soap and warm running water is one of the best and easiest things you can do to stay healthy.

But I wash my hands a lot – We are all in a hurry – to eat, get back to work, make that important meeting or class. Too often we forget or "don't have time," or we think a quick cold-water rinse will do. But that doesn't "cut it"…literally!

Oils, and any attached germs, must be removed from the skin. A splash of cold water and a quick rub with a towel doesn't do much good. You need to use warm water and soap to get the oil and germs off your skin.

When you've been touching things many people have handled, routine hand washing can help reduce your chances of getting an infection.

Should I use antibacterial soap? The most important thing to remember is to wash with warm running water and soap. If you want to use antibacterial soap, keep in mind that it helps kill some germs – but not all. Some germs can't be killed, no matter how strong the soap is or how long it is on your hands. You may not always have special soap with you. That is why it's very important to spend enough time and care to wash germs away.

You may wish to use an antimicrobial soap or alcohol based hand rub if you are ill or caring for someone who is, or has a weakened immune system.

To do the most good, washing your hands has to become a habit. You're more likely to learn a new habit and stick with it if it's easy. Most of the time, proper hand washing is easy.

Should I use towelettes? Antimicrobial towelettes may be used in place of regular soap and water. They are not as effective as alcohol based hand rubs or antimicrobial soaps, so are not a substitute for them.

Can I use a waterless hand sanitizer lotion or gel with alcohol? Using this type of product is ok, except when hands look dirty; then washing your hands with soap and warm water is a must. Alcohol is not as effective at killing germs when dirt is present.

When using, use the amount of an alcohol-based hand rub recommended by the manufacturer. Apply the product to the palm of one hand, and rub hands together, covering all surfaces of the hands and fingers, until hands are dry.

Adapted and used with permission from the Iowa Department of Public Health, Center for Acute Disease Epidemiology factsheet on Hand washing.

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