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Assessment and mitigation of airborne transmission of methicillin-resistant Staphylococcus Aureus in animal feeding operations and the outdoor environment

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ASSESSMENT AND MITIGATION OF AIRBORNE TRANSMISSION OF METHICILLIN-RESISTANT STAPHYLOCOCCUS AUREUS IN ANIMAL FEEDING OPERATIONS AND THE OUTDOOR ENVIRONMENT

by

Dwight Deon Ferguson

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

December 2012

Thesis Supervisor: Professor Kelley J. Donham

ABSTRACT

Methicillin-resistant *Staphylococcus aureus* (MRSA) was originally recognized as a hospital acquired infection. However, it is now recognized that MRSA infections are frequently acquired in the community and agricultural settings as well. As epidemiological studies and surveillance of MRSA continued over the past decade, agricultural sources of MRSA have also been recognized. Although direct person-to-person transmission of MRSA has been recognized as a major known route of transmission, a preliminary study has shown that aerosol exposures may also be an important mechanism of transmission, both occupationally to workers inside animal feeding operations and environmentally via exhaust ventilation to the outside. In this study I aimed to 1) determine the concentration of viable MRSA inside and outside swine buildings known to be positive for MRSA, 2) determine the efficiency of the N-95 respirator for potentially protecting workers inside swine buildings, and 3) determine the efficiency of a biofilter unit for mitigating emissions of MRSA from a swine building. I hypothesize that remediation and control of airborne MRSA in animal feeding operations can be achieved by the appropriate use of N-95 respirators to protect workers and the addition of biofilters to the exhaust ventilation system to mitigate transmission of this emerging environmental contaminant to the outdoor environment. The results of the study indicate that aerosolized MRSA in the respirable size range can be detected inside a swine building and 215 m downwind of the swine building. Aim 2 results indicated that the N95 respirator was efficient at potentially protecting workers exposed to MRSA particles greater than 5 µm but not as effective with MRSA particles less than 5 µm. The

results of aim 3 indicated that hardwood chips and western red cedar chips are efficient biofilter media for mitigating the emission of MRSA from a swine building. These studies showed that workers inside swine buildings and the outdoor environment can be potentially protected against the transmission of MRSA with a respiratory program which includes the use of N95 respirators and biofilters as mitigation control measures.

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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

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To Janice, Zandria, Elliot, Rachael, Sabrina, my mom and dad, and my family for their loving, faithful support as they continue to encourage me to look to the ant.

For God so loved the world that he gave his one and only Son, that whoever believes in him shall not perish but have eternal life.

Jesus
John 3:16

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Methicillin-resistant *Staphylococcus aureus* (MRSA) was originally recognized as a hospital acquired infection. However, it is now recognized that MRSA infections are frequently acquired in the community and agricultural settings as well. As epidemiological studies and surveillance of MRSA continued over the past decade, agricultural sources of MRSA have also been recognized. Although direct person-to-person transmission of MRSA has been recognized as a major known route of transmission, a preliminary study has shown that aerosol exposures may also be an important mechanism of transmission, both occupationally to workers inside animal feeding operations and environmentally via exhaust ventilation to the outside. In this study I aimed to 1) determine the concentration of viable MRSA inside and outside swine buildings known to be positive for MRSA, 2) determine the efficiency of the N-95 respirator for potentially protecting workers inside swine buildings, and 3) determine the efficiency of a biofilter unit for mitigating emissions of MRSA from a swine building. I hypothesize that remediation and control of airborne MRSA in animal feeding operations can be achieved by the appropriate use of N-95 respirators to protect workers and the addition of biofilters to the exhaust ventilation system to mitigate transmission of this emerging environmental contaminant to the outdoor environment. The results of the study indicate that aerosolized MRSA in the respirable size range can be detected inside a swine building and 215 m downwind of the swine building. Aim 2 results indicated that the N95 respirator was efficient at potentially protecting workers exposed to MRSA particles greater than 5 µm but not as effective with MRSA particles less than 5 µm. The

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CHAPTER I
INTRODUCTION AND LITERATURE REVIEW

Since 1945 when penicillin began to be commonly used in treating infections, the general use of antibiotics has greatly expanded. Applications of antibiotics include widespread use in hospitals, outpatient prescriptions, and widespread availability to lay persons for usage in livestock production and crop production. Accompanying the expanded usage of antibiotics has been an increasing growth in the prevalence of antibiotic-resistant bacteria. Dispersion of antibiotic-resistant bacteria in animal hosts may be through the environment via sewage, drinking water, direct contact among infected animals and people, through contaminated food, and air pollution. Expanded usage and dispersion may indicate a perfect storm of increased selection pressure for enhanced development of resistant organisms. Methicillin-resistant *Staphylococcus aureus* (MRSA) may be one result of such selection pressures. MRSA was first recognized after the initial use of penicillin antibiotics to treat *Staphylococcus aureus* infections in hospital settings. S.aureus infections include skin and soft tissue infections, endocarditis, pneumonia, foreign body infection and bloodstream infections[1]. As antibiotics usage increased, other microorganisms including MRSA evolved into multidrug- resistant pathogens.

Although MRSA was originally believed an issue only in human populations, MRSA has emerged over the past decade as a zoonotic pathogen [2]. The occurrence of MRSA in companion animals [2,3]livestock animals [4] and in the environment [5] has changed the perspective of the medical and public health community that MRSA infections are not just solely due to hospital visits. With MRSA identified in the general environment as

well as in hospitals, it is imperative that scientists and infection control professionals understand how MRSA is transmitted to assist in the implementation of control measures for MRSA as well as other multidrug-resistant pathogens. As there appears to be multiple hosts and reservoirs of MRSA outside the human body, MRSA can be also be considered a potential occupational and environmental illness. To understand the ecology of MRSA, a study of the identification of different strains of the organism and their environments is important. Therefore an understanding and practice of proper sampling and typing methods is critical.

In this review, antibiotics in the environment, ecology of MRSA and MRSA as an occupational and environmental illness will be addressed. Furthermore I will focus on airborne transmission of MRSA in swine production facilities. I will describe general MRSA identification tests, sampling methods and prevention measures. In so doing my intent is to address mitigation strategies for MRSA and other multidrug-resistant pathogens in swine production facilities.

MRSA in the world of resistant pathogens

Prior to the use of antibiotics to treat *Staphylococcus aureus* infections, resistant strains outside of hospital settings were rarely recognized. After World War II and the more widespread use of antibiotics, penicillin-resistant *Staphylococcus aureus* prevalence increased .Within six years of the introduction of penicillin in hospitals; resistant strains reached a prevalence rate of twenty five percent. The introduction of methicillin in 1959 led to a drop in the prevalence of penicillin-resistant *S.aureus* [6]. However, in less than a year of its introduction methicillin-resistant *S. aureus* was identified[6-8].

As resistance to methicillin increased in hospitals, gentamicin became the preferred antibiotic to treat *S.aureus* infections. However, similar to the trend observed with penicillin and methicillin, antibiotic resistance to gentamicin also emerged in hospital settings. Surveillance of hospitals from the 1970s to 1980s detected a trend with MRSA; hospitals associated with medical schools and more than six hundred beds had higher infection rates than hospitals with fewer beds [6]. Some risk factors noted for the emergence of antibiotic resistance in large hospitals are treatment in special units, inter-hospital transfer, number of beds, length of stay and insurance [6,9]. A ten year study from 1997 to 2006 identified that over ninety percent of hospitalized infections were due to antibiotic resistant organisms. A concerning trend in the 10 year study demonstrated that antibiotic resistance infections for patients between the ages of 18 and 64 had greatly increased since the year 1997 to 2006 from 0.68% to 3.64%, an increase of 435 percent. Also, infection among patients younger than 18 has dramatically increased from 0.21% to 5.67% an increase of 2600 percent [9].

With people having close contact with animals and the emergence of zoonotic diseases, a better understanding is needed to determine the relatedness of antibiotic resistant bacteria in animals, especially pigs due to the focus of this review. A Pub Med search using "prevalent antibiotic resistant bacteria in pigs" and a study conducted by Gibbs et al 2004 [10] identified *Enterococci*, *Salmonella* and *Escherichia coli* as the predominant antibiotic resistant bacteria in addition to MRSA found to be associated with swine CAFOs. I will briefly review those organisms in the sections which follow.

Mechanisms of Antibiotic Resistance

Antibiotic Resistant Bacteria and MRSA Resistance Mechanisms

Antibiotics (depending on the specific class of antibiotics) act on bacteria by inhibition (1) of cell wall synthesis, (2) of protein synthesis, (3) of nucleic acid synthesis, and (4) of metabolic pathways [11]. Antibiotic resistant bacteria prevent the action of antibiotics by innate resistance and acquired resistance. Innate resistance occurs when the bacteria has intrinsic resistance to a particular antibiotic. On the other hand, acquired resistance occurs when a change in nucleic acid allows the bacteria to prevent the actions of antibiotics on the bacteria. Mechanisms involved with acquired resistance are (1) transformation (i.e., the exchange of nucleic acid), (2) transduction (i.e., bacteriophage inserts resistance gene into bacteria), (3) conjugation with plasmids or transposons and 4) mutation [12]. The acquired resistance mechanism of MRSA involves the chromosomal mutation of the DNA that encodes penicillin-binding-protein (PBP2')[13]. Many different mechanisms among bacteria confer resistance. The specific resistance factor attacks the specific mechanism of action of the particular antibiotic class. Regarding MRSA, the production of PBP2' is encoded by the *mecA* gene. PBP2' acts by binding beta-lactams to the cell wall and inactivating the beta-lactam which prevents the antibiotic from acting on the bacteria [14].

MRSA role in occupational and environmental illness

Pathogenesis of MRSA

MRSA has numerous virulence factors which enable it to cause diseases. Virulence factors such as adhesins (allows the pathogen to bind to host cells), enzymes and toxins provide the bacterium with the ability to evade, invade and destroy host defense mechanisms [15]. The Staphylococcal chromosome cassette (SCC) *mec* complex, a unique mobile genetic element, confers methicillin resistance. SCC*mec* is composed of three regions , (i) *ccr* gene complex which is responsible for the mobility of the SCC*mec* gene by the action of DNA recombinase enzymes, (ii) *mec* gene complex regulates gene expression and the (iii) Joining (J) region may provide sites for plasmids and transposons. Currently there are eleven identified types of SCC*mec* (types I- XI) and other designated types [16]. Hospital acquired MRSA (HA-MRSA) and community acquired MRSA (CA-MRSA) have been identified to be associated with different SCC*mec* types. HA-MRSA has been shown to carry SCC*mec* types I, II, III and CA-MRSA carries SCC*mec* types IV and V[17-19].

A toxin of significant importance in the virulence of MRSA is Panton-Valentine Leukocidin (PVL). PVL is a cytotoxin that targets host defense response such as polymorphonuclear cells, monocytes and macrophages [20]. PVL is associated with skin, soft tissue infections and necrotizing community acquired pneumonia and is commonly detected in CA-MRSA[21,22]. Although PVL is associated with virulence in MRSA, its exact role in virulence is controversial [23].

Adhesion of MRSA to host cell surface has been linked to a gene called *sasX* which encodes for a protein which enables the pathogen to bind to the host cell surface [24].

SasX has also been shown to cause aggregation leading to formation of biofilm. Common types of infections associated with *sasX* are respiratory infections, pleural cavity infections and abscesses. It is also known to be associated with nasal carriage[24,25]. Other toxin genes associated with virulence factor for S.aureus includes *tsst* which encodes for toxic shock syndrome, *et* an exfoliative toxin gene which encodes for staphylococcal scaled syndrome and *se* a heat-stable enterotoxin which encodes for the toxin causing staphylococcal food poisoning[26]

Molecular typing of MRSA

Genetic typing of MRSA can be categorized into two major methods, "band typing" and "sequence typing". Band typing methods include Pulse Field Gel Electrophoresis (PFGE) and SCC*mec* typing. PFGE is the gold standard for typing *S.aureus* during hospital outbreaks[27-29]. This method uses a restriction enzyme *SmaI* which cleaves the DNA and gel electrophoresis which separates the DNA segments into bands detected on the agarose gel. SCC*mec* typing uses PCR methods such as Multiplex PCR assay to detect *mecA* and other loci on the *SCCmec* element and several PCR assays to detect the *mec* and *ccr* complex[30,31]. Sequence typing methods include Multilocus sequence typing (MLST) and *S.aureus* protein A (*spa*) typing. MLST uses multiple loci to identify the *S.aureus* strain based on the sequence of housekeeping genes[32,33]. This method is used to assess the molecular evolution of *S.aureus*. *spa* typing determines the type of *S.aureus* by the hypervariable *spa* locus, which encodes the protein A gene [34]. *spa* typing has been shown to be advantageous over MLST and PFGE methods since it can evaluate both molecular evolution and hospital outbreaks [35].

Other prevalent zoonotic resistant bacteria found in pigs

Vancomycin Resistant Enterococci

Various *Enterococci* species/subspecies occur as normal bacterial flora of the alimentary tract. However, various *Enterococci* species/subspecies may also cause infections anywhere in the body. They are common infections of the female urinary tract. To help treat outbreaks of *Enterococci* infections, vancomycin became the antibiotic of choice for these infections in hospitals. In the United States (U.S.), vancomycin-resistant *Enterococci* (VRE) emerged in hospitals at higher prevalence rates than in Europe. The difference in the prevalence of VRE in the U.S. and Europe is postulated to be due to the use of Vancomycin in the U.S. and avoparcin in Europe [36]. The emergence of VRE since the mid 1980s in the U.S. has mainly occurred in hospitals and is relatively uncommon in the community outside of health care facilities [37],whereas in Europe VRE is prevalent in healthy people and farm animals and not as common in hospital settings [38]. Of particular concern with VRE is that it can be transmitted to humans through direct contact and through various links in the food chain [39].

Salmonella

Antibiotic-resistant *Salmonella* is a zoonotic pathogen with its emerging linked to antibiotics used in animal feed for treatment or growth promotion of livestock and poultry. In production animals, antibiotic resistant *Salmonella* were more commonly found in poultry than in pork and cattle [40]. In humans, illness due to *Salmonella* is predominantly due to food-borne illness from consuming undercooked meat and water contaminated with feces [41]. The presence of multiple drug resistant *Salmonella* is a public

health concern due to its ubiquitous nature in the environment. Multiple drug resistant Salmonella has been identified in cattle [42] processing plants. As the cattle are processed the detection of multiple drug resistant *Salmonella* decreases which indicates that practices used decontaminate meat products at the facility being tested was successful. In addition to identification in cattle processing plants, multiple drug resistant Salmonella has been detected in animal feedlots[43,44]. The presence of multiple drug resistant Salmonella in feedlot pens presents public health concern for the potential transmission of persons working in animal feedlots.

Escherichia coli

Escherichia coli can cause food-borne illness in humans and can lead to potentially fatal hemolytic uremic syndrome as caused by *E.coli* serotype O157:H7 [45]. Human infections results from eating contaminated food products and drinking water [46]. The overuse of extended spectrum cephalosporins and extended spectrum beta lactams in companion animals may have led to antibiotic resistant *E. coli* being detected in companion animals which present a cause for concern for veterinary therapeutic use[47]. With companion animals being in close contact with humans the risk for zoonotic transmission of antibiotic resistant *E. coli* from animals to people is increased. As noted above, the use of antibiotics in human and veterinarian practices presents the potential for selective pressure for the evolution and growth of antibiotic resistance organisms, including *E. coli* [48]. Antibiotics in feed, have been shown to be associated with an increase in prevalence of resistant bacteria in the intestinal flora of animals and persons living on the farm [49]. The potential transmission of *E. coli* resistance as the result of antibiotic use in farm animals to farmers may present a concern for public health.

Widespread use of antibiotics

Hospitals

Antibiotics were commonly used to treat bacterial infections in patients in hospitals. Intensive Care Units (ICUs) were at the center of the fight against bacterial infections due to the poor health status of the patients as well as the procedures patients went through that made them more susceptible to infections. As antibiotic usage in ICUs increased, the emergence of antibiotic resistant bacteria in ICUs also increased [50].

Several factors influence the development of antibiotic resistant bacteria in hospitals; use of antibiotics, the effect of antibiotics on patients [51], bacteria ability to transfer resistance via plasmids [52] and routes of transmission [53,54]. The rate of the occurrence of MRSA in hospital infections has been shown to be related to the use of ciprofloxacin [55]. It has also been noted that persons with recent hospitalization within one year are more likely to be colonized with MRSA[56]. The use of antibiotics can lead to the elimination of susceptible strains and can promote the development of antibiotic resistant strains in patients. Plasmids can transfer antibiotic resistance from resistant strains to non-resistant strains and in so doing confer antibiotic resistance to previously susceptible bacteria. As with other agents of disease, antibiotic resistance is influenced by routes of transmission. For example, patients in various types of treatment centers (ICUs, Burn Units, Coronary Care Unit and General Surgery) are supported with medical devices, such as catheters, which can promote antibiotic resistant bacteria growth.

Community

The general public has access to antibiotics either by prescription medication or over the counter (OTC). In the United States most antibiotics are only available through prescriptions except for topical antibiotics such as Neomycin, Bacitracin and Diabecline. Diabecline is a new topical ointment which contains tetracycline (3%) hydrochloride and is used as a first aid antibiotic. Antibiotics such as bacitracin and neomycin used for OTC have been linked to the occurrence of community-associated MRSA. The relational link between community-associated MRSA (CA-MRSA) and OTC bacitracin and neomycin appeared to be the result of selective pressure for the USA300 (a human adapted MRSA) clone [57]. Antibiotic use in children and adults prior to hospitalization has also been linked to development of MRSA[58-61]. A study in Denmark found that ninety seven percent of antibiotics were prescribed after consultation with physicians [62]. Physicians in the Denmark study generally prescribed antibiotics for urinary infections and respiratory illness. People in countries other than the U.S. also have access to antibiotics as OTC medication. In Malta persons obtained OTC antibiotics from their local pharmacies [63]. Self-medicated use of OTC antibiotics has been identified as prevalent in the rural population in Greece[64]. It has also been noted that self-medication of OTC antibiotics in Mexico occurs at a high rate[65]. The finding in Mexico potentially has significant public health implications due to the possibility of migrant workers developing antibiotic resistance from self medication and then working in close contact with livestock which can potentially lead to zoonotic transmission of antibiotic resistance from livestock to people or vice versa . Academic communities are not immune to the over-use of OTC antibiotics. A study of academic staff in Turkey identified overuse of antibiotics in this

population for the common cold [66]. Although antibiotic use in the U.S. is generally limited through prescription, there is a concern for the possible transport of antibiotics across the US border from countries where access to antibiotics can be obtained without prescriptions.

Livestock

In animal production antibiotics have traditionally been used to treat sick animals, prevent infections at therapeutic levels and promote animal growth at subtherapeutic levels. The use of antibiotics in animal production has been shown to increase the presence of bacteria with resistant genes in the intestine of swine herds [67]. Antibiotic use has also been shown to influence the effect of bacterial prophages, bacteria with viral genetic material inserted into its genome[68,69]. Antibiotics resistant prophages have been found to increase the production of antibiotic resistant genes through gene transfer. Additionally, pig exposure to antibiotics has greatly influenced antibiotic resistance in herds with low resistance (not exposed to antibiotics) and herds with high resistance (exposed to antibiotics). A study by Dawson (1984) suggested that Chlortetracycline administered to low resistance swine (i.e., animals not exposed to antibiotics in feed for 8 years) and high resistance swine (i.e., animals constantly exposed to antibiotics in feed) was associated with increased prevalence of chlortetracycline resistant anaerobic and coliform bacteria in both the low resistance and high resistance herds [70]. The use of antibiotics also has a prolonged effect even after antibiotic use has been discontinued. It was shown that antibiotic resistance to chlortetracycline and tylosin (a macrolide antibiotic) was still evident after two and half years of non-use of antibiotics at a swine complex facility [71]. Fecal samples collected from cattle fed with antibiotics

(chlortetracycline plus sulfamethazine (TET-SUL), chlortetracycline (TET), virginiamycin, monensin, and tylosin) were found to have increased prevalence of tetracycline and ampicillin resistant *E. coli* in cattle. The same study also noted that the diet of the cattle can also be a factor in the development of antibiotic resistance[72]. It was found that feeding the cattle TET-SUL increased the prevalence of tetracycline and ampicillin resistant *E.coli* whereas using only TET did not increase the prevalence of tetracycline and ampicillin resistant *E.coli*. Likewise in pigs, a dietary additive (oregano in this case) resulted in a lower concentration of antibiotic resistance *E. coli* in pigs using the antibiotics as compared to controls [73]. The use of antibiotics has also been suggested to be a risk factor to the development of livestock associated MRSA[74]. It has been suggested in a recent study that the use of aminocyclitol (a aminoglycoside) to treat *E. coli* infections was related to the development of livestock associated MRSA in cattle and swine [75].

Crops and Plants

The initial success of antibiotics in human and veterinarian medicine led to antibiotics use in crop and plant agriculture. In crops and plants, antibiotics were used to control bacterial disease. In the United States streptomycin and oxytetracycline are the predominant antibiotics used on crops (and those only EPA-approved). Fire blight in high end fruit crops[76] is an example of a disease where antibiotics are used in crops. Antibiotics such as gentamicin were banned from crop use in the US. As a result, plants treated with gentamicin from other countries were banned from being imported into the US [77]. Although streptomycin resistant bacteria have been identified in maize roots, its etiology is unknown [78]. Streptomycin was discovered in 1944 by Schatz and Waksman

and was used as the first drug to treat tuberculosis[79] Streptomycin antibiotics use in agricultural systems to treat animals may have led to the dissemination of streptomycin in soil from animal manure[80]. The land application of streptomycin via animal manure on crops and plants may have led to the development of streptomycin resistant bacteria in crops and plants. Brown (2008) conducted a study of herbal supplements in the US and found antibiotic resistant bacteria in the supplements tested were contaminated with antibiotic resistant bacteria such as *Staphylococcus spp.* , *Bacillus spp*., and *Enterobacteriaceae* [81]. The presence of antibiotic resistant bacteria in herbal supplements warrants further investigation to determine the origin of these antibiotic resistant bacteria. Crop farms in Latin America that used oxytetracycline and gentamicin have shown to carry antibiotic resistance bacteria in crops which when eaten raw can potentially lead to the transfer of antibiotic resistance genes and antibiotic resistant bacteria to people[82]. With the identification of antibiotic resistance in crop plants, additional concern was recognized when plasmids and integrons (both of which can be used to disseminate and transfer antibiotic resistant genes to other ecosystems including people) were detected in crop plants which can be eaten raw in retail[83]. The potential of antibiotic resistance bacteria and antibiotic resistant genes being ingested through raw crop is a concern to public health.

Source of antibiotics in the environment

Hospital sewage

Antibiotic resistant bacteria and antibiotics have been detected in the sewage from hospitals. Antibiotic resistant bacteria such as *E. coli* and *Salmonella spp.* were detected in raw and treated sewage at high levels [84]. In addition to antibiotic resistant bacteria

being isolated from hospital sewage, antibiotic resistant bacteria such as coliforms are sometimes detected at higher percentage than the rates of antibiotic resistant coliforms in clinical isolates [85]. Yang (2009) found that the percentage of multidrug resistant coliforms (32%) in a regional hospital waste water treatment facility was higher than the percentage of multidrug resistant coliforms (19.1%) found in clinical isolates collected at the same regional hospital in Taiwan. A study in Sweden evaluating the use of antibiotics and the detection of antibiotic resistant bacteria in hospital sewage identified VRE at high percentages. It was noted that the hospital with higher prevalence of VRE also used vancomycin at ten times the percentage of use at the hospital with lower prevalence of VRE [86].

Hospitals with water treatment plants also had high prevalence of *Klebsiella pneumoniae* in sewage. *Klebsiella pneumoniae* can be found in environmental and human isolates and is an opportunistic nosocomial pathogen causing bloodstream and urinary tract infections and shows resistance to cephalosporins as a result of the production extended spectrum beta lactamases [87,88]. The detection of antibiotic resistant *K. pneumoniae* after waste water treatment showed that the treatment facility was not effective at removing antibiotic resistant bacteria from the environment. With *K. pneumoniae* being able to produce extended spectrum beta lactamase (ESBL) it has the ability to transfer genes via plasmids to other antibiotic resistant bacteria to promote the development of multiple drug resistant bacteria in the environment[88,89]. The detection of antibiotic resistant *K. pneumoniae* in the chlorine contact effluent tank, i.e. process where treated sewage is disinfected before disposal, in a hospital waste water treatment facility

is a cause for concern due to the potential dissemination of antibiotics and antibiotic resistant bacteria to the environment [90].

Municipal sewage

The increased use of antibiotics in human medicine, veterinarian medicine, crop production and other areas has led to studies resulting in detection of antibiotic resistant genes and antibiotic resistant bacteria in municipal sewage. Antibiotic-resistant *E. coli* have been detected in the receiving water source at waste water treatment facilities at higher concentrations than the inflow water source. The identification of antibiotic resistant *E. coli* at the inflow source indicated the possibility of dissemination of antibiotic resistance to the environment [91]. Antibiotic resistant *Enterococci spp.* was detected with higher prevalence in municipal waste water treatment facilities which had a higher number of hospitals in the municipality[92,93]. A study which evaluated the outcome of tetracycline resistant bacteria at biological waste water treatment facilities which used activated sludge showed that the normal operating conditions for these facilities increased the growth rate and presence of tetracycline resistant bacteria in the environment [94]. Of great public health concern is the identification of antibiotic resistant genes and antibiotic resistant bacteria which were released into the environment from waste water treatment facilities. The biosolids from such facilities can be used in agricultural applications which can lead to further dissemination into the environment [95].

Livestock manure

With the use of antibiotics in animal production to prevent disease, treat sickness and promote animal growth, the presence of antibiotics in animal waste is not surprising.

In a study which compared the detection of antibiotic resistant bacteria in separated (liquid separated from solids) and unseparated (liquid and solid) pig slurry, it was found that pig fattening houses waste samples identified *Salmonella spp.* with multiple drug resistance [96]. Separated pig slurry can be used for land application in an environmentally friendly waste management manner[97-99]. Environmental factors such as the number of animals (frequency of the introduction of animals), rodent control (rodents being reservoirs for *Salmonella spp.)* and water contaminated with sewage also influenced the detection of *Salmonella spp.* with multiple drug resistance[100]. Tetracycline-resistant bacteria were identified in samples from cattle operation waste water facilities and municipal waste water. The prevalence of tetracycline-resistant bacteria was higher in the waste water from the cattle operation than from the municipal waste water samples[101].

Antibiotic resistant *E. coli* has also been identified in pig slurry. The presence of antibiotic resistant *E. coli* in pig slurry has great implication in the spread of antibiotic resistant *E. coli* through the use of pig slurry in agricultural manure which can spread to the environment[102]. Of particular public health concern is the dissemination of antibiotic resistant bacterial strains through pig slurry in field applications.

Antibiotic resistant strains of VRE were detected in pig slurry similar to the strains of VRE found in municipal sewage[103]. The same study also found erythromycin resistant *Enterococci* strains in the pig slurry similar to strains found in municipal sewage. Multiple drug resistant VRE were also detected in farm water run-off[104]. MRSA strains have been identified in fecal samples of poultry houses. One hundred percent of the strains of MRSA detected were similar to MRSA strains detected in aerosol samples from inside and downwind of the poultry house [105]. The identification of MRSA

downwind of the poultry hen house presents a particular challenge to public health since the airborne MRSA particles can colonize the nasal passages of persons and workers on the farm and can travel to nearby communities leading to potential MRSA infections.

Drinking water

The detection of antibiotics, antibiotic resistant genes and antibiotic resistant bacteria in municipal waste water treatment facilities has created a concern for the potential of these substances circulating in drinking water. Drinking water from a water treatment facility tested positive for multiple drug resistant bacteria. The drinking water was obtained from communities with varying levels of complex water treatment. In that study, a correlation was identified in the rate of multiple antibiotic resistant bacteria prevalence and the extent of the water treatment. It was shown that the more complex the water treatment, the higher the prevalence of multiple antibiotic resistant bacteria [106]. In a separate study antibiotic resistant *E. coli* were found in the drinking water sampled. The antibiotic resistant *E. coli* identified were multiple drug resistant and transferred antibiotic resistance genes through conjugation [107].

In another study, antibiotic resistant genes were detected in tap water at concentrations higher than finished water (ready for distribution to the consumer) and source water (untreated water entering the water treatment process). Additionally antibiotic resistant bacteria concentrations were higher in tap water than in finished water from the water treatment facilities [108]. Other studies have also identified antibiotic resistant *S. aureus* in coastal communities in their tap water and fresh water[109]. Of concern with the findings in this study was that the highest prevalence of antibiotic resistant bacteria was found in seawater, a harsh environment for most bacteria. Samples

taken from well water have also identified antibiotic resistant bacteria. Antibiotic resistant *S. aureus, E. coli* , and *Enterococcus spp.* were identified in the water taken from well in villages in Nigeria [110]. The occurrence of antibiotic resistant genes and antibiotic resistant bacteria in drinking water is of special concern due to the number of persons and animals who may be potentially affected by consuming water containing antibiotic resistant bacteria that can transfer antibiotic resistance to the normal bacterial flora in the intestines of people and animals.

MRSA in healthcare workers

Intimate contact of healthcare workers (HCWs) with patients can lead to person-person transmission of MRSA due to the prevalence of nasal carriage of *S. aureus* in both HCWs and patients [111]. Active surveillance for MRSA among HCWs at a German hospital showed that 33% percent of HCWs were colonized with *S. aureus* and 1.6% of the HCWs were positive for MRSA. The HCWs identified as MRSA positive were only the nursing staff [112]. Saiman (2003) conducted an epidemiologic study of a MRSA outbreak in a neonatal intensive care unit (ICU) which identified 1.3% of HCWs to be colonized with MRSA and nineteen percent with methicillin-susceptible *Staphylococcus aureus* (MSSA) [113]. A ten year study conducted in a hospital in the Netherlands identified fifty nine out of 840 HCWs were colonized with MRSA. It was determined that sixty eight percent of the HCWs MRSA positive were nurses. Furthermore, the investigators in this ten year study found that fifty one percent of the HCWs who were MRSA positive became MRSA positive after treating MRSA positive patients[114]. Kluytmans (1995) authored the first study which identified food borne transmission of MRSA in a hematology unit at a hospital in the Netherlands. The MRSA outbreak which occurred

was linked to food contamination by a dietary worker in the hospital who did not have direct contact with the index patient. It was determined through epidemiologic investigation that the dietary worker, who provided a peeled banana to patients on the ward of the index patient, had the same epidemiologic strain as the index patient and other MRSA cases. A nurse who worked in the hematology unit was identified as MRSA positive and was responsible for transmission of the epidemiologic MRSA strain to other wards[115].

Lu (2008) conducted a study at a dialysis unit of a hospital in Taiwan to determine MRSA colonization, infection and transmission among patients, HCWs and the family members of dialysis patients and HCWs. This study showed that the nasal carriage rate of MRSA in the family members of HCWs was higher than the nasal carriage rate of MRSA in the family members of dialysis patients[116]. A study conducted at a tertiary hospital in the Northeast US found that nineteen patients in a burn unit who were involved in the same fire event became MRSA positive. During this outbreak in the trauma ICU, fifty eight percent of the burn patients became MRSA positive and four percent of the HCWs treating the burn patients became MRSA positive[117]. Roberts (2011) conducted an epidemiological study at seven dental clinics at the University of Washington and found that MRSA was on the surfaces inside the dental clinics and the nasal passages of dental students. It was found that twenty-one percent of dental student's nasal passages were colonized with MRSA and 8.4% of the surfaces tested were MRSA positive. These studies illustrate the importance of active surveillance of HCWs to prevent transmission of MRSA to patients (vice versa) and to their family members.

MRSA in Schools

Outbreaks of MRSA have occurred in schools of various age groups, student athletes, and athletic settings and in university settings[118-123]. Lo (2007) conducted a study among kindergarten students in Taiwan and found that twenty five percent of the student's nasal passages were colonized with *S. aureus*. Nine of the sixty eight students sampled were positive for CA-MRSA and 88.9% of CA-MRSA was genetically related[124]. In a later study, Lo (2010) compared the nasal colonization rates for *S.aureus* and MRSA for 2004-2006 and 2007-2009. The investigators found that *S.aureus* nasal colonization rates decreased from 28.1% to 23.3% during the time period of 2004-2006 and 2007-2009 respectively. On the other hand, MRSA nasal colonization increased from 8.1% to 15.1% during the same time period that *S.aureus* nasal colonization decreased [125]. A study conducted among students and faculty at a university in Hawaii found that thirty three percent of the study population were carriers of *S.aureus* and among those carriers, 3% were colonized with MRSA[126].

Kassem (2007) conducted an environmental survey of computers at a metropolitan university which were swabbed for contamination with MRSA. Of the twenty four computer keyboards swabbed, 2 of 24 (8.3%) were MRSA positive [127]. Montgomery (2010) investigated the prevalence of MRSA at an athletic health care facility in schools in Ohio. Environmental samples were taken from water coolers, lockers, training room, treatment tables and other areas of the facilities. The environmental survey identified that forty six percent of the surfaces sampled were MRSA positive [128]. Buss (2009) conducted a study among Nebraska high school student athletes during 2006-2007 and 2007-2008[129]. This study determined that the incidence of

MRSA infections increased during that time period. Overall MRSA infections increased from 4.4% to 14% and specifically among wrestlers (from 19.6% to 60.1%) and football players (from 5% to 25.1%). The findings of the aforementioned studies showed that MRSA can be transmitted in school settings and presents a public health concern for transmission via person-person and person-fomite (or vice versa) in various public settings.

MRSA in Agricultural Settings

The occurrence of MRSA in food producing animals presents a public health challenge to prevent the transmission of MRSA to consumers. Persoons (2009) investigated the presence of MRSA in broiler chickens and laying hens on poultry farms in Belgium. It was determined that eight out of seventy five broiler chickens were MRSA positive and the laying hens were negative for MRSA [130]. In addition to the Persoons (2009) study, Nemati (2008) showed that poultry samples from the 1970's and 2006 (91.7% and 82.2% respectively) were MRSA positive[131]. Dairy cows have also been shown to be colonized with MRSA [132]. Spohr (2011) evaluated the occurrence of MRSA in dairy cows and milk in Southwest Germany. MRSA was detected in the three herds tested and milk samples from the three herds. MRSA was found in bulk milk tanks, 7 out of 15 cows, and in environmental samples in pig areas and nasal samples from the farm staff[133]. Of special concern was the identification of *spa* type *t*011 in samples from cattle, pig environments and staff. The identification of spa type *t*011 on all three farms tested even without direct or indirect contact may suggest an environmental source of contamination. Graveland (2010) investigated the occurrence of MRSA in veal calves in the Netherlands and found that 28% of the veal calves were MRSA positive. Human

MRSA was associated with hours worked in the stables and use of antibiotics[134].

Numerous studies in various locations around the world have identified MRSA in swine[132,135-139]. Recently Alt (2011) conducted a study in Germany to evaluate the prevalence of MRSA in fattening pigs. The investigators found that fifty two percent of the farms tested were MRSA positive with herd size (with sizes of small, middle and large) and production type facility being risk factors for MRSA on the farms[140]. Increases in the number of pigs in a herd may lead to an increase in having MRSA colonized pigs. Additionally as the size of the herd increases, it may lead to having to purchase pigs from different sources, was shown to increase the association with MRSA positive pigs. Farm production types described either as wean-to-finish or farrow-to-finish. It was also found that purchasing patterns differed in the two production types. Wean-to-finish farms purchased piglets at a rate six times greater than the farrow-to-finish. In the Netherlands, de Neeling (2007) sampled 540 pigs for MRSA and found 39% of the nasal samples were MRSA positive and all of the slaughter facilities had MRSA positive pigs [141].

Several studies in the Netherlands have identified MRSA in pigs as an emerging pathogen of public health concern[142-144]. With MRSA being detected in pigs in the Netherlands the potential for MRSA to be a zoonotic pathogen needed to be investigated. Huijsdens (2006) conducted an epidemiological investigation to determine the origin of MRSA infection in an individual. The study involved one pig farm and determined that three family members, three co-workers and eighty percent of the pigs sampled as MRSA positive. All of the isolates were of the same *spa* type t108 and ST398 which have been shown to be associated with pig farm[139]. In a larger study in the Netherlands, Van den Broek (2009) evaluated the prevalence of MRSA in pig farmers and pigs. The

investigators found that thirty percent of the persons' nasal samples tested were MRSA positive. An interesting finding in their study was that only on farms with either MRSA positive pig or dust samples were persons found to be MRSA carriers[145]. Van de Giessen (2009) sampled black rats on pig farms in the Netherlands and Belgium for the presence of MRSA. Sixty six percent of the farms sampled were found to have MRSA positive for rats and 5 out of 40 black rats were MRSA positive[146].

Compared to Europe, there have been fewer studies in North America evaluating MRSA in pigs and pig workers. In Canada, Khanna (2008) collected nasal swabs from pigs and pig farmers to determine MRSA prevalence in pigs and pig workers. MRSA prevalence in pigs was 24.9% and MRSA prevalence in pig farmers was 20% with the predominant strain in both pigs and pig farmers of the *spa* type *t*034 [136]. Smith (2009) conducted the first study to evaluate MRSA carriage in pigs and pig workers in the US. In their study MRSA prevalence among pigs and pig workers was forty nine percent and forty five percent respectively. The only MRSA strain identified was ST398[138]. MRSA positive pigs have also been identified at a state fair in the US. Dressler (2012) found 15.9% of the pigs tested at a state fair to be *S. aureus* positive and among the *S.aureus* samples identified 8% were MRSA (overall MRSA prevalence in the study was 1.3%) [147]. Additional *spa* types identified in the Dressler (2012) study included t034, t337, t002 and several other *spa* types. Leedom (2011) found MRSA (spa type t034) in the shower facilities of two different pork production systems. A range of three to six percent of the swab samples taken was positive. Pork production workers were surveyed through the National Pork Board database and 3.7% of the pork producers self-reported MRSA infections [148] .

Although there are only a few studies of MRSA carriage in pigs and pig workers in the United States, studies in the US have documented the presence of MRSA in retail meat. Kelman (2011) conducted a study on raw retail meat in the Washington, D.C. and found that 17% of the ground pork tested was MRSA positive[149]. In another study, O'Brien (2012) identified MRSA in retail pork meat in the US. MRSA positive pork samples was 6.6% with the predominant MRSA *spa* type *t*002 and *t*008 at 46.8% and the LA-MRSA *spa* type *t*034 and *t*011 at 26.9% among the MRSA isolates. Hanson (2010) surveyed retail meat products in Iowa and found that 1.2% of the pork meat sampled was MRSA positive with the *spa* types *t*034, *t*008, *t*002 and *t*337 being the common types isolated [150]. Pu (2009) evaluated 30 grocery stores in Louisiana collecting 120 samples of retail meat and identified that 73% of the grocery stories were had *S.aureus* positive samples with 5% of the meat samples being MRSA positive with *spa* types *t*002 and *t*008 [151]. The identification of MRSA in meat products raises concern for the potential MRSA being transmitted via food. With MRSA being detected in food producing animals and in rats living on farms with MRSA positive animals the potential for MRSA to be transmitted to people via direct or indirect contact presents a major challenge to public health.

Shimizu (1997) conducted a study in Japan which identified MRSA positive horses[152]. In a separate study, Weese et al (2005), investigated the occurrence of MRSA in horses in the Netherlands from 2000-2002. The investigators found that seventy-nine out of approximately six thousand horses were MRSA positive[153].

Airborne MRSA inside and outside Swine Buildings

Few studies have evaluated the presence of MRSA inside and outside swine buildings[154,155]. Chapin (2004) was able to find airborne antibiotic resistant bacteria in high levels inside a swine building [156]. Gibbs (2004) also identified airborne antibiotic resistant bacteria inside swine facilities [157]. Studies have also identified airborne antibiotic resistant bacteria downwind of swine facilities. Gibbs (2006) and Green (2006) evaluated the air exhausted from a swine facility and determined that airborne antibiotic bacteria were found downwind of the facility at 150 m[158,159]. The identification of airborne antibiotic resistant bacteria inside and outside swine facilities presents a public health concern to protect swine workers and persons living near swine facilities from the possible airborne transmission of antibiotic resistant bacteria.

With airborne antibiotic resistant bacteria being detected inside swine buildings and downwind of swine facilities it is apparent there is a possible risk of MRSA being transmitted via airborne transmission. In a preliminary study to determine if MRSA can be detected inside and outside a swine facility, I was able to identify viable MRSA isolates using a six stage cascade impactor (Andersen Sampler). This preliminary finding warranted my dissertation research to quantify the concentration of MRSA detected inside a swine building and downwind a swine facility.

Protection Methods for Swine Workers and People living near Swine CAFOs against MRSA

Respirators

With the detection of MRSA in the nasal swabs of persons working inside swine facilities respirators, need to be evaluated for protection for swine workers [138,145]. NIOSH has approved three categories of filtering facepiece respirators based on oil resistance according to 42 CFR Part 84 (Respiratory Protection Devices; Final Rules and Notice. Federal Register, 60 8 June: 110pp. 30335-30398). The non-resistant to oil (N), partially resistant to oil (R) and strongly resistant to oil (P) filter facepiece categories have three level of filter efficiency at 95, 99 and 100[160]. Filtering facepiece respirators (FFR) have been used as respiratory protection devices to protect against inhalation hazards and infection control. Types of respiratory protection devices include surgical masks, dust mask, dust fume mask, N95 respirators and High Efficiency Particulate Air (HEPA) filters[161]. Surgical masks are designed to prevent the wearer of the mask from spreading potential airborne pathogens to persons in their environment. On the other hand dust mask, N95 filters and HEPA filters are designed to protect the wearer from the environment [162].

Several studies have been conducted on the efficiencies of surgical masks and FFRs [161,163,164]. Chen (1994) found that surgical masks and dust masks had efficiencies of ninety seven percent and HEPA filters had efficiencies of ninety nine percent when tested with aerosolized *Mycobacteria chlelonae* [161,164]. McCullough (1997) tested aerosolized *Bacillus subtilis*, *Mycobacterium abscessus* and *Staphylococcus epidermis* and found that surgical masks and dust mask had efficiencies lower than HEPA filters[164]. Studies

have identified N95 respirators as effective means to protect workers from airborne contaminant exposure[163,165]. Qian (1998) tested polydisperse sodium chloride (NaCl) particles, monodisperse polystyrene latex particles (PSL), and airborne bacteria to evaluate the efficiencies of surgical masks (71%), dust masks (82%) and N95 filtering facepiece respirators (96%) and found that N95 filters had the highest efficiency.

Although there is a large body of information on the efficiency of respirators (both viable and non-viable protection), there is no information at the present time regarding the effectiveness of a N95 respirator in the protection of livestock production workers from viable pathogenic organisms. MRSA is an emerging threat in livestock production, where respirators are not commonly worn. A positive result would assist producers in their consideration of the use of respirators by their workers. Presently no study has evaluated the effectiveness of N95 respirators to protect swine workers from airborne MRSA.

Biofilters

The detection of antibiotic resistant bacteria downwind of swine facilities suggests that persons living near swine CAFOs can be potentially exposed to airborne MRSA [159,166]. Biofilters are used as mechanical barriers to clean the air exhausting from swine facilities from airborne particles, odors and gases[167-169]. Two factors aid in the reduction of odors passing through biofilters: 1) the media provide a mechanical barrier to reduce emissions; and 2) the media contain microorganisms which can break down the gases and vapors entering the biofilters. The use of biofilters to reduce emissions of odors, ammonia, hydrogen sulfide and volatile organic/inorganic compounds has been evaluated. In such studies biofilters have been shown to reduce the emissions of odors

and volatile compounds up to 90 percent reduction efficiency[169,170]. Hartung (2001) evaluated several types of filter media for biofilters for odor reduction and found that biochips and coconut peat had a reduction of odor emissions of eighty one percent and wood bark odor reduction of sixty two percent[171]. Chen (2009) evaluated odor, ammonia and hydrogen sulfide reduction efficiencies of two types of woodchips (western red cedar and hardwood) using a pilot biofilter model at a swine facility. It was found that both western red cedar and hardwood chips had high odor reduction efficiency on average of ninety nine percent[170]. Seedorf (1999) evaluated bioscrubbers (artificial media using a biological process generally in a closed vessel) and biofilters and found that biofilters were able to reduce the emissions of airborne particles up to 96 percent, bacteria and fungi up to seventy one percent reduction efficiency. On the other hand the bioscrubber was able to reduce airborne particles up to twenty two percent reduction efficiency [172]. To date the number of studies designed to evaluate the efficiency of biofilters at reducing emissions of airborne bacteria from swine confinement facilities is sparse.

With the potential of MRSA and other antibiotic resistant bacteria being exhausted from the ventilation system of swine CAFOs; the effectiveness of biofilters at mitigating airborne MRSA transmission to communities near swine CAFOs warrants evaluation. Reductions in the airborne emission of MRSA from CAFOs by using biofilters may greatly aid producers in reducing the spread of MRSA on their farm, between farms, and into the environment and the communities surrounding CAFOs.

With little or no information on airborne MRSA inside and outside of swine facilities and the potential effects on persons working in swine facilities and people in

communities near swine facilities my dissertation was designed to address the following questions:

1. Determine the viable airborne MRSA concentration and particle size both inside and downwind over 200 meters from a swine feeding operation
    a. *My dissertation research hypothesis is that MRSA will be present in the air of CAFOs where animal carriers of the organism reside. Furthermore, I hypothesize that MRSA will be present in the air over 200 m downwind from animal feeding facilities that house carrier animals.*
2. Determine the effectiveness of an N95 respirator for protecting workers from airborne MRSA within swine buildings
    a. *My dissertation research hypothesis is that NIOSH approved N95 respirators will be efficient at reducing or eliminating the exposure of airborne transmission of MRSA to persons working inside swine buildings*.
3. Determine the effectiveness of biofilter media for protecting communities near swine facilities
    a. *My dissertation research hypothesis is that biofilters will be efficient at reducing or eliminating the discharge of airborne MRSA in exhaust air from CAFOs housing carrier pigs*.

My overarching aim is to investigate methods to reduce airborne MRSA exposures to workers inside swine facilities, and airborne MRSA contamination of the outdoor environment from confined swine feeding operations where MRSA is present with the potential of being transmitted to neighboring communities via bacterial plume.

CHAPTER II
AIRBORNE METHICILLIN RESISTANT STAPHYLOCOCCUS AUREUS INSIDE AND DOWNWIND FROM A SWINE FEEDING FACILITY

Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an antibiotic resistant bacterium which has developed resistance to currently available beta-lactams and cephalosporin and has emerged as a pathogen of great public health concern[1,11,31,173-175]. Over the past several years, increasing evidence has been generated regarding the presence of MRSA carriage among both livestock and the workers caring for these animals. This research suggests that MRSA is a zoonotic agent and potential pathogen of importance to occupational and environmental health significance[176]. In a recent study in the Netherlands, pig and cattle farmers had increased odds of being carriers of MRSA relative to comparison groups (OR 12.2 and 19.7 respectively). Further, MRSA of livestock origin ("livestock-associated MRSA," or LA-MRSA) has been recognized as causing more than 20% of the cases of MRSA in the Netherlands[143]. Of special note, MRSA occurrences in the study population clustered predominantly around areas of dense pig farms. In another Dutch study, it was found that in farms where there was a high rate of MRSA positive samples from pigs and settled dust inside pig houses, there was also a high MRSA carriage rate among the workers, suggesting that transmission may occur through the environment [145].LA- MRSA has also been detected in healthcare workers in the Netherlands in contact with livestock [177]. This has led to screening of healthcare workers in the Netherlands to determine if they are carriers of MRSA to prevent transmission to patients. MRSA has also been reported to have been transmitted

between cows and humans [178]. This was the first study to isolate MRSA from bovines and a human on the same farm, with the implication of zoonotic transmission. Other studies have identified MRSA in unpasteurized cow milk, suggesting another potential source of MRSA transmission[179,180]. However, additional studies are needed to elucidate the full potential of milk being a reservoir for MRSA.

In addition to the findings of MRSA in pigs and cattle, it has also been identified in poultry. A study of broiler flocks and workers in slaughterhouses in the Netherlands indicated that 5.6% of the personnel were positive for MRSA[181]. In this study broiler hangers were at a higher risk than other personnel in the slaughterhouse. An interesting finding was that MRSA contamination increased in different areas of the slaughterhouse during the production day. In Belgium, a group of investigators were able to isolate LA-MRSA in broiler chickens [130].The finding that MRSA can be found in poultry suggests that MRSA transmission to humans is a potential concern for public health[150].

Although the early work on LA-MRSA was conducted in the Netherlands, more recent studies have revealed LA-MRSA also exists in North America. Khanna (2008) showed that LA-MRSA in pigs correlated with human MRSA carriers on the pig farm. Pig farmers had an LA-MRSA prevalence of 20% and the spa type for MRSA had a similar clone [136]. Smith (2009) was the first group in the U.S. to identify and study human associations with LA-MRSA. In a pilot study, Smith (2009) reported that almost half of the swine workers and pigs studied on two farming systems in Iowa and Illinois were colonized with MRSA[138]. Both LA-MRSA and human MRSA strains have been shown to be associated with animals, however the LA-MRSA strains are predominantly found among livestock and poultry species.

Since veterinarians work in close contact with livestock, they may act as carriers of MRSA. Hanselman (2006) identified MRSA isolates in 23 out of 345 veterinarians' nasal samples from a veterinarian conference. The predominant MRSA strains identified were ST8-MRSA IV and ST5-MRSA II. MRSA isolates from veterinarians in large animal practice were found mainly with the ST8-MRSA IV strain (15/96)[182]. Wulf (2006) conducted a study in the Netherlands and also found MRSA in veterinarians. MRSA identified were *spa* type *t*011, *t*108, and *t*034[183]. In another study, Moodley (2008) found that veterinarians had an occupational exposure risk to MRSA. Veterinarians showed significantly increased odds of MRSA (OR 6, 95% CI: 1.23-54) as compared to persons not professionally exposed to animals. *Spa* types identified were *t*008, *t*020, *t*022, *t*011 and *t*034 [184]. Garcia-Graells (2012) conducted a study of veterinarians in Belgium and Denmark and found that veterinarians had increased risk of MRSA. It was found that 13 out of 16 MRSA isolated were of the multilocus sequence type (MLST) ST398, and the other types identified included ST1, ST5 and ST45. All of the ST398 were found to be resistant to tetracycline. Additionally, veterinarians working with livestock had an odds ratio of 5.6 for LA-MRSA and an odds ratio of 6.7 for total MRSA (ST398 and non-ST398)[185]. Working with livestock appears to present an occupational exposure risk of MRSA.

The detection of MRSA in the nasopharynx of livestock, farmers, veterinarians and others occupationally exposed persons suggests airborne transmission as another possible route of transmission [121].Furthermore, a study conducted in animal feeding operations detected *S. aureus* as the predominant viable bacterial species present in air samples [157]. A later study found that aerosolized *S. aureus* accounted for 76% of viable

bacteria detected within 150 meters downwind from an animal feeding operation [159]. Antibiotic-resistant strains of *S. aureus* were also isolated from air samples taken downwind at dairy cattle feeding operations[186].

A study in Texas suggested that residential houses in the vicinity of livestock facilities may also be contaminated with antibiotic resistant bacteria [187]. Gandara (2006) were able to recover antibiotic resistant bacteria in concentrations higher inside the residential homes than outside the homes. This finding along with the Gibbs (2006) detection of antibiotic resistant bacteria being recovered 150 m downwind of swine feeding operations illustrates the potential of the transmission of antibiotic resistant bacteria from swine feed operations to communities nearby [158].

To date in the literature there is no study evaluating the airborne concentration and particle size of MRSA inside a swine facility and downwind of a swine facility over 200 meters. The main objective of this study was to determine the viable airborne MRSA concentration and particle size both inside and downwind over 200 meters from a swine feeding operation. The findings of this study can help determine if the present separation distances of swine facilities from residential areas are adequate.

Materials and Methods

Sample site

The study site was selected as it was representative of modern swine production facilities, and we had previously documented that the workers and swine at the facility were culture positive for MRSA[138]. The producers were willing to cooperate for this study, and informed consent was obtained and all requirements of IRB were followed. The veterinarian for the facility was helped facilitate the study, providing consultation in

the conduct of sampling at the facility. The study site consisted of two buildings and produced approximately 48,000 feeder pigs/yr. Pigs entered the buildings at 14 days of age and left at the age of 60 days and weighing approximately 50 lbs. The stocking density of the two buildings was one pig per 4 $ft^{2.}$.

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

Air sampling

The concentration of viable MRSA and total particulates inside the swine feeding facility and 215 meters downwind of the facility was determined by collecting air samples using a six stage viable Andersen Cascade Impactor (Andersen Sampler In., Atlanta, GA, USA) and a Optical Particle Counter (GRIMM Technologies Inc., Douglas, GA, USA). The same instruments were used in the study for quality assurance and quality control.

The basic principles of bio-security protocol (standard practices to keep disease out) of the facility management was practiced throughout the facility study [188]. Prior to and before entry into the buildings, all of the sampling instruments were disinfected either by autoclave, or by spraying with 70% ethanol. All sampling instruments were

calibrated before field sampling. The Q-Trak and VelociCalc were sent to the manufacturer for calibration prior to the study using the methods outlined in Edimansyah et al 2009 [189]. The Optical Particle Counter (OPC) was calibrated at a flow rate of 1.2 liters per minute (LPM) each day prior to sampling [190]and the vacuum pump for the six stage Andersen Cascade Impactor was calibrated at a flow rate of 28.3LPM according to the methods of Middendorf (2001) with the Sensidyne Gilibrator ™ (Clearwater, Fla.) [191].

Air samplings were collected on three days, November 9, 10 and 11 of 2010. The sampling dates were chosen based on having access to the facility. Based on preliminary studies, sampling times inside the facility were set at 30 seconds, 1 minute and 3 minutes. Sampling was conducted in triplicate, i.e. sampling was done three times, at each sampling time to increase data reliability. Air sampling instruments were set at a height of 1.3 m on a table inside the animal feeding operation (see figure 2). Sampling locations inside the swine facility were conducted inside an empty pen in the middle of the building. Selective media used for MRSA isolation were CHROMagar plates (Becton, Dickinson and Company, Sparks, MD, USA). The plates were placed on stage one, two and five of the Andersen Cascade Impactor to collect aerosolized MRSA.

Outside environmental conditions were measured including temperature, relative humidity, carbon monoxide (CO) concentration, carbon dioxide (CO_2) concentration using the Q-trak instrument (TSI, Inc., Minneapolis, MN, USA), air velocity using the Velocicalc Air Velocity Meter (TSI, Inc., Minneapolis, MN, USA), and solar intensity (RadioShack Auto Range Multimeter, RadioShack Corp., Forth Worth, TX, USA). Solar intensity and wind speed were used to determine atmospheric stability classes (categories

of the stability of the atmosphere which can influence plume dispersion). Outside the animal feeding operation, sampling was conducted at a distance of 215 meters downwind from the animal feeding operations on a table at a height of 1.3 m with sampling times set at 3 minutes, 5 minutes and 10 minutes. The distance of 215 meters was chosen due to limitation of energy source beyond 215 meters. Additionally, wind direction downwind of the swine facility and boundary limitations was used to determine sample collection location.

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

Bacterial diagnostics

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

Molecular typing

Additional molecular diagnostics were performed on a random selection of isolates. Molecular diagnostics conducted included antibiotic susceptibility testing (CLSI 2006 and CLSI 2009), *mec*A PCR [192], *spa* typing [193], and PVL PCR [22]. Positive and negative controls were used for all tests.

Results

The meteorological conditions for the sampling days are shown in Tables 1 and 2. Table 1 shows the results for atmospheric conditions outside the facility on the sampling day. Sampling day three had the lowest temperature, wind speed and solar intensity readings. This sampling day had the highest relative humidity and the most unstable atmospheric stability class. Atmospheric stability decreased as solar intensity, wind speed and temperature decreased.

Table 2 lists the average measures inside the swine facility for temperature, relative humidity, CO and CO_2. The average measures were the highest on sampling day three. Sampling day one had the lowest relative humidity (61.9%) and CO_2 (1589 ppm). As the relative humidity increased on each sampling day, the concentration of CO_2 also increased. Both the concentration of CO and CO_2 increased on sampling day three from their levels on sampling day two.

Table 3 shows the median and range for total particles detected with the GRIMM Dust Particle counter. Non-respirable particles and respirable particles were measures to determine the concentration of particles inside relative to the concentration of particles downwind of the facility. It was noted that other potential sources of outdoor particles

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

The sampling time of 30 seconds resulted in the highest mean concentration of total viable particles in the non-respirable range (particles > 5um) at 23,191 cfu/m^3 on sampling day three inside the swine facility (Table 4). Sampling day two sampling time of 30 seconds resulted in the highest mean concentration of total viable particles in the non-respirable range at 47cfu/m^3 downwind from the swine facility. The lowest mean concentration of 1cfu/m^3 for total viable particles in the non-respirable size range with a sampling time of 600 seconds occurred on sampling day three. The respirable particles (<5um) inside the swine facility ranged from 11.6 x 10^3 cfu/m^3 to 15.9 x10^3 cfu/m^3 with the highest mean of 13.8 cfu/m. Downwind of the facility the concentration of total viable respirable particles (<5 um) ranged from 15 cfu/m^3 to 111 cfu/m^3, with the highest mean of 63 cfu/m^3. The non-respirable MRSA particles (> 5um) inside the swine facility ranged from 547 cfu/m^3 to 1,103 cfu/m^3, with the highest mean of 825 cfu/m^3. Respirable MRSA particles inside the swine facility ranged from 74 cfu/m^3 to 302 cfu/m^3, the highest mean concentration 188 cfu/m^3. Downwind of the facility respirable MRSA particles was detected with the highest mean concentration of 5 cfu/m^3. A general

trend with MRSA particles recovered was that as time increased the concentration of MRSA particles recovered decreased.

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

Table 7 shows the results for antibiotic susceptibility testing and molecular typing. Twelve isolates (100 percent) were resistant to methicillin; 8/12 (67 percent) were resistant to tetracycline and clindamycin, and 4/8 (33 percent) were resistant to erythromycin. All the isolates were *mec*A PCR and *spa* PCR positive and 1/12 (0.08 percent) were PVL PCR positive. *spa* type identified were *t*034, *t*5706 and *t*008.

Discussion

Our results show that viable MRSA can be detected at both the non-respirable size range (> 5um) and respirable size range (< 5um) inside, as illustrated in figure 1, and downwind of a swine feeding facility. This study utilized a swine facility where the swine workers and swine have been previously confirmed to be MRSA positive [138]. Our results indicated that the highest concentration of viable airborne MRSA at the study site was from within the swine facility.

In this study, meteorological and environmental conditions outside and inside the swine facility affected the concentrations of viable particles and non-viable particles detected. We observed that as relative humidity increased, the concentration of the total particulates (viable and non-viable) increased inside and downwind of the swine facility. Increased relative humidity is known to affect the size of particles through adsorption of

water molecules on the surface of airborne particles. We hypothesize that as the size of particles increased due to hydroscopic growth; the large particles were deposited in the cascade impactor as a result of impaction and remained viable for longer time periods as compared to particles with decreased relative humidity. As outside temperature decreased, the detected concentration of particles downwind of the swine facility increased. The increased particle concentration as a result of decreased temperature may have been due to condensation of water on particles in the air leading to their detection with the samplers.

In this, study higher wind speeds corresponded to lower concentrations of viable particles. We speculate that wind speed affected the dispersion and dilution of particles in the air. We further speculate that increased wind speed led to the particles being mixed and dispersed further from the facility, albeit in lower concentrations [155]. We found that solar intensity was related to the relative concentration of viable and non-viable bacterial particles in the following ways: 1) decreased solar intensity was associated with lower concentrations of both total viable and total non-viable particulates; and 2) increased concentrations of total viable MRSA particulates increased with solar intensity. In a previous study, solar intensity appeared to affect the concentration of viable particulates detected downwind of a sample point speculatively due to inactivation of the microbe by the effects of the sun with increased travel time [87]. We speculate that increased solar intensity can lead to the dehydration of airborne particles in a bacterial plume and lead to a decrease in the size of the particle. As the particle size decreases it can travel farther from the source of the sample before impaction occurs. Thus wind speed and solar intensity have a dynamic relationship with MRSA plume dispersion.

Wind speed and solar intensity were used to determine the atmospheric stability class. High wind speeds, and increased solar intensity create unstable atmospheric conditions resulting in increased dispersion of respirable sized particles. We speculate this was associated with being able to detect MRSA 215 meters downwind of the swine facility (the furthest isolations reported to date).

We found that inside the swine facility the concentration of CO_2 increased as the relative humidity increased. The concentration of CO_2 inside swine buildings is due to the number of animals and the ventilation rate of the exhaust system. Increased CO_2 levels inside the facility from the respiration of the pigs potentially due to increased activity may have led to increased water content in the air and hence increased the relative humidity. The presence of CO suggested a point source for the emission of CO [11]. A propane gas heater inside the facility was the likely source of the CO detected.

We showed that the concentration of non-respirable MRSA particulates were higher than the respirable MRSA particulates inside the swine facility (Table 9). On the other hand, viable MRSA particles detected downwind of the swine facility showed a higher concentration of respirable particles (< 5um). It is speculated that the non-respirable particles inside the facility may have resulted from dust and feed, whereas the respirable particles including MRSA detected downwind may be of pig origin (dander, dried fecal matter or epithelial cells). The viable MRSA particles were detected at 215 meters away from the swine facility , a distance farther than the Gibbs' (2006) recommended separation distance of 200 meters from residential homes [158,159]. According to Iowa Code Law for Confined Animal Feeding Operations[194] summarized in table 6, there is no current separation distance requirement for the swine facility tested.

The swine facility tested had an AU of 400 for which the Iowa Code Law does not have a separation distance for any swine facility with less than 500 AU. With the detection of MRSA at least 215 m downwind of the swine facility this distance is only 14 m less than what is required for CAFOs built prior to 1999. The separation distances for swine CAFOs built between 1999 and 2003 is less than 90 m from the distance of MRSA detected downwind from the swine facility tested. The results of MRSA detected 215 m downwind of the swine facility indicate are within the present Iowa Code Law for separation distances albeit very close to the required Iowa Code Law. It is noted that the separation distances for swine CAFOs vary from state to state.

We determined that as sampling time increased the detection of viable MRSA particle decreased. The inverse relationship of sampling time and detected viable MRSA particles observed possibly resulted from desiccation of MRSA particles. As the particles were impacted on the media and air was pulled through the Cascade Impactor the viability of the MRSA decreased due to the loss of water content. This study indicated that the optimal sampled time of 30 seconds inside a swine CAFO and 3 minutes downwind for airborne viable MRSA particles with the Andersen Cascade Impactor.

We speculate that the activities of the pigs and decreased ventilation led to increased CO_2 levels which correlated with the respirable MRSA particles detected. It is plausible that increased pig activity may have increased the release of MRSA particles from the floor and pig feed. Our study also found that when the swine CAFO was emptied and disinfected, no MRSA particles were detected in the air. This finding suggested pigs and other factors associated with their activities can influence the

detection of airborne MRSA in swine feeding facilities and that the all-in, all-out method and disinfecting the swine buildings could reduce airborne MRSA.

We found that all isolates identified were resistant to methicillin which was similar to results of Dressler (2012)[147]. Animal feed were the only isolates which showed susceptibility to tetracycline and clindamycin. Furthermore, all of the animal feed tested was resistant to erythromycin. Cavaco (2011) showed that metal supplements such as zinc oxide and copper sulphate used as supplements in animal feed may promote selective pressure for MRSA emergence[195]. We also found *sp*a types *t*008 and *t*034 which have been found in other studies to be associate with MRSA in veterinarians and pig farming[184,196,197]. A unique *spa* type *t*5706 was also identified in air samples inside the swine facility and exhausted from the swine facility. These findings suggests that MRSA associated with livestock and human colonization are similar to *spa* types of airborne MRSA

Our study showed results similar to Gibbs (2004) [157] and Green (2006) [159]. Gibbs (2004) also showed that antibiotic resistant bacteria in the respirable and non-respirable size ranges were recovered inside swine buildings and downwind of swine facilities. Gibbs used a control facility (without pigs) to determine that the source of microorganisms detected were the pigs. We were able to detect MRSA particles on various stages of the six stage Andersen sampler which indicated that MRSA possibly originated from multiple sources inside the swine feeding facility. Donham (1986) showed that smaller particulates in swine buildings were from animal source such as dried fecal matter and the larger particulates were from feed material [198].We speculate that MRSA particles detected in the respirable size range originated from the pigs

(dander, dried fecal matter and epithelial cells) and MRSA particles in the non-respirable size range originated from feed and dusts. The sizes of MRSA particles detected inside the swine building were similar to the size of particles in other studies causing respiratory symptoms.

Donham (1989) reported respiratory symptoms such as colds, chest illness and pneumonia due to respirable dust particles, total dust particles and the endotoxin in total dust particles [199]. Andersen (2004) reported similar respiratory symptoms which were also associated with increased number of hours worked inside a swine CAFO [200]. Green (2006) showed that antibiotic-resistant *S. aureus* can be recovered from the air exhausted from swine CAFOs at distances of 150 m. In our study we recovered viable airborne MRSA at 215 meters downwind of a swine CAFO. This suggested that smaller respirable particles, of pig origin, can be transmitted downwind and potentially to communities nearby swine feeding facilities. It is further suggested that people within the radius of 215 meters of a swine CAFO can also be impacted by airborne viable MRSA particles. In addition to respiratory symptoms, airborne viable MRSA particles can potentially lead to MRSA infections of persons working on the farm outside of swine buildings or nearby communities. The detection of airborne viable MRSA particles downwind of the swine facility suggests that a comprehensive MRSA surveillance program should include both air sampling and collecting nasal swabs from workers on swine farms.

MRSA detected in the nasal passages of a swine worker and in the air may suggest contamination rather than biological colonization which can assist in control measures for the worker and the swine facility. In terms of contamination versus colonization, MRSA contamination refers to a short term occupational or environmental

exposure which leads to the nares being initially MRSA positive, but after removal from the exposure for a time period becoming MRSA negative, whereas MRSA colonization refers to a person persistently having their nares colonized even after removal from the exposure for a period of time due to replication of the organism in the host [201]. This suggests implementation of a respiratory program to protect swine workers from occupational and environmental contamination of nasal passages and the use of a ventilation system which can dilute the air inside the swine building and a biofilter system to remove MRSA from the exhaust ventilated air.

This study had several strengths. It was the first study to evaluate respirable and non-respirable dust particles with the OPC and the six stage Andersen Cascade Impactor. The OPC allowed real time dust counts to be performed and the Andersen Cascade Impactor allowed viable dust particles to be recovered and cultured. The six stage Andersen sampler was able to indicate the different potential dust particle sources and location within the respiratory tract where viable MRSA can be deposited. This information helps to define potential health risk and location of respiratory illness. This study also detected viable MRSA further downwind of a swine facility than any prior studies. This study also indicated that when a swine CAFO is power washed and disinfected with biocides, airborne viable MRSA and total viable particles can be reduced to prevent potential transmission of antibiotic-resistant bacterial infections. Our study was conducted at a facility which our group had identified to have swine workers and swine to be carriers of MRSA[138].

The study also had limitations. Our study was based on sampling one swine farm. This prevents generalization of results to other farms, especially in different geographical

locations dissimilar from the settings in our study. Additionally, we tested over a few days during the fall season. However, the results of the regression models for both respirable and non-respirable viable MRSA particles indicated there are independent variables that are associated with concentrations of MRSA that were statistically significant and not due to chance.

Our study indicated that further studies need to be done to identify various potential sources of airborne MRSA inside buildings other than the pigs. For example, a future study to evaluate animal feed as an additional source of MRSA needs to be conducted. Identification of animal feed as a potential source of MRSA can assist swine producers and swine veterinarians in decision making relative to the use of and type of antibiotics to use for livestock production and health purposes. Additionally, further studies are needed to further elucidate whether MRSA is an occupational or environmental problem related to swine production. Furthermore, a study to determine the effectiveness of N95 respirators at reducing the amount of MRSA particles a worker in a swine CAFO can inhale needs to be assessed. With viable MRSA being detected downwind of the swine CAFO, a study to evaluate the ability of biofilters at reducing MRSA particles from being exhausted from a swine CAFO needs to be assessed.

Conclusion

This study supports earlier studies that airborne viable MRSA can be detected inside swine CAFOs. The concentrations of viable MRSA particles suggested a potential occupational health risk to swine workers. The identification of airborne MRSA suggests that hygienic controls such as hand washing as used in hospital settings for infection control may not be adequate in agricultural settings. Inside a swine facility, respiratory

protection devices such as N95 respirators needs to be assessed to determine its effectiveness to provide protection to workers from airborne MRSA and act as an infection control method. Furthermore, viable MRSA particles were detected at 215 meters downwind of the swine facility, the farthest downwind detection published to date. This finding can be used to aid community health prevention in areas near swine CAFOs. Engineering controls associated with the ventilation system needs to be evaluated to determine its effectiveness to mitigate emission of airborne MRSA to nearby communities. With the detection of MRSA less than 90 m downwind of the separation distance for swine CAFOs built prior to 2003 in Iowa, further studies are warranted to determine if MRSA can be detected beyond the separation distance of swine CAFOs built prior to and after 2003. Additionally, MRSA concentration was identified to be related to the environmental conditions inside the swine facility such as CO_2, CO and temperature. Airborne MRSA *spa* types *t*034 and *t*008 found in this study was similar to MRSA *spa* types identified in other studies associate with nasal colonization in farm workers, veterinarians and animals. This finding suggests airborne transmission as a route for MRSA dissemination in farm settings. Our findings showed that swine workers and people living near swine facilities are at risk for inhaling airborne MRSA particles.

Table 1. Meteorological conditions outside (taken between 9am and 12pm)

Sampling Date	Temperature (°F)	Relative Humidity (%)	Wind Speed (mph)	Solar intensity (w/m^2)	Wind Direction	Atmospheric Stability Class
11/9/2010	67	48.5	6-12	515	S	B-C
11/10/2010	67.8	61.9	8-14	519	SSE	B-C
11/11/2010	51.9	72.6	2-5	490	WNW	A-B

Table 2. Conditions inside barn

Sampling Date	Temperature (°F)	Relative Humidity (%)	CO (ppm)	CO_2 (ppm)
11/9/2010	81	61.9	1	1589
11/10/2010	75.2	70.2	0	2357
11/11/2010	89.8	74	1	4362

Table 3. Particles detected with GRIMM Dust Particle Counter

	Non-respirable (particles > 5 um)		**Respirable (particles < 5 um)**	
Sampling Date	Inside M (R) (particles/liter)	Downwind M (R) (particles/liter)	Inside M (R) (particles/liter)	Downwind M (R) (particles/liter)
11/9/2010	278 (80-619)	2 (0-31)	720 (307-21250)	150 (0-15,679)
11/10/2010	325 (84-920)	2 (0-60)	705 (234-42150)	332 (0-120,927)
11/11/2010	1,000 (106-3095)	6 (0-6238)	2470 (1170-1,119,0093)	363 (10-42,738)

*Downwind distance 215 meters.

* M= median, R= range

Table 4**.** MRSA and total viable particles recovered with N-6 Andersen Sampler

	Non-respirable (particles > 5um)						**Respirable (particles < 5um)**					
Sampling Date	Inside (M/SD) (cfu/m^3)			Downwind (M/SD) (cfu/m^3)			Inside (M/SD) (cfu/m^3)			Downwind (M/SD) (cfu/m^3)		
	Time (sec)			Time (sec)			Time (sec)			Time (sec)		
	30	60	180	180	300	600	30	60	180	180	300	600
Total viable particles												
11/9/2010	15276± 11943	3003± 4668	3906± 559	39± 38	24± 31.50	10± 18	12508± 10858	3498± 6059	3647± 453	63± 48	61± 43	0± 0
11/10/2010	16489± 3999	10995± 1739	3829± 479	47± 61	19± 16	16± 14	13757± 2136	9222± 2594	3824± 529	27± 18	31± 35	29± 11
11/11/2010	23191± 2061	612. ± 434	363± 133	4± 6	2± 4	1± 2	471± 163	188± 114	255± 54	4± 7	5± 8	0± 0
MRSA												
11/9/2010	0± 0	6± 14	29± 24	0± 0	2± 6	0± 0	47± 41	24± 41	31± 7	0± 0	5± 8	0± 0
11/10/2010	318± 322	412± 368	192± 108	0± 0	0± 0	2± 2	188± 178	106± 0	86± 59	0± 0	0± 0	1± 2
11/11/2010	825± 278	612± 434	363± 133	4± 6	2± 4	1± 2	471± 163	188± 114	255± 54	4± 7	5± 8	0± 0

*Downwind distance 215 meters

**M= mean, SD= standard deviation

Table 5. MRSA concentration after swine building emptied and disinfected

	Non-respirable (particles > 5um (cfu/m^3)			**Respirable (particles < 5um) (cfu/m^3)**		
	Time (sec)			Time (sec)		
	30	60	180	30	60	180
Total viable particles	2261	459	2826	353	282	2190
MRSA	0	0	0	0	0	0

Table 6. Separation distances for swine CAFOs in Iowa

Year Built	Animal Units	Incorporated Regions	Unincorporated Regions
Prior to 1/1/1999	501 AU to < 625,000 lbs	750 ft (228.6 m)	1,250 ft (381 m)
	625,000 to < 1,250,000 lbs	1,000 ft (304.8 m)	1,875 ft (571.5 m)
	≥ 1,250,000 lbs	1,500 ft (457.2 m)	2,500 ft (762 m)
Between 1/1/1999 to 3/1/2003	501 AU to < 1,600,000 lbs	1,000 ft (304.8 m)	1,250 ft (381 m)
	1,600,000 lbs to < 4,000,000 lbs	1,250 ft (381 m)	1,875 ft (571.5 m)
	≥ 4,000,000 lbs	1,875 ft (571.5 m)	2,500 ft (762 m)
After 3/1/2003	501 AU to < 1,000 AU	1,875 ft (571.5 m)	1,250 ft (381 m)
	1,000 AU to < 3,000 AU	2,500 ft (762 m)	1,875 ft (571.5 m)
	≥ 3,000 AU	3,000 ft (914.4 m)	2,375 ft (723.9 m)

Table 7. Antibiotic Susceptibility Testing and Molecular Typing of isolates

Sample #	**Sample Collected from**	**Oxacillin**	**Tetracycline**	**Clindamycin**	**Erythromycin**	***mec*A PCR**	**pvl PCR**	***spa* PCR**	***spa* types**
1	Inside Room	>16	>16	>8	0.5	Positive	Negative	Positive	t034
2	Feed in room	>16	<=0.12	0.12	>32	Positive	Negative	Positive	t034
3	Truck feed	>16	<=0.12	<=0.06	32	Positive	Negative	Positive	t034
4	Feed in room	>16	<=0.12	<=0.06	>32	Positive	Negative	Positive	t034
5	Truck feed	>16	<=0.12	<=0.06	32	Positive	Negative	Positive	t034
6	Inside Room	>16	>16	>8	0.5	Positive	Negative	Positive	t034
7	Inside Room	>16	>16	>8	0.5	Positive	Negative	Positive	t034
8	Inside Room	>16	>16	>8	0.5	Positive	Negative	Positive	t5706
9	Wall Exhausted Air	>16	>16	>8	0.5	Positive	Negative	Positive	t5706
10	Pit Fan Exhausted Air	>16	>16	>8	0.5	Positive	Negative	Positive	t034
11	Wall Exhausted Air	>16	>16	>8	0.5	Positive	Negative	Positive	t034
12	Pit Fan Exhausted Air	>16	>16	>8	0.5	Positive	Positive	Positive	t008

Figure 1. MRSA Size Distribution inside Swine Facility

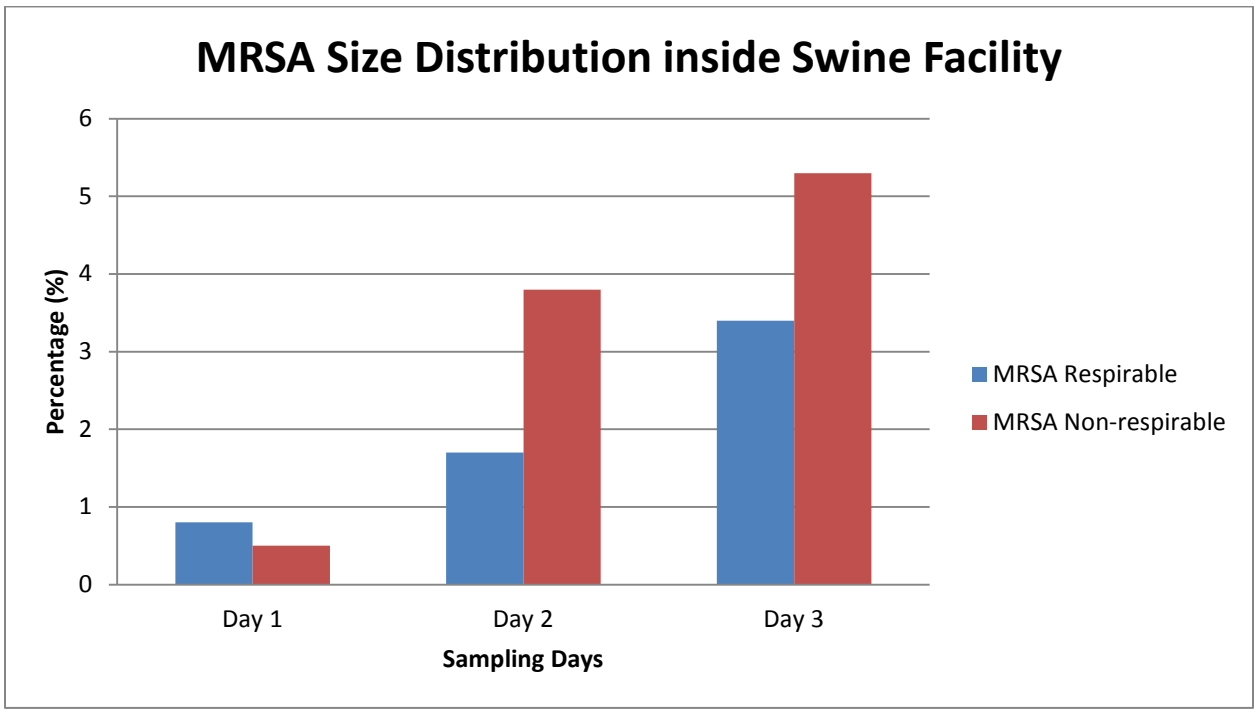
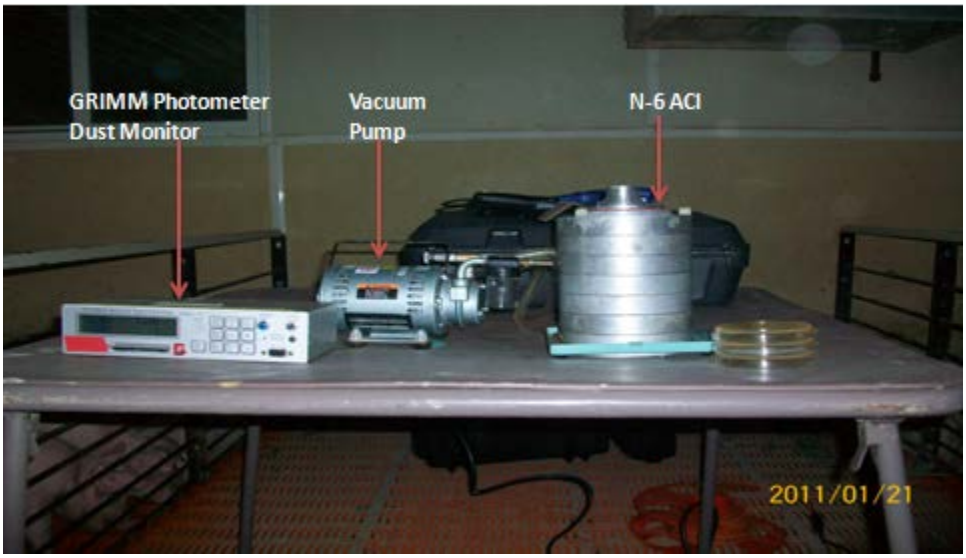


Figure 2. Air sampling inside swine feeding facility



CHAPTER III
THE EFFICIENCY OF THE N95 RESPIRATOR AT PROTECTING ANIMAL WORKERS FROM EXPSOURE TO AIRBORNE MRSA

Introduction

Numerous research publications have documented a variety of aerosol exposures which resulted in respiratory symptoms in producers and workers in swine buildings. Aerosolized particulate is one of those hazardous exposures. Animal feeding operations have been found to have high concentrations of dust particles. Dust particles in swine feeding facilities consist of predominantly organic material such as pig dander, animal feed, feces, fungi, bacteria and gases[198,202-204]. High levels of bioaerosols in swine feeding facilities have been linked to animal and human activities. The activity of feeding pigs has been identified to increase exposure to airborne dust to swine workers[205,206]. Bioaerosols inside swine feeding facilities can lead to potential respiratory health hazards[202,207]. Respiratory symptoms or conditions such as non-allergic asthma, organic toxic dust syndrome and bronchitis have been identified in swine workers[199,200,203].

An additional potentially hazardous exposure is antibiotics that are added to the feed or water. They are often added in growing animals at sub-therapeutic levels for the economic advantage of increased rate of gains feed efficiency and possibly disease prevention. However, using antibiotics in feed can present a risk of using antibiotics in feed of unintended environmental consequences. Antibiotics have been detected in the air in swine facilities[208,209]. This suggests the possibility of the development of antibiotic resistant organisms in this environment and the potential adverse health effects of resistant infections on swine and swine workers [210]. Antibiotic resistant bacteria have

been detected in the nasal passages of swine workers[138,211]. Methicillin resistant *Staphylococcus aureus* (MRSA) has been identified as a zoonotic pathogen occurring in swine feeding facilities with swine workers, veterinarians and swine as carriers[148,212]. Infections caused by hospital or community acquired MRSA include upper respiratory infections, pneumonia, skin lesions and nosocomial infections[135,213,214]. MRSA can spread through the environment by direct contact between swine workers and swine, contact with fomites, and through airborne transmission [121]. The spread of MRSA via airborne transmission in swine facilities presents a respiratory hazard to swine workers and veterinarians[121,138,148].

The National Institute of Occupational Safety and Health (NIOSH) approved a standard to regulate the testing, certification and use of respiratory devices to protect workers in environments where the source of inhalation hazards are unable to be engineered out of the air [215]. NIOSH has designated three types of face filtering respirators (FFR), not resistant to oil (N), resistant to oil (R) and oil proof (P). The three types are certified at classifications of 95, 99 and 100 which represent 95%, 99% and 99.97% filtering efficiencies. The FFRs are pretreated at and tested under various conditions to simulate working conditions[216]. Some of the conditions include testing at 38 degrees and 85% relative humidity for forty two days[215]. To improve the efficiency of the FFRs fit testing is also required by the NIOSH standard [215]. N95 filtering face respirators and surgical masks have been used for infection control in hospital settings. Surgical masks are used to protect patients from inhalation hazards from healthcare workers (HCW) and N95 respirators are used to protect the wearer from inhalation hazards from the environment[161-164].

To help protect workers in swine feeding facilities from airborne transmission of MRSA, a mitigation program protecting workers from aerosolized substances needs to be implemented. Although source control is the best approach, a respirator or personal respiratory protective device (RPD) may need to be used as an adjunct to source control, and may be the only protection perceived as possible and affordable by swine producers. When recommending and selecting a respirator for this purpose, one needs to choose an efficient respiratory protection device (RPD). Two strapped N95 dust filtering respirators have been identified as an effective RPD to help prevent exposure to airborne contaminants including infectious agents. [163,217,218] Although respirators for use in swine production have been evaluated for effectiveness in protection from dusts, there has not been an evaluation of respirator efficiency for protection against infectious agents in swine buildings [219]. The purpose of this study is to evaluate the efficiency of N95 filtering face respirator to protect against MRSA exposure in a swine feeding facility. It is hypothesized that the N95 filtering face respirator will have an efficiency of at least ninety five percent.

Materials and Methods

The efficiency of the N95 respirator was determined first by testing in the laboratory in an exposure test chamber. After the test chamber was refined in the laboratory it was taken to the swine facility where air was sampled gravimetrically and by particle counts before and after flowing through the N95 respirator.

Sampling site

The study site was selected as it was representative of modern swine production facilities, and we had previously documented the workers and swine at the facility were culture positive for MRSA[138]. The producers were willing to cooperate for this study, and informed consent was obtained and all requirements of IRB were followed. The veterinarian for the facility helped facilitate the study, providing consultation in the conduct of sampling at the facility tested. The study site facility consisted of two buildings and produced approximately 48,000 feeder hogs/yr. Pigs entered the site at 14 days of age and leave at the age of 60 days and weighing 50 lbs. The stocking density of the two buildings was one pig per 4 ft^2.

Ventilation for the facility was provided by sixteen 24" and eight 14" wall fans (both thermostat controlled) and eight 9" continuous pit fans. The facility had double-sided curtain for increased ventilation during warm seasons. The volume of the study room was 12847 ft^3. The sampled facility was power washed with detergent and biocide between cycling of hogs due to the all in all out nature of the site. Topography of the area surrounding the facility was flat without any wind buffers.

Exposure test chamber

We refined the N95 respirator exposure test chamber at the Environmental Modeling and Exposure Assessment Facility at the Institute for Rural and Environmental Health, the University of Iowa. The respirator exposure test chamber was a modified version of a test chamber which had been used in a previous pilot study [220].The following describes the laboratory set up and is depicted in figures 5 and 6. N95 respirators (Model

7130N95, North by Honeywell, Cranston, RI) were placed between two polymethyl methacrylate covers and placed inside a dust chamber with a metal tube (1/4" outer diameter) inserted at the back of the chamber. The N95 respirator was attached to this metal tube. An inlet tube was placed inside this metal tube to sample airborne particles which passed through the N95 respirator (filtered air). A second inlet tube was inserted through the back of the test chamber and was positioned next to and in front of the N95 respirator to sample unfiltered air in the test chamber. Tygon tubes were attached to both sampling air inlets (filtered and unfiltered) which were connected to a six stage viable Andersen Cascade Impactor (N-6 ACI) (Andersen Sampler Inc., Atlanta, GA, USA) at a flow rate of 28.3 liters per minute (LPM) which sampled unfiltered air and a second N-6 ACI sampled filtered air. The Tygon tube was also ported to an OPC (GRIMM Technologies Inc., Douglasville, GA, USA) which sampled unfiltered air and to a second OPC which sampled filtered air [221]. Air was pulled through the sample inlets in the test chamber at 85 LPM flow rate via a stationary air mover (a work shop Vacuum) which was monitored by an inclined-vertical manometer and a Venturi flowmeter (Dwyer Instruments Inc., Michigan City, IN, USA). Arizona Test Dust Fine, ISO 12103-1 A2 (Powder Technology Inc., Burnsville, MN, USA) in the size range of 1 um to 10 um were used to represent the size distribution of *S. aureus* and was aerosolized using a Wright Dust Feeder (BGI, Waltham, MA, USA) in the dust chamber to simulate airborne particles. The OPC and the N-6 ACI were set at a sampling rate of 1.2 LPM and 28.3 LPM respectively and monitored with the digital manometer (Dwyer Instruments Inc., Michigan City, IN, USA). Viable airborne particles were collected with the N-6 ACI and deposited on CHROMagar plates (stages one, two and five) in triplicate runs. At the end

of the preliminary test the test chamber was taken into the field for sampling. All instruments were calibrated according to manufacturer instructions.

For use in the field, the chamber (figures 7 and 8) was modified. The test chamber was then placed in the center of swine facility sampled (in an empty pen to prevent damage from the hogs) at a height of 1.3 m from the ground within the "breathing zone" region. Samples downstream (filtered) the respirator were taken at 15 and 20 minutes to account for low concentration (cfu/m^3) counts, whereas samples from upstream (unfiltered) the respirator were taken at 30 and 60 seconds to account for the expected high concentration (cfu/m^3) of particles. A commercial air mover (vacuum cleaner designed for use in workshop) provided a constant flow rate of 85 LPM of pulled air through the N95 respirator and was monitored by a manometer. Flow rate for the viable N-6 ACI was set to 28.3LPM and the OPC was set at 1.2 LPM. Each sampling time was conducted in triplicate for data reliability. The count per liter of particles determined by the OPC was stored on the instrument and was analyzed at the laboratory for efficiency percent. The CHROMagar culture plates were sealed with tape, labeled, placed in a Ziploc bag and finally placed (upside down) into a cooler with ice packs for transport to the laboratory. Environmental conditions such as of temperature, relative humidity, CO concentration, CO2 concentration were measured using the Q-trak instrument (TSI, Inc., Minneapolis, MN, USA), air velocity were measured using Velocicalc Air Velocity Meter (TSI, Inc., Minneapolis, MN, USA).

Bacterial diagnostics

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

Results

Efficiency of a respirator is defined the ratio of the difference of the concentration of MRSA particles in unfiltered air outside the respirator and concentration of MRSA particles in filtered air divided by the unfiltered particles. The efficiency is reported as percentage.

Efficiency = (unfiltered particles – filtered particles)/ (unfiltered particles) * 100

Figure 3 shows the laboratory trial results for the N95 respirator using the OPC. The N95 respirator had efficiencies greater than 98.61 percent for Arizona dust particles. Table 8 shows the field results for the efficiency of the N95 respirator for viable MRSA particles using the N-6 ACI. Only 0.75 percent of MRSA particles with mean size of 5.85 µm penetrated the filter media resulting in an efficiency of 99.25 percent.

The field results for the efficiency of the N95 respirator for dust particles using the OPC for the N95 respirator are shown in figure 4. The mean particle size of 0.45 µm

had a penetration of 50.66 percent and 49.34 percent efficiency. The mean particle size of 1.8 µm had a penetration of 27.42 percent and 72.57 percent efficiency. Particles above the mean particle size of 2.5 µm had N95 respirator efficiency greater than 93.52 percent.

Discussion

The study results showed that the N95 respirator had efficiency greater than 99 percent with the six stage Andersen Cascade Impactor for viable MRSA particles. This result indicated that the N95 respirator filtered out over 99percent of viable MRSA particles. With an effective seal, the N95 respirator is capable of providing protection. This finding is significant to help justify a respiratory protection program in swine facilities to prevent the transmission of airborne MRSA to workers. Both respirable and non-respirable viable MRSA particles were reduced from being inhaled. In addition to the results from the Andersen Cascade Impactor, the OPC was used to determine the efficiency of the N95 respirator for non-viable MRSA particles. The OPC identified that particles smaller than 4 µm (respirable size range) had efficiencies less than 95percent efficiency. Furthermore it was identified that N95 respirator had efficiency less than 50percent for particles with a mean diameter of 0.45 µm. It was shown that as the particle mean diameter increased, the efficiency of the N95 respirator also increased. These findings indicated that the size of the particles affected the efficiency of the N95 respirator.

Respiratory protection devices (RPD) such as surgical masks and N95 respirators have been evaluated for their performance and efficiency for infection control in hospitals. Chen (1994) assessed the efficiencies of surgical masks, dust mist respirators (DM), dust mist fume respirators (DMF) and HEPA respirators challenged with

aerosolized viable *Mycobacterium chlelonae* (0.5µm in size) and latex spheres (0.804 µm in size)[161]. The results showed that the surgical masks had a mean efficiency of 97%, DM and DFM respirators had efficiencies of 99% and 99.9% respectively and HEPA respirators had a mean efficiency of >99.99 percent. Qian (1998) tested the efficiencies of N95 filtering facepiece respirator, DM, DFM and non-certified surgical mask. It was found that only the N95 filtering facepiece respirator meet the minimum 95% efficiency NIOSH requirement[163]. Lawrence (2006) conducted a study to compare the performance of surgical masks, N95 filtering facepiece respirators and N95 elastomeric respirators[222]. The surgical masks had the lowest performance among the respiratory devices tested. It is speculated that low performance of the surgical masks relates to its original purpose of preventing the wearer of the mask from transmitting potential microbial agents to patients rather than protect the wearer from potential inhalation hazards. Additionally, the difficulty in adjusting the surgical mask to offer a better seal may have presented the wearer with an insufficient seal of protection. The results from the present study also suggest that the N95 filtering facepiece respirator can be used as an effective RPD for infection control in hospital settings.

This study had several strengths. The respirators were evaluated both in the laboratory and an actual swine barn. Furthermore the swine barn had been previously identified as positive for MRSA in the air and in the nasopharynx of swine workers and pigs in the building[138]. In addition to using a direct reading instrument to sample particle concentration, i.e. OPC, we simultaneously used the Andersen Cascade Impactor which is a standard for viable air sampling techniques. The design of the plate covers and the how the plate covers were sealed to the N95 respirators prevented leakage.

There were also limitations with this study. The sample size of the study was small. We only performed three time trials. This study did not address the effect of relative humidity on the effectiveness of the N95 respirator and was not tested on human workers.

As Donham (1989) reported exposure to dust particles, endotoxins and other pathogenic organisms can lead to respiratory illnesses.[199]. O'Shaughnessy (2009) identified that various tasks inside a swine building increased the concentration of airborne dust[175]. Dust particles inside swine facilities are composed of various substances [198]. This suggests a wide size range of particles to which workers inside a swine facility maybe exposed. The N95 respirator showed efficiencies greater than 99 percent for airborne MRSA particles in both the respirable and non-respirable size range. However, total dust particles in the respirable size showed efficiencies less than 95 percent. Leakage of total dust particles may have led the respirable dust particles having efficiencies less than 95 percent. A leak was found in the cheek area of the plate covers which may have allowed the penetration of particles due to the lack of a perfect seal. With the inadequate protection provided by the N95 respirator for total dust particles of the respirable size range using an OPC, further studies are needed to assess N95 respirator efficiencies for total dust the respirable size range of less than 5 um using an OPC.

The N95 respirator was shown to have efficiencies greater than 95 percent at filtering MRSA (Figure 9). This would suggest implementation of N95 respirators in agricultural settings for infection control. However, compliance to a respiratory program in agricultural settings may not be widely accepted due to requirement of fit testing

(workers will have to shave their beards) and uncomfortability. To ensure compliance producers will need to promote and enforce the use of N95 respirator

Conclusion

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

Figure 3. N95 Respirator Efficiency Laboratory Trials using the OPC

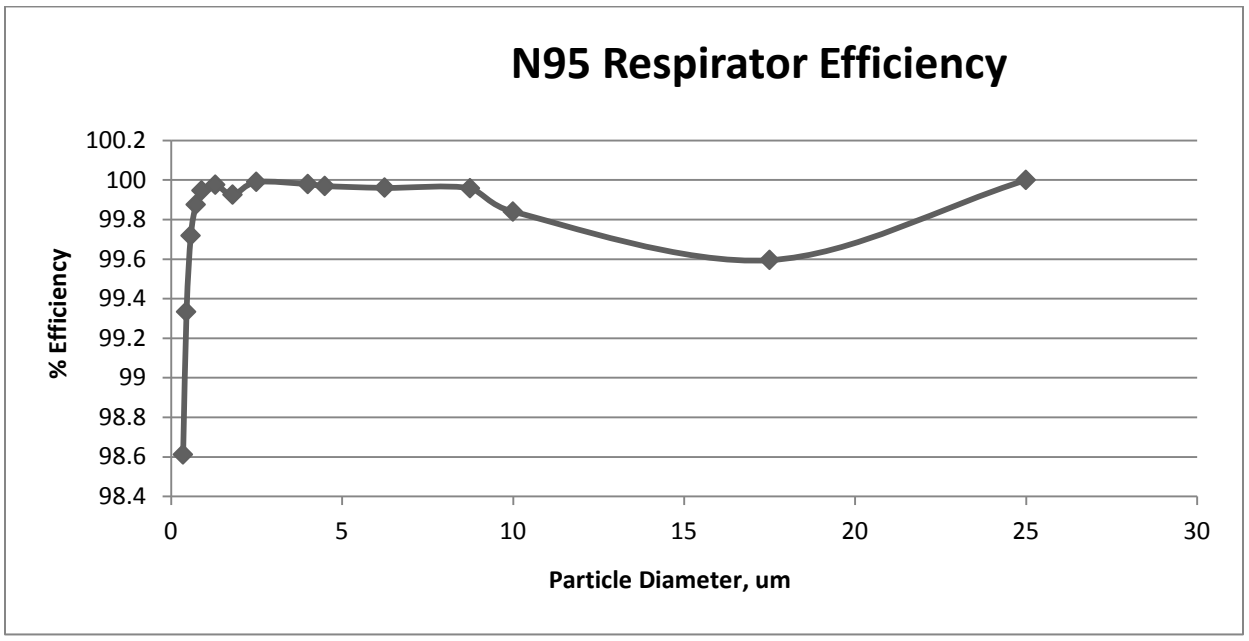


Table 8. N95 Respirator Efficiency Field Trials using the N-6 Andersen Sampler

Particle diameter Lower limit (µm)	Particle diameter Upper limit (µm)	Particle diameter Average (µm)	Unfiltered (CFU/m^3)	Filtered (CFU/m^3)	Penetration	Efficiency
1.1	2.1	1.6	9187.28	36.12	0.39	99.61
4.7	7	5.85	2826.86	21.20	0.75	99.25
7		20	18374.56	37.69	0.20	99.80

Figure 4. N95 Respirator Efficiency Field Trials using the OPC

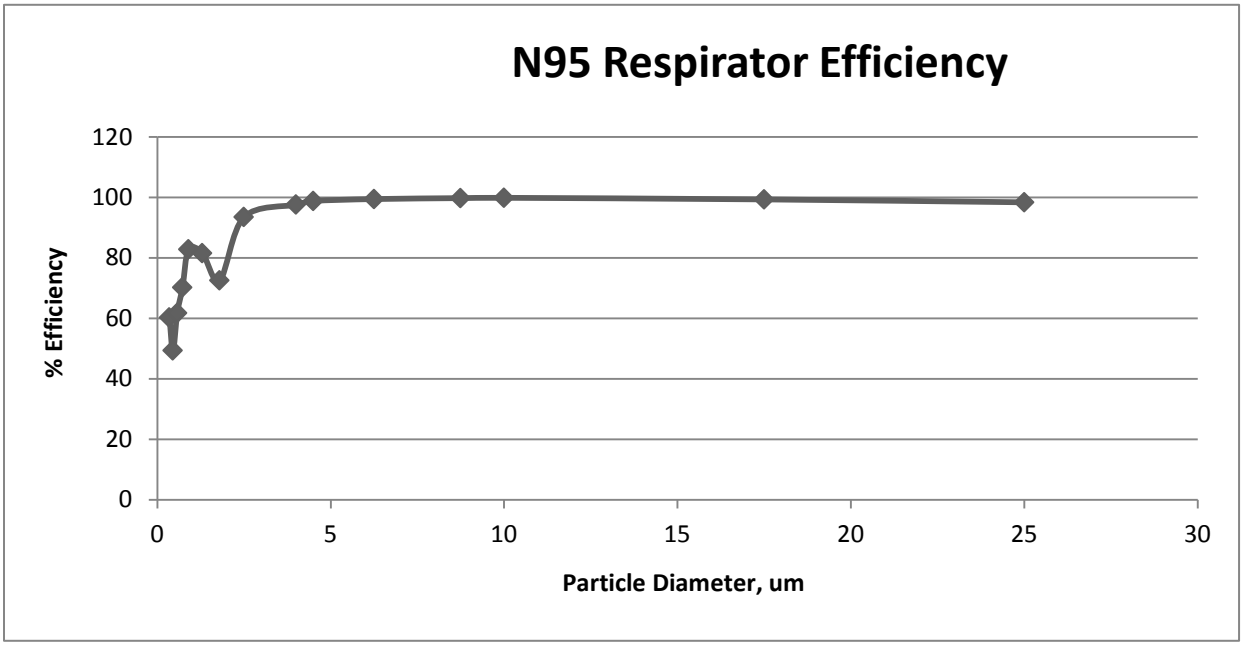


Figure 5. N95 respirator laboratory test chamber (front view)

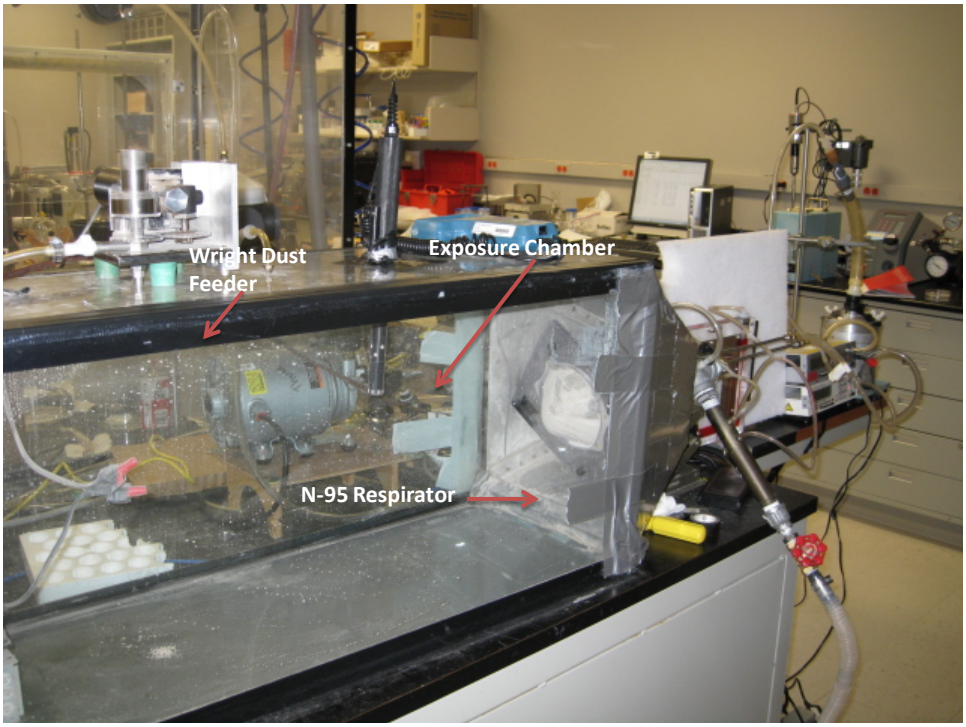


Figure 6. N95 Respirator laboratory test chamber (rear view)

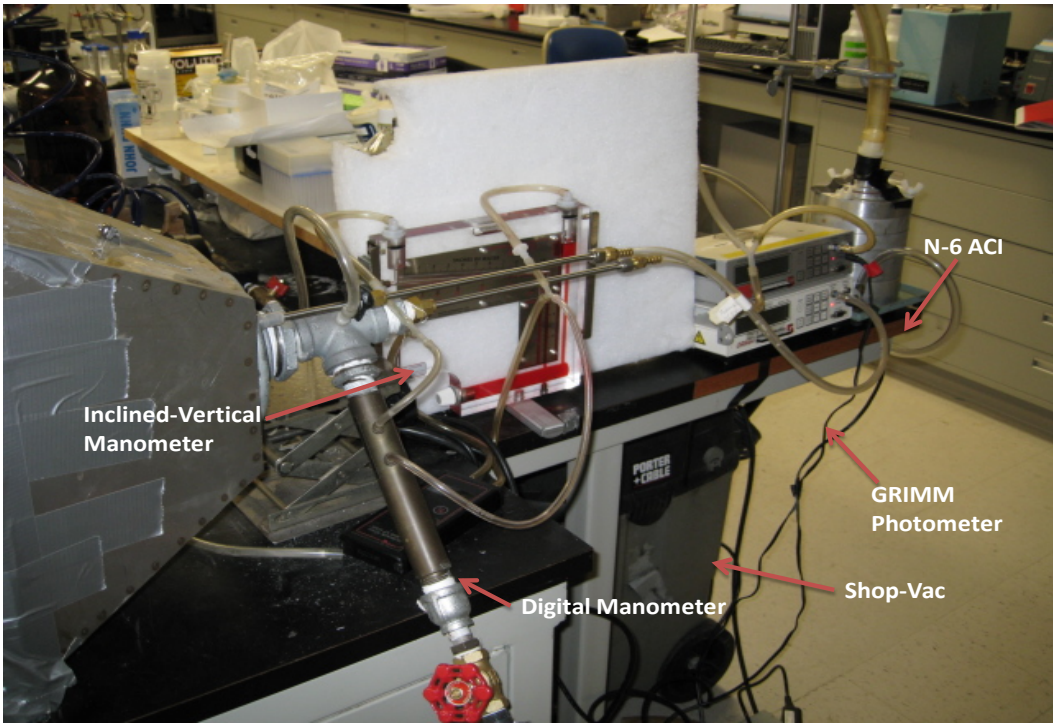


Figure 7. N95 respirator field chamber (front view)

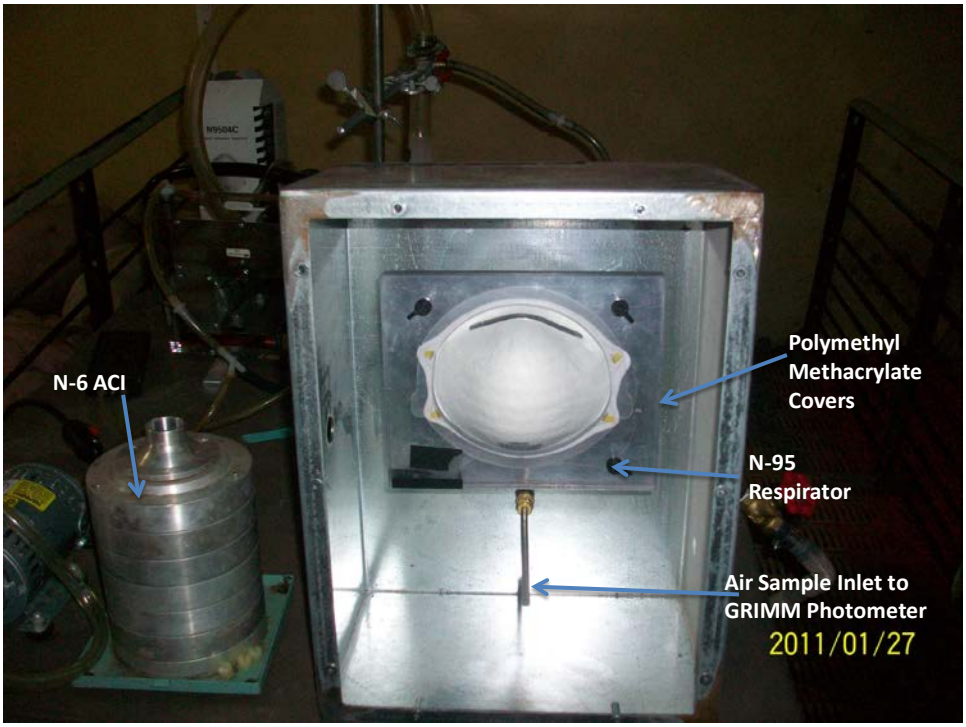


Figure 8. N95 respirator field chamber (rear view)

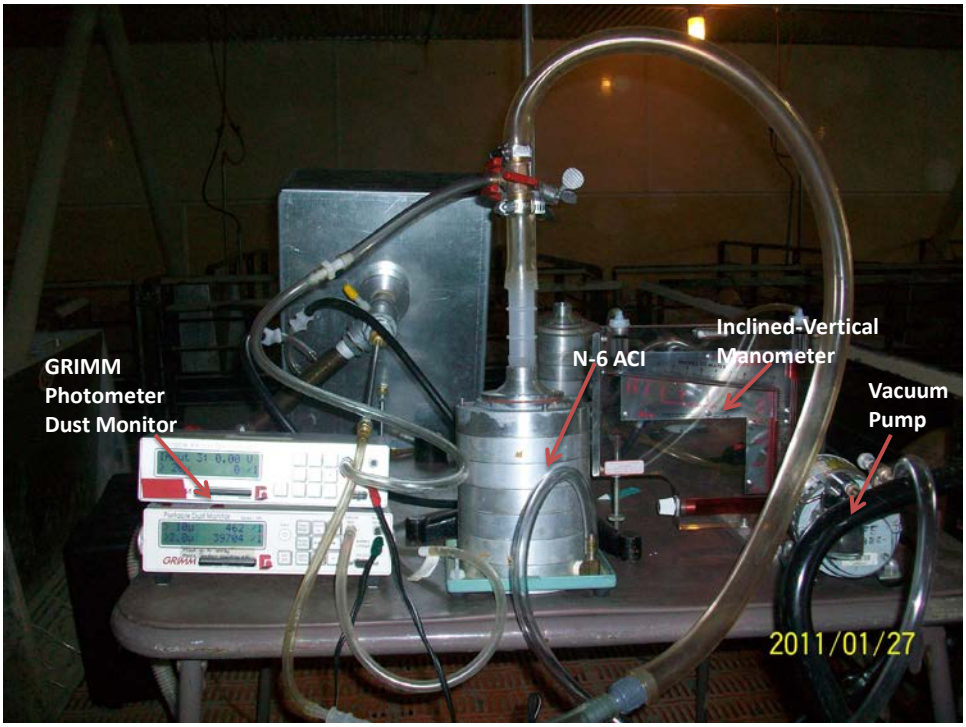
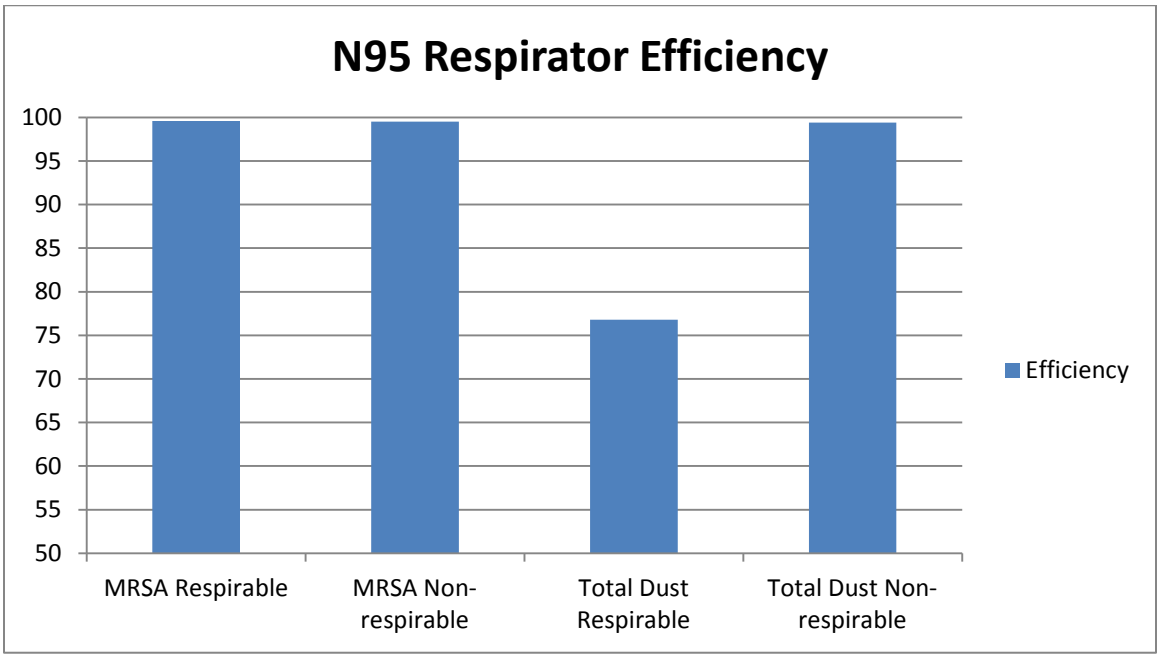


Figure 9. N95 Respirator Efficiency for MRSA and Total Dust



CHAPTER IV
THE EFFICIENCY OF HARDWOOD CHIPS AND WESTERN RED CEDAR MEDIA BIOFILTERS AT MITIGATING AIRBORNE MRSA DISPERSION FROM ANIMAL FEEDING OPERATIONS TO THE OUTDOOR ENVIRONMENT

Introduction

Agricultural feeding operations have been shown to be sources of air contaminants such as odors, gases, dust, endotoxins, bacteria and antibiotic resistant bacteria[68,204,223]. Workers inside animal feeding operations are exposed to concentrations of these air contaminants that result in risk of respiratory illnesses.[4-8 69,199,201,224,225]. Potential symptoms of health hazards associated with working in animal feeding operations include chest tightness, wheezing, cough and excess sputum production. Further, the condition organic toxic dust syndrome, gastrointestinal illness and immunologic problems have been associated with work inside swine buildings[200,203,226,227]. Swine workers and pigs inside confined feeding operations have also been found to be colonized with antibiotic resistant bacteria such as methicillin resistant *Staphylococcus aureus* (MRSA)[138]. Historically MRSA was identified as a hospital acquired infection, and it was not until the past decade that MRSA was also determined to exist in the general community and in livestock facilities. Air contaminants from swine confined feeding operations have also been identified to be emitted from the exhaust ventilation system into nearby communities[150,228]. The transmission of airborne antibiotic resistant bacteria as well as other air contaminants emitted from swine CAFOs can pose a public health concern[229-234].

Donham (2006) and Gibbs (2006) conducted studies which have identified that air sampled in residential areas near (within 15,840 ft and 492.12 ft respectively) swine CAFOs have air contaminants from swine CAFOs including antibiotic resistant bacteria[234,235]. Persons living near swine CAFOs have reported respiratory symptoms similar to that of swine workers and veterinarians[236,237]. Of special concern is the potential public health risk to young people in agricultural communities populated by swine CAFOs. Children in schools near swine CAFOs may be exposed to airborne contaminants which may include MRSA emitted from swine CAFOs. Students in schools near swine CAFO's have higher rates of wheezing than schools with greater distances from swine CAFOs[238,239]. Antibiotic resistant Staphylococcus aureus has been detected downwind from CAFOs and in residential homes (492.12 ft and 262.47 ft respectively)[187,235]. Although MRSA exposure from livestock has undefined public health or occupational health consequences at this time, the precautionary principle suggests control methods need to be investigated to mitigate the emission of MRSA and other contaminants from exhaust air of swine CAFOs.

Biofilters, a system which generally uses compost and wood chips to biologically degrade odors, have been used to reduce odor emissions from swine CAFOs[170]. Evaluation of these biofilters have shown them to be efficient at mitigating odor emissions from swine CAFOs[167,240,241]. Biofilters have also been shown to reduce the concentration levels of dust, endotoxins and bacteria from CAFOs[169,242].

The objective of this study was to determine the efficiency of biofilters to mitigate airborne MRSA emitted from a swine CAFO. The efficiency of biofilters to reduce the concentration of airborne MRSA particles was tested using a mobile biofilter unit. A

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

Materials and Methods

Sampling site

The study site was selected as representative of modern swine production facilities. Further, we had previously documented that the workers and swine at the facility were culture positive for MRSA[138]. The producers were willing to cooperate for this study, and informed consent was obtained and all requirements of IRB were followed. The veterinarian for the facility helped facilitate the study, providing consultation in the conduct of sampling at the facility. The study site consisted of two buildings and produced approximately 48,000 feeder pigs/yr. Pigs entered the buildings at 14 days of age and left at the age of 60 days and weighing approximately 50 lbs. The stocking density of the two buildings was one pig per 4 ft^2.

Ventilation for the facility was operated by sixteen 24” and eight 14” wall fans (both thermostat controlled) and eight 9” continuous pit fans. The facility had double-sided curtains for increased ventilation during warm seasons. The volume of the study room was 12,847 ft^3. The sampled facility was power washed with detergent and Keno

X5 (CID Lines, Belgium, Europe) between cycling of hogs (46 days) due to the all-in, all-out nature of the site. The active ingredients of the disinfectant Keno X5 were hydrogen peroxide and peroxyacetic acid. Topography of the area surrounding the facility was flat without any wind buffers.

Biofilter unit

We used a modified version of Hoff (200 9) biofilter design in collaboration with the Air Dispersion Laboratory (under the direction of Dr. Steve Hoff) at Iowa State University [243]. The modified biofilter design was tested and refined at the Air Dispersion Laboratory before field testing was performed to verify that constant air flow and pressure was being maintained. For the field test, the six stage Andersen Sampler (Andersen Sampler Inc., Atlanta, GA, USA) and an Optical Particle Counter(GRIMM Technologies Inc., Douglasville, GA, USA) were used to assess the particulate and viable MRSA content of the air, inside the building, the filtered air, and a negative control filter [190,244,245]. A plenum (duct) was connected to the exhaust fan of the CAFO and the biofilters. The mobile biofilter unit was composed of eight 50 gallon barrels with one of two biofilter media treatments (see figure 16) consisting of Hardwood chips (HWC) of 5 cm and Western Red Cedar (WRC) less than 5 cm[170]. The media dept was 25 cm for both media treatments. Prior to biofilter use, media chips were evaluated for MRSA and were found to be negative.

We assessed the air for presence of viable MRSA inside the CAFO in comparison to exhausted air. The air exhausted from the CAFO was assessed for viable MRSA,

comparing the efficiency of the two different media (HWC, and WRC). The retention time of the air within the biofilters was adjusted to 4 seconds as determined by Chen et al 2009. N-6 ACI (using only 3 stages for collection) was used to sample air at three locations: 1) the center inside the CAFO in an empty pen, 2) the exhaust of the biofilters (figure 17), and 3) the control biofilter (which contained no filter media). Air sampling times of 30 seconds and 1 minute were conducted inside the CAFO. Sampling of filtered air and the negative control unit were 15 and 20 minutes. The air sampling times were selected based on preliminary trials. Environmental conditions inside the CAFO and outside the CAFO atmospheric conditions such as temperature, relative humidity, CO concentration, and CO_2 concentration were measured[189-191]. Each trial was conducted in triplicate for data reliability.

After each sampling period the culture plates were sealed with tape, labeled, placed in a Ziploc bag and finally placed (upside down) into a cooler with ice packs for transport to the laboratory. Air was sampled using CHROMagar plates as the collection media on stages one, two and five. Concentration (colony forming units, cfu/m^3) was determined by multiplying sampling time and volume of air collected.

Bacterial diagnostics

At the laboratory the CHROMagar MRSA plates were incubated at 35°C for 48 hrs. Representative colonies from the CHROMagar plates were subcultured on Columbia CNA (Remel, Lenexa, KS, USA) for diagnostic testing. Identification tests for S. *aureus* Isolates included the catalase test, the coagulase test and the *S. aureus* latex agglutination

assay (Pastorex Staph-plus, Bio-Rad). Methicillin resistance was confirmed by testing for the presence of penicillin binding protein (PBP2') (MRSA latex agglutination test, Oxoid Ltd). . Positive and negative controls were used for all tests. .

Results

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

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

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

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

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

Discussion

Our results showed that HWC and WRC media were highly efficient biofilters to prevent the emission of viable MRSA particles in the exhaust air from swine feeding facilities. The HWC media had an efficiency of 77 percent for particles with mean particle size of 1.6 µm. The efficiency of the HWC media increased as the bioaerosol particle size increased. Western Red Cedar was highly effective for particles with mean diameters of 1.6 µm to 5.85 µm. We speculate that the difference in efficiency shown by the two different biofilter media may have been due to the size of media mesh which may have affected the biofilter porosity [246]. The HWC (> 5 cm) media were larger than the WRC (< 5 cm) media which may have prevented the HWC from intertwining and forming a mesh with smaller pores. We also speculate that the larger size mesh for the HWC media allowed the various sized dust and MRSA particles to pass through the HWC mesh. However, as the size of the particles increased, the larger particles were apparently impacted on the HWC and were prevented from passing through the outer

layer of the HWC media. On the other hand, the WRC biofilter media were shredded chips intertwined closely forming a smaller mesh thus making the WRC biofilter less porous than the HWC biofilter. As a result of the WRC biofilter being less porous than the HWC biofilter, the WRC biofilter had higher filtering efficiencies for the smaller size particles compared to the HWC biofilter. The results of the two sample t test showed that the efficiencies of the two different biofilter media were not due to the difference in the concentration of the dust particles filtered by HWC and WC. Instead we postulate that the difference in the efficiencies may have been due to the biofilter media used [170]. These findings showed that HWC and WRC were highly efficient biofilter media for reducing emissions of MRSA from swine feeding facilities.

Although this is the first study of effectiveness of biofilters on emission of MRSA, other studies have reported on the effectiveness of filtering other contaminants. Tymczyna (2007) found that biofilter media were efficient at retaining dust, gram-negative bacteria and endotoxins exhausted from a chicken hatchery room [242]. Martens (2001) found that biofilters were efficient at reducing bioaerosols from pig facilities [247]. In addition to the first study of biofilter effectiveness regards to MRSA, our study has advanced the field by evaluating the efficiency of different media (HWC and WRC) as shown in figure 13 and 14. Both HWC and WRC were efficient at mitigating emissions of total dust particles. WRC was the more efficient media at mitigation respirable MRSA. Prior research findings[169,241,242], along with the results from our study, indicated that biofilters can be efficient at reducing emissions of airborne MRSA, gram-negative bacteria, endotoxins and various gases from ventilation exhaust systems of swine feeding facilities.

Our study had several strengths. The study was conducted at a swine facility previously identified to have swine workers and pigs which had been tested positive for MRSA[138]. In addition the particular biofilter design we used was previously tested and found to be efficient in the field [170]at reducing odors from swine facilities. We also conducted simultaneous assessment of real time dust concentrations and viable sampling.

There were also limitations in this study. This one-month study duration prevented more extensive sampling from being conducted. The all-in, all-out nature of the swine facility prevented a longer study period. The study small sample size precludes generalization to different types of buildings and different geographical and climatic regions.

Despite the limitations of our study, we believe there are important findings relative to community and public health. Green (2006) found that antibiotic resistant bacteria were emitted from the ventilation exhaust system of swine feeding facilities at concentrations which can cause potential health problems living within 150 meters of the facility [159]. Of special concern in a study conducted by Gibbs (2006), *Staphylococcus aureus* was detected to be the most recovered species downwind of the swine facility tested [158]. A study by Gandara (2006) found that antibiotic resistant *Staphylococcus aureus* were also detected and recovered in residential homes[248] although the source was not identified. The results of our studies suggest that airborne viable MRSA particles can be emitted from the ventilation exhaust systems of animal feeding facilities and can potentially travel in the airstream to nearby outdoor worksites, rural residences, and communities.

Biofilters are relatively inexpensive and can be potentially used to mitigate airborne MRSA. It is estimated to cost $150 to $250 per 1000 cubic feet per minute (CFM) to install a new biofilter to a CAFO. The annual preventative maintenance of the biofilter is estimated to cost $5 to $15 per 1000 CFM[249]. However, the implementation of biofilter may be too costly for some producers. For example, a facility using 150,000 CFM, at the low capital end would cost $22,500 and at the high capital end cost $37,500. The initial cost of installing a biofilter may be prohibitive to some producers. Additional concern with the biofilter media is the proper disposal of contaminated media chips after use.

Our results showed that biofilters can be efficiently used to reduce the emission of viable MRSA particles from swine facilities to mitigate transmission of this antibiotic resistant pathogen and total dust particles (see figures 14 and 15).

Conclusion

Our study was the first study to evaluate the effectiveness of biofilters at mitigating the emission of MRSA particles from a swine facility which tested positive for MRSA in pigs and swine workers. We have previously shown that MRSA can be detected at 705.38 ft from a swine CAFO which is greater than the downwind emission detection found by Green (2006)[235] who assessed at a distance of at least 656. 17 ft. Our findings show that biofilters can be used to mitigate airborne transmission of MRSA particles from MRSA positive swine facilities to the nearby communities. Although biofilters have been shown previously to be effective at reducing the emissions of odors and gases from swine facilities, our study adds to the body of evidence for the use of

biofilters as a means to mitigate MRSA particles from these facilities to the surrounding areas. We recommend that future studies be done to determine if MRSA can be detected beyond the regulated separation distances recommend for CAFOs presently. These future studies can help determine if the separation distances of CAFOs from communities is presently adequate or if the separation distances needs to re-evaluate. The findings of our study can be used to advise swine producers to add biofilters for swine facility ventilation system. Biofilters have the potential to ease the concerns of persons living nearby a swine feeding facility.

Figure 10. Hardwood biofilter efficiency using OPC

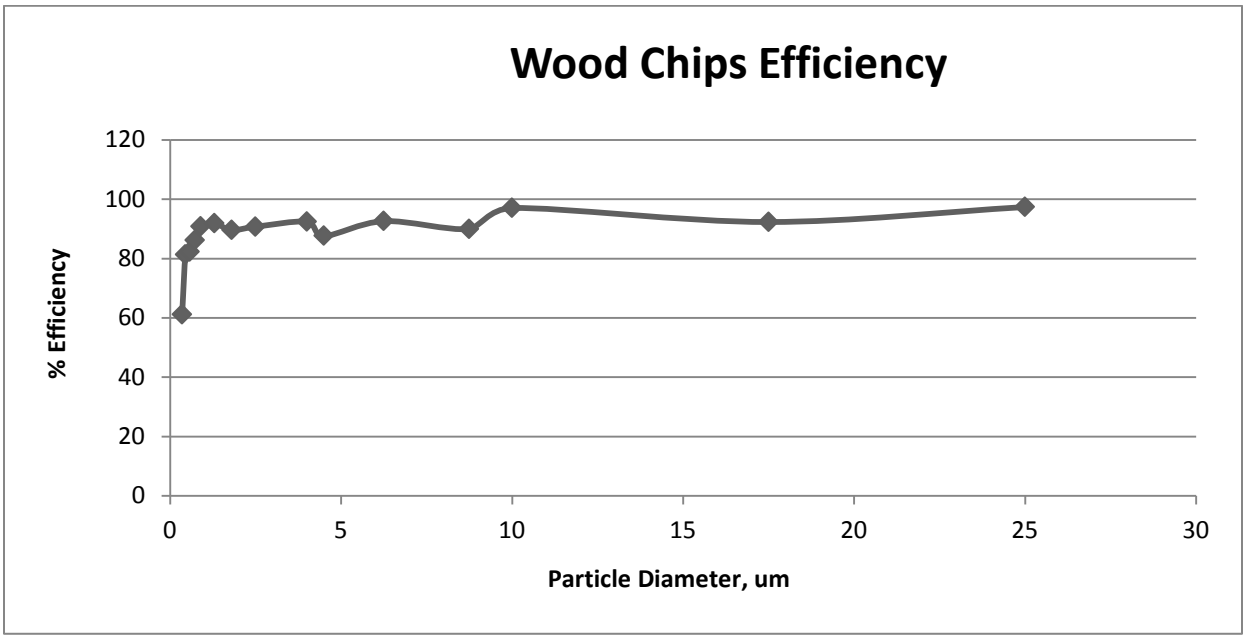


Figure 11. Western Red Cedar biofilter efficiency using OPC

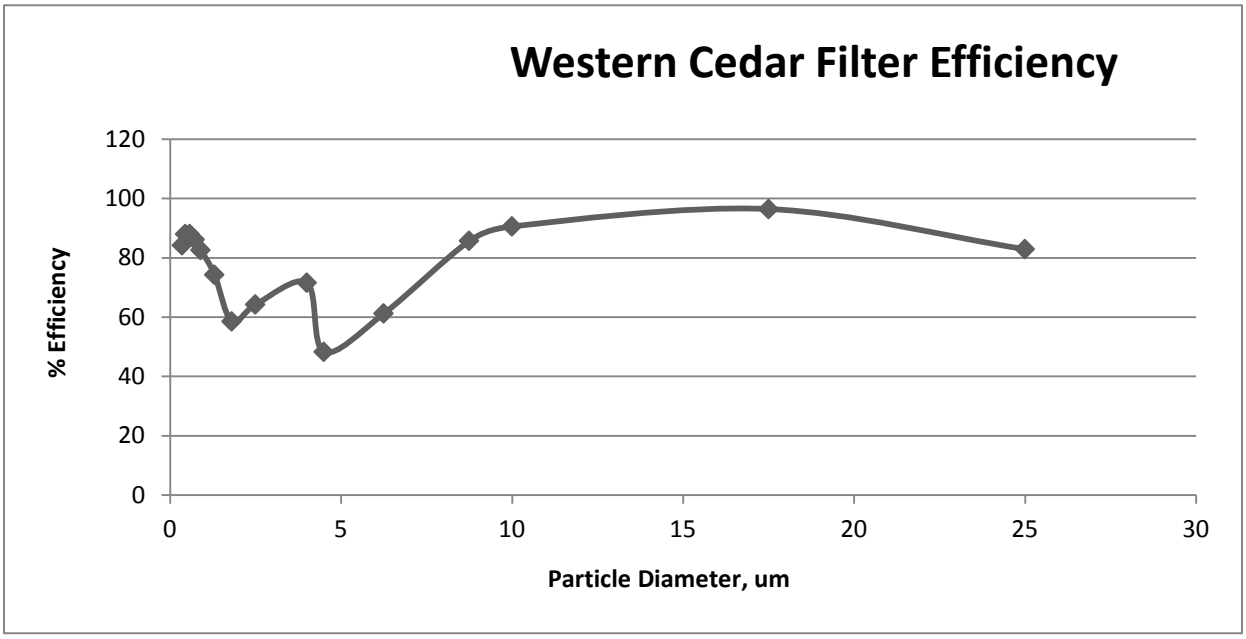


Figure 12. Hardwood biofilter efficiency using Andersen Sampler

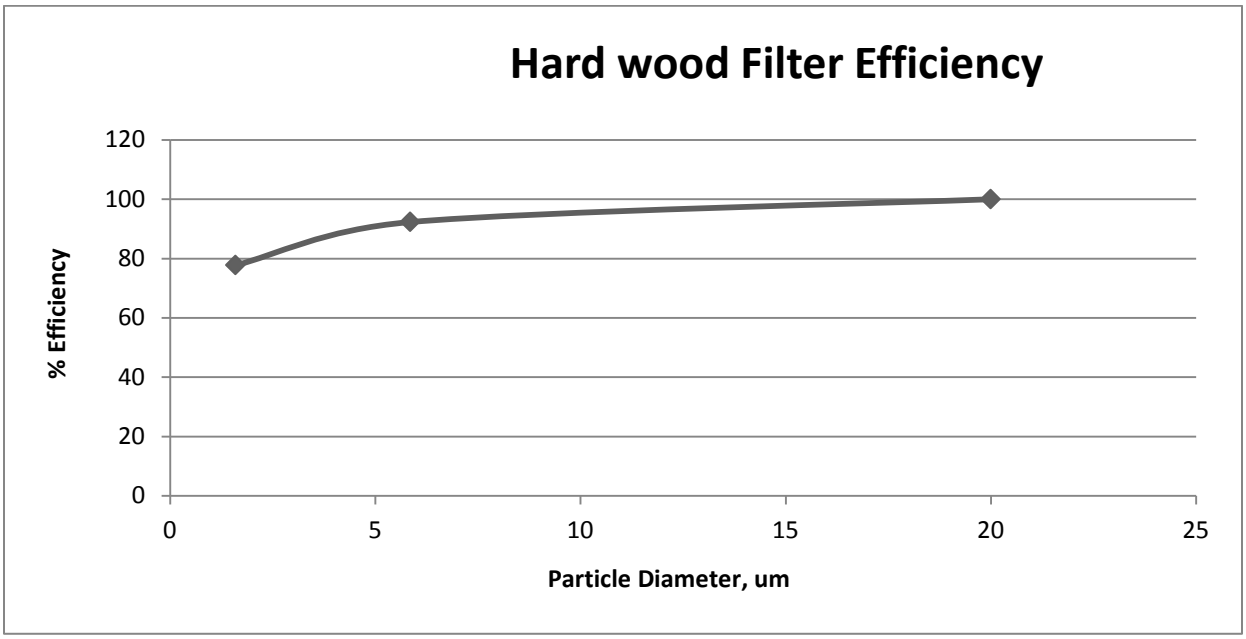


Figure 13. Western Red Cedar biofilter efficiency using Andersen Sampler

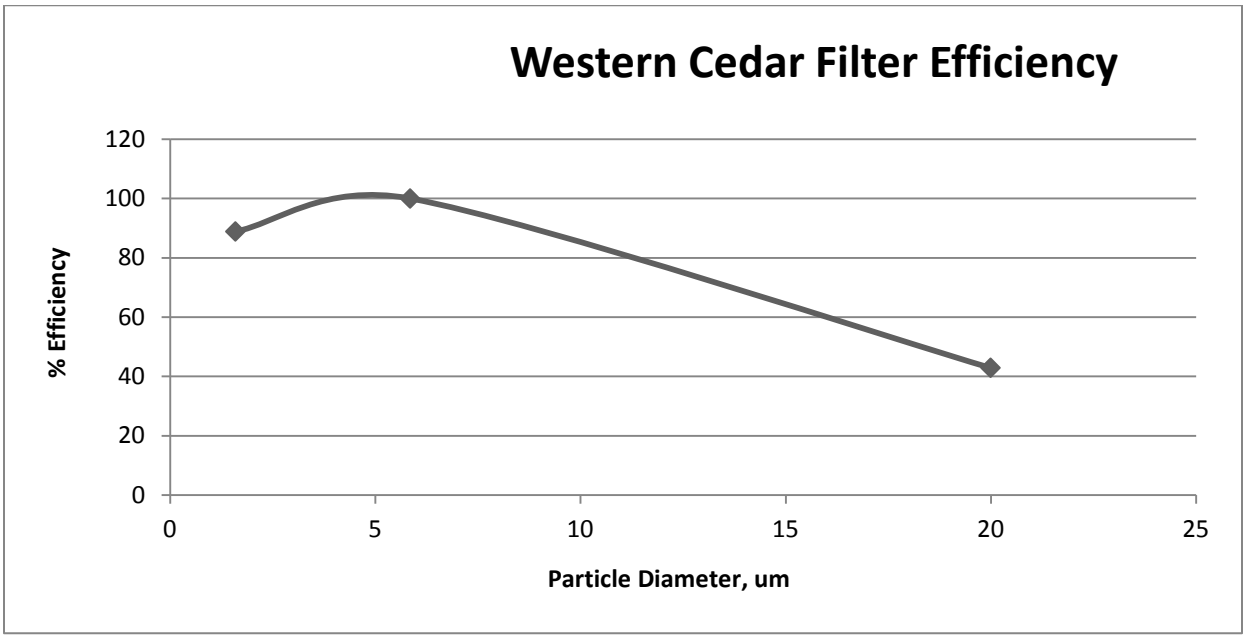


Figure 14. Biofilter Total Dust Efficiency

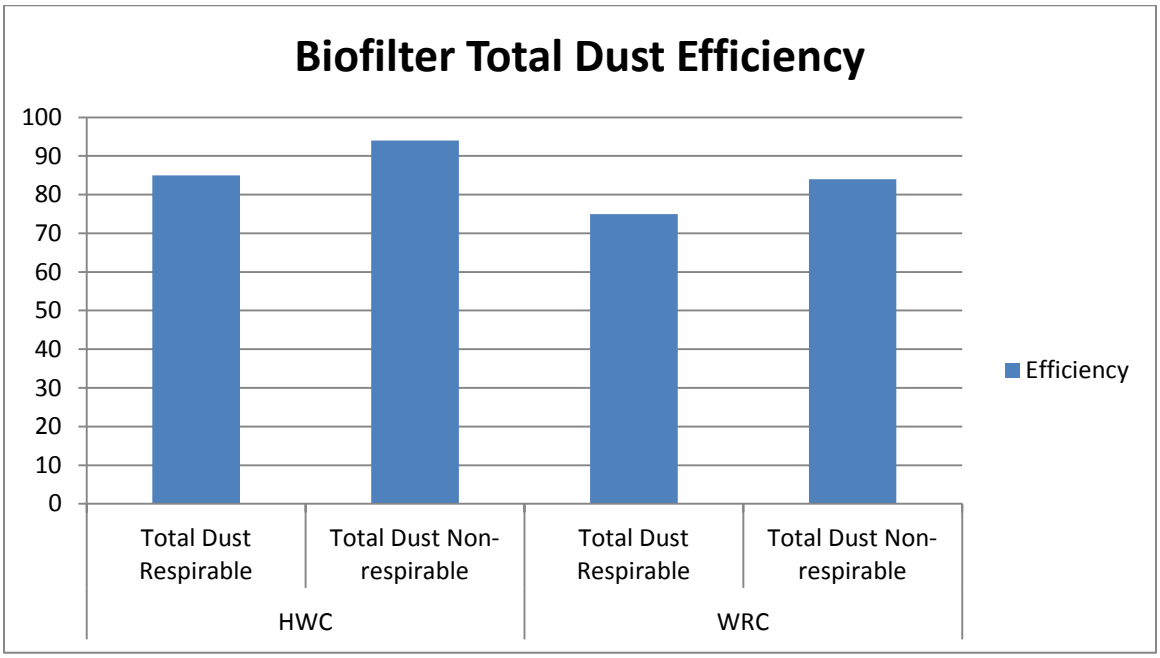


Figure 15. Biofilter MRSA Efficiency

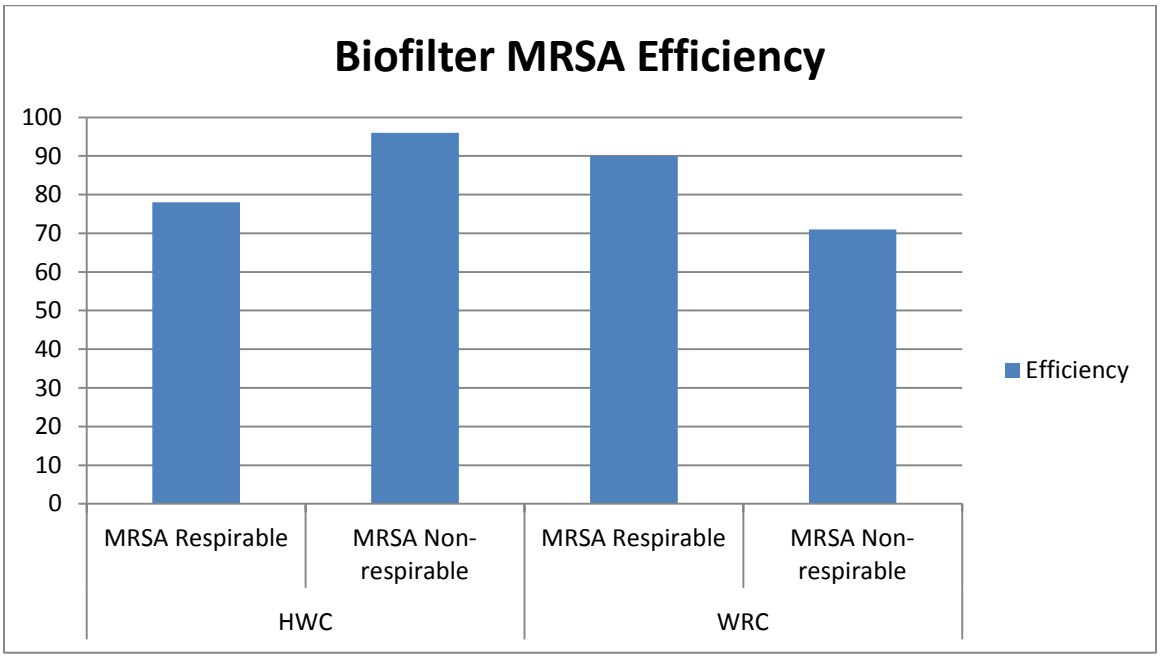
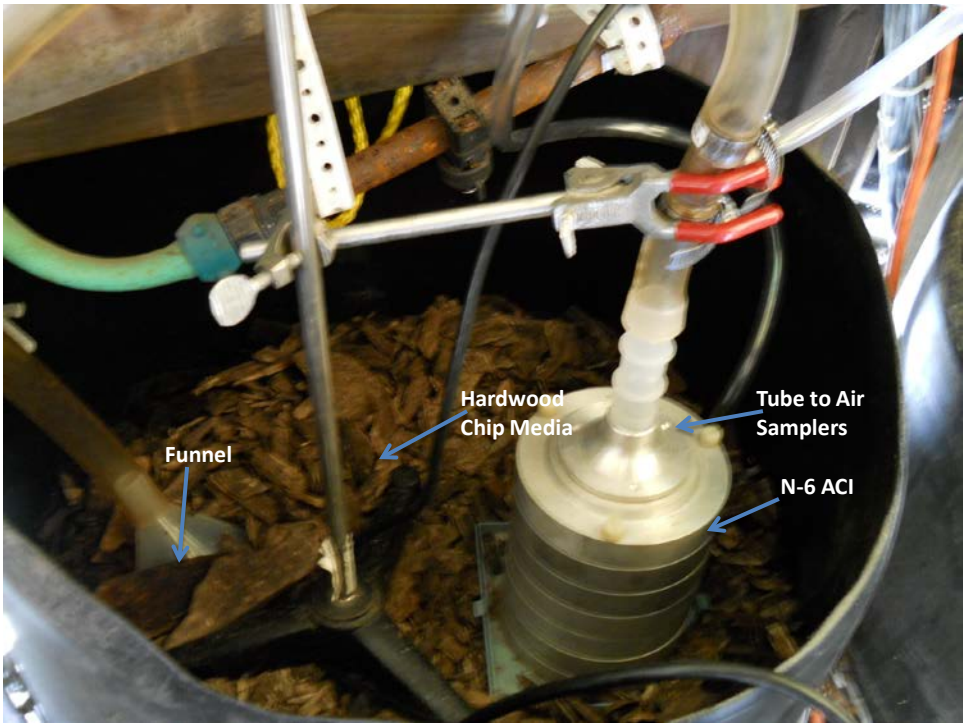


Figure 16. Biofilter mobile unit

Figure 17. Sampling air passing through biofilter media



CHAPTER V
CONCLUSIONS

The overarching goal of this research project was to investigate the risk and mitigation methods to protect workers inside swine facilities and persons living near swine facilities from airborne MRSA transmission. Through this research project I was able to confirm that viable MRSA organisms are present in the air inside a swine facility and in the exhausted air from a swine facility. MRSA particles detected inside the swine facility were predominantly in the non-respirable size range (larger particles) indicating that the potential source of contamination from animal feed and dust. The predominant MRSA particles detected outside the swine building were in the respirable size range (smaller particles) indicating that the potential source of contamination was of swine origin. MRSA organisms were detected at the farthest distance assessed, which was 215 meters. The study was limited in evaluating farther distances due to the lack of a power source and interference with other obstacles. It is well documented that swine workers and others having swine exposure are at risk of carrying MRSA in their nares, but the full individual and public health risk of LA-MRSA carriage is not fully known. However, with significant clinical risk of LA-MRSA infection in the Netherlands, the precautionary principle suggests that management and control methods of LA-MRSA exposure should be pursued. The findings of this research project have several implications relevant to the protection of persons working in or near swine CAFOs.

Our detection of viable LA MRSA in the air inside the swine facility at high concentrations suggests that it would be prudent for workers to use respiratory protective devices (RPDs). We suggested the use of N95 respirators. The efficiency of

the N95 was evaluated in a test chamber as an indicated protective measure. The N95 respirators assessed were efficient at mitigating exposure to viable MRSA in both the non-respirable size and respirable size ranges. We suggest these findings imply that a respiratory program using N95 respirators (if fitted and worn correctly) may be used to protect workers from MRSA (and although not tested for) could be protective for other airborne antibiotic resistant zoonotic bacteria in addition to MRSA. The finding that the N95 was not as effective at mitigating dust particles in the respirable size range less than 3 µm would suggest additional research is needed to further assess the protective function of the N95 against smaller size particles (< 3 µm).

Other studies have suggested that large dust particles in swine facilities are due to the bacteria being attached to materials such as dust, feed and epithelial cells. With *S.aureus* having a size range of 0.5-1.5 µm, this can suggest MRSA detected <3 µm were individual MRSA cells. It is unknown what concentration of MRSA is required to cause an infection or colonization of the nasal passages. Future studies with N95 respirators to evaluate its effectiveness with providing protection against smaller size particles (respirable range) in swine facilities are needed to determine if N95 respirators are to be recommended for smaller size dust particles and MRSA. In addition studies to compare the filtering effectiveness of the N95 respirator to that of the R95 (oil resistant),P95 (oil proof), powered air purifying respirators (PAPR) and other half-faced respirators against MRSA to determine which type of filtering respirator offers the best protection against MRSA.

A potential method for both future studies to evaluate the various RPDs efficiency for particles <3 µm would be to aerosolize monodisperse particles of the size range of

S.aureus in a laboratory test chamber to determine which type of respirator provided the highest efficiency. In terms of the hierarchy of controls RPDs are the last resort and needs to be used in conjunction with source control and engineering controls. In this study, relative humidity was shown to impact the detection of dust particles inside the swine facility. Moisture in the air may have led to an increase in the size of dust particles. The concept of hydroscopic growth can be used to mitigate the smaller MRSA CFUs in the air. Water and or oil emulsion mists have been evaluated to reduce total dust particles and endotoxins in swine facilities[250-252]. It has been found that spraying oil mist on the pen floor, pen dividers, and animals of enclosed swine facilities can reduce airborne dust to 52% of the original dust concentration [250]. Although another study found that oil mist reduced total dust by 86% and total endotoxin by 82.5% of the larger dust particles (>5 um) but did not effectively reduce smaller dust particles (<5 um) [251]. Further studies are needed to evaluate oil mist effectiveness in controlling respirable dust particles, particularly antibiotic resistant bacteria such as MRSA inside swine facilities to determine its effectiveness of mitigating airborne dust exposure in conjunction with N95, R95 and P95 respirators. In addition, the mechanical ventilation system of a swine facility needs to be evaluated for its ability at diluting respirable MRSA CFUs from the air inside swine facilities.

The isolation of viable MRSA at 215 m downwind suggests there may be a significant public health implication in terms of persons living or working near swine facilities. However, it is to be noted that minimum separation distances of CAFOs and private homes or public places are usually further than 215 m. Regulations vary from state to state, but for Iowa, the separation distances vary from 577 mto926 m, depending

on the concentration of animal units on site. However, family members or workers may live or work within this zone. In order to mitigate MRSA emissions from the exhaust vents from a swine facility, the efficacy of biofilters was studied. Our study found that the biofilters used were effective at mitigating MRSA exhausted from the swine facility. The ability of biofilters to mitigate respirable MRSA suggests they may be an effective engineering solution to mitigate the airborne transmission of MRSA to nearby communities**.** Future studies with biofilters using HWC and WRC can be carried out over longer periods than was done in the present study. In some biofilter applications, a mixture of compost and wood chips are used. We did not use compost in our biofilter. Specific types of biofilter media need to be evaluated to determine the most efficient ratio of compost and wood chip product, and only wood chips. Further studies are also needed to determine if MRSA exhausted from swine facilities can be detected at distances greater than the separation distances of 1250 ft to 1875 ft (or 318 meters to 572 meters) required for construction of a CAFO near communities. The detection of airborne MRSA which originate from a swine facility beyond the required separation distance may lead to further re-evaluation of the separation distances of CAFOs from nearby communities.

This study had several strengths. The swine facility used in the study was previously documented as MRSA positive for both swine workers and swine. In the previous study the nares of the swine workers and swine were swabbed and the samples were positive for MRSA. The detection of MRSA in the air of the swine facility suggests there is a risk of airborne transmission of MRSA. This was the first study to evaluate the effectiveness of the N95 respirator against MRSA in a swine facility. The N95 respirator test was pre-tested in the lab and showed similar efficiency in the lab as it did during the

testing at the swine facility. Additionally, this was the first study to evaluate the effectiveness of biofilters at mitigation the emission of MRSA from a swine facility. The mobile biofilter unit used in this study was previously tested and proved to be effective at mitigating odors and gases from swine facilities.

Several limitations existed in this study. The study sample size was small. Only one swine facility was evaluated in the study which limits comparisons to other swine facilities and generalization to swine facilities in different geographical locations. The number of trials for both the N95 respirator testing and the biofilter testing was small. The small number of trials for the N95 respirator and the biofilter may lead to uncertainty in the efficiencies detected with both filtration devices.

The N95 respirator was shown to have efficiencies greater than 95 percent at filtering MRSA. This would suggest implementation of N95 respirators in agricultural settings for infection control. However, compliance to a respiratory program in agricultural settings may not be widely accepted due to requirement of fit testing (workers will have to shave their beards) and uncomfortability. To ensure compliance producers will need to promote and enforce the use of N95 respirators.

Biofilters are relatively inexpensive and can be potentially used to mitigate airborne MRSA. It is estimated to cost $150 to $250 per 1000 cubic feet per minute (CFM) to install a new biofilter to a CAFO. The annual preventative maintenance of the biofilter is estimated to cost $5 to $15 per 1000 CFM[249]. However, the implementation of biofilter may be too costly for some producers. For example, a facility using 150,000 CFM, at the low capital end would cost $22,500 and at the high capital end cost $37,500. The initial cost of installing a biofilter may be prohibitive to some producers.

In conclusion, this research project determined methods to mitigate the risk of occupational and outdoor environmental exposure to airborne transmission to workers inside swine facilities and to mitigate the risk of environmental exposure working or living outside swine facilities to airborne MRSA due to the emission of MRSA from the exhaust system of swine facilities. Further studies are warranted on a larger scale to evaluate the mitigation of airborne MRSA in swine facilities using biofilters as engineering control. Such studies should also evaluate the use of antibiotics in feed, have a larger sample size, evaluate during different seasons, conducting air sampling beyond the required separation distances of CAFOs from incorporated and unincorporated areas and longer duration of the biofilter test. Antibiotics in feed needs to be evaluated to determine if the use of antibiotics is associated with the presence of MRSA in swine buildings. Animal feed and water in the building needs to be evaluated as potential sources of MRSA. The feed and water source needs to be cultured and genotyped to determine if the MRSA detected is the same *spa* and MLST as isolated from the air in the building and nasal isolated from swine and people. The findings of this study and the proposed future studies can alleviate occupational, environmental and public health concerns of the airborne transmission of MRSA from swine facilities.

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