PERSISTENCE OF *STAPHYLOCCOCUS AUREUS* NASAL CARRIAGE AMONG INDUSTRIAL HOG OPERATION WORKERS AND THEIR HOUSEHOLD CONTACTS IN NORTH CAROLINA

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ABSTRACT

Elizabeth Pierce: Persistence of *Staphylococcus aureus* Nasal Carriage among Industrial Hog Operation Workers and Their Household Contacts in North Carolina
(Under the direction of Jill Stewart)

Industrial hog operations (IHOs) are a potential source of exposure to antibiotic-resistant *Staphylococcus aureus* including methicillin-resistant *S. aureus* (MRSA). However, the duration of nasal colonization among IHO workers is unknown. In order to better understand nasal carriage patterns, nasal swabs from IHO workers and their household contacts were analyzed for up to four months for the presence of *S. aureus* and MRSA. Isolates also underwent spa typing and antibiotic resistance testing to determine potential livestock association among isolated bacteria. Results indicate that forty percent of the 175 participants were intermittent carriers and twenty-seven were persistent carriers for *S. aureus*. None of the participants were persistent carriers for MRSA and eight percent were intermittent carriers. This study demonstrates that nasal carriage of *S. aureus* can persist among IHO workers and their household contacts over a 4 month period that includes time away from work.
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<tr>
<td>CA</td>
<td>Community associated</td>
</tr>
<tr>
<td>CAFO</td>
<td>Confined animal feeding operation</td>
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<td>CC</td>
<td>Clonal complex</td>
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<tr>
<td>CFU</td>
<td>Colony forming unit</td>
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<td>HA</td>
<td>Hospital associated</td>
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<tr>
<td>IHO</td>
<td>Industrial hog operation</td>
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<td>LA</td>
<td>Livestock associated</td>
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<tr>
<td>MDRSA</td>
<td>Multi-drug resistant <em>Staphylococcus aureus</em></td>
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<tr>
<td>MRSA</td>
<td>Methicillin-resistant <em>Staphylococcus aureus</em></td>
</tr>
<tr>
<td>MSSA</td>
<td>Methicillin-susceptible <em>S. aureus</em></td>
</tr>
<tr>
<td>PCR</td>
<td>Polymerase chain reaction</td>
</tr>
<tr>
<td>REACH</td>
<td>Rural Empowerment for Community Help</td>
</tr>
<tr>
<td>Spa</td>
<td>Surface protein A</td>
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<tr>
<td>ST</td>
<td>Sequence type</td>
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<tr>
<td>TNTC</td>
<td>Too numerous to count</td>
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CHAPTER 1: INTRODUCTION

*Staphylococcus aureus* is a bacterium found in the nares of approximately one third of Americans and can result in minor skin infections (Gorwitz et al, 2008). Exposure can also cause dangerous infections that can lead to mortality if the bacteria enter an individual’s body and invade the bloodstream, lungs, joints, bones, or heart (Lowy et al, 1998). In the 1960s, a strain of *S. aureus* emerged that had evolved resistance to methicillin, a common antibiotic used to treat *S. aureus* infections. Methicillin-resistant *S. aureus* (MRSA) poses a health risk as the infection spreads rapidly and is not easily treatable. Furthermore, some strains of *S. aureus* are multi-drug resistant (MDRSA), with resistance to three or more classes of antibiotics. This acquired antibiotic resistance makes treatment of infection increasingly difficult (Wu et al, 2010).

Strains of MRSA are often classified according to the ecology of infections. Hospital associated MRSA is related to the spread of *S. aureus* and MRSA within health care environments. Community associated strains of MRSA have also emerged. These strains are spread in public places such as gyms and daycares and are now classified as endemic within the United States (Zetola et al, 2005). Recently, new strains of MRSA have emerged as livestock associated (LA) MRSA. This type of MRSA has been linked to confined breeding practices of livestock such as horses, cattle, poultry, and hogs (Mulders et al, 2010; Graveland et al, 2011; Cuny et al, 2008). In 2004, livestock associated clonal complex 398 (CC 398) was first recognized in the Netherlands and has since been identified in several European counties as well as China, Canada, and parts of South America (Salmenlinna et al, 2010; Arriola et al, 2011, Khanna et al, 2008; Wagenaar et al., 2009). Subsequent genetic analysis shows that this
antibiotic resistant strain is exchanged between humans and animals with the use of a livestock reservoir (Armand-Lefevre et al., 2005, Price et al, 2012). Although CC 398 is frequently identified around Europe, it has only been observed in the United States as recently as 2009, first in Iowa and later in North Carolina. (Smith et al, 2009; Rinsky et al., 2013).

Recent studies have related the use of antibiotics in livestock to a higher prevalence of antibiotic resistant MRSA and \textit{S. aureus} within the animals in industrial livestock settings (Waters et al, 2011; Pu et al, 2009). Due to the high livestock exposure workers experience while working in confined animal feeding operations (CAFOs), workers are likely to be exposed to LA-MRSA and \textit{S. aureus}. One study concluded that workers on CAFO hog farms in North Carolina were more likely to be positive for LA-MRSA and \textit{S. aureus} than antibiotic free farmers (Rinsky et al, 2013).

Although nasal carriage has been repeatedly demonstrated among industrial farm workers, it is unknown if workers whose nasal swabs test positive for \textit{S. aureus} or MRSA are either briefly contaminated or persistently colonized with the bacteria. This information is important in understanding the potential health risks to the workers. This information is also important to understanding potential transport and transmission pathways of these bacteria. The objective of this study is to examine the dynamics of nasal colonization with \textit{S. aureus} among people who work at industrial hog operations (IHOs) in Eastern North Carolina, and their household members, over four months.

Objectives:

The objectives for this research are as follows:

1. To determine the carriage status of \textit{S. aureus} and MRSA for IHO workers and their household members over a four month period;
2. To determine whether persistence of nasal colonization with \textit{S. aureus} is associated with concentration of \textit{S. aureus};

3. To determine the prevalence of MDRSA within \textit{S. aureus} isolates; and

4. To determine the potential presence of LA \textit{S. aureus} and MRSA through antibiotic resistance testing, \textit{spa} typing, and presence of the \textit{scn} gene.

The findings contained in this master’s thesis contribute to a larger study which aims to analyze factors which influence carriage rates of \textit{S. aureus}, MRSA, and MRDSA in the population of interest over a four month period.

BACKGROUND

\textit{Staphylococcus aureus}:

\textit{Staphylococcus aureus}, commonly known as staph, is a bacterium that is present in the nares of approximately one third of Americans. Humans and animals are the primary reservoir of \textit{S. aureus} but it has also been observed in water, soil, and air which can further spread the bacteria. \textit{S. aureus} also can persist on dry surfaces for up to months (Kramer et al, 2006). There is also some evidence that \textit{S. aureus} can exchanged between humans as well as between animals and humans (Graveland, 2010). One study found evidence that \textit{S. aureus} can also be transferred in the form of insect vectors such as mosquitos that transfer the bacteria from one subject to another (Eby, 2010). The bacterium is oftentimes harmless, however strains that are resistant to antibiotics, including methicillin resistant \textit{Staphylococcus aureus} (MRSA), can be difficult to treat and have caused mortality in some instances (Cosgrove et al, 2003). The increase in methicillin resistance is acquired through horizontal gene transfer of the \textit{mecA} gene or through random mutations of existing genes. Selective pressure from the environment within animal and human hosts, such as exposure to antibiotics, allows for the transfer of the existing methicillin
resistance and multiply with succeeding generations and replace bacteria that lacks resistance. *S. aureus* can also be multi-drug resistant (MDRSA), which is classified as being resistant to three or more classes of antibiotics and is acquired in similar manner to methicillin resistance (Rinsky et al., 2013). MDSRA makes treating infections extremely difficult as many of the commonly used antibiotics to treat *S. aureus* are ineffective, such as cephalosporins and clidamycin.

*S. aureus* is found most frequently on the skin of individuals as well as within the nasal cavities. Routes of transmission of *S. aureus* are not well understood however many pathways have been found as humans can become exposed to the staphylococci bacteria. Hospital associated (HA) *S. aureus* is related to the transfer of the bacteria in hospital settings. Community associated (CA) *S. aureus* has reservoirs in human environments such as through parks, wrestling mats, and other commonly used areas that multiple people have access to and the area is not adequately sanitized (Niami et al, 2003). Despite the infection control measures of health care facilities, the rise of HA-MRSA is much higher than CA-MRSA because the individuals present within hospitals are more likely to have weakened immune systems making them more susceptible to harmful health effects from the exposure. Livestock associated (LA) *S. aureus* is found in livestock farms such as confined feeding operations that use antibiotics both therapeutically and for non-therapeutic purposes.

Strains of *S. aureus* are molecularly typed to help determine their genetic properties and relatedness and their epidemiological origins. Typing of the surface protein A, *spa* typing, involves the typing of a single locus. The *spa* typing technique uses sequences within the polymorphic X region of the *S. aureus* specific staphylococcal protein A (*spa*). This technique identifies polymorphic repeats, typically 24 base pairs long, for a given strain and then identifies the *spa* type associated with the strain of *S. aureus* (Hallin et al, 2009). This technique is
commonly used for investigation of outbreak settings of *S. aureus*, as it helps to identify the origin by inferring the possible clonal complex (CC) to which it belongs.

**Relationship of *S. aureus* to Farming Practices:**

Over the past sixty years, major livestock practices have shifted from smaller family owned farms to large industrial livestock operations. The conventional practice of livestock farming in the United States is known as confined animal feeding operations (CAFOs) in which livestock animals such as hogs, chickens, and cattle are densely packed into small areas (Vos et al, 2000). The animals are typically denied access to areas to roam and participate in physical activity and are fed a diet heavy in grains to increase their weight quickly. Also confined animal practices supply antibiotics to prevent diseases and for growth enhancement. It is estimated that 87 percent of all antibiotic use in the United States is administrated to livestock, primarily for growth enhancement (Gilchrist et al, 2007). The exposure to low dosages of antibiotics actually ends up not killing the pathogens but instead promotes resistance by selecting for populations that are resistant to the antibiotic. The fecal matter of the livestock also contains antibiotic resistant bacteria and it is estimated that up to 75% of administered antibiotics are excreted (Anderson et al, 2006; Chee-Sanford et al, 2009).

The manure of the hogs is sometimes poorly treated and is typically stored in a lagoon on the farm before being sprayed onto nearby fields. During instances of flooding or breaches, the lagoons can leach their contents into streams and waterways nearby. In a study conducted in eastern North Carolina, 96 percent of the *E. coli* isolates found within tested hog lagoons were resistant to multiple antibiotics (Liwimbi, 2009). Land application of bio-solids from farms have also seen instances where antibiotic pathogens have leached into the affected soil which can also
be transferred out into groundwater and local water ways (Borjesson, 2009; Anderson et al, 2006). Antibiotic resistant strains of *S. aureus* also have been found in waterway systems as well as the soil surrounding hog farms, further spreading them into other environments (Schulz et al, 2012).

Not only have antibiotic resistant bacteria from livestock been identified in the surrounding environments, they are also found in retail meat and dairy products. The presence of LA-MRSA within retail meat has been found in the United States, Canada, and Europe (Hanson et al, 2011). One study in Switzerland determined that MRSA was present in 1.4 percent of samples of mastitis milk (N=142) and 1.3 percent (N=800) of the sampled pigs prior to slaughter (Huber et al, 2010). In Canada, retail meat products from pork, ground beef, and chicken were purchased from stores in four provinices and tested for the prevalence of MRSA. According to the reported results, MRSA was present in 9.6 percent of pork (N=230), 5.6 percent of beef (N=198), and 1.2 percent of chicken samples (N=250) (Weese et al, 2010). Although the percent of food samples that contain MRSA are relatively small, it does provide evidence that MRSA can be transferred into food which is produced and sold to the general public who may not come into close contact with the livestock animals.

As an alternative to the conventional livestock practices, organic farming grew rapidly since the 1990s and in 2011 the organic market size in the United States was estimated to be 29 billion dollars (Willer, 2011). Since 2008, the sale of organic meat has been one of the fastest growing of the organic industries. To be considered a certified organic hog farm, farmers are required to only feed the pigs natural feed that most likely came from the same land on which the farm animals are raised. In organic farm settings the manure also has to be handled and treated in a fashion that does not negatively harm the soil, crops, and the surrounding environment.
The hogs are also required have space to roam around and supplies to build bedding areas which have been observed to harbor and reduce the spread of pathogens. Organic practices can be beneficial in the livestock setting as the spread of bacteria such as \textit{S. aureus} can be reduced under conditions which have a lack of confinement as well as an absence of antibiotics to lower the chance of strains developing antibiotic resistance (McEwen et al, 2002).

The presence of LA-MRSA and \textit{S. aureus} within the livestock may serve as a risk to IHO workers as it can be transferred to humans. In one study based in North Carolina which compared workers from antibiotic-free hog farms to CAFOs, it was found that MRSA and MDRSA were present in comparable percentages in workers from both of the types of animal production practices. Despite the similar prevalence, \textit{scn} negative LA-MDRSA and LA-MRSA, were only found in workers from CAFOs and not in antibiotic-free farm workers (Rinsky et al, 2013). These results support the growing concern that practices that use antibiotics within livestock and confinement promote the spread of antibiotic resistant bacteria such as MRSA, which are difficult to treat and can be dangerous to humans. Another study from the Netherlands showed that calves were more likely to be carriers of MRSA when treated with antibiotics. Also, the use of hygienic practices that were more likely to be associated with antibiotic free farms than with confined practices, such as the use of bedding for animals, had a negative association with the prevalence of MRSA within the calves (Graveland et al., 2010).

Characteristics of LA \textit{S. aureus} and MRSA:

Livestock associated MRSA and \textit{S. aureus} have been found in a variety of livestock farms such as poultry, cattle, and hogs. First identified in 2003, some livestock associated strains of \textit{S. aureus} belong to the lineage clonal complex 398 (CC398) and can cause infection in both
humans and farm animals (Lewis et al, 2008). First identified in the Netherlands, CC398 has also been identified in Hong Kong, Germany, Belgium, Spain, Italy, Austria, Canada, Australia, and the United States (Monecke et al, 2011). Livestock association is also commonly associated with the absence of the \textit{scn} gene. The absence of the \textit{scn} gene has suggested that the strain of \textit{S. aureus} is livestock in origin. The presence of the \textit{scn} gene is found in low frequencies of \textit{S. aureus} isolates obtained from livestock (2-35%) compared to humans (90-100%) (Price et al., 2012). Another characteristic of livestock association is complete resistance to tetracycline. The tetracycline resistance gene, \textit{tet}(M), has been observed in one study to be present in 99 percent of livestock associated isolates (N=70) and none of the human-associated isolates (Price et al, 2012).

LA-MRSA has been observed in hogs in the United States in rates as high as 49 percent (N=200), with all of the isolates identified as CC398 (Smith et al, 2009). As the livestock animals are contaminated with MRSA, studies have shown that the transfer from animal to human of MRSA is possible through either direct contact, air, or in the animal byproduct used for food. Human exposures to LA-MRSA can occur through occupational exposures and through environmental transmission. Studies have shown that MRSA can become airborne and trapped within dust particles and can be inhaled by individuals (Wilson et al, 2004). Once MRSA has become airborne, the air can be transported downwind from industrial livestock farms and can potentially affect neighbors, although studies have only shown transport downwind to be in small doses 50 m and 150 m from farm sites (Schulz et al, 2012).

Exposure to MRSA may be a risk for employees of livestock operations such as the workers, slaughterhouse employees, and veterinarians who come within close contact with the animals. One study conducted in the Netherlands determined that slaughterhouse workers on hog
farms had a high prevalence of MRSA. The study found that 17 percent of human subjects (N=118) tested positive for MRSA after one day of work, with the most important risk factor being direct contact with live pigs (Van Cleef et al, 2011). Veterinarians that work within the livestock farms are also at high risk for being contaminated with MRSA. In Belgium and Danish farms, it was found that veterinarians were positive for MRSA and LA-MRSA at a rate of 9.5 and 7.5 percent, respectively (Garcia-Graells et al, 2012). Despite the high prevalence rate of MRSA colonization among these occupations, the workers within the livestock farms are of the highest concern as they are typically exposed for longer periods and to larger numbers of animals. A study conducted in Iowa and Illinois determined that MRSA was detected in 45 percent of participating farm workers (Smith et al, 2009).

Although the presence of S. aureus and MRSA has been examined since the 1960s, the presence of LA-MRSA has only recently been studied. First reported in the Netherlands, LA-MRSA has now also been identified in Canada as well as the United States (Golding et al, 2010). In the Netherlands, LA-MRSA has been documented to be present in approximately 71 percent of conventional pig herds (Vijver et al, 2013). In the United States, the first pilot study was conducted in Iowa in 2008 which identified CC398 LA-MRSA in 49 and 45 percent of swine (N=299) and farmworkers (N=20), respectively (Smith et al., 2009). Despite initially high percentages of LA-MRSA being reported, another study in the United States that assessed farms from five states found lower rates of occurrence at 4.6 and 20.9 percent of swine (N=1058) and workers (N=148), respectively (Smith et al, 2013). Although instances of LA-MRSA within the United States have not been determined at the same elevated levels as found within the Netherlands, the problem is still relevant and needs to be explored in more detail to gain a better understanding of its magnitude and the potential health risk.
Nasal Carriage Patterns:

Studies that test for prevalence of *S. aureus* among livestock workers typically test nasal swabs of individuals. However, this test does not distinguish whether an individual is contaminated with *S. aureus*, or is colonized with the bacterium. To be contaminated by *S. aureus* means that an individual is positive for *S. aureus* within their nasal cavities for a single instance but does not necessarily means that they are a persistent carrier. The individual that is classified as being contaminated will most likely test negative for *S. aureus* or MRSA within the first couple days of being exposed to the bacterium such as from a bio-aerosol. An individual is classified to be colonized with *S. aureus* if they test positive for *S. aureus* within their nasal cavity over a longer period of time and have a pattern of *S. aureus* positivity that proves to be persistent despite changes in exposure (Hardy et al, 2006).

Risk Factors for Exposure:

Risk factors associated with contamination or colonization of LA-MRSA are not well understood. In the Netherlands, a study was conducted that enrolled 52 veal farmers to identify possible factors for MRSA colonization. Results showed a positive relationship between the presence of animal reservoirs, such as free range cats and dogs, around the subjects and positive contamination of MRSA. There was also evidence of a positive relationship in the duration of human exposure to veal calves and human contamination with MRSA. The study also analyzed dust samples from the stables and determined that there was a significantly higher MRSA load in farms which had MRSA human carriers (Dorado-Garcia et al, 2013). The major findings of this
study suggest that environmental factors are key risk factors in human contamination with MRSA.

Another potential risk factor is the duration of workers’ exposure to the livestock as well as the intensity of the exposure. In a study which looked at two farms in the mid-western United States, the farm classified as having approximately twice the number of swine as the other farm showed a 64 percent prevalence of MRSA in the workers while the other farm exhibited none (Smith et al, 2009). A related study found that farmers who were classified as persistent carriers for MRSA worked on average 35.2 hours per week while intermediate and never-carriers worked 15.8 and 12.7 hours per week, respectively (Graveland et al, 2011). The correlation of the number of animals an individual is exposed to and the prevalence of MRSA, however, is dependent on MRSA being presence in the animals and environmental media of the farms. These two studies suggest that the longer a worker is exposed and the higher number of animals a worker is exposed to, the greater the likelihood that the worker will be contaminated with MRSA.

It has also been hypothesized that the age of the livestock to which the worker is exposed also plays a factor in the likelihood of worker contamination with MRSA. One study based in Spain looked at the rates of LA-MRSA between the youngest classification of pigs, suckling piglets, and the oldest, finishing pigs. MRSA was reported to be present in 49 percent of the 53 sampled suckling piglets and only 21 percent of the 53 sampled finishing pigs (Gomez-Sans et al, 2010). These findings suggest that workers who are exposed largely to the suckling piglets may be more likely to be contaminated with MRSA as opposed to workers who work with older pigs because suckling pigs have a higher prevalence of MRSA.
Duration of Colonization:

While studies have found that in some cases hospital associated MRSA can colonize a human for up to a year, it is not well understood how long LA-MRSA can persist within humans (Robicsek et al, 2009; Sanford et al, 1994). It may be that once exposed, individuals are able to go home and take a shower and no longer be contaminated. One study that tested showers within hog farms, where workers were sometimes required to shower after work, found up to 26 percent of the shower samples tested positive for MRSA (Larson et al, 2010). This study did not test human workers but did test the swine for prevalence of MRSA so the contribution of MRSA to humans from livestock still remains unclear. It is also unclear whether or not long-term exposure leads to persistent carriage of the LA strain of MRSA or S. aureus.

With the discovery of farm workers carrying LA-MRSA, it is important to determine if the persistence of MRSA colonization is associated with exposure to livestock. Workers who have long-term exposure to livestock have not shown consistent results on the relationship between exposure and colonization. One study conducted on veal calf farmers concluded there was a strong animal exposure association for the colonization of MRSA within the farmer (Graveland et al, 2011). The study found that MRSA was not present after an individual had been away from work and out of contact with the veal calves for a period as long as two weeks, suggesting that LA-MRSA might not be a persistent colonizer within humans. However, one study of hog farmers (N=25) conducted in Germany found that during the farmers’ vacation time of approximately two weeks, 59 percent of farmers did not clear MRSA colonization during their leave from work (Köck et al, 2012). These two longitudinal studies provide conflicting information on the relationship between exposure time and colonization of MRSA.
Colonization of human by LA-MRSA over short-term periods of exposure is also unclear. Researchers at the University of Iowa conducted a study where veterinary students visiting local swine farms were tested for presence of LA-MRSA after a short exposure period of up to four hours. Results showed that it was possible for persons visiting contaminated farms to test positive for MRSA in a nasal swab, with 22 percent (N=30) being positive after the visit. All students were negative 24 hours after the visit which suggests the duration of carriage was brief and that short term exposure does not lead to colonization of the bacteria (Frana et al, 2013). One study analyzed MRSA colonization of field workers who helped take samples from animals and animal houses as short term exposure subjects. Although MRSA was found in 17 percent of the workers (N=34) shortly after the initial visits, results showed that 94 percent of these subjects appeared to be MRSA negative 24 hours after they were first exposed (van Cleef et al, 2011). Results from this study also suggest that brief exposure to livestock farms positive for MRSA does not cause persistent carriage within human subjects.

In order to determine how LA-MRSA strains interact with human strains within the nares of humans, one study based in the Netherlands artificially inoculated human subjects once with a combination of treatable and low risk MSSA (methicillin-susceptible Staphylococcus aureus) using equal amounts of both human (ST931) and bovine (ST398) strains within their nasal cavities. The subjects were first treated with soap seven weeks prior in the inoculation to ensure that any previous infections were cleared and then the subjects were followed for 21 days to determine the duration of colonization of the two strains. Of the 14 participants, four were capable of completely clearing both of the stains within 21 days. Of the remaining 10 participants which still carried S. aureus, five had no differences in the quantity of the two strains and five subjects exhibited a far greater number of the ST398 strain than the human strain of S.
MRSA and S. aureus (Slingerland et al, 2012). Results from this study provide evidence that ST398 is able to outcompete human strains and may not necessarily be a poor colonizer as previously observed. The study also showed no differences in the mobile genetic elements of either strain, suggesting that the human and bovine strains did not interact with one another and alter their genetic characteristics.

Transfer of S. aureus to Household Contacts:

Although there is overwhelming evidence suggesting that LA-MRSA and S. aureus can transfer from livestock to humans (Price et al, 2012; Graveland et al, 2010; Golding et al, 2010), little is understood about the potential to transfer from livestock workers to their family members. In one study, although family members were found to have a lower prevalence rate of MRSA than farmers, family members were more likely to test positive to MRSA if the farmer also tested positive for MRSA (Graveland et al., 2011). Another study found clusters of MRSA in three families, which suggests transmission from person to person as the original farmer first was exposed to CC398 on the farm and transmitted it to their family members (Lewis et al, 2008). Despite the potential for human to human transfer of LA-MRSA to family members of hog farmers, there is still a large difference between MRSA carriage among farmers and family members as not all family members test positive. This suggests that human to human transfer is infrequent and may not be a major route of transmission. Although the transmission from person to person among family members is possible, it is not known how long a family member will remain colonized with MRSA once they are exposed. It is also not uncommon for livestock workers and their families to live on the same land as the farm itself, so the transfer of MRSA can occur not just from the worker by from other environmental exposure sources. The risk of
family members being exposed and potentially contaminated by MRSA greatly increases when they also have the threat of environmental exposure if living on the farm itself.

The potential for farm workers to transmit MRSA to others within their household is especially concerning for households with children. Children are more susceptible to being colonized with MRSA. Children have shown to be at a higher risk for MRSA due to certain unhygienic habitats such as picking their nose and lack of preventative hand washing (Finn, 2005). In studies that have looked at LA-MRSA within families, children have shown to be more likely colonized with MRSA than other family members (Graveland et al, 2011). Children have weaker immune systems typically than older adults making them more likely to be harmed if there was an outbreak by a dangerous strain of LA-MRSA.

**RESEARCH RATIONAL**

*S. aureus* is a bacterium of health concern as it can cause infections in humans. IHOs have been identified as a source of human exposure to livestock associated *S. aureus* with observed antimicrobial resistance in MRSA and MDRSA, possibly due to the use of non-therapeutic drugs for livestock production. Previous studies have identified this exposed human population as a concern, however the nasal carriage rates of individuals for *S. aureus* is still unknown. North Carolina is the second highest producer of hogs in the United States producing 10.1 million hogs annually. The two largest hog producing counties in North Carolina, Duplin and Sampson, are ideal areas to conduct research of occupational exposure to livestock associated *S. aureus*. This study aimed to follow IHO workers and their household contacts for a four month period in order to determine rates of nasal carriage for *S. aureus* and MRSA and to determine indicators of livestock association.
CHAPTER 2: METHODS

A four month longitudinal study was conducted to evaluate persistence of *S. aureus* and MRSA nasal carriage among industrial livestock workers and their family members. Genetic analysis was also conducted to determine if the strains of *S. aureus* detected among this study population were livestock associated. Figure 1 reflects a flow diagram of the laboratory techniques used for sample collection, bacteria isolation, and characterization by biochemical, antibiotic susceptibility, and nucleic acid methods.

Sample Population:

Individuals who were eligible for participation in the study consist of workers from industrial hog operation (IHO) farms who volunteered to participate and up to three household members who also volunteered. Recruitment for this study was conducted with a partnership through REACH NC (Rural Empowerment for Community Help). The workers on the industrial hog farm had to have been at least 18 years old. Adult and children (age 7 to 17) household members who are non-IHO workers were also included. Participants were recruited from counties in eastern North Carolina, primarily Duplin and Sampson counties, the two largest hog producing counties in North Carolina. Participants went through an initial baseline enrollment session where they filled out a questionnaire with information such as their duration of exposure to hogs, how many hours per day they worked around hogs, and type of hog with which they work. During the enrollment session, a nasal swab was self-collected for each participant as a baseline sample. Each participant was then followed for up to four months, where a nasal swab
was self-collected every two weeks in follow-up visits, resulting in up to a total of nine nasal swabs per participant. The four month period was selected as it was thought to capture potentially important occupational changes (e.g. quit job at hog operation, take a vacation from job at hog operation, start working at a different hog operation, or a new replacement herd comes into the current hog operation) which could affect presence/levels of *S. aureus* in the nose, as the previous 14-day study did not show many changes from following workers for only two weeks. Also a four month period was thought to be a short enough period to retain participants for the entire duration.

Quantification of *S. aureus*:

Up to nine nasal swabs per participant were analyzed for *S. aureus*. The nasal samples were taken using BD BBL™ CultureSwab™ in and transported in Liquid Stuart Medium Transport which contains sodium glycerophosphate, calcium chloride, mercaptoacetic acid, and distilled water. A trip blank swab was included in batches of nasal swabs to control for any possible contamination while in transport. Once swabs were taken, they were placed into a cooler or refrigerator (4-8 °C) to minimize die-off of any bacteria. The methods used to store and transfer the swabs as well as quantify *S. aureus* have been previously verified in a lab method survival experiment (Appendix). Swabs were clipped into 1 mL of 0.01 M phosphate buffered saline (PBS). In order to quantify the amount of bacteria present on the nasal swab, 100 µL of the liquid was pipetted on a plate of a *S. aureus* selective chromogenic media, CHROMagar™ *S. aureus* medium (BD, Franklin Lakes, NJ), and then spread evenly via plate spinning. Those plates were incubated overnight at 37 °C.
The number of colonies after one day of incubation was counted and recorded. Plates are considered TNTC (too numerous to count) if they appeared to have more than 300 colony forming units (CFUs). For these samples, a 1 to 10 dilution was made of the original swab in PBS and then re-plated with the same technique used with the neat sample. If the plate still appeared to be TNTC, a 1 to 100 dilution or 1 to 1000 dilution was plated to increase the accuracy of colony counting.

Phenotypic Characteristics Confirmation:

For each of the samples that initially appeared negative during the quantification step, the mixture including PBS and the swab was transferred into a tube of 10 mL of Mueller Hinton Broth with 6.5% NaCl and then incubated overnight at 37 °C. The culture was then streaked onto two different media for confirmation of *S. aureus*: Baird-Parker and CHROMagar™ (BD, Franklin Lakes, NJ). Using two different media to streak the culture has shown to increase identification of *S. aureus* positive samples by 29 percent (Nadimpalli et al., 2013). The plates were incubated again overnight at 37 °C. If the cultures on the plates grew up positive for *S. aureus* (mauve, matte, halo for CHROMagar; black, shiny, halo for Baird-Parker) two positive isolates from either medium were streaked onto the same type of media for isolation.

Molecular Confirmation:

DNA extraction of the isolates was done using the Qiagen’s DNeasy Blood & Tissue kit (Qiagen, Valencia, CA) or by crude extraction of the isolates as detailed in Reischl et al. (2000). Following DNA extraction, multiplex PCR was conducted for three genes, 16S, *nuc*, and *mecA*. The 16S gene is characteristic for all staphylococci. The *nuc* gene codes specifically for *S.*
*aureus* while *mecA* is specific for methicillin resistance. Therefore, if an isolate has both the *nuc* and 16S gene, the isolate is characterized as *S. aureus*. If the isolate contains all three genes, then the isolate is characterized as MRSA. PCR was also conducted for the presence of the *scn* gene. The presence of the *scn* gene serves as another marker to classify isolates obtained during the study as livestock associated. Amplified gene products were confirmed by gel electrophoresis in duplicate along with a positive and negative control for MRSA.

Biochemical Confirmation:

For all isolates on which DNA extraction was performed, biochemical testing for both catalase and coagulase enzymes was conducted to confirm the results from the gel electrophoresis. Testing for the presence of the catalase enzyme was done by placing the colony on a drop of hydrogen peroxide to ensure the bacteria can convert hydrogen peroxide to hydrogen gas. This presence of the catalase enzyme is specific to all strains of staphylococci. Testing for the presence of the coagulase enzyme was done by using Rabbit Plasma (BD BBL, Franklin Lakes, NJ) and adding a sample of the isolate into the mixture. A positive result was shown by the vial appearing to have coagulated after 24 hours of incubation, indicating the presence of the coagulase enzyme, which is *S. aureus* specific.

*Spa* Typing and MDRSA Testing:

*Spa* typing was conducted on all isolates that are positive for *S. aureus* based on multiplex-PCR and gel electrophoresis. *Spa* types of livestock origin that are of interest are ones that are indicative of LA *S. aureus*, primarily CC 398.

Pure isolates that tested positive for *S. aureus* also underwent resistance testing to determine if they classify as MDRSA. Isolates were tested on a range of 12 antibiotic classes.
comprised of 16 antibiotics. Isolates are considered to be MDRSA if completely resistant to three or more classes of antibiotics (Table 1). Antibiotic resistance was determined by using the BD Phoenix™ Automated Microbiology System (BD, Franklin Lakes, NJ) which incorporates the BACTEC™ System (BD, Franklin Lakes, NJ) in automatic identification and susceptibility testing of pure isolates. Isolates that exhibit complete resistance to three or more classes of antibiotics were classified as MDRSA. This portion of the testing was conducted at Johns Hopkins University.

**Table 1. Antibiotics used in susceptibility testing**

<table>
<thead>
<tr>
<th>Antibiotic</th>
<th>Class</th>
<th>Concentration</th>
</tr>
</thead>
<tbody>
<tr>
<td>Moxifloxacin</td>
<td>Fluoroquinolone</td>
<td>5 µg</td>
</tr>
<tr>
<td>Ceftriaxone</td>
<td>Cephalosporin</td>
<td>30 µg</td>
</tr>
<tr>
<td>Clindamycin</td>
<td>Lincosamide</td>
<td>2 µg</td>
</tr>
<tr>
<td>Daptomycin</td>
<td>Lipopetid</td>
<td>4 µg</td>
</tr>
<tr>
<td>Erythromycin</td>
<td>Macrolide</td>
<td>15 µg</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>Aminoglycoside</td>
<td>10 µg</td>
</tr>
<tr>
<td>Linezolid</td>
<td>Oxazolidonone</td>
<td>30 µg</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>Nitrofuran</td>
<td>100 µg</td>
</tr>
<tr>
<td>Rifampin</td>
<td>Rifampicin</td>
<td>5 µg</td>
</tr>
<tr>
<td>Quinupristin/dalfopristin</td>
<td>Other</td>
<td>15 µg</td>
</tr>
<tr>
<td>Sulfamethoxazole with Trimethoprim</td>
<td>Sulfonamide</td>
<td>23.75/1.25 µg</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>Tetracycline</td>
<td>30 µg</td>
</tr>
<tr>
<td>Minocycline</td>
<td>Tetracycline</td>
<td>30 µg</td>
</tr>
<tr>
<td>Vancomycin</td>
<td>Glycopeptide</td>
<td>5 µg</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>Penicillin</td>
<td>10 µg</td>
</tr>
<tr>
<td>Oxacillin</td>
<td>Penicillin</td>
<td>1 µg</td>
</tr>
<tr>
<td>Penicillin</td>
<td>Penicillin</td>
<td>10 units</td>
</tr>
</tbody>
</table>
Statistical Analysis:

Descriptive statistics was performed on the presence/absence of *S. aureus* and MRSA within the nares of each individual over the study period to determine the rate of carriage on the individual and household level. Averages, quartiles, and ranges were calculated on the quantification of *S. aureus* on both the household and individual level. All significance testing was conducted at a significant level of $p=0.05$. To test the difference of overall prevalence for *S. aureus* and MRSA among groups, an exact chi-square test was conducted. A chi-square test was conducted to determine significant difference among carrier status for each participant type. For testing significance of concentration between carriage statuses within groups a one-way analysis of variance was calculated. In testing significance for differences of concentration between participant types, an analysis of variance (ANOVA) was performed. In comparing results to expected values observed in the US population, a chi-squared test was conducted. Significance testing was conducted in comparing results found in the study to those in similar populations by using a two-tailed $Z$-test on proportion. All statistical analysis was performed using SAS, version 9.3 (SAS Institute Inc, Cary, North Carolina).

IRB Approval:

All procedures were reviewed and approved by the Johns Hopkins Bloomberg School of Public Health institutional review board (Baltimore, Maryland) under IRB number 00004608 with reliance approval from the University of North Carolina at Chapel Hill. Before enrollment, all adult participants provided written informed consent and minors consented under the permission of their parent or legal guardian. All participant swab identifications were de-identified prior to arrival at the lab.
Figure 1. Protocol of procedures for *S. aureus* isolations and characterization from nasal swab samples.
Adapted from: (Nadimpalli, 2012).
CHAPTER 3: RESULTS

Study Population:

A total of 183 individuals from 75 households were enrolled in this study. Of the 183 individuals who participated, 100 were industrial hog farm workers, 35 were adult household members, and 48 were minors. Of the 183 participants, approximately half were male (49.2 percent) and majority were Hispanic (89.6 percent). The characteristics of the participants are displayed in Table 2. Individuals enrolled in the study were followed for up to four months and carriage results are only reported on 175 individuals who were followed for at least one month.

Table 2. Distribution of characteristics among 183 participants enrolled

<table>
<thead>
<tr>
<th></th>
<th>Workers % (N=100)</th>
<th>Adult Non-IHO Workers % (N=35)</th>
<th>Minors % (N=48)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Gender</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Male</td>
<td>55 (55)</td>
<td>31 (11)</td>
<td>50 (24)</td>
</tr>
<tr>
<td>Female</td>
<td>45 (45)</td>
<td>69 (24)</td>
<td>50 (24)</td>
</tr>
<tr>
<td><strong>Ethnicity</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hispanic</td>
<td>90 (90)</td>
<td>83 (29)</td>
<td>94 (45)</td>
</tr>
<tr>
<td>African-American</td>
<td>10 (10)</td>
<td>14 (5)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Caucasian</td>
<td>0</td>
<td>3 (1)</td>
<td>0</td>
</tr>
</tbody>
</table>

Prevalence of *S. aureus* and MRSA per Household:

Of the 75 households, 17 percent (n=13) contained individuals who never exhibited *S. aureus* during the study and were classified as non-carrying households (Table 3). Sixty-four percent (n=48) contained individuals described as intermittent carriers meaning some individuals were positive for *S. aureus* for at least one data point in the study but for less than 80 percent of the data points tested. Nineteen percent (n=14) of households were classified as persistent...
carriers where all individuals were positive for *S. aureus* in at least 80 percent of the data points.

The definitions used to outline the different carrier statuses are commonly used as thresholds (VandenBergh et al, 1999).

Eighty-five percent (n=64) of households were never-carrying for MRSA, and fifteen percent (n=11) were intermittent carriers (Table 3). None of the households were persistent carriers for MRSA.

**Table 3. Carriage status of *S. aureus* and MRSA on the household level**

<table>
<thead>
<tr>
<th>Carriage Status</th>
<th><em>S. aureus</em> % (N= 75)</th>
<th>MRSA % (N= 75)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never-Carrying</td>
<td>17 (13)</td>
<td>85 (64)</td>
</tr>
<tr>
<td>Intermittent</td>
<td>64 (48)</td>
<td>15 (11)</td>
</tr>
<tr>
<td>Persistent</td>
<td>19 (14)</td>
<td>0 (0)</td>
</tr>
</tbody>
</table>

*a* Refers to all individuals within the household exhibiting characteristic 0% of data points  
*b* Refers to all individuals within household exhibiting characteristics >0% but ≤80% of data points  
*c* Refers to all individuals within a household exhibiting characteristics >80% of data points

Prevalence of *S. aureus* and MRSA in Individuals:

The overall prevalence of *S. aureus* in the swab analyzed was 48 percent. There appeared to be a significant difference of *S. aureus* prevalence between the participant groups as workers had the highest prevalence at 52.4 percent (p=0.0024). For the 175 participants, thirty-three percent (n=58) were classified as non-carrying for *S. aureus* as they never tested positive for *S. aureus* on all data points (Table 2). Forty percent (n=70) of the individuals exhibited *S. aureus* between >0 to 80 percent of data points and were classified as intermittent carriers. Twenty-seven percent (n=47) of individuals were categorized as persistent workers as they were positive for *S. aureus* on over 80 percent of data points. In comparing participant type by carriage status, minors appear to significantly have the highest percentage of persistent carriers out of the three
types at 34 percent \( (p=0.0043) \). Workers significantly had the highest percentage of intermittent carriers at 50 percent \( (p=0.024) \). Adult household members had the highest percentage of never-carrying participants out of the three at 52.9 percent however this was not statistically significant \( (p=0.1321) \).

![Figure 2. Distribution of S. aureus carriage status in participants.](image)

The overall prevalence in the swabs analyzed for MRSA was 2.4 percent. There appeared to be a significance difference in the prevalence of MRSA between participant types as workers had the highest with 3.4 percent \( (p=0.0448) \). Ninety-two percent \( (n=161) \) of participants were never-carrying for MRSA and eight percent \( (n=14) \) were intermittent carriers (Table 4). None of the participants were persistent carriers for MRSA.
Table 4. Carriage status of *S. aureus* and MRSA for all participants

<table>
<thead>
<tr>
<th>Individual Categories</th>
<th>S. aureus</th>
<th>MRSA</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>% (N=175)</td>
<td>% (N=175)</td>
</tr>
<tr>
<td><strong>Workers</strong></td>
<td>N=94</td>
<td>N=94</td>
</tr>
<tr>
<td>Non-carrier a</td>
<td>23 (22)</td>
<td>90 (85)</td>
</tr>
<tr>
<td>Intermittent b</td>
<td>50 (47)</td>
<td>10 (9)</td>
</tr>
<tr>
<td>Persistent c</td>
<td>27 (25)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Adult non-IHO workers</strong></td>
<td>N=34</td>
<td>N=34</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>53 (18)</td>
<td>94 (32)</td>
</tr>
<tr>
<td>Intermittent</td>
<td>29 (10)</td>
<td>6 (2)</td>
</tr>
<tr>
<td>Persistent</td>
<td>18 (6)</td>
<td>0</td>
</tr>
<tr>
<td><strong>Minors</strong></td>
<td>N=47</td>
<td>N=47</td>
</tr>
<tr>
<td>Non-carrier</td>
<td>38 (18)</td>
<td>94 (44)</td>
</tr>
<tr>
<td>Intermittent</td>
<td>28 (13)</td>
<td>6 (3)</td>
</tr>
<tr>
<td>Persistent</td>
<td>34 (16)</td>
<td>0</td>
</tr>
</tbody>
</table>

| a Refers to all individuals exhibiting characteristic on 0% of data points |
| b Refers to all individuals exhibiting characteristics >0% but ≤80% of data points |
| c Refers to all individuals exhibiting characteristics >80% of data points |

Quantification of *S. aureus* in Nasal Swabs:

A total of 1016 nasal swabs were analyzed for presence of *S. aureus* for 175 individuals. Quantification results are for individuals that were followed for at least one month. Of the 58 individuals that were classified as never-carrying of *S. aureus*, zero CFUs per swab were found (Table 5). Individuals who exhibited an intermittent carriage status (n=70) had on average of 8.5 x 10^4 CFUs/swab (range: 0 to 3.3 x 10^6 CFUs/swab). Persistent carriers of *S. aureus* (n=47) had on average 7.0 x 10^5 CFUs/swab (range: 0 to 1.2 x 10^7).

Table 5. Quantification of *S. aureus* CFUs/swab for all participants

<table>
<thead>
<tr>
<th>Carriage Status</th>
<th>N=175</th>
<th>Average (CFUs/swab)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never-carrying a</td>
<td>58</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intermittent b</td>
<td>70</td>
<td>8.5 x 10^4</td>
<td>0</td>
<td>3.3 x 10^6</td>
</tr>
<tr>
<td>Persistent c</td>
<td>47</td>
<td>7.0 x 10^5</td>
<td>0</td>
<td>1.2 x 10^7</td>
</tr>
</tbody>
</table>

| a Refers to all individuals exhibiting characteristic 0% of data points |
| b Refers to all individuals exhibiting characteristics >0% but ≤80% of data points |
| c Refers to all individuals exhibiting characteristics >80% of data points |
For the 94 industrial hog farmers, twenty-two individuals exhibited characteristics of never-carrying *S. aureus* had zero CFUs/swab (Table 6). Forty-seven of the workers were intermittent carriers and on average displayed $1.0 \times 10^5$ CFUs/swab (range: 0 to $3.3 \times 10^6$ CFUs/swab). Twenty-five individuals were persistent carriers of *S. aureus* and on average displayed $1.0 \times 10^6$ CFUs/swab (range: 0 to $1.2 \times 10^7$ CFUs/swab).

### Table 6. Quantification of *S. aureus* CFUs/swab for workers only

<table>
<thead>
<tr>
<th>Carriage Status</th>
<th>N= 94</th>
<th>Average (CFU/swab)</th>
<th>Min</th>
<th>Max</th>
</tr>
</thead>
<tbody>
<tr>
<td>Never-carrying</td>
<td>22</td>
<td>0</td>
<td>0</td>
<td>0</td>
</tr>
<tr>
<td>Intermittent</td>
<td>47</td>
<td>$1.0 \times 10^5$</td>
<td>0</td>
<td>$3.3 \times 10^6$</td>
</tr>
<tr>
<td>Persistent</td>
<td>25</td>
<td>$1.0 \times 10^6$</td>
<td>0</td>
<td>$1.2 \times 10^7$</td>
</tr>
</tbody>
</table>

*a* Refers to all individuals exhibiting characteristic $\leq 20\%$ of data points  
*b* Refers to all individuals exhibiting characteristics $>20\%$ but $\leq 80\%$ of data points 
*c* Refers to all individuals exhibiting characteristics $>80\%$ of data points

The distribution of quantification of presumptive *S. aureus* isolates for intermittent and persistent carriers is displayed in Figure 3 and is displayed by participant type. There does not appear to be a significant difference in assessing the difference in the mean *S. aureus* concentration between the three participant types (worker: $p=0.5493$; adults: $p=0.0924$; minors: $p=0.4102$)
a. All Participants

![Box plot for All Participants]

b. Workers

![Box plot for Workers]
c. Adult Household Members

![Box plot showing quantification of S. aureus by CFU/swab by intermittent and persistent for adult household members.]

**Figure 3.** Quantification of *S. aureus* by CFU/swab by intermittent and persistent for all participants (a), workers (b), adult non-IHO workers (c), and minors (d).
Quantification and Carriage Relationship:

The relationship between quantification of *S. aureus* in CFUs/swab versus carriage status is displayed in Figure 4 below. There is a positive association between carrier index and concentration of *S. aureus* ($R^2=0.6782$).

![Figure 4. Average *S. aureus* CFU versus carriage index for all participants. Note: Carrier index is an individual’s proportion of swabs which are positive for *S. aureus* out of the total swabs samples.](image)

Antibiotic Resistance:

A total of 203 presumptive *S. aureus* isolates from 61 participants were analyzed for antibiotic resistance testing of sixteen different antibiotics from fourteen different classes. Of the 203 isolates, forty-three percent (n=87) demonstrated complete resistance to three or more antibiotics and are thereby classified as MDRSA (Figure 5). One hundred ninety-three isolates exhibited complete resistance to β-lactams with the second highest group exhibiting resistance to the macrolides. None of the isolates demonstrated complete resistance to lipopeptides, oxazolidonoes, nitrofurans, rifampicin, sulfonamides, or glycopeptides.
Livestock Association:

Livestock-association was is characterized by absence of the *scn* gene and complete resistance to tetracycline. A total of 369 isolates from 118 individuals underwent PCR for the presence of the *scn* gene. Forty percent of the isolates (n=147) did not demonstrate presence of the *scn* gene. Of the 203 isolates tested for antibiotic susceptibility, 33 percent of the isolates (n=67) demonstrated complete resistance to tetracycline.

**Figure 5.** Percent of isolates demonstrating complete resistance to antibiotic class by sub-group.

- Livestock-association was characterized by absence of the *scn* gene and complete resistance to tetracycline. A total of 369 isolates from 118 individuals underwent PCR for the presence of the *scn* gene. Forty percent of the isolates (n=147) did not demonstrate presence of the *scn* gene. Of the 203 isolates tested for antibiotic susceptibility, 33 percent of the isolates (n=67) demonstrated complete resistance to tetracycline.
CHAPTER 4: DISCUSSION

This study is the first longitudinal study to track industrial hog farmers and their family members for an extended period of four months to determine carriage status of *S. aureus*. Results showed that 27 percent of the participants were persistent carriers of *S. aureus* (n=47), 40 percent were intermittent (n=70), and 33 percent were never-carrying (n=58) which differs from the 20-60-20 percent distribution of carriage status reported among all healthy Americans (Peacock et al, 2001). The distribution of carriage status observed in this study population in comparison to the US population appears to be significantly different ($X^2=30.895$, p<0.001). This provides evidence that the population targeted by this study has higher rates of *S. aureus* carriage than those without livestock exposure.

Overall, MRSA carriage in the study population was lower than *S. aureus* carriage with none of the participants being persistent carriers for MRSA. Eight percent of the study population were intermittent carriers for MRSA (n=14) with the prevalence of 2.4 percent which is higher than the 2002 estimated MRSA prevalence in the US of 0.8 percent in a 2.3 million person study (Kuehnert et al, 2004). The prevalence of MRSA within this study population is significantly different than the observed prevalence in the US population ($X^2=32.880$, p<0.001).

Results from this longitudinal four month study can be compared to those found in a 14-day longitudinal study conducted by members of our research team on a similar participant group of industrial hog farmers in Eastern NC. In the 14-day study, 45.5 percent of the workers were determined to be persistent carriers of *S. aureus* compared to the twenty-seven percent workers
in this study (Nadimpalli et al, 2014). Twenty seven percent of workers in the 14-day study were described as intermittent carriers for S. aureus compared to the fifty percent (n=47) found in this four month study. The difference between the S. aureus carriage rates between the 14-day study and the workers in this study were all found to not be statistically significant (persistent carriers: Z=1.74, p=0.082; intermittent carriers: Z=1.95, p=0.051; non-carrying: Z= 0.404, p=0.689).

None of the workers were persistent carriers for MRSA in this study compared to the 4.5 percent of the workers in the 14-day study. In comparing MRSA carriage rate, this was the only difference found to be significantly significant (persistent: Z=2.07, p=0.038; intermittent: Z=-1.55, p=0.121; non-carrying: Z=0.813, p=0.418).

One of the interesting findings from this study was that minors were the group with the highest percentage of persistent carriers of S. aureus at thirty-four percent (n=16) which was significantly different than the rate observed in workers and their adult household contacts (Table 5). This agrees with a previous study that identified minors as more likely to be persistent carriers than adults, and that carriage patterns tend to change between the ages of 10 and 20 years old (Armstrong-Ester, 1976). Workers had the lowest percentage of never-carrying individuals at twenty-three percent (n=22) which was to be expected due to the fact that the workers have the highest level of exposure compared to the other participants in the study population. Over half of the adult household contacts were never-carrying for S. aureus suggesting that the group is the least likely exposed to S. aureus than the other two, however the difference was not significant (p=0.1321).

There also appears to be a trend exhibited in the quantification of S. aureus isolates and carriage rate of participants. The average CFU/swab increases with the increase in carriage index with a slope of 77574 determined by a linear line of best fit (R²=0.6782) (Figure 4). This suggests that individuals who are prevalent carriers of S. aureus are more likely to carry a larger
concentration of *S. aureus* in the nares and are unlikely to be temporarily contaminated or eradicate the bacterium after a short period of time. Also workers had a higher average of CFU/swab for both persistent and intermittent carriers compared to the all study participants however this was not proven to be significantly different.

Approximately 42 percent (n=79) of tested isolates were MDRSA. Of the commonly used antibiotics used to treat specific *S. aureus* and MRSA infections, the only one to which isolates demonstrated resistance was clindamycin. Isolates tested in this study have not developed resistance to other common antibiotics such as vancomycin, rifampin, trimethoprim-sulfamethoxozol, linezolid, and daptomycin (Wagener et al, 2014). Interestingly enough of the nine different classes in which any of the isolates demonstrated complete resistance to, eight of them are used in the livestock setting. Only one of the five antibiotic classes that none of the isolates demonstrated resistance to, sulfunonamides, had been used in the livestock setting where the other four are only administered to humans.

Out of the 187 isolates which underwent antibiotic resistance testing, 32 percent (n=59) demonstrated complete resistance to tetracycline, a characteristic of livestock association. The percentage of tetracycline resistance is lower than previously reported values in similar study populations of IHO workers and their family members (46.2 percent); however, it still remains larger than expected outside IHO settings (Rinsky et al, 2012). Additionally, the absence of the *scn* gene was demonstrated in 39 percent of *S. aureus* isolates (n=123), suggesting another marker of livestock association.

There were several limitations with this study and study design. First, participants were not chosen randomly due to difficulty recruiting. Therefore the study relied on volunteers that may not be an accurate depiction of the desired study population. This study may be improved if
a randomization of participants could be obtained. However, even then, restrictions due to employees frequently changing jobs and transitory employment on the industrial hog farms would undermine the ability to access accurate work records of employees and their current addresses. Another limitation to this study is the lack of appropriate reference groups such as those within the community or other demographically similar farm populations. Having a reference group would be beneficial for drawing conclusions of the study results.

Another limitation to this study was that we were unable to measure the exposure conditions of individuals. The degree to which individuals are exposed to sources of antibiotic resistant bacteria varies due to lifestyle factors. This study would have benefited from being able to analyze working conditions such as through sampling environmental conditions of industrial hogs operations, the livestock the workers are exposed to, as well as any household exposures. While another study is currently being performed to test household environmental samples from some of the participating households, we were not permitted to sample in industrial animal operations of workers. Future studies would benefit from incorporating environmental samples from both the home and work environment to better link sources and exposure pathways that lead to high risk of contamination or colonization of S. aureus.

Conclusion:

Results from this study showed significant differing MRSA and S. aureus carriage to the general US population which implies livestock exposure may be altering carriage patterns. The resistance to antibiotics administered to livestock observed in isolates from this study suggests that antibiotics used in industrial hog production may be altering the resistance profiles of S. aureus colonizing in IHO workers and their household members. The presence of livestock
association characteristics in *S. aureus* isolates in this population suggest that there may be an exchange of *S. aureus* between hogs and humans exposed to them. These results could help inform policies and practices aimed at lowering occupational exposure risk experienced by workers on industrial animal farms.
APPENDIX: *STAPHYLOCOCCUS AUREUS* SURVIVAL EXPERIMENT

Introduction:

Due to logistical constraints imposed by the design of this persistence study, participants’ nasal swabs were stored for up to eight days prior to laboratory analysis. In order to determine whether false negative swabs could result from an eight-day holding time, we conducted a *Staphylococcus aureus* survival experiment prior to beginning the study. In this experiment, we examined the effect of (a) holding times between one to ten days, (b) storage temperature, and (c) initial inoculation concentration on *S. aureus* survival.

Methods:

Nasal Swab Seeding

An outline of the study design is provided in Figure 6. On Day 0 of the study, we prepared two inoculation solutions with concentrations of $10^5$ colony forming units (CFUs)/ml and $10^3$ CFU/ml, respectively, using freshly grown *S. aureus* (ATCC 25923) diluted in sterile phosphate buffered saline (PBS). Both solutions were quantified by overnight culture at 37°C on tryptic soy agar (TSA) prior to use. Using sterile conditions, 63 BD BBL™ CultureSwabs™ were inoculated with 100 µl of the $10^5$ CFU/ml solution and 63 nasal swabs were inoculated with 100 µl of the $10^3$ CFU/ml solution. This resulted in a final concentration of $10^4$ CFU/swab among 63 nasal swabs, to mimic concentrations that may be detected among persistent nasal colonizers (Iwase et al, 2010), and a final concentration of $10^2$ CFU/swab among the other 63 nasal swabs, to mimic concentrations that may be detected among individuals whose nasal passages are contaminated with *S. aureus*. 
Three swabs seeded with $10^4$ CFU and three seeded with $10^2$ CFU were immediately quantified using procedures described below, in order to obtain baseline counts. For the remaining 120 swabs, half of the swabs seeded with $10^4$ CFU (n=30) and half of the swabs seeded with $10^2$ CFU (n=30) were stored at room temperature (25°C), in ambient light. The remaining 60 swabs were stored at 4-8°C.

Quantification of *S. aureus* on seeded nasal swabs

We assayed three swabs from each of the four experimental groups (Figure 7) on days 1 through 10. Swabs were clipped into 500 µl of PBS and vortexed for 30-60 seconds at high speed. 100 µl of the neat sample and serial 10-fold dilutions thereof were spread on TSA plates using a sterilized spreader and incubated for 24 hours at 37°C. Colonies with *S. aureus* morphology were counted manually or by a destructive counter. The detection limit using this method was 5 *S. aureus* CFU/swab.

Statistical Analyses

We examined the effect of storage time on *S. aureus* survival by constructing time series curves for each initial inoculation concentration. To assess the effect of storage temperature on *S. aureus* survival, we used unpaired, two-sided student t-tests and the Satterthwaite approximation to evaluate the hypothesis that the average *S. aureus* CFU/swab recovered from refrigerated swabs was equivalent to the average *S. aureus* CFU/swab recovered from swabs stored at room temperature ($H_0$: $\mu_1=\mu_2$) for each day elapsed. We evaluated this hypothesis separately for each initial inoculation concentration. All analyses were conducted using SAS version 9.3 (SAS Institute, Cary, NC).
Results:

Ten-day survival curves for *S. aureus* seeded onto nasal swabs at concentrations of $10^4$ CFU and $10^2$ CFU are presented in Figure 7. *S. aureus* was recovered throughout the ten-day period from swabs seeded with $10^4$ CFU, regardless of storage temperature. However, *S. aureus* was only consistently recovered (*S. aureus* CFU/swab ≥ detection limit for all three replicates) from swabs seeded with $10^2$ CFU through days 1-4.

The effect of storage temperature on survival of *S. aureus* was unclear among swabs inoculated with $10^2$ CFU. However, among swabs inoculated with $10^4$ CFU, we observed that storage at 4-8°C resulted in greater survival of *S. aureus* compared to storage at room temperature. This effect was statistically significant at p=0.05 on day 5 and after day 7 (Table 7).

Conclusions:

We conclude that swabs inoculated with $10^4$ CFU or higher will reliably be detected by culture following a holding time of up to eight days whether stored at 4-8°C or 25°C. However, swabs inoculated with $10^2$ CFU or lower may not be reliably detected by culture after five or more days of storage whether stored at 4-8°C or 25°C. To minimize *S. aureus* die-off before laboratory analysis, we determined that nasal swabs should be stored at 4-8°C following participant collection.
Figure 6. Outline of *S. aureus* survival study design.
Figure 7. Survival of *S. aureus* seeded onto nasal swabs over a ten-day period.\textsuperscript{a}

\textsuperscript{a}Markers indicate average *S. aureus* CFU/swab among the three replicates. Error bars indicate standard deviation.
Table 7. Differences in survival of *S. aureus* seeded onto nasal swabs when stored at room temperature (25°C) versus refrigeration (4-8°C) over a 10-day period.

<table>
<thead>
<tr>
<th>Day</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
<th>p-value&lt;sup&gt;a&lt;/sup&gt;</th>
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<td>5</td>
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<td>0.6244</td>
</tr>
<tr>
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<td>-&lt;sup&gt;b&lt;/sup&gt;</td>
</tr>
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<td>-</td>
</tr>
<tr>
<td>9</td>
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<td>-</td>
</tr>
<tr>
<td>10</td>
<td>0.0054*</td>
<td>-</td>
</tr>
</tbody>
</table>

<sup>a</sup>P-value comparing average *S. aureus* CFU/swab recovered from swabs stored at room temperature versus refrigeration using unpaired, two-sided student t-test and the Satterthwaite approximation.

<sup>b</sup>P-value cannot be computed because observations are too few or because there is not enough variation among observations within a group.

*Statistically significant difference in survival at the 0.05 level.


