ENVIRONMENTAL PRESENCE OF AND POTENTIAL OCCUPATIONAL EXPOSURE TO ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* IN REGIONS OF HIGH INDUSTRIAL HOG OPERATION DENSITY

Sarah M. Hatcher

A dissertation submitted to the faculty at the University of North Carolina at Chapel Hill in partial fulfillment of the requirements for the degree of Doctor of Philosophy in the Department of Environmental Sciences and Engineering in the Gillings School of Global Public Health.

Chapel Hill
2015

Approved by:

Jill Stewart

Mark Sobsey

Rebecca Fry

Melissa Miller

Christopher Heaney

ABSTRACT

Sarah M. Hatcher: Environmental presence of and potential occupational exposure to antibiotic-resistant *Staphylococcus aureus* in regions of high industrial hog operation density
(Under the direction of Jill Stewart)

Since the 1980s, hog production in the United States has been characterized by a shift from small, independently owned operations to large, vertically integrated operations often referred to as industrial hog operations (IHOs). This change has been especially pronounced in North Carolina, with most IHOs concentrated in the eastern part of the state. Prophylactic use of antibiotics for growth promotion and disease prevention in these operations may contribute to the selection of antibiotic-resistant (ABR) bacteria in and around IHOs. A growing body of literature has documented the emergence of ABR *Staphylococcus aureus* that is unique to livestock sources; and carriage of these ABR *S. aureus* strains have been documented in hogs and IHO workers. Yet, research regarding dissemination of these bacteria to the off-farm environment is lacking. Important questions also remain regarding potential community exposures and the effects of IHO worker exposure on household members, especially among children who may have enhanced susceptibility to *S. aureus* infection.

To better understand routes of exposure to ABR *S. aureus* originating from IHOs in NC, we investigated 1) the presence of ABR *S. aureus* in surface water proximal to IHO spray fields; 2) associations between occupational exposure to IHOs and ABR *S. aureus* carriage in adult workers and their child (<7 yr old) household members; and 3) associations between work-related activities of IHO workers and ABR *S. aureus* carriage in adult workers and their child household members. Study results document the presence of ABR *S. aureus* in surface water near IHO spray fields. We also observed a higher prevalence of ABR *S. aureus* among IHO workers and their child household members than among community referent participants.

Interestingly, carriage of *S. aureus* strains characteristic of the IHO environment was observed in community referent participants, albeit at lower rates than in occupationally exposed households. Among IHO households, mask use at work was associated with lower carriage prevalence in workers and adult workers bringing protective gear home was associated with ABR *S. aureus* carriage in children. These results suggest that there are potential occupational and environmental routes of exposure to ABR *S. aureus* from IHOs.

To Papa.

ACKNOWLEDGEMENTS

First, I would like to thank my advisor, Dr. Jill Stewart, and my committee members, Dr. Mark Sobsey, Dr. Rebecca Fry, Dr. Melissa Miler, and Dr. Chris Heaney for their guidance and support throughout the PhD program.

I am indebted to the Rural Empowerment Association for Community Help (REACH) for their tireless work on these research projects and the North Carolina Environmental Justice Network (NCEJN) for the training they have provided me in community-based research methods. In particular, I would like to thank Naeema Muhammad, Steve Wing, Devon Hall, and Dothula Baron for their contributions to this research and for their years of patient mentorship. In addition, I would like to thank the REACH community organizers that were responsible for sample collection, recruitment, and enrollment.

I am grateful to all past and present members of the Stewart lab for their support and encouragement. Kevin Myers was responsible for processing the water samples from which all of the presumptive MRSA isolates in Chapter 2 were collected. In addition, I would like to thank Sarah Rhodes for her significant contributions to laboratory sample processing for Chapters 3 and 4. I would also like to thank Maya Nadimpalli for her assistance. A big thanks to the undergraduates who provided laboratory assistance, including Katie Overbey, Sharon Jiang, Thao Le, Preetha Naidu, Daira Melendez, Amy Guo, Sarah Menz, and Grace Marshall.

Thank you to our collaborators at the Johns Hopkins University Bloomberg School of Public Health: Dr. Chris Heaney, Dr. Nora Pisanic, Dr. Ellen Silbergeld, Dr. Karen Caroll, and many others for their contributions to this work. I would especially like to thank Amanda Krosche

for questionnaire data entry. Thanks to Statens Serum Institut, especially Jesper Larsen, for conducting *spa* type analysis.

I would like to thank Dr. Melissa Miller and her lab for their assistance with MALDI-TOF MS, especially Melissa Jones.

I am also grateful to Dr. Jessica Rinsky, Alan Kinlaw, and Dr. Steve Wing from the UNC Department of Epidemiology for their assistance with epidemiologic data analysis and interpretation.

The funding sources that made this research possible include the NIH pre-doctoral Training Grant (T32ES007018), the National Science Foundation Graduate Research Fellowship Program (under Grant No. DGE-1144081), the NSF-NIH-USDA Ecology and Evolution of Infectious Diseases Program (Grant No. 1316318), and the Thrasher Research Fund.

TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

LIST OF ABBREVIATIONS

ABR	Antibiotic resistant
AFO	Animal feeding operation
CA	Community-associated
CAFO	Concentrated animal feeding operation
CC	Clonal complex
CR	Community referent
HA	Healthcare-associated
IHO	Industrial hog operation
MDRSA	Multidrug-resistant *Staphylococcus aureus*
MLST	Multi-locus sequence typing
MRSA	Methicillin-resistant *Staphylococcus aureus*
MSSA	Methicillin-susceptible *Staphylococcus aureus*
PFGE	Pulsed-field gel electrophoresis
PCR	Polymerase chain reaction
ST	Sequence type (Multi-locus sequence type)
VISA	Vancomycin-intermediate *Staphylococcus aureus*
VRSA	Vancomycin-resistant *Staphylococcus aureus*
WGST	Whole genome sequence typing

CHAPTER ONE: INTRODUCTION

1. *Staphylococcus aureus*

Staphylococcus aureus is a gram-positive, opportunistic bacterial pathogen belonging to the family *Micrococcaceae*. *S. aureus* asymptomatically colonizes about one-third of the United States population [1, 2] and although individuals may be colonized without ever becoming infected by the organism, nasal carriage of *S. aureus* is a well-described risk factor for subsequent infection, especially in the hospital setting [3]. *S. aureus* can cause a diverse array of infections, ranging in severity from skin and soft tissue (SSTI) infections to bacteremia, toxic shock syndrome, and sepsis [4].

Development of antibiotic resistance

Since the 1940s, *S. aureus* infections have become increasingly difficult to treat due to the organism's acquisition of antibiotic resistance. Soon after the introduction of penicillin, Kirby [5] documented the presence of a penicillinase in *S. aureus* from infected patients who had not been treated with penicillin. Subsequently, these penicillinase-producing *S. aureus* became pandemic in hospitals and the community [6]. *S. aureus* has since acquired resistance to several other antibiotic classes. Only two years after the introduction of methicillin for clinical use in 1959, methicillin-resistant *S. aureus* (MRSA) emerged in United Kingdom hospitals [7]. *S. aureus* remained susceptible to vancomycin—a drug of last resort against MRSA infections—until 1996 when the first documented case of vancomycin-intermediate *S. aureus* (VISA) was reported [8]; reports of vancomycin-resistant *S. aureus* (VRSA) emerged shortly after, in 2002 [9]. *S. aureus* has therefore evolved resistance to all major antibiotics that have been produced by humans to combat its infections.

Because one of the primary concerns regarding *S. aureus* is its resistance to antibiotics, it is often categorized according to the type and number of antibiotics to which it has exhibited resistance. Methicillin-susceptible *S. aureus* (MSSA) is *S. aureus* that is susceptible to methicillin, but can be resistant to other antibiotics. Methicillin-resistant *S. aureus* (MRSA) is *S. aureus* with broad beta-lactam antibiotic class resistance, including penicillins, cephalosporins, and carbapenems. This resistance is conferred by either *mecA* or *mecC* ($mecA_{LGA251}$) [10, 11] and can also be identified by phenotypic resistance to oxacillin and ceftriaxone/cefoxitin. Multidrug-resistant *S. aureus* (MDRSA) is *S. aureus* exhibiting resistance to three or more antibiotic classes.

***S. aureus* typing methods**

In addition to classifying *S. aureus* by its antibiotic resistance pattern, *S. aureus* is commonly categorized using genetic typing. One of the most useful tools supporting sound epidemiologic investigation of *S. aureus* colonization, infection, and transmission is genetic sequence typing. The most common methods for typing *S. aureus* include pulsed-field gel electrophoresis (PFGE), Staphylococcal Protein A (*spa*) typing, multi-locus sequence typing (MLST), whole genome sequence typing (WGST) and, among MRSA, *SCCmec* typing. In contrast to *spa* typing, MLST, and WGST, pulsed-field gel electrophoresis (PFGE) and SCCmec typing (MRSA only) are able to identify large-scale genetic changes in *S. aureus*. PFGE and SCCmec typing are unable to detect, for example, the single-nucleotide polymorphisms that allow the organism to adapt to new or multiple host species (i.e., identify livestock- versus human-associated *S. aureus*).

In recent years, the preferred typing methods of researchers studying zoonotic exchange of *S. aureus* and the emergence of *S. aureus* in new reservoirs have been those that provide more detailed information regarding the molecular evolution and epidemiology of *S. aureus*. These methods include *spa* typing, MLST, and WGST. It is common for sequence types to be

assigned to clonal complexes (CCs), which are groups of closely related *spa* or sequence types that have a common ancestral genotype.

Staphylococcal protein A (*spa*) typing (*spa* typing) is performed by sequencing the polymorphic region X of the conserved *spa* gene—which is characterized by 24-bp repeats—using a technique developed by Frenay et al. [12]. [13]. Shopsin et al. [14] demonstrated that *spa* typing compares favorably to and is able to group isolates in congruence with PFGE and RLFP (restriction fragment length polymorphism) analysis. The advantages of this method include its unambiguous sequence data, which facilitates inter-laboratory sharing and the development of large-scale databases for national and international epidemiology investigations [13, 14]. It is more discriminatory and simpler than MLST because it only involves the sequencing of a single locus and can be used to study the molecular evolution of *S. aureus* and hospital outbreaks [13-15]. Using based upon repeat pattern (BURP) software, *spa* types can be assigned to *spa* clonal complexes (CCs), which are reasonably congruent with CCs assigned by MLST [16]. Alternatively, CCs can be assigned based on the existing scientific literature.

In 1998, Maiden et al. described **multi-locus sequence typing (MLST)**, which is based on the sequence analysis of the *S. aureus* housekeeping genes *arc*, *aroE*, *glpF*, *gmk*, *pta*, *tpi* and *yqiL,* yielding an allelic profile or sequence type (ST) of distinct alleles of each housekeeping gene. BURST (based upon related sequence types) software can then be used to assign sequence types to CCs. This typing method and software are suitable for studying evolutionary relationships and events, but is not as useful in studying outbreaks as other typing techniques because of its lower discriminatory power [13, 14, 18].

Whole genome sequence typing (WGST) is a relatively new technology that maps genome-wide single nucleotide polymorphisms (SNPs) to a reference sequence using next-generation sequencing technologies. Of the many advantages of this method, one is that whole-genome sequence data can be generated for multiple bacterial isolates at once. Furthermore, discriminatory power of WGST is sufficient to study micro-evolutionary events that cannot be

identified by PFGE, MLST and *spa* typing. This SNP data can be used to inform epidemiological analysis of transmission events that occur in clinical settings, with the discriminatory power to distinguish between isolates recovered only days apart. [19]

Pulsed-field gel electrophoresis (PFGE) typing for *S. aureus* is performed by the digestion of bacterial DNA by the SmaI restriction enzyme, followed by agarose gel electrophoresis during which alternating electric fields are applied at different angles [18]. Banding patterns are interpreted according to the scheme proposed by Tenover et al. [20], which is based on the number of 'genetic events' considered to be associated with differences in bands between bacterial isolates.

***SCCmec* typing** is based on the characterization of different structural properties of the staphylococcal cassette chromosome *mec*, which harbors the *mecA* gene conferring methicillin resistance. SCCmec types are determined using their ccr gene complex type and the mec gene complex class, as described by the International Working Group on the Classification of Staphylococcal Cassette Chromosome [21]. To date, 46 *SCCmec* variants have been identified, but the epidemiological relevance of all types is unknown [22]. SCCmec types I-V are most well characterized and studied, with SCCmec type II predominating in hospitals and thus limiting the discriminatory power of the technique for measuring differences among isolates [23]. The combination of the SCCmec classification and the sequence type from MLST and epidemiological information are commonly used to better understand the source of MRSA outbreaks.

2. Antibiotic-resistant *S. aureus* in healthcare and community settings

When MRSA first emerged in the United Kingdom in the 1960s [7] and subsequently became endemic in the United States and worldwide, most MRSA infections were confined to the hospital and healthcare setting. However, in the early 1990s, MRSA infections became more common in otherwise healthy individuals without a history of recent healthcare contact [24]. In the United States, MRSA infections outside of the hospital setting were identified in the early

1980s [25] but the first cases among those with no risk factors for MRSA emerged in otherwise healthy children in the late 1990s [26, 27]. Molecular epidemiology investigations revealed that these isolates were genetically distinct—and often more virulent—than their hospital-origin counterparts [28].

Epidemiological and microbiological definitions

Two categories of MRSA, often termed “healthcare associated” (HA-MRSA) and “community associated” (CA-MRSA) have been distinguished by both epidemiologic and microbiologic characteristics. A community-associated MRSA (CA-MRSA) infection, in contrast to a healthcare-associated (HA-MRSA) one, is an infection that emerges from “a strain isolated in an outpatient setting, or from patients within 48 h of hospital admission” in a patient with “no history in the previous year of either hospitalization, admission to a nursing home, dialysis or surgery” and with no permanent indwelling or temporary medical devices that pass through the skin [13]. Microbiologically, CA-MRSA is more likely to harbor SCC*mec* type IV [29] and HA-MRSA is more likely to harbor SCC*mec* types I, II, or III. CA-MRSA is often positive for the virulence gene Panton-Valentine Leukocidin (*pvl*) while HA-MRSA is *pvl*-negative, respectively [30], but CA-MRSA is less likely to be multidrug-resistant.

Risk factors for infection

Using the traditional definition of CA- and HA-MRSA described above, several risk factors for both colonization and infection have been documented. Risk factors of HA-MRSA colonization include: prior hospitalization [31, 32]; severity of disease classification and prolonged hospital stay [31]; and high numbers of MRSA carriers at the time of hospital admission [33]. Risk factors of HA-MRSA infection include: colonization prior to hospital admission [34]; recent antibiotic therapy [35]; undergoing invasive procedures while colonized [31, 36]; and stay in an intensive care unit [35].

Risk of CA-MRSA infection has been associated with a variety of populations, including Blacks [37]; Alaska Natives, Native Americans and Pacific Islanders [38]; athletes [39] and

sports teams [40]; military recruits [41]; prisoners [42]; and children [43], specifically those attending child care centers [44] and under two years of age [45]. Among incarcerated persons, prior antibiotic use was shown to be a risk factor for CA-MRSA infection [46]. Risk factors for infection also include contact with a person in the same household colonized and/or infected with MRSA, history of colonization or recent infection with community-associated MRSA, and concurrent skin and soft tissue infection [45]. Risk factors for CA-MRSA colonization are not well documented in the literature but likely result from close contact with those colonized or infected with MRSA, especially among the above risk groups.

Antibiotic-resistant *S. aureus* among children

Children have been identified as a group at increased risk for many infections, including CA-MRSA [47]. Their increased susceptibility relative to adults can be attributed to their underdeveloped immune systems and generally less-sanitary interactions with their environment. In addition, risk factors for CA-MRSA infection among children include recent use of antibiotics, a history of MRSA infection or symptoms in the family, and parental occupation in a school or daycare [48]; and child care attendance [49].

In the United States, the CA-MRSA first emerged among otherwise healthy children [27] and prevalence of CA-MRSA in children without previously recognized risk factors has since become more common [50, 51]. For example, between 1988-1990 and 1993-1995, Herold et al. [26] documented a nearly 26-fold increase in CA-MRSA infection among children admitted to the hospital. Furthermore, CA-MRSA colonization – which is considered a risk factor for infection in adults and children – has also increased in pediatric intensive care unit patients [52].

In addition to this increase in CA-MRSA carriage and infection among healthy children, MDRSA carriage – including multidrug-resistant MRSA – has emerged in young children in some regions [53, 54]. Similarly to MSSA and MRSA carriage and infection risk factors, MDRSA carriage may be affected by recent antibiotic use, number of household members, and parental smoking [53]. However, pediatric MDRSA carriage and infection prevalence and risk factors

have not been extensively explored.

Increasing burden of CA-MRSA in the healthcare environment

Although it has been well established that HA- and CA-MRSA are distinct both epidemiologically and microbiologically, these characteristics are becoming increasingly less distinct in hospitals [55] and communities [30]. Additionally, CA-MRSA infections have occurred in patients with healthcare-associated risk factors [56]. Recently, David et al. [57] demonstrated that, by 2008, under one quarter of their epidemiologically-defined HA-MRSA belonged to traditional microbiologically-defined HA-MRSA clones (defined as ST5 or USA100 in their study), and their MRSA infections in their hospital were replaced by characteristic CA-MRSA clones (defined as ST8 or USA300 in their study). The studies demonstrate the need to complement epidemiologic definitions with phenotypic and genotypic analyses of isolates as well as the importance of periodic reevaluation of the relevance of categories of MRSA infection origin. Furthermore, the replacement of hospital infections by an organism that was previously thought to have its epidemiologic onset in the community highlights the importance of monitoring emerging strains of *S. aureus* both within and outside of the hospital setting, especially among individuals that have a higher risk of exposure to antibiotic-resistant *S. aureus*.

3. Industrial hog production

Since the 1980s, food animal production in the United States has been characterized by a shift from small, biologically diverse, and independently owned farms to large, vertically integrated operations [58, 59]. This system of animal production is characterized by the high-throughput production of hundreds to thousands of food animals (i.e., swine, layer hens, broiler chickens, etc.) in partial or complete confinement. To illustrate this shift, between 1978 and 1994 alone, there were 63% fewer operations, but 30% more operations with at least 500 hogs [60]. Through the practice of producing more animals in a smaller place, companies enjoy the benefits of lower production cost per animal [59].

These industrial animal production facilities are often referred to as animal feeding operations (AFOs) or concentrated animal feeding operations (CAFOs). The Environmental Protection Agency (EPA) defines an AFO as “a lot or where animals have been, are, or will be stabled or confined and fed or maintained for a total of 45 days or more in any 12-month period, and crops, vegetation, forage growth, or post-harvest residues are not sustained in the normal growing season over any portion of the lot or facility” [61]. An AFO may be designated as a CAFO on a case-by-case basis. In addition to the number of animals, CAFO designation by the EPA is partially based on whether or not a facility declares waste discharge into surface waters. [61] This proposal focuses on the potential impacts of hog AFOs and CAFOs and for the purposes of simplicity, facilities where hogs are raised in confinement will be referred to as industrial hog operations (IHOs).

Industrial hog production in North Carolina

Changes in hog production practices have been especially pronounced in North Carolina, which is second only to Iowa in pork production in the US, with the majority of swine CAFOs concentrated in the eastern part of the state [62, 63]. According to agricultural census data, in 2012 approximately 9 million hogs were grown on 2,217 farms in North Carolina. Roughly 8 million of these hogs were grown on 936 integrator or contract grower farms, while 1 million of were grown on 1,281 independent farms [64]. According to the 2013 North Carolina Agricultural Statistics [63], the top ten hog producing counties in the state, in order from most to least dense are: Duplin, Sampson, Bladen, Wayne, Jones, Greene, Robeson, Pender, Lenoir, and Pitt, all of which are located in eastern NC.

Antibiotic use in industrial hog production

In the industrial hog production system, antibiotics are administered for three reasons: (1) therapeutic treatment of sick animals; (2) prevention of disease via prophylaxis; and (3) to promote growth by increasing feed efficiency [58]. In 2011, approximately 13.5 million kg of antibiotics were sold for use in food-producing animals in the United States, of which 8.2 million

kg consists of antibiotics deemed important for use in human medicine. Of this 8.2 million kg of medically important antibiotics, 97% are available without veterinary prescription. Furthermore, the FDA reports that nearly 7.7 million kg of the medically important antibiotics sold for use in food producing animals were administered via feed or water. [65] Administration of antibiotics in feed and water means that there is little control over the dose each animal receives. Such antibiotic use may contribute to the selection of antibiotic-resistant bacteria in and around CAFOs [66].

Waste management in industrial hog production

Most large IHOs in the United States treat their waste using an anaerobic lagoon [67]. Regardless of the confinement building type, waste that falls through the slatted floors is usually transported to an anaerobic lagoon. These lagoons are deep, built in low-permeability soils or over clay or plastic liners, and designed to provide enough storage to withstand a 25-year, 24-hour rainfall event. They have a hydraulic residence time of 3 months or more and fill to capacity within 2-3 years of construction. As their name suggests, the lagoons are constructed to be anaerobic throughout most of the water volume to allow for the treatment of their high organic loads by anaerobic bacterial digestion. The sludge layer must cleared out by periodically applying it to land with liquid waste or using a manure slurry spreader to land-apply the sludge [68]. Land application of the waste is the final step in this waste treatment and management system. Waste from anaerobic lagoons is applied to fields and croplands based on agronomic loading rates as described in the NRCS Code 633 [69].

4. Zoonotic *S. aureus* in the industrial hog operation setting

In addition to healthcare- and community-associated *S. aureus*, the industrial livestock operation setting can serve as a potential source of human exposure to antibiotic-resistant *S. aureus*. Antibiotic resistance likely emerged in this reservoir as a result of the animal husbandry practices (antibiotic use) involved in vertically integrated industrial livestock production. While zoonotic *S. aureus* associated with industrial hog operations has been studied extensively in

both humans and animals in Europe, research in the United States has been limited. Furthermore, it appears that the molecular epidemiology of *S. aureus* associated with livestock production in the United States differs from that of Europe, where MRSA CC398 appears to be the dominant *S. aureus* strain circulating in the industrial hog production environment [70].

Markers of livestock association

Although antibiotic-resistant *S. aureus* was first identified in livestock in 1972 [71] researchers have since discovered that industrial livestock operations can serve as a reservoir of antibiotic-resistant *S. aureus* and that *S. aureus* originating from this reservoir are genetically distinct. In keeping with the terminology used to distinguish *S. aureus* from the healthcare vs. community setting, the term “livestock-associated” is often used to refer to *S. aureus* belonging to a clonal complex that has been detected in industrial livestock. However, within these livestock-associated CCs, there are human- and animal-adapted strains (human-adapted or livestock-adapted *S. aureus*). While *S. aureus* genotype and phenotype provide insight into the human or animal origin of a *S. aureus* isolate, there is currently no established universal definition of “livestock-associated” *S. aureus*. This is further complicated by the diversity of genotypes that appear to be circulating in the industrial hog operation and the lack of surveillance for these strains in industrial hogs, IHO workers, and in rural communities [70]. However, several proposed markers of livestock adaptation have been suggested, including tetracycline resistance, absence of *scn*, and clonal complex (genotype).

When the first non-typeable (NT) by PFGE *S. aureus* strains emerged in pigs, pig farmers, and their household contacts in France and the Netherlands, MLST revealed that these and other accounts of pig-associated *S. aureus* belonged to clonal complex (CC) 398, which consists of the closely related sequence types ST398, ST752, and ST753 [72]. Several other clonal complexes have been associated with pigs, including CC5, CC9, CC30, and CC45 [73, 74], although CC398 appears to be the most common among pigs and humans who work in direct contact with pigs [75]. While early accounts of ABR *S. aureus* classified all CC398 as

"livestock-associated", recent research has revealed that there are human- and livestock-adapted clades within CC389 [76]; these genetic distinctions have also been observed among additional CCs, including those associated with cattle [77, 78] and poultry [79]. Therefore, it is not sufficient to rely solely upon clonal complex as an indicator of human vs. livestock origin of a *S. aureus* isolate.

In an effort to distinguish between livestock- and human-adapted *S. aureus* among livestock-associated CCs, other markers of livestock association have been suggested in the literature. These include the absence of genes that modulate the ability of *S. aureus* to colonize and infect humans as well as the presence of phenotypic and genotypic antibiotic resistance to antibiotics commonly used in industrial animal production. By weight, tetracycline is one of the most heavily produced antibiotics for use in industrial animal production [65, 80] and is a feed supplement due to its growth-promoting effect [81]. Tetracycline resistance, which can be conferred by *tet(M)*, is frequently documented among livestock-adapted *S. aureus* [76]. In addition to the presence of *tet(M)* among CC398 of animal origin, Price et al. [76] demonstrated that absence of *scn*, which encodes a staphylococcal complement inhibitor that is a part of the immune evasion cluster (IEC) in *S. aureus*, is strongly associated with *S. aureus* CC398 isolates of animal origin. Similarly, McCarthy et al. [82] and Verkaik et al. [83] investigated differences in carriage of the *S. aureus* IEC in *S. aureus* isolates from human and animal sources by detection of *scn* and found a greater prevalence of *scn* in human compared to animal *S. aureus* strains. Furthermore, Sung et al. (2008) determined that *scn* is often absent in *S. aureus* of livestock origin.

Both absence of *scn* and the presence of tetracycline resistance conferred by *tet(M)* have been validated as markers of livestock-adapted *S. aureus*, but only among CC398 [76]. Although phenotypic tetracycline resistance has yet to be validated as a marker of livestock-adaptation, recent research in our study area documented widespread phenotypic tetracycline resistance among *scn*-negative *S. aureus* belonging to CC398 [84]. These studies provide a

supplemental framework to strain typing for understanding the potential origins of *S. aureus* isolates from livestock and humans.

It is worth noting that, in contrast to research conducted in Europe, no comprehensive surveillance of strains circulating in the United States industrial hog operation setting have been conducted [70]. Therefore, in the United States, interpretations regarding whether or not a *S. aureus* isolate is of livestock origin is often based on European studies and a limited number of studies conducted in the United States. Some United States-based research has investigated carriage within the pig host [85, 86], but much of the remaining evidence base in the United States relies on human carriage in individuals with regular and intense industrial livestock contact [84, 87, 88] due to restricted researcher access to industry-owned hogs [70].

Antibiotic-resistant and livestock-associated *S. aureus* in hogs and humans

Antibiotic resistant *S. aureus* with markers of livestock association have received substantial attention in Europe (most commonly reported as CC398 MRSA) and, more recently, the United States. The earliest reports of colonization, infection, and transmission of CC398 MRSA were case reports in the Netherlands [89] and retrospective investigations conducted in France [90]. In 2003, CC398 MRSA was discovered in a hog farmer, his family, workers, and hogs on his farm in the Netherlands [89]. Later, Voss et al. [91] investigated hog farming as a potential source of MRSA in the Dutch community – where prevalence of MRSA was extremely low at the time – and found that not only was the family of their patient of interest colonized by the same *spa* type (t108) as their hogs, but that other hog farmers in the area were colonized by *spa* type t108 as well. In France, Armand-Lefevre et al. [90] used MLST to retrospectively investigate the sources of *S. aureus* colonizing healthy farmers and healthy non-farmers and compared these strains to *S. aureus* collected from infected hogs in the same regions in France. The majority of *S. aureus* isolates collected from healthy hog farmers were most genetically similar to isolates of hog origin and belonged to sequence types not found in the collection of *S. aureus* from healthy non-farmers. These reports of zoonotic antibiotic-resistant *S. aureus*

among livestock and farmers in Europe stimulated a field of research investigating the prevalence of the antibiotic resistant *S. aureus* circulating in hogs and humans occupationally exposed to hogs, as well as potential transmission to familial and other household contacts of hog workers and nearby communities.

Antibiotic-resistant *S. aureus* in hogs

Since the first reports of antibiotic-resistant *S. aureus* colonizing hogs, farmers, and their families in the Netherlands [89], antibiotic-resistant *S. aureus* has been documented in hogs globally [89, 92-99], including Canada [100] and the United States [85, 86]. While CC398 is the most common strain of *S. aureus* among hogs [75] other CCs that have been collected from hogs include CC1, CC5, CC8, CC9, CC30, CC45, CC97, CC49, and CC133 [73, 94, 101, 102]. A limited number of studies have investigated the persistence and transmission of antibiotic-resistant *S. aureus* within and between swine herds [103-106], but several studies have addressed the potential impacts of this reservoir on occupational and community health.

Human exposure to ABR *S. aureus* of potential livestock origin

Humans with the highest risk of exposure to and infection with *S. aureus* associated with the livestock reservoir are those whose occupation involves direct contact with hogs, such as livestock veterinarians [102, 107-109] or IHO employees [89, 102, 110]. It is unclear the extent to which carriage of these strains of *S. aureus* in occupationally-exposed individuals can lead to exposure among individuals with whom they are in close contact, such as household members. Although workers may be persistently colonized by *S. aureus* [88] the transmissibility of livestock-adapted *S. aureus* appears to be lower than that hospital- and community-associated strains [111]. Additionally, individuals living in communities with a high density of intensive hog production may be disproportionately exposed to *S. aureus* characteristic of livestock sources [112], but the mechanisms for this exposure are not well understood.

Occupational exposure. Industrial hog operation workers, slaughterhouse workers, livestock veterinarians, and others in close contact with hogs and pork products at work are

most likely to be exposed to ABR *S. aureus* with markers of livestock association. Over the last decade, research both internationally [89, 91, 99, 102, 111, 113-117], and in the United States [84-86, 88] has documented carriage of *S. aureus* with markers of livestock association in hog workers, establishing that individuals in direct contact with hogs are more likely to carry these strains. Furthermore, workplace exposures such as intensity of animal contact and farm hygiene tend to be associated with risk of carriage of MRSA with markers of livestock association [118, 119]. Other occupations that carry a risk for carriage of these strains of *S. aureus* are livestock veterinarians [109, 111, 120] and slaughterhouse workers [87, 121, 122]. Among all of these occupationally exposed groups, it is unclear how long carriage persists [88, 109, 113, 123].

Although a large amount of research has characterized this occupational exposure in Europe, different conditions are being found in North Carolina and elsewhere in the United States [70]. In contrast to observations in European countries, research in NC suggests NC IHO workers infrequently carry MRSA and that LA-MDRSA is the more prevalent strain in the region's IHO workers [84]. These differences may be due in part to the focus of European surveillance on MRSA carriage and infection, rather than all strains of antibiotic resistant *S. aureus* characteristic of livestock sources. In the absence of access to source samples and information regarding feed additives in the United States, further research characterizing *S. aureus* carried by IHO workers will contribute to our understanding of the impact of antibiotic use in IHOs on exposure to antibiotic-resistant *S. aureus* among those in closest contact with industrial hogs and among non-occupationally exposed community members living near these operations.

Community exposure. Non-occupationally exposed individuals may be exposed to livestock-associated *S. aureus* by (1) contact with household members who are employed in an IHO; (2) contact with or consumption of retail meat contaminated with LA-*S. aureus;* and (3) residential proximity to a high density of industrial livestock production.

In addition to exchange of *S. aureus* between humans and animals, human-to-human transmission of *S. aureus* characteristic of the IHO environment may occur between individuals living in the same household [124]. Such transmission pathways have been investigated but it is often unclear whether homology between hog farmers and their household members is due to household member livestock contact, environmental contamination, or human-to-human transmission. Garcia-Graells et al. [125] documented a high level of homology between farmers and their household members, but household member MRSA carriage was significantly associated with exposure to pigs and administering pig antibiotics; their direct contact with pigs makes it difficult to draw strong conclusions about human-to-human transmission events. In another study, *spa* type homology was observed 4.3% of the time between hog workers and their household members that did not have direct contact with livestock; however, households were often located on the same property as the farm and this homology may therefore be due to environmental contamination [111]. In North Carolina, IHO workers often do not live on the same property as the hog operation where they work. Research to date has documented few cases of *S. aureus* genotype being carried in workers and their household members at the same time [84], implying that transmission of *S. aureus* from livestock sources is low, as has been suggested by several European studies [111, 126, 127]. However, pilot studies suggest that NC IHO workers are persistently colonized, even after up to 96 h away from work [88], which provides more opportunities for transmission. In addition, previous studies of IHO workers and their household members in NC may not have been of adequate sample size to capture *S. aureus* isolate homology between workers and their household members [84].

Industrially produced meat is distributed nationally and globally, thus serving as a potential route of exposure to those geographically removed from the industrial livestock reservoir. In a study conducted in rural Iowa, 18.2% of commercially available pork was contaminated with antibiotic-resistant *S. aureus* [128]. While one study in Canada found that 5.8% of retail pork was contaminated with MRSA and that 32% of isolates were a strain

commonly associated with livestock [129], another quantified only low levels of MRSA contamination and no livestock-associated strains [130]. Additionally, MDRSA belonging to sequence types that have been detected in industrial livestock has been detected in retail meat and poultry in the United States [131]. The risk of *S. aureus* infection from retail meat is considered to be low [132], but there is a lack of evidence to evaluate this route of exposure. There have been no documented cases of carriage of or infection with *S. aureus* due to contact with industrially-produced meat products and contact with retail meat has not been epidemiologically linked with carriage of or infection with *S.aureus* with markers of livestock association.

In addition to occupational and household exposures, individuals living in regions where industrial livestock production is most concentrated appear more likely to be exposed to antibiotic-resistant *S. aureus* and *S. aureus* with markers of livestock association. In the Netherlands, doubling pig density increases the odds that an individual carries MRSA belonging to CCs or sequence types associated with pigs – rather than other strains of MRSA – by nearly 25% [112]. Additionally, hospitals in the Netherlands in areas with a high density of pig farming observed a significant increase in their detection of MRSA carriers between 2002-2006 and 2006-2008; 82% of the newly identified carriers were colonized with CC398 MRSA. While this was partially due the inclusion of patients in direct contact with livestock, the majority of patients with CC398 MRSA infections did not have direct contact with livestock [133]. Such research is possible in the Netherlands due to their nationwide surveillance systems for MRSA that are not just focused on hospital and community-associated strains, but also strains that are characteristic of livestock sources. Evidence is more limited in the United States, but a few studies have attempted to investigate associations between livestock density and antibiotic-resistant *S. aureus* carriage or infection using hospital-based surveillance systems. Among veterans in rural Iowa, living within one mile of an IHO with >1,000 animal units increased the risk of being colonized with MRSA [134]. Residential proximity to IHOs and IHO spray fields

may also be associated with CA-MRSA as well as skin and soft-tissue infection, although by strains that are uncommon in livestock [135, 136].

A limited number of cases of carriage of *S. aureus* with markers of livestock association have been documented in non-occupationally exposed individuals who live or work near IHOs. Moritz and Smith [137] reported carriage of MSSA *spa* type t571 in a childcare worker in Iowa and Neyra et al. [87] reported an elevated level of MDRSA and MRSA among non-occupationally exposed individuals from communities with a high density of IHOs in eastern North Carolina. These studies suggest that there is a need to better characterize potential non-occupational exposures in regions of the United States where IHOs are heavily sited.

Although these United States-based studies did not document strains characteristic of livestock sources, much of our interpretation of what classifies a *S. aureus* isolate as "livestock-associated" is based on European surveillance of strains circulating in their livestock production systems [70] and on the limited number of studies that have been conducted in the United States. Even though the aforementioned hospital-based studies investigating proximity to IHOs as a risk factor for infection and carriage did not detect strains that are commonly recognized as characteristic of pig sources, we lack a sufficient evidence base in the United States to state with certainty that none of the ABR *S. aureus* carriage or infection documented in these studies are of livestock origin. These studies additionally suggest that there may also be potential for the transport of antibiotic residues or antibiotic resistance genes from IHOs to the off-farm environment that could alter the natural flora of individuals living in close proximity to IHOs [70]. While evidence is limited, these studies highlight the potential emerging public health impacts in communities where IHOs are numerous, as well as the need to better understand the community health impacts of environmental contamination with ABR *S. aureus* with markers of livestock association.

5. Potential environmental routes of exposure to *S. aureus* characteristic of IHOs

A limited number of studies have examined the environmental mechanisms for dissemination of ABR *S. aureus* characteristic of pig sources from IHOs to the community. Although few have documented potential mechanisms for their dispersal into the environment, antibiotic-resistant – including methicillin- and multidrug-resistant – *S. aureus* have been recovered from the environment surrounding industrial livestock operations.

Air

While some studies have documented the presence of bioaerosols and endotoxin in and around IHOs [138, 139], airborne *S. aureus* with markers of livestock association within and outside of operations has not been studied extensively. Schulz et al. [140] demonstrated that MRSA could be detected in soil at distances of up to 300 m from pig barns inside which MRSA was collected from air, swine, or plastic work boots. Furthermore, air samples within and downwind of MRSA-positive confinement buildings were the same *spa* types and belonged livestock-associated CCs. Additionally, pooled dust samples from barn surfaces in another IHO in Germany also tested positive for MRSA, while samples from organic hog farms did not [116]. More recently, airborne MSSA and MRSA has been detected within hog confinement buildings at a mean concentration of 1564 CFU/m^3 and 300 CFU/m^3, respectively [141] on farms where hogs and hog workers previously tested positive for MRSA and MDRSA carriage [102]. Though understudied, airborne deposition of antibiotic resistant and livestock-associated *S. aureus* remains a plausible mechanism of transport to nearby environments.

Insect, rodent, and wildlife vectors

Antibiotic-resistant *Staphylococcus* have been isolated from insects in industrial animal operations [142, 143] and MRSA, including MRSA belonging to livestock-associated CCs, was isolated from black and brown rats on pig farms in the Netherlands [144]. These vectors may transmit ABR *S. aureus* from confinement buildings to nearby environments, but the presence of ABR *S. aureus* colonizing vectors beyond the immediate farm environment has not been

documented. Antibiotic-resistant *S. aureus* has been detected in wildlife [145] but the significance of wildlife as a reservoir, its impact on human health, and the likelihood of wildlife vectors to interact with the various components of the IHO reservoir (i.e., waste, spray fields, confinement buildings) is unclear.

Antibiotic-resistant *S. aureus* in waste

Waste from IHOs can serve as a reservoir for antibiotic resistant bacteria and genes conferring antibiotic resistance (including multidrug resistance) via three conceivable mechanisms that may be influenced by antibiotic use in agriculture: (1) shedding of antibiotic-resistant organisms by hogs; (2) shedding of unabsorbed antibiotics by hogs; and (3) antibiotic residues from feed and water that may enter the waste stream prior to administration to hogs. Sobsey et al (2005) demonstrated that antibiotic-resistant *E. coli* and *Salmonella* could be detected from fresh and treated waste on conventional farms and that there was no significant difference in antibiotic resistance in the isolates collected from treated versus untreated waste. Additionally, Binh et al. [146] demonstrated that pig manure can serve as a reservoir for genes conferring multidrug resistance. Tetracycline resistance genes have also been detected in hog waste [147]. Antibiotic residues of tetracyclines, sulfamethazine, lincomycin, penicillin, and erythromycin have all been detected in manure from hog waste management systems [148]. Horizontal gene transfer may then increase the number of antibiotic-resistant bacteria in waste. Antibiotic-resistant bacteria have also been detected in soil where industrial animal wastes are land applied [149].

Antibiotic-resistant *S. aureus* have not yet been detected in hog waste in the United States; however, *S. aureus* and MRSA have been isolated from human wastewater treatment plants (WWTP) [150, 151]. Additionally, Friese et al. [152] detected MRSA in approximately half of fecal waste samples collected on MRSA-positive German pig farms. Better characterization of antibiotic-resistant MSSA, MRSA, and MDRSA in IHO waste would advance our understanding of one potential source of *S.aureus* from IHOs.

Water

Conventional IHOs in North Carolina commonly practice a waste disposal method whereby waste from several hundred to thousands of hogs is collected in anaerobic lagoons and sprayed onto nearby cropland as fertilizer. During extreme flooding events, lagoons can overflow into nearby waterways [153]. If heavy rain storms cause land-applied waste to run off of fields and into nearby creeks and streams, microbial water quality could be affected [154]. Documentation of groundwater contamination by antibiotic resistant enteric bacteria has been documented on NC swine farms [155]. The detection of swine-specific microbial markers has recently been reported in surface waters near AFOs and CAFOs in southeastern NC [156], demonstrating the presence of swine waste in surface waters adjacent to spray fields. Flooding of lagoons and runoff from spray fields both represent potential mechanisms by which *S. aureus* from IHOs can enter surface waters.

While *S. aureus* is not typically considered a waterborne pathogen, recent research has demonstrated that both clinical and environmental strains of MRSA can survive in marine and freshwater for up to ten and five days, respectively [157]. On the west coast of the United States, waterborne *S. aureus* and MRSA have been detected in recreational fresh [158] and marine waters [158-160]. Waterborne *S. aureus* has also been recovered from coastal streams in O'ahu [159]. On the east coast, three studies at sub-tropical, non-point source recreational marine beaches in South Florida have investigated the presence of *S. aureus* and MRSA in ambient surface water [161-163], but only two confirmed the presence of *S. aureus* [162] and MRSA [163]. While some have suggested the source of *S. aureus* to coastal or recreational waters and beaches is beach-goer shedding [162-166], Viau et al. [159] found that *S. aureus* displayed a statistically significant positive association with agricultural land covers in O'ahu, suggesting that environmental reservoirs in agricultural settings may contribute *S. aureus* to surface waters.

For their studies conducted in the Northwestern US fresh water and South Florida marine water, both Levin-Edens et al. [158] and Plano et al. [163], reported source-related genetic and antibiotic susceptibility characteristics for *S. aureus*. Although Levin-Edens et al. [158] detected sequence types associated with animal sources, including ST133 and ST1946, markers that help distinguish between livestock- and human-derived strains of *S. aureus* such as the lack of *scn* and presence of tetracycline resistance were not evaluated. Presence and characterization of antibiotic-resistant *S. aureus* in surface waters near IHOs and their associated spray fields has not been evaluated.

6. Public health significance of *S. aureus* with markers of livestock-association

Staphylococcus aureus is one of the most well-known and persistent pathogens in the healthcare and community setting globally [167]. Although an estimated 18,650 deaths were attributed to MRSA in the United States in 2005 [37], infections due to strains of *S. aureus* commonly associated with livestock or humans in direct contact with livestock (i.e., CC398 and CC9) remain low [136]. Livestock-adapted *S. aureus* is currently considered less virulent [76] and less transmissible [111, 126, 127] than its hospital- and community-associated counterparts.

Surveillance efforts in European countries show that CC398 MRSA infection is growing [168] and that MRSA CC398 may have begun circulating among people in the general Dutch population [169]. Analysis of the 2008-2009 National MRSA Surveillance data revealed that approximately 26% of MRSA with unknown origin (MUO; MRSA from individuals with no established risk factors for colonization or infection) in Dutch hospitals belonged to CC398, and the majority of these CC398 MUO were lacking a link to livestock [169]. Compared to livestock-adapted CC398, human-adapted MSSA CC398 appears to be a source of more virulent infections globally, is more transmissible, has a genotype that may be more likely to acquire mobile genetic elements like virulence genes, and has been detected in individuals with and without livestock contact [170, 171]. Therefore, it is important to distinguish between these

human- and livestock-adapted CC389 clades when commenting on the burden of disease caused by “livestock-associated” *S. aureus* [70].

No surveillance efforts for livestock-associated *S. aureus* have been implemented in the United States, but occasional CC398 *S. aureus* infections have been reported [171-173] in individuals without livestock contact. However, in these and other cases of CC398 infection, it is important to note that the infection was caused by strains that are more characteristic of the human-adapted than livestock-adapted lineage within CC398 [76, 171]. Yet, studies linking MRSA infection to high-density livestock production in the United States suggest that it is also possible that MRSA strains other than ST398 (CC398) that may be found on IHOs are more important for infection [136].

While it remains unclear the extent to which human-to-human transmission of livestock-adapted *S. aureus* occurs, a metapopulation model concluded that the current trends of low transmissibility could be maintained only in the scenario of <10% persistent carriage [174]. This calculation highlights the importance of vigilant monitoring of carriage of a diversity of both MRSA and MSSA strains among individuals with and without direct livestock contact, especially in communities where intensive livestock production is prevalent. Better surveillance of the *S. aureus* strains colonizing hogs and colonizing or infecting occupationally-exposed humans and individuals living in rural communities where IHOs are numerous is a crucial but thus far unimplemented component of mitigating the potential public health impacts of antibiotic use on IHOs in the United States [70].

SPECIFIC AIMS

The goal of this research was to advance the understanding of potential routes of occupational and environmental exposure to antibiotic-resistant *S. aureus* originating from industrial hog operations. To achieve this goal, we investigated the presence of ABR *S. aureus* strains characteristic of IHO sources in surface water, IHO workers and their child household members, and in non-occupationally exposed community members in eastern North Carolina – one of the densest regions of hog production in the United States. This was completed by addressing the following specific aims, which correspond to Research Chapters One, Two, and Three:

Aim 1: Investigate the presence of methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant (MDRSA) strains in surface waters near IHO spray fields in eastern North Carolina by addressing the following sub-aims:

Sub-aim 1.1: Test surface waters near IHOs for the presence of MRSA using culture-based and molecular methods.

Sub-aim 1.2: Investigate the phenotypic antibiotic-resistance profiles of *S. aureus* isolated from these surface waters, classifying strains as MRSA and MDRSA.

Sub-aim 1.3: Characterize waterborne *S. aureus* isolates using genotypic methods to understand their potential origins (human versus animal adaptation).

Sub-aim 1.4: Identify non-*S. aureus* isolates using matrix-assisted laser desorption time-of-flight mass spectrometry (MALDI-TOF MS).

Aim 2. Investigate the association between occupation in an IHO and carriage of *S. aureus*, MRSA, MDRSA, and *S. aureus* with markers of livestock association in adults and their child (<7 yr. old) household members by addressing the following sub-aims:

Sub-aim 2.1: Evaluate nasal swabs collected from IHO workers, community referent workers, and children living in the same household as these workers for the presence of *S. aureus*, MRSA, and MDRSA.

Sub-aim 2.2: Investigate the presence of markers of livestock association among *S. aureus*-positive isolates collected from nasal swabs.

Sub-aim 2.3: Among adults, examine the association of occupation with binary *S. aureus*-related outcomes.

Sub-aim 2.4: Among children, examine the association of adult household member occupation with binary *S. aureus*-related outcomes.

Sub-aim 2.5: Evaluate the phenotypic and genotypic homology of *S. aureus* strains within IHO worker households and community referent worker households.

Aim 3: Among IHO households, evaluate associations between specific work-related activities and carriage of of *S. aureus*, MRSA, MDRSA, and *S. aureus* with markers of livestock association by addressing the following sub-aims:

Sub-aim 3.1: Among adult IHO workers, examine the association of specific occupational activities with binary *S. aureus*-related outcomes.

Sub-aim 3.2: Among children living in IHO worker households, examine the association of specific occupational exposures of their IHO worker household members with binary *S. aureus*-related outcomes of the child.

CHAPTER TWO: MULTIDRUG- AND METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* IN SURFACE WATERS NEAR INDUSTRIAL HOG OPERATION SPRAY FIELDS IN NORTH CAROLINA

1. Introduction

Since the 1980s, food animal production in the United States has been characterized by a shift from small, independently owned operations to large, vertically integrated operations [58] often referred to as industrial hog operations (IHOs). This change in production practices has been especially pronounced in North Carolina, which is second only to Iowa in pork production, with the majority of IHOs concentrated in the eastern part of the state [62]. One of the characteristic animal husbandry practices of IHOs is the prophylactic use of antibiotics for growth promotion and disease prevention [58]. This antibiotic use may contribute to the selection of antibiotic-resistant bacteria in and around IHOs [66].

A growing body of literature has documented the emergence of antibiotic-resistant *S. aureus* originating from livestock production, including cases of methicillin-resistant *S. aureus* (MRSA) colonization in humans with direct or indirect exposure to livestock. Since the first reports of novel MRSA strains colonizing pigs, pig farmers, and their families in the Netherlands [89-91], strains of *S. aureus* with genetic and phenotypic markers of livestock origin have been identified in hogs and IHO workers globally, including the Netherlands [89, 175], Belgium [117], Canada [100], and the United States [84-86]. This lineage of *S. aureus* not only has an important reservoir in pigs and other livestock [176, 177], but has also emerged in the community in areas with a high density of pig farming [178].

The emergence and dissemination of one particular *S. aureus* strain found in livestock, known as clonal complex (CC) 398, has received substantial attention [110], especially around

hog farms. However, researchers have since documented that this lineage is not the only *S. aureus* strain circulating in the IHO environment. Furthermore, among these and other lineages, there is evidence that a number of genetic markers may distinguish livestock-adapted clades from human-adapted clades among genotypes that are commonly associated with livestock.

In addition, genes conferring resistance to antibiotics commonly used in industrial livestock production as well as genes thought to aid *S. aureus* in the colonization of human hosts have been suggested as supplemental evidence for the origin of *S. aureus* isolates. Price et al. [76] demonstrated that among a group of *S. aureus* CC398 isolates, presence of the *tet(M)* gene and absence of the *scn* gene, which encodes a staphylococcal complement inhibitor that is a part of the immune evasion cluster (IEC) in *S. aureus*, were strongly associated with *S. aureus* CC398 isolates of animal origin. Similarly, the findings of McCarthy et al. [82], Verkaik et al. [83], and Sung et al. [179] suggest that *scn* can serve as a marker of non-human origin. These studies provide a supplemental framework to strain typing for understanding the potential origins of *S. aureus*.

While the emergence and characterization of *S. aureus* with markers of livestock origin among hogs and IHO workers has become better documented and described, evidence for the dissemination of these strains to the off-farm environment is limited. Antibiotic-resistant, including methicillin- and multidrug-resistant, *S. aureus* have been recovered from the environment surrounding industrial livestock operations and some studies have documented potential mechanisms for their dispersal into the environment. Schulz et al. [140] demonstrated that methicillin-resistant *S. aureus* (MRSA) could be detected in soil at distances of up to 300 m from pig barns inside which MRSA was collected from air, swine, or plastic work boots. Furthermore, air samples within and downwind of MRSA-positive confinement buildings were the same *spa* types and belonged to *spa* types commonly associated livestock. Antibiotic-resistant *Staphylococcus* have been isolated from insects in industrial animal operations [142, 143] and MRSA, including ST398 MRSA, was isolated from black and brown rats on pig farms

in the Netherlands [144]. These vectors, as well as airborne deposition, may transmit antibiotic-resistant *S. aureus* from confinement buildings to nearby environments.

Conventional industrial hog operations in North Carolina commonly practice a waste disposal method whereby waste from several hundred to thousands of livestock is collected in waste lagoons and sprayed onto nearby cropland as fertilizer. If heavy rain storms cause land-applied waste to run off of fields and into nearby creeks and streams, microbial water quality could be affected. The detection of swine-specific microbial markers has recently been reported in surface waters near IHOs in southeastern NC [180], demonstrating the likely presence of swine waste in surface waters adjacent to spray fields. In addition to airborne deposition [140] or potential spread to nearby environments by insects [143] and rodents [144], runoff from spray fields into nearby surface waters represents another potential mechanism by which *S. aureus* from IHOs can enter surface waters.

Staphylococcus aureus is not typically considered a waterborne pathogen, but recent research has demonstrated that both clinical and environmental strains of MRSA can survive in marine and freshwater for up to ten and five days, respectively [157]. On the west coast of the United States, waterborne *S. aureus* and MRSA have been detected in recreational fresh [158] and marine waters [158-160]. Waterborne *S. aureus* has also been recovered from coastal streams in O'ahu [159]. On the east coast, three studies at sub-tropical, non-point source recreational marine beaches in South Florida have investigated the presence of *S. aureus* and MRSA in ambient surface water [161-163], but only two of the studies confirmed the presence of *S. aureus* [162] and MRSA [163]. Some have suggested that the source of this *S. aureus* in coastal or recreational waters and beaches is beach-goer shedding [162-166]; however, Viau et al. [159] found that waterborne *S. aureus* presence displayed a statistically significant positive association with agricultural land covers in O'ahu, suggesting that other *S. aureus* sources in this setting may contribute *S. aureus* to surface waters.

Although antibiotic-resistant *S. aureus* has been detected from industrial swine and workers, dust and surface samples within IHOs, and air and soil in the surrounding environment, surface waters near IHOs have yet to be evaluated for the presence of antibiotic resistant *S. aureus*. In this study, we characterized 698 presumptive *S. aureus* isolates collected from 179 samples from surface waters near IHO spray fields in southeastern NC—one of the densest areas of industrial hog production in the United States. The goals of this study were to: 1) test surface waters near swine CAFOs in eastern NC for the presence of MRSA; 2) investigate phenotypic antibiotic resistance profiles of *S. aureus* isolated from these surface waters; and 3) characterize waterborne *S. aureus* isolates using genotypic methods to understand their potential origins (i.e., human or livestock). This is the first study to report the presence of *S. aureus* in surface waters in North Carolina, with targeted sampling in surface waters adjacent to swine CAFO spray fields.

2. Methods

2.1 Study area and sample collection

The study area was located in southeastern North Carolina, a region where there is a high density of swine and poultry CAFOs. Liquid waste management systems are used in the majority of swine CAFOs in the area, whereby waste is collected in open-pit lagoons and sprayed onto nearby cropland periodically. Poultry CAFOs in the area primarily use a dry litter waste management system, which is also applied to fields periodically. Beef cattle are also raised on pasture in the area, and septic systems are a common method for household sewage disposal. Sampling locations were selected as described in Heaney et al. [156]; a total of nine sampling locations were included in this study.

Surface water samples were collected from public access waters near swine lagoon spray fields from mid-February 2010 to mid-January 2011 as described in Heaney et al. [156]. Samples were transported on ice to UNC-Chapel Hill by a courier and were analyzed for presumptive MRSA within 24 hours of sample collection.

2.2 Presumptive MRSA isolation

Surface water samples were analyzed for antibiotic-resistant *Staphylococcus* following the experimental procedure reporteded by Goodwin and Pobuda [181]. Water samples were filtered using a 0.45µm membrane, placed onto CHROMagar™ MRSA (BD BBL™) plates, and incubated at 37°C overnight. Due to a high amount of growth on CHROMagar™ MRSA media, samples were filtered in duplicate and in multiple dilutions. Colonies with morphological characteristics of MRSA (e.g. mauve with a matte halo) were counted after 18-24 hours of incubation. Up to ten of these colonies were selected from each sample site and streaked onto CHROMagar™ Staph aureus (BD BBL™) plates for isolation and morphology verification. After incubation at 37°C for 18-24 hours, all mauve with a matte halo streaks were inoculated in 0.75mL of Brain Heart Infusion Broth (BHIB) with 15% glycerol, and stored at -80°C until further characterization.

These original archived cultures are referred to as presumptive MRSA because they were originally isolated on MRSA-specific culture medium. Subsequent laboratory workflow is described below and presented in Figure 1.

2.3 *S. aureus* and MRSA confirmation

In order to identify the true positives from the archived presumptive MRSA cultures, we first performed culture-based and biochemical testing to screen cultures for *S. aureus*.

Archived isolates were regrown in 1 ml BHIB enrichment at 37°C overnight. A loopful of inoculum was streaked for isolation on Baird-Parker (BP) agar and incubated for 48 h at 37°C. Colonies with characteristic *S. aureus* morphology (i.e., shiny, black colonies) at 48 h were then streaked for isolation on trypticase soy (TS) agar and incubated overnight at 37°C. Colonies were streaked again on TS agar until a pure culture was obtained.

These pure cultures were first tested for the production of catalase. Catalase-positive isolates were then subjected to the direct tube coagulase test (BBL Coagulase Plasma, Rabbit

with EDTA) according to the manufacturer's protocol. Catalase- and coagulase-positive isolates were considered presumptive *S. aureus* and subjected to molecular confirmation by PCR.

2.4 Molecular confirmation of presumptive *S. aureus*

A crude DNA extraction was performed on fresh, pure cultures of presumptive *S. aureus* according to the protocol described in Reischl et al. [182]. All PCR reactions were then performed immediately following extraction to identify and characterize isolates.

A multiplex PCR described in Poulsen et al. [183] was used to confirm the presence of the 16S rDNA, *nuc*, and *mecA* genes in each of the presumptive *S. aureus* isolates using the primers listed in Table 1 and according to the following thermal cycling parameters: initial 15 minutes at 95°C; 30 cycles of 30 s at 94°C, 30 s at 55°C, and 60 s at 72°C; and a final extension at 72°C for 10 min.

2.5 Matrix assisted laser desorption ionization – time-of-flight mass spectrometry (MALDI-TOF MS)

MALDI-TOF MS was performed on non-*S. aureus* isolates (by PCR) that regrew on BP agar with characteristic *S. aureus* morphology (Figure 1). From archived cultures, isolates were streaked onto TS agar and incubated overnight at 37°C. MALDI-TOF MS was performed using the FDA-cleared VITEK MS per manufacturer's recommendations for direct colony spotting (bioMerieux, Durham, NC) [184].

2.6 Molecular characterization

2.6.1 Molecular markers of livestock association

Three molecular measures were used to identify livestock association of the *S. aureus* isolates in this study – lack of the *scn* gene, presence of the *tetM* gene, and Staphylococcal protein A (*spa*) type were all considered when determining the livestock-independence or -association of *S. aureus* isolates. A duplex PCR targeting the *scn* and *tetM* genes [185] was used to measure the first two markers of livestock association. The PCR was performed

according to the following cycle conditions: initial 11 minutes at 95°C; 35 cycles of 30 s at 95°C, 30 s at 61°C, and 60 s at 72°C; and a final extension at 72°C for 10 min.

2.6.2 *spa* typing

Staphylococcal protein A (*spa*) typing was performed by amplifying the *spa* gene using the primers listed in Table 1 and using methods described previously [186]. The *spa* PCR was performed according to the following thermal cycling parameters: initial 5 minutes at 94°C; 35 cycles of 45 s at 94°C, 45 s at 62°C, and 90 s at 72°C; and a final extension at 72°C for 10 min. Staphylococcal protein A *(spa)* typing was performed using the Ridom Staph Type standard protocol (http://www.ridom.com) and the Ridom SpaServer (http://spa.ridom.de/index.shtml). Clonal complexes were assigned based on the existing scientific literature.

2.7 Antibiotic susceptibility profiles

Confirmed *S. aureus* isolates were tested for phenotypic susceptibility to 16 antibiotics from 11 distinct antibiotic classes, including aminoglycosides (gentamicin), β-lactams (ampicillin, oxacillin, penicillin), cephalosporins (ceftriaxone), fluorquinolones (ciprofloxacin, gatifloxacin, levofloxacin), lincosamides (clindamycin), macrolides (erythromycin), oxazolidones (linezolid), rifamycin (rifampin), streptogramins (quinupristin/dalfopristin), sulfonamides (sulfamethoxazole/trimethoprim), tetracyclines (tetracycline), and glycopeptides (vancomycin) (Appendix 1). Antibiotic susceptibility testing to all antibiotics except vancomycin was performed using the Kirby-Bauer disk diffusion method according to the protocol published by the Clinical Laboratory Standards Institute [187].

Vancomycin susceptibility was investigated by first screening isolates on Brain-Heart Infusion agar containing 5 mg/L teicoplanin (BHIT5) [188]. Isolates were grown overnight on TS agar at 37°C and diluted to a 0.5 McFarland standard in TS broth before streaking 10 µl on BHIT5 at 35°C for 24 to 48 h.

Multidrug resistance was defined as complete resistance to ≥3 antibiotic classes and phenotypic methicillin resistance was verified by resistance to oxacillin and non-susceptibility to ceftriaxone.

2.8 Statistical analysis

We also investigated associations between *S. aureus*, MDRSA, or MRSA positive samples and 24- and 48- hour rainfall, fecal indicator bacteria (FIB) levels, and spray field acreage within 500 and 1000 meters by using an analysis of covariance (ANCOVA) model, controlling for season (FIB analysis). All statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC).

3. Results

3.1 Presumptive methicillin-resistant *S. aureus* (MRSA) in surface waters

A total of 183 surface water samples were collected from nine sites adjacent to swine CAFO spray fields in southeastern North Carolina. Of the 183 surface water samples tested, 179 (98%) had at least one colony that grew on CHROMagar™ MRSA (BD BBL™) and CHROMagar™ Staph aureus (BD BBL™) media with characteristic MRSA morphology, yielding 698 archived isolates of presumptive MRSA that were archived until further characterization. We refer to these original archived isolates as presumptive MRSA because they were first isolated on a MRSA-specific culture medium but this medium yielded a high rate of false positives in our study.

From the original 698 archived presumptive MRSA isolates, 24 were confirmed *S. aureus* (Figure 1.2). Of these 24 *S. aureus* isolates, 12 were confirmed MRSA. These represent 20 and 9 of the original 179 presumptive MRSA-positive surface water samples, respectively. *S. aureus* was detected in surface water most frequently during the spring and summer months and was detected every month except January and February (data not shown).

These 24 confirmed *S. aureus* were identified by a combination of two confirmation methods – PCR and MALDI-TOF MS – which are described below.

3.2 Presumptive *S. aureus* screening

Of the original 698 archived presumptive MRSA isolates, 263 regrew on BP agar after 48 h at 37°C with characteristic *S. aureus* morphology. These were considered presumptive *Staphylococcus*. When streaking onto BP agar from an archived culture and when streaking for isolation on TS agar, sometimes more than one putative *S. aureus* morphology was observed per original archived isolate. Therefore, a total of 305 isolates were tested, of which 101 isolates from 91 original archived isolates were catalase- and coagulase-positive. These 101 catalase- and coagulase-positive isolates were then considered presumptive *S. aureus* and subjected to molecular confirmation by PCR. (Figure 1.1)

3.3 Molecular confirmation of MSSA and MRSA by PCR

From the original 698 presumptive MRSA isolates, a total of 16 isolates collected from 16 distinct surface water samples were confirmed *S. aureus* by the presence of the *nuc* gene in a multiplex PCR assay targeting the 16S rDNA, *nuc*, and *mecA* genes [183]. These 16 *S. aureus* isolates were recovered from 9 distinct sampling events and 7 distinct sites. Five of these *S. aureus* harbored *mecA* and were classified as MRSA (Figure 1.2).

3.4 Matrix-assisted laser desorption ionization – time-of-flight mass spectrometry (MALDI-TOF MS) analysis

Due to the low number of *S. aureus*-positive isolates detected by culture-based screening and PCR, isolates were screened using MALDI-TOF MS in an attempt to more rapidly identify any additional *S. aureus*-positive isolates and to investigate the identity of non-*S. aureus* bacteria originally isolated from CHROMagar™ MRSA. Isolates included in MALDI-TOF MS analysis were those that were archived from the original culture-based screening process described above. Ultimately, 205 of the original 698 archived isolates were identified to at least the genus level. However, as described above, during the culture-based screening process, multiple colonies were sometimes obtained from a single original archived culture. Therefore, a total of 227 isolates were identified to at least the genus level.

From these 227 isolates, an additional eight *S. aureus* isolates were identified by MALDI-TOF MS analysis. This brought the count of confirmed waterborne *S. aureus* in this study to 24 isolates from 20 distinct surface water samples. Therefore, four of the eight MALDI-TOF MS confirmed *S. aureus* were from additional surface water samples. Seven of these eight additional *S. aureus* isolates were *mecA* positive by PCR, bringing the total number of MRSA-positive water samples to 9.

In this collection of 227 isolates, non-*aureus Staphylococcus* and other bacterial genera were detected. The most common non-*aureus Staphylococcus* were *S. epidermidis* (66/227), *S. warneri* (14/227), and *S. saprophyticus* (11/227). Other identified species included *S. arlettae, S. capitis, S. caprae, S. cohnii, S. haemolyticus, S. hominis,* and *S. lugdunensis*. *Staphylococcus* was the most frequently detected genus (155/227), followed by *Bacillus* (55/227), *Enterococcus* (9/227), *Morganella* (4/227), *Acinetobacter* (1/227), *Comamonas* (1/227), *Micrococcus* (1/227), and *Prevotella* (1/227). Three species of *Bacillus—B. cereus*, *B. mycoides*, and *B. thuringiensis*—were consistently indistinguishable by MALDI-TOF MS; therefore, all *Bacillus* results are reported only to the genus level. Genus- and species-level results are summarized in Table 1.2.

3.5 Molecular characterization

3.5.1 Markers of livestock association

In addition to *spa* types, two molecular markers were considered when determining the potential association of an isolate to livestock: lack of *scn* and presence of *tet(M)*. The latter gene conferring tetracycline resistance has been associated with *S. aureus* of livestock origin [76, 185]. Seven *S. aureus* isolates from seven distinct samples were *scn*-negative. All *S. aureus*-confirmed isolates were *tetM* negative and approximately 70% (17/24) were *scn*-positive (Figure 1.2).

3.5.2 *spa* typing

Staphylococcal protein A (*spa*) typing was performed to better understand potential sources of waterborne *S. aureus* isolates. Figure 2 lists the *spa* type of each confirmed *S. aureus* isolate and their corresponding clonal complexes. The most common *spa* types represented in this study were t008 (12/24) and t021 (7/24), which belong to clonal complex (CC) 8 and 30, respectively (http://spa.ridom.de/mlst.shtml). Other *spa* types represented include t190, t216, t267, and t338 (Figure 1.2).

3.6 Antibiotic susceptibility profiles

Fifteen of the 24 confirmed *S. aureus* isolates were resistant to at least one of the 16 tested antibiotics. All antibiotics to which these isolates exhibited resistance have been approved for use in animals [65]. Eleven of the 24 *S. aureus* isolates exhibited phenotypic multidrug resistance, which we defined as resistance to ≥ 3 antimicrobial classes. All of the multidrug-resistant isolates were also resistant to methicillin. Nine isolates from eight distinct samples exhibited phenotypic tetracycline resistance and all tetracycline-resistant isolates were also methicillin-resistant. Non-susceptibility was also observed to the antibiotics ampicillin (15/24), penicillin (14/24), oxacillin (13/24), ceftriaxone (12/24), erythromycin (11/24), ciprofloxacin (10/24). No resistance to gatifloxacin, gentamycin, levofloxacin, linezolid, rifampin, quinupristin/dalfopristin, trimethroprim/sulfamethoxazole, or vancomycin was observed. (Figure 1.2).

3.7. Waterborne *S. aureus* presence and site characteristics

S. aureus, MRSA, and MDRSA presence was not associated with spray field acreage within 1000 meters, 24- or 48-hour rainfall, fecal indicator bacteria concentration, or swine-specific microbial source tracking markers (data not shown).

4. Discussion

To our knowledge, this is the first report of multidrug- and methicillin-resistant *S. aureus* recovered from surface waters adjacent to IHO spray fields. Others have reported waterborne

S. aureus, MRSA, and MDRSA in recreational fresh and marine waters in the United States [157, 158, 160, 162, 163, 189]. Some have attributed waterborne *S. aureus* to bather shedding [162, 163], but that does not appear to be the case in the surface waters tested. These waters are not used for recreational purposes.

Of the original 698 presumptive MRSA isolates archived, only 24 were confirmed *S. aureus* by PCR. One explanation for the low number of confirmed *S. aureus* from surface waters in this study compared to previous studies may be the difference in the selective media used; many other waterborne *S. aureus* studies employed methodology involving an enrichment step or media selective for *S. aureus* while we used CHROMagar™-MRSA as the first line of bacterial selection from water samples. This likely limited both the amount of *S. aureus* recovered and our observed genotypes and phenotypes. Furthermore, both Goodwin et al. [160] and Abdelzaher et al. [161] reported difficulty using CHROMagar™ plates in environmental samples in which *S. aureus* is not the dominant bacterial species present in the sample. Up to 61% of samples in our study exceeded federal recreational microbiological water quality standards [156], and it was common for filters on CHROMagar™ MRSA to be overgrown with non-*S. aureus* bacteria. Although Goodwin and Pobuda [181] reported a % positive predictive accuracy of 92% for CHROMagar™-MRSA using colony appearance on a filter combined with isolate appearance, combining membrane filtration with an alternate selective enrichment method, such as that employed by [189] may have improved our ability to recover *S. aureus* from surface water.

Many have speculated that beachgoer shedding contributes *S. aureus* to coastal recreational waters since Charoenca and Fujioka [166] demonstrated a statistically significant association between *Staphylococcus* and bather density in marine waters in Hawaii [162, 163, 165]. More recently, Levin-Edens et al. [158] reported that children were frequently found playing during sampling at the freshwater stream sites where MRSA was most frequently isolated. In our study, however, research team members did not observe people using any of

the sample sites for recreation. The presence of antibiotic-resistant *S. aureus* in surface waters not routinely used for recreation and in a rural agricultural setting suggests that other sources likely contribute *S. aureus* to this environment.

In rural agricultural settings, *S. aureus* may enter surface waters through a variety of sources, including human waste [151], wildlife [145], pets [190], or industrial animal production [140]. Many rural North Carolinians rely on private septic systems rather than public sewer services. Although *S. aureus* and MRSA have not been evaluated in private septic system influent and effluent, *S. aureus* and MRSA have been isolated from human wastewater by sampling wastewater treatment plants (WWTP) [151]. Our study area is also influenced by industrial animal operations, and Heaney et al. [156] detected swine-specific microbial source tracking (MST) markers in surface water samples evaluated in this study, although none of the *S. aureus* positive samples were also positive for validated swine-specific MST markers (data not shown). Furthermore, the presence of *S. aureus*, MRSA, and MDRSA in surface water were not associated with FIB concentration or 24- or 48-hr rainfall (data not shown). Other ways in which *S. aureus* of potential livestock origin could surface water may include air [140] and rodent or insect vectors [143, 144], neither of which were examined in our research. Recent research in our study area has also documented MRSA and MDRSA carriage in individuals who work in industrial livestock operations [84].

Because this study was uniquely designed to evaluate the presence of *S. aureus*, MRSA, and MDRSA in an environment dominated by industrial animal production, it is one of the few studies to evaluate not only the presence of *S. aureus*, MRSA, and MDRSA in surface water, but to also provide information regarding the genotypes of the recovered isolates as well as markers of livestock association. The most prevalent *spa* types in our study were t008 (CC8) and t021 (CC30). These *spa* types are typically associated with human-derived clones, although CC30 has previously been associated with livestock [94]. In our collection of isolates, *spa* types

associated with CC30 were mostly *scn*-negative, which is a common genetic characteristic of *S. aureus* isolates from non-human sources [76, 82, 83, 179].

Of the eight isolates belonging to *spa* types associated with CC30 (t021, t338), seven lacked the *scn* gene. However, all but one of the *scn*-negative isolates were susceptible to all 16 antibiotics. CC30, *spa* type t021 MSSA and MRSA has been identified in pigs in Portugal [95] and CC30 MRSA has been described in breeding pigs in Europe [94]. Additionally, *scn*-negative CC30 MSSA was recently detected in antibiotic-free livestock workers in North Carolina [84]. While lack of the *scn* gene in our CC30 isolates suggests non-human origins of these waterborne *S. aureus*, their *spa* type (t021) and susceptibility to tetracycline do not align with previously described characteristics of *S. aureus* found in livestock production. Levin-Edens et al. [158] detected sequence types associated with animal sources, including ST133 and ST1959, and some sequence types previously associated with both humans and animals or humans and meat products, including ST8 and ST30. However, this and other studies reporting genotypes did not consider other markers of non-human or livestock association, such as *scn* and *tetM*.

In contrast to the isolates of potential non-human origin, *S. aureus* belonging to *spa* type t008—which is a common human strain of *S. aureus*—were often methicillin- and multidrug-resistant. Multidrug-resistant *spa* type t008 MRSA was also detected in isolates from an injured Eastern cottontail rabbit (*Sylvilagus floridanus*) from a wildlife care clinic in central Iowa [145]. In a study designed to better understand the contribution of bathers to *S. aureus* in marine water in South Florida, the sequence typing performed by Plano et al. [163] revealed that most of their MRSA isolates were of typically hospital-associated genotypes, including *spa* type t008. Similarly, Soge et al. [189] recovered sequence types commonly associated with hospital clones at beaches in the Pacific Northwest. Since *S. aureus* and MRSA *spa* type t008 have previously been recovered from both human- and non-human sources, we are unable to draw conclusions regarding the specific source of these *S. aureus* in our study area.

Fifteen of the twenty-four confirmed *S. aureus* isolates were resistant to at least one antibiotic. All of the antibiotics to which our collection of isolates exhibited resistance belong to antibiotic classes that have been sold or distributed for use in food-producing animals [65]. This includes one of the *scn*-negative isolates (i.e., non-human associated), which was resistant to erythromycin and ampicillin. All of the methicillin- and multidrug-resistant isolates belonged to *spa* type t008 (CC8), which is a common human strain of *S. aureus.*

Interpretation of these results is limited by a lack of information regarding antibiotics used in food animal production in the United States. One of the only antibiotics for which there is evidence of common use in food animal production in the United States is tetracycline [65]. Tetracycline resistance was observed in nine of our twenty-four *S. aureus* isolates and all tetracycline-resistant isolates were also MRSA and MDRSA; however, all of the tetracycline-resistant isolates belong to *spa* type t008 (CC8), which is commonly associated with humans.

Because our initial screening process yielded only 16 confirmed *S. aureus* isolates out of the original 698 presumptive MRSA isolates, MALDI-TOF MS was performed to identify the unknown bacteria that were originally isolated on MRSA-selective media. Results revealed that the majority (68%) belonged to the *Staphylococcus* genus, with ten non-*aureus Staphylococcus* species identified. Among other staphylococci identified, seven have been identified as *mecA* gene carriers [191]. *S. saprophyticus* has been identified in poultry flocks in Japan [192] and pig farms in China [193]. Additionally, *S. cohnii*, *S. arlettae*, *S. haemolyticus*, and *S. hominis* have previously been documented in pig farms in China [193]. While potential poultry and swine sources of these bacteria exist in our study area, these non-*S. aureus* bacteria have not been investigated in swine or poultry operations in our study area, nor does our study confirm their origins.

A limitation of this study is that *S. aureus* and MRSA were not analyzed from waste, nor were samples from spray fields or industrial animal operations themselves collected due to lack of access. Furthermore, the density of IHOs in the region prevented us from being able to

identify a control site not in proximity to one or more IHOs within our study area. It is possible that antibiotic-resistant *S. aureus*—including the strains identified in this study—are present in surface waters that are not located near IHOs. We did not observe an association with spray field acreage, rainfall, or microbial water quality data that would suggest that this waterborne *S. aureus* is linked to IHOs. Therefore, we cannot identify the sources of waterborne *S. aureus* in this study, despite their proximity to IHO spray fields. Recent research has documented nasal carriage of livestock associated, antibiotic-resistant *S. aureus* in workers of industrial animal operations in this area [84]. Future research should focus on better characterization of source samples to better understand the mechanisms by which methicillin- and multidrug-resistant *S. aureus* enters surface waters in these settings, and to better evaluate the impact of industrial animal agriculture on microbial surface water quality.

5. Conclusions

This research demonstrated that *S. aureus* as well as methicillin- and multidrug-resistant *S. aureus* are sometimes present in surface waters in southeastern NC and is the first study to report waterborne *S. aureus*, MRSA, and MDRSA in fresh water in the southeastern U.S. Our findings are limited by our choice of a MRSA-selective media, difficulties associated with applying media adopted for clinical use to environmental samples, and our lack of a control site and IHO samples. Although the specific sources of the *S. aureus* in this study are unknown, genetic typing revealed that non-human sources may contribute *S. aureus* to surface waters in our study area. Further research is necessary to evaluate potential mechanisms for spread of MRSA and MDRSA into the environment.

Table 1.1 PCR assays, primers, and primer sequences

PCR Assay	Primers	Primer sequence	Source
Multiplex			[183]
	16S (F) (16Sup1)	5′-GTGCCAGCAGCCGCGGTAA-3'	
	16S (R) (16Sup2)	5′-AGACCCGGGAACGTATTCAC-3'	
	nuc (F) (nucPCR1)	5′-TCAGCAAATGCATCACAAACAG-3′	
	nuc (R) (nucPCR2)	5′-CGTAAATGCACTTGCTTCAGG-3′.	
	mecA (F) (mecup1)	5′-GGGATCATAGCGTCATTATTC-3'	
	mecA (R) (mecup2)	5′-AACGATTGTGACACGATAGCC-3′	
scn/tet(M) duplex			[185]
	scn (F)	5'-AGCACAAGCTTGCCAACATCG-3'	
	scn (R)	5'-TTAATATTTACTTTTTAGTGC-3'	
	tet(M) (F)	5'-GTGGACAAAGGTACAACGAG-3'	
	tet(M) (R)	5'-CGGTAAAGTTCGTCACACAC-3'	
spa			[14]
	SPA 1095F new (1794)	5'-AGACGATCCWTCAGTGAGC-3'	
	SPA extend:f (1827)	5'-TAATCCACCAAATACAGTTGTACC-3'	

Table 1.2 Bacterial genus and species identified by MALDI-TOF MS.

Genus	Species	N	Percent
Acinetobacter		1	0.4
	Acinetobacter baumannii complex	1	0.4
Bacillus		55	24
Comamonas		1	0.4
	Comamonas testosteroni	1	0.4
Enterococcus		9	4.0
	Enterococcus casseliflavus	4	1.8
	Enterococcus durans	1	0.4
	Enterococcus faecalis	2	0.9
	Enterococcus hirae	2	0.9
Micrococcus		1	0.4
	Micrococcus luteus/lylae	1	0.4
Morganella		4	1.8
	Morganella morganii	4	1.8
Prevotella		1	0.4
	Prevotella buccalis	1	0.4
Staphylococcus		155	68
	S. arlettae	1	0.4
	S. aureus	24	11
	S. capitis	9	4.0
	S. caprae	1	0.4
	S. cohnii ssp cohnii	2	0.9
	S. cohnii ssp cohnii and *S. haemolyticus*	1	0.4
	S. cohnii ssp urealyticus	5	2.2
	S. epidermidis	66	29
	S. haemolyticus	10	4.4
	S. hominis ssp hominis	9	4.0
	S. lugdunensis	2	0.9
	S. saprophyticus	11	4.8
	S. warneri	14	6.2

Figure 1.1 Laboratory methods used to identify *S. aureus* from presumptive MRSA cultures.

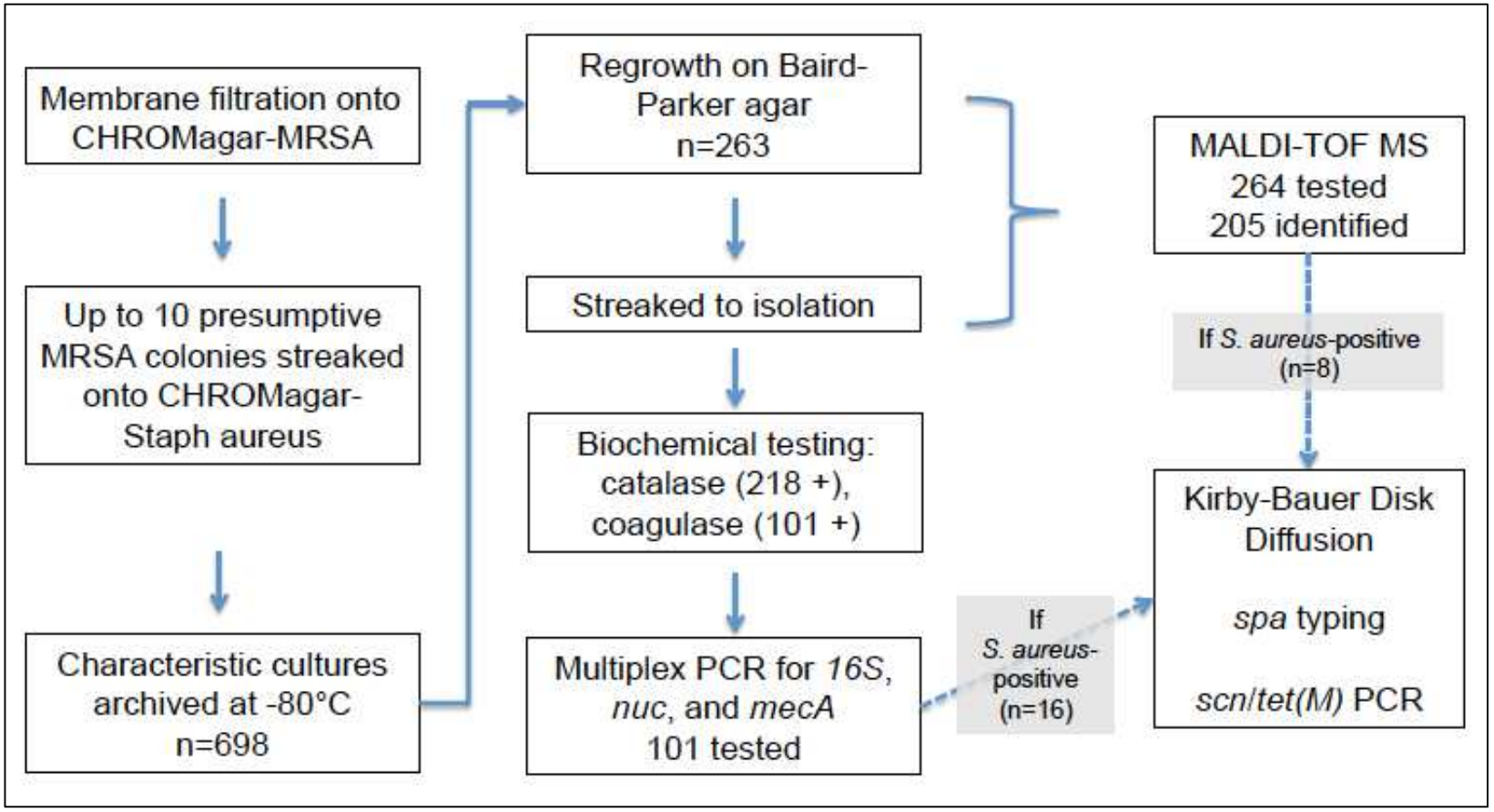
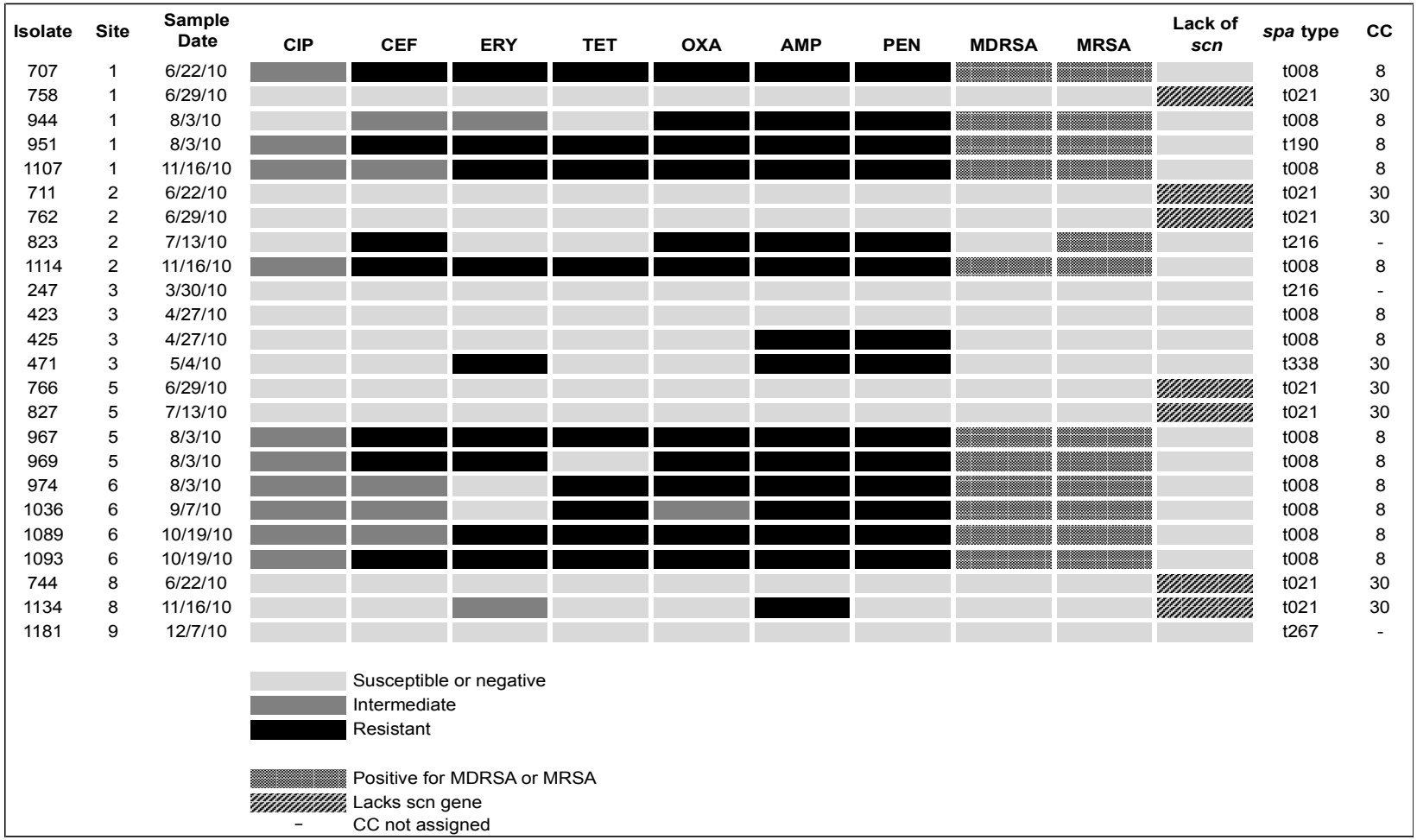


Figure 1.2. Genotype and antibiotic resistance profiles of confirmed *S. aureus* isolates.[1]



[1] All isolates were susceptible to gatifloxacin, gentamycin, levofloxacin, rifampin, quinupristin/dalfopristin, trimethroprim/sulfamethoxazole, and vancomycin (teicoplanin).

CHAPTER THREE: EXPOSURE TO ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* IN ADULT INDUSTRIAL HOG OPERATION WORKERS AND THEIR HOUSEHOLD MEMBERS UNDER SEVEN YEARS OLD

1. Introduction

In recent years, the industrial hog operation (IHO) environment has emerged as a potential source of antibiotic-resistant (ABR) *Staphylococcus aureus*, including methicillin-resistant *S. aureus* (MRSA) [86, 91, 176, 194] and multidrug-resistant *S. aureus* (MDRSA) [84, 88]. The speculated cause of this emergence is the use of antibiotics in hogs for the purposes of disease prevention and growth promotion in these facilities [80, 195]. Previous research has revealed that ABR *S. aureus* circulating in pig herds is genetically distinct from ABR *S. aureus* carried by humans that are not occupationally exposed to industrial livestock [89, 90].

Early studies of ABR circulating in pigs focused on sequence type as the defining genetic characteristic of *S. aureus* from industrial hog and other livestock operations; much of this literature describes multi-locus sequence type 398 (ST398) as “livestock-associated” [110]. Since these early studies, additional STs and *S. aureus* clonal complexes (CCs) as well as the gain or loss of mobile genetic elements (MGEs) have been used to characterize *S. aureus* from the IHO environment. For example, presence of the tetracycline resistance gene *tet(M)* is commonly found in CC398 *S. aureus* from livestock sources [76, 185]. Several studies have also documented the near-ubiquitous lack of *scn* in *S. aureus* from non-human sources such as livestock [76, 82, 185]. Although there is no established universal definition of livestock-association in the literature, these characteristics (CC previously identified in livestock, tetracycline resistance, and lack of *scn*) can be considered markers of livestock association in *S. aureus* [88].

It has also been established that these ABR *S. aureus* with markers of livestock association can be exchanged between pigs and humans with occupational exposure to pigs (e.g., IHO workers, livestock veterinarians) [76, 86, 100, 175]. It appears that methicillin-resistance is dominant antibiotic resistance phenotype of *S. aureus* carried by pigs and workers in the IHO environment in Europe [110]. A limited number of studies in the United States have documented the presence of ABR *S. aureus* with markers of livestock association in IHO pigs [85, 86] and in humans with occupational exposure to pigs and other livestock [84, 88]. However, in North Carolina, MRSA carriage prevalence in IHO workers appears to be similar to that of the United States adult population; currently, industrial livestock operation employees in NC predominately carry MDRSA and other ABR but methicillin-susceptible *S. aureus* (MSSA) with markers of livestock association [84, 88]. Samples from pigs within North Carolina IHOs have not been reported, but evidence from the Midwestern United States suggests that MRSA carriage swine herds in Iowa is common [86]. Therefore, it is important to consider both MRSA and other ABR *S. aureus* (e.g., MDRSA) when investigating carriage prevalence among industrial hog operation workers in the United States.

Household members of IHO workers are sometimes exposed to ABR *S. aureus* characteristic of the livestock production environment [84, 89]. Recent research also suggests that IHO workers can persistently carry *S. aureus* with markers of livestock association, even after 96 hours away from work [88], which provides more evidence of potential household exposures for those living with IHO workers. One study in North Carolina additionally investigated household member carriage of ABR *S. aureus* with markers of livestock association [84]; however, only children aged older than seven years were eligible to participate.

Children are known to be an at risk population for ABR *S. aureus* infection [196]. MRSA carriage among children has increased in the past decade [52] and MRSA carriage has also been associated with a 4-fold increase in risk of infection compared to methicillin-susceptible *S. aureus* (MSSA) carriage [197]. Recent antibiotic use, recent hospitalization, participation in

contact sports, close (household member) contact with individuals with a history of MRSA infections [198], and—among children—child care attendance [199], are all considered risk factors for MRSA carriage and infection. Parental or caregiver occupation in a healthcare setting has been investigated as a risk factor for child carriage [30, 200]; however, other occupations with a high level of exposure to antibiotic-resistant pathogens, such as those working in IHOs, have not been considered.

Furthermore, previous studies examining exposure to ABR *S. aureus* in the industrial livestock production workplace in North Carolina were not designed to investigate potential community exposures in areas of dense IHO production. Rinsky et al. [84] compared ABR *S. aureus* prevalence in livestock workers with exposure to industrial versus antibiotic-free livestock production. However, since most of the industrial livestock operations in North Carolina are located in the eastern part of the state [62], this study was unable to comment on the prevalence of ABR *S. aureus* carriage in individuals who may be exposed via the environment or other community members. Exposure to ABR *S. aureus* and *S. aureus* with markers of livestock association is currently thought to be limited to IHO workers and their familial contacts [111]. However, researchers have documented an increased prevalence of ABR *S. aureus* with markers of livestock association in pig-dense regions of the Netherlands [178] and hospital-based studies in the United States have found associations between proximity to large IHOs or IHO spray fields and MRSA carriage [134] and skin and soft tissue infection [136], respectively. Therefore, it is also important to investigate carriage prevalence in a referent population within the same community in order to better understand whether livestock worker exposures are purely occupational or if they are a reflection of community and environmental exposures that are unique to regions of dense livestock production.

In this study, we aimed to examine the nasal carriage prevalence of *S. aureus*, MRSA, MDRSA, and *S. aureus* with one or more markers of livestock association in adults (≥18 years old) and children (<7 years old) with and without occupational or caregiver occupational

exposure to IHOs, respectively. We conducted this study among individuals residing in the top ten hog-producing counties in North Carolina [201]. This study design allowed us also examined evidence of strain concordance within households and evidence of non-occupational exposure to *S. aureus* with markers of livestock association in communities of high IHO density.

2. Methods

2.1 Ethics Statement

This community-based study was a collaboration between the Johns Hopkins Bloomberg School of Public Health, the Rural Empowerment Association for Community Help (REACH) and the University of North Carolina at Chapel Hill (UNC). The Johns Hopkins University (JHU) institutional review board (IRB) approved this study; the UNC IRB conceded reliance upon the JHU IRB. Prior to participation, adult participants provided written informed consent. Parents or legal guardians provided written informed assent for their participating child under seven years old and provided questionnaire responses for their participating child.

Data were collected between March and October 2014 in North Carolina, USA by trained researchers from UNC in collaboration with trained organizers from REACH.

2.2 Study population

Using a snowball sampling approach, participants were recruited and enrolled from the top ten hog-producing counties in North Carolina according to 2010 NC agricultural statistics [201]. In order of highest to lowest density of hogs, these counties were: Duplin, Sampson, Bladen, Wayne, Greene, Pender, Robeson, Lenoir, Jones, and Columbus [201]. One adult (≥ 18 years old) worker and one child (< 7 years old) were recruited from the following types of households: 1) households with at least one adult employed at an industrial hog operation (IHO) (IHO group); and 2) households whose adult residents were not employed in the livestock production industry within the last 12 months – a community referent (CR) group (CR group). Other eligibility criteria are described in Table 2.1.

2.3 Questionnaire and nasal swab collection

Nasal swabs and questionnaire responses were collected during the same study visit. Adult participants and parents or legal guardians of child participants reported occupational activities and workplace exposures, personal and household member contact with livestock, environmental exposures, personal and household member healthcare exposures, personal and household member childcare attendance, personal and household member medical history, and demographic information.

Study personnel obtained a nasal swab from adult participants by rotating a sterile, double-tipped BD BBL™ CultureSwab™ five times clockwise and five times counter-clockwise in both nares. To minimize discomfort of child participants, two single, mini-tipped BD BBL™ CultureSwab™ nasal swabs were collected in the same manner from children under seven years old.

A set of trip blanks for all swabs were collected prior to transport from REACH to UNC and stored with samples during transport. Swabs were stored in Stuart's medium at 4°C and transported to UNC within five days of sample collection for processing.

2.4 Detection of *S. aureus* and MRSA

We aimed to obtain two presumptive *S. aureus* colonies per swab. One of the two nasal swabs collected (one tip of the double-tipped adult swab and one of the mini-tipped children's swabs) were aseptically clipped into 1 ml sterile 0.01M phosphate buffered saline (PBS). After vortexing for one minute, 100 µl PBS eluate was pipetted directly onto CHROMagar™ Staph aureus (CA) media (BD, Franklin Lakes, NJ). A sterilized stainless steel spreader and a petri dish inoculating turntable were used to evenly distribute the 100 µl PBS eluate throughout the plate until dry. CA plates were then incubated at 37°C for 24 hours. If two or more morphologically characteristic colonies grew on a CA after 24 h, two colonies were picked and streaked to isolation on CA media for biochemical and molecular confirmation. If less than two colonies grew on CA after 24 hours, the original swab and entire PBS swab eluate volume were

inoculated into 10 ml Mueller-Hinton broth supplemented with 6.5% NaCl and incubated overnight at 37°C. This enrichment was then streaked on the CA and Baird-Parker (BP) plates and incubated for 24 h and 48 h, respectively, at 37°C to improve recovery of *S. aureus* from the nasal swab [202]. Up to two pure, morphologically characteristic *S. aureus* colonies were archived at -80°C in brain-heart infusion broth (BHIB) with 15% glycerol until further characterization. Catalase and tube coagulase testing with rabbit plasma (BD BBL™, Franklin Lakes, NJ) were used to confirm *S. aureus* biochemical characteristics prior to molecular testing.

Following crude DNA extraction [182], a multiplex polymerase chain reaction (PCR) assay was employed to detect the *S. aureus*-specific gene *spa*, as well as *mecA, mecC* (*mecA*$_{LGA251}$*), scn,* and *pvl*. This PCR was performed according to the protocol described by Stegger et al. [203] and the presence of each of the five amplified genes was confirmed by gel electrophoresis on a 3% agarose gel. Among morphologically and biochemically characteristic *S. aureus* isolates that lacked *spa* by multiplex PCR, we attempted to amplify alternate *S. aureus*-specific genes by PCR for an alternate *spa* primer [204] and for *nuc* [183] and *femA* [205].

Finally, MALDI-TOF MS was performed on isolates that were negative for both *spa* primers but positive for *nuc* and/or *femA* to confirm *S. aureus* identity. From archived cultures, isolates were streaked onto Tryptic Soy Agar with 5% Sheep Blood and incubated overnight at 37ºC. MALDI-TOF MS was performed using the FDA-cleared VITEK MS per manufacturer's recommendations for direct colony spotting (bioMerieux, Durham, NC) [184].

Isolates that met the following criteria were classified as *S. aureus*: 1) *spa*-positive; 2) positive for *nuc* and *femA;* or 3) identified as *S. aureus* by MALDITOF-MS.

2.5 Molecular typing

Staphylococcal protein A (*spa*) typing was performed by amplifying the *spa* gene as described above. PCR products were sequenced by Eton Biosciences, Inc. (Research Triangle

Park, NC). Staphylococcal protein A *(spa)* types were assigned using the Ridom Staph Type standard protocol (http://www.ridom.com) and the Ridom SpaServer (http://spa.ridom.de/index.shtml). *spa* types were assigned to putative MLST clonal complexes (CCs) on the scientific literature. The clonal complexes that were considered in this study were CCs 398 and 9.

2.6 Antibiotic susceptibility testing

Both confirmed *S. aureus* isolate(s) from each *S. aureus*-positive nasal swab were tested for susceptibility to the following 12 classes of antibiotics at UNC: aminoglycosides, beta-lactams, cephalosporins, floroquinolones, lincosamides, macrolides, oxazolidones, rifamycin, streptogramins, sulfonamide, nitrofuran and tetracyclines (Appendix 1). The Kirby-Bauer disk diffusion method was used to test each isolate's susceptibility to all antibiotic classes. Interpretation of zones of inhibition was reported as susceptible, resistant, or intermediately resistant (where applicable) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [187]. In erythromycin-resistant isolates, inducible clindamycin resistance was assessed using the D-zone test [206]. *S. aureus* isolates that exhibited intermediate or complete phenotypic resistance to at least three antibiotic classes were classified as multidrug-resistant *S. aureus* (MDRSA).

2.7 Markers of livestock association

Based on the rationale provided by Rinsky et al. [84] and Nadimpalli et al. [88], three markers of livestock association were examined among *S. aureus*-positive isolates: 1) presence of CC398 or CC9 by *spa* type; 2) lack of *scn*; and 3) presence of phenotypic tetracycline resistance.

2.8 Carriage outcomes

The nasal carriage outcomes examined were: *S. aureus*, MRSA, MDRSA, CC398, and CC9; *scn*-negative *S. aureus*, MRSA, MDRSA, CC398, and CC9; tetracycline-resistant *S. aureus*, MRSA, MDRSA, CC398, and CC9; and, *scn*-negative and tetracycline-resistant *S.*

aureus, MRSA, MDRSA, CC398, and CC9. For each carriage outcome, an individual was considered positive for the outcome if either of the two isolates from the participant's nasal swab met the criteria for that outcome. For example, a participant was considered positive for *S. aureus* carriage if either the first or the second isolate from his or her nasal swab was confirmed *S. aureus*.

2.9 Statistical Analysis

We compared the distribution of demographic characteristics and potential risk factors for *S. aureus* carriage among the IHO and CR groups for both adult and child participants. Where sample size was sufficient, we examined the association between demographic characteristics and potential *S. aureus* nasal carriage risk factors with *S. aureus*-related nasal carriage outcomes.

The crude prevalence of carriage in each group was calculated for adults and children separately.

Using log-binomial models, we compared carriage of each outcome among adults and children in the IHO and CR groups by calculating crude prevalence ratios (PRs) and 95% confidence intervals (CIs). Based on previous literature we considered the following variables as potential confounders: age, gender, education level, household pet, pet type (outdoor only vs. indoor or indoor and outdoor), antibiotic use within 3 months, healthcare contact within 3 months, participation in contact sports within 3 months, use of a workout gym within 3 months, personal and household smoking, childcare attendance, and school attendance. Those variables that were found to be associated with exposure (IHO vs. CR) and *S. aureus*-related carriage outcomes and for which sufficient sample size existed were considered for inclusion in log binomial models to control confounding. Putative confounders varied by outcome but included age, gender, education level, and healthcare contact within 3 months. These variables were further evaluated using a backwards elimination approach, whereby confounders were removed one-by-one from a fully adjusted model. Putative confounders remained in the final

adjusted model if removing them resulted in a >10% change in the PR estimate of the fully adjusted model. Where sample size allowed, adjusted PRs are presented.

Within-household concordance was investigated by examining the presence of the same *spa* type in an adult and child participant living in the same household. We also examined concordance of other *S. aureus*-related outcomes. All statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC).

3. Results

3.1 Participant Characteristics

Adult and child demographic and environmental exposure characteristics are described in Tables 2.2 and 2.3, respectively. A total of 198 IHO and 202 CR households were enrolled, for a total of 400 participating households. The percentage of male and female adults was roughly the same among IHO households, while the CR group had more female (80%) than male (20%) adult participants. The majority of IHO participating adults self-identified as Hispanic (93%) and the majority of CR participating adults self-identified as Black (62%). In general, IHO adult participants reported lower proportions of antibiotic use, healthcare contact, and gym attendance in the 3 months prior to sample collection compared to CR adult participants. However, CR adult participants reported less participation in contact sports within 3 months of sample collection.

Among child participants, the percentage of males and females was roughly the same in CR households, while more male (62%) than female (38%) children participated in the IHO group. The majority of children in the IHO group were identified as Hispanic (94%) by their assenting caregiver, while in the CR group the majority of children were identified as Black (60%). Few children were reported to have used antibiotics within 3 months of sample collection; however, more children within the CR group (42%) reported exposure to a healthcare facility in the 3 months prior to sample collection, compared to children in the IHO group (13%).

Reported childcare attendance was also higher among CR children (11%) compared to IHO children (3%).

Gender- and race-stratified prevalence is presented for adults and children in Appendices 2 and 3.

3.2 Prevalence of *S. aureus*, MRSA, and MDRSA carriage among adults and children

S. aureus, MRSA, and MDRSA carriage prevalence patterns are described in Tables 2.3 and 2.4 and Figures 2.1 and 2.2. In the IHO population, the prevalence of *S. aureus* carriage was 52% for adults and 49% for children. In the CR population, *S. aureus* carriage prevalence was 31% for adults and 31% for children. Among IHO participants, 2% of adults carried MRSA while 14% of children carried MRSA. In contrast, 4% of adults and 2% of children in the CR group carried MRSA. The prevalence of MDRSA carriage was higher among IHO adults (13%) and IHO children (23%) compared to CR adults (8%) and CR children (8%), respectively.

While the crude prevalence of *S. aureus* was significantly higher among IHO adults compared to CR adults (Prevalence Ratio [PR]: 1.7; 95% Confidence Interval [CI]: 1.3, 2.2), adjusting for differences in gender and education level resulted in a PR of 1.2 (95% CI: 0.9, 1.6). Differences in carriage of MRSA and MDRSA between IHO and CR adults were also not statistically significant.

The crude and adjusted prevalence of *S. aureus*, MRSA, and MDRSA was significantly higher among children living with IHO workers compared to CR children. Prevalence ratios comparing carriage of these outcomes in children were adjusted for the education level of the participating adult, as education level was our best proxy for socio-economic status for the entire household and it was associated with exposure and *S. aureus*, MRSA, and MDRSA carriage in child participants. After adjusting for adult education level, children living with IHO workers had a greater carriage prevalence of MRSA (Adjusted PR: 2.0; 95% CI: 1.1, 3.5) and MDRSA (Adjusted PR: 2.4; 95% CI: 1.4, 4.1) than those not living with IHO workers (CR children).

3.3 Prevalence of markers of livestock association among adults and children

The prevalence of markers of livestock association was higher among IHO adults compared to CR adults, but these markers were uncommon in children in both exposure groups (Tables 2.3 and 2.4). In the IHO group, prevalence of *scn*-negative *S. aureus* was 13% in adults and 4% in children. In the CR group, prevalence of *scn*-negative *S. aureus* was 3% in adults and 2% in children. Similarly, *scn*-negative MDRSA carriage prevalence was higher in adult IHO workers (10%) and their child household members (3%) compared to CR adults (2%) and children (1%). *scn*-negative, tetracycline-resistant *S. aureus* and MDRSA prevalence was higher among IHO adults (8% and 7%, respectively) compared to CR adults (2% for *S. aureus* and MDRSA).

After adjusting for differences in education level, we observed a greater prevalence of *scn*-negative *S. aureus* carriage in IHO workers compared to CR adults (Adjusted PR: 3.8, 95% CI: 1.2, 11.8). While the education- and gender-adjusted prevalence of *scn*-negative MDRSA was greater among IHO workers compared to CR adult participants, the estimate was imprecise (Adjusted PR: 4.8; 95% CI: 1.0, 23.3). However, among adult participants, we observed a higher crude prevalence in IHO workers compared to CR participants for *scn*-negative and tetracycline-resistant *S. aureus* (PR: 5.4; 95% CI: 1.6, 18.4) and MDRSA (PR: 4.8; 95% CI: 1.4, 16.3). Although carriage prevalence of *S. aureus* belonging to CC398 and CC9—including CC398 and CC9 with markers of livestock association—was higher among IHO workers compared to CR adults, PR estimates were imprecise.

Although the prevalence of *scn*-negativity and presence of tetracycline resistance was higher among children in the IHO group compared to the CR group, the presence of these markers in children were uncommon and PR estimates were imprecise. Carriage of CC398 and CC9 *S. aureus* was roughly equal in children living in the same household as an IHO worker compared to those living in CR households (Tables 2.3 and 2.4).

3.4 Prevalence of markers of livestock association in non-occupationally exposed households

Three adults and one child in the CR group carried *S. aureus* with all three markers of livestock association (Tables 2.3 and 2.4). Among adults, one carried *scn*-negative, tetracycline-resistant CC398 *S. aureus* while two carried *scn*-negative, tetracycline-resistant CC9 *S. aureus*. The *S. aureus* isolates carried by all of these adult participants was also methicillin-susceptible but multidrug-resistant. None of these CR adults reported antibiotic use or healthcare contact within 3 months of sample collection, direct contact with livestock of any kind, or living on the same property as an industrial hog operation. The self-reported occupations of these workers were “landscaping” (CC9), “field worker” (CC9), and “saw operation worker” (CC398) (data not shown).

One child in the CR group carried *S. aureus* with all three markers of livestock association. The strain carried by this child was genetically (by PCR and *spa* type) and phenotypically identical to the strain carried by the adult participant living in the same household as the child (Table 2.5). Like the adult participant from this household, the child was not reported to have had direct contact with livestock, nor was the household reported to be on the same property as a livestock operation.

3.5 Within-household *S. aureus* concordance

Twenty of the 400 participating households had adults and children carrying concordant *S. aureus* strains, defined as at least one of the *S. aureus* isolates collected from an adult nasal swab having an identical *spa* type as at least one of the *S. aureus* isolates collected from nasal swab of a child living in the same household (Table 2.5). With the exception of one household, antibiotic resistance profiles were also identical in *S. aureus* from adults and children carrying concordant *S. aureus* and living in the same household. All household participants with identical *S. aureus spa* types additionally had concordant *scn* and *mecA* results.

The majority of these *S. aureus* isolates were *scn*-positive, methicillin-susceptible, and resistant to fewer than 3 antibiotic classes (non-MDRSA). However, one CR household and one IHO household had an adult and child carrying *scn*-negative *S. aureus*. In the IHO household, this *S. aureus* was methicillin- and multidrug-resistant and belonged to *spa* type t002. In the CR household the *S. aureus* belonged to *spa* type t034 and was tetracycline- and multidrug-resistant. Another adult-child pair in the IHO group carried tetracycline-resistant *S. aureus*; however, this *S. aureus* was *scn*-positive and did not belong to a *spa* type commonly found in livestock.

We also investigated the probability of a child carrying *S. aureus*, MRSA, and MDRSA if an adult in the household is carrying *S. aureus*, MRSA, and MDRSA (Table 2.6). The probability of a child carrying these outcomes given positive adult carriage for the outcome was similar between IHO and CR households.

4. Discussion

To our knowledge, this is the first study to investigate exposure to antibiotic-resistant *S. aureus* in children under seven years old whose adult household members are employed in an IHO. We found that children living in households with an IHO worker had a greater crude nasal carriage prevalence of both MRSA and MDRSA. However, this association was not observed among adults. Additionally, children living in IHO households and CR households had a similar carriage prevalence of *S. aureus* with one or more markers of livestock association. In our study population, adult household member employment in an IHO was not associated with child carriage of *S. aureus* strains characteristic of the IHO environment. Nonetheless, the elevated crude prevalence of MRSA and MDRSA in children in the IHO group suggests that adult household member employment in an IHO may be associated with ABR *S. aureus* carriage in child household members under seven years old.

In addition, this study contributes to the body of scientific literature characterizing potential occupational exposures to ABR *S. aureus* in the IHO workplace. The crude prevalence

of *scn*-negative and tetracycline-resistant *S. aureus* and *scn*-negative MDRSA carriage in IHO workers appeared to be greater than that of individuals living in the same geographic community but not employed in the industrial livestock industry. While these results are similar to those of previous studies conducted with North Carolina industrial livestock workers [84], we compared IHO workers to those who are not occupationally exposed but who – due to the density of IHO production in eastern North Carolina [62] – may experience environmental exposures to bacteria originating from IHO facilities.

We also observed carriage of *S. aureus* with one or more markers of livestock association in individuals who were not occupationally exposed to livestock production in North Carolina. Four individuals (three adults and one child) in the CR group carried *S. aureus* with all three markers of livestock association (i.e., CC398 or CC9, *scn*-negative, and tetracycline resistant). Surprisingly, an adult and child in a non-occupationally exposed household were carrying the same strain (*spa* type t034) of *scn*-negative, tetracycline-resistant MDRSA. Moritz and Smith [137] reported carriage of *S. aureus* t571 in a day care worker in Iowa; however, this isolate was characteristic of human- rather than livestock-derived lineages within CC398. These results suggest that unmeasured non-occupational exposures to *S. aureus* characteristic of industrial livestock production may exist in communities with high IHO density.

Children are an at-risk population for MRSA infection [43-45, 47] and the risk of infection has been shown to increase 4-fold among individuals carrying MRSA compared to MSSA [197]. Risk factors for MRSA carriage among adults and among healthy pediatric populations include but are not limited to recent hospitalization, age under 2 years, male gender, and black race [45, 198, 207]. Although we observed a higher prevalence of these risk factors in children in the CR group compared to the IHO group, prevalence of MRSA carriage was greater in IHO children compared to CR children in our study. This suggests that in our study population, adult household member occupation in an IHO may be more important than these known carriage risk factors when considering MRSA nasal carriage prevalence. Close contact with household

members with a history of MRSA infection or carriage is also considered a risk factor for MRSA carriage [198]. While we gathered information regarding household member *S. aureus* and MRSA infection history, we were unable to collect swabs from all household members to gather data on MRSA carriage within the household. It is also important to note that due to sample size, we were unable to control for the many demographic and risk factor differences between IHO and CR children. When we were able to produce adjusted prevalence ratio (PR) estimates, adjusted PRs were closer to the null than crude PRs.

Those that have reported MRSA carriage among pediatric populations have reported carriage prevalence ranging from <1% to 7.6% in the community [200, 208, 209]. While the prevalence of MRSA carriage in children living in CR households (6%) falls within this range of pediatric MRSA carriage prevalence in the community, the prevalence of MRSA among children living in IHO households (13.6%) was much greater than other studies. In particular, one study conducted in 24 child care centers in eastern North Carolina and Virginia between 2007 and 2010 documented an overall MRSA prevalence of 1.3% (1.4% in North Carolina) in pre-school aged children [30]. Although the Miller et al. [30] study was conducted in a similar geographic region, differences in demographic characteristics—especially with respect to race—make comparisons difficult. For example, among the subset of participants included in the case-control analysis conducted by Miller et al. [30], children colonized by MRSA were more likely to be nonwhite; however, only 15/75 (20%) children included in this analysis were nonwhite. In our study population, 100% of IHO children are nonwhite and 98% of CR children are nonwhite. But since both IHO and CR children are demographically dissimilar to those included in the Miller et al. [30] study and since we observed a 6% prevalence of MRSA carriage in CR children, it is unlikely that the difference in demographics between the two studies could explain entire magnitude of the high prevalence among IHO children (14%).

We collected information regarding traditional risk factors for infection and exposure in adults and children, including child care attendance, school attendance, prior infections,

healthcare exposure, and antibiotic use. More children in the CR group were reported to have healthcare contact, antibiotic use, and attend childcare than those in the IHO group. Household education level, determined by the reported highest level of education completed by the adult participant, was different between the IHO and CR group. Despite the greater prevalence of these risk factors in CR children and after controlling for differences in household education level, the prevalence of MRSA and MDRSA carriage was significantly greater in children living in IHO households compared to children living in CR households.

It is still possible that the greater prevalence of MRSA and MDRSA carriage observed in the IHO group compared to the CR group may be a reflection of demographic differences between the two groups. For example, the majority of child participants in the IHO group were Hispanic while the majority of participants in the CR group were Black, and we did not collect information regarding country of origin, immigration status, or time since arrival in the United States. Because sample size limited our ability to adjust for race as a confounder, we were unable to determine if our observed associations were a reflection of an exposure related to household member occupation or other unmeasured demographic characteristics related to race. After accounting for adult education level, the association between MRSA and MDRSA carriage and household member occupation in an IHO remained significant but the adjusted PR was closer to the null. Because education level may be associated with SES and race, this suggests that differences in the race/ethnicity characteristics between the two groups may influence the crude associations observed between household member occupation in an IHO and child carriage of ABR *S. aureus* and that crude associations should be interpreted with caution.

Few studies have investigated nasal carriage of MDRSA in healthy children under seven years old. We reported a crude prevalence of MRSA carriage of 22% in children living with an IHO worker and 8% in CR children. Children in IHO households had a higher prevalence of MDRSA carriage than CR children, which suggests that differences between these two

populations – adult household member occupation or otherwise – may be associated with greater MDRSA exposure. Due to the lack of scientific literature on MDRSA carriage in children, it is unclear how this prevalence compares to population-based prevalence estimates in the United States or globally. One multicenter study conducted in Iran reported a 6% (20/350) prevalence of MDRSA carriage among children ≤7 years old [53]. Our findings not only contribute novel findings of childhood exposure to MDRSA among children living with IHO workers, but also represent one of the few studies to investigate MDRSA nasal carriage among children.

Although living in a household with an IHO worker rather than a CR adult was associated with MRSA and MDRSA carriage in children, the same association was not observed among adults. This is in contrast to research conducted by Graveland et al. [118] that found that children of veal calf farmers were more often MRSA carriers when the farmer was a MRSA carrier. It is possible that IHO adults are exposed to MRSA and MDRSA in the workplace without becoming nasal carriers of the bacteria. For example, IHO workers could carry these and other antibiotic-resistant bacteria, antibiotic resistance genes, or antibiotic residues on other parts of their bodies or clothing and subsequently introduce it to their household environment. In this scenario, their child household members may be exposed to the ABR *S. aureus* and become carriers, or exposure to antibiotic residues or antibiotic resistance genes could alter their natural flora to a more antibiotic-resistant profile. Because we did not collect swabs from any other body sites, clothing, workplace protective equipment, the household environment, or nearby environmental samples, we are unable to determine the exact mechanism of exposure to MRSA and MDRSA among the children in our study. Future research should be designed to better characterize these potential mechanisms of childhood exposure.

Recent research conducted in the United States has investigated ABR *S. aureus* carriage among industrial [84-86, 88] and antibiotic-free livestock workers [84, 85]. Our observed prevalence of MRSA (2%) among IHO workers is similar to estimates for the general

U.S. population [1] but lower than the prevalence observed in IHO workers in the Midwest [86] and Europe [175]. Research conducted in eastern North Carolina has reported a similarly low prevalence of MRSA carriage among industrial livestock workers (3%; 3/99) [84] and IHO workers (5%; 1/22) [88]. In the absence of access to samples from IHO pig herds in North Carolina, we are unable to determine if the low MRSA prevalence observed among NC industrial livestock workers reflects workplace MRSA exposures.

Three studies in the United States have investigated prevalence of MDRSA among industrial livestock or slaughterhouse workers, all of which were conducted in North Carolina. These studies reported a MDRSA carriage prevalence of 15% (15/99) in industrial livestock workers (ILO) [84] and 45% (10/22) in IHO workers [88]. However, in the latter study, the prevalence of MRSA and MDRSA were reported as ever-carriage over a 14-day sampling period, which limits comparisons to our cross-sectional analysis. The MDRSA prevalence reported in another NC-based cross-sectional study [84] is similar to our observed MDRSA prevalence (13%) in IHO workers. Population-based surveillance studies in the United States have not investigated the prevalence of MDRSA nasal carriage and therefore we are unable to interpret our observed MDRSA prevalence in the context of the larger U.S. population. However, Neyra et al. [87] documented a 5% prevalence of MDRSA in community members in our study area, which is consistent with our observed prevalence in community referent adults.

The data suggest that IHO workers may have a higher prevalence of *scn*-negative *S. aureus* and MDRSA as well as *scn*-negative and tetracycline-resistant *S. aureus* and MDRSA compared to CR adults. This is consistent with research conducted in North Carolina [84, 88] and elsewhere in the United States [85, 86]. In North Carolina, a higher prevalence of *scn*-negative and tetracycline-resistant *S. aureus* CC398 was observed among industrial livestock workers and their household members compared to antibiotic-free livestock workers and their household members [84]. The presence of ST398 (a *S. aureus* MLST belonging to CC398) was also reported in swine and swine workers in the Midwestern U.S. [85, 86]. However,[85], Smith

et al. [86] did not investigate other markers of livestock association such as the lack of *scn*. All of the above studies focused on sequence types belonging to CC398, but it is also important to consider the diversity of other CCs that may be circulating in the IHO environment.

In addition to a higher observed crude prevalence of *scn*-negative CC398 *S. aureus*, the crude prevalence of *scn*-negative CC9 *S. aureus* in IHO workers was greater than that of CR adults. The prevalence of CC9 (6%) in IHO adults was also greater than the prevalence of CC398 (3.5%) among IHO workers in our study. While CC9 was uncommon (3/99 ILO participants; 0/105 AFLO participants) in the Rinsky et al. [84] study, which included workers from other industrial livestock industries (e.g., poultry), Nadimpalli et al. [88] reported that CC9 was the second most frequently detected *S. aureus* CC detected in North Carolina IHO workers over a 14-day sampling period. Much of the literature on *S. aureus* strains circulating in the industrial hog production environment has focused on CC398, especially in Europe [72, 117, 210]. However, the greater prevalence of CC9 (6%) compared to CC398 (3.5%) among hog workers in our study and the reported frequent detection of CC9 among IHO workers by Nadimpalli et al. [88] suggest that CC9 may be another strain of *S. aureus* in the IHO environment. Future studies should focus on characterizing the diversity of *S. aureus* strains being exchanged between industrial hogs and IHO workers.

A similar prevalence of *S. aureus* with at least one marker of livestock association was observed in non-occupationally exposed individuals in our study (CR group) and in Rinsky et al. [84] (antibiotic-free livestock operation worker group). However, Rinsky et al. [84] reported that no antibiotic free livestock operation (AFLO) participants carried *scn*-negative and tetracycline-resistant CC398 *S. aureus* or MDRSA, while we observed carriage of MDRSA with all three of these characteristics in one adult and child without exposure to industrial hog or other livestock operations. Our study was conducted only in the top ten hog-producing counties in NC, while the occupationally unexposed (AFLO) participants in Rinsky et al. [84] were mainly recruited from regions with a comparatively low amount of industrial livestock production. Recent

research has documented a high prevalence of antibiotic-resistant *S. aureus* with markers of livestock association in pig-dense regions of the Netherlands [112, 178, 211]. In the United States, there appears to be an increased risk of MRSA carriage among those who live near large IHOs in Iowa [134] and one study linked MRSA skin and soft tissue infection to residential proximity to IHO spray fields and facilities in Pennsylvania [136]. Although these United States studies have not investigated markers of livestock association, these studies and our results suggest that in IHO-dense areas, there may be an elevated level of community exposure to antibiotic-resistant *S. aureus*. To better understand these potential environmental exposures, future work will investigate the potential association between IHO proximity and density on household antibiotic-resistant *S. aureus* carriage.

We identified twenty households with an adult-child pair carrying *S. aureus* with identical *spa* types. In most concordant households, participants were carrying methicillin-susceptible, non-MDRSA that was *scn*-positive and with *spa* types that belong to CCs commonly found in humans. However, in two concordant households, adult and child participants were carrying *scn*-negative MDRSA that belongs to *spa* types that have been identified in hogs and in humans in direct contact with hogs [85]. *S. aureus* with markers of livestock association are thought to be less transmissible than human-adapted *S. aureus* strains [111], but these two cases of within-household concordance provide some evidence of potential bacterial exchange between individuals living in the same household..

Our study design prevents us from determining whether or not within-household concordance represents true human-to-human transmission. A repeated-measures study would be required to determine temporal directionality. Additionally, our lack of household and environmental samples prevent us from determining the potential role of these factors in mediating bacterial exchange between participants sharing the same household environment. It is also worth noting that recent *S. aureus* diversity and transmission studies demonstrate considerable within-host and temporal diversity of *S. aureus* strains [212], suggesting that our

and other studies' reliance upon the geno- and phenotypes of two isolates per person may not capture the full complexity of bacterial exchange that may exist within-household.

We used a snowball sampling approach and relied upon local knowledge to recruit and enroll participants in this study. This community-based approach has been identified as an effective method of recruitment in industrial livestock operation workers [84, 88] and communities [213] of dense livestock production in North Carolina. Although this approach and partnership with REACH allowed for effective recruitment and enrollment of participants, we are unable to generalize our results to what would be seen in all IHO workers and residents of the top ten hog-producing counties in North Carolina, or to the hog production industry in the United States.

We were unable to consider race as a covariate in our models due to differences in the self-reported race of participants in each group. In particular, all of the IHO participants and 98% of the CR participants were nonwhite, and the majority of IHO participants were Hispanic (93%), while the majority of CR participants were Black (62%). The lack of and low percentage of white participants in IHO and CR groups, respectively, likely reflects the siting of IHOs in low-income communities of color in North Carolina [62]. Because we lack employment rosters, we are unable to comment on whether the proportion of Hispanic and Black participants in the IHO group is representative of the true IHO worker population. The demographic characteristics of the IHO group in this study are similar to those of the industrial livestock worker participants in another North Carolina-based study [84], although we have a slightly higher proportion of self-identified Hispanic workers. The differences in demographic characteristics of the two groups may account for some of the difference in prevalence of *S. aureus* carriage outcomes; however, many other known risk factors for carriage—such as antibiotic use, healthcare contact, and gym use—were observed to be higher in the CR group than in the IHO group. In addition, although some studies have documented that Blacks may have a higher prevalence of ABR *S. aureus* carriage and infection compared to Whites [1, 37, 214], individuals of a Hispanic background

appear to have a similar or lower prevalence of ABR *S. aureus* carriage compared to Whites [1, 215]. The greater prevalence of *S. aureus* with one or more markers of livestock association among IHO adults and the greater prevalence of MRSA and MDRSA carriage in children living with IHO workers was observed despite the greater prevalence of non-race related established risk factors for carriage in the CR group.

While the scientific literature has not identified a biological or immunological mechanism behind associations between race and *S. aureus* carriage, race has been associated with other potential covariates, such as socioeconomic status, that may affect *S. aureus*-related carriage outcomes in a biologically-plausible manner (e.g., crowded living conditions [198]). The distribution of the education level of the adult participant was different between the IHO (70% <High school) and CR (22% <High school) groups. Given our inability to include race as a putative confounder in our models, adult participant's education level was considered a proxy for household SES and was considered in both adult and child statistical models. Adult education level was associated with *S. aureus*, *scn*-negative *S. aureus* and MDRSA, and tetracycline-resistant *S. aureus* and MDRSA in adults and with *S. aureus*, MRSA, and MDRSA in children. Therefore, education level (i.e., SES) was an important confounder in all models for which adjustment was possible. It is also possible that differences in carriage between the two groups may be due in part to unmeasured race-related confounders such as country of origin. Strains with some characteristics of *S. aureus* of livestock origin have been documented among individuals of Caribbean nationality [172] and among families in New York who immigrated from the Dominican Republic [216]. Community surveillance data is not available for the countries from which many livestock workers immigrate and we did not collect information on country of origin, immigration status, or time of arrival in the United States; without adjusting for race, we are unable to determine how this affects our crude and adjusted estimates.

In addition to our inability to produce race-adjusted estimates, our ability to produce adjusted estimates for outcomes of interest was limited by sample size so adjusted estimates

are only available for a limited number of outcomes. It is important to note that adjusted models resulted in a PR closer to the null value in all cases, which means that confounding bias contributed to an elevated crude PR. Therefore, crude PRs should be interpreted with caution.

5. Conclusions

Despite its limitations, this research contributes to our knowledge of the potential occupational exposures of IHO workers and their household members under seven years old. Our data suggest that children living in households with IHO workers have a greater nasal carriage prevalence of antibiotic-resistant *S. aureus* of public health concern, and that non-occupationally exposed individuals who live in communities of dense industrial hog production also carry *S. aureus* characteristic of the industrial hog operation environment. The exact route and mechanism of exposure remain unclear, and the lack of source samples in addition to our inability to control for all putative confounders prevent us from determining with great certainty that our observed associations are due entirely to occupational exposure. Future research efforts should aim to better characterize exposure pathways, especially in communities of high IHO density and among vulnerable populations including young children.

Table 2.1. Eligibility and exclusion criteria for participation by exposure group.

	Both	IHO	CR
Eligibility criteria	Household located in the top ten hog-producing counties in NC At least one child (<7 yr) lives in the same household as the worker	During 3 months prior to enrollment, at least one household member has worked full time at an industrial hog operation	During 12 months prior to enrollment, no household member has worked at an industrial livestock facility, including industrial hog or poultry operations, meat processing plants, or animal rendering plants
Exclusion criteria	Households with anyone who works in a healthcare or day care setting. Households without an adult (≥18 yr) parent/caregiver who can respond to the survey in English or Spanish	Workers who have contact with animals other than hogs at work	Workers who have contact with livestock at work

Table 2.2 Description of study population characteristics stratified by exposure group.

	IHO		CR		Chi-square (df)	
	Adult N (%)	**Child N (%)**	**Adult N (%)**	**Child N (%)**	**Adult**	**Child**
Age	198	198	202	202	39.2 (3)	25.3 (2)
0-2	-	32 (16)	-	75 (37)		
3-5	-	116 (59)	-	100 (50)		
6-7	-	50 (25)	-	27(13)		
18-27	47 (24)	-	108 (54)			
28-37	88 (44)	-	62 (31)			
38-47	42 (21)	-	24 (12)			
≥ 47	21 (11)	-	8 (4)			
Gender	198	198	202	202	48.8 (1)	12.4 (1)
Male	107 (54)	123 (62)	41 (20)	90(45)	.	
Female	91 (46)	75 (38)	161 (80)	112 (54)		
Race/Ethnicity	198	198	202	202	158.6 (3)	163.3 (3)
White	0 (0)	0 (0)	8 (4)	3 (2)		
Black	12 (6)	11(6)	125 (62)	122 (60)		
Hispanic	185 (93)	186 (94)	66 (33)	65 (32)		
Multi-racial	1 (<1)	1 (<1)	3 (2)	12 (6)		
Education	198	-	201	-	92.5 (1)	-
< High school	139 (70)	-	45 (22)	-		
≥ High school	59 (30)	-	156 (78)	-		
Household pet	198	198	201	201	9.3 (1)	9.3 (1)
Yes	65 (33)	65 (33)	39 (19)	39 (19)		
No	133 (67)	133 (67)	162 (81)	162 (81)		
Pet type	64	64	39	39	8.7 (2)	8.7 (2)
Indoor only	4 (6)	4 (6)	10 (26)	10 (26)		
Outdoor only	49 (76)	49 (76)	21 (54)	21 (54)		
Indoor & outdoor	11 (17)	11 (17)	8 (21)	8 (21)		
Antibiotic use[1]	198	198	202	202	12.1 (2)	5.3 (1)
No	196 (99)	196 (99)	185 (92)	192 (95)		
Yes	2 (1)	2 (1)	17 (8)	10 (5)		
Healthcare	198	198	202	202	83.2 (2)	46.0 (1)

	IHO		CR		Chi-square (df)	
	Adult N (%)	Child N (%)	Adult N (%)	Child N (%)	Adult	Child
contact[1]						
No	188 (95)	173 (87)	112 (55)	115 (57)		
Yes	10 (5)	25 (13)	90 (45)	87 (42)		
Sports[1]	198	198	202	202	7.6 (2)	0.4 (1)
No	186 (94)	196 (99)	200 (99)	201 (>99)		
Yes	12 (6)	2 (1)	2 (1)	1 (<1)		
Gym[1]	198	-	202	-	15.0 (2)	-
No	193 (97)	-	176 (87)	-		
Yes	5 (3)	-	26 (13)	-		
Smoking	198	-	202	-	20.9 (2)	-
No	167 (84)	-	130 (64)	-		
Yes	31 (16)	-	72 (36)	-		
Household smoking	198	198	202	202	24.6 (1)	24.6 (1)
No	167 (83)	167 (83)	126 (62)	126 (62)		
Yes	31 (16)	31 (16)	76 (38)	76 (38)		
Childcare attendance	-	198	-	202	-	12.1 (1)
No	-	193 (97)	-	179 (89)		
Yes	-	5 (3)	-	23 (11)		
School attendance[1]	-	198	-	202	-	30.8 (1)
No	-	89 (45)	-	146 (72)		
Yes	-	109 (55)	-	56 (28)		
Time outside	-	198	-	202		4.4 (1)
< 3 hr	-	158 (80)	-	143 (71)		
≥ 3 hr	-	40 (20)	-	59 (29)		

[1] Within 3 months of sample collection.

Table 2.3 Crude prevalence (%), prevalence ratios (PR) and 95% confidence intervals (95% CI) stratified by exposure group (IHO vs. CR) for adult and child participants.

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
S. aureus						
IHO	104/198	53	1.7 (1.3, 2.2)	97/198	49	1.6 (1.2, 2.1)
CR	63/202	31	Ref	62/202	31	Ref
MRSA						
IHO	4/198	2	0.6 (0.2, 2.0)	27/198	14	2.5 (1.3, 4.9)
CR	7/202	4	Ref	11/202	6	Ref
MDRSA						
IHO	25/198	13	1.5 (0.8, 2.7)	45/198	23	2.7 (1.6, 4.6)
CR	17/202	8	Ref	17/202	8	Ref
CC398						
S. aureus						
IHO	7/198	4	7.1 (0.9, 57.5)	2/198	1	1.02 (0.2, 1.2)
CR	1/202	1	Ref	2/202	1	Ref
MDRSA						
IHO	7/198	4	7.1 (0.9, 57.5)	2/198	1	2.0 (0.2, 22.3)
CR	1/202	1	Ref	1/202	1	
CC9						
S. aureus						
IHO	12/198	6	6.1 (1.4, 27.0)	1/198	1	1.0 (0.1, 16.2)
CR	2/202	1	Ref	1/202	1	Ref
MDRSA						
IHO	9/198	5	4.6 (1.0, 21.0)	0/198	0	-[1]
CR	3/202	1	Ref	0/202	0	Ref
***scn*-negative**						
S. aureus						
IHO	25/198	13	5.1 (2.0, 13.1)	7/198	4	1.8 (0.5, 6.0)
CR	5/202	3	Ref	4/202	2	Ref
MRSA						
IHO	2/198	1	-[2]	1/198	1	-[2]
CR	0/202	0	Ref	0/202	0	Ref
MDRSA						

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
IHO	20/198	10	6.8 (2.0, 22.5)	5/198	3	5.1 (0.6, 43.3)
CR	3/202	2	Ref	1/202	1	Ref
CC398						
IHO	7/198	4	7.1 (0.9, 57.5)	1/198	1	0.5 (0.1, 5.6)
CR	1/202	1	Ref	2/202	1	Ref
CC9						
IHO	12/198	6	6.1 (1.4, 27)	0/198	0	-[1]
CR	2/202	1	Ref	0/202	0	Ref
tet-resistant						
S. aureus						
IHO	18/198	9	3.7 (1.4, 9.7)	4/198	2	1.4 (0.3, 6.0)
CR	5/202	3	Ref	3/202	2	Ref
MRSA						
IHO	0/198	0	-[1]	0/198	0	-[1]
CR	0/202	0	Ref	1/202	1	Ref
MDRSA						
IHO	15/198	8	3.8 (1.3, 11.3)	2/198	1	0.7 (0.1, 4.0)
CR	4/202	2	Ref	3/202	2	Ref
CC398						
IHO	7/198	4	7.1 (0.9, 57.5)	1/198	1	1.0 (0.1, 16.2)
CR	1/202	1	Ref	1/202	1	Ref
CC9						
IHO	6/198	3	3.1 (0.6, 15.0)	0/198	0	-[1]
CR	2/202	1	Ref	0/202	0	Ref
***scn*-negative and tet-resistant**						
S. aureus						
IHO	16/198	8	5.4 (1.6, 18.4)	1/198	1	1.0 (0.1, 16.2)
CR	3/202	2	Ref	1/202	1	Ref
MDRSA						
IHO	14/198	7	4.8 (1.4, 16.3)	1/198	1	1.0 (0.1, 16.2)
CR	3/202	2	Ref	1/202	1	Ref

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
CC398 or CC9 *S. aureus*						
IHO	13/198	7	4.4 (1.3, 15.3)	1/198	1	1.0 (0.1, 16.2)
CR	3/202	2	Ref	1/202	1	Ref
CC398 *S. aureus*						
IHO	7/198	4	7.1 (0.9, 57.5)	1/198	1	1.0 (0.1, 16.2)
CR	1/202	1	Ref	1/202	1	Ref
CC9 *S. aureus*						
IHO	6/198	3	3.1 (0.6, 15.0)	0/198	0	-[1]
CR	2/202	1	Ref	0/202	0	Ref

[1] PR estimates not computed due to zero observations in at least one exposure category.

[2] PR estimates not computed due to zero observations in the referent category.

Table 2.4 Crude prevalence and crude and adjusted prevalence ratios (PR) and 95% confidence intervals (95% CI) stratified by exposure group (IHO vs. CR) for adult and child participants.

Carriage outcome	Adults				Children			
			Crude	Adjusted			Crude	Adjusted
	No. Pos/ Total	%	PR (95% CI)	PR (95% CI)	No. Pos/ Total	%	PR (95% CI)	PR (95% CI)
S. aureus								
IHO	104/198	53	1.7 (1.3, 2.2)	1.2 (0.9, 1.6)[1]	97/198	49	1.6 (1.2, 2.1)	1.4 (1.1, 2.0)[2]
CR	63/202	31	Ref	Ref	62/202	31	Ref	Ref
MRSA								
IHO	4/198	2	0.6 (0.2, 2.0)	–[3]	27/198	14	2.5 (1.3, 4.9)	2.0 (1.1, 3.5)[2]
CR	7/202	4	Ref	Ref	11/202	6	Ref	Ref
MDRSA								
IHO	25/198	13	1.5 (0.8, 2.7)	–[3]	45/198	23	2.7 (1.6, 4.6)	2.4 (1.4, 4.1)[2]
CR	17/202	8	Ref	Ref	17/202	8	Ref	Ref
CC398								
S. aureus								
IHO	7/198	4	7.1 (0.9, 57.5)	–[3]	2/198	1	1.02 (0.2, 1.2)	–[3]
CR	1/202	1	Ref	Ref	2/202	1	Ref	Ref
MDRSA								
IHO	7/198	4	7.1 (0.9, 57.5)	–[3]	2/198	1	2.0 (0.2, 22.3)	–[3]
CR	1/202	1	Ref	Ref	1/202	1	Ref	Ref
CC9								
S. aureus								
IHO	12/198	6	6.1 (1.4, 27.0)	–[3]	1/198	1	1.0 (0.1, 16.2)	–[3]
CR	2/202	1	Ref	Ref	1/202	1	Ref	Ref
MDRSA								

Carriage outcome	Adults				Children			
			Crude	Adjusted			Crude	Adjusted
	No. Pos/ Total	%	PR (95% CI)	PR (95% CI)	No. Pos/ Total	%	PR (95% CI)	PR (95% CI)
IHO	9/198	5	4.6 (1.0, 21.0)	–[3]	0/198	0	– 33[4]	–[3]
CR	3/202	1	Ref	Ref	0/202	0	Ref	Ref
***scn*-negative**								
S. aureus								
IHO	25/198	13	5.1 (2.0, 13.1)	3.8 (1.2, 11.8)[2]	7/198	4	1.8 (0.5, 6.0)	–[3]
CR	5/202	3	Ref	Ref	4/202	2	Ref	Ref
MRSA								
IHO	2/198	1	–[5]	–[3]	1/198	1	–[5]	–[3]
CR	0/202	0	Ref	Ref	0/202	0	Ref	Ref
MDRSA								
IHO	20/198	10	6.8 (2.0, 22.5)	4.8 (1.0, 23.3)[1]	5/198	3	5.1 (0.6, 43.3)	–[3]
CR	3/202	2	Ref	Ref	1/202	1	Ref	Ref
CC398								
IHO	7/198	4	7.1 (0.9, 57.5)	–[3]	1/198	1	0.5 (0.1, 5.6)	–[3]
CR	1/202	1	Ref	Ref	2/202	1	Ref	Ref
CC9								
IHO	12/198	6	6.1 (1.4, 27)	–[3]	0/198	0	–[4]	–[3,4]
CR	2/202	1	Ref	Ref	0/202	0	Ref	Ref
tet-resistant								
S. aureus								
IHO	18/198	9	3.7 (1.4, 9.7)	2.2 (0.6, 7.9)[2]	4/198	2	1.4 (0.3, 6.0)	–[3]
CR	5/202	3	Ref	Ref	3/202	2	Ref	Ref
MRSA								
IHO	0/198	0	–[4]	–[3]	0/198	0	–[4]	–[3]
CR	0/202	0	Ref	Ref	1/202	1	Ref	Ref
MDRSA								
IHO	15/198	8	3.8 (1.3, 11.3)	2.1 (0.4, 9.8)[6]	2/198	1	0.7 (0.1, 4.0)	–[3]
CR	4/202	2	Ref	Ref	3/202	2	Ref	Ref
CC398								

Carriage outcome	Adults				Children			
			Crude	Adjusted			Crude	Adjusted
	No. Pos/ Total	%	PR (95% CI)	PR (95% CI)	No. Pos/ Total	%	PR (95% CI)	PR (95% CI)
IHO	7/198	4	7.1 (0.9, 57.5)	–[3]	1/198	1	1.0 (0.1, 16.2)	–[3]
CR	1/202	1	Ref	Ref	1/202	1	Ref	Ref
CC9								
IHO	6/198	3	3.1 (0.6, 15.0)	–[3]	0/198	0	–[4]	–[3]
CR	2/202	1	Ref	Ref	0/202	0	Ref	Ref
scn*-negative and tet-resistant *S. aureus								
IHO	16/198	8	5.4 (1.6, 18.4)	–[3]	1/198	1	1.0 (0.1, 16.2)	–[3]
CR	3/202	2	Ref	Ref	1/202	1	Ref	Ref
MDRSA								
IHO	14/198	7	4.8 (1.4, 16.3)	–[3]	1/198	1	1.0 (0.1, 16.2)	–[3]
CR	3/202	2	Ref	Ref	1/202	1	Ref	Ref
CC398 *S. aureus*								
IHO	7/198	4	7.1 (0.9, 57.5)	–[3]	1/198	1	1.0 (0.1, 16.2)	–[3]
CR	1/202	1	Ref	Ref	1/202	1	Ref	Ref
CC9 *S. aureus*								
IHO	6/198	3	3.1 (0.6, 15.0)	–[3]	0/198	0	–[4]	–[3,4]
CR	2/202	1	Ref	Ref	0/202	0	Ref	Ref

[1] Adjusted for gender and education of adult participant.

[2] Adjusted for education of adult participant.

[3] Adjusted PR not available due to sample size.

[4] PR estimates not computed due to zero observations in at least one exposure category.

[5] PR estimates not computed due to zero observations in the referent category.

[6] Adjusted for healthcare contact and education.

Table 2.5. Isolate and household exposure characteristics of households with *S. aureus* nasal carriage concordance.

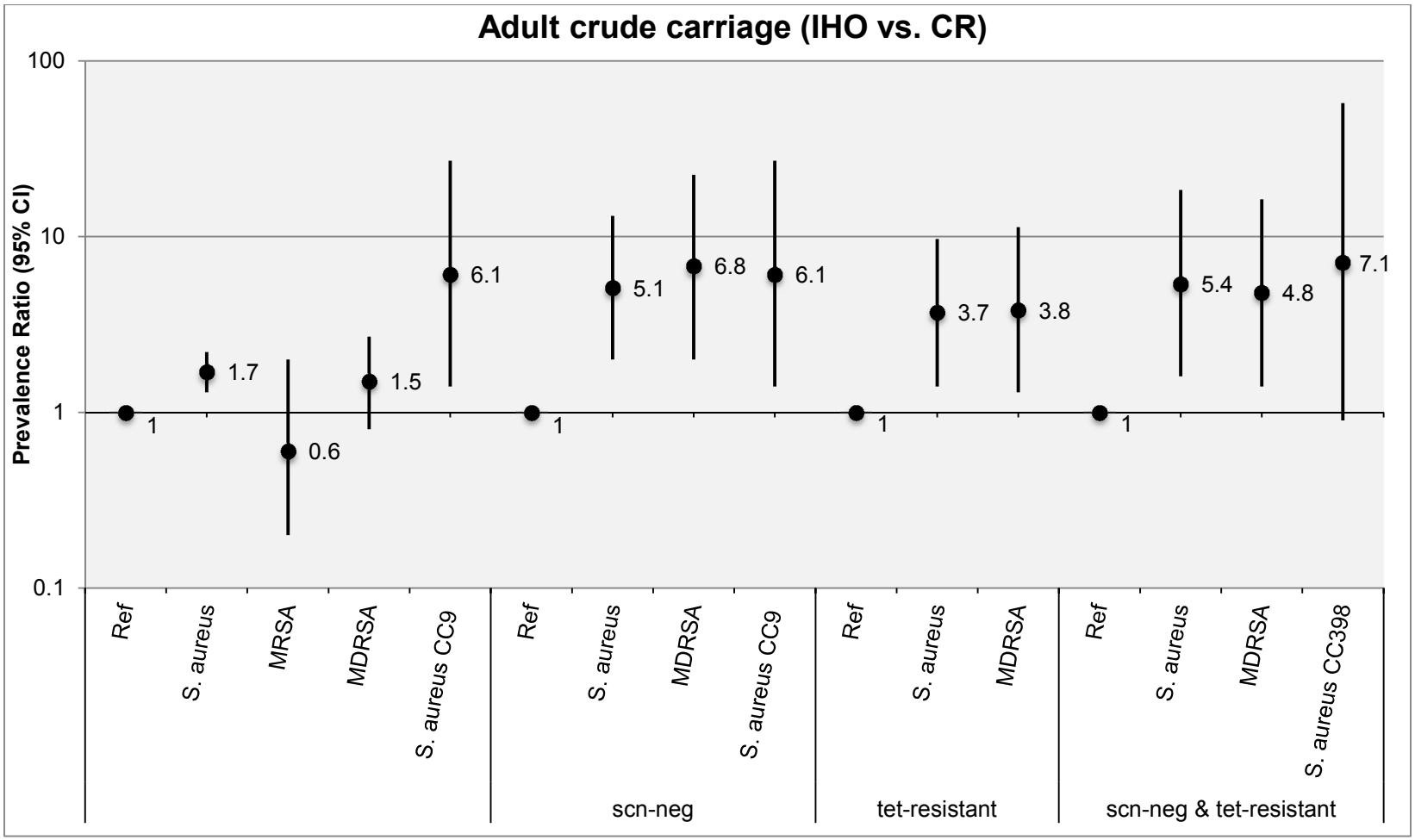
		Isolate characteristics				Household characteristics	
House No.	**Group**	***spa* type**	***scn***	**ABR profile**	***S. aureus* type**	**Pets**	**Pet direct contact**
1	CR	t034	−	CC, E, TET, AMP, PEN	MDRSA	None	-
2	CR	t688	+	AMP, PEN	MSSA	Indoor pet	Yes
3	CR	t015	+	AMP, PEN	MSSA	None	-
4	CR	t688	+	AMP, PEN	MSSA	Outdoor pet	Yes (adult)
5	CR	t008	+	CIP, CRO, GAT, LVX, OX, AMP, PEN	MRSA & MDRSA	None	-
6	CR	t493	+	AMP, PEN	MSSA	None	-
7	CR	t688	+	AMP, PEN	MSSA	None	-
8	CR	t493	+	AMP, PEN	MSSA	None	-
9	CR	t688	+	AMP, PEN	MSSA	None	-
10	CR	t2949	+	AMP, PEN	MSSA	None	-
11	CR	t088	+	CRO, E, OX, AMP, PEN	MRSA MDRSA	None	-
12	CR	t185	+	AMP, PEN	MSSA	None	-
13	CR	t089	+	NONE[1]/AMP, PEN	MSSA	None	-
14	IHO	t189	+	AMP, PEN	MSSA	None	-
15	IHO	t1077	+	TET, AMP, PEN	MSSA	None	-
16	IHO	t1937	+	AMP, PEN	MSSA	None	-
17	IHO	t688	+	AMP, PEN	MSSA	None	-
18	IHO	t002	−	CRO, CC, E, OX, AMP, PEN	MRSA & MDRSA	None	-
19	IHO	t688	+	AMP, PEN	MSSA	None	-
20	IHO	t008	+	AMP, PEN	MSSA	Outdoor pet	Yes

[1] *S. aureus* from adult swab was susceptible to all 12 tested antibiotics.

Table 2.6 Prevalence of child *S. aureus*, MRSA, and MDRSA carriage among households with adults positive for *S. aureus*, MRSA, and MDRSA carriage.

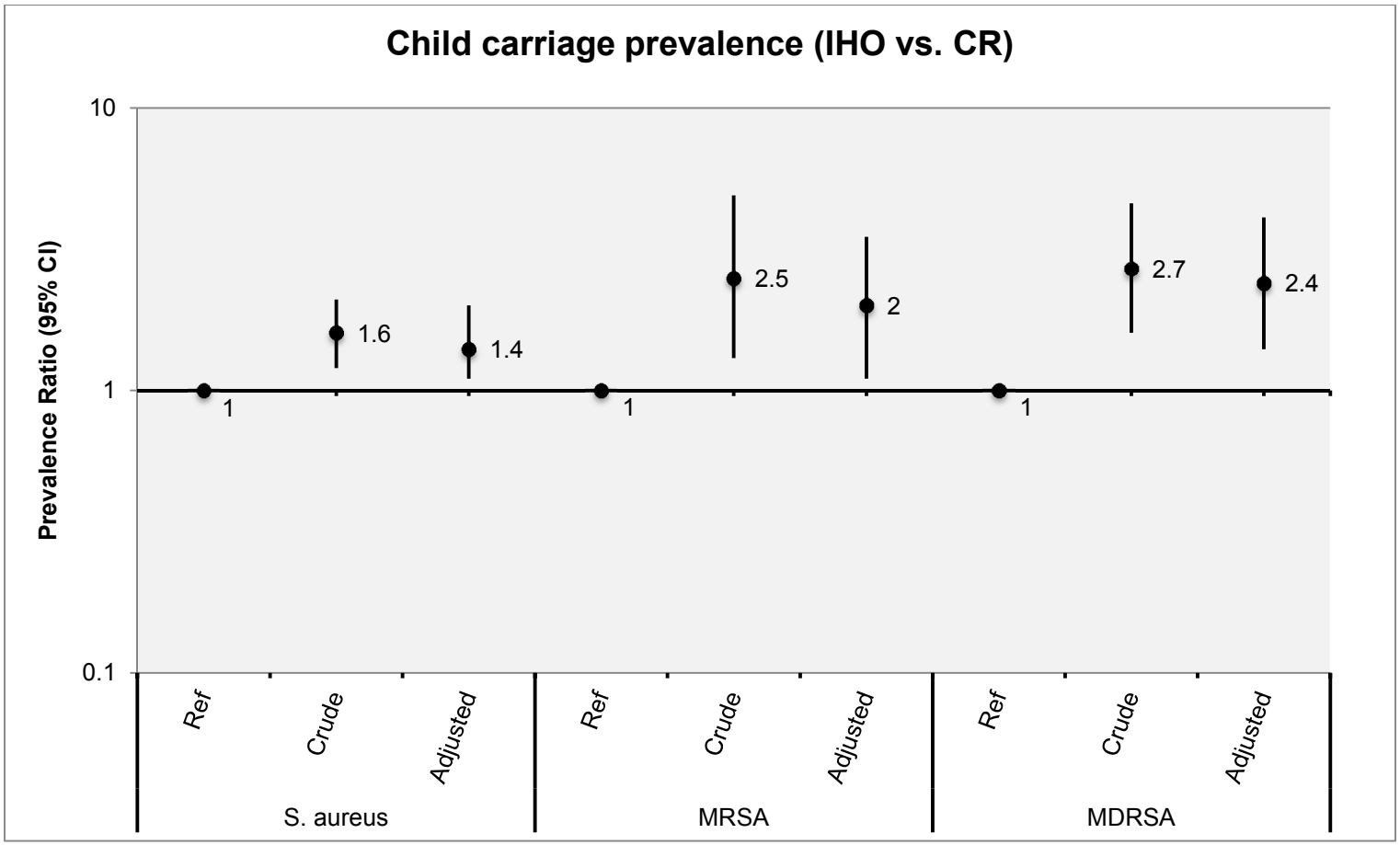
Carriage outcome	No. pos/Total N	%	PR (95% CI)
S. aureus			
IHO	58/104	56	1.2 (0.9, 1.6)
CR	30/63	48	Ref
MRSA			
IHO	1/4	25	0.9 (0.1, 6.9)
CR	2/7	29	Ref
MDRSA			
IHO	2/25	8	0.2 (0.1, 0.8)
CR	6/17	35	Ref

Figure 2.1. Prevalence ratios (PR) and 95% Confidence Intervals (95% CI) for *S. aureus*-related carriage outcomes[1] comparing industrial hog operation (IHO) adult participants to community referent (CR) adult participants.



[1] All *S. aureus* CC398 were *scn*-negative and tetracycline-resistant.

Figure 2.2. Prevalence ratios (PR) and 95% Confidence Intervals (95% CI) for *S. aureus*, MRSA, and MDRSA comparing children living with an industrial hog operation (IHO) worker compared to children in community referent (CR) households.



CHAPTER FOUR: INFLUENCE OF SPECIFIC WORK-RELATED EXPOSURES ON ANTIBIOTIC-RESISTANT *STAPHYLOCOCCUS AUREUS* CARRIAGE IN ADULT INDUSTRIAL HOG OPERATION WORKERS AND THEIR CHILD HOUSEHOLD MEMBERS

1. Introduction

Staphylococcus aureus is an opportunistic pathogen that is carried in the anterior nares of approximately 30% of the adult United States population [1]. While highly antibiotic-resistant strains of these bacteria like methicillin-resistant *S. aureus* (MRSA) were largely confined to the hospital environment until the mid 1990s, genetically-distinct MRSA strains have since emerged and spread in the community [28]. In recent years, an additional and genetically distinct reservoir of ABR *S. aureus* has been identified in industrial hog operations (IHOs) where non-therapeutic antibiotic use has created a selective pressure for the emergence of ABR *S. aureus* [76] that can be exchanged between hogs and their caretakers [89, 100].

The epidemiology of *S. aureus* from the industrial livestock environment has evolved since Huijsdens et al. [89] first reported multi-locus sequence type (MLST or ST) 398 MRSA colonizing a pig farmer and his family members, co-workers, and pigs. Since then, the clonal complex (CC) to which this ST belongs – CC398 – has been identified as the dominant strain circulating in hogs and being exchanged between hogs and humans in Europe [94], Canada [100], and the United States [85, 86]. In addition, other *S. aureus* CCs and genetic markers have been identified that are unique to *S. aureus* from livestock sources. Among CC398 *S. aureus*, tetracycline resistance has been identified as a marker of *S. aureus* of livestock origin [76]. In addition, the lack of *scn* – a marker of a mobile genetic element (MGE) thought to play a role in human colonization and infection – has been identified as a reliable marker of *S. aureus* from a non-human source [76, 82, 185]. However, there is no established universal definition of livestock-associated *S. aureus* in the scientific literature. Although many have termed *S. aureus*

with these characteristics “livestock-associated” *S. aureus*, others have considered *S. aureus* belonging to a CC previously identified in livestock, tetracycline resistance, and lack of *scn* to be markers of livestock association rather than defining an isolate as “livestock-associated” [88].

Although it has been established that IHO workers and their familial contacts are an at-risk group for carriage of ABR *S. aureus* [91, 111], much uncertainty remains regarding the impact of specific work-related activities on carriage of these bacteria among IHO workers and their household members. Furthermore, the few studies that have examined the association between work activity-related exposures and ABR *S. aureus* carriage in IHO workers yielded conflicting results. Workers who drew blood or collected other fluids from pigs were less likely to carry MRSA than those who did not in an Iowa and Illinois-based study conducted by Smith et al. [86], but the opposite association was observed in North Carolina IHO workers [84]. It has also been reported that intensity of exposure and use of protective gear were not significantly associated with MRSA carriage, although MRSA carriage was generally lower in workers who used protective gear at work [85]. It is likely that ABR *S. aureus* carriage in humans is driven by positivity of the herd with which they have had contact [86, 100, 175].

In North Carolina, where access to industrial livestock including hogs is restricted, researchers have documented nasal carriage of ABR *S. aureus* and *S. aureus* with markers of livestock association in industrial livestock workers [84], slaughterhouse workers [87] and IHO workers [88]. We recently reported that IHO workers from the top ten hog producing counties in North Carolina had a greater prevalence of MDRSA and *S. aureus* with one or more markers of livestock association, compared to others living in the same community but not occupationally-exposed to IHOs (Chapter 3).

Household members of IHO workers may also be exposed to ABR *S. aureus* from the IHO environment [111]. We found that children (< 7 yrs old) living in the same household as IHO workers had a greater prevalence of MRSA and MDRSA carriage compared to children whose adult household members were not occupationally exposed to IHOs (Chapter 3). However, this

elevated prevalence of MRSA carriage among children in IHO households was not consistent with the MRSA carriage prevalence of their adult household members; adult IHO workers had a similar and slightly lower prevalence of MRSA carriage compared to non-occupationally exposed community members. Children living in IHO households had less reported contact with healthcare and day care environments and a lower prevalence of other traditional risk factors for exposure to ABR *S. aureus*. This suggests that another exposure related to living in the same household as an adult IHO worker might influence MRSA and MDRSA carriage in children. It is possible that children could be exposed to bacteria from the IHO environment that are contaminating other body sites or the clothing of adult IHO workers, but are not colonizing the nares of workers. For example, IHO protective gear like boots has been shown to be contaminated with MRSA [152], but there is otherwise a lack of information regarding contamination of IHO workers' protective gear in the literature.

Given the scarcity of information regarding the influence of work-related activities on IHO worker carriage of *S. aureus* and the absence of information regarding work activities that may influence household member *S. aureus* exposure in the literature, we examined the association between self-reported work-related activities and carriage of ABR *S. aureus* and *S. aureus* with one or more markers of livestock association in adults and their child household members under seven years old. This allowed us to further investigate the potential mechanisms of exposure to ABR *S. aureus* in young (<7 yr old) children living in the same household as IHO workers.

2. Methods

2.1 Ethics Statement

This community-based study was a collaboration between the Johns Hopkins Bloomberg School of Public Health, the Rural Empowerment Association for Community Help (REACH) and the University of North Carolina at Chapel Hill (UNC). The Johns Hopkins University (JHU) institutional review board (IRB) approved this study; the UNC IRB conceded reliance upon the JHU IRB. Prior to participation, adult participants provided written informed consent. Parents or

legal guardians provided written informed assent for their participating child under seven years old and provided questionnaire responses for their participating child.

Data were collected between March and October 2014 in North Carolina, USA by trained researchers from UNC in collaboration with trained organizers from REACH.

2.2 Study population

Using a snowball sampling approach, participants were recruited and enrolled from the top ten hog-producing counties in North Carolina according to 2010 NC agricultural statistics [201]. In order of highest to lowest density of hogs, these counties were: Duplin, Sampson, Bladen, Wayne, Greene, Pender, Robeson, Lenoir, Jones, and Columbus [201]. One adult (≥ 18 years old) worker and one child (< 7 years old) were recruited from the households with at least one adult employed at an industrial hog operation (IHO). To be eligible for participation in this study, household members could not be employed in day care or health care facilities of any kind.

2.3 Questionnaire and nasal swab collection

Nasal swabs and questionnaire responses were collected during the same study visit.

Adult participants and parents or legal guardians of child participants reported occupational activities and workplace exposures, personal and household member contact with livestock, environmental exposures, personal and household member healthcare exposures, personal and household member childcare attendance, personal and household member medical history, and demographic information. Occupational activities reported included life stage of pig at work, interaction with pigs and medication administration, cleaning activities and use of protective gear, direct contact with pigs, size of operation, work hours, length of employment, and time since last work shift, and others.

Study personnel obtained a nasal swab from adult participants by rotating a sterile, double-tipped BD BBL™ CultureSwab™ five times clockwise and five times counter-clockwise in both nares. To minimize discomfort of child participants, two single, mini-tipped BD BBL™

CultureSwab™ nasal swabs were collected in the same manner from children under seven years old.

A set of trip blanks for all swabs were collected prior to transport from REACH to UNC and stored with samples during transport. Swabs were stored in Stuart's medium at 4°C and transported to UNC within five days of sample collection for processing.

2.4 Detection of *S. aureus* and MRSA

We aimed to obtain two presumptive *S. aureus* colonies per swab. One of the two nasal swabs collected (one tip of the double-tipped adult swab and one of the mini-tipped children's swabs) were aseptically clipped into 1 ml sterile 0.01M phosphate buffered saline (PBS). After vortexing for one minute, 100 µl PBS eluate was pipetted directly onto CHROMagar™ Staph aureus (CA) media (BD, Franklin Lakes, NJ). A sterilized stainless steel spreader and a petri dish inoculating turntable were used to evenly distribute the 100 µl PBS eluate throughout the plate until dry. CA plates were then incubated at 37°C for 24 hours. If at least two morphologically characteristic (i.e., mauve with matte halo) colonies grew on a CA after 24 h, two colonies were picked and streaked to isolation on CA media for biochemical and molecular confirmation. If fewer than two colonies grew on CA after 24 hours, the original swab and entire PBS swab eluate volume were inoculated into 10 ml Mueller-Hinton broth supplemented with 6.5% NaCl and incubated overnight at 37°C. This enrichment was then streaked on the CA and Baird-Parker (BP) plates and incubated for 24 h and 48 h, respectively, at 37°C to improve recovery of *S. aureus* from the nasal swab [202]. Up to two pure, morphologically characteristic *S. aureus* colonies were archived at -80°C in brain-heart infusion broth (BHIB) with 15% glycerol until further characterization. Catalase and tube coagulase testing with rabbit plasma (BD BBL™, Franklin Lakes, NJ) were used to confirm *S. aureus* biochemical characteristics prior to molecular testing.

Following crude DNA extraction [182], a multiplex polymerase chain reaction (PCR) assay was employed to detect the *S. aureus*-specific gene *spa*, as well as *mecA, mecC*

(*mecA*$_{LGA251}$), *scn,* and *pvl*. This PCR was performed according to the protocol described by Stegger et al. [203] and the presence of each of the five amplified genes was confirmed by gel electrophoresis on a 3% agarose gel. Among morphologically and biochemically characteristic *S. aureus* isolates that lacked *spa* by multiplex PCR, we attempted to amplify alternate *S. aureus*-specific genes by PCR for an alternate *spa* primer [204] and for *nuc* [183] and *femA* [205].

Finally, MALDI-TOF MS was performed on isolates that were negative for both *spa* primers but positive for *nuc* or *femA* to confirm *S. aureus* identity. From archived cultures, isolates were streaked onto Tryptic Soy Agar with 5% Sheep Blood and incubated overnight at 37ºC. MALDI-TOF MS was performed using the FDA-cleared VITEK MS per manufacturer's recommendations for direct colony spotting (bioMerieux, Durham, NC) [184].

Isolates that met the following criteria were classified as *S. aureus*: 1) *spa*-positive; 2) positive for *nuc* and *femA;* or 3) identified as *S. aureus* by MALDI-TOF MS.

2.5 *spa*-typing

Staphylococcal protein A (*spa*) typing was performed by amplifying the *spa* gene as described above. PCR products were sequenced by Eton Biosciences, Inc. (Research Triangle Park, NC). Staphylococcal protein A *(spa)* types were assigned using the Ridom Staph Type standard protocol (http://www.ridom.com) and the Ridom SpaServer (http://spa.ridom.de/index.shtml). *spa* types were assigned to putative MLST clonal complexes (CCs) on the scientific literature. The clonal complexes that were considered in this study were CCs 398 and 9.

2.6 Antibiotic susceptibility testing

Both PCR-confirmed *S. aureus* isolates from each *S. aureus*-positive nasal swab were tested for susceptibility to the following 12 classes of antibiotics at UNC: aminoglycosides, beta-lactams, cephalosporins, floroquinolones, lincosamides, macrolides, oxazolidones, rifamycin, streptogramins, sulfonamide, nitrofuran and tetracyclines (Appendix 1). The Kirby-Bauer disk

diffusion method was used to test each isolate's susceptibility to all antibiotic classes. Interpretation of zones of inhibition was reported as susceptible, resistant, or intermediately resistant (where applicable) according to the Clinical and Laboratory Standards Institute (CLSI) guidelines [187]. In erythromycin-resistant isolates, inducible clindamycin resistance was be assessed using the D-zone test [206]. *S. aureus* isolates that exhibited intermediate or complete phenotypic resistance to ≥ three antibiotic classes were classified as multidrug-resistant *S. aureus* (MDRSA).

2.7 Markers of livestock association

Based on the rationale provided by Rinsky et al. [84] and Nadimpalli et al. [88], three markers of livestock association were examined among *S. aureus*-positive isolates: 1) presence of CC398 or CC9 by *spa* type; 2) lack of *scn*; and 3) presence of phenotypic tetracycline resistance.

2.8 Carriage outcomes

The nasal carriage outcomes examined were: *S. aureus*, MRSA, MDRSA, CC398, and CC9; *scn*-negative *S. aureus*, MRSA, MDRSA, CC398, and CC9; tetracycline-resistant *S. aureus*, MRSA, MDRSA, CC398, and CC9; and *scn*-negative and tetracycline-resistant *S. aureus*, MRSA, MDRSA, CC398, and CC9. For each binary carriage outcome, an individual was considered positive for the outcome if either of the two isolates from the participant's nasal swab met the criteria for that outcome. For example, a participant was considered positive for *S. aureus* carriage if either the first or the second isolate from his or her nasal swab was confirmed *S. aureus*.

2.8 Statistical Analysis

We examined the distribution of demographic characteristics among IHO adults and children and the distribution of adult gender by reported work activity. Carriage prevalence stratified by adult work activity was calculated for adults and children separately.

Using log-binomial models, we compared carriage of each outcome among adults and children by work activity by calculating crude prevalence ratios (PRs) and 95% confidence intervals (CIs). We also examined reported size of operation as a confounder in adult and child work-activity models; however, sample size did not allow for the calculation of adjusted PR estimates.

All statistical analyses were performed in SAS 9.4 (SAS Institute Inc., Cary, NC).

3. Results

3.1 Participant Characteristics

Adult and child demographic and environmental exposure characteristics are described in Table 1. A total of 198 households participated in this study, with one adult IHO worker and one child under seven years old per household. The majority of children were between three and seven years old and approximately 90% of adults were between 18 and 47 years old. The percentage of male and female workers was roughly equal (54% and 46%, respectively), but the majority of children were male. Most households self-identified as Hispanic (93% of adults and 94% of children). Other personal and environmental exposures were uncommon among adults and children; the majority of households did not report having a pet, had not used antibiotics within the last 3 months, had not been to a healthcare facility within the last 3 months, did not participate in contact sports or attend a gymnasium, and most children did not attend childcare (Table 3.1).

The distribution of work activities reported by adult IHO participants is presented in Table 3.2. The most commonly reported work activities included working with wean, farrow, or feeder pigs (47%), administering shots (71%) or antibiotics (62%), performing cleaning activities (74%) and cleaning with chemicals (57%), working at an IHO of a size between 1000 and 4000 hogs (40%), and handling dead pigs (72%). The majority of adult participants worked over 40 hours per week and had worked at their current job for less than five years. Some participants reported that the IHO where they were employed had fewer than 250 hogs. These individuals

were excluded from analysis for this variable (size of IHO) because animals with fewer than 250 hogs are not considered to be industrial-scale operations in North Carolina according to Senate Bill 1217 [217].

Most work activities were similar by gender (Table 3.3). More females worked with wean, farrow, or feeder pigs than males, and fewer females worked with sows or boars (4%) and finishing pigs (6%). Females also reported contacting fewer pigs per day and reported working at smaller operations. However, it is possible that these latter two variables may be explained by a tendency to over- or under-estimate by gender.

3.2 Prevalence of *S. aureus*-related carriage outcomes among adults by work activity

The overall prevalence of *S. aureus*-related carriage outcomes among IHO adults is reported in Chapter 3. Among adults, few work activities were associated with a greater prevalence of *S. aureus*-related carriage outcomes in IHO workers (Appendix 4, Tables 3.4a-3.4c). Only individuals who reported working with wean, farrow, or feeder pigs were positive for MRSA nasal carriage, but pig life stage was not associated with any other carriage outcomes. In addition, MRSA carriage was more common among workers who reported using a pressure washer at work (5%) than those who did not. Individuals who worked at their current job or any IHO for over five years had a greater prevalence of all *S. aureus* outcomes except MRSA; the prevalence ratio was greatest for carriage of CC398 or CC9 (Tables 3.4a-3.4c).

Adult workers who reported never wearing a mask had a greater prevalence of all *S. aureus*-related outcomes (Tables 3.4a-3.4c, Figures 3.1 and 3.2). All MRSA-positive workers reported never wearing a mask at work. Compared to adults who reported direct contact with ≤200 pigs on a typical day at work, those who reported direct contact with >1000 pigs at had a greater prevalence of most *S. aureus*-related outcomes, with a significantly greater prevalence of *scn*-negative and tetracycline resistant *S. aureus* (PR: 3.3; 95% CI: 1.2, 8.6), MDRSA (PR: 3.0; 95% CI: 1.3, 7.2), *scn*-negative MDRSA (PR: 2.9; 95% CI: 1.1, 7.4) and *scn*-negative and

tetracycline-resistant MDRSA (PR: 3.7; 95% CI: 1.4, 10.2) (Tables 3.4a-3.4c, Figures 3.3 and 3.4).

3.3 Prevalence of *scn*-negative *S. aureus*, MRSA, and MDRSA among children by work activity of adult household members

The overall prevalence of *S. aureus*-related carriage outcomes in children is reported in Chapter 3. Although the prevalence of *S. aureus*-related outcomes differed by contact with wean, farrow, or feeder pigs, mask use, and number of pigs directly contacted among adults, these variables were not associated with *S. aureus*-related outcomes in IHO children. Prevalence of *scn*-negative *S. aureus*, MRSA, and MDRSA was lower among children whose adult household members reported never wearing a mask at work and prevalence of MRSA and MDRSA was higher among children whose household members contacted 200 or fewer pigs per day (Appendix 5, Table 3.5).

However, some work-related activities were associated with elevated carriage prevalence in children. Children whose adult household member reported contact with pig manure at work had a greater prevalence of MRSA (PR: 19.2; 95% CI: 4.7, 78.9) and MDRSA (PR: 2.3; 95% CI: 1.4, 3.9). Additionally, children living with an adult household member who reported bringing protective gear home from the IHO had a greater prevalence of MRSA (PR: 19.2; 95% CI: 4.7, 78.9) and MDRSA (PR: 2.1; 95% CI: 1.3, 3.5). Chemical use at work was also associated with MRSA (PR: 9.6; 95% CI: 2.3, 39.4) and MDRSA (PR: 2.4; 95% CI: 1.3, 4.4) prevalence in children. (Table 3.5, Figures 3.5-3.7)

4. Discussion

Although the majority of IHO work activities did not appear to impact nasal carriage of ABR *S. aureus* and *S. aureus* with markers of livestock association, we found that never using a mask was associated with a greater prevalence of MDRSA and *S. aureus* with markers of livestock association in IHO workers, compared to those who reported using a mask at least sometimes. This finding suggests that mask use may influence nasal carriage of ABR *S. aureus*

in the IHO workplace, which may decrease risk of infection among workers since *S. aureus* nasal carriage has associated with an increased risk of infection with the bacterium [197]. However, adult household member mask use was not associated with greater carriage prevalence among children in our study. Therefore, while mask usage may be associated with nasal carriage of ABR *S. aureus* in workers, it did not appear to influence carriage in child household members.

Others have reported conflicting results regarding the influence of protective gear on MRSA carriage prevalence in industrial livestock settings [85, 117, 218]. Smith et al. [85] found that among workers at operations with MRSA-positive herds, 65% of workers who reported rarely or never wearing a mask carried MRSA, compared to 35% of workers who at least sometimes wore a mask. However, reported use of protective clothing was associated with a greater MRSA carriage prevalence among swine farm personnel in Belgium [117] and it has also been reported that protective gear was ineffective in preventing nasal carriage in livestock veterinarians [218]. In our study population, it appears that wearing a mask at work may decrease nasal carriage among IHO workers. We did not collect information regarding the type of mask used by workers, which may also impact nasal carriage outcomes. Furthermore, it is possible that different mask types may be provided for certain activities performed at work, such as cleaning with chemicals or disinfectants. Since we did not collect this information and were unable to directly observe the type and frequency of mask use at the workplace, it is unclear whether the associations we observed are truly due to mask use or if they represent an unmeasured workplace exposure that is associated with reported mask use. This finding are provides interesting preliminary information that suggests that the frequency or type of mask use warrant further investigation. Alternatively, it may suggest that other workplace activities performed while wearing a mask may be important to explore in future studies.

In addition, reported direct contact with >1000 pigs was associated with a greater prevalence of MDRSA and *S. aureus* with one or more markers of livestock association among

IHO workers in our study population. In two swine production systems in Iowa, Smith et al. [86] did not observe a difference in ABR *S. aureus* carriage prevalence by number of pigs contacted on a typical day at work. Since we did not observe an association between only the IHO size and *S. aureus*-related carriage outcomes, these results suggest that intensity or frequency of animal contact may play a role in zoonotic exchange of ABR *S. aureus* on IHOs.

In Chapter 3, we observed that IHO children had a higher prevalence of MRSA than community referent (CR) children. However, this finding was not supported by elevated MRSA prevalence in their adult household members, as only 4/198 adult IHO workers carried MRSA at the time of sample collection. One potential explanation for this discrepancy is that, although workers are not nasally colonized by MRSA, they may carry it on other parts of their bodies or on their work clothing when they return home. Among 27 IHOs in Germany, 74% of IHO worker boot swabs were MRSA positive [152], demonstrating that work clothing or protective gear may be another important site of contamination by ABR *S. aureus* through which household members could be exposed if they come into contact with it in the home environment. Interestingly, our data suggest that MRSA and MDRSA carriage prevalence was higher among children whose adult household members reported bringing protective equipment home. We did not sample other body sites, clothing, or protective equipment of workers and do not know the prevalence of ABR *S. aureus* contaminating protective equipment. Furthermore, workers did not specify the type of protective gear they brought home from IHOs, so this information may not have been reported consistently. Future studies examining household exposure should consider sampling boots, clothing, or other protective equipment of IHO workers to examine this potential route of exposure for household members.

Adult household member contact with manure was also associated with greater prevalence of ABR *S. aureus* carriage in children. It is likely that all individuals working in a confined space with hundreds to thousands of hogs come into contact with their waste at some point during their work shift, as high concentrations of antibiotic-resistant fecal bacteria have

been detected within confinement buildings [219]. Reported manure contact may provide a reflection of a perceived dirtiness of the work environment and at best, it may capture a true work activity involving hog waste, such as flushing waste from beneath the hogs into the lagoon. Although there is uncertainty associated with the interpretation of this variable and what it represents, this finding suggests that exposure to ABR *S. aureus* in young children living in the same household as IHO workers may be associated with waste-related work activities of adult household members. Most research investigating presence of ABR *S. aureus* in environmental samples from IHOs has focused on air and dust sampling [140, 152, 219, 220]. However, Friese et al. [152] detected MRSA in 56% of hog feces samples and others have demonstrated that MRSA can be detected in waste water treatment plants [151]. While the specific mechanism of child exposure via adult IHO household member reported hog waste contact is not clear, it is feasible that reported contact with waste at work at the IHO by adults could influence ABR *S. aureus* carriage of children living in the same household as workers.

These work activities provide insight into the potential routes of ABR *S. aureus* exposure in the workplace and home environment. However, this study is limited by our inability to sample IHO herds. Concordant herd and worker CC398 MRSA positivity has been documented in in IHOs in Europe [89, 117] and the Midwestern United States [86]. Among 20 farms in Canada, workers were only positive for MRSA carriage on farms where pig herds were MRSA positive [100]. Because we did not have access to IHOs, we do not have data regarding ABR *S. aureus* carriage among IHO herds contacted by workers. Therefore, it is possible that herd positivity is driving or influencing our observed associations between work activities and adult or child carriage. Lack of access to the workplace also prevents us from directly observing IHO work activities. Therefore, we must rely upon reported values of work activities that may reflect intensity of contact – such as number of pigs contacted, administering shots, and inseminating sows – but cannot observe the full range of activities performed by workers or verify the accuracy of reported continuous exposures.

In addition, we rely on worker nasal carriage as a proxy for a sample from the IHO environment. However, we do not know how many participants shared a specific IHO work location. Questionnaires requested the name, address, or permit number of IHOs where adults were employed, but nearly all participants refused to answer this question (data not shown). This is likely due to fear of retaliation by their employers, as intimidation tactics of the hog industry have been documented in the past [221]. Agricultural census data reports that among IHOs in NC that reported hired labor, there are an average of approximately 7 employees per operation [222], so we do not expect that a large number of workers were employed at the same IHO. Nonetheless, the lack of this information prevents us from investigating the number of farms represented by workers that are positive for certain outcomes.

We did not observe an association between reported administration of antibiotics on the farm and antibiotic-resistant *S. aureus* carriage in workers. Researchers have previously investigated the animal husbandry practice of non-therapeutic antibiotic use in North Carolina, comparing workers on antibiotic-free livestock farms to those on industrial livestock operations [84]. They found that workers at industrial livestock operations had a higher prevalence of multidrug resistant bacteria and *scn*-negative, tetracycline-resistant CC398 *S. aureus* than workers on farms that did not practice non-therapeutic antibiotic use. It is possible that antibiotics are administered at most IHOs included in our study but that some workers were not responsible for administering medication and therefore did not report it, especially if those antibiotics are administered via industry-provided feed. Therefore, the observed lack of association between reported antibiotic administration and worker carriage may be due to a lack of variability in antibiotic use in the IHOs represented in this study.

5. Conclusions

This research has increased our understanding of the workplace exposures of IHO adult workers and their young (< 7 yr old) household members, which can inform the design of future research investigating the public health impacts of IHOs on children's' health. Years of

collaboration with REACH have allowed our partnership to build the community trust and organizational capacity to conduct a study of this size. It is important to note that addressing the limitations of this and other research involving occupational exposures of IHO workers in the United States depends upon acquiring access to and samples from IHOs. In addition, rather than relying solely on nasal carriage as a representation of the bacteria that are transported off of the IHO environment via workers, samples from the household and from clothing, protective gear, and other body sites may provide a more complete picture of the routes of exposure for household members.

Table 3.1. Characteristics of industrial hog operation (IHO) study participants, stratified by participant type (adult vs. child).

Participant Characteristic	Adult[1]	Child[2]
	N (%)	N (%)
Age	198	198
0-2	-	32 (16)
3-5	-	116 (59)
6-7	-	50 (25)
18-27	47 (24)	-
28-37	88 (44)	-
38-47	42 (21)	-
≥ 47	21 (11)	-
Gender	198	198.0
Male	107 (54)	123 (62)
Female	91 (46)	75 (38)
Race/Ethnicity	198	198.0
White	0 (0)	0 (0)
Black	12 (6)	11(6)
Hispanic	185 (93)	186 (94)
Multi-racial	1 (<1)	1 (<1)
Education	198	-
≤ High school	139 (70)	-
> High school	59 (30)	-
Household pet	198	198.0
Yes	65 (33)	65 (33)
No	133 (67)	133 (67)
Pet type	64	64.0
Indoor only	4 (6)	4 (6)
Outdoor only	49 (76)	49 (76)
Indoor & outdoor	11 (17)	11 (17)
Antibiotic use[3]	198	198
No	196 (99)	196 (99)
Yes	2 (1)	2 (1)
Healthcare contact[3]	198	198
No	188 (95)	173 (87)

Participant Characteristic	Adult[1]	Child[2]
	N (%)	N (%)
Yes	10 (5)	25 (13)
Participation in contact sports[3]	198	198
No	186 (94)	196 (99)
Yes	12 (6)	2 (1)
Gym attendance[3]	198	-
No	193 (97)	-
Yes	5 (3)	-
Smoking	198	-
No	167 (84)	-
Yes	31 (16)	-
Household smoking	198	198
No	167 (83)	167 (83)
Yes	31 (16)	31 (16)
Childcare attendance	-	198
No	-	193 (97)
Yes	-	5 (3)
School attendance	-	198
No	-	89 (45)
Yes	-	109 (55)
Time outside	-	198
< 3 hr	-	158 (80)
≥ 3 hr	-	40 (20)

[1] Eligibility criteria required that all adult participants were at least 18 years of age at the time of sample collection.

[2] Eligibility criteria required that all children were less than seven years old at the time of sample collection.

[3] Within 3 months of sample collection.

Table 3.2. Distribution of work activities among adult industrial hog operation (IHO) worker participants.

Work Activity	Total N	N pos	%
Pig life stage[1]			
Nursery	198	39	20
Finishing	198	36	18
Wean, farrow, or feeder	198	94	47
Sow or Boar	198	31	16
Interaction with pigs and medication use			
Administer shots	198	140	71
Inseminate sows	198	26	13
Administer antibiotics	198	123	62
Cleaning activities and protective gear			
Chemical use	198	112	57
Any cleaning	198	147	74
Pressure washer use	198	67	34
Mask use	198	22	11
Take protective gear home	198	78	40
Amount of direct contact with pigs			
Hours/day in direct contact with pigs			
>8	198	25	13
≤8	198	173	87
Total pigs per day			
≤200	198	113	57
>200 to 1000	198	59	30
>1000	198	26	13
Size of IHO			
<250[2]	198	8	4
≥250 to 1000	198	36	18
>1000 to 4000	198	80	40
≥4000	198	74	37
Other work activities			
Handle dead pigs	198	143	72
Contact pig manure	198	78	40
Other employment characteristics			
Hours worked per day			
>8 h	198	43	22
≤8 h	198	155	78
Hours worked per week			
>40 h	198	181	91
≤40 h	198	17	9
Years worked at current IHO			
> 5	198	52	26
≤ 5	198	146	74

Work Activity	Total N	N pos	%
Years worked at any IHO			
>5	198	63	32
≤5	198	135	68
Time since last work shift			
≤3 h	198	81	41
>3 to 12 h	198	67	34
>12 h	198	50	25

[1] Categories were developed based on age/life stage and substantial overlap among individuals who reported working with sows and boars or wean, farrow, and feeder pigs. Additional overlap was observed between the following categories: sow and wean pigs (2 individuals), nursery and finish pigs (1 individual), wean pigs and boars (1 individual), and farrow pigs and boars (1 individual).

[2] This exposure category was not included in analysis.

Table 3.3. Distribution of work activities by gender among adult industrial hog operation worker participants.

Work activity	Gender		Chi-squared
	Females	Males	
	N/Total (%)	N/Total (%)	
Life stage of pig[1]			
Nursery	22/91 (24)	17/107 (16)	2.1
Finishing	5/91 (6)	31/107 (29)	18.2
Wean, farrow, or feeder	59/91 (65)	35/107 (33)	20.4
Sow or Boar	4/91 (4)	27/107 (25)	16.2
Interaction with pigs and medication use			
Administer shots	81/91 (89)	59/107 (55)	27.2
Inseminate sows	3/91 (3)	23/107 (22)	14.3
Administer antibiotics	65/91 (71)	58/107 (54)	6.2
Cleaning activities and protective gear			
Chemical use	49/91 (54)	63/107 (59)	0.5
Any cleaning	63/91 (69)	84/107 (79)	2.2
Pressure washer use	29/91 (32)	38/107 (36)	0.3
Mask use	84/91 (92)	92/107 (86)	2.0
Take protective gear home	29/91 (32)	49/107 (46)	4.0
Amount of direct contact with pigs			
Hours/day in direct contact with pigs			1.1
>8	9/91 (10)	16/107 (15)	-
≤8	82/91 (90)	91/107 (85)	-
Total pigs per day			
≤200	52/91 (57)	91/107 (57)	0.0
>200 to 1000	34/91 (37)	25/107 (23)	4.6
>1000	5/91 (6)	21/107 (20)	8.6
Size of IHO			
<250[2]	7/91 (8)	1/107 (1)	5.8
≥250 to 1000	17/91 (30)	9/107 (8)	14.9
>1000 to 4000	39/91 (43)	41/107 (38)	0.4
≥4000	18/91 (20)	56/107 (52)	22.2
Other work activities			
Handle dead pigs	57/91 (62)	86/107 (80)	7.7
Contact pig manure	28/91 (31)	50/107 (47)	5.2
Other employment characteristics			
Hours worked per day			5.5
>40 h	13/91 (14)	30/107 (28)	-
≤40 h	78/91 (86)	77/107 (72)	-
Years worked at current IHO			0.9
> 5	21/91 (23)	31/107 (29)	-
≤ 5	70/91 (77)	76/107 (71)	-
Years worked at any IHO			0.8
>5	26/91 (29)	37/107 (35)	-
≤5	65/91 (71)	70/107 (65)	-
Time since last work shift			

Work activity	Gender		Chi-squared
	Females	Males	
	N/Total (%)	N/Total (%)	
≤3 h	40/91 (44)	41/107 (38)	0.6
>3 to 12 h	31/91 (34)	36/107 (34)	0.0
>12 h	20/91 (22)	30/107 (28)	0.9

[1] Categories were developed based on age/life stage and substantial overlap among individuals who reported working with sows and boars or wean, farrow, and feeder pigs. Additional overlap was observed between the following categories: sow and wean pigs (2 individuals), nursery and finish pigs (1 individual), wean pigs and boars (1 individual), and farrow pigs and boars (1 individual).

[2] This category was not included in analysis.

Table 3.4a. Prevalence (%), prevalence ratios (PR) and 95% confidence intervals (95% CI) for *S. aureus*-related carriage outcomes in adult IHO workers, stratified by work activity (Part 1).

Work Activity	*scn*-neg *S. aureus*		scn-neg, tet-R *S. aureus*		MRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage						
Wean, farrow, or feeder						
Yes	15/94 (16)	1.7 (0.8, 3.5)	7/94 (8)	0.9 (0.3, 2.2)	4/94 (4)	-[1]
No	10/104 (10)	Ref	9/104 (9)	Ref	0/104 (0)	Ref
Administer antibiotics to pigs						
Yes	17/123 (14)	1.2 (0.6, 2.9)	11/123 (9)	1.3 (0.5, 3.7)	3/123 (2)	1.8 (0.2, 17.3)
No	8/75 (11)	Ref	5/75 (7)	Ref	1/75 (1)	Ref
Use a pressure washer						
Yes	10/67 (15)	1.3 (0.6, 2.7)	6/67 (9)	1.2 (0.4, 3.1)	3/67 (5)	5.9 (0.6, 55.3)
No	15/131 (12)	Ref	10/131 (8)	Ref	1/131 (1)	Ref
Frequency of mask use						
Ever use	18/176 (10)	Ref	12/176 (7)	Ref	0/22 (0)	Ref
Never	7/22 (32)	3.1 (1.5, 6.6)	4/22 (18)	2.7 (0.9, 7.6)	4/176 (2)	-[1]
Total pigs contacted per day						
≤200	11/113 (10)	Ref	8/113 (7)	Ref	0/113 (0)	Ref
>200 to 1000	8/59 (14)	1.4 (0.6, 3.3)	2/59 (3)	0.5 (0.1, 2.2)	4/59 (7)	-[1]
>1000	6/26 (23)	2.4 (1.0, 5.8)	6/26 (23)	3.3 (1.2, 8.6)	0/26 (0)	-[1]
Size of IHO[2]						
≥250 to 1000	4/36 (11)	Ref	2/36 (6)	Ref	1/36 (3)	Ref

Work Activity	*scn*-neg *S. aureus*		scn-neg, tet-R *S. aureus*		MRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
>1000 to 4000	11/80 (14)	1.2 (0.4, 3.6)	6/80 (8)	1.4 (0.3, 6.4)	3/80 (4)	1.4 (0.1, 12.5)
≥4000	9/74 (12)	1.1 (0.4, 3.3)	8/74 (11)	1.9 (0.4, 8.7)	0/74 (0)	-[3]
Length of employment						
Years worked at current IHO						
> 5	12/52 (23)	2.6 (1.3, 5.3)	8/52 (15)	2.8 (1.1, 7.1)	0/52 (0)	-[3]
≤5	13/146 (9)	Ref	8/146 (6)	Ref	4/146 (3)	Ref
Years worked at any IHO						
>5	16/63 (25)	3.8 (1.8, 8.1)	9/63 (14)	2.8 (1.1, 7.1)	1/63 (2)	0.7 (0.1, 6.7)
≤5	9/135 (7)	Ref	7/135 (5)	Ref	3/135 (2)	Ref

Table 3.4b. Prevalence (%), prevalence ratios (PR) and 95% confidence intervals (95% CI) for *S. aureus*-related carriage outcomes in adult IHO workers, stratified by work activity (continued).

Work Activity	MDRSA		scn-neg MDRSA		scn-neg, tet-R MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage						
Wean, farrow, or feeder						
Yes	12/94 (13)	1.0 (0.5, 2.1)	10/94 (11)	1.1 (0.5, 2.5)	5/94 (5)	0.6 (0.2, 1.8)
No	13/104 (13)	Ref	10/104 (10)	Ref	9/104 (9)	Ref
Administer antibiotics to pigs						
Yes	17/123 (14)	1.3 (0.6, 2.9)	13/123 (11)	1.1 (0.5, 2.7)	9/123 (7)	1.1 (0.4, 3.2)
No	8/75 (11)	Ref	7/75 (9)	Ref	5/75 (7)	Ref
Use a pressure washer						
Yes	11/67 (16)	1.5 (0.7, 3.2)	8/67 (12)	1.3 (0.6, 3.0)	4/67 (6)	0.8 (0.3, 2.4)
No	14/131 (11)	Ref	12/131 (9)	Ref	10/131 (8)	Ref
Frequency of mask use						
Ever use	19/176 (11)	Ref	14/176 (8)	Ref	10/176 (6)	Ref
Never	6/22 (27)	2.5 (1.1, 5.6)	6/22 (27)	3.4 (1.5, 8.0)	4/22 (18)	3.2 (1.1, 9.3)
Total pigs contacted per day						
≤200	10/113 (9)	Ref	9/113 (8)	Ref	7/113 (6)	Ref
>200 to 1000	8/59 (14)	1.5 (0.6, 3.7)	5/59 (9)	1.1 (0.4, 3.0)	1/59 (2)	0.3 (0.0, 2.2)
>1000	7/26 (27)	3.0 (1.3, 7.2)	6/26 (23)	2.9 (1.1, 7.4)	6/26 (23)	3.7 (1.4, 10.2)
Size of IHO2						
≥250 to 1000	3/36 (8)	Ref	3/36 (8)	Ref	1/36 (3)	Ref
>1000 to 4000	10/80 (13)	1.5 (0.4, 5.1)	8/80 (10)	1.2 (0.3, 4.3)	5/80 (6)	2.3 (0.3, 18.6)

Work Activity	MDRSA		scn-neg MDRSA		scn-neg, tet-R MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
≥4000	12/74 (16)	1.9 (0.6, 6.5)	9/74 (12)	1.5 (0.4, 5.1)	8/74 (11)	3.9 (0.5, 29.9)
Length of employment						
Years worked at current IHO						
> 5	11/52 (21)	2.2 (1.1, 4.5)	11/52 (21)	3.4 (1.5, 7.8)	8/52 (15)	3.7 (1.4, 10.3)
≤5	14/146 (10)	Ref	9/146 (6)	Ref	6/146 (4)	Ref
Years worked at any IHO						
>5	13/63 (21)	2.3 (1.1, 4.8)	13/63 (20)	4.0 (1.7, 9.5)	8/63 (13)	2.9 (1.0, 7.9)
≤5	12/135 (9)	Ref	7/135 (5)	Ref	6/135 (4)	Ref

Table 3.4c. Prevalence (%), prevalence ratios (PR) and 95% confidence intervals (95% CI) for *S. aureus*-related carriage outcomes in adult IHO workers, stratified by work activity (continued).

Work Activity	CC398 or CC9[4]	
	N pos/Total N (%)	PR (95% CI)
Pig life stage		
Wean, farrow, or feeder		
Yes	10/94 (11)	1.2 (0.5, 2.9)
No	9/104 (9)	Ref
Administer antibiotics to pigs		
Yes	13/123 (11)	1.3 (0.5, 3.3)
No	6/75 (8)	Ref
Use a pressure washer		
Yes	6/67 (9)	0.9 (0.4, 2.3)
No	13/131 (10)	Ref
Frequency of mask use		
Ever use	13/176 (7)	Ref
Never	6/22 (27)	3.7 (1.6, 8.7)
Total pigs contacted per day		
≤200	10/113 (9)	Ref
>200 to 1000	4/59 (7)	0.8 (0.3, 2.3)
>1000	5/26 (19)	2.2 (0.8, 5.8)
Size of IHO[2]		
≥250 to 1000	3/36 (8)	Ref
>1000 to 4000	7/80 (9)	1.1 (0.3, 3.8)
≥4000	8/74 (11)	1.3 (0.4, 4.6)
Length of employment		
Years worked at current IHO		
> 5	12/52 (23)	4.8 (2.0, 11.6)
≤5	7/146 (5)	Ref
Years worked at any IHO		
>5	14/63 (22)	6.0 (2.3, 15.9)
≤5	5/135 (4)	Ref

[1] PR estimate not computed due to zero observations in the referent category.

[2] Restricted to individuals who reported ≥250 pigs at operation.

[3] PR estimate not computed due to zero observations in at least one exposure category.

[4] All CC398 and CC9 were *scn*-negative.

Table 3.5. Prevalence (%), prevalence ratios (PR) and 95% Confidence Intervals (95% CI) for *scn*-negative *S. aureus*, MRSA, and MDRSA carriage in child household members of adult industrial hog operation (IHO) workers, stratified by work activity of the adult participant.

Work activity	*scn*-neg *S. aureus*		MRSA		MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage						
Nursery						
Yes	0/39 (0)	-[1]	10/39 (26)	2.4 (1.2, 4.8)	11/39 (28)	1.3 (0.7, 2.4)
No	7/159 (4)	Ref	17/159 (11)	Ref	34/159 (21)	Ref
Sow or Boar						
Yes	1/31 (3)	0.9 (0.1, 7.2)	0/31 (0)	-[1]	6/31 (19)	0.8 (0.4, 1.8)
No	6/167 (4)	Ref	27/167 (16)	Ref	39/167 (23)	Ref
Inseminate sows						
Yes	0/26 (0)	-[1]	0/26 (0)	-[1]	5/26 (19)	0.8 (0.4, 1.9)
No	7/172 (4)	Ref	27/172 (16)	Ref	40/172 (23)	Ref
Administer antibiotics						
Yes	6/123 (5)	3.7 (0.4, 29.8)	8/123 (7)	0.3 (0.1, 0.6)	24/123 (20)	0.7 (0.4, 1.2)
No	1/75 (1)	Ref	19/75 (25)	Ref	21/75 (28)	Ref
Cleaning activities						
Chemical use						
Yes	1/112 (1)	0.1 (0.0, 1.0)	25/112 (22)	9.6 (2.3, 39.4)	34/112 (30)	2.4 (1.3, 4.4)
No	6/86 (7)	Ref	2/86 (2)	Ref	11/86 (13)	Ref
Any cleaning						
Yes	3/147 (2)	0.3 (0.1, 1.1)	26/147 (18)	9.0 (1.3, 64.8)	39/147 (27)	2.3 (1.0, 5.0)
No	4/51 (8)	Ref	1/51 (2)	Ref	6/51 (12)	Ref
Frequency of mask use						

Work activity	*scn*-neg *S. aureus*		MRSA		MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Ever use	7/176 (4)	Ref	27/176 (15)	Ref	43/176 (24)	Ref
Never	0/22 (0)	-[1]	0/22 (0)	-[1]	2/22 (9)	0.4 (0.1, 1.4)
Take protective gear home						
Yes	1/78 (1)	0.3 (0.0, 2.1)	25/78 (32)	19.2 (4.7, 78.9)	26/78 (33)	2.1 (1.3, 3.5)
No	6/120 (5)	Ref	2/120 (2)	Ref	19/120 (16)	Ref
Total pigs contacted per day						
≤200	1/113 (1)	Ref	25/113 (22)	Ref	29/113 (26)	Ref
>200 to 1000	4/59 (7)	7.7 (0.9, 67.0)	2/59 (3)	0.2 (0.0, 0.6)	11/59 (19)	0.7 (0.4, 1.3)
>1000	2/26 (8)	8.7 (0.8, 92.3)	0/26 (0)	-[1]	5/26 (19)	0.7 (0.3, 1.7)
Size of operation[2]						
≥250 to 1000	1/36 (3)	Ref	2/36 (6)	Ref	5/36 (14)	Ref
>1000 to 4000	3/80 (4)	1.4 (0.1, 12.5)	6/80 (8)	1.4 (0.3, 6.4)	17/80 (21)	1.5 (0.6, 3.8)
≥4000	3/74 (4)	1.5 (0.2, 13.5)	19/74 (26)	4.6 (1.1, 18.8)	23/74 (31)	2.2 (0.9, 5.4)
Other work activities						
Handle dead pigs						
Yes	7/143 (5)	-[3]	24/143 (17)	3.1 (1.0, 9.8)	30/143 (27)	2.5 (1.1, 5.6)
No	0/55 (0)	Ref	3/55 (6)	Ref	6/55 (11)	Ref
Contact pig manure						
Yes	1/78 (1)	0.3 (0.0, 2.1)	25/78 (32)	19.2 (4.7, 78.9)	27/78 (35)	2.3 (1.4, 3.9)
No	6/120 (5)	Ref	2/120 (2)	Ref	18/120 (15)	Ref

[1] PR estimates not computed due to zero observations in at least one exposure category.

[2] Restricted to individuals who reported ≥250 pigs at operation.

[3] PR estimates not computed due to zero observations in referent category.

Figure 3.1. Plot of prevalence ratios (PR) and 95% confidence intervals (95% CI) for *S. aureus*-related outcomes among adult industrial hog operation (IHO) workers, stratified by mask use (comparing those who reported never using a mask compared to those who reported ever using a mask).

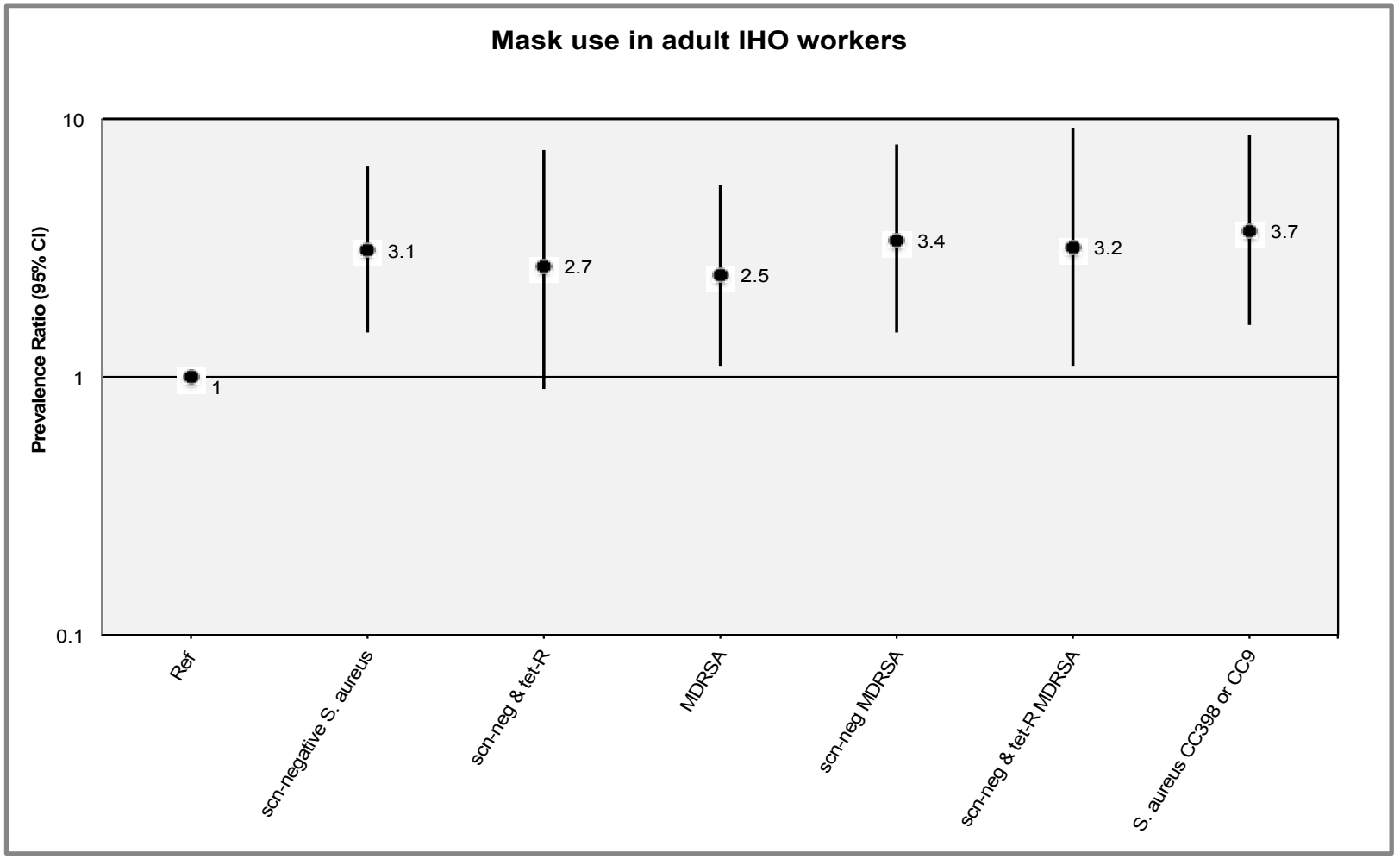


Figure 3.2. Prevalence (%) of mask use at work for *S. aureus* related outcomes among adult industrial hog operation (IHO) workers.

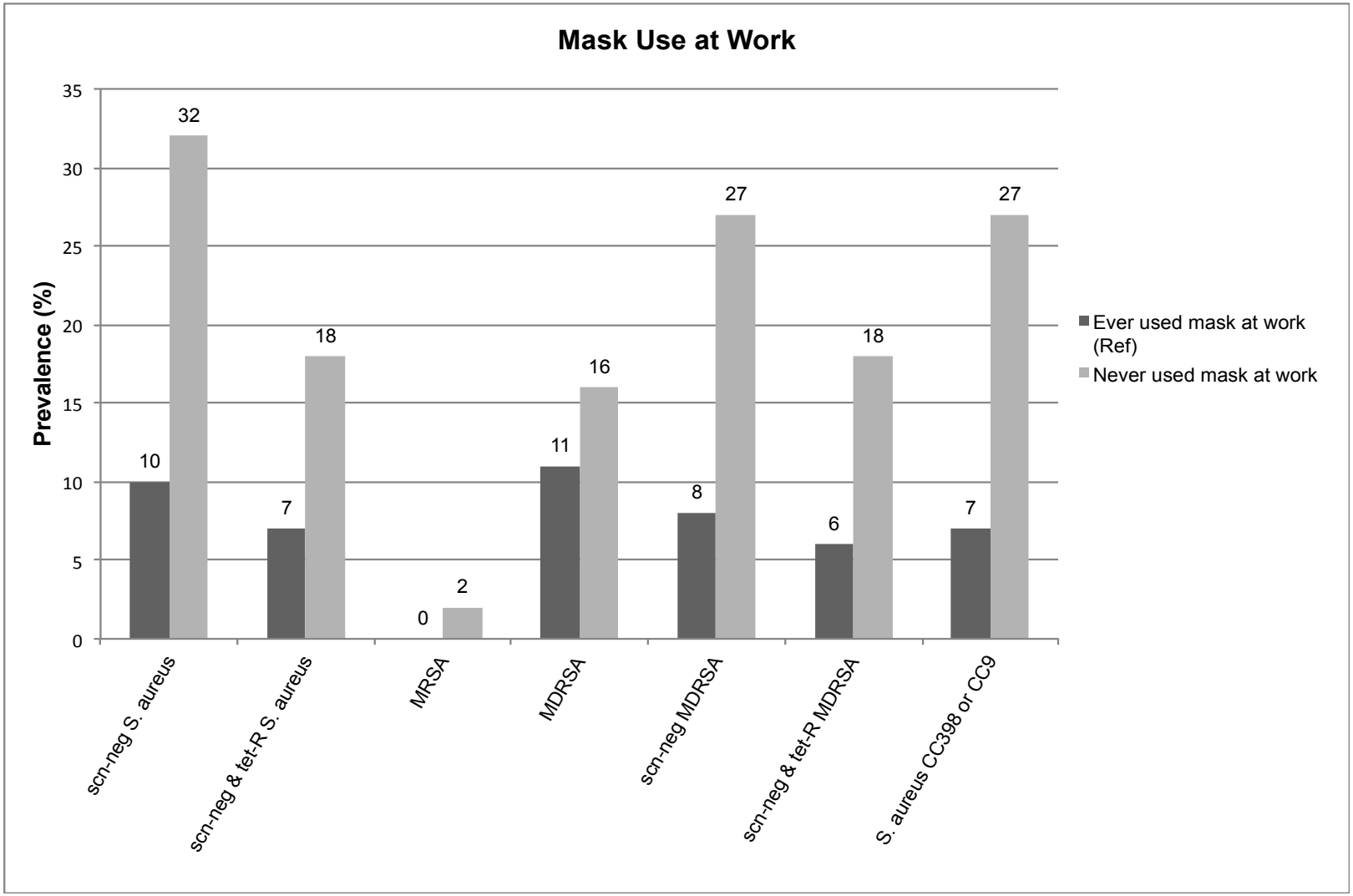
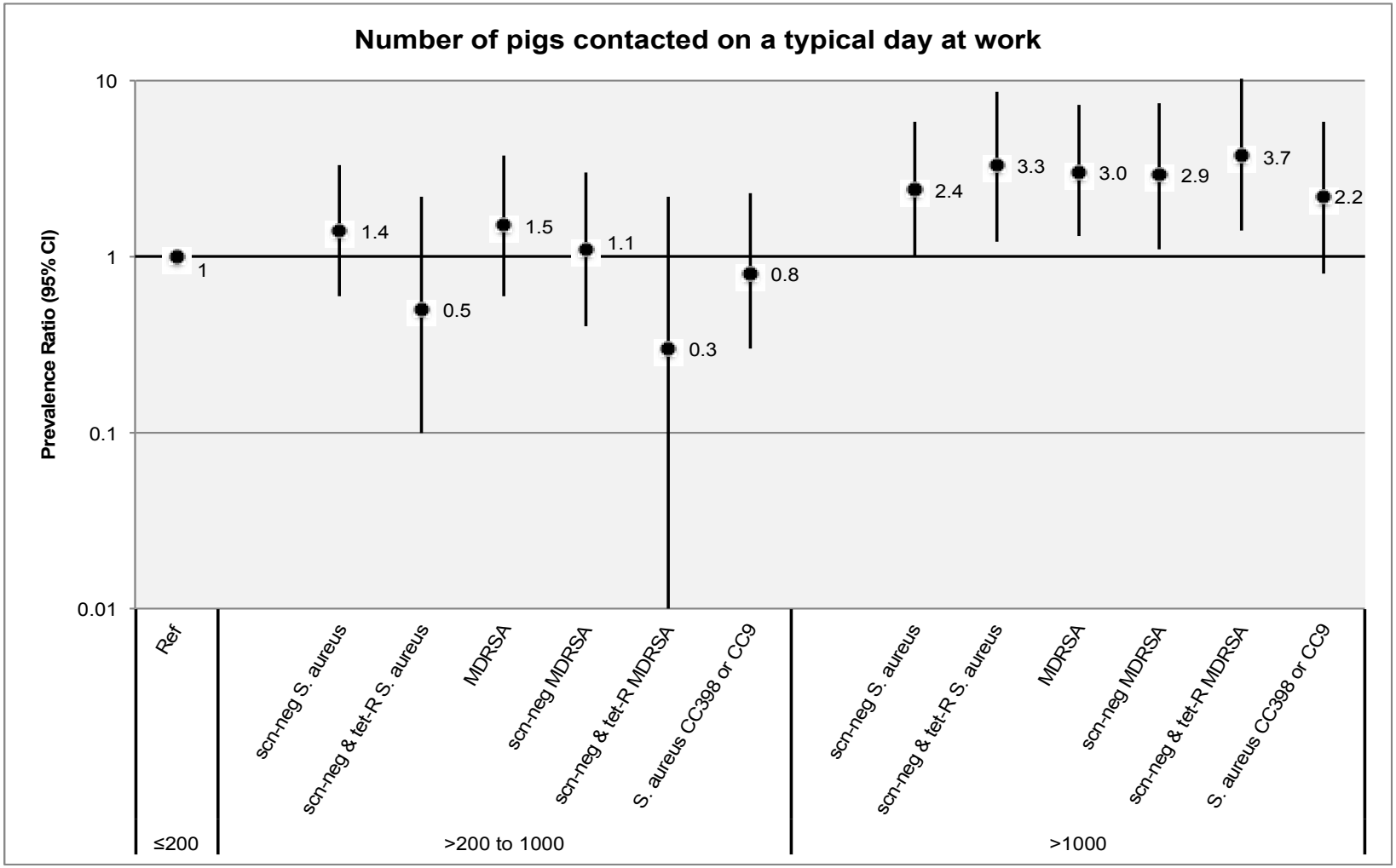


Figure 3.3. Plot of prevalence ratios (PR) and 95% confidence intervals (95% CI) for *S. aureus* related outcomes[1,2] among adult industrial hog operation (IHO) workers, stratified by reported number of pigs contacted on a typical day at work.



[1] Lower 95% CI for *scn*-neg and tet-R MDRSA is zero (0).

[2] MRSA statistics not computed due to zero cases in the referent and >1000 group.

Figure 3.4. Prevalence (%) of *S. aureus*-related outcomes among adult industrial hog operation (IHO) workers, stratified by reported number of pigs contacted on a typical day at work.

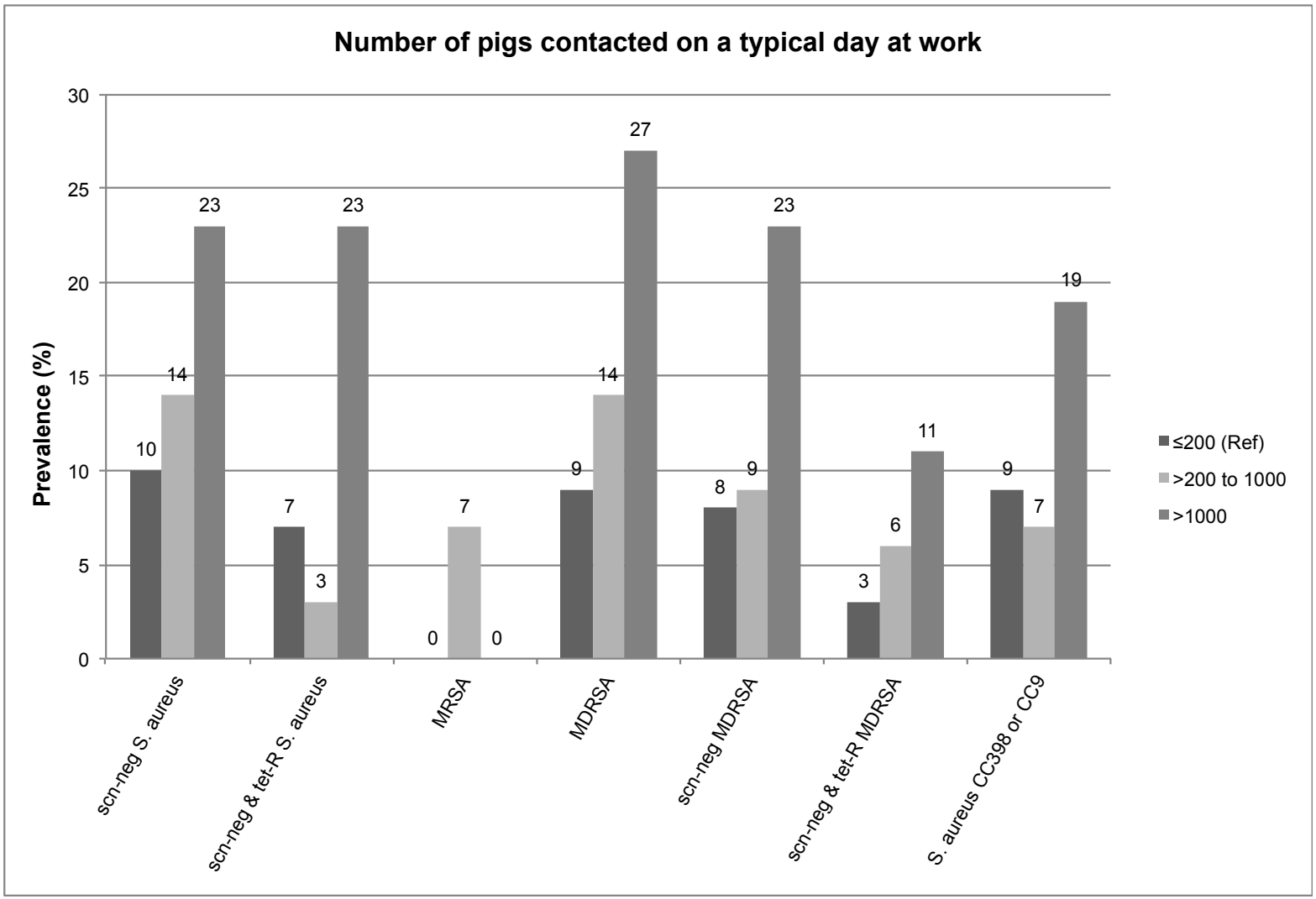


Figure 3.5. Prevalence (%) of MRSA carriage among children living in the same household as an adult industrial hog operation (IHO) worker, stratified by work activity of the adult IHO worker.

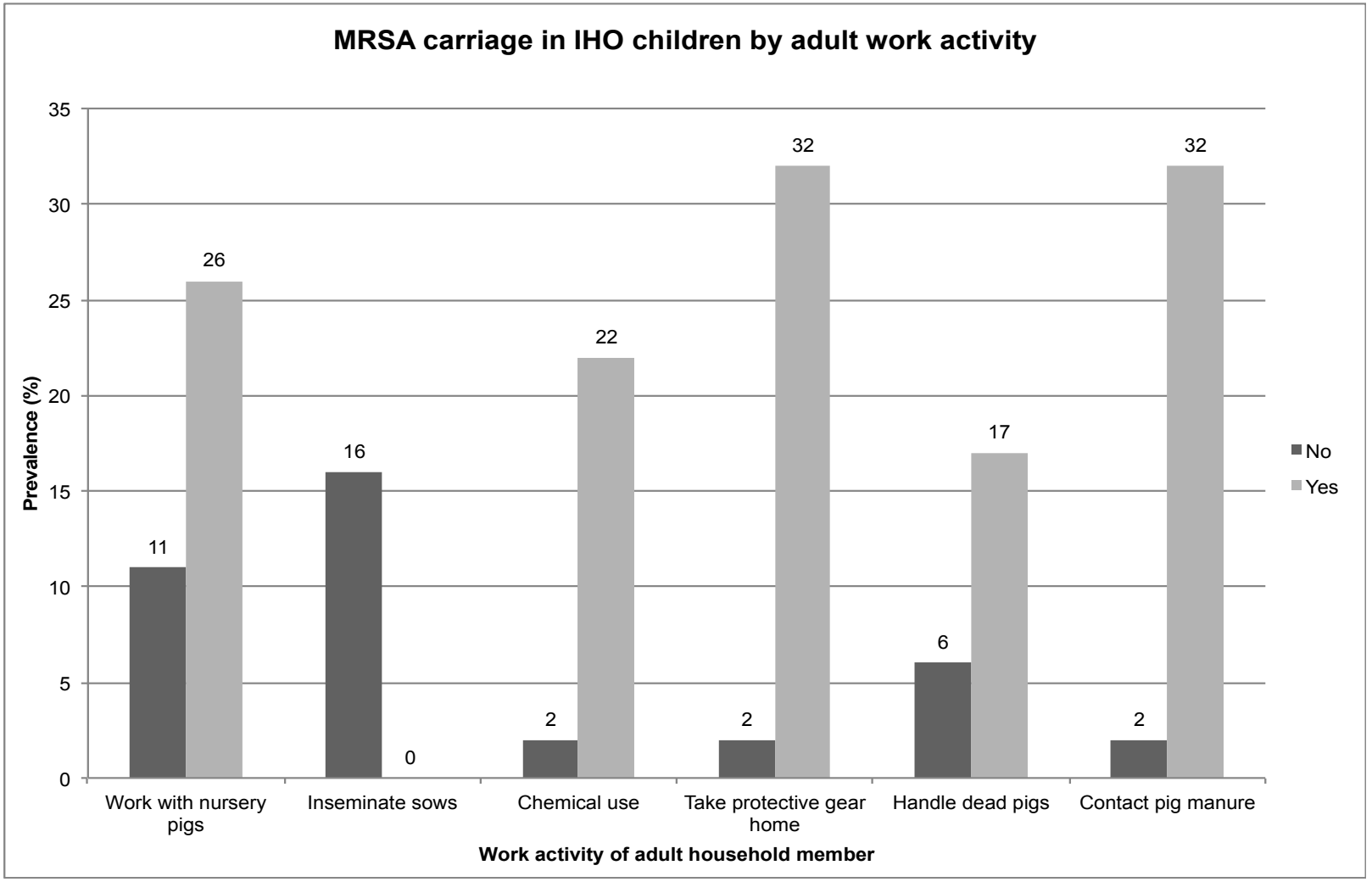
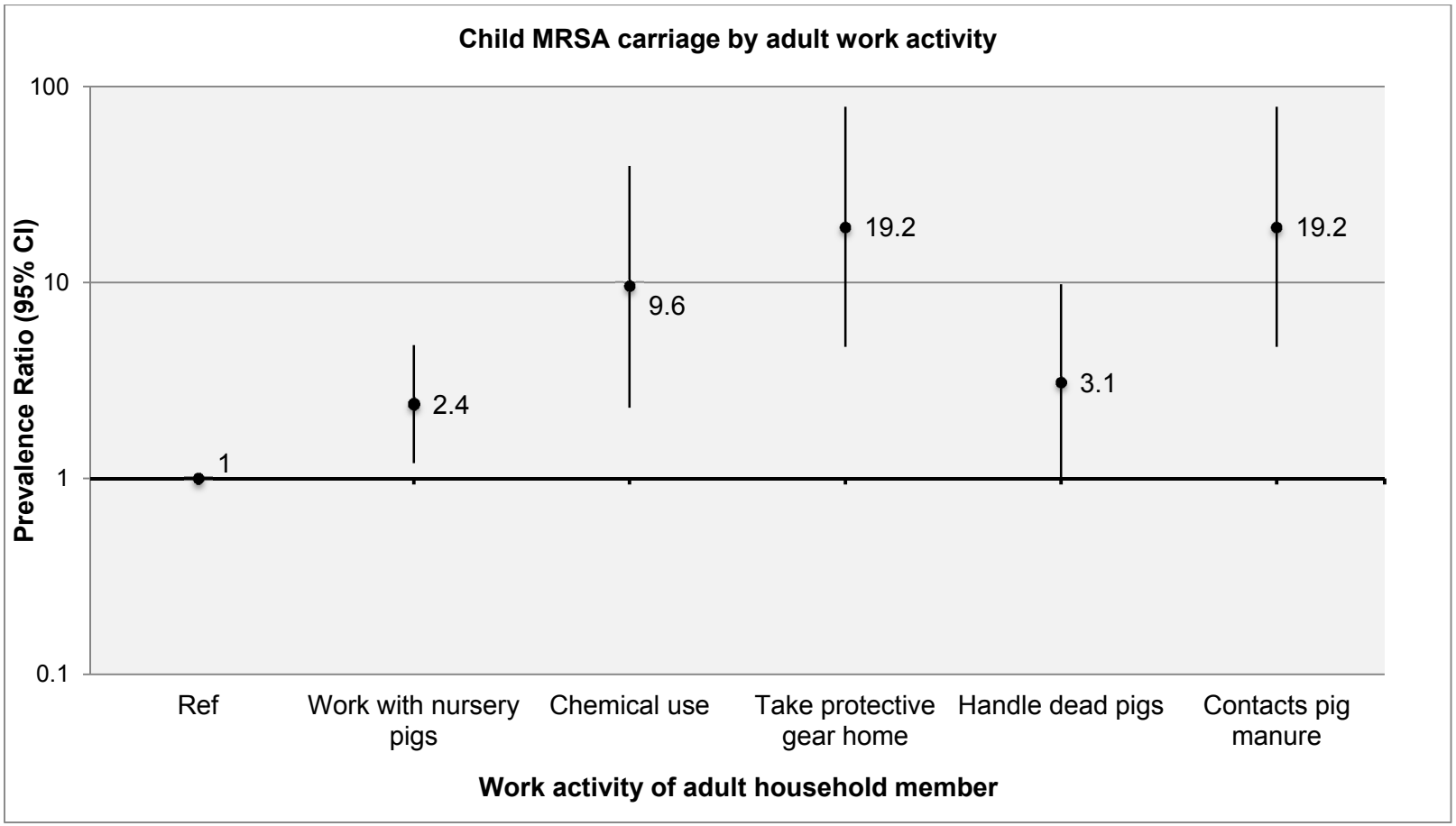


Figure 3.6. Plot of prevalence ratios (PR) and 95% confidence intervals (95% CI) of MRSA carriage among children, stratified by work activity of their adult IHO household member.



CONCLUSIONS

The goal of this research was to advance the understanding of the environmental presence of and potential occupational and community exposure to antibiotic-resistant *S. aureus* originating from IHOs. To achieve this goal, we investigated the presence of antibiotic-resistant *S. aureus* strains characteristic of IHO sources in surface water, IHO workers and their child household members, and in non-occupationally exposed community members in eastern North Carolina – one of the densest regions of hog production in the United States. Our findings demonstrate that there exist potential environmental, occupational, and community exposures to antibiotic resistant *S. aureus* in our study area.

In Chapter Two, we sought to better understand presence of these antibiotic-resistant *S. aureus* of livestock origin from IHOs in the environment by investigating the presence of MRSA in surface waters adjacent to IHO spray fields. This rationale for this study was influenced in part by the growing scientific evidence that IHOs may serve as a reservoir for antibiotic-resistant *S. aureus*, the paucity of empirical evidence for the presence of these strains of *S. aureus* in the United States at the time, and growing evidence that agricultural non-point sources were affecting water quality in eastern NC [154, 156, 223]. Despite significant methodological challenges, this study demonstrated that *S. aureus*, including methicillin- and multidrug-resistant *S. aureus* is sometimes present in surface waters adjacent to IHO spray fields in eastern NC. The genotypes of these isolates are not among the most common CCs reported in European studies, but some markers of livestock association – specifically, the lack of *scn* – were observed in a some isolates.

Our data suggest that ABR *S. aureus* is present in surface water adjacent to IHO spray fields. Although others have reported the presence of ABR *S. aureus* in the environment surrounding IHOs [140, 219, 220], and in other areas dominated by agricultural land cover [159]. We are not able to identify the source of the waterborne ABR *S. aureus* with any certainty, nor do we know the mechanism for dispersal of these bacteria into surface waters. Others who reported the presence of *S. aureus* in surface water have attributed its presence to humans via beachgoer shedding [162, 163, 165]. However, none of the surface waters included in our study are used for human recreation. Additional potential sources of *S. aureus* exist in our study area. The sources include industrial hog operations, as well as industrial poultry operations, private septic systems, wildlife, domestic pets, and others. We also detected *scn*-negative *S. aureus* in surface water, which is indicative of a non-human source but does not necessarily indicate source in the absence of validated source-specific genetic markers. Therefore, we are unable to conclude with certainty the source of our waterborne *S. aureus* isolates. We are also unable to comment on the presence and characteristics of waterborne *S. aureus* in non-IHO impacted surface waters. This research demonstrates that ABR *S. aureus* can be detected in surface waters in land covers dominated by industrial animal agriculture and provides important insight for future research efforts.

In particular, the methodological challenges described in Chapter Two demonstrate that future studies designed to investigate environmental presence of antibiotic resistant bacteria near IHOs – especially water-based studies – should consider evaluating more common environmental targets for which laboratory methods have been optimized. For example, we experienced difficulty isolating *S. aureus* from an environmental matrix using a media developed for clinical use, but optimized environmental sampling methods exist for fecal indicator bacteria like *E. coli*, which are also present in IHOs. It may be more appropriate to evaluate antibiotic resistance in the environment using *E. coli* as the bacterium of interest, rather than *S. aureus*. Alternatively, it may be useful to use a combination of the methods described in Chapter Two

with an enrichment approach. While this would eliminate the possibility of quantitative data, it may reduce the number of false positives collected from environmental samples.

In Chapters Three and Four, we investigated occupational, household, and community exposures to ABR *S. aureus* with markers of livestock association. A number of European studies have demonstrated occupational exposure to ABR *S. aureus* in IHO workers [72, 89, 175], but it has become evident that the exposures experienced by workers and communities in the United States differ [70]. The studies described in Chapters Three and Four therefore contribute to the existing body of evidence regarding adult occupational exposure in the United States and additionally address unique research questions related to household member and community exposures that have not been adequately explored in the literature.

Our data suggest that industrial hog operation workers may be more exposed to ABR *S. aureus* and *S. aureus* that are genetically and phenotypically characteristic of livestock sources. Industrial hog operation workers appeared to have a greater crude prevalence of multidrug-resistant *S. aureus* carriage than non-occupationally exposed adults living in the same community, but this association did not reach statistical significance. In addition, IHO workers appeared to have a greater prevalence of carriage of *S. aureus* with one or more markers of livestock association. This result is consistent with the results of similar research conducted in North Carolina and provides evidence that IHOs are a source of antibiotic-resistant *S. aureus* that is genetically distinct from common community- or hospital-associated strains of *S. aureus* and that these bacteria can be exchanged between workers and pigs or the IHO environment. Previous studies have documented this trend by comparing industrial livestock workers to individuals who worked on antibiotic-free livestock farms [84]; however, the latter group did not reside within areas of high IHO density. It is important to note that, for *S. aureus* with one or markers of livestock association, sample size was limited and adjusted estimates are unavailable for many of the livestock-associated outcomes. In addition, among those outcomes for which an adjusted prevalence ratio was produced, the resulting PR was always closer to the

null value. We were also unable to adjust for race as a confounder, which prevents us from concluding with certainty that observed differences are due solely to differences in occupation. Therefore, these results should be interpreted with caution. Nonetheless, this is the first study in North Carolina to compare carriage in non-slaughterhouse, IHO workers to non-occupationally exposed individuals living in the same community.

Children under seven years old living in the same household as IHO workers also appear to experience exposures that lead to a greater carriage prevalence of MRSA and MDRSA carriage than children living in households with individuals who do not work in livestock production. While children are more susceptible to carriage and infection than adults, children in the IHO group had a much higher MRSA carriage prevalence than previously reported estimates of MRSA prevalence in children in the community [200, 208, 209]. Since we observed a MRSA carriage prevalence in IHO adults that was similar to that observed in the United States adult population [1], unmeasured exposures may be contributing to the observed elevated prevalence in children. These could be unrelated to the occupation of the adult worker, but they may also include IHO-related exposures such as contamination of clothing or other body sites with MRSA or other antibiotic-resistant bacteria, antibiotic-resistance genes, or antibiotic residues. These results both demonstrate potential risks to children's health and a need for future studies that better characterize these unmeasured exposures. Sampling may include the collection of additional samples from workers or the household environment.

This work also demonstrates that non-occupational exposures to *S. aureus* characteristic of the IHO environment exist in communities where IHOs are numerous. This finding suggests that these strains either naturally circulate at low levels in the community or that community members are exposed via environmental contamination. This finding represents a significant contribution to the current literature which suggests that exposure to strains that are characteristic of livestock-adapted *S. aureus* is largely limited to livestock workers and their familial (household) contacts [111]. Furthermore, it provides an argument for future research to

better characterize community and environmental exposures via geospatial analyses and additional environmental sampling. These analyses were beyond the scope of this work; however, others will conduct geospatial analyses with this data to investigate associations between residential proximity to IHOs and the *S. aureus*-related nasal carriage outcomes described here. Investigating potential environmental exposures via geospatial analysis is also important because our results suggest that within-household strain concordance is uncommon for *S. aureus* with markers of livestock association.

Providing adequate protective gear such as masks and enforcing use of masks may decrease workers' exposure to *S. aureus* from the IHO environment. Our data suggest that IHO workers who reported ever wearing a mask at work had a lower prevalence of MDRSA and *S. aureus* with one or more markers of livestock association. However, our sample size was limited and we were unable to directly observe the type of mask and proper use of masks. This finding suggests that mask use or other work-related activities that are associated with the type of mask and frequency of mask use may be worth further investigation. In addition, the observed association between bringing protective gear home and child MRSA carriage supports the theory that investigating unmeasured IHO-related household exposures may be an important component of future studies. It is worth noting that we did not observe an association between most work activity related exposures and carriage outcomes in adults and children. Our lack of samples from pigs and the IHO environment where our participants are employed limits our ability to draw strong conclusions from these associations since it is possible that IHO herd positivity may be driving or attenuating the observed associations, given that others have demonstrated that herd positivity is a predictor of worker positivity [86, 89, 100, 117].

Our study design prevents us from examining whether or not individuals who were positive for a given *S. aureus*-related carriage outcome are persistently colonized by *S. aureus* or if carriage is representative of contamination due to exposure to a source of *S. aureus*; persistent carriage has also been associated with a greater risk of infection compared to

intermittent or non-carriage [224]. In addition, it is possible that the lack of association observed between most work activities and carriage outcomes in adults is related to our examination of binary rather than continuous (e.g., *S. aureus* colony forming units (CFU)/swab) outcomes. However, associations observed between work activities and *S. aureus* CFU/swab would be difficult to interpret due to our study design and our inability to control for persistent versus intermittent carriage. Persistent carriers of *S. aureus* carry a greater number of CFUs per sample than intermittent carriers [225]; therefore, it would be inappropriate to draw conclusions from any observed associations in a cross-sectional study.

A common limitation to all of the results reported here is the lack of access to on-farm samples. This limitation extends to all of the research on this topic that has been conducted in North Carolina to date and others have commented on the challenges presented by our inability to widely and comprehensively collect and characterize samples from the on-farm environment [70]. While we are able to interpret our results within the context of scientific studies conducted outside of the United States and the few studies that report results from on-farm samples within the United States, there is a great need to characterize *S. aureus* circulating in United States livestock. In addition, our lack of access to industry data requires that we recruit and enroll participants using a snowball sampling approach, which limits the generalizability of our findings. Moving forward, priority should be placed on collecting samples from hogs and other industrial livestock as well as the on-farm environment in order to strengthen our ability to better identify the source of ABR circulating in IHO workers and their household members, the community, and the environment.

This research contributes to a growing body of scientific literature regarding the potential public and environmental health impacts of antibiotic use in industrial animal agriculture. In summary, the results of this research suggest that human-to-human exchange between IHO workers and their household members is not the only route through which ABR *S. aureus* from the IHO environment can be disseminated to the community. We reported evidence that

antibiotic resistant *S. aureus* with some genetic characteristics of the IHO environment can be detected in surface waters near IHO spray fields although the source of these bacteria is unknown.. In addition, it appears that individuals lacking occupational exposure also carry these strains of *S. aureus*, suggesting that community or environmental exposures may exist in IHO-dense regions of North Carolina. Future research efforts should focus on better characterizing household exposures among households where IHO workers reside, especially for young children. In addition, our data suggest that further evaluation of environmental exposures is warranted in communities where IHOs are densely sited.

APPENDIX 1: ANTIBIOTIC CONCENTRATIONS

List of antibiotic concentrations used in antibiotic susceptibility testing

Antibiotic class	Antibiotic	Concentration
Aminoglycosides	Gentamicin	10 µg
β-lactams	Ampicillin	10 µg
	Penicillin	10 units
	Oxacillin	1 µg
Cephalosporins	Ceftriaxone	30 µg
Floroquinolones	Ciprofloxacin	5 µg
	Gatifloxacin	5 µg
	Levofloxacin	5 µg
Nitrofuran[1]	Nitrofurantoin	30 µg
Glycopeptides[2]	Vancomycin	Teicoplanin 5 µg/mL
Lincosamides	Clindamycin	2 µg
Macrolides	Erythromycin	15 µg
Oxazolidones	Linezolid	30 µg
Rifamycin	Rifampin	5 µg
Streptogramins	Quinupristin/dalfopristin	15 µg
Sulfanomide/methroprim	Sulfamethoxazole/trimethroprim	23.75/1.25 µg
Tetracycline	Tetracycline	30 µg

[1] Chapters 3 and 4 only.

[2] Chapter 2 only.

APPENDIX 2: GENDER-STRATIFIED CHAPTER 3 RESULTS

Crude prevalence ratios (PR) and 95% confidence intervals (95% CI) comparing IHO to CR participants, stratified by gender.

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
S. aureus						
IHO	104/198	53	1.7 (1.3, 2.2)	97/198	49	1.6 (1.2, 2.1)
Male	69/107	65	1.6 (1.1, 2.3)	70/123	57	1.4 (1.1, 2.0)
Female	35/91	39	1.3 (0.9, 1.9)	27/75	36	1.5 (1.0, 2.3)
CR	63/202	31	Ref	62/202	31	Ref
Male	17/41	42	Ref	35/90	39	Ref
Female	46/161	29	Ref	27/112	24	Ref
MRSA						
IHO	4/198	2	0.6 (0.2, 2.0)	27/198	14	2.5 (1.3, 4.9)
Male	2/107	2	-	25/123	20	1.8 (0.9, 3.6)
Female	2/91	2	0.5 (0.1, 2.4)	2/75	3	3.0 (0.3, 32.4)
CR	7/202	4	Ref	11/202	6	Ref
Male	0/41	0	Ref	10/90	11	Ref
Female	7/161	4	Ref	1/112	1	Ref
MDRSA						
IHO	25/198	13	1.5 (0.8, 2.7)	45/198	23	2.7 (1.6, 4.6)
Male	16/107	15	3.1 (0.7, 12.7)	33/123	16	2.2 (1.2, 4.1)
Female	9/91	10	1.1 (0.5, 2.3)	12/75	16	3.0 (1.2, 7.6)
CR	17/202	8	Ref	17/202	8	Ref
Male	2/41	5	Ref	11/90	12	Ref
Female	15/161	9	Ref	6/112	5	Ref
***scn*-negative**						
S. aureus						
IHO	25/198	13	5.1 (2.0, 13.1)	7/198	4	1.8 (0.5, 6.0)
Male	13/107	12	5.0 (0.7, 36.9)	4/123	2	-

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
Female	12/91	13	5.3 (1.8, 16.0)	3/75	4	1.1 (0.3, 4.9)
CR	5/202	3	Ref	4/202	2	Ref
Male	1/41	2	Ref	0/90	0	Ref
Female	4/161	3	Ref	4/112	4	Ref
tet-resistant						
S. aureus						
IHO	18/198	9	3.7 (1.4, 9.7)	4/198	2	1.4 (0.3, 6.0)
Male	10/107	9	1.9 (0.4, 8.4)	1/123	1	0.7 (0.0, 11.5)
Female	8/91	9	4.7 (1.3, 17.3)	3/75	4	2.2 (0.4, 13.1)
CR	5/202	3	Ref	3/202	2	Ref
Male	2/41	5	Ref	1/90	1	Ref
Female	3/161	2	Ref	2/112	2	Ref

APPENDIX 3: RACE-STRATIFIED CHAPTER 3 RESULTS

Crude prevalence ratios (PR) and 95% confidence intervals (95% CI) comparing IHO to CR participants, stratified by race.

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
S. aureus						
IHO	104/198	53	1.7 (1.3, 2.2)	97/198	49	1.6 (1.2, 2.1)
White	0/0	-	-	0/0	-	-
Black	3/12	25	1.1 (0.4, 3.1)	3/8	27	1.2 (0.4, 3.4)
Hispanic	101/185	54.6	1.2 (0.9, 1.7)	93/198	50	1.1 (0.8, 1.5)
Multi-racial	0/1	0	-	1/1	100	-
CR	63/202	31	Ref	62/202	31	Ref
White	5/8	63	Ref	2/3	67	Ref
Black	28/125	20	Ref	27/122	22	Ref
Hispanic	29/66	44	Ref	30/65	46	Ref
Multi-racial	1/3	33	Ref	3/12	25	Ref
MRSA						
IHO	4/198	2	0.6 (0.2, 2.0)	27/198	13.6	2.5 (1.3, 4.9)
White	0/0	-	-	0/0	-	-
Black	1/12	8.3	1.7 (0.2, 13.3)	1/11	9.1	2.8 (0.3, 22.7)
Hispanic	3/185	1.6	-	26/186	14	1.3 (0.6, 2.8)
Multi-racial	0/1	0	-	0/1	0	-
CR	7/202	3.5	Ref	11/202	5.5	Ref
White	1/8	12.5	Ref	0/3	0	Ref
Black	6/125	4.8	Ref	4/122	3.3	Ref
Hispanic	0/66	0	Ref	7/58	10.8	Ref
Multi-racial	0/3	0	Ref	0/12	0	Ref
MDRSA						
IHO	25/198	12.6	1.5 (0.8, 2.7)	45/198	22.7	2.7 (1.6, 4.6)

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
White	0/0	0	-	0/0	0	-
Black	2/12	16.7	2.1 (0.5, 8.4)	2/11	18.2	3.2 (0.7, 13.4)
Hispanic	23/185	12.4	1.6 (0.7, 4.1)	42/186	22.6	1.5 (0.8, 2.8)
Multi-racial	0/1	0	-	1/1	100	-
CR	17/202	8.4	Ref	17/202	8.4	Ref
White	2/8	25	Ref	0/3	0	Ref
Black	10/125	8	Ref	7/122	5.7	Ref
Hispanic	5/66	7.6	Ref	10/65	15.4	Ref
Multi-racial	0/3	0	Ref	0/12	0	Ref
***scn*-negative**						
S. aureus						
IHO	25/198	12.6	5.1 (2.0, 13.1)	7/198	3.5	1.8 (0.5, 6.0)
White	0/0	0	-	0/0	0	-
Black	1/12	8.3	10.4 (0.7, 156.2)	0/11	0	-
Hispanic	24/185	13	2.1 (0.8, 5.9)	7/186	3.8	2.4 (0.3, 19.5)
Multi-racial	0/1	0	-	0/1	0	-
CR	5/202	2.5	Ref	4/202	2	Ref
White	0/8	0	Ref	0/3	0	Ref
Black	1/125	0.8	Ref	3/122	2.5	Ref
Hispanic	4/66	6.1	Ref	1/65	1.5	Ref
Multi-racial	0/3	0	Ref	0/12	0	Ref
tet-resistant						
S. aureus						
IHO	18/198	9.1	3.7 (1.4, 9.7)	4/198	2	1.4 (0.3, 6.0)
White	0/0	0	-	0/0	0	-
Black	1/12	8.3	10.4 (0.7, 156.2)	0/11	0	-
Hispanic	17/185	9.2	1.5 (0.5, 4.3)	4/186	2.2	0.7 (0.1, 3.7)

Carriage outcome	Adults			Children		
	No. Pos/Total	%	PR (95% CI)	No. Pos/Total	%	PR (95% CI)
Multi-racial	0/1	0	-	0/1	0	-
CR	5/202	2.5	Ref	3/202	1.5	Ref
White	0/8	0	Ref	0/3	0	Ref
Black	1/125	0.8	Ref	1/122	0.8	Ref
Hispanic	4/66	6.1	Ref	2/65	3.1	Ref
Multi-racial	0/3	0	Ref	0/12	0	Ref

APPENDIX 4: CHAPTER 4 RESULTS FOR ALL ACTIVITES, ADULTS

Prevalence of all *S. aureus* carriage outcomes by work activity among adult IHO workers.

APPENDIX 4, Table 1a. Prevalence of *S. aureus*, *scn*-negative *S. aureus*, and *scn*-negative and tetracycline-resistant *S. aureus* by work activity among adult IHO workers.

Work Activity	*S. aureus*		*scn*-neg *S. aureus*		*scn*-neg, tet-R *S. aureus*	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage[1]						
Nursery						
Yes	24/39 (62)	1.2 (0.9, 1.6)	3/39 (8)	0.6 (0.2, 1.8)	3/39 (8)	0.9 (0.3, 3.1)
No	80/159 (50)	Ref	22/159 (14)	Ref	13/159 (8)	Ref
Finishing						
Yes	22/36 (61)	1.2 (0.9, 1.6)	2/36 (6)	0.4 (0.1, 1.6)	2/36 (6)	0.6 (0.2, 2.7)
No	82/162 (51)	Ref	23/162 (14)	Ref	14/162 (9)	Ref
Wean, farrow, or feeder						
Yes	44/94 (47)	0.8 (0.6, 1.1)	15/94 (16)	1.7 (0.8, 3.5)	7/94 (8)	0.9 (0.3, 2.2)
No	60/104 (58)	Ref	10/104 (10)	Ref	9/104 (9)	Ref
Sow or Boar						
Yes	16/31 (52)	1.0 (0.7, 1.4)	5/31 (16)	1.3 (0.5, 3.3)	4/31 (13)	1.8 (0.6, 5.2)
No	88/167 (53)	Ref	20/167 (12)	Ref	12/167 (7)	Ref
Interaction with pigs and medication use						
Administer shots						
Yes	70/140 (50)	0.9 (0.6, 1.1)	22/140 (16)	3.0 (0.9, 9.8)	14/140 (10)	2.9 (0.7, 12.4)
No	34/58 (59)	Ref	3/58 (5)	Ref	2/58 (4)	Ref
Inseminate sows						
Yes	13/26 (50)	0.9 (0.6, 1.4)	4/26 (15)	1.2 (0.5, 3.4)	2/26 (8)	0.9 (0.2, 3.9)

Work Activity	*S. aureus*		*scn*-neg *S. aureus*		*scn*-neg, tet-R *S. aureus*	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
No	91/172 (53)	Ref	21/172 (12)	Ref	14/172 (8)	Ref
Administer antibiotics						
Yes	55/123 (45)	0.7 (0.5, 0.8)	17/123 (14)	1.2 (0.6, 2.9)	11/123 (9)	1.3 (0.5, 3.7)
No	49/75 (65)	Ref	8/75 (11)	Ref	5/75 (7)	Ref
Cleaning activities						
Chemical use						
Yes	70/112 (63)	1.6 (1.2, 2.1)	13/112 (12)	0.8 (0.4, 1.7)	10/112 (9)	1.3 (0.5, 3.4)
No	34/86 (40)	Ref	12/86 (14)	Ref	6/86 (7)	Ref
Performed any cleaning activities						
Yes	89/147 (61)	2.1 (1.3, 3.2)	19/147 (13)	1.1 (0.5, 2.6)	12/147 (8)	1.0 (0.4, 3.1)
No	15/51 (29)	Ref	6/51 (12)	Ref	4/51 (8)	Ref
Pressure washer use						
Yes	30/67 (45)	0.8 (0.6, 1.1)	10/67 (15)	1.3 (0.6, 2.7)	6/67 (9)	1.2 (0.4, 3.1)
No	74/131 (56)	Ref	15/131 (12)	Ref	10/131 (8)	Ref
Frequency of mask use						
Ever use	12/22 (55)	1.0 (0.7, 1.6)	18/176 (10)	Ref	12/176 (7)	Ref
Never	92/176 (53)	Ref	7/22 (32)	3.1 (1.5, 6.6)	4/22 (18)	2.7 (0.9, 7.6)
Take protective gear home						
Yes	57/78 (73)	1.9 (1.4, 2.4)	9/78 (12)	0.9 (0.4, 1.9)	7/78 (9)	1.2 (0.5, 3.1)
No	47/120 (39)	Ref	16/120 (13)	Ref	9/120 (8)	Ref
Amount of direct contact with pigs						
Hours/day in direct contact						
>8	11/25 (44)	0.8 (0.5, 1.3)	5/25 (20%)	1.7 (0.7, 4.2)	1/25 (4)	0.5 (0.1, 3.3)

Work Activity	*S. aureus*		*scn*-neg *S. aureus*		*scn*-neg, tet-R *S. aureus*	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
≤8	93/173 (54)	Ref	20/173 (12)	Ref	15/173 (9)	Ref
Total pigs contacted per day						
≤200	67/113 (59)	Ref	11/113 (10)	Ref	8/113 (7)	Ref
>200 to 1000	24/59 (41)	0.7 (0.5, 1.0)	8/59 (14)	1.4 (0.6, 3.3)	2/59 (3)	0.5 (0.1, 2.2)
>1000	13/26 (50)	0.8 (0.6, 1.3)	6/26 (23)	2.4 (1.0, 5.8)	6/26 (23)	3.3 (1.2, 8.6)
Size of IHO[2]						
≥250 to 1000	10/36 (28)	Ref	4/36 (11)	Ref	2/36 (6)	Ref
>1000 to 4000	39/80 (49)	1.8 (1.0, 3.1)	11/80 (14)	1.2 (0.4, 3.6)	6/80 (8)	1.4 (0.3, 6.4)
≥4000	52/74 (70)	2.5 (1.5, 4.4)	9/74 (12)	1.1 (0.4, 3.3)	8/74 (11%)	1.9 (0.4, 8.7)
Other work activities						
Handle dead pigs						
Yes	86/143 (60)	1.8 (1.2, 2.7)	20/143 (14)	1.5 (0.6, 3.9)	13/143 (9)	1.7 (0.5, 5.6)
No	18/55 (33)	Ref	5/55 (9)	Ref	3/55 (6)	Ref
Contact pig manure						
Yes	56/78 (72)	1.8 (1.4, 2.3)	9/78 (12)	0.9 (0.4, 1.9)	7/78 (9)	1.2 (0.5, 3.1)
No	48/120 (40)	Ref	16/120 (13)	Ref	9/120 (8)	Ref
Other employment characteristics						
Hours worked per day						
>8 h	21/43 (49)	0.9 (0.6, 1.3)	5/43 (12)	0.9 (0.4, 2.3)	1/43 (2)	0.2 (0.0, 1.8)
≤8 h	83/155 (54)	Ref	20/155 (13)	Ref	15/155 (10)	Ref
Years worked at current IHO						
> 5	28/52 (54)	1.0 (0.8, 1.4)	12/52 (23)	2.6 (1.3, 5.3)	8/52 (15)	2.8 (1.1, 7.1)

Work Activity	*S. aureus*		*scn*-neg *S. aureus*		*scn*-neg, tet-R *S. aureus*	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
≤5	76/146 (52)	Ref	13/146 (9)	Ref	8/146 (6)	Ref
Years worked at any IHO						
>5	34/63 (54)	1.0 (0.8, 1.4)	16/63 (25)	3.8 (1.8, 8.1)	9/63 (14)	2.8 (1.1, 7.1)
≤ 5	70/135 (52)	Ref	9/135 (7)	Ref	7/135 (5)	Ref
Time since last work shift						
≤3 h	37/81 (46)	0.7 (0.5, 0.9)	11/81 (13)	1.7 (0.6, 5.0)	6/81 (7)	1.9 (0.4, 8.8)
>3 to 12 h	32/67 (48)	0.7 (0.5, 0.9)	10/67 (15)	1.9 (0.6, 5.6)	8/67 (12)	3.0 (0.7, 13.5)
>12 h	35/50 (70)	Ref	4/50 (8)	Ref	2/50 (4)	Ref

APPENDIX 4, Table 1b. Prevalence of MDRSA, *scn*-negative MDRSA, and *scn*-negative and tetracycline-resistant MDRSA by work activity among adult IHO workers.

Work Activity	MDRSA		*scn*-neg MDRSA		*scn*-neg, tet-R MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage[1]						
Nursery						
Yes	4/39 (10)	0.8 (0.3, 2.1)	3/39 (8)	0.7 (0.2, 2.3)	3/39 (8)	1.1 (0.3, 3.8)
No	21/159 (13)	Ref	17/159 (11)	Ref	11/159 (7)	Ref
Finishing						
Yes	4/36 (11)	0.9 (0.3, 2.3)	2/36 (6)	0.5 (0.1, 2.1)	2/36 (6)	0.8 (0.2, 3.2)
No	21/162 (13)	Ref	18/162 (11)	Ref	12/162 (7)	Ref
Wean, farrow, or feeder						
Yes	12/94 (13)	1.0 (0.5, 2.1)	10/94 (11)	1.1 (0.5, 2.5)	5/94 (5)	0.6 (0.2, 1.8)
No	13/104 (13)	Ref	10/104 (10)	Ref	9/104 (9)	Ref
Sow or Boar						
Yes	5/31 (16)	1.3 (0.5, 3.3)	5/31 (16)	1.8 (0.7, 4.6)	4/31 (13)	2.2 (0.7, 6.4)
No	20/167 (12)	Ref	15/167 (9)	Ref	10/167 (6)	Ref
Interaction with pigs and medication use						
Administer shots						
Yes	20/140 (14)	1.7 (0.7, 4.2)	17/140 (12)	2.3 (0.7, 7.7)	12/140 (9)	2.5 (0.6, 10.8)
No	5/58 (9)	Ref	3/58 (5)	Ref	2/58 (4)	Ref
Inseminate sows						
Yes	3/26 (12)	0.9 (0.3, 2.8)	3/26 (12)	1.2 (0.4, 3.7)	2/26 (8)	1.1 (0.3, 4.6)
No	22/172 (13)	Ref	17/172 (10)	Ref	12/172 (7)	Ref
Administer antibiotics						
Yes	17/123 (14)	1.3 (0.6, 2.9)	13/123 (11)	1.1 (0.5, 2.7)	9/123 (7)	1.1 (0.4, 3.2)
No	8/75 (11)	Ref	7/75 (9)	Ref	5/75 (7)	Ref
Cleaning activities						
Chemical use						
Yes	11/112 (10)	0.6 (0.3, 1.3)	10/112 (9)	0.8 (0.3, 1.8)	8/112 (7)	1.0 (0.4, 2.8)

Work Activity	MDRSA		*scn*-neg MDRSA		*scn*-neg, tet-R MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
No	14/86 (16)	Ref	10/86 (12)	Ref	6/86 (7)	Ref
Performed any cleaning activities						
Yes	20/147 (14)	1.4 (0.5, 3.5)	16/147 (11)	1.4 (0.5, 4.0)	10/147 (7)	0.9 (0.3, 2.6)
No	5/51 (10)	Ref	4/51 (8)	Ref	4/51 (8)	Ref
Pressure washer use						
Yes	11/67 (16)	1.5 (0.7, 3.2)	8/67 (12)	1.3 (0.6, 3.0)	4/67 (6)	0.8 (0.3, 2.4)
No	14/131 (11)	Ref	12/131 (9)	Ref	10/131 (8)	Ref
Frequency of mask use						
Ever use	19/176 (11)	Ref	14/176 (8)	Ref	10/176 (6)	Ref
Never	6/22 (27)	2.5 (1.1, 5.6)	6/22 (27)	3.4 (1.5, 8.0)	4/22 (18)	3.2 (1.1, 9.3)
Take protective gear home						
Yes	10/78 (13)	1.0 (0.5, 2.2)	9/78 (12)	1.3 (0.5, 2.9)	7/78 (9)	1.5 (0.6, 4.2)
No	15/120 (13)	Ref	11/120 (9)	Ref	7/120 (6)	Ref
Amount of direct contact with pigs						
Hours/day in direct contact						
>8	4/25 (16)	1.3 (0.5, 3.5)	3/25 (12)	1.2 (0.4, 3.9)	1/25 (4)	0.5 (0.1, 3.9)
≤8	21/173 (12)	Ref	17/173 (10)	Ref	13/173 (8)	Ref
Total pigs contacted per day						
≤200	10/113 (9)	Ref	9/113 (8)	Ref	7/113 (6)	Ref
>200 to 1000	8/59 (14)	1.5 (0.6, 3.7)	5/59 (9)	1.1 (0.4, 3.0)	1/59 (2)	0.3 (0.0, 2.2)
>1000	7/26 (27)	3.0 (1.3, 7.2)	6/26 (23)	2.9 (1.1, 7.4)	6/26 (23)	3.7 (1.4, 10.2)
Size of IHO[2]						
≥250 to 1000	3/36 (8)	Ref	3/36 (8)	Ref	1/36 (3)	Ref
>1000 to 4000	10/80 (13)	1.5 (0.4, 5.1)	8/80 (10)	1.2 (0.3, 4.3)	5/80 (6)	2.3 (0.3, 18.6)

Work Activity	MDRSA		*scn*-neg MDRSA		*scn*-neg, tet-R MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
≥4000	12/74 (16)	1.9 (0.6, 6.5)	9/74 (12)	1.5 (0.4, 5.1)	8/74 (11)	3.9 (0.5, 29.9)
Other work activities						
Handle dead pigs						
Yes	21/143 (15)	2.0 (0.7, 5.6)	17/143 (12)	2.2 (0.7, 7.1)	12/143 (8)	2.3 (0.5, 10.0)
No	4/55 (7)	Ref	3/55 (6)	Ref	2/55 (4)	Ref
Contact pig manure						
Yes	10/78 (13)	1.0 (0.5, 2.2)	9/78 (12)	1.3 (0.5, 2.9)	7/78 (9)	1.5 (0.6, 4.2)
No	15/120 (13)	Ref	11/120 (9)	Ref	7/120 (6)	Ref
Other employment characteristics						
Hours worked per day						
>8 h	5/43 (12)	0.9 (0.4, 2.3)	3/43 (7)	0.6 (0.2, 2.1)	1/43 (2)	0.3 (0.0, 2.1)
≤8 h	20/155 (13)	Ref	17/155 (11)	Ref	13/155 (8)	Ref
Years worked at current IHO						
> 5	11/52 (21)	2.2 (1.1, 4.5)	11/52 (21)	3.4 (1.5, 7.8)	8/52 (15)	3.7 (1.4, 10.3)
≤5	14/146 (10)	Ref	9/146 (6)	Ref	6/146 (4)	Ref
Years worked at any IHO						
>5	13/63 (21)	2.3 (1.1, 4.8)	13/63 (21)	4.0 (1.7, 9.5)	8/63 (13)	2.9 (1.0, 7.9)
≤ 5	12/135 (9)	Ref	7/135 (5)	Ref	6/135 (4)	Ref
Time since last work shift						
≤3 h	8/81 (10)	0.8 (0.3, 2.2)	7/81 (9)	1.1 (0.3, 3.5)	5/81 (6)	1.5 (0.3, 7.7)
>3 to 12 h	11/67 (16)	1.4 (0.5, 3.4)	9/67 (13)	1.7 (0.5, 5.1)	7/67 (11)	2.6 (0.6, 12.0)
>12 h	6/50 (12)	Ref	4/50 (8)	Ref	2/50 (4)	Ref

APPENDIX 4, Table 1c. Prevalence of MRSA and CC398/CC9 *S. aureus* by work activity among adult IHO workers.

Work Activity	MRSA		CC398 or CC9[3]	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage[1]				
Nursery				
Yes	0/39 (0)	-[4]	2/39 (5)	0.5 (0.1, 2.0)
No	4/159 (3)	Ref	17/159 (11)	Ref
Finishing				
Yes	0/36 (0)	-[4]	2/36 (6)	0.5 (0.1, 2.2)
No	4/162 (3)	Ref	17/162 (11)	Ref
Wean, farrow, or feeder				
Yes	4/94 (4)	-[5]	10/94 (11)	1.2 (0.5, 2.9)
No	0/104 (0)	Ref	9/104 (9)	Ref
Sow or Boar				
Yes	0/31 (0)	-[4]	5/31 (16)	1.9 (0.7, 5.0)
No	4/167 (2)	Ref	14/167 (9)	Ref
Interaction with pigs and medication use				
Administer shots				
Yes	3/140 (2)	1.2 (0.1, 11.7)	17/140 (12)	3.5 (0.8, 14.8)
No	1/58 (2)	Ref	2/58 (4)	Ref
Inseminate sows				
Yes	0/26 (0)	-[4]	4/26 (15)	1.8 (0.6, 4.9)
No	4/172 (2)	Ref	15/172 (9)	Ref
Administer antibiotics				
Yes	3/123 (2)	1.8 (0.2, 17.3)	13/123 (11)	1.3 (0.5, 3.3)
No	1/75 (1)	Ref	6/75 (8)	Ref
Cleaning activities				
Chemical use				
Yes	0/112 (0)	-[4]	10/112 (9)	0.9 (0.4, 2.0)
No	4/86 (5)	Ref	9/86 (11)	Ref
Performed any cleaning activities				
Yes	3/147 (2)	1.0 (0.1, 9.8)	13/147 (9)	0.8 (0.3, 1.9)
No	1/51 (2)	Ref	6/51 (12)	Ref

Work Activity	MRSA		CC398 or CC9[3]	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pressure washer use				
Yes	3/67 (5)	5.9 (0.6, 55.3)	6/67 (9)	0.9 (0.4, 2.3)
No	1/131 (1)	Ref	13/131 (10)	Ref
Frequency of mask use				
Ever use	0/22 (0)	Ref	13/176 (7)	Ref
Never	4/176 (2)	-[5]	6/22 (27)	3.7 (1.6, 8.7)
Take protective gear home				
Yes	0/78 (0)	-[4]	8/78 (10)	1.1 (0.5, 2.7)
No	4/120 (3)	Ref	11/120 (9)	Ref
Amount of direct contact with pigs				
Hours/day in direct contact				
>8	2/25 (8)	6.9 (1.0, 46.9)	3/25 (12)	1.3 (0.4, 4.1)
≤8	2/173 (1)	Ref	16/173 (9)	Ref
Total pigs contacted per day				
≤200	0/113 (0)	Ref	10/113 (9)	Ref
>200 to 1000	4/59 (7)	-[5]	4/59 (7)	0.8 (0.3, 2.3)
>1000	0/26 (0)	-[4,5]	5/26 (19)	2.2 (0.8, 5.8)
Size of IHO[2]				
≥250 to 1000	1/36 (3)	Ref	3/36 (8)	Ref
>1000 to 4000	3/80 (4)	1.4 (0.1, 12.5)	7/80 (9)	1.1 (0.3, 3.8)
≥4000	0/74 (0)	-[4]	8/74 (11)	1.3 (0.4, 4.6)
Other work activities				
Handle dead pigs				
Yes	3/143 (2)	1.2 (0.1, 10.9)	14/143 (10)	1.1 (0.4, 2.8)
No	1/55 (2)	Ref	5/55 (9)	Ref
Contact pig manure				
Yes	0/78 (0)	-[4]	8/78 (10)	1.1 (0.5, 2.7)
No	4/120 (3)	Ref	11/120 (9)	Ref
Other employment characteristics				

Work Activity	MRSA		CC398 or CC9[3]	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Hours worked per day				
>8 h	2/43 (5)	3.6 (0.5, 24.9)	3/43 (7)	0.7 (0.2, 2.2)
≤8 h	2/155 (1)	Ref	16/155 (10)	Ref
Years worked at current IHO				
> 5	0/52 (0)	-[4]	12/52 (23)	4.8 (2.0, 11.6)
≤5	4/146 (3)	Ref	7/146 (5)	Ref
Years worked at any IHO				
>5	1/63 (2)	0.7 (0.1, 6.7)	14/63 (22)	6.0 (2.3, 15.9)
≤ 5	3/135 (2)	Ref	5/135 (4)	Ref
Time since last work shift				
≤3 h	1/81 (1)	0.6 (0.0, 9.4)	9/81 (11)	1.4 (0.5, 4.3)
>3 to 12 h	2/67 (3)	1.5 (0.1, 16.0)	6/67 (9)	1.1 (0.3, 3.8)
>12 h	1/50 (2)	Ref	4/50 (8)	Ref

[1] Categories were developed based on age/life stage and substantial overlap among individuals who reported working with sows and boars or wean, farrow, and feeder pigs. Additional overlap was observed between the following categories: sow and wean pigs (2 individuals), nursery and finish pigs (1 individual), wean pigs and boars (1 individual), and farrow pigs and boars (1 individual).

[2] Restricted to individuals who reported ≥250 pigs at operation.

[3] All CC398 and CC9 were *scn*-negative.

[4] PR estimate not computed due to zero observations in at least one exposure category.

[5] PR estimate not computed due to zero observations in the referent category.

APPENDIX 5: CHAPTER 5 RESULTS FOR ALL ACTIVITIES, CHILDREN

Prevalence of all *S. aureus* carriage outcomes by work activity among children living in the same household as an adult IHO worker.

Appendix 5, Table1a. Prevalence of *S. aureus* and *scn*-negative *S. aureus* carriage outcomes by work activity among children living in the same household as an adult IHO worker.

Work activity	*S. aureus*		*scn*-neg *S. aureus*	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage[1]				
Nursery				
Yes	22/39 (56)	1.2 (0.9, 1.6)	0/39 (0)	-[2]
No	75/159 (47)	Ref	7/159 (4)	Ref
Finishing				
Yes	14/36 (39)	0.8 (0.5, 1.2)	2/36 (6)	1.8 (0.4, 8.9)
No	83/162 (51)	Ref	5/162 (3)	Ref
Wean, farrow, or feeder				
Yes	48/94 (51)	1.1 (0.8, 1.4)	4/94 (4)	1.5 (0.3, 6.4)
No	49/104 (47)	Ref	3/104 (3)	Ref
Sow or Boar				
Yes	13/31 (42)	0.8 (0.5, 1.3)	1/31 (3)	0.9 (0.1, 7.2)
No	84/167 (50)	Ref	6/167 (4)	Ref
Interaction with pigs and medication use				
Administer shots				
Yes	70/140 (50)	1.1 (0.8, 1.5)	5/140 (4)	1.0 (0.2, 5.2)
No	27/58 (47)	Ref	2/58 (4)	Ref
Inseminate sows				
Yes	13/26 (50)	1.0 (0.7, 1.5)	0/26 (0)	-[2]
No	84/172 (49)	Ref	7/172 (4)	Ref
Administer antibiotics				
Yes	58/123 (47)	0.9 (0.7, 1.2)	6/123 (5)	3.7 (0.4, 29.8)
No	39/75 (52)	Ref	1/75 (1)	Ref
Cleaning activities				

Work activity	*S. aureus*		*scn*-neg *S. aureus*	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Chemical use				
Yes	60/112 (54)	1.2 (0.9, 1.7)	1/112 (1)	0.1 (0.0, 1.0)
No	37/86 (43)	Ref	6/86 (7)	Ref
Any cleaning				
Yes	77/147 (52)	1.3 (0.9, 1.9)	3/147 (2)	0.3 (0.1, 1.1)
No	20/51 (39)	Ref	4/51 (8)	Ref
Pressure washer use				
Yes	35/67 (52)	1.1 (0.8, 1.5)	2/67 (3)	0.8 (0.2, 3.9)
No	62/131 (47)	Ref	5/131 (4)	Ref
Frequency of mask use				
Yes	90/176 (51)	Ref	7/176 (4)	Ref
No	7/22 (32)	0.6 (0.3, 1.2)	0/22 (0)	-[2]
Take protective gear home				
Yes	45/78 (58)	1.3 (1.0, 1.8)	1/78 (1)	0.3 (0.0, 2.1)
No	52/120 (43)	Ref	6/120 (5)	Ref
Amount of direct contact with pigs				
Hours/day in direct contact				
>8	11/25 (44)	0.9 (0.6, 1.4)	2/25 (8)	2.8 (0.6, 13.5)
≤8	86/173 (50)	Ref	5/173 (3)	Ref
Total pigs contacted per day				
≤200	57/113 (50)	Ref	1/113 (1)	Ref
>200 to 1000	31/59 (53)	1.0 (0.8, 1.4)	4/59 (7)	7.7 (0.9, 67.0)
>1000	9/26 (35)	0.7 (0.4, 1.2)	2/26 (8)	8.7 (0.8, 92.3)
Size of IHO[3]				
≥250 to 1000	14/36 (39)	Ref	1/36 (3)	Ref
>1000 to 4000	43/80 (54)	1.4 (0.9, 2.2)	3/80 (4)	1.4 (0.1, 12.5)
≥4000	39/74 (53)	1.4 (0.9, 2.2)	3/74 (4)	1.5 (0.2, 13.5)
Other work activities				
Handle dead pigs				

Work activity	*S. aureus*		*scn*-neg *S. aureus*	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Yes	74/143 (52)	1.2 (0.9, 1.8)	7/143 (5)	-[4]
No	23/55 (42)	Ref	0/55 (0)	Ref
Contact pig manure				
Yes	44/78 (56)	1.3 (1.0, 1.7)	1/78 (1)	0.3 (0.0, 2.1)
No	53/120 (44)	Ref	6/120 (5)	Ref
Other employment characteristics				
Hours worked per day				
>8 h	17/43 (40)	0.8 (0.5, 1.1)	2/43 (1)	1.4 (0.3, 7.2)
≤8 h	80/155 (52)	Ref	5/155 (3)	Ref
Years worked at current IHO				
> 5	21/52 (40)	0.8 (0.5, 1.1)	1/52 (2)	0.5 (0.1, 3.8)
≤5	76/146 (52)	Ref	6/146 (4)	Ref
Years worked at any IHO				
>5	29/63 (46)	0.9 (0.7, 1.3)	2/63 (3)	0.9 (0.2, 4.3)
≤5	68/135 (50)	Ref	5/135 (4)	Ref
Time since last work shift				
≤3 h	35/81 (43)	0.7 (0.5, 1.0)	5/81 (6)	3.1 (0.4, 25.7)
>3 to 12 h	32/67 (48)	0.8 (0.6, 1.1)	1/67 (2)	0.7 (0.0, 11.6)
>12 h	30/50 (60)	Ref	1/50 (2)	Ref

Appendix 5, Table1b. Prevalence of MRSA and MDRSA carriage outcomes by work activity among children living in the same household as an adult IHO worker.

Work activity	MRSA		MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
Pig life stage[1]				
Nursery				
Yes	10/39 (26)	2.4 (1.2, 4.8)	11/39 (28)	1.3 (0.7, 2.4)
No	17/159 (11)	Ref	34/159 (21)	Ref
Finishing				
Yes	7/36 (19)	1.6 (0.7, 3.4)	11/36 (31)	1.5 (0.8, 2.6)
No	20/162 (12)	Ref	34/162 (21)	Ref
Wean, farrow, or feeder				
Yes	10/94 (11)	0.7 (0.3, 1.3)	17/94 (18)	0.7 (0.4, 1.1)
No	17/104 (16)	Ref	28/104 (27)	Ref
Sow or Boar				
Yes	0/31 (0)	-[2]	6/31 (19)	0.8 (0.4, 1.8)
No	27/167 (16)	Ref	39/167 (23)	Ref
Interaction with pigs and medication use				
Administer shots				
Yes	13/140 (9)	0.4 (0.2, 0.8)	28/140 (20)	0.7 (0.4, 1.1)
No	14/58 (24)	Ref	17/58 (29)	Ref
Inseminate sows				
Yes	0/26 (0)	-[2]	5/26 (19)	0.8 (0.4, 1.9)
No	27/172 (16)	Ref	40/172 (23)	Ref
Administer antibiotics				
Yes	8/123 (7)	0.3 (0.1, 0.6)	24/123 (20)	0.7 (0.4, 1.2)
No	19/75 (25)	Ref	21/75 (28)	Ref
Cleaning activities				
Chemical use				
Yes	25/112 (22)	9.6 (2.3, 39.4)	34/112 (30)	2.4 (1.3, 4.4)
No	2/86 (2)	Ref	11/86 (13)	Ref
Any cleaning				
Yes	26/147 (18)	9.0 (1.3, 64.8)	39/147 (27)	2.3 (1.0, 5.0)

Work activity	MRSA		MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
No	1/51 (2)	Ref	6/51 (12)	Ref
Pressure washer use				
Yes	3/67 (5)	0.2 (0.1, 0.8)	13/67 (19)	0.8 (0.4, 1.4)
No	24/131 (18)	Ref	32/131 (24)	Ref
Frequency of mask use				
Yes	27/176 (15)	Ref	43/176 (24)	Ref
No	0/22 (0)	-[2]	2/22 (9)	0.4 (0.1, 1.4)
Take protective gear home				
Yes	25/78 (32)	19.2 (4.7, 78.9)	26/78 (33)	2.1 (1.3, 3.5)
No	2/120 (2)	Ref	19/120 (16)	Ref
Amount of direct contact with pigs				
Hours/day in direct contact				
>8	1/25 (4)	0.3 (0.0, 1.9)	5/25 (20)	0.9 (0.4, 2.0)
≤8	26/173 (15)	Ref	40/173 (23)	Ref
Total pigs contacted per day				
≤200	25/113 (22)	Ref	29/113 (26)	Ref
>200 to 1000	2/59 (3)	0.2 (0.0, 0.6)	11/59 (19)	0.7 (0.4, 1.3)
>1000	0/26 (0)	-[2]	5/26 (19)	0.7 (0.3, 1.7)
Size of IHO[3]				
≥250 to 1000	2/36 (6)	Ref	5/36 (14)	Ref
>1000 to 4000	6/80 (8)	1.4 (0.3, 6.4)	17/80 (21)	1.5 (0.6, 3.8)
≥4000	19/74 (26)	4.6 (1.1, 18.8)	23/74 (31)	2.2 (0.9, 5.4)
Other work activities				
Handle dead pigs				
Yes	24/143 (17)	3.1 (1.0, 9.8)	30/143 (27)	2.5 (1.1, 5.6)
No	3/55 (6)	Ref	6/55 (11)	Ref
Contact pig manure				
Yes	25/78 (32)	19.2 (4.7, 78.9)	27/78 (35)	2.3 (1.4, 3.9)
No	2/120 (2)	Ref	18/120 (15)	Ref
Other employment characteristics				
Hours worked per day				

Work activity	MRSA		MDRSA	
	N pos/Total N (%)	PR (95% CI)	N pos/Total N (%)	PR (95% CI)
>8 h	3/43 (7)	0.5 (0.1, 1.4)	9/43 (21)	0.9 (0.5, 1.7)
≤8 h	24/155 (16)	Ref	36/155 (23)	Ref
Years worked at current IHO				
> 5	9/52 (17)	1.4 (0.7, 2.9)	12/52 (23)	1.0 (0.6, 1.8)
≤5	18/146 (12)	Ref	33/146 (23)	Ref
Years worked at any IHO				
>5	10/63 (16)	1.3 (0.6, 2.6)	16/63 (25)	1.2 (0.7, 2.0)
≤5	17/135 (13)	Ref	29/135 (22)	Ref
Time since last work shift				
≤3 h	4/81 (5)	0.2 (0.1, 0.5)	11/81 (14)	0.4 (0.2, 0.7)
>3 to 12 h	9/67 (13)	0.5 (0.2, 1.0)	16/67 (24)	0.7 (0.4, 1.2)
>12 h	14/50 (28)	Ref	18/50 (36)	Ref

[1] Categories were developed based on age/life stage and substantial overlap among individuals who reported working with sows and boars or wean, farrow, and feeder pigs. Additional overlap was observed between the following categories: sow and wean pigs (2 individuals), nursery and finish pigs (1 individual), wean pigs and boars (1 individual), and farrow pigs and boars (1 individual).

[2] PR estimate not computed due to zero observations in at least one exposure category.

[3] Restricted to individuals who reported ≥250 pigs at operation.

[4] PR estimate not computed due to zero observations in the referent category.

REFERENCES

1. Gorwitz, R.J., et al., *Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004.* J Infect Dis, 2008. 197(9): p. 1226-34.

2. Graham, P.L., S.X. Lin, and E.L. Larson, *A US population-based survey of Staphylococcus aureus colonization.* Ann Intern Med, 2006. 144(5): p. 318-325.

3. Kluytmans, J., A. Van Belkum, and H. Verbrugh, *Nasal carriage of Staphylococcus aureus: epidemiology, underlying mechanisms, and associated risks.* Clin Microbiol Rev, 1997. 10(3): p. 505-520.

4. Lowy, F.D., *Staphylococcus aureus Infections.* N Engl J Med, 1998. 339(8): p. 520-532.

5. Kirby, W.M., *Extraction of a highly potent penicillin inactivator from penicillin resistant staphylococci.* Science, 1944. 99(2579): p. 452-453.

6. Rountree, P.M. and B.M. Freeman, *Infections caused by a particular phage type of Staphylococcus aureus.* Med J Aust, 1955. 42(5): p. 157-61.

7. Jevons, M., *"Celbenin"-resistant staphylococci.* Br Med J, 1961. 1(5219): p. 124-125.

8. Hiramatsu, K., et al., *Methicillin-resistant Staphylococcus aureus clinical strain with reduced vancomycin susceptibility.* J Antimicrob Chemother, 1997. 40(1): p. 135-6.

9. Chang, S., et al., *Infection with Vancomycin-Resistant Staphylococcus aureus Containing the vanA Resistance Gene.* N Engl J Med, 2003. 348(14): p. 1342-1347.

10. García-Álvarez, L., et al., *Meticillin-resistant< i> Staphylococcus aureus with a novel mecA homologue in human and bovine populations in the UK and Denmark: a descriptive study.* The Lancet Infect Dis, 2011. 11(8): p. 595-603.

11. Ballhausen, B., et al., *The mecA homolog mecC confers resistance against beta-lactams in Staphylococcus aureus irrespective of the genetic strain background.* Antimicrob Agents Chemother, 2014. 58(7): p. 3791-8.

12. Frenay, H., et al., *Molecular typing of methicillin-resistantStaphylococcus aureus on the basis of protein A gene polymorphism.* Eur J Clin Microbiol Infect Dis, 1996. 15(1): p. 60-64.

13. Deurenberg, R., et al., *The molecular evolution of methicillin‐resistant Staphylococcus aureus.* Clin Microbiol Infec, 2007. 13(3): p. 222-235.

14. Shopsin, B., et al., *Evaluation of protein A gene polymorphic region DNA sequencing for typing of Staphylococcus aureus strains.* J Clin Microbiol, 1999. 37(11): p. 3556-63.

15. Malachowa, N., et al., *Comparison of multiple-locus variable-number tandem-repeat analysis with pulsed-field gel electrophoresis, spa typing, and multilocus sequence typing for clonal characterization of Staphylococcus aureus isolates.* J Clin Microbiol, 2005. 43(7): p. 3095-3100.

16. Mellmann, A., et al., *Based Upon Repeat Pattern (BURP): an algorithm to characterize the long-term evolution of Staphylococcus aureus populations based on spa polymorphisms.* BMC Microbiol, 2007. 7: p. 98.

17. Maiden, M.C., et al., *Multilocus sequence typing: a portable approach to the identification of clones within populations of pathogenic microorganisms.* Proc Natl Acad Sci USA, 1998. 95(6): p. 3140-3145.

18. Li, W., D. Raoult, and P.E. Fournier, *Bacterial strain typing in the genomic era.* FEMS Microbiol Rev, 2009. 33(5): p. 892-916.

19. Harris, S.R., et al., *Evolution of MRSA during hospital transmission and intercontinental spread.* Science, 2010. 327(5964): p. 469-74.

20. Tenover, F.C., et al., *Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing.* J Clin Microbiol, 1995. 33(9): p. 2233.

21. International Working Group on the Classification of Staphylococcal Cassette Chromosome, E., *Classification of staphylococcal cassette chromosome mec (SCCmec): guidelines for reporting novel SCCmec elements.* Antimicrob Agents Chemother, 2009. 53(12): p. 4961-7.

22. Oliveira, D.C., H. de Lencastre, and A. Tomasz, *Evolution of molecular techniques for the characterization of MRSA clones*, in *Antibiotic Discovery and Development*. 2012, Springer. p. 571-592.

23. Vindel, A., et al., *Methicillin-resistant Staphylococcus aureus in Spain: molecular epidemiology and utility of different typing methods.* J Clin Microbiol, 2009. 47(6): p. 1620-7.

24. Udo, E.E., J.W. Pearman, and W.B. Grubb, *Genetic analysis of community isolates of methicillin-resistant Staphylococcus aureus in Western Australia.* J Hosp Infect, 1993. 25(2): p. 97-108.

25. Saravolatz, L.D., et al., *Methicillin-resistant Staphylococcus aureus. Epidemiologic observations during a community-acquired outbreak.* Ann Intern Med, 1982. 96(1): p. 11-6.

26. Herold, B.C., et al., *Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk.* JAMA-J Am Med Assoc, 1998. 279(8): p. 593-598.

27. CDC, *Four pediatric deaths from community-acquired methicillin-resistant Staphylococcus aureus—Minnesota and North Dakota, 1997-1999.* MMWR Morb Mortal Wkly Rep,1999. 48(32): p. 707.

28. Chambers, H.F. and F.R. DeLeo, *Waves of resistance: Staphylococcus aureus in the antibiotic era.* Nat Rev Microbiol, 2009. 7(9): p. 629-641.

29. Lu, P.-L., et al., *Risk factors and molecular analysis of community methicillin-resistant Staphylococcus aureus carriage.* J Clin Microbiol, 2005. 43(1): p. 132-139.

30. Miller, M.B., et al., *Prevalence and risk factor analysis for methicillin-resistant Staphylococcus aureus nasal colonization in children attending child care centers.* J Clin Microbiol, 2011. 49(3): p. 1041-7.

31. Asensio, A., et al., *Colonization and infection with methicillin-resistant Staphylococcus aureus: associated factors and eradication.* Infect Control Hosp Epidemiol, 1996. 17(1): p. 20-8.

32. Warshawsky, B., et al., *Hospital- and community-based surveillance of methicillin-resistant Staphylococcus aureus: previous hospitalization is the major risk factor.* Infect Control Hosp Epidemiol, 2000. 21(11): p. 724-7.

33. Merrer, J., et al., *"Colonization pressure" and risk of acquisition of methicillin-resistant Staphylococcus aureus in a medical intensive care unit.* Infect Control Hosp Epidemiol, 2000. 21(11): p. 718-23.

34. Huang, S.S. and R. Platt, *Risk of methicillin-resistant Staphylococcus aureus infection after previous infection or colonization.* Clin Infect Dis, 2003. 36(3): p. 281-285.

35. Pujol, M., et al., *Risk factors for nosocomial bacteremia due to methicillin-resistant Staphylococcus aureus.* Eur J Clin Microbiol Infect Dis, 1994. 13(1): p. 96-102.

36. Coello, R., et al., *Risk factors for developing clinical infection with methicillin-resistant Staphylococcus aureus (MRSA) amongst hospital patients initially only colonized with MRSA.* J Hosp Infect, 1997. 37(1): p. 39-46.

37. Klevens, R.M., et al., *Invasive methicillin-resistant Staphylococcus aureus infections in the United States.* JAMA-J Am Med Assoc, 2007. 298(15): p. 1763-1771.

38. Weber, J.T., *Community-associated methicillin-resistant Staphylococcus aureus.* Clin Infect Dis, 2005. 41 Suppl 4: p. S269-72.

39. Kazakova, S.V., et al., *A clone of methicillin-resistant Staphylococcus aureus among professional football players.* N Engl J Med, 2005. 352(5): p. 468-475.

40. CDC, *Methicillin-resistant staphylococcus aureus infections among competitive sports participants--Colorado, Indiana, Pennsylvania, and Los Angeles County, 2000-2003.* MMWR Morb Mortal Wkly Rep, 2003. 52(33): p. 793-5.

41. Zinderman, C.E., et al., *Community-acquired methicillin-resistant Staphylococcus aureus among military recruits.* Emerg Infect Dis, 2004. 10(5): p. 941-4.

42. CDC, *Outbreaks of community-associated methicillin-resistant Staphylococcus aureus skin infections--Los Angeles County, California, 2002-2003.* MMWR Morb Mortal Wkly Rep, 2003. 52(5): p. 88.

43. Milstone, A.M., et al., *Community-associated methicillin-resistant Staphylococcus aureus strains in pediatric intensive care unit.* Emerg Infect Dis, 2010. 16(4): p. 647.

44. Adcock, P.M., et al., *Methicillin-resistant Staphylococcus aureus in two child care centers.* J Infect Dis, 1998. 178(2): p. 577-80.

45. Fridkin, S.K., et al., *Methicillin-resistant Staphylococcus aureus disease in three communities.* N Engl J Med, 2005. 352(14): p. 1436-44.

46. Maree, C.L., et al., *Risk factors for infection and colonization with community-associated methicillin-resistant Staphylococcus aureus in the Los Angeles County jail: a case-control study.* Clin Infect Dis, 2010. 51(11): p. 1248-57.

47. Naimi, T.S., et al., *Comparison of community-and health care–associated methicillin-resistant Staphylococcus aureus infection.* JAMA-J Am Med Assoc, 2003. 290(22): p. 2976-2984.

48. Shapiro, A., et al., *Community-Acquired MRSA Infections in North Carolina Children.* NC Med J, 2009. 70(2).

49. Dietrich, D.W., D.B. Auld, and L.A. Mermel, *Community-acquired methicillin-resistant Staphylococcus aureus in southern New England children.* Pediatrics, 2004. 113(4): p. e347-e352.

50. Hussain, F.M., et al., *Current trends in community-acquired methicillin-resistant Staphylococcus aureus at a tertiary care pediatric facility.* Pediatr Infect Dis J, 2000. 19(12): p. 1163-1166.

51. Kaplan, S.L., et al., *Three-Year Surveillance of Community-AcquiredStaphylococcus aureus Infections in Children.* Clin Infect Dis, 2005. 40(12): p. 1785-1791.

52. Hermos, C.R., et al., *Changing epidemiology of methicillin-resistant Staphylococcus aureus colonization in paediatric intensive-care units.* Epidemiol Infect, 2013. 141(9): p. 1983-1992.

53. Erami, M., et al., *Nasal Carriage and Resistance Pattern of Multidrug Resistant Staphylococcus aureus Among Healthy Children in Kashan, Iran.* Iran Red Crescent Med J, 2014 16(9): p. e21346.

54. Lo, W.-T., et al., *Changes in the nasal colonization with methicillin-resistant Staphylococcus aureus in children: 2004-2009.* PLoS One, 2010. 5(12): p. e15791.

55. Jenkins, T.C., et al., *Epidemiology of Healthcare‐Associated Bloodstream Infection Caused by USA300 Strains of Methicillin‐Resistant Staphylococcus aureus in 3 Affiliated Hospitals.* Epidemiology, 2009. 30(3): p. 233-241.

56. Klevens, R.M., et al., *Community-associated methicillin-resistant Staphylococcus aureus and healthcare risk factors.* Emerg Infect Dis, 2006. 12(12): p. 1991-3.

57. David, M.Z., et al., *Replacement of HA-MRSA by CA-MRSA Infections at an Academic Medical Center in the Midwestern United States, 2004-5 to 2008.* PloS One, 2014. 9(4): p. e92760.

58. MacDonald, J.M. and W.D. McBride, *The transformation of U.S. Livestock Agriculture: Scale, Efficiency, and Risks*, in *Economic Information Bulletin No. 43*, U. Economic Research Service, Editor. 2009.

59. Pew Commission on Industrial Farm Animal Production, *Putting Meat on the Table: Industrial Farm Animal Production in America*. 2008: http://www.ncifap.org/_images/PCIFAPFin.pdf.

60. Office, U.S.G.A., *Animal agriculture: information on waste management and water quality issues*, C. Resources, and Economic Development Division, Editor. 1995.

61. EPA. *Animal Feeding Operations Overview*. 2014 September 9, 2014 [cited 2014 October 22]; Available from: http://water.epa.gov/polwaste/npdes/afo/index.cfm.

62. Wing, S., D. Cole, and G. Grant, *Environmental Injustice in North Carolina's Hog Industry.* Environ Health Persp, 2000. 108(3): p. 225-231.

63. USDA and NCDA&CS, *North Carolina Agricultural Satatistics*. 2013, U.S. Dept. of Agricultural National Agricultural Statistics Service, North Carolina Dept. of Agriculture & Consumer Services: http://www.ncagr.gov/stats/2013AgStat/.

64. USDA, *2012 Census of Agriculture - State Data*, National Agricultural Statistics Service, Editor. 2012.

65. FDA, *2011 Summary Report on Antimicrobials Sold or Distributed for use in Food-Producing Animals*, Department of Health and Human Services, Editor. 2011: http://www.fda.gov/AnimalVeterinary/NewsEvents/CVMUpdates/ucm338178.htm.

66. Love, D.C., et al., *Dose imprecision and resistance: free-choice medicated feeds in industrial food animal production in the United States.* Environ Health Perspect, 2011. 119(3): p. 279-83.

67. Ziemer, C.J., et al., *Fate and transport of zoonotic, bacterial, viral, and parasitic pathogens during swine manure treatment, storage, and land application.* J Anim Sci, 2010. 88(13 Suppl): p. E84-94.

68. Hill, V.R., *Prospects for pathogen reductions in livestock wastewaters: A review.* Crit Rev Env Sci Technol, 2003. 33(2): p. 187-235.

69. Sobsey, M., et al., *Pathogens in animal wastes and the impacts of waste management practices on their survival, transport and fate.* White Papers on Animal Agriculture and the Environment. MidWest Plan Service (MWPS), Iowa State University, Ames, IA, 2001.

70. Smith, T.C., *Livestock-Associated Staphylococcus aureus: The United States Experience.* PLoS Pathog, 2015. 11(2): p. e1004564.

71. Devriese, L., L.v. Damme, and L. Fameree, *Methicillin (Cloxacillin) ‐Resistant Staphylococcus aureus strains Isolated from Bovine Mastitis Cases.* Zentralblatt für Veterinärmedizin Reihe B, 1972. 19(7): p. 598-605.

72. Van Loo, I., et al., *Emergence of methicillin-resistant Staphylococcus aureus of animal origin in humans.* Emerg Infect Dis, 2007. 13(12): p. 1834.

73. Hasman, H., et al., *spa type distribution in Staphylococcus aureus originating from pigs, cattle and poultry.* Vet Microbiol, 2010. 141(3–4): p. 326-331.

74. Neela, V., et al., *Prevalence of ST9 methicillin-resistant Staphylococcus aureus among pigs and pig handlers in Malaysia.* J Clin Microbiol, 2009. 47(12): p. 4138-4140.

75. Cuny, C., R. Kock, and W. Witte, *Livestock associated MRSA (LA-MRSA) and its relevance for humans in Germany.* Int J Med Microbiol, 2013.

76. Price, L.B., et al., *Staphylococcus aureus CC398: host adaptation and emergence of methicillin resistance in livestock.* MBio, 2012. 3(1).

77. Spoor, L.E., et al., *Livestock origin for a human pandemic clone of community-associated methicillin-resistant Staphylococcus aureus.* MBio, 2013. 4(4): p. e00356-13.

78. Resch, G., et al., *Human-to-bovine jump of Staphylococcus aureus CC8 is associated with the loss of a β-hemolysin converting prophage and the acquisition of a new staphylococcal cassette chromosome.* PloS one, 2013. 8(3): p. e58187.

79. Lowder, B.V., et al., *Recent human-to-poultry host jump, adaptation, and pandemic spread of Staphylococcus aureus.* Proc Natl Acad Sci USA, 2009. 106(46): p. 19545-19550.

80. Love, D.C., et al., *Dose imprecision and resistance: free-choice medicated feeds in industrial food animal production in the United States.* Environ Health Perspect, 2011. 119(3): p. 279.

81. Schnappinger, D. and W. Hillen, *Tetracyclines: antibiotic action, uptake, and resistance mechanisms.* Arch Microbiol, 1996. 165(6): p. 359-369.

82. McCarthy, A.J., et al., *The distribution of mobile genetic elements (MGEs) in MRSA CC398 is associated with both host and country.* Genome Biol Evol, 2011. 3: p. 1164-74.

83. Verkaik, N.J., et al., *Immune evasion cluster-positive bacteriophages are highly prevalent among human Staphylococcus aureus strains, but they are not essential in the first stages of nasal colonization.* Clin Microbiol Infect, 2011. 17(3): p. 343-8.

84. Rinsky, J.L., et al., *Livestock-Associated Methicillin and Multidrug Resistant Staphylococcus aureus Is Present among Industrial, Not Antibiotic-Free Livestock Operation Workers in North Carolina.* PLoS One, 2013. 8(7): p. e67641.

85. Smith, T.C., et al., *Methicillin-Resistant Staphylococcus aureus in Pigs and Farm Workers on Conventional and Antibiotic-Free Swine Farms in the USA.* PLoS One, 2013. 8(5): p. e63704.

86. Smith, T.C., et al., *Methicillin-resistant Staphylococcus aureus (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers.* PLoS One, 2009. 4(1): p. e4258.

87. Neyra, R.C., et al., *Multidrug-resistant and methicillin-resistant Staphylococcus aureus (MRSA) in hog slaughter and processing plant workers and their community in North Carolina (USA).* Environ Health Persp, 2014. 122(5): p. 471.

88. Nadimpalli, M., et al., *Persistence of livestock-associated antibiotic-resistant Staphylococcus aureus among industrial hog operation workers in North Carolina over 14 days.* Occup Environ Med, 2014: p. oemed-2014-102095.

89. Huijsdens, X.W., et al., *Community-acquired MRSA and pig-farming.* Ann Clin Microbiol Antimicrob, 2006. 5: p. 26.

90. Armand-Lefevre, L., R. Ruimy, and A. Andremont, *Clonal comparison of Staphylococcus aureus isolates from healthy pig farmers, human controls, and pigs.* Emerg Infect Dis, 2005. 11(5): p. 711-714.

91. Voss, A., et al., *Methicillin-resistant Staphylococcus aureus in pig farming.* Emerg Infect Dis, 2005. 11(12): p. 1965-1966.

92. Guardabassi, L., M. Stegger, and R. Skov, *Retrospective detection of methicillin resistant and susceptible Staphylococcus aureus ST398 in Danish slaughter pigs.* Vet Microbiol, 2007. 122(3): p. 384-386.

93. Gómez-Sanz, E., et al., *Detection, molecular characterization, and clonal diversity of methicillin-resistant Staphylococcus aureus CC398 and CC97 in Spanish slaughter pigs of different age groups.* Foodborne Pathog Dis, 2010. 7(10): p. 1269-1277.

94. EFSA, *Analysis of the baseline survey on the prevalence of methicillin-resistant Staphylococcus aureus (MRSA) in holdings with breeding pigs, in the EU, 2008, Part A: MRSA prevalence estimates; on request from the European Commission.* EFSA Journal, 2009. 7(11): p. 82.

95. Pomba, C., et al., *First description of meticillin-resistant Staphylococcus aureus (MRSA) CC30 and CC398 from swine in Portugal.* Int J Antimicrob Agents, 2009. 34(2): p. 193-4.

96. Lim, S.-K., et al., *The first detection of methicillin-resistant Staphylococcus aureus ST398 in pigs in Korea.* Vet Microbiol, 2012. 155(1): p. 88-92.

97. Arriola, C.S., et al., *Presence of methicillin-resistant Staphylococcus aureus in pigs in Peru.* PloS One, 2011. 6(12): p. e28529.

98. Huber, H., et al., *Prevalence and characteristics of meticillin-resistant Staphylococcus aureus in humans in contact with farm animals, in livestock, and in food of animal origin, Switzerland, 2009.* Euro Surveill, 2010. 15(6).

99. Morcillo, A., et al., *Prevalence and characteristics of methicillin-resistant Staphylococcus aureus in pigs and pig workers in Tenerife, Spain.* Foodborne Pathog Dis, 2012. 9(3): p. 207-210.

100. Khanna, T., et al., *Methicillin resistant Staphylococcus aureus colonization in pigs and pig farmers.* Vet Microbiol, 2008. 128(3): p. 298-303.

101. Wagenaar, J.A., et al., *Unexpected Sequence Types in livestock-associated methicillin-resistant Staphylococcus aureus (MRSA): MRSA ST9 and a Single Locus Variant of ST9 in pig farming in China.* Vet Microbiol, 2009. 139(3-4): p. 405-9.

102. Oppliger, A., et al., *Antimicrobial resistance of Staphylococcus aureus strains acquired by pig farmers from pigs.* Appl Environ Microb, 2012. 78(22): p. 8010-8014.

103. Broens, E.M., et al., *Longitudinal study on transmission of MRSA CC398 within pig herds.* BMC Vet Res, 2012. 8(1): p. 58.

104. Espinosa-Gongora, C., et al., *Transmission of MRSA CC398 strains between pig farms related by trade of animals.* Vet Rec, 2012. 170(22): p. 564-564.

105. Merialdi, G., et al., *Environmental methicillin-resistant Staphylococcus aureus contamination in pig herds in relation to the productive phase and application of cleaning and disinfection.* Res Vet Sci, 2013. 94(3): p. 425-427.

106. Moodley, A., F. Latronico, and L. Guardabassi, *Experimental colonization of pigs with methicillin-resistant Staphylococcus aureus (MRSA): insights into the colonization and transmission of livestock-associated MRSA.* Epidemiol Infect, 2011. 139(10): p. 1594-1600.

107. Verkade, E., et al., *Dynamics and Determinants of Staphylococcus aureus Carriage in Livestock Veterinarians: A Prospective Cohort Study.* Clin Infect Dis, 2013. 57(2): p. e11-7.

108. Moodley, A., et al., *High risk for nasal carriage of methicillin-resistant Staphylococcus aureus among Danish veterinary practitioners.* Scand J Work Env Hea, 2008. 34(2): p. 151-157.

109. Frana, T.S., et al., *Isolation and characterization of methicillin-resistant Staphylococcus aureus from pork farms and visiting veterinary students.* PloS One, 2013. 8(1): p. e53738.

110. Smith, T.C. and N. Pearson, *The emergence of Staphylococcus aureus ST398.* Vector Borne Zoonotic Dis, 2011. 11(4): p. 327-39.

111. Cuny, C., et al., *Nasal colonization of humans with methicillin-resistant Staphylococcus aureus (MRSA) CC398 with and without exposure to pigs.* PLoS One, 2009. 4(8): p. e6800.

112. Feingold, B.J., et al., *Livestock density as risk factor for livestock-associated methicillin-resistant Staphylococcus aureus, the Netherlands.* Emerg Infect Dis, 2012. 18(11): p. 1841.

113. Köck, R., et al., *Persistence of nasal colonization with livestock-associated methicillin-resistant Staphylococcus aureus in pig farmers after holidays from pig exposure.* Appl Environ Microbiol, 2012. 78(11): p. 4046-4047.

114. Cui, S., et al., *Isolation and characterization of methicillin-resistant Staphylococcus aureus from swine and workers in China.* J Antimicrob Chemoth, 2009. 64(4): p. 680-683.

115. Huang, E., et al., *Detection of livestock-associated methicillin-resistantStaphylococcus aureus among swine workers in Romania.* J Infect Public Health, 2014.

116. Dahms, C., et al., *Occurrence of methicillin-resistant Staphylococcus aureus in farm workers and the livestock environment in Mecklenburg-Western Pomerania, Germany.* Acta Veterinaria Scandinavica, 2014. 56(1): p. 53.

117. Denis, O., et al., *Methicillin-resistant Staphylococcus aureus ST398 in swine farm personnel, Belgium.* Emerg Infect Dis, 2009. 15(7): p. 1098.

118. Graveland, H., et al., *Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact.* PLoS One, 2011. 6(2): p. e16830.

119. Graveland, H., et al., *Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans.* Int J Med Microbiol, 2011. 301(8): p. 630-4.

120. Garcia-Graells, C., et al., *Livestock veterinarians at high risk of acquiring methicillin-resistant Staphylococcus aureus ST398.* Epidemiol Infect, 2012. 140(03): p. 383-389.

121. Van Cleef, B., et al., *High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in The Netherlands.* Epidemiol Infect, 2010. 138(5): p. 756-763.

122. Gilbert, M.J., et al., *Livestock-associated MRSA ST398 carriage in pig slaughterhouse workers related to quantitative environmental exposure.* Occup Environ Med, 2012. 69(7): p. 472-478.

123. van Cleef, B.A., et al., *Persistence of livestock-associated methicillin-resistant Staphylococcus aureus in field workers after short-term occupational exposure to pigs and veal calves.* J Clin Microbiol, 2011. 49(3): p. 1030-1033.

124. Rafee, Y., et al., *Increased prevalence of methicillin-resistant Staphylococcus aureus nasal colonization in household contacts of children with community acquired disease.* BMC Infect Dis, 2012. 12(1): p. 45.

125. Garcia-Graells, C., et al., *Dynamic of Livestock-Associated Methicillin-Resistant Staphylococcus aureus CC398 in Pig Farm Households: A Pilot Study.* PLoS One, 2013. 8(5).

126. Bootsma, M.C., et al., *The nosocomial transmission rate of animal-associated ST398 meticillin-resistant Staphylococcus aureus.* J Roy Soc Interface, 2011. 8(57): p. 578-584.

127. Wassenberg, M., et al., *Transmissibility of livestock‐associated methicillin‐resistant Staphylococcus aureus (ST398) in Dutch hospitals.* Clin Microbiol Infec, 2011. 17(2): p. 316-319.

128. Hanson, B., et al., *Prevalence of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus (MRSA) on retail meat in Iowa.* J Infect Public Health, 2011. 4(4): p. 169-174.

129. Weese, J.S., et al., *Methicillin-resistant Staphylococcus aureus (MRSA) contamination of retail pork.* Canadian Vet J, 2010. 51(7): p. 749.

130. Weese, J., B. Avery, and R. Reid‐Smith, *Detection and quantification of methicillin‐resistant Staphylococcus aureus (MRSA) clones in retail meat products.* Lett Appl Microbiol, 2010. 51(3): p. 338-342.

131. Waters, A.E., et al., *Multidrug-Resistant Staphylococcus aureus in US Meat and Poultry.* Clin Infect Dis, 2011. 52(10): p. 1227-30.

132. Andreoletti, O., et al., *Assessment of the Public Health significance of meticillin resistant Staphylococcus aureus (MRSA) in animals and foods.* Scientific Opinion of the Panel on Biological Hazards. 2009.

133. Wulf, M.W., et al., *Infection and colonization with methicillin resistant Staphylococcus aureus ST398 versus other MRSA in an area with a high density of pig farms.* Eur J Clin Microbiol Infect Dis, 2012. 31(1): p. 61-5.

134. Carrel, M., et al., *Residential proximity to large numbers of swine in feeding operations is associated with increased risk of methicillin-resistant Staphylococcus aureus colonization at time of hospital admission in rural Iowa veterans.* Infect Control Hosp Epidemiol, 2014. 35(2): p. 190-3.

135. Casey, J.A., et al., *High-density livestock operations, crop field application of manure, and risk of community-associated methicillin-resistant Staphylococcus aureus infection in Pennsylvania.* JAMA Intern Med, 2013. 173(21): p. 1980-1990.

136. Casey, J.A., et al., *High-density livestock production and molecularly characterized MRSA infections in Pennsylvania.* Environ Health Persp, 2014. 122(5): p. 464.

137. Moritz, E.D. and T.C. Smith, *Livestock-associated Staphylococcus aureus in childcare worker.* Emerg Infect Dis, 2011. 17(4): p. 742-744.

138. Ko, G., et al., *Investigation of Bioaerosols Released from Swine Farms using Conventional and Alternative Waste Treatment and Management Technologies.* Environ Sci Technol, 2008. 42(23): p. 8849-8857.

139. Ko, G., et al., *Endotoxin levels at swine farms using different waste treatment and management technologies.* Environ Sci Technol, 2010. 44(9): p. 3442-3448.

140. Schulz, J., et al., *Longitudinal Study of the Contamination of Air and of Soil Surfaces in the Vicinity of Pig Barns by Livestock-Associated Methicillin-Resistant Staphylococcus aureus.* Appl Environ Microbiol, 2012. 78(16): p. 5666-71.

141. Masclaux, F.G., et al., *Concentration of Airborne Staphylococcus aureus (MRSA and MSSA), Total Bacteria, and Endotoxins in Pig Farms.* Ann Occup Hyg, 2013. 57(5): p. 550-557.

142. Graham, J.P., et al., *Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations.* Sci Total Environ, 2009. 407(8): p. 2701-10.

143. Ahmad, A., et al., *Insects in confined swine operations carry a large antibiotic resistant and potentially virulent enterococcal community.* BMC Microbiol, 2011. 11(1): p. 23.

144. Van de Giessen, A., et al., *Occurrence of methicillin-resistant< i> Staphylococcus aureus</i> in rats living on pig farms.* Prev Vet Med, 2009. 91(2): p. 270-273.

145. Wardyn, S.E., L.K. Kauffman, and T.C. Smith, *Methicillin-resistant Staphylococcus aureus in Central Iowa Wildlife.* J Wildl Dis, 2012. 48(4): p. 1069-73.

146. Binh, C.T., et al., *Piggery manure used for soil fertilization is a reservoir for transferable antibiotic resistance plasmids.* FEMS Microbiol Ecol, 2008. 66(1): p. 25-37.

147. Schwaiger, K., et al., *Tetracycline in liquid manure selects for co-occurrence of the resistance genes tet(M) and tet(L) in Enterococcus faecalis.* Vet Microbiol, 2009. 139(3): p. 386-392.

148. Chee-Sanford, J.C., et al., *Fate and transport of antibiotic residues and antibiotic resistance genes following land application of manure waste.* J Environ Qual, 2009. 38(3): p. 1086-1108.

149. Sobsey, M., et al., *Phase 3: Evaluation of Alternative Technologies for Pathogens*, in *Development of Environmentally Superior Technologies for Swine Waste Management*. 2005: http://www.cals.ncsu.edu/waste_mgt/smithfield_projects/smithfieldsite.htm.

150. Borjesson, S., et al., *A seasonal study of the mecA gene and Staphylococcus aureus including methicillin-resistant S. aureus in a municipal wastewater treatment plant.* Water Res, 2009. 43(4): p. 925-32.

151. Borjesson, S., et al., *Methicillin-resistant Staphylococcus aureus (MRSA) in municipal wastewater: an uncharted threat?* J Appl Microbiol, 2010. 108(4): p. 1244-51.

152. Friese, A., et al., *Occurrence of MRSA in air and housing environment of pig barns.* Vet Microbiol, 2012. 158(1–2): p. 129-135.

153. Casteel, M.J., M.D. Sobsey, and J.P. Mueller, *Fecal contamination of agricultural soils before and after hurricane-associated flooding in North Carolina.* J Environ Sci Health A Tox Hazard Subst Environ Eng, 2006. 41(2): p. 173-184.

154. Mallin, M.A. and L.B. Cahoon, *Industrialized animal production: a major source of nutrient and microbial pollution to aquatic ecosystems.* Popul Environ, 2003. 24(5): p. 369-385.

155. Anderson, M.E. and M.D. Sobsey, *Detection and occurrence of antimicrobially resistant E. coli in groundwater on or near swine farms in eastern North Carolina.* Water Sci Technol, 2006. 54(3): p. 211.

156. Heaney, C.D., et al., *Source tracking swine fecal waste in surface water proximal to swine concentrated animal feeding operations.* Sci Total Environ, 2015. 511C: p. 676-683.

157. Levin-Edens, E., et al., *Survival of environmental and clinical strains of methicillin-resistant Staphylococcus aureus [MRSA] in marine and fresh waters.* Water Res, 2011. 45(17): p. 5681-6.

158. Levin-Edens, E., et al., *Methicillin-resistant Staphylococcus aureus from Northwest marine and freshwater recreational beaches.* FEMS Microbiol Ecol, 2011. 79(2): p. 412-20.

159. Viau, E.J., et al., *Bacterial pathogens in Hawaiian coastal streams--associations with fecal indicators, land cover, and water quality.* Water Res, 2011. 45(11): p. 3279-90.

160. Goodwin, K.D., et al., *A multi-beach study of Staphylococcus aureus, MRSA, and enterococci in seawater and beach sand.* Water Res, 2012. 46(13): p. 4195-207.

161. Abdelzaher, A.M., et al., *Presence of pathogens and indicator microbes at a non-point source subtropical recreational marine beach.* Appl Environ Microbiol, 2010. 76(3): p. 724-732.

162. Plano, L.R., et al., *Shedding of Staphylococcus aureus and methicillin-resistant Staphylococcus aureus from adult and pediatric bathers in marine waters.* BMC Microbiol, 2011. 11(1): p. 5.

163. Plano, L.R., et al., *Human-Associated Methicillin-Resistant Staphylococcus aureus from a Subtropical Recreational Marine Beach.* Microb Ecol, 2013. 65(4): p. 1039-51.

164. Enns, A.A., et al., *Spatial and temporal variation in indicator microbe sampling is influential in beach management decisions.* Water Res, 2012. 46(7): p. 2237-46.

165. El-Shenawy, M.A., *Staphylococcus aureus and fecal indicators in Egyptian coastal waters of Aqaba Gulf, Suez Gulf, and Red Sea.* Egypt J Aquat Res, 2005. 31(2): p. 113-124.

166. Charoenca, N. and R.S. Fujioka, *Assessment of Staphylococcus bacteria in Hawaii's marine recreational waters.* Water Sci Technol, 1993. 27(3-4): p. 283-289.

167. Uhlemann, A.C., et al., *Evolution of community- and healthcare-associated methicillin-resistant Staphylococcus aureus.* Infect Genet Evol, 2013.

168. Statens Serum Institute, et al., *DANMAP 2010 - Use of antimicrobial agents and occurence of antimicrobial resistance in bacteria from food animals, food and humans in Denmark*. 2010: http://www.danmap.org.

169. Lekkerkerk, W.S.N., et al., *Emergence of MRSA of unknown origin in the Netherlands.* Clin Microbiol Infec, 2012. 18(7): p. 656-661.

170. Verkade, E. and J. Kluytmans, *Livestock-associated< i> Staphylococcus aureus</i> CC398: Animal reservoirs and human infections.* Infect Genet Evol, 2014. 21: p. 523-530.

171. Uhlemann, A.C., et al., *Identification of a highly transmissible animal-independent Staphylococcus aureus ST398 clone with distinct genomic and cell adhesion properties.* MBio, 2012. 3(2).

172. Mediavilla, J.R., et al., *Methicillin-susceptible Staphylococcus aureus ST398, New York and New Jersey, USA.* Emerg Infect Dis, 2012. 18(4): p. 700-702.

173. Orscheln, R.C., et al., *Contribution of genetically restricted, methicillin-susceptible strains to the ongoing epidemic of community-acquired Staphylococcus aureus infections.* Clin Infect Dis, 2009. 49(4): p. 536-542.

174. Porphyre, T., et al., *A Metapopulation Model to Assess the Capacity of Spread of Meticillin-Resistant <italic>Staphylococcus aureus</italic> ST398 in Humans.* PLoS One, 2012. 7(10): p. e47504.

175. Van den Broek, I.V.F., et al., *Methicillin-resistant Staphylococcus aureus in people living and working in pig farms.* Epidemiol Infect, 2009. 137(5): p. 700-8.

176. de Neeling, A.J., et al., *High prevalence of methicillin resistant Staphylococcus aureus in pigs.* Vet Microbiol, 2007. 122(3-4): p. 366-72.

177. Verkade, E. and J. Kluytmans, *Livestock-associated Staphylococcus aureus CC398: animal reservoirs and human infections.* Infect Genet Evol, 2013.

178. Van Cleef, B.A., et al., *Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands.* PLoS One, 2010. 5(2): p. e9385.

179. Sung, J.M.L., D.H. Lloyd, and J.A. Lindsay, *Staphylococcus aureus host specificity: comparative genomics of human versus animal isolates by multi-strain microarray.* Microbiology, 2008. 154(7): p. 1949-1959.

180. Myers, K.W., *Microbial water quality assessment near industrial swine operations in North Carolina*, in *Environmental Sciences and Engineering*. 2011, University of North Carolina at Chapel Hill. p. 75.

181. Goodwin, K.D. and M. Pobuda, *Performance of CHROMagar Staph aureus and CHROMagar MRSA for detection of Staphylococcus aureus in seawater and beach sand--comparison of culture, agglutination, and molecular analyses.* Water Res, 2009. 43(19): p. 4802-11.

182. Reischl, U., et al., *Rapid identification of methicillin-resistant Staphylococcus aureus and simultaneous species confirmation using real-time fluorescence PCR.* J Clin Microbiol, 2000. 38(6): p. 2429-33.

183. Poulsen, A.B., R. Skov, and L.V. Palleson, *Detection of methicillin resistance in coagulase-negative staphylococci and in staphylococci directly from simulated blood cultures using the EVIGENE MRSA Detection Kit.* J Antimicrob Chemoth, 2003. 51(2): p. 419-421.

184. Rychert, J., et al., *Multicenter evaluation of the Vitek MS matrix-assisted laser desorption ionization–time of flight mass spectrometry system for identification of Gram-positive aerobic bacteria.* J Clin Microbiol, 2013. 51(7): p. 2225-2231.

185. Stegger, M., et al., *Rapid differentiation between livestock-associated and livestock-independent Staphylococcus aureus CC398 clades.* PLoS One, 2013. 8(11): p. e79645.

186. European Union Reference Laboratory for Antimicrobial Resistance, *Protocol for spa Typing.*, D.N.F.I. Kongens Lyngby, Technical University of Denmark, Editor. 2009: http://www.crl-ar.eu/data/images/tc_april-2009/7-protocols for spa typing.pdf.

187. Clinical and Laboratory Standards Institute, *Performance standards for antimicrobial susceptibility testing; 20th information supplement (M100-S200)*. 2010, Clinical and Laboratory Standards Institute: Wayne, PA.

188. Fitzgibbon, M.M., A.S. Rossney, and B. O'Connell, *Investigation of reduced susceptibility to glycopeptides among methicillin-resistant Staphylococcus aureus isolates from patients in Ireland and evaluation of agar screening methods for detection of heterogeneously glycopeptide-intermediate S. aureus.* J Clin Microbiol, 2007. 45(10): p. 3263-9.

189. Soge, O.O., et al., *Characterization of methicillin-resistant Staphylococcus aureus and methicillin-resistant coagulase-negative Staphylococcus spp. isolated from US West Coast public marine beaches.* J Antimicrob Chemoth, 2009. 64(6): p. 1148-1155.

190. Baptiste, K.E., et al., *Methicillin-resistant staphylococci in companion animals.* Emerg Infect Dis, 2005. 11(12).

191. Suzuki, E., K. Hiramatsu, and T. Yokota, *Survey of methicillin-resistant clinical strains of coagulase-negative staphylococci for mecA gene distribution.* Antimicrob Agents Ch, 1992. 36(2): p. 429-434.

192. Kawano, J., et al., *Isolation of methicillin-resistant coagulase-negative staphylococci from chickens.* J Clin Microbiol, 1996. 34(9): p. 2072-2077.

193. Wang, Y., et al., *Distribution of the multidrug resistance gene cfr in Staphylococcus species isolates from swine farms in China.* Antimicrob Agents Ch, 2012. 56(3): p. 1485-1490.

194. Lewis, H.C., et al., *Pigs as source of methicillin-resistant Staphylococcus aureus CC398 infections in humans, Denmark.* Emerg Infect Dis, 2008. 14(9): p. 1383.

195. Silbergeld, E.K., J. Graham, and L.B. Price, *Industrial food animal production, antimicrobial resistance, and human health.* Annu Rev Public Health, 2008. 29: p. 151-69.

196. Fridkin, S.K., et al., *Methicillin-resistant Staphylococcus aureus disease in three communities.* New Engl J Med, 2005. 352(14): p. 1436-1444.

197. Safdar, N. and E.A. Bradley, *The risk of infection after nasal colonization with Staphylococcus aureus.* Am J Med, 2008. 121(4): p. 310-5.

198. Boucher, H.W. and G.R. Corey, *Epidemiology of methicillin-resistant Staphylococcus aureus.* Clin Infect Dis, 2008. 46 Suppl 5: p. S344-9.

199. Churchill, R.B. and L.K. Pickering, *Infections in child care centers.* Curr Opin Infect Dis, 1996. 9(3): p. 176-180.

200. Nakamura, M.M., et al., *Prevalence of methicillin-resistant Staphylococcus aureus nasal carriage in the community pediatric population.* Pediatr Infect Dis J, 2002. 21(10): p. 917-921.

201. USDA and NCDA&CS, *North Carolina Agricultural Statistics*, U.S. Dept of Agricultural National Agricultural Statistics Service and NC Dept of Agriculture and Consumer Services, Editors. 2010: http://ncagr.gov/stats/2010AgStat/index.htm.

202. Nadimpalli, M., C. Heaney, and J.R. Stewart, *Identification of Staphylococcus aureus from enriched nasal swabs within 24 hours is improved with use of multiple culture media.* J Med Microbiol, 2013.

203. Stegger, M., et al., *Rapid detection, differentiation and typing of methicillin‐resistant Staphylococcus aureus harbouring either mecA or the new mecA homologue mecALGA251.* Clin Microbiol Infec, 2012. 18(4): p. 395-400.

204. Institute, D.N.F., *Protocol for spa typing.* http://www.crl-ar.eu/data/images/tc_april-2009/7-protocols for spa typing.pdf, 2009.

205. Paule, S.M., et al., *Direct detection of Staphylococcus aureus from adult and neonate nasal swab specimens using real-time polymerase chain reaction.* J Mol Diagn, 2004. 6(3): p. 191-6.

206. Steward, C.D., et al., *Testing for induction of clindamycin resistance in erythromycin-resistant isolates of Staphylococcus aureus.* J Clin Microbiol, 2005. 43(4): p. 1716-1721.

207. Fritz, S.A., et al., *Prevalence of and risk factors for community-acquired methicillin-resistant and methicillin-sensitive staphylococcus aureus colonization in children seen in a practice-based research network.* Pediatrics, 2008. 121(6): p. 1090-8.

208. Creech, C.B., et al., *Increasing rates of nasal carriage of methicillin-resistant Staphylococcus aureus in healthy children.* Pediatr Infect Dis J, 2005. 24(7): p. 617-621.

209. Shopsin, B., et al., *Prevalence of methicillin-resistant and methicillin-susceptible Staphylococcus aureus in the community.* J Infect Dis, 2000. 182(1): p. 359-362.

210. Köck, R., et al., *Prevalence and molecular characteristics of methicillin-resistant Staphylococcus aureus (MRSA) among pigs on German farms and import of livestock-related MRSA into hospitals.* Eur J Clin Microbiol, 2009. 28(11): p. 1375-1382.

211. van Rijen, M.M.L., et al., *Livestock-Associated MRSA Carriage in Patients without Direct Contact with Livestock.* PLoS One, 2014. 9(6): p. e100294.

212. Paterson, G.K., et al., *Capturing the cloud of diversity reveals complexity and heterogeneity of MRSA carriage, infection and transmission.* Nat Commun, 2015. 6: p. 6560.

213. Wing, S., et al., *Air pollution and odor in communities near industrial swine operations.* Environ Health Perspect, 2008. 116(10): p. 1362.

214. King, M.D., et al., *Emergence of community-acquired methicillin-resistant Staphylococcus aureus USA 300 clone as the predominant cause of skin and soft-tissue infections.* Ann Intern Med, 2006. 144(5): p. 309-17.

215. Moran, G.J., et al., *Methicillin-resistant S. aureus infections among patients in the emergency department.* N Engl J Med, 2006. 355(7): p. 666-674.

216. Meera, B., et al., *Staphylococcus aureus ST398, New York City and Dominican Republic.* Emerg Infect Dis, 2009. 15(2): p. 285.

217. *An act to implement recommendations of the blue ribbon study commission on agricultural waste*, in *SB1217*. 1995.

218. Wulf, M., et al., *Prevalence of methicillin‐resistant Staphylococcus aureus among veterinarians: an international study.* Clin Microbiol Infec, 2008. 14(1): p. 29-34.

219. Chapin, A., et al., *Airborne multidrug-resistant bacteria isolated from a concentrated swine feeding operation.* Environ Health Perspect, 2005. 113(2): p. 137.

220. Gibbs, S.G., et al., *Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation.* Environ Health Perspect, 2006. 114(7): p. 1032.

221. Wing, S., *Social responsibility and research ethics in community-driven studies of industrialized hog production.* Environ Health Persp, 2002. 110(5): p. 437.

222. USDA, *United States Department of Agriculture Census of Agriculture*. 2007: http://www.agcensus.usda.gov/Publications/2007/Full_Report/.

223. Mallin, M.A., et al., *Effect of human development on bacteriological water quality in coastal watersheds.* Ecol Appl, 2000. 10(4): p. 1047-1056.

224. Wertheim, H.F., et al., *The role of nasal carriage in< i> Staphylococcus aureus</i> infections.* The Lancet Infect Dis, 2005. 5(12): p. 751-762.

225. van Belkum, A., et al., *Reclassification of Staphylococcus aureus nasal carriage types.* J Infect Dis, 2009. 199(12): p. 1820-1826.