

ENVIRONMENTAL, OCCUPATIONAL, AND MEDICAL EXPOSURES ASSOCIATED
WITH METHICILLIN RESISTANT *STAPHYLOCOCCUS AUREUS* NASAL CARRIAGE IN
PATIENTS ADMITTED TO AN EASTERN NORTH CAROLINA HOSPITAL

Leah Hope Schinasi

A dissertation submitted to the faculty of 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 Epidemiology.

Chapel Hill
2012

Approved by:

Steve Wing

Pia MacDonald

Keith Ramsey

David Richardson

Jill Stewart

ABSTRACT

LEAH HOPE SCHINASI: Environmental, occupational, and medical exposures associated with methicillin resistant *Staphylococcus aureus* nasal carriage in patients admitted to an eastern North Carolina hospital
(Under the direction of Steve Wing)

Methicillin resistant *Staphylococcus aureus* (MRSA) is a versatile human pathogen. Originally acquired in medical settings, strains later emerged in the community and, most recently, within the context of industrial livestock production. Epidemiologic research on sources of MRSA acquisition and transmission is important for designing effective infection prevention measures. The objective of this work was to investigate medical, household, environmental, and occupational exposures associated with MRSA nasal carriage identified at admission among patients at a rural tertiary care hospital.

I conducted a hospital based case control study at Vidant Medical Center (VMC), the largest hospital in eastern North Carolina, which is a region with intensive livestock production. VMC screens all admitted patients for MRSA using duplicate nasal swabs of the anterior nares. I interviewed 117 cases and 119 controls about occupational, household, and environmental exposures, abstracted information from medical charts, and used geographic mapping tools and publicly available data to estimate environmental exposures to livestock facilities. I used conditional logistic regression models to derive estimates of associations between MRSA carriage and medical, household, environmental, and occupational exposures.

In this hospitalized population, community and household exposures were important predictors of MRSA carriage. Also, MRSA nasal carriage was associated with living near moderate densities of swine. This work represents an important baseline investigation and demonstrates the need for further research of environmental and occupational exposures that could be related to MRSA carriage with healthier populations.

ACKNOWLEDGEMENTS

I have many people to thank for their contributions and support. Amy Ising, the program manager at the NC Disease Event Tracking and Epidemiologic Tool, first connected me, via Bill Cleave, with Keith Ramsey from Vidant Medical Center's (VMC) Division of Infection Control, and thus put the forces in motion for me to conduct this research at VMC. I am grateful to Keith Ramsey for supporting my research at VMC. Also, the staff in VMC's Division of Infection Control provided essential project support and assistance. In particular, Delores Nobles helped design the participant recruitment protocol; Kerri Augustino conducted the laboratory work and provided critical input regarding interpretation of molecular results; and Darlene King provided daily administrative assistance. I am also grateful to Meghan Davis, Amy Peterson, Ellen Silbergeld, and Christopher Heaney for their recommendations during the preliminary planning and designing of this study. In addition, I am grateful to Devon Hall, Dothula Baron, Jennifer Hall, Clifton Smith, Norma Mejia, and Amy Lowman for their advice when I designed the structured interview questions.

My father, Michael Schinasi, assisted with the translation of the interview materials. He and my mother, Meryl Besterman, provided support as I completed the doctoral program. They also provided me with room and board and much needed extra support while I conducted in-hospital interviews in Greenville. Members of my dissertation support group--Kirstin Huiber, Kim Angelon Gaetz, Margaret Adgent, Quaker Harmon, Petra Sander, Steve Lippman, Sarah Rhea, Anne Hakenworth, and Jonathon Todd--provided critical support and

thoughtful and creative input throughout all stages of my dissertation work. Jessica Rinsky provided thoughtful input on the presentation and interpretation of information related to MRSA. Ghassan Hamra served as my unofficial second dissertation support group; he offered helpful analytic suggestions, patiently listened to me talk about MRSA, livestock and the trials and tribulations of data collection on countless occasions, and cooked me many delicious dinners along the way. I am grateful to all of my committee members, who provided input and support throughout various stages of the planning and implementation of this research. In particular, I owe a special thank you to Steve Wing; he advised me even before and throughout graduate school and taught me the importance and power of careful language and of maintaining a critical perspective.

TABLE OF CONTENTS

LIST OF TABLES	x
LIST OF FIGURES	xi
LIST OF EQUATIONS	xii
LIST OF ABBREVIATIONS.....	xiii
Chapter	
I. Background.....	1
Overview.....	1
<i>S. aureus</i> biology	2
History of MRSA.....	3
Molecular typing.....	4
<i>S. aureus</i> as a commensal organism	8
Duration of carriage	9
Community and healthcare associated MRSA	10
Epidemiology of MRSA infections	11

Epidemiology of MRSA carriage	12
Animals, livestock, and MRSA	15
Phenotypic characteristics of MRSA CC398.....	19
Antibiotic use and industrial animal production.....	20
Transmission of MRSA in the environment	22
Public health relevance of livestock associated MRSA.....	25
Livestock production in eastern North Carolina.....	26
Summary.....	26
Figure	27
II. Specific aims	28
III. Materials and methods	33
Study design and setting	33
MRSA screening and typing.....	33
Inclusion criteria	35
Participant identification.....	35
Enrollment and interviews	36
Medical record review	37

Data checking and cleaning	38
Geocoding	38
Identification of concentrated animal feeding operations near participant homes	39
Human and swine population densities and rural area classifications	39
Statistical analysis	40
Variables	43
Table	51
 IV. Medical and household exposures associated with methicillin resistant <i>Staphylococcus aureus</i> nasal carriage in patients hospitalized at an eastern North Carolina hospital	 52
Introduction	52
Methods	53
Results	58
Discussion	62
Figure	66
Tables	67
 V. Environmental and occupational exposures associated with methicillin resistant <i>Staphylococcus aureus</i> nasal carriage in patients hospitalized at an eastern North Carolina hospital	 72

Introduction.....	72
Methods	73
Results.....	81
Discussion.....	87
Tables.....	92
VI. Conclusion.....	95
Background.....	95
Summary of work conducted.....	96
Discussion and implications for future work.....	99
Conclusions.....	103
APPENDIX.....	105
REFERENCES	118

LIST OF TABLES

Table

3.1	Comparison of information derived from medical charts and in-hospital interviews describing antibiotic use in the past year.....	51
4.1	Characteristics and diagnoses of methicillin resistant <i>Staphylococcus aureus</i> nasal carriers and their matched controls	67
4.2	Distribution of first-listed diagnoses for the current hospitalization among methicillin resistant <i>Staphylococcus aureus</i> nasal carriers and their matched controls, categorized according to chapters from the International Classification of Diseases, ninth edition (ICD-9).....	68
4.3	Estimates of association of methicillin resistant <i>Staphylococcus aureus</i> nasal carriage with medical and household exposures from a multivariable conditional logistic model	69
4.4	Estimates of association of methicillin resistant <i>Staphylococcus aureus</i> nasal carriage with medical and household exposures, derived from multivariable conditional logistic model, stratified by culture status	70
4.5	Estimates derived from univariable logistic models conditioned on age and gender, comparing community associated and healthcare associated methicillin resistant <i>Staphylococcus aureus</i> (MRSA) nasal carriers to their matched controls.....	71
5.1	Characteristics of methicillin resistant <i>Staphylococcus aureus</i> nasal carriers and their matched controls	92
5.2	Estimates of associations of methicillin resistant <i>Staphylococcus aureus</i> nasal carriage with environmental and occupational exposures, derived from conditional logistic regression models	93
5.3	Estimates of association of methicillin resistant <i>Staphylococcus aureus</i> nasal carriage with environmental and occupational exposures from conditional logistic models adjusted for education, stratified by culture status	94

LIST OF FIGURES

Figure

1.1. Permitted swine in each North Carolina block group 27

4.1. Numbers of cases and controls enrolled and included in
the conditional analysis..... 66

LIST OF EQUATIONS

$\log(p/1-p)=\alpha_i + \sum c_i \gamma_i$	40
$\log(p/1-p)= 4.9E-5 x (a-b) - 1.8 E-9 x (a^2-b^2) + 1.2E-14 x (a^3-b^3)$	84
$\log(p/1-p)= 4.8E-4 x (a-b) - 9.5E-8 x (a^2-b^2)$	84
$\log(p/1-p)=1.6E-3 x (a-b) - 1.9E-9 x (a^2-b^2) + 4.4E-10 x (a^3-b^3)$	84

LIST OF ABBREVIATIONS

β	Beta
CA-MRSA	Community associated MRSA
CAFO	Concentrated animal feeding operation
CC	Clonal complex
DWQ	Division of Water Quality
HA-MRSA	Healthcare associated MRSA
ICD-9	International Classification of Diseases, 9 th edition
Ln	Natural logarithm
MLST	Multi locus sequence typing
MGE	Mobile genetic elements
MRSA	Methicillin resistant Staphylococcus aureus
MSSA	Methicillin susceptible Staphylococcus aureus
NHAMES	National Health and Nutrition Examination Survey
NT-MRSA	Non-typeable MRSA
OR	Odds ratio
PBP	Penicillin binding protein
PFGE	Pulsed field gel electrophoresis
rep-PCR	Repetitive Polymerase Chain Reaction
ROS	Reactive oxygen species
S. aureus	Staphylococcus aureus
SCC	staphylococcal cassette chromosomes

SD	Standard deviation
SE	Standard error
Spa	staphylococcal protein A
ST	Sequence type
VMC	Vidant Medical Center

CHAPTER 1

Background

Overview

Methicillin resistant *Staphylococcus aureus* (MRSA) is a versatile human pathogen that has evolved resistance to methicillin and other beta (β)-lactam antibiotics [1]. In the United States in 2005, MRSA caused an estimated 94,360 invasive infections and 18,650 deaths [2].

MRSA has a long history that continues to evolve. MRSA was first identified in the United States in the 1960s in tertiary care hospitals. At this time, MRSA predominantly affected elderly and sick patients [1]. Then, in the early 1990s, MRSA was detected in younger and healthier people who did not have any of the traditional medical exposures, such as surgery or hospitalization, associated with MRSA acquisition [3]. Around this time, companion animals were recognized as potential vectors for MRSA transmission [4]. Most recently, new strains of MRSA were identified in humans and associated with livestock [5, 6]. Community associated (CA), health care associated (HA), and livestock associated (LA) strains of MRSA are genotypically and phenotypically distinct [7]. However, CA strains are now causes of HA infections [2, 8] and people without any recent medical exposures might be carrying CA strains [9].

In the United States, compared to methicillin susceptible *S. aureus* (MSSA), the prevalence of MRSA has increased over time. This trend has been attributed to the

emergence of new CA strains. The decrease in MSSA might be due to increased use of antimicrobials at the population level, which promotes resistant strains [10]. In parts of Europe, increased prevalence of MRSA carriage and infection has been attributed to the emergence of LA MRSA [11].

S. aureus has a remarkable ability respond to environmental pressures [12, 13]; this is demonstrated by the bacteria's history and biology. Epidemiologic research and surveillance are essential for identifying novel strains of MRSA and for designing effective interventions and prevention strategies. In the sections below, I outline MRSA's history, biology, and epidemiology, introduce key terms and concepts, and identify remaining knowledge gaps and research needs that support the importance of my dissertation work.

***S. aureus* biology**

S. aureus is a member of the *staphylococci* genus [14]; it is a prokaryotic cell [15] and a gram positive bacteria [14] that appears as clusters of cocci under a microscope [1]. *S. aureus* has a cell wall that is 50% peptidoglycan in weight. The peptidoglycan chains that make up the cell wall are cross-linked by tetrapeptide chains that are bound to N-acetylmuramic acid and a pentaglycine bridge, the latter of which is exclusive to *S. aureus* [1].

S. aureus has a circular chromosome that contains core and accessory genomes [1]. The core genome contains genes that are necessary for cell survival--genes that encode molecules that are involved in DNA and RNA synthesis and cellular replication, for example. The accessory genome consists of mobile genetic elements (MGEs)--plasmids, transposons, insertion sequences, bacteriophages, pathogenicity islands, and staphylococcal cassette chromosomes (SCC). MGEs encode proteins, such as resistance and virulence factors, that

allow the bacteria to adapt to different ecological niches [16]. Bacteria sometimes transfer MGEs from one to another via horizontal gene transfer [16]. Horizontal gene transfer allows *S. aureus* to survive in new environments [16]. Benign, antibiotic resistant bacteria can transfer resistance genes to pathogenic bacteria; therefore, even selection for resistant, commensal bacteria can be dangerous [17].

S. aureus reproduce through the process of binary fission, which is an asexual process that results in genetically identical offspring. Sometimes mutations occur; these cause offspring to differ from their parents in terms of their genetic make-up [18].

These structural and reproductive characteristics allow the bacteria to respond to exposures to antibiotics [16]. Bacteria that harbor resistance genes are able to survive in the presence of antibiotics, while susceptible strains die. The resistant bacteria then produce genetically identical bacteria. In addition to naturally selecting for resistant bacteria, low levels of antibiotic treatments may stimulate bacteria to form reactive oxygen species (ROS), which lead to the development of mutations that allow resistance [19].

History of MRSA

Penicillin is beta (β)-lactam antibiotic, meaning that it has a β -lactam nucleus in its molecular structure. It was introduced into clinical practice in the 1940s. Soon after its introduction, *S. aureus* developed resistance to the antibiotic by producing penicillinase, which is a β -lactamase enzyme. Penicillinase destroys penicillin by hydrolyzing the amide bond of the β -lactam ring of penicillin. Production of penicillinase is encoded by the structural gene *blaZ*, which is controlled by the regulatory genes *blaI* and *blaRI*[20].

Methicillin and other β -lactam antibiotics were developed in response to penicillin resistance [20]. Methicillin is a semi-synthetic penicillin that was designed to resist

hydrolysis by penicillinases. Methicillin and other β -lactam antibiotics are substrate analogs of penicillin-binding proteins (PBP), which catalyze the construction of the cross-links of peptide that occur between the glycan chains in the cell wall. These antibiotics inhibit PBPs and cause the bacterial cell wall to weaken, resulting in eventual lysis and death [21].

Methicillin resistance in *S. aureus* is attributable to the bacteria's expression of an alternative penicillin-binding protein, known as PBP2a or PBP2'. PBP2a has low affinity for all β -lactam antibiotics. Therefore, methicillin resistance refers to the bacteria's ability to resist all β -lactam antibiotics. Today, use of methicillin has by in large been replaced by other antibiotics such as oxacillin. However, methicillin resistant *S. aureus* is the term that is most commonly used to describe resistance to β -lactam antibiotics [20].

The *mecA* gene, which is located on the chromosome in a MGE called the staphylococcal cassette chromosome *mec* (*SCCmec*), encodes PBP2a. The *SCCmec* is located in the staphylococcal chromosome, inside a gene called the *orfX*, which has two recombinase genes, *ccrA* and *ccrB*. These genes aid the integration and excision of *SCCmec*. *SCCmec* can also integrate other mobile elements or resistance genes [20]. There are different types of *SCCmec*, denoted using a number, which vary depending on their structural composition [14].

Molecular typing

Because MRSA is clonal and infectious and because it is able to transfer genetic material and respond to different environments, studying the genetic make-up of different MRSA strains is useful for identifying the evolutionary history and sources for colonizing or infecting bacteria.

Numerous methods for molecular typing of MRSA exist. Techniques for genotyping of MRSA include the following: pulsed field gel electrophoresis (PFGE), repetitive polymerase chain reaction (rep-PCR), multilocus sequence typing (MLST), staphylococcal protein A gene typing (*spa*-typing), *SCCmec* typing [1, 22] and whole genome sequencing (WGS) [23]. I briefly describe each of these below.

Pulsed field gel electrophoresis

Until recently, pulsed field gel electrophoresis (PFGE) using the restriction enzyme *SmaI* was considered the gold standard for ascertaining relatedness of MRSA isolates. With PFGE, bacteria are set in agarose and lysed in situ. The chromosomal DNA is then digested (cut into small fragments) using *SmaI*. Portions of agarose, which have the chromosomal DNA fragments, are inserted into wells of an agarose gel. On the basis of size, the restriction patterns of the isolates are situated into a pattern of discrete bands in the gel. These restriction patterns can then be compared to one another to determine the relatedness of isolates [24]. However, livestock associated MRSA strains were not typeable using PFGE with *smaI* digestion [6]. Bens et al. [25] later found this non-typeability was due to a DNA methylation system. LA MRSA is typeable using PFGE with the APAI [26] or EAGI enzymes [27].

Repetitive polymerase chain reaction

The Diversilab® Staphylococcus kit for DNA fingerprinting (bioMerieux, Boxtel, The Netherlands) is a commercial package that is useful for typing within clinical laboratories since it provides results in shorter time periods compared to PFGE. Additionally,

the system does not require DNA sequencing facilities, as does the use of MLST or *spa*-typing. The system works by amplifying repetitive non-coding sequences in the genome, separating fragments using electrophoresis, and comparing the size of these fragments to determine genetic relatedness between strains and to compare strains to the DiversiLab® MRSA library, which contains 70 samples of 14 representative USA pulsed field gel electrophoresis types [28]. The Diversilab® system has been shown to be useful for typing MRSA isolates for potential outbreaks; however, the system is not considered to be as discriminatory as PFGE [29].

Multi locus sequence typing

With MLST, bacterial isolates are characterized based on sequence analysis of fragments of 7 housekeeping genes (*arcC*, *aroE*, *glpF*, *gmk*, *pta*, *tpi*, and *yqiL*); these housekeeping genes are about 500 base pairs in length. For each fragment, the sequences are assigned distinct alleles. The alleles of the genes are used to characterize the lineage of *S. aureus* and to assign a sequence type (ST) that corresponds to the allelic profile. The ST for an allelic profile can be accessed from the MLST website (<http://www.mlst.net/>) [30]. There are numerous alleles at each of the 7 loci; therefore, it is unlikely that isolates will have identical allelic profiles [31, 32].

Isolates with the same allelic profile may be designated as members of the same clone [31, 32]. The algorithm based upon related sequence types (BURST) can be used to characterize the clonal complex (CC) to which the isolate belongs (<http://eburst.mlst.net>) [30]. A CC represents a more general classification; grouping of STs is based on sharing an allelic identity with at least one other ST. Strains of *S. aureus* can be defined as belonging to

the same CC based on a similarity threshold set by the user. The ancestor of a CC is the ST with the most single locus variants [30].

Spa-typing

Spa-typing is simpler than MLST, since it involves DNA sequencing of only one gene, the polymorphic X, or short sequence repeat region (SSR) of the protein A gene (*spa*). The polymorphic X region contains a variable number of 24-base pair (bp) repeats. The SSR region is biologically diverse, which might be due to deletion and duplication of repetitive units and point mutations [33]. *Spa* types can be found at a public *spa* type database (<http://tools.eugenomics.com/>) and at the Ridom Spa Server (<http://spaserver.ridom.de/>). These 2 databases provide slightly different *spa* type assignments. For example, the same isolate would be described as *spa1* by the public *spa* type database but as *spa008* by the Ridom Spa Server [31]. It is possible to classify *spa* types into clonal complexes using the algorithm based upon repeat pattern (BURP). A *spa* type may correspond to several STs, but the *spa* types remain within an assigned clonal cluster [30].

Because *spa*-typing involves sequencing of a single locus, it is a cheaper, less labor intensive, and less time consuming compared to MLST. An overall good concordance between PFGE, MLST, and *spa*-typing combined with BURP analysis has been observed [30]. However, a disadvantage of *spa*-typing is that it sometimes lacks discriminatory power, since the same or related *spa* loci might be in different clonal lineages, or because there may be related *spa* repeat successions in different *S. aureus* lineages.

SCCmec typing

Typing of the *SCCmec* element is common. This method is based on identifying the different structural characteristics of *SCCmec* chromosome in order to characterize its type [15]. Detection of the *SCCmec* chromosome is also used to identify methicillin resistance.

Whole genome typing

Whole genome typing has been described as superior to *spa*-typing and MLST, especially for source tracking and evolutionary studies. It better characterizes variations within ST and CC groups. In contrast, other methods, like *Spa*-typing and MLST, may be limited due to homoplasy, lateral gene transfer, and/or homologous recombination [23, 34].

***S. aureus* as a commensal organism**

In addition to being a dangerous pathogen that causes invasive human infections, *S. aureus* is also commensal [35]. An asymptomatic person with MRSA on their body is known as a MRSA carrier, or as being MRSA colonized. Carriers of MRSA are more likely to develop bacterial infections; they may also spread the bacteria to other people with whom they come into contact [1]. Based on data from the National Health and Nutrition Examination Survey (NHANES), approximately 84 million and 2 million non-institutionalized people living in the United States between 2001 and 2002 were colonized with MSSA and MRSA, respectively [36].

Someone who is identified as a MRSA carrier might either be a persistent or intermittent carrier, and a person who appears to be a non-carrier might actually be an intermittent carrier [37]. The distinction between persistent and intermittent carriage is important not only from a research perspective but also from a public health practice one;

those who are persistently colonized carry higher loads of bacteria and are more likely to develop *S. aureus* infections [37].

In humans, the main ecological niche for *S. aureus* is the anterior nares [1]. Approximately 20% of people are chronically nasally colonized by *S. aureus* and 30% are intermittently colonized. Persistent nasal carriage of *S. aureus* might result from the bacteria's introduction into the nose via nose picking, for example, in combination with nasal trauma [37]. Other parts of the body might be colonized by *S. aureus*--the skin, perineum, pharynx, for example, and less commonly, the gastrointestinal tract, vagina, and axillae [37].

Hands are the main vector for transmitting *S. aureus* from the environment into the nose, and vice versa [37]. For example, many nosocomial *S. aureus* infections are acquired from the hands of health care workers [1]. Although less common, *S. aureus* can also reach the nose directly through the air; this is an important mechanism since it causes dispersal of the bacteria to many different sources, which the hands might then touch and subsequently introduce into the nose [37].

Duration of carriage

Relatively few longitudinal studies of MRSA have been conducted; therefore, information on duration of carriage is sparse. In a study in Pennsylvania, among 8 index cases, defined as patients who presented to the hospital with soft tissue MRSA infections, the average duration of colonization was 33 days and ranged from 14 to 104 days. Mean duration of colonization among 3 household members who were also MRSA colonized was 54 days, with a range of 12 to 94 days [38]. In a study of admitted and readmitted patients in Chicago, there was a 50% decrease in prevalence of colonization in less than 1 month. After

300 days, however, the prevalence of colonization had not declined much below 50%, and prevalence of colonization never decreased much below 20%. This finding suggests that some people are decolonized quickly whereas others are chronic MRSA carriers [39]. In another hospital-based study, for patients who were readmitted at least once, the half-life time for persistence of MRSA carriage was 566 days [40]. Factors that have been shown to predict longer duration of carriage include having a household member who is concurrently colonized [38], being colonized at multiple anatomical sites, and anatomical site of colonization [40].

Recent research has suggested that the predominant strain of LA MRSA in the United States, CC398, is not as persistent of a human colonizer compared to others [7]. For example, workers who are carriers of MRSA ST398 have been shown to become decolonized during periods of non-contact with livestock [41, 42].

Community and healthcare associated MRSA

HA MRSA was detected in the 1960s in United States hospitals but did not become a serious problem until the 1980s [43]. Then, in 1993, Western Australia residents who had not visited a health care facility in the previous year were found to be infected or colonized with new strains of MRSA [44], representing the identification of CA MRSA. Around this same time, CA MRSA was detected in the United States [3, 45, 46]. Overall, since the late 1990s, the proportion of *S. aureus* infections that are resistant to methicillin have increased in the United States, largely because of increases in the prevalence of CA MRSA strains [47].

HA and CA MRSA strains differ genetically. CA MRSA is believed to have emerged as a result of CA-strains of MSSA acquiring *SCCmec*. CA MRSA strains tend to carry *SCCmecIV* or *SCCmecV*, which are the smallest of the *SCCmecs*. These two types of *SCCmec* tend to be susceptible to a number of non- β -lactam antibiotics. In contrast, HA MRSA strains generally carry larger *SCCmec* types that are multi-drug resistant [35].

Over time, there has been a mixing of HA and CA strains. For example, hospital acquired infections have been attributed to strains that were classified as CA [48], and a recent hospital-based study found that a high proportion of patients who had not been hospitalized in the past 6 months were carrying HA strains [9]. The most common CA strain in the United States, USA300, has developed unusual plasmid-mediated resistance phenotypes, probably due to its introduction into human medicine settings [49]. Because of this mixing, CA and HA MRSA are sometimes defined based on the type of exposures a person has received. A commonly used definition is that a MRSA infected or carrying person with any of the following types of exposures is classified as having acquired their MRSA in a healthcare setting—as a result of current or recent hospitalization, use of an indwelling venous catheter, residence in a long term care or rehabilitation hospital, having recently had surgery, or dialysis [2, 50].

Epidemiology of MRSA infections

MRSA infections have been associated with a number of exposures, including the following: among children, having a parent who works at a school or daycare; use of antibiotics in the six months prior to a positive MRSA culture; having a family member with

history of skin boils [51], presence of a wound [52], contact with school athletic facilities such as locker rooms or training areas [53], participation in athletics, especially contact sports [54, 55], incarceration [56], men who have sex with men [57, 58], being a member of the military [59], being over the age of 65 [60] or under the age of 2, injection drug use [61], having a recent influenza like illness or severe pneumonia, having a concurrent skin and soft-tissue infection [62], having a history of MRSA colonization or infection [62], or having had close contact with a person (eg. living in the same household) who was infected with MRSA [62]. In Hong Kong, CA MRSA infections were positively associated with sharing of personal items (towels, razors, nail clippers, for example) and negatively associated with hand-washing and with acne [63]. In the Southeastern United States, Ferreira et al. [64] compared visitors to an outpatient clinic who had MRSA infections (cases) with pet-owners utilizing a veterinary clinic. They found that the following variables were associated with MRSA infection: living with children, having a family member who was diagnosed with MRSA in the past year, being hospitalized in the past year, being diagnosed with a disease or having taken a medication that affects immune function, and having been treated with antibiotics in the past year. Companion animals inside the home have also been implicated as potential sources of MRSA infection or re-infection. Ferreira et al. identified identical strains of MRSA in pets and their owners [64].

Epidemiology of MRSA carriage

Variables related to MRSA carriage as opposed to infection are not as well researched. Research has suggested that *S. aureus* carriage is not related to seasonality,

temperature, or relative humidity [37]. In a study using data from the National Health and Nutrition Examination Survey (NHANES) in 2001-2002, the following characteristics were related to MSSA carriage: being younger than age 65, male, having less than a high school education, and having asthma. The following characteristics were associated with MRSA carriage: being age 65 or older, female, diabetic, and residing in a long-term care facility in the previous 12 months. Compared to whites, a lower proportion of Hispanic individuals were MRSA colonized. The authors of this study speculate that some of the differences in risk factors for MRSA versus MSSA carriage might reflect traditional HA MRSA risk factors [36]. Kuehnert performed a similar analysis using NHANES data from 2001-2002 and found similar results [62].

Gorwitz et al. performed an updated analysis that included data from NHANES from 2003-2004 [10]. For the years 2003-2004, MSSA carriage was more prevalent among non-Hispanic whites and Mexican Americans compared to non-Hispanic blacks, and among people under the age of 20 compared to older adults. MSSA carriage was also associated with being overweight. For the years 2001-2004 combined, compared to non-foreign born women, a lower proportion of adult females who were foreign born were MSSA carriers. Comparing years 2003-2004 to 2001-2002, the prevalence of MRSA carriage increased among males but not among females. Whereas in the years 2001-2002 the prevalence of MRSA carriage was lower among men than women, this gender difference essentially disappeared in the years 2003-2004. For the years 2001-2004 combined, MRSA carriage was more common in people aged 60 years or older. Neither BMI nor education was associated with MRSA colonization. Among males, MRSA carriage was associated with

being hospitalized in the previous year. Among females, MRSA carriage was associated with being 60 or older, diabetic, and having a household income below the poverty level [10].

More recently, a multi-center study of hospital admissions in Scotland showed MRSA carriage was associated with older age, having a high frequency of prior hospital admissions, having been admitted from someplace other than home, and having been admitted for a medical emergency rather than for an elective surgery [65]. A study of long term dialysis patients in Taiwan found that nasal MRSA carriage was strongly associated with nursing home admission, nasogastric tube feeding, and congestive heart failure [66]. Other underlying conditions, such as diabetes, COPD, cellulitis, folliculitis, being long-term bed ridden, and having a previous hospitalization were not as predictive of MRSA colonization; however, sample sizes were very small for some exposures, making it difficult to draw conclusions. In a hospital-based study in Georgia, place of residence (residential vs. alternative housing), skin or soft tissue infection diagnosis at admission, history of MRSA colonization or infection in the past 12 months, HIV infection, incarceration in the past 12 months, hospitalization history, and previous antibiotic use were related to MRSA colonization [67].

Animals such as horses [68], poultry [69-71], cats [72-74], dogs [72-74], pigs [5, 54, 75-77], veal calves [78], and cows [6] have also been shown to be vectors for MRSA carriage. MRSA has also been found in animal food products, including milk and cheese [79] and retail meat [80-82]. However, the extent to which meat handling plays a role in human MRSA carriage and transmission remains unclear.

Occupational exposures within medical settings have been shown to be strongly associated with MRSA carriage and transmission. In a study of 256 health care practitioners,

the prevalence of MSSA and MRSA nasal carriage was 43.8% and 15.2%, respectively [83]. When paramedics, physicians, nurses, and clerical or non-clinical workers were compared, paramedics had the highest prevalence of MSSA carriage but physicians had the lowest prevalence (57.7% vs. 38.5%). The highest prevalence of MRSA carriage was in nurses and the lowest was in paramedics (10.5% vs. 1.9%). In another study at 5 different teaching hospitals in Pittsburgh, PA [84], among 255 emergency department workers receiving nasal cultures, there was a 4.3% prevalence of MRSA, which is higher than the national estimates. Interestingly, all MRSA positive health care workers were nurses, nursing assistants, or patient care technicians; none worked as physicians, physician's assistants, or in clerical or social services positions.

Veterinary personnel may also be exposed to MRSA. Among 417 individuals who attended a veterinary conference, 6.5% had MRSA in their nasal cavities. Of those with MRSA, 15.6% worked in large-animal and 4.4% in small-animal practices [74]. In a more recent study, 17.3% of 341 veterinary personnel (veterinarians and technicians) screened positive for MRSA [85]. Most recently, contact with animals through work in slaughterhouses [71, 86], pig stables [87], with horses [68], and more generally as pig farmers [54, 75, 76] has been associated with MRSA carriage.

Animals, livestock, and MRSA

The connection between livestock and MRSA represents the newest development in the bacteria's epidemiology. In the first years of the 21st century, new strains of MRSA were identified and associated with animal production. The new strains are known as livestock

associated (LA). In North America and Europe, the predominant LA MRSA strain belongs to CC398 [88].

In one of the first studies to identify CC398, Armand-Levre found identical MRSA sequence types on pigs and pig farmers, suggesting potential exchange of MRSA between the groups [89]. Around this same time, in the Netherlands a 6 month old and her 2 parents who lived on a farm and raised pigs were colonized with MRSA strains that were non typeable (NT) using PFGE with *SmaI* restriction; this finding suggested a newly emergent strain of MRSA [5]. Subsequently, a survey in the Netherlands showed that 23% of 26 pig farmers were colonized with NT MRSA [5]. Also in the Netherlands, 8 of 10 pigs on a farm were colonized with the same MRSA strains as family members who lived on the farm, suggesting pig to human transmission of the bacteria [76]. In an important study, also in the Netherlands, Van Loo et al. found that individuals colonized with NT MRSA had approximately 9 times the odds of contact with pigs and 13.5 times the odds of contact with cattle, compared to individuals colonized with MRSA that was typeable with PFGE [6]. In this study, MLST showed that 32 of the 35 study participants with NT MRSA were colonized with MRSA ST398. Van rijen et al. reported an increase in MRSA incidence in a Netherlands hospital between 2002 and 2006, and they attributed this increase to the emergence of MRSA CC398 [90].

MRSA CC398 has been observed on pigs [77], chickens [71], turkeys [91], cattle [26], and horses [92], and in retail meat products [81, 93]. It has been found on workers at pig [77, 86] and broiler slaughterhouses [71].

Other genetic strains of MRSA, aside from CC398, have been associated with livestock contact—for example, *spa*-type t1430, which belongs to MLST ST9 [71], and *spa*-

type t3992 and ST1379, which is a member of CC97 [94]. In Asia, the predominant LA-strain of MRSA is ST9 [7].

LA MRSA has been detected in numerous countries, including Spain [94], Southeast Austria [95], Belgium [54, 69, 70], Denmark [96], Germany [97], Italy [98], and Korea [99]. Compared to Europe, research in North America on the relationship between human MRSA carriage and animals is less extensive. In a cross-sectional survey of 20 farms in Ontario, Canada [75], Khanna et al. found that 25% of 285 pigs and 20% of 25 workers were MRSA colonized. The most frequently detected MRSA strains were genetically related and belonged to CC398. In the first published study of this relationship in the United States, Smith et al. [27] surveyed 2 swine production facilities in Iowa and Illinois and observed an overall 45% prevalence of MRSA colonization in pig caretakers and a 49% prevalence in pigs. MLST analysis on a subset of isolates indicated that workers and pigs were carrying MRSA ST398. Interestingly, the authors only detected MRSA in humans and pigs at one of the 2 swine production facilities that they surveyed. The authors speculate that this could be due differences in the source of the pigs at the 2 production facilities (Canada for the facility at which MRSA was detected and Michigan for the other) and/or the age of the facility (the facility at which MRSA was detected was older and raised more pigs than the other). The 2 facilities also raised different breeds of pig.

More recently in the United States, Larson et al. sampled showers at 2 pig production systems in Iowa and Illinois. The authors detected MRSA in 1 of 30 samples at one system and in 18 of 70 at the second. Interestingly, at one site within the second production facility, 50% of the swine were colonized with MRSA, but none of the shower samples cultured positive for the bacteria. The shower was separate from the swine barn. This might imply that

the separation of the barns from the dust prevents airborne spread of MRSA [100]. In an anonymous survey of pork producers in the United States that was conducted in 2008, 3.7% of respondents reported a MRSA skin or soft tissue infection [101].

Researchers have investigated characteristics that predict MRSA carriage in animals and in human workers. For example, spending more hours working in the stable, more time feeding calves, conducting veterinary care, and managing the stable have been associated with LA MRSA carriage in humans [78]. Job tasks have also been investigated as factors that could be related to MRSA carriage. Smith et al reported that workers who did not obtain blood or other specimens from pigs had higher odds of MRSA carriage compared to those who participated in these tasks [27]. Factors such as animal age (younger) [27, 78, 102], antibiotic use [103], and group treatment with antimicrobials [102] have been shown to be associated with MRSA carriage in the animals.

Research findings have also suggested that MRSA CC398 is less transmissible compared to other CA and HA strains. This implies that, at this point at least, LA MRSA might represent a greater threat to those who have occupational rather than environmental or community exposures to the bacteria. For example, in Dutch hospitals, transmission of MRSA ST398 was shown to occur less frequently compared to other MRSA strains [104]. A German study observed minimal transmission of MRSA CC398 from workers to their household members. In this study, 86% of pig farmers and 45% pig veterinarians were carriers of MRSA CC398; respectively, only 4% and 9% of their family members without contact with pigs were carrying the LA-MRSA strain [97]. In a study in the Netherlands, MRSA was observed in 33% of 97 veal calf farmers and in 28% of 2,151 veal calves on 102 farms, but in only 8% of the farmers' 259 family members [78]. A Canadian study observed

some but infrequent transmission of MRSA ST398 from workers to household members [97]. A study in the Netherlands found MRSA ST398 carriage in 1 of 534 people without livestock contact. The prevalence in this group was much lower than that among those with livestock contact (13 of 49) [86].

The epidemiology of LA MRSA could be changing, however. For example, recently in Iowa, MSSA ST398 was cultured from the nose and throat of a childcare worker who did not have any contact with livestock [105]. Some have argued that lower rates of transmissibility of MRSA CC398 might have partially to do with patient-related factors. A recent study of admitted patients in Germany showed that a higher proportion of patients carrying MRSA CC398 upon admission were younger, had shorter lengths of stay in the hospital, were men, had invasive measures (endoscopies, catheterizations, etc) and had a lower mean number of International Classification of Diseases (ICD) diagnoses [106]. However, low transmissibility of the strain might have also to do with phenotypic characteristics of LA strains, which I discuss below.

Phenotypic characteristics of MRSA CC398

Generally, MRSA CC398 is resistant to tetracycline and beta-lactam antibiotics. However, other resistance profiles have been observed [26]. For example, MRSA CC398 has been found to be resistant to zinc chloride [107]. Numerous *SCCmec* types have been identified in MRSA CC398—including types II, III, IV, IV1, and V, as well as nontypeable *SCCmec* cassettes. CC398 generally lacks the toxin genes, such as Panton-Valentine leukocidin (PVL), found in other strains of MRSA [23, 88, 108]. A recent whole genome sequencing phylogenetic analysis suggested that ancestors of MRSA CC398 were human

strains of MSSA that acquired the *SCCmec* cassette after being transmitted to livestock. Furthermore, human MSSA strains carried human innate immunomodulatory genes, but LA MRSA CC398 lacked these genes, which play an important role in human niche adaptation. This analysis suggests that the MRSA strains lost the human niche genes after they were introduced to nonhuman hosts [23]. Another recent study compared the genome of MSSA ST398, a virulent resistant strain of MSSA that has recently caused dangerous infections and is easily transmissible between humans, to that of the LA MRSA ST398 [109]. The comparison showed that the genome of the human associated MSSA strain was better adapted to the human host compared to LA MRSA ST398; the human associated strain carried human-specific immune evasion cluster genes. In addition, the human strain demonstrated enhanced adhesion to human skin keratinocytes and keratin.

Phenotypic heterogeneity has been observed within CC398. Recently, researchers observed different resistance and PFGE patterns among MRSA CC398 isolates collected from the same farm. The investigators speculate that this might result from importation of animals from different places, or diversification of the strain through horizontal gene transfer, genetic rearrangements, or changes of *spa* types resulting from the loss or acquisition of single *spa* repeats [26].

Antibiotic use and industrial animal production

In industrialized animal production facilities, livestock are raised indoors in confinement, creating situations that allow for easy transmission of pathogens [110]. In North Carolina for example, thousands of confined swine produce massive amounts of waste, which falls through slats in the house floor. The waste is then flushed into on-site, open

cesspools, called lagoons, where it is stored until being sprayed onto nearby farmland. These conditions provide an ideal environment for bacteria to thrive and allow easy animal-to-animal transmission of pathogens [110].

Industrial animal producers administer antimicrobials to livestock for several reasons—for therapeutic purposes, to promote animal growth, and to prevent disease in susceptible flocks [110, 111]. Subtherapeutic use of antimicrobials in animal production facilitates growing animals in confinement by preventing sickness in the animals [112]. It also promotes lower production costs since the antibiotics allow the animals to grow faster, feed for shorter amounts of time, and reach slaughter weight sooner [111]. Food producers say that antimicrobial use is essential because it maintains animal health and protects the economics of this food production system [113].

A 2009 United States Food and Drug Administration report demonstrated that multiple classes of antimicrobials that are medically important for humans are also distributed for use in food animal production [114]. This shared use could promote bacterial resistance to medically important antimicrobials, thus rendering these drugs ineffective and creating challenges for treating infections in humans [115]. In the European Union [111], the use of medically important antimicrobials for nontherapeutic purposes in livestock production has been banned; however, it continues in the United States.

Connections between antibiotic use in animal production and prevalence of antibiotic resistant bacteria have been shown previously. For example, Hayes et al. [116] showed that after several European countries banned the use of the glycopeptide antibiotic avoparcin from use as a feed additive in animal meat production, the prevalence of vancomycin resistant enterococci (VRE) in poultry meat samples and in the gut flora of healthy German human

residents decreased. Similarly, Skot-Rasmussen compared antibiotic resistance trends of *Campylobacter jejuni* in broiler chicken meat imported from other countries to meat from Denmark, where the use of fluorquinolones in animals is restricted. The authors found that bacteria on Danish broiler chicken meat had lower resistance patterns to ciprofloxacin, nalidixic acid and tetracycline compared to meat from other countries. In addition, they found that, for the most part, bacterial isolates collected from people who traveled outside Denmark had higher levels of resistance patterns to ciprofloxacin and nalidixic acid compared to isolates from people who had not traveled outside the country [117].

Investigators have also compared the prevalence of bacteria in animals raised on antibiotic free farms to that in animals raised on farms that routinely administer antibiotics. In a recent German study, neither MRSA nor MSSA was found in the nares of pigs raised on alternative farms (smaller farms that provide room for the pigs to run and do not apply antibiotics to animals that exceeded a body mass of 25 kg). MSSA was found in approximately 35% of 89 people who worked or lived on the farms; however, only one person was nasally colonized with MRSA CC398 [103].

Transmission of MRSA in the environment

There are various mechanisms by which MRSA may be transmitted in the environment near CAFOs. Perhaps the most plausible route is via community members who have direct contact with animals [118]. However, as described above, current research findings suggest that person to person transmission of LA strains occurs less frequently compared to other types of MRSA. LA MRSA has also been identified in retail meat, and so it is possible that humans could come into contact with MRSA via this mechanism [81, 93].

There is some evidence that antibiotic resistant bacteria may exist in the waste that livestock secrete; people who inhale the air within or near swine facilities could be exposed to drug resistant bacteria. For example, Nandi et al. found antibiotic resistance genes in gram positive bacteria in poultry litter [119]. Similarly, in the south eastern United States, *enterococci* isolated from poultry litter or transport containers in processing production facilities showed resistance to a number of antimicrobial classes [116]. Resistant bacteria have also been found in areas near concentrated animal feeding operations (CAFOs), which might be due to the emission of particulate matter from inside confinement houses into the external environment [110]. Gibbs et al. [120] sampled bioaerosols for antibiotic resistant bacteria located inside, downwind, and upwind from a swine CAFOs. The investigators found organisms that were resistant to at least 2 classes of antibiotics at all locations; *S. aureus* was the most prevalent organism. The amount of antibiotic resistant organisms inside the confinement houses was 2 times that which was found upwind of the facility. The prevalence of antibiotic resistant bacteria was higher downwind compared to upwind of the facility. At the time of sampling the pigs were not receiving nontherapeutic doses of antibiotics, but had been treated with oxy-tetracycline 4 weeks prior to sampling. The investigators' ability to detect antibiotic resistant bacteria after treatment had ceased suggests that these antibiotic resistant bacteria persist in the environment. In another study, Hamscher et al. [121] found high concentrations of antibiotics in dust collected inside a pig-fattening farm facility. Similarly, Chapin et al. isolated 124 bacteria in the air of a swine confinement house and found that 98% of these were resistant to high levels of at least 2 types of the following antibiotics commonly used in swine production: erythromycin, clindamycin, virginiamycin, or tetracycline [122].

Antibiotic resistant organisms may also be transported in water. Anderson et al. [123] sampled groundwater at four sites in eastern North Carolina and found antibiotic resistant *Escherichia coli* (*E. coli*) more frequently in water on or near industrial swine farms than on farms without swine. This suggests that water near swine operations could be an important environmental reservoir for antibiotic resistant bacteria and antibiotic resistance genes. Sapkota et al. [124] sampled surface and ground waters up- and down-stream from a swine CAFO in eastern North Carolina. They found higher levels of *enterococci* and *E. coli* in waters up gradient from the CAFO. The *enterococci* that they found in down gradient waters expressed higher levels of resistance to antibiotics used in swine production compared to isolates that were collected from up-gradient waters. Interestingly, *enterococci* that were recovered from down and up gradient water samples were susceptible to vancomycin, which is an antibiotic that is not approved for use in swine production in the United States.

Antibiotic resistant bacteria have also been isolated in sewage sludge and pig slurry [125] and wastewater treatment plants have been shown to be a potential reservoir for MRSA [126]. There is also emerging evidence that fish might be a vector for transmission of MRSA. In Malaysia, MRSA was found in the brains, eyes, and kidneys of farm-raised Tilapia fish [127].

An additional mechanism for transmission of antibiotic resistant bacteria is via animals that come into contact with livestock or livestock waste. For instance, Graham et al. [128] found evidence to suggest that near intensive poultry production areas, flies might transfer antibiotic resistant bacteria from poultry litter and contribute to human exposures. Canadian geese have also been shown to be potential vectors for the transfer of antimicrobial

resistant genes from swine waste to other environments, such as water, crops, and other wildlife [129].

Public health relevance of livestock associated MRSA

MRSA ST398 does not generally carry the toxin genes that contribute to the pathogenicity of MRSA. Recently, however, a Chinese study identified a high prevalence of MSSA ST398 that harbored the toxin gene Panton-Valentine Leukocidin [130]. In this study, the patients carrying MSSA ST398 did not have any recent contact with livestock.

LA MRSA strains could acquire toxin genes through horizontal gene transfer, or might already possess as of yet unidentified toxin genes [88]. There have already been cases of LA MRSA strains causing invasive infections, including endocarditis [131], ventilator-associated pneumonia [132], respiratory tract infections[133], pyomyositis, cellulitis, abscesses [15], other skin and soft-tissue infections [96], and more [14, 88].

Also concerning is the potential for these strains to be introduced into clinical settings. Under environmental pressures from heavy use of antibiotics, the bacteria could acquire new resistance and toxin genes. Already, there was an outbreak of the LA strain in a Dutch residential care facility [134], a Dutch hospital [135], and a Dutch nursing home [136]. Given the history of MRSA and our understanding of the remarkable ability of these bacteria to evolve, it is essential that we continuously study their activity, especially in regions with intensive animal production.

Livestock production in eastern North Carolina

In North Carolina, as of August of 2009 there were 2,166 swine animal production facilities with active wastewater discharge permits from the NC Division of Water Quality. The majority of these operations are located in the southeastern region of the state [137] (Figure 1.1). The state produces about 10 million hogs and produces approximately 14.4% of US pork [138]. The Environmental Defense Fund estimated that in NC, three million pounds of antibiotics, the same amount used in human medicine, are used annually in animal production. NC ties with Iowa for using the most antibiotics in animal feed in the USA [139]. Eastern NC is also home to the world's largest pork processing plant, Smithfield Packing Incorporated [140].

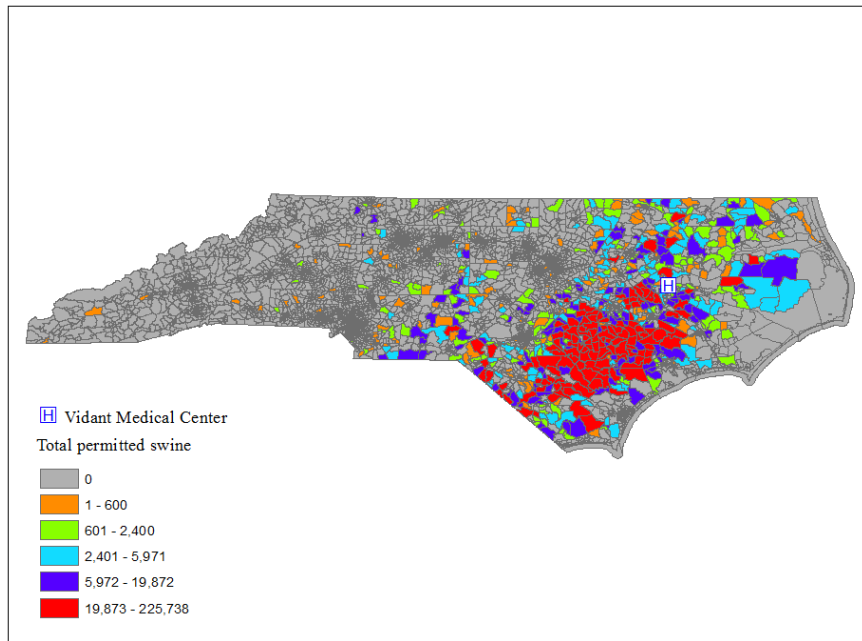
Summary

MRSA is a resilient organism that evolves in response to environmental pressures. Once confined to clinical settings, it later emerged in the community, affecting people without recent medical exposures. Even more recently, strains of MRSA emerged that were associated with industrial livestock production. The potential for these strains to be introduced into clinical settings is concerning, since the medical environment could impose new selective pressures on the bacteria. There remains a relative lack of epidemiologic investigation of the relationship between MRSA carriage and environmental and occupational exposures in eastern NC. The objective of my dissertation work was to investigate the relationship of MRSA carriage with environmental, household, medical, and occupational exposures among patients admitted to a tertiary care hospital in eastern North

Carolina. I conducted a hospital-based case control study of inpatients at the Vidant Medical Center (VMC), which is the largest hospital in eastern North Carolina.

Figure

Figure 1.1. Permitted swine in each North Carolina block group



CHAPTER 2

Specific aims

Methicillin resistant *Staphylococcus aureus* (MRSA) is a pathogenic bacteria that has evolved resistance to methicillin and other β -lactam antibiotics [1]. Infections from *Staphylococcus aureus* cause severe clinical conditions that are associated with increased medical costs [141] and may sometimes culminate in patient death. People may also asymptotically carry MRSA on their bodies. Historically, MRSA was nosocomial; later, genetically and phenotypically distinct strains emerged in the community [8]. Healthcare associated (HA) and community associated (CA) MRSA strains are genetically distinct [35, 142]; however, they have started to mix in terms of their epidemiology. Indeed, the epidemiology of MRSA is constantly evolving.

Factors associated with MRSA carriage or infection include demographic [10, 65] and residential characteristics [67], medical exposures [10, 65, 67], history of MRSA colonization or infection, past antibiotic use [67, 143], living with companion animals, with children, with someone infected with MRSA, or in crowded or substandard housing [64, 144, 145], or playing contact sports [55, 146]. Recently, meat animals were recognized as potential reservoirs, as novel strains of MRSA were detected [5] and associated with livestock, especially pigs [133]. Researchers believe that livestock associated MRSA may have developed in response to subtherapeutic administration of antibiotics to healthy animals

[110]. Identification of environmental sources for MRSA is important for infection prevention, especially within hospitals where heavy use of antimicrobials could impose selective pressures on CA or LA strains.

The overall objective of this research was to investigate environmental, household, and occupational exposures associated with MRSA nasal carriage among patients admitted to Vidant Medical Center (VMC), a tertiary care hospital in eastern North Carolina. VMC is the largest and only academic medical center in eastern North Carolina, a region that is densely populated by animal production facilities. Since February 2007, VMC has screened all admitted patients for MRSA using duplicate swabs of the anterior nares [147]. This screening program presents a unique opportunity to study MRSA carriage at the time of hospital admission. Using data from VMC's screening program and information on occupational, environmental, and medical exposures that I ascertained through structured in-hospital interviews, geographic mapping, and medical record abstraction; I addressed the following specific aims:

- 1) Investigate associations between medical and household exposures and MRSA nasal carriage by:
 - a. Reviewing medical charts and conducting structured interviews to identify information on previous medical exposures;
 - b. Conducting structured interviews to ascertain information about household member presence and medical exposures;
 - c. Conducting structured interviews to ascertain information about smoking history, indoor pets, demographic information, and playing contact sports or attending a public gym; and

- d. Developing a multivariable logistic regression model to derive estimates of association between MRSA nasal carriage and medical and household exposures

Rationale: The scientific literature suggests that the following factors may be associated with MRSA carriage: previous medical contact (i.e. surgery, hospitalization, antibiotic use, etc.), history of MRSA carriage, prior hospitalization of household members, prior antibiotic use by household members, living with pets, cigarette smoking, and playing contact sports or attending a public gym. I investigated the relationship between these variables and MRSA carriage among patients who were screened for MRSA nasal carriage at the time of admission to VMC.

Hypothesis: The following factors will be positively associated with MRSA carriage: recent medical contact, recent antibiotic use, indoor pets, gym use or sports participation, cigarette smoking, household member presence, and household member medical exposures (i.e. antibiotic use and past hospitalization).

- 2) Examine associations between environmental exposures to livestock, horses, or meat and MRSA carriage by:
 - a. Characterizing participants' environmental exposures through the following scenarios:
 - i. Living within 1 mile of swine or poultry concentrated animal feeding operations (CAFOs),
 - ii. Living in block groups with medium or high densities of permitted swine,

- iii. Ability to smell odors from animal farms when at home,
 - iv. Handling meat at home or at work,
 - v. Having contact with livestock or horses in the community,
 - vi. Living with household members who work with livestock and/or on farms; and
- b. Comparing the log-odds of MRSA nasal carriage in participants with the above environmental exposures to the log-odds of MRSA nasal carriage in participants without the above exposures.

Rationale: Handling of uncooked meat may be associated with MRSA carriage.

People who live near CAFOs may experience exposure to the bacteria via the following routes: Coming into contact with dust from CAFOs; drinking well water that could be contaminated by waste from CAFOs; wading, swimming, or fishing in surface waters that might be contaminated by waste from CAFOs; or coming into contact with other humans who have contact with livestock.

Additionally, living with someone who works with CAFOs may be a risk factor for MRSA carriage.

Hypothesis: Higher proportions of cases than controls will have recent environmental exposures to livestock, horses, and meat.

- 3) Investigate associations between occupational exposures and MRSA carriage by:
- a. Characterizing participants' occupations and the industries for which they work,
 - b. Characterizing the type and the extent of study participants' occupational exposures to livestock, horses, and meat,

- c. Comparing the log-odds of MRSA carriage across industry categories,

Rationale: As a result of workplace exposures, people may become carriers of MRSA and vectors for transmission of this pathogen into the community.

Hypothesis: Higher proportions of cases than controls will work in medical related settings, with children, and/or with livestock.

- 4) Examine associations between MRSA strain carriage and environmental, occupational, and household exposures by:

- a. Characterizing the strain of MRSA that cases are carrying as CA or HA, and
- b. Comparing the log-odds of occupational, environmental, household, and medical exposures in CA MRSA carriers versus controls and HA MRSA carriers versus controls.

Rationale: Traditionally, HA and CA MRSA carriage has been associated with different epidemiologic exposures.

Hypothesis: HA and CA MRSA carriers will differ in terms of the log-odds of exposure to various medical, environmental, household, and occupational exposures.

This work represents an important contribution to the epidemiologic literature on the sources for MRSA nasal carriage. This study provides information on the epidemiology of MRSA nasal carriage at the time of admission among eastern North Carolina patients.

CHAPTER 3

Materials and methods

Study design and setting

This was a hospital based case-control study. Vidant Medical Center (VMC) is an 861 bed teaching hospital of the Brody School of Medicine at East Carolina University. VMC is the tertiary care center for 29 counties in eastern North Carolina. In February of 2007, VMC implemented a universal MRSA screening program; all admitted patients are screened for MRSA by duplicate swabs from the anterior nares [147]. Patients are screened for MRSA within 24 hours of their hospital admission.

MRSA screening and typing

The clinical microbiology laboratory at VMC processes the swabs. One swab is tested for MRSA using the BD GenOhm MRSA Polymerase Chain Reaction (PCR) [148]. The BD GenOhm works by targeting a single locus that includes the right portion of SCC*mec* that is downstream of *mecA* and a section of the *orfX* gene, which is specific to *S. aureus* [149]. Every patient who screens positive for MRSA is put on contact isolation (anyone visiting their room must wear gloves and a gown), prescribed a 5 day course of mupirocin to decolonize their nares, and bathed with chlorhexidine soap [150].

For each case, approximately 24 to 48 hours after the screening was performed, the duplicate nasal swab was transferred to the infection control laboratory, streaked onto a CHROMagar® MRSA plate (CHROM agar Microbiology, Paris, France) and incubated for 24-48 hours at 37 °C. According to manufacturer recommendations, rose or mauve colored colonies were identified as MRSA.

After 24 hours of incubation on blood agar plates, DNA was extracted using an UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA). The NanoDrop® ND-1000 Spectrophotometer (Isogen, Ijssel stein, The Netherlands) was used to estimate the genomic DNA concentrations. Extracts were diluted to give a final DNA concentration of 35 ng/μl.

The Diversilab® Staphylococcus kit for DNA fingerprinting (bioMerieux, Boxtel, The Netherlands), a repetitive sequence-based PCR (rep-PCR), was used to amplify regions between repetitive, noncoding sequences in DNA samples [147]. The protocol was run according to manufacturer's specifications. Software from the DiversiLab® system (version v.r.3.3.40) was used for typing analysis. The rep-PCR profiles were compared to the DiversiLab® MRSA library containing 70 samples of the 14 representative USA pulsed field gel electrophoresis types [28]. At VMC, the MRSA library also includes one sample of livestock associated (LA) MRSA, which was isolated from a pig in Iowa and confirmed to be multi-locus sequence type 398. A sample with an indistinguishable fingerprint was matched to one in the library and assigned that type. Based on this analysis, MRSA isolates were defined as community associated (CA), healthcare associated (HA), livestock associated (LA), or non-matches.

Inclusion criteria

To be eligible to participate, patients were required to live in one of the top swine producing zip codes in NC. I identified the list of eligible zip codes using data from the North Carolina Division of Water Quality (NC DWQ). The NC DWQ publishes a publicly available data base that provides information on the geographic locations, numbers and types of animals allowed to be produced at each production facility in North Carolina that has been issued a non-discharge wastewater permit. I used this information to characterize top swine producing zip codes in North Carolina. For each zip code that contains at least one facility, I calculated the total number of swine that were produced in that zip code. I then calculated the median number of swine that were produced in the North Carolina zip codes; this was equal to 1,032,750 swine. Any zip code in which 1,032,750 or more swine were produced was designated to be an eligible zip code. In total, 176 zip codes met this criterion.

Other eligibility criteria included the following: 1) ages 18 to 65 2) screened for MRSA at VMC, 3) present in the hospital at a time when interviews were being conducted, 4) English or Spanish speaker, and 5) able to answer questions during an interview.

Participant identification

To identify patients who were eligible to participate in the study, I downloaded daily reports from the hospital electronic medical record. These reports provided the following information on each patient admitted to the hospital: date of hospital admission, name, address, medical record number, MRSA screening result (positive or negative), admitting physician, hospital room number, date of birth, and gender. Cases were defined as patients

who screened positive for MRSA nasal carriage at the time of admission. Controls were people who screened negative for MRSA.

The daily reports were input into Statistical Analysis Software version 9.3. For each eligible case, I identified patients who could serve as a matching control; these were people who screened negative for MRSA, were admitted to the hospital within 24 hours of the case, were ± 5 years of the case, and were the same gender as the case. Each potential matching control was assigned a unique random number. I attempted to enroll the lowest randomly numbered control.

Enrollment and interviews

For every potential participant, I paged the physician taking care of that person; I introduced the study to the medical provider and made sure that it was appropriate that I approach the patient about participating. After receiving approval, I visited each potential participant in their room, introduced the study, and invited them to participate. If the patient was sleeping or receiving a medical treatment when I approached them, I returned to their room at a later time. If the patient was unconscious but a family member was present, I invited the family member to participate on behalf of the patient.

If the patient agreed to participate, I administered a brief, structured interview. The interview included questions about place, industry, and job title if they were currently employed, occupational exposures to animals, household member occupational exposures, medical and antibiotic use history for the patient and his/her household members, pet ownership and contact with animals, recreational activities, smoking status, home

environment, meat handling, and demographic information. The complete questionnaire that I used for the interviews is presented in Appendix 1.

Medical chart review

To check that the exposures that I asked about during the interview occurred prior to the MRSA screen, I reviewed the microbiology lab results to identify the date that the MRSA nasal swab that defined case/control status was taken. I reviewed medical charts to see if the participant had surgery within the past year. I also identified the primary diagnosis for the current hospitalization. I classified the primary diagnoses according to the International Classification of Diseases, 9th (ICD-9) edition.

I reviewed medical records to confirm participant reports about hospitalizations within the past year. I also identified the last date within a year of the current hospital admission that the patient was prescribed an antibiotic, if at all. If, during the interview, the participants reported not using antibiotics in the past year but the medical chart indicated that they were prescribed an antibiotic, I adjusted the variable coding to reflect the information from the medical chart. If participants reported antibiotic use but there was no evidence of prescription in the medical chart, I coded the variable according to the participant report.

For participants who were admitted to VMC previously, I identified the dates and results of MRSA screenings that occurred within 1 year of the current hospitalization. I checked the electronic pharmacy records charts to determine if the patient was prescribed mupirocin after a previous positive MRSA screen.

Data checking and cleaning

To check the data entry, I randomly selected and compared 10% of the paper copies of the interviews with the data that were entered into the Qualtrics® system. There were data entry errors in fewer than 1% of the paper copies. Where possible, I also compared variables that were collected from the interview with the information in the medical charts (age, address, previous hospitalization, and previous antibiotic use). I corrected the data on age, previous hospitalization, and previous antibiotic use based on the information in the medical charts. If the address that the participant reported was outside the list of eligible zip codes, I excluded the person from the study.

For the medical chart review, I began by checking only 10% of the records. I identified inconsistencies and so, with the exception of the data on previous MRSA screenings, I checked all of the records against the medical records a second time. For the data on MRSA screenings, I checked only 10% of all records against the medical records, since I found minimal mistakes in those that I checked.

I imported all of the data into SAS 9.3. I performed cross-checks on relevant variables to identify inconsistencies, outlying values, and other suspicious values.

Geocoding

I used ArcMap10® (ESRI, Inc., Redlands, CA, USA) to geocode participants' home and work addresses. If the home address that the participant reported could not be geocoded but the one listed in the medical chart could, I assigned coordinates according to the address in the medical record.

Identification of concentrated animal feeding operations near participant homes

I used satellite imagery in Google Earth™ to identify 1 or more swine or poultry CAFO within 1 mile radii of participants' home and work addresses. In North Carolina, swine CAFOs store animal waste in open air pits, euphemistically called lagoons, but most poultry operations do not utilize these liquid waste management systems. Therefore, I identified images of animal barns beside small bodies of water, the lagoons, as swine and images of barns without lagoons as poultry CAFOs.

Human and swine population densities and rural area classifications

I downloaded topically integrated geographic encoding and referencing (Tiger)® shapefiles showing census block groups and urban areas from the 2010 United States Census [151]. I used SAS version 9.3's GINSIDE procedure to define each home address as an urban area, urban cluster, or rural area. I combined urban areas and clusters into a single "urban" category. Urban clusters contain at least 2,500 people and urbanized areas contain 50,000 or more people. I also used the GINSIDE procedure to identify the census block group to which each home address belonged.

The publicly available NC DWQ database presents information on the total number of swine, and the developmental stage (farrowing, weaning, feeding, finishing) permitted at each facility in NC. I used this information to assign totals and densities of the following in each block group: 1) total permitted swine, 2) permitted farrowing swine (these are pigs between birth and weaning), and 3) permitted non-farrowing swine. I classified densities by developmental stage because of evidence that LA MRSA is more prevalent among youngest

pigs [27, 152]. Swine densities were defined as the number of permitted swine divided by the number of square miles in a block group.

I also used 2010 United States Census data to assign human population densities to each block group. I defined human population densities as the number of people living in a block group divided by the number of square miles in the area.

Statistical analysis

I used Statistical Analysis Software version 9.3 to conduct all analyses.

Investigation of medical and household exposures associated with MRSA carriage

I developed a multivariable model to estimate associations between medical and household exposure variables and MRSA carriage (case/control status). This model was a conditional logistic regression model. The equation for the conditional logistic regression can be expressed as:

$$\log(p/1-p) = \alpha_i + \sum c_i \gamma_i ,$$

where p represents the probability of being a case, α_i represents the overall level of the log-odds of the outcome within each matched set (stratum) that is not being estimated by the conditional model fitting, c represents the i variables that were included in the models to predict case status, and γ_i represents the estimates for the change in the log-odds of being a case that were associated with incremental changes in the coding for each term.

The multivariable model included the following variables: 1) education (less than high school vs. high school or more), 2) race/ethnicity (black or other vs. non-Hispanic white), 3) hospitalization and MRSA screening history in the past 12 months, 4) visiting a

gym or playing sports in the past 2 weeks, 5) smoking cigarettes in the past 12 months, 6) household member prior hospitalization in the past 12 months and antibiotic use in the past 4 weeks, and 7) living with cats or dogs inside the home. Variables were selected *a priori* based on evidence from the scientific literature that they might be related to MRSA carriage. I selected time windows and variable coding schemes that provided the most predictive model with the fewest degrees of freedom, as indicated by deviance and Akaike information criteria (AIC) statistics. I also ran the multivariable model using data for only cultured cases and their matched controls.

Based on results from the molecular typing analysis, I also examined the relationship between HA or CA MRSA carriage and the variables in the model; I compared the log-odds of exposure in HA or CA MRSA carriers to their matched controls. Because sample sizes were small, I ran separate models for each predictor variable and conditioned only for the matching variables, gender and age.

Investigation of environmental and occupational exposures associated with MRSA carriage

I used conditional logistic regression models to derive estimates of associations between MRSA nasal carriage and variables related to environmental exposure to meat, livestock, and horses. Specifically, I examined relationships between MRSA carriage and the following: 1) residence within 1 mile of a swine or poultry CAFO, 2) counts and densities of swine (total, farrowing, and non-farrowing) in the census block group of residence, 3) reported ability to ever smell odor from an animal farm when at home, 4) handling of uncooked meat at work and/or at home, 5) indirect contact at work or direct contact at home

with horses, and 6) indirect contact at work or direct contact at home with livestock, defined as pigs, cows, chickens, turkeys. To provide a comparison of relationships between MRSA carriage and environmental or occupational contact with livestock, I also examined relationships between densities of humans living in the census block group area of residence, residence in a rural or urban area, and employment status. To be defined as employed, participants had to have worked in the 2 weeks that preceded the current hospital admission.

All exposure variables were coded as binary terms, except those representing swine head count total, swine densities, and human population density. I coded the human population density variable as a linear term; this coding produced a smaller AIC statistic compared to quadratic, cubic, or categorical coding.

Variables representing densities of total, farrowing, and non-farrowing swine were coded as 3-level categorical variables (0 swine/square mile of block group, referent vs. 149 swine/square mile vs. > 149 swine/square mile). Zero was the median and mode for the distribution of total swine density and 149 was the 25% of the distribution of observations with non-zero values for swine density. Using the 25% rather than 50% as the cut-point provided superior model fit, as indicated by a comparison of AIC statistics. Coding the density variables using three categories led to improved model fit compared to binary coding, to other categorical coding schemes and to using linear, quadratic or cubic terms. I also explored the relationship between total swine count and case status. Coding total swine count using linear, quadratic, and cubic terms produced the smallest AIC statistic.

All models were conditioned on the matching variables age and gender. I also adjusted for potential confounding by education (< high school degree vs. high school degree or higher), which was selected *a priori* based on the belief that this variable serves a proxy-

measure for socioeconomic status and unmeasured environmental factors that could confound the relationship between the exposure and outcome variables. In addition, I included the education term in the model based on the assumption that it was not on the causal pathway between exposures and case status.

After running the models on the full data set, I reran them using a stratified data set; I compared cases whose MRSA swabs grew colonies to their matched controls and cases whose swabs did not grow colonies to their matched controls. In addition, I ran the models to compare CA MRSA carriers and HA MRSA carriers to each of their matched controls.

Below, I describe in detail the variables that I included in the analyses.

Variables

Age

Cases and controls were matched on the basis of age (± 5 years). Age was calculated by subtracting the patient's date of birth from the day on which I identified them as a potential case or control.

Gender

Cases and controls were matched based on gender, which was identified in the medical records.

Race/ethnicity

This variable was self-reported by the participants during the interview. The following options were presented to the participants: Black, White, American Indian/Alaska Native, Asian, Hispanic/Latino, or Other. Since there were fewer than 10 people in all

categories of race/ethnicity other than black and white, I collapsed the variable to be dichotomously coded: black or other vs. non-Hispanic white.

Education

This variable was self-reported by the participant during the interview. The participant was presented with the following options to describe the highest degree that they had earned in school: more than bachelor's degree, bachelor's degree, associate's degree, high school diploma, less than high school diploma, and other. Based on the distribution of this variable, I began by considering this is a three-level variable: 1) less than high school diploma, 2) high school diploma or GED 3) More than high school diploma or GED. Ultimately, this variable coded as a 2-level variable (High school/GED or more vs. less than high school).

Hospitalized in the past year

Hospitalization was defined as staying in a hospital for 8 hours or more. During the interview, I asked participants if, starting the day before they were admitted to the hospital, they were hospitalized in the past 4 weeks, six months, and year. However, comparison of participant reports with information in their medical records suggested that people misreported this variable. Therefore, I reviewed all participant medical records to ascertain the information for this variable, and always coded that variable according to the information recorded in the chart. I collapsed the variable to be dichotomously coded (hospitalized within the past year vs. not hospitalized in the past year). This variable coding is consistent with much of the literature on the relationship between medical contact and MRSA carriage and infection.

Surgery in the past year

Information on this variable was derived by reviewing the doctor's notes in the patient medical charts for the year preceding their current hospital admission. This variable was dichotomously coded.

Primary diagnosis

Information on each of these variables was derived from the primary diagnosis noted in the discharge notes for the current hospitalization.

Positive MRSA screen within the past year

I identified those patients who had screened positive for MRSA within the past year by reviewing microbiological lab results in the participants' electronic medical charts. This variable was coded as a dichotomous variable.

Antibiotic use in the past year:

During the interview, I asked participants if, starting the day before they were admitted to the hospital, they had used antibiotics in the past 4 weeks, 6 months, and year. However, after reviewing electronic medical records I identified instances where chart information and participant reports were discordant. Twenty-three people reported that they had not used antibiotics in the past year; however, the medical chart review indicated that they had used antibiotics. Fifty-nine people reported having used antibiotics in the past year, even though there was no evidence of this in their medical charts. An additional 5 people reported that they did not remember if they had used antibiotics in the past year; I found evidence in their medical charts that 3 of these 5 people had used antibiotics in the past year (Table 3.1).

People could have received antibiotics from various sources other than the hospital; therefore, I based the classification of antibiotic use in the past year on participant reports during the interview. However, because of evidence in their medical charts, I reclassified those 23 people who reported that they *had not* used antibiotics in the past year as people who *had*. For the 5 people who had reported that they did not know if they had used antibiotics in the past year, I filled in this missing data using information from their medical charts. To maintain consistency with much of the literature on MRSA carriage, I coded this variable dichotomously (used antibiotics in the past year vs. did not use antibiotics in the past year).

Household member antibiotic use and prior hospitalization

Participants without household members could not live with someone who had been hospitalized or had used antibiotics. Therefore, I combined these variables into a three-level indicator variable (0=no household members, 1=had household member(s), none of whom were hospitalized or used antibiotics, 2=had household member(s) who used antibiotics in the past 4 weeks or was/were hospitalized in the past year).

Below, I describe the origin of each of the variables that were used to derive this composite variable. I explored various time windows for the variables describing household member antibiotic use and hospitalization; I selected those that were most predictive of case status in univariable and multivariable models.

Number of household members

During the interview, I asked participants if they had any household members, and if so, how many household members they had.

Household member antibiotic use

During the interview, I asked participants if, starting the day before they were admitted to the hospital, any member of their household had used antibiotics in the past 4 weeks, 6 months, or year.

Previous hospitalization by household member

During the interview, I asked participants if any member of their household was hospitalized within the past 4 weeks, 6 months, or year.

Household member employment

During the interview, I asked participants who had any household members if those household members were currently employed. I combined this information with information on household member presence to create a 3-level categorical variable: (0=no household member present vs. 1=household member(s) present but none were currently working vs. 2=household member present and at least one was currently working).

Smoking in the past year

During the interview, I asked participants when the last time was that they smoked tobacco cigarettes. The participants selected one of the following options to describe their smoking status: 1) never, 2) more than a year ago, 3) more than a month but less than a year ago, 4) more than a week but less than a month ago, 5) more than a day but less than a week ago, and 6) less than a day ago.

Very few people responded “yes” to options 2-4, and only about 20% of participants responded that they smoked cigarettes less than a day before they were admitted. First, I collapsed smoking history into a three-level variable: (never vs more than a year ago vs. within the past year). I also explored coding this variable dichotomously: (never/more than a

year ago vs. within the past year). Ultimately, this variable was entered into the model as a dichotomous variable, since it provided superior model prediction, based on the AIC statistic (0=never/more than a year ago, 1=within the past year).

Visited a gym or played contact sports in the past 2 weeks

I asked participants how often in the 2 weeks before their current hospital admission they had visited a gym to work out or exercise. I also asked participants if they had played contact sports in the past 2 weeks. Because so few people indicated that they had participated in either of these activities, I combined this information in a dichotomously coded variable, where 1=visited a gym and/or played contact sports in the past 2 weeks, and 0=the participant did not engage in either of these activities in the past 2 weeks.

Work outside the home

Information on current employment outside the home was derived from the interview question, “Do you currently work outside the home?” I coded this variable dichotomously.

Rural/urban classification

The United States Census Bureau characterizes places as urban areas (UA), urban clusters (UC), or rural. These designations are made at the census block or tract level. For the 2010 census, the Bureau classified UCs as those territories with a population of at least 2,500 and fewer than 50,000 and UAs as those with 50,000 or more people. Rural areas are population, housing, and territory not included within an urban area or cluster.

I used SAS’s PROC GINSIDE to over-lay participants’ geocoded addresses with shape files showing the United States Census Bureau’s urban classifications and to determine whether participants’ addresses occurred within an UA or UC. The shape files were downloaded from the US Census 2010 webpage.

Handled raw meat in the 2 weeks prior to the current hospital admission

I asked participants how often, in the past 2 weeks before the current hospital admission, they had handled raw meat. The response options for this variable were: 1) never, 2) less than 1 day each week, 3) about 1 day each week, 4) 2-5 days each week, 5) 6-7 days each week. Because I was interested in 2-week exposures prior to the MRSA screening, and because I framed this question as asking about exposures within 2 weeks before the current hospital admission, I coded this variable as missing in the participant was screened for MRSA 9 days or more before the current admission. I combined information on handling meat at work with the information on meat handling outside of work. During the interview, participants were asked: “Do you handle raw meat products at work? Raw meat products are defined as meat products that have not been cooked?” If participants reported handling meat at work then they were coded as being exposed to meat. This variable was finally entered into the model dichotomous coding (ever vs. never).

Human population densities

I also used 2010 United States census data to assign human population densities to each block group. Human population densities were defined as the number of people living in a block group divided by the number of square miles in the area. I explored various coding schemes for this variable, including use of categorical, cubic, and quadratic terms. I entered the variable into the model as a linear term because this coding provided the smallest AIC statistic.

Residence within 1 mile of a swine or poultry CAFO

As described above, I determined residence within 1 mile of a swine or poultry CAFO based on examination of the geocoded address in GoogleEarth, and by visually

identifying CAFOs within 1 mile radii of the home address. This variable was coded as a dichotomous term.

Densities of swine (total, farrowing, and non-farrowing) in the census block group of residence

As I described above, I used data from the publicly available NC DWQ database to calculate the total swine, total farrowing swine, and total non-farrowing swine in each block group. I then calculated densities of swine weight by dividing the totals by the number of square miles in a census block group.

Reported ability to ever smell odor from an animal farm when at home

This variable was based on participant interview responses about frequency of ability to smell odor from a farm when at home (daily, several times each week, several time each month, less than once a month, never). I investigated various coding schemes. Because it was the most predictive, as indicated by examination of AIC statistics, I coded this variable dichotomously (ever vs. never).

Indirect contact at work or direct contact at home with horses

During the interview, I asked participants if they ever had direct or indirect contact with horses, pigs, cows, chickens, or turkeys at work. I also asked participants if they had direct contact with these animals outside of work. No participants reported having direct contact with horses or with livestock at work. I created 2 variables, one describing indirect contact at work or direct contact with horses, and another describing indirect contact at work or direct contact with livestock (pigs, cows, chickens, and turkeys). Because of small cell sizes, each of these variables were coded dichotomously (ever vs. never).

The research protocol and all consent procedures and interview forms were approved by the Institutional Review Boards at the University of North Carolina at Chapel Hill and East Carolina University.

Table

Table 3.1. Comparison of information derived from medical charts and in-hospital interviews describing antibiotic use in the past year.

Self-reported antibiotic use in past year	Antibiotic use in the past year based on medical chart review	
	No	Yes
No	48	23
Yes	59	101
Do not know	2	3

CHAPTER 4

Medical and household exposures associated with methicillin resistant *Staphylococcus aureus* nasal carriage in patients hospitalized at an eastern North Carolina hospital

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) causes dangerous, sometimes life-threatening infections [2]. MRSA is also commensal [35]; it lives on the human body without making the host sick. Asymptomatic MRSA carriers are more likely to develop infections from the bacteria or to spread it to other people [1, 13]. Identification of epidemiologic predictors of MRSA carriage is important for preventing transmission of this dangerous bacterium.

MRSA was first identified in the 1960s in tertiary care hospitals. At the time, MRSA predominantly affected elderly and sick patients [1, 2]. In the early 1990s, the bacteria were detected in younger and healthier people without medical exposures (surgery, hospitalization, etc.) [3]. MRSA that is acquired in the community is known as community associated or acquired (CA), rather than hospital or health care associated (HA). Since the late 1990s, the proportion of *S. aureus* infections that are resistant to methicillin has increased in the United States, largely because of increases in the prevalence of CA MRSA strains [47].

Historically, HA and CA MRSA differed genetically [28, 35, 142]. CA MRSA strains tended to be susceptible to classes of antibiotics to which HA strains were resistant, probably because of fewer selective pressures in the community [142]. Over time, HA and

CA strains have mixed. CA strains have caused hospital acquired infections [48, 153], newly admitted hospital patients have been found carrying CA MRSA strains into hospitals [67], and people without recent hospitalizations have been shown to be carriers of HA strains [9]. As a result, there are growing concerns about the introduction of CA MRSA into hospitals and about selection for enhanced resistance in traditionally CA strains [48].

In addition to medical related exposures [2], household and community exposures are gaining recognition as potentially important determinants of transmission and acquisition [145, 154]. The prevalence of nasal MRSA carriage in the United States has increased [10] and in hospitals the bacteria continues to represent a danger to patients [155]. Understanding sources of acquisition is essential for effective infection prevention efforts. The goal of this study was to identify household and medical exposures associated with MRSA carriage in recently admitted patients to a hospital in eastern North Carolina. MRSA carriage was determined through a hospital-based MRSA screening program that used polymerase chain reaction (PCR) to identify nasal carriers and cultured swabs from positive screen cases.

Methods

Study Setting

Vidant Medical Center (VMC) is an 861-bed teaching hospital of the Brody School of Medicine at East Carolina University, and serves as the tertiary care center for 29 counties in eastern North Carolina. Since February of 2007, all patients admitted to VMC are screened for MRSA using duplicate nasal swabs of the anterior nares [147]. Patients are screened within 24 hours of their hospital admission. Every patient who screens positive for MRSA is

put on contact isolation (anyone visiting their room must wear gloves and a gown), prescribed a 5 day course of mupirocin to decolonize their nares, and bathed with chlorhexidine soap [150].

Inclusion criteria

Cases and controls were identified from lists of the electronic medical records of all admitted patients at VMC. Cases were carrying MRSA in the anterior nares at the time of screening; controls were not carrying MRSA. To be eligible to participate, cases and controls had to be adults between the ages of 18 and 65, screened for MRSA at VMC, speak English or Spanish, and present in the hospital when interviews were being conducted.

Because an original study objective was to investigate relationships between livestock and MRSA, participants (cases and controls) were restricted to residents of North Carolina zip codes in which the number of swine permitted for production by the North Carolina Division of Water Quality was equal to or greater than the median for the state. There were 176 eligible zip codes.

Participant identification

To identify eligible patients, daily reports were downloaded from the hospital's electronic medical record service. These reports provided the following information on each patient: name, date of hospital admission, address, date of birth, gender, medical record number, MRSA screening result (positive or negative), and hospital room number. For each eligible case, I identified one potential control who was: 1) age \pm 5 years of the case, and 2)

the same gender as the case. In analysis, cases and controls were pooled to avoid double loss of information [156].

I received approval to approach each potential participant from the attending physician or his/her designee. I visited each person in their hospital room to invite them to participate. If the patient was sleeping or receiving medical treatment, I returned at a later time. If the patient was unconscious but a family member was present, I invited the family member to participate on behalf of the patient.

In-hospital interviews

I conducted brief, structured interviews with case and control participants in their hospital rooms (Appendix 1). The interview included questions about home environment, medical and antibiotic use history for the patient and his/her household members, indoor pets, recreational activities, smoking status, and demographic information. Data from the interview was entered into the Qualtrics® system (Provo, Utah).

Medical record review

I reviewed medical records to determine if the participant had surgery within the past year. I also identified the first listed and all other diagnoses listed in the discharge notes for the current hospitalization. I categorized the first-listed diagnoses according to the International Classification of Diseases, 9th edition (ICD-9).

I reviewed medical records to see if patients were hospitalized within the past year. I also identified the last date within a year of the current hospital admission that the patient was prescribed any antibiotics, if at all. If the participants reported not using antibiotics in the

past year but the medical chart indicated that they were prescribed an antibiotic, I adjusted the variable coding to reflect the information from the medical chart. People may obtain antibiotics from a variety of sources other than the hospital. Therefore, if participants reported antibiotic use but there were no prescriptions listed in the medical chart, I coded the variable according to the participant report.

For participants who were admitted to VMC previously, I identified the dates and results of MRSA screenings that occurred within one year of the current hospitalization. For all participants, I also identified the date of the MRSA screen for the current hospitalization. I checked the electronic pharmacy records to determine if the patient was prescribed mupirocin after a previous positive MRSA screen.

MRSA culturing and molecular typing

VMC's microbiology laboratory used the BD GenOhm® MRSA polymerase chain reaction (PCR) to test nasal swabs for MRSA [148]. Swabs were stored at 4°C for 24 - 48 hours. They were transferred to the infection control laboratory and streaked onto a CHROMagar® MRSA plate (CHROM agar Microbiology, Paris, France). CHROMagar® plates were incubated for 24 - 48 hours at 37°C. Mauve-colored colonies were identified as positive for MRSA.

After 24 hours of incubation on blood agar plates, DNA was extracted using an UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA). The NanoDrop® ND-1000 Spectrophotometer (Isogen, Ijssel stein, The Netherlands) was used to estimate the genomic DNA concentrations. Extracts were diluted to give a final DNA concentration of 35 ng/μl.

The Diversilab® Staphylococcus kit for DNA fingerprinting (bioMerieux, Boxtel, The Netherlands), a repetitive sequence-based PCR (rep-PCR), was used to amplify regions between repetitive, noncoding sequences in DNA samples [147]. The protocol was run according to manufacturer's specifications. Software from the DiversiLab® system (version v.r.3.3.40) was used for typing analysis. The rep-PCR profiles were compared to the DiversiLab® MRSA library containing 70 samples of the 14 representative USA pulsed field gel electrophoresis types [28]. At VMC, the MRSA library also includes one sample of livestock-associated (LA) MRSA, which was isolated from a pig in Iowa and confirmed to be multi-locus sequence type 398. A sample with an indistinguishable fingerprint was matched to one in the library and assigned that type. Based on this analysis, MRSA isolates were defined as CA, HA, LA, or non-matches.

Statistical methods

I examined variable distributions using tabular analyses. Conditioning on age and gender, I developed a multivariable logistic regression model to derive adjusted estimates of association between MRSA carriage and medical and household exposure variables. The following variables were examined: education (less than high school vs. high school or more), race (black or other vs. non-Hispanic white), 3) hospitalization and MRSA screening history, having visited a gym or played sports in the past 2 weeks, having smoked cigarettes within the past year, living with someone who was hospitalized or used antibiotics, and living with cats or dogs inside the home. I selected these variables *a priori* based on evidence in the scientific literature that they might be related to MRSA carriage.

For the following variables, I explored various exposure time-windows: smoking status, household member antibiotic use, and household member hospitalization. I selected time windows and variable coding schemes that provided the most predictive model with the fewest degrees of freedom, as indicated by comparisons of deviance and Akaike Information Criteria (AIC) statistics.

I also ran the multivariable model to compare cultured cases to their matched controls and cases whose MRSA swabs did not culture to their matched controls. Based on results from the sequence typing analyses, I also examined the relationship between HA or CA MRSA carriage and the variables in the multivariable model; I compared HA or CA MRSA carriers to their matched controls. Because sample sizes were small, for the analyses of HA and CA MRSA carriers, I ran a separate model for each variable, conditioning only on the matching variables age and gender.

I report results from conditional logistic regression models as beta coefficients (β) \pm standard error (SE). In the tables I also report odds ratios (OR) and Wald statistics as indicators of the contribution of the variables to model fit.

This study was approved by the Institutional Review Boards at the University of North Carolina at Chapel Hill and East Carolina University. Each participant signed consent and Health Information Portability and Accountability Act (HIPAA) authorization forms.

Results

From July 26, 2011 - December 15, 2011, I conducted in-hospital interviews with patients at VMC. I invited 164 cases and 190 controls to participate; 26% and 36% declined,

respectively. In total, I interviewed 122 controls and 121 cases. Four cases and 3 controls who participated in the interview reported living at an address that was outside the eligible zip codes; they were excluded from the analyses (Figure 4.1).

In total, 117 cases and 119 controls are included in the analysis. One hundred and five (93.8%) matched sets had 1 case and 1 control; 7 (6.3%) had 2 controls per case; and 5 (4.5%) had 2 cases per control. All participants lived in eastern North Carolina or the eastern-most portion of central North Carolina.

Sixty-eight (57.1%) controls and 67 (57.3%) cases were female (Table 4.1). In the past 12 months, 93 controls (78.2%) and 93 cases (79.5%) used or were prescribed antibiotics, and 46 (39.3%) cases and 40 controls (33.6%) had surgery.

Five cases had concomitant MRSA clinical infections—abscesses (n=2), cellulitis/abscess, pneumonia, and bacteremia (Table 4.1). Thirteen (11.1%) cases and 3 (3.4%) controls had cellulitis or soft tissue infection diagnoses listed in any position. Seven (6.0%) cases and 2 (2.5%) controls had diarrhea. Twenty-nine (24.8%) cases and 34 (28.6%) controls had diabetes.

Participants' first-listed diagnoses spanned a large range of ICD-9 codes (Table 4.2). Eight (6.8%) cases and 1 (0.8%) control had a primary diagnosis for an infectious or parasitic disease. Two (1.7%) cases and 10 (8.4%) controls were in the hospital for factors influencing health status and contact with health professionals, meaning they had diseases or injuries other than those classifiable in the other disease or injury categories--dialysis catheter placement, pre-operative examinations, chemotherapy, surgery, organ donation, for example. Otherwise, the distribution of diagnoses was not markedly different for cases versus controls.

Table 4.3 shows coefficients from the multivariable model, conditioned on the age and gender matched sets. Higher proportions of cases than controls had less than a high school degree (0.71 ± 0.51), indoor cats or dogs (0.58 ± 0.36), smoked cigarettes within the past 12 months (0.47 ± 0.35), and visited a gym (0.97 ± 0.72). Being hospitalized in the past 12 months with a positive MRSA screen was associated with higher log-odds of current carriage (1.17 ± 0.54), whereas being hospitalized without a positive screen was associated with lower log-odds (-0.92 ± 0.39). Living with one or more people who used antibiotics in the past 4 weeks and/or were hospitalized in the past year was positively associated with MRSA carriage (1.18 ± 0.49).

Of the 117 cases, 108 duplicate swabs were available for culturing and of these 108 swabs, 49 (45%) successfully grew colonies. I ran the multivariable model on the subgroups of culture positive cases and their 52 matched controls, and 68 culture-negative cases and their 69 matched controls. Results from these analyses are presented in Table 4.4. Most estimates were imprecise. Among culture positive cases, being hospitalized and never screening positive for MRSA was negatively associated with MRSA carriage (-1.22 ± 0.79). The following were positively associated with MRSA carriage: living with someone who used antibiotics in the past four weeks or was hospitalized in the past 12 months (1.56 ± 0.86); having less than a high school degree (0.97 ± 0.82); and having indoor cats or dogs (0.23 ± 0.59). In contrast to the full analysis, similar proportions of cultured cases and controls had prior hospitalization plus prior MRSA carriage (0.03 ± 0.72). Also, lower proportions of culture positive cases than controls had the following: non-white race (-0.11 ± 0.63), smoked tobacco cigarettes in the past year (-0.50 ± 0.75), and lived with someone who did not use antibiotics and was not hospitalized (-0.51 ± 0.72). Because of sparse data, the

effect of gym use or sports participation in the past 2 weeks was not estimable for the comparison of culture positive cases versus controls.

Eighteen culture negative cases and 0 controls were previously hospitalized and identified as a MRSA carrier. This effect estimate was not estimable due to sparse data. Compared to the full analysis, Wald statistics for all but the following 3 terms increased in magnitude, suggesting a higher level of prediction: cigarette smoking, which remained the same, education, and previous hospitalization and previous positive MRSA screen.

Sequence typing results

Of 49 cultured isolates, 7 (14.3%) were non-matches, 0 were LA, 21 were HA and 21 were CA strains (data not shown). Fifteen (30.6%) were USA100, 2 (4.1 %) were USA500, 3 (6.1%) were USA800, 1 (2.0%) was USA200, 1 (2.0%) was USA600, and 20 (40.8%) were USA300.

Results from conditional univariable models comparing CA and HA MRSA carriers to controls are presented in Table 4.5. Compared to their matched controls, CA MRSA carriers had 2.17 ± 1.10 higher log-odds of being hospitalized and screening positive for MRSA in the past 12 months (Wald=3.39). In contrast, HA MRSA carriers had 1.24 ± 1.03 lower log-odds of being hospitalized in the past 12 months (Wald=1.46), compared to controls. All other effect estimates were either close to the null and/or very imprecise.

Discussion

Of 117 MRSA nasal carriers identified by PCR (cases) in this study, 5 had MRSA infections. As in previous reports [67, 157], a higher proportion of cases than controls were diagnosed with soft tissue infections or cellulitis; otherwise, comorbidities were similar. For the most part, cases and controls were selected from patients who were hospitalized for a variety of reasons. Also comparable to previous studies [67, 158, 159], MRSA carriage identified by PCR predicted nasal carriage at later hospital admission. VMC prescribes mupirocin to MRSA carriers to decolonize their nares, either while hospitalized or completed as outpatients. This suggests that MRSA carriers who retested positive were re-colonized in the community.

Results from this study contribute to evidence that the household environment may affect MRSA acquisition [145, 154]. Living with someone who used antibiotics or was hospitalized predicted MRSA carriage. MRSA carriage was also positively associated with having indoor cats or dogs, and with having less than a high school degree, which could be related to exposures previously shown to be risk factors for MRSA such as crowded or subsidized housing [145]. These findings are similar to previous studies, which found evidence of MRSA transmission between humans and pets [64, 160] and positive associations between MRSA infections and family members' previous antibiotic use and MRSA diagnoses [64]. Also similar to our findings, a higher prevalence of MRSA carriage has been reported in United States residents with less than a high school diploma [10]. Based on small numbers, prevalence of gym use or sports participation among participants was associated with MRSA carriage. This finding supports past reports of MRSA infections and outbreaks in athletics participants [161]. The positive association between smoking and

MRSA carriage could reflect higher susceptibility, greater exposure, or both among smokers. A recent population-based study in Pennsylvania reported an association between smoking and MRSA infections [157].

Prior hospitalization without a positive MRSA screen appeared protective against current MRSA carriage. This was surprising, since previous research suggests that MRSA carriage is associated with medical contact [2, 50]. Patients previously hospitalized with a negative MRSA PCR on admission may be less susceptible or exposed in the community.

It was surprising that similar proportions of cases and controls used antibiotics in the past year. This contradicts results from several [64, 143, 153, 159] but not all studies [63]. I attempted to correct participant responses to the question about prior antibiotic use based on information in their medical charts; however, there might be residual misclassification. This could at least partially explain the finding of no relationship.

Twenty-one of 49 MRSA isolates matched CA types in the DiversiLab® MRSA library; 20 of these were USA300 [28]. Also, a higher proportion of CA carriers than controls were hospitalized and screened positive for MRSA previously. These findings correspond to reports that CA strains may be carried by people with a history of hospitalization [9] and indicate the potential for CA MRSA to enter the hospital.

Results from this study must be interpreted cautiously. MRSA carriers were identified using the BD GenOhm® real-time PCR; however, only 45% of the MRSA swabs from cases cultured. This could raise concerns about false positives in the analysis of cases identified by PCR. The BD GeneOhm® real-time PCR works by targeting a single locus that includes the *SCCmec* right extremity (SRE) that is downstream of *mecA* and a section of the

orfX gene, which is specific to *S. aureus* [149]. Even when they lack the *mecA* gene, strains of MSSA with remnants of *SCCmec* might be identified as MRSA [149, 162].

Also, the PCR might have detected non-viable, non-culturable bacteria [163-165] or DNA residue that remained after MRSA decolonization [149, 164]. Cases who screened positive for MRSA during a previous hospital admission might have had DNA residue in their nares and not been true current positives. This could be part of the reason that, upon running the multivariable model on only culture positive MRSA cases, similar proportions of cases and controls had previous MRSA carriage.

The low proportion of culture positive cases could also be partially attributable to VMC having cultured the swabs without salt enrichment, which has been shown to improve the sensitivity of cultures in some [163, 166] but not all studies [167].

This work had several limitations. First, because VMC only routinely performs swabs of the anterior nares, a patient who entered the hospital with MRSA on a region of their body besides their nose would be identified as a control. While *S. aureus* is most commonly found on the nares [1], the bacteria can live on other places--for example, the skin, perineum, pharynx, the gastrointestinal tract, vagina, and axillae [37]. Second, since these data derive from a hospital-based study and inpatients make up the study population, results are not generalizable to non-hospitalized, healthier populations, or even to certain segments of hospitalized patients. The most severely sick or injured patients were less likely to participate since they were unconscious, in too much pain, and or/ too medicated to respond to questions. The least sick patients were often discharged before I was able to approach them. A third limitation is the potential for recall bias. When they participated, study participants knew if they screened positive or negative for MRSA. In particular, cases were aware of and

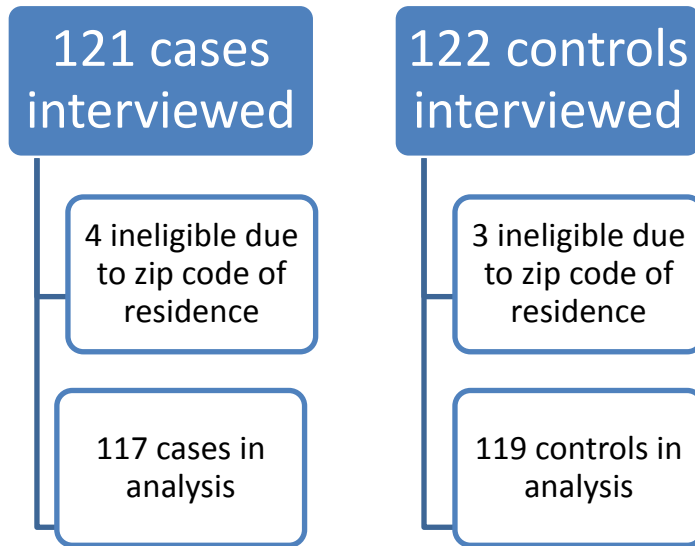
concerned by the screening results, especially since they were exposed to decolonization therapies and put on contact precautions.

This work had a number of strengths. This research captured information on members of the rural eastern North Carolina community, an under-studied segment of the population. By conducting in-hospital interviews, I collected information that would otherwise be unavailable via chart review only. I improved the quality of the interview data by checking certain responses against medical records. There were small amounts of missing data and the participation rate was relatively high. Using results from the rapid-PCR to identify MRSA carriers and controls allowed me to approach potential participants in a timely manner, some of whom might have been discharged had I waited for culture results. I was the only person to conduct interviews and abstract data from medical records, which should have provided more internal consistency within the data.

In conclusion, community and household exposures may be important predictors of MRSA carriage. This work points to the need for further research with healthier, non-hospitalized groups in the eastern NC community; longitudinal studies of hospitalized patients who are screened and treated for MRSA; and investigations of environmental exposures that could be related to MRSA carriage.

Figure

Figure 4.1. Numbers of cases and controls enrolled and included in the conditional analysis



Tables

Table 4.1. Characteristics and diagnoses of methicillin resistant *Staphylococcus aureus* nasal carriers and their matched controls

Characteristics	No. (%)			
	Controls (n=119)		Cases (n=117)	
Female	68	57.1	67	57.3
Age, y:				
18-29	29	24.4	24	20.5
30-39	15	12.6	16	13.7
40-49	17	14.3	22	18.8
50-59	40	33.6	37	31.6
60-65	18	15.1	18	15.4
Antibiotic use, past 12 mo.	93	78.2	93	79.5
Surgery, past 12 mo.	40	33.6	46	39.3
Hospitalized, past 12 mo.	70	58.8	71	60.7
<i>Diagnoses, current hospitalization^a</i>				
End-stage renal disease	5	4.2	7	6.0
Cancer	7	5.9	5	4.3
Diabetes mellitus	34	28.6	29	24.8
HIV or AIDS	2	1.7	4	3.4
MRSA infection	0	-	5	4.3
Sepsis or bacteremia	1	0.8	5	4.3
Pneumonia	2	1.7	1	0.9
Urinary tract infection	4	3.4	2	1.7
Necrotizing fasciitis	0	-	1	0.9
Cellulitis or soft tissue infection	3	3.4	13	11.1
Abscess	8	6.7	8	6.8
Fever	3	2.5	3	2.6
Diarrhea	2	2.5	7	6.0
Nausea or vomiting	7	5.9	7	6.0
Shortness of breath	3	2.5	4	3.4
Chest pain	7	5.9	6	5.9

Abbreviations: y, year; human immunodeficiency virus, HIV; acquired immune deficiency syndrome, AIDS

^aDiagnoses are based on any diagnoses listed in the discharge notes for the current hospitalization. The categories listed are not mutually exclusive.

Table 4.2. Distribution of first-listed diagnoses for the current hospitalization among methicillin resistant *Staphylococcus aureus* nasal carriers and their matched controls, categorized according to chapters from the International Classification of Diseases, ninth edition (ICD-9)

	ICD-9 codes	No. (%)			
		Controls (n = 119)		Cases (n = 117)	
Infectious and parasitic diseases	001-139	1	0.8	8	6.8
Neoplasms	140-239	4	3.4	7	6.0
Endocrine, nutritional, and metabolic diseases, and immunity disorders	240-279	13	10.9	8	6.8
Diseases of the blood and blood-forming organisms	280-289	1	0.8	1	0.9
Mental disorders	290-319	0	-	1	0.9
Diseases of the nervous system and sense organs	320-389	3	2.5	5	4.3
Diseases of the circulatory system	390-459	15	12.6	14	12.0
Diseases of the respiratory system	460-519	9	7.6	4	3.4
Diseases of the digestive system	520-579	10	8.4	9	7.7
Disease of the genitourinary system	580-629	5	4.2	8	6.8
Pregnancy complications, childbirth, or conditions originating in the perinatal period	630-679, 760-779	14	11.8	10	8.6
Diseases of the skin and subcutaneous tissue	680-709	7	5.9	11	9.4
Diseases of the musculoskeletal and connective tissue	710-739	3	2.5	3	2.5
Congenital anomalies	740-759	0	-	0	-
Symptoms, signs, and ill-defined conditions	780-799	15	12.6	20	17.1
Injury and poisoning	800-999	9	7.6	6	5.1
Factors influencing health status and contact with health professionals	V01-V89	10	8.4	2	1.7

Table 4.3. Estimates of association of methicillin resistant *Staphylococcus aureus* nasal carriage identified by a rapid Polymerase Chain Reaction screen with medical and household exposures from a multivariable conditional logistic model

	No. (%)		β	SE	Wald	OR		
	Controls (n=119)	Cases (n=117)						
Less than high school diploma	13	10.9	24	20.5	0.71	0.51	1.96	2.0
Cats or dogs inside the home	40	33.6	45	38.5	0.58	0.36	2.58	1.79
Non-white race or ethnicity	61	51.3	63	53.9	0.39	0.38	1.07	1.48
Smoked tobacco cigarettes within one year of the current hospital admission	43	36.1	52	44.4	0.47	0.35	1.76	1.60
Visited a gym or participated in sports within the past 2 weeks ^a	5	4.2	9.0	7.7	0.97	0.72	1.72	2.64
Prior hospitalization and MRSA nasal carriage in past year ^b								
Hospitalized in past year, never screened positive for MRSA	64	53.8	41	35.0	-0.92	0.39	5.39	0.40
Hospitalized in past year, screened positive for MRSA at least once	6	5.0	30	25.6	1.17	0.54	4.69	3.22
Household members ^c								
Did not use antibiotics in the past weeks, not hospitalized in the past year	71	59.7	58	49.6	0.35	0.45	0.60	1.42
Used antibiotics in the past 4 weeks and/or was hospitalized in past year	25	21.0	44	37.6	1.18	0.49	5.77	3.25

Abbreviation: months, mo.; odds ratio, OR; β , beta or log-odds estimate; SE, standard error

^aTwo cases and one control was screened for MRSA nine or more days before their current admission; however, information on gym visitation or sports participation reflects the 2 week period prior to the hospital admission, not the 2 weeks prior to the date of the most recent MRSA screen. To keep participants from dropping out of the analysis due to missing data, information for this variable was based on their report of the exposure 2 weeks prior to the hospital admission, even though it does not reflect the 2 weeks preceding the MRSA screen.

^bThis variable was entered into the model as a 3-level categorical variable. Participants not hospitalized in the past year make up the referent category.

^cThis variable was entered into the model as a 3-level categorical variable. Participants living alone make up the referent category.

Table 4.4. Estimates of association of methicillin resistant *Staphylococcus aureus* nasal carriage with medical and household exposures, derived from multivariable conditional logistic model, stratified by culture status

	Culture Positive Cases and Matching Controls							
	Controls		Cases		B	SE	Wald	OR
	N	%	N	%				
Total	52	100	49	100				
Less than high school	5	9.6	9	18.4	0.97	0.82	1.40	2.64
Cats or dogs inside the home	18	34.6	16	32.7	0.23	0.59	0.15	1.26
Non-white race	29	55.8	26	53.1	-0.11	0.63	0.03	0.89
Smoked tobacco cigarettes within one year of the current hospital admission	16	30.8	18	36.7	-0.50	0.75	0.45	0.61
Visited a gym or participated in sports within the past 2 weeks ^a	1	1.9	3	6.1	NE	NE	NE	NE
Prior hospitalization and MRSA nasal carriage in past 12 mo.^b								
Hospitalized in past year never screened positive for MRSA	21	40.4	14	28.6	-1.22	0.79	2.38	0.29
Hospitalized in past year, screened positive for MRSA at least once	6	11.5	12	24.5	0.03	0.72	0.00	1.03
Household members^c								
Did not use antibiotics in the past weeks, not hospitalized in the past 12 mo.	37	71.2	20	40.8	-0.51	0.72	0.50	0.60
Used antibiotics in the past 4 weeks and/or was hospitalized in past 12 mo.	7	13.5	21	42.9	1.56	0.86	3.29	4.76
	Culture Negative Cases and Matching Controls							
	Controls		Cases		B	SE	Wald	OR
	N	%	N	%				
Total	69	100	68	100				
Less than high school	8	11.6	15	22.1	0.52	0.73	0.51	1.69
Cats or dogs inside the home	22	31.9	29	42.7	1.38	0.68	4.09	3.98
Non-white race	33	47.8	37	54.4	1.68	0.75	5.06	5.37
Smoked tobacco cigarettes within one year of the current hospital admission	29	42.0	34	50.0	0.97	0.55	3.05	2.63
Visited a gym or participated in sports within the past 2 weeks ^d	4	5.8	6	8.8	1.69	0.90	3.56	5.45
Prior hospitalization and MRSA nasal carriage in past 12 mo.^b								
Hospitalized in past year but not screened for MRSA	44	63.8	27	39.7	-1.29	0.61	4.50	0.28
Hospitalized in past year, screened positive for MRSA at least once	0	0.0	18	26.5	NE	NE	NE	NE
Household members^c								
Did not use antibiotics in the past weeks, not hospitalized in the past 12 mo.	34	49.3	38	55.9	1.64	0.86	3.68	5.18
Used antibiotics in the past 4 weeks and/or was hospitalized in past 12 mo.	19	27.5	23	33.8	1.61	0.79	4.09	4.99

Abbreviation: months, mo.; odds ratio, OR; β , beta or log-odds estimate; SE, Standard error; non-estimable effect estimate, NE

^aOne control was screened for MRSA nine or more days before their current admission; however, information on gym visitation or sports participation reflects the 2 week period prior to the hospital admission, not the 2 weeks prior to the date of the most recent MRSA screen. To keep participants from dropping out of the analysis due to missing data, information for this variable was based on their report of the exposure 2 weeks prior to the hospital admission, even though it does not reflect the 2 weeks preceding the MRSA screen.

^bThis variable was entered into the model as a 3-level categorical variable. Participants not hospitalized in the past year make up the referent category.

^cThis variable was entered into the model as a 3-level categorical variable. Participants living alone make up the referent category.

^dTwo cases screened for MRSA nine or more days before their current admission.

Table 4.5. Estimates derived from univariable logistic models conditioned on age and gender, comparing community associated and healthcare associated methicillin resistant *Staphylococcus aureus* (MRSA) nasal carriers to their matched controls

	No. %		B	SE	Wald	OR		
	Controls	Cases						
Healthcare associated MRSA carriers and their matching controls								
Total	22	51.2	21	48.84				
Less than high school	1	4.6	3	14.3	1.10	1.15	0.91	3.00
Cats or dogs inside the home	7	31.8	5	23.8	-0.51	0.73	0.49	0.60
Non-white race	14	63.6	11	52.4	-0.51	0.73	0.49	0.60
Smoked tobacco cigarettes within one year of the current hospital admission	5	22.7	7	33.3	0.51	0.73	0.49	1.67
Prior hospitalization and MRSA nasal carriage in past 12 mo.^a								
Hospitalized in past year but never screened positive for MRSA	8	36.4	6	28.6	-1.02	0.88	1.32	0.36
Hospitalized in past year, screened positive for MRSA at least once	5	22.7	3	14.3	-1.24	1.03	1.46	0.29
Household members^b								
Did not use antibiotics in the past weeks, not hospitalized in the past 12 mo.	16	72.7	5	23.8	-0.11	0.82	0.02	0.89)
Used antibiotics in the past 4 weeks and/or was hospitalized in past 12 mo.	3	13.6	13	61.9	NE	NE	NE	NE
Community associated MRSA carriers and their matching controls								
Total	24	53.33	21	46.67				
Less than high school	4	16.7	5	23.8	0.49	0.77	0.40	1.63
Cats or dogs inside the home	8	33.3	9	42.9	0.66	0.70	0.90	1.9)
Non-white race	11	45.8	11	52.4	0.40	0.78	0.26	1.49
Smoked tobacco cigarettes within one year of the current hospital admission	8	33.3	9	42.9	0.86	0.85	1.01	2.36
Prior hospitalization and MRSA nasal carriage in past 12 mo.^a								
Hospitalized in past 12 mo. but never screened positive for MRSA	9	37.5	5	23.8	-0.30	0.92	0.11	0.74
Hospitalized in past 12 mo., screened positive for MRSA at least once	1	4.2	8	38.1	2.17	1.10	3.89	8.76
Household members^b								
Did not use antibiotics in the past 4 weeks, not hospitalized in the past 12 mo.	17	70.8	12	57.1	0.32	0.91	0.12	1.37
Used antibiotics in the past 4 weeks and/or was hospitalized in past 12 mo.	2	8.3	6	28.6	1.56	1.12	1.91	4.74

Abbreviation: months, mo.; β , beta or log-odds estimate; SE, Standard error; OR, Odds ratio; non-estimable effect estimate, NE

^aTwo cases and one control was screened for MRSA nine or more days before their current admission; however, information on gym visitation or sports participation reflects the 2 week period prior to the hospital admission, not the 2 weeks prior to the date of the most recent MRSA screen. To keep participants from dropping out of the analysis due to missing data, information for this variable was based on their report of the exposure 2 weeks prior to the hospital admission, even though it does not reflect the 2 weeks preceding the MRSA screen.

^bThis variable was entered into the model as a 3-level categorical variable. Participants not hospitalized in the past year make up the referent category.

^cThis variable was entered into the model as a 3-level categorical variable. Participants living alone make up the referent category.

CHAPTER 5

Environmental and occupational exposures associated with methicillin resistant *Staphylococcus aureus* nasal carriage in patients hospitalized at an eastern North Carolina hospital

Introduction

Methicillin resistant *Staphylococcus aureus* (MRSA) is a resilient, dangerous human pathogen [168]. When it was first identified in the 1960s in United States hospitals, MRSA was healthcare associated (HA), meaning that it was acquired in hospitals, long-term care facilities, and other medical settings [142, 169]. In the 1990s, the epidemiology of MRSA changed, as it was detected in healthy people without recent medical contact [170, 171]. Community associated (CA) and HA strains are genetically and phenotypically distinct. However, strains that were once CA have caused hospital onset infections [48, 153] and people without any recent medical contact have been identified as carrying HA MRSA [9].

Aside from medical exposures, a number of variables have been associated with MRSA carriage. These include age and poverty level [10, 65]; occupation as a veterinarian [85] or health care practitioner [83]; participation in contact sports [54, 55]; and living with a household member who has been infected with MRSA [62], with companion pets, or with children [64].

Recently, distinct, livestock associated (LA) MRSA strains were identified [5, 27, 89] and direct contact with livestock was recognized as an additional risk factor for MRSA carriage [11]. LA MRSA strains appear to have evolved in response to selective pressures within industrial animal production systems; thousands of animals are stored in concentrated animal feeding operations (CAFOs) and administered subtherapeutic levels of antibiotics [110, 172]. The predominant LA strains belong to clonal complex (CC) 398 in the Americas and Europe and CC9 in Asia [7, 88]. Most research on LA MRSA has focused on CC398, which has been identified on livestock, horses, and meat products [173] and been shown to be especially prevalent in young pigs [27, 152]. In humans, the strain has been strongly related to direct but not indirect contact with livestock [90, 97], and does not appear to be a persistent colonizer [41], probably because it lacks human niche specific genes [23]. Aside from CC398 and CC9, other strains, some human associated, have been identified in animals [173]. The emergence of LA MRSA suggests that could be an important environmental source for the bacteria.

Sections of eastern North Carolina are densely populated by CAFOs (Figure 1.1). However, little is known about environmental and occupational variables that are related to MRSA carriage in this region. The objective of this work was to investigate the relationship between MRSA carriage and exposures to livestock, horses, and meat among inpatients at Vidant Medical Center (VMC), which is an 861-bed teaching hospital of the Brody School of Medicine at East Carolina University, serving as the tertiary care center for 29 eastern North Carolina counties.

Methods

Identification of MRSA carriers

VMC screened all admitted patients for MRSA by taking duplicate swabs of the anterior nares. Each patient who screens positive is put on contact isolation (visitors to their room must wear gloves and a gown), bathed with chlorhexadine soap, and treated with the topical antibiotic mupirocin [147, 150].

As part of routine hospital procedures, the clinical microbiology laboratory at VMC used the BD GenOhm® MRSA polymerase chain reaction (PCR) to perform a rapid test for MRSA on 1 of the duplicate swabs [148]. The second swab was stored at 4°C, and then streaked onto a CHROMagar® MRSA plate (CHROM agar Microbiology, Paris, France) and incubated for 24 to 48 hours at 37 °C. According to manufacturer recommendations, rose or mauve colored colonies were identified as MRSA.

Case and control selection

I identified eligible cases and controls using data from patients' electronic medical records. Cases had MRSA in their anterior nares, as indicated by the rapid PCR; controls did not. Cases were matched to controls based on age (± 5 years) and gender.

Eligibility

Any person who met the following criteria was eligible to participate: inpatient at VMC and present at the time of the interview, screened for MRSA, English or Spanish speaker, able to respond to questions during an interview or had a family member available

and willing to do so, ages 18 to 65, and resident of 1 of the top swine producing zip codes of North Carolina. The age and geographic restrictions were intended to increase the probability of enrolling participants who worked and/or lived near CAFOs.

Top swine producing zip codes were defined as those at or above the median number of swine permitted to be produced by the NC Division of Water Quality (NC DWQ). In total, 176 zip codes met this criterion. Although eastern North Carolina is also home to high concentrations of intensive poultry production facilities, most are not represented in the DWQ database; they do not hold DWQ-issued non-discharge waste water permits because they do not use liquid waste management systems. Therefore, I did not include poultry operations in this calculation.

Interviews

After receiving permission from hospital-based medical providers, I visited patients in their hospital rooms to administer a short, structured interview. The interview included questions about the following: current employment, job title, employer, work address; number of household members; household member antibiotic use, previous hospitalizations, and occupation; home address; direct (defined as touching) and indirect (defined as working near but not touching) contact with cows, pigs, chickens, turkeys, and horses at work or outside of work; cats and dogs living inside the home; antibiotic use within the past year, demographic information; ability to smell odor from animal farms when at home; residence on a farm with animals; and handling of meat at home and at work. Legally authorized representatives could respond to questions on behalf of the patient, if they were willing.

Chart review

I reviewed medical records to ascertain whether, within 1 year of the current hospitalization, the participant was previously hospitalized. I checked participant interview reports about prior antibiotic use against information in the medical charts. If participants reported using antibiotics within the past year, I left the variable coded as such. If the medical charts indicated that the patient was prescribed an antimicrobial within the past year but the participant reported that they had not used antibiotics in the past year, I coded the variable to reflect the data in the medical chart.

Geocoding

I used ArcMap10® (ESRI, Inc., Redlands, CA, USA) to geocode participants' home and work addresses. If the home address that the participant reported could not be geocoded but the address listed in the medical chart could, I assigned coordinates according to the address in the medical record.

Identifying CAFOs near participant homes

I used satellite imagery in Google Earth™ to identify swine or poultry CAFO within 1 mile radii of participants' home and work addresses. In North Carolina, swine CAFOs store animal waste in open air pits, euphemistically called lagoons, but most poultry operations do not utilize these liquid waste management systems. Therefore, I identified images of animal barns beside small bodies of water, the lagoons, as swine. I classified images of barns without lagoons as poultry CAFOs.

Human and swine population densities and rural area classifications

I downloaded topically integrated geographic encoding and referencing (Tiger)® shapefiles showing census block groups and urban areas from the 2010 United States Census [151]. I used SAS version 9.3's GINSIDE procedure to define each home address as within an urban area, urban cluster, or rural area. I combined urban areas and clusters into a single "urban" category. I also used the GINSIDE procedure to identify the census block group to which the home address belonged.

The publicly available North Carolina Division of Water Quality (DWQ) database presents information on the total counts of swine, and the developmental stage of the swine (farrowing, weaning, feeding, finishing) at each permitted facility in North Carolina. I used this information to assign counts and densities of the following in each block group: permitted swine, permitted farrowing swine, and permitted non-farrowing swine. Densities were defined as number of swine divided by the number of square miles in the block group of residence. I classified densities by developmental stage because of evidence that LA MRSA is more prevalent among the youngest pigs [27, 152].

I also used 2010 United States Census data to assign human population densities to each block group. Human population densities were defined as the number of people living in a block group divided by the number of square miles in the area.

Molecular typing

The UltraClean™ Microbial DNA Isolation Kit (Mo Bio Laboratories, Solana Beach, CA) was used to extract the MRSA DNA, and the NanoDrop® ND-1000 Spectrophotometer

(Isogen, Ijssel stein, The Netherlands) was used to estimate the genomic DNA concentration. Sample concentrations were set to 35 ng/μl.

The Diversilab® Staphylococcus kit for DNA fingerprinting (bioMerieux, Boxtel, The Netherlands), a repetitive sequence-based polymerase chain reactin (rep-PCR), was used to amplify regions between repetitive, noncoding sequences in the DNA samples [147, 150]. The system was run according to manufacturer specifications, and analysis was conducted using the DiversiLab® software (version v.r.3.3.40).

Software from the DiversiLab® system (version v.r.3.3.40) was used for typing analysis. The rep-PCR profiles were compared to the DiversiLab® MRSA library containing 70 samples of the 14 representative USA pulsed field gel electrophoresis types [28]. At VMC, the MRSA library also includes one sample of livestock-associated (LA) MRSA, which was isolated from a pig in Iowa and confirmed to be multi-locus sequence type 398. A sample with an indistinguishable fingerprint was matched to one in the library and assigned that type. Based on this analysis, MRSA isolates were defined as CA, HA, LA, or non-matches.

Data checking and cleaning

I checked the data for entry mistakes, inconsistencies and outliers. I checked identification of CAFOs within 1 mile radii of participants' households against the DWQ spreadsheet.

Statistical analyses

I used SAS version 9.3 (Cary, NC) to conduct the statistical analyses. I used conditional logistic regression models to derive estimates of associations between MRSA carriage and the following variables: residence within 1 mile of a swine or poultry CAFO, swine counts (total, farrowing, and non-farrowing) in the block group of residence, swine densities (total, farrowing, and non-farrowing) in the block group of residence, reported ability to ever smell odor from an animal farm when at home, reported handling of uncooked meat at work and/or at home in the 2 weeks preceding the current hospitalization, indirect contact at work or direct contact at home with horses, and indirect contact at work or direct contact at home with livestock (pigs, cows, chickens, turkeys). To provide a comparison of relationships between MRSA carriage and environmental or occupational contact with livestock, I also examined relationships between MRSA carriage and the following: densities of humans living in the census block group, residence in a rural or urban area, employment status of the participant, and employment status of household members, if present. To be defined as employed, participants had to have worked in the 2 weeks preceding the current hospital admission.

I made coding decisions based on variable distributions and comparison of Akaike Information Criteria (AIC) statistics. All exposure variables were coded as binary terms, except those for household member presence and employment; swine head counts; swine densities; and human population density. I coded the human population density variable as a linear term; this coding produced a smaller AIC statistic compared to quadratic, cubic, or categorical coding.

I coded variables representing densities of total, farrowing, and non-farrowing swine as 3-level categorical variables (0 swine per square mile, referent vs. > 0-149 swine per square mile vs. > 149 swine per square mile). Zero was the median and mode of the distribution of total swine density and 149 was the 25th percentile of the distribution of observations with non-zero values for total swine density. Making the cut-point at the 25th rather than 50th percentile produced superior model fit. Coding the density variables using three categories led to improved model fit compared to binary coding, to other categorical coding schemes and to using linear, quadratic or cubic terms.

Because there were small numbers of observations within the categories of non-farrowing and farrowing swine density variables, I also investigated the effect of re-categorizing these according to their own distributions: (0 vs. > 0-77 vs. > 77 for farrowing and 0 vs. > 0-616 vs. > 616 for non-farrowing swine). The cut-points 77 and 616 were the median of the distribution of non-zero values for farrowing and non-farrowing densities, respectively.

Variables representing swine counts were entered into the models as linear, quadratic, and cubic terms for total and non-farrowing swine, and linear and quadratic terms for non-farrowing swine. This coding caused model fit to improve compared to use of categorical terms.

All models were conditioned on the matching variables age and gender. I also adjusted the models for education (< high school degree vs. high school degree or higher), selected *a priori* based on the belief that this could confound the relationship between MRSA carriage and the exposure variables.

Because of the potential for the rapid PCR test to identify false positives, I reran the conditional logistic regression models using a stratified dataset, examining relationships within the matched sets containing cases whose MRSA swabs grew colonies when cultured, and the matched sets with cases whose swabs did not culture. In addition, I ran the models to compare CA MRSA carriers and HA MRSA carriers to each of their matched controls.

I report results as beta coefficients \pm 1 Standard Error (SE). In the tables I also report odds ratio (OR) estimates to aid interpretation and Wald statistics as an indicator of the variable's contribution to the fit of the model.

The Institutional Review Boards at both the University of North Carolina at Chapel Hill and East Carolina University approved this research. All participants provided written informed consent and signed Health Information Portability and Accountability Act authorization forms.

Results

From July - December 2011, I invited 164 cases and 190 controls to participate. In total, 121 (73.8%) cases and 122 (64.2%) controls participated. Of these participants, 4 (3.3%) cases and 3 (2.5%) controls reported an address that was outside the eligible zip codes. They were excluded from the analysis, leaving 117 cases and 119 controls. The analysis included 100 (89.3%) matched sets with one case and one control; 7 (6.3%) with 2 controls per case; and five (4.5%) with 2 cases per control. Participants lived in 152 block groups in eastern North Carolina or the most eastern section of central North Carolina.

Participant characteristics

Table 5.1 presents descriptive statistics for the study population. More than half of study participants were female, and nearly half were 50 – 65 years of age. Cases had lower levels of education than controls; 24 (20.5%) cases and 12 (10.9%) controls reported their highest degree earned in school as less than a high school diploma. Cases and controls had similar races; 61 (51.3%) controls and 63 (53.9%) cases were non-white Hispanic. Five cases (4.3%) and 4 (3.4%) controls reported living on a farm where animals were raised, but none reported living on a farm where animals were raised in confinement.

Zero participants reported working directly with livestock. Two (1.7%) cases and 8 (6.7%) controls worked within a 1 mile radius of a swine or poultry CAFO. Four (3.4%) cases and 13 (10.9%) controls worked in a census block group with permitted swine. Four (3.4%) controls and 1 (0.9%) case reported having indirect contact with livestock at work; 0 were employed at a livestock farm or slaughterhouse. Five (4.2%) controls and 4 (3.4%) cases reported working as a medical provider; 4 (3.4%) controls and 5 (4.3%) cases worked a job that involved contact with children.

Four (3.4%) controls and 1 (0.9%) case reported living with a person who worked on a farm with animals. Of these, 2 controls and 1 case reported that the animals on the farm lived in confinement. Proportions of controls and cases living with household members who worked in health care were similar; 15 (12.8%) controls and 12 (10.3%) cases.

Permitted swine in the block group of residence and MRSA nasal carriage

In total, 58 (49.6%) cases and 47 (39.5%) controls lived in a block group with any permitted swine. However, 23 (19.7%) cases and 30 (25.2%) controls lived within a 1 mile

radius of a swine or poultry CAFO (Table 5.2). The mean \pm 1 standard deviation (SD) for densities of total, farrowing, and non-farrowing swine in the census block groups of residences were 400.2 ± 760.7 , 44.9 ± 128.6 , and 355.3 ± 709.5 , respectively.

Adjusted for education, cases had 1.56 ± 0.64 higher log-odds of living in a census block group with more than 0 and up to 149 swine per square mile. Similarly, case status was positively associated with living in block groups with medium densities of farrowing swine (0.69 ± 0.36). The relationship between non-farrowing swine density and MRSA carriage was also positive, although the effect estimate was similar in magnitude to its SE (0.71 ± 0.62). The relationships between swine densities and case status were non-linear. The associations between residence in a block group with the highest densities of swine and case status were negative with small Wald statistics.

Because there were small numbers of observations within categories of non-farrowing and farrowing swine density variables, I re-ran the models, categorizing these according to their own distributions: (0 vs > 0-77 vs > 77 for farrowing and 0 vs > 0-616 vs > 616 for non-farrowing swine). After re-categorizing these variables, the non-linear relationships between case status and swine densities remained; the relationship between median densities of farrowing swine and case status became stronger and more positive. The other estimates remained in the same direction but were less predictive compared to those based on the variable coding scheme reported in Table 5.2. The effect estimates for living in block groups with > 0-77 and >77 permitted farrowing swine/square mile were 1.05 ± 0.48 (Wald=4.74) and -0.38 ± 0.37 (Wald=1.04), respectively. The effect estimates for living in block groups with >0-616 and >616 permitted non-farrowing swine/square mile were 0.28 ± 0.35 (Wald=0.63) and -0.12 ± 0.34 (Wald=0.12), respectively (data not shown).

In addition to considering swine densities in the block group of residence, I also considered the relationship between swine counts and MRSA nasal carriage. Compared to swine density, swine counts were not as predictive. The means \pm 1 SD of total, farrowing, and non-farrowing swine counts were $9,870.5 \pm 22,057.5$, $940.8 \pm 2,135.3$, and $8,929.6 \pm 20,834.6$, respectively. The relationship between case status and total swine head was best fit by a model with linear, quadratic, and cubic terms for total swine head counts. Based on this model, adjusted for education, the equation for estimating the natural logarithm (ln) of the odds of case status ($p/1-p$, where p is the probability of being a case), comparing participants living in block groups with 2 different values for swine count represented by a and b , was:

$$\log(p/1-p) = 4.9E-5 \times (a-b) - 1.8 E-9 \times (a^2-b^2) + 1.2E-14 \times (a^3-b^3).$$

The relationship between farrowing swine and case status was best fit with a model with linear and quadratic terms for number of farrowing swine. The equation from the adjusted model for estimating the ln-odds of case status, comparing participants living in block groups with different values of farrowing swine was:

$$\log(p/1-p) = 4.8E-4 \times (a-b) - 9.5E-8 \times (a^2-b^2).$$

The relationship between non-farrowing swine and case status was best fit with a linear-quadratic-cubic term model. The equation for estimating the ln-odds of case status, based on a comparison of participants living in block groups with different values of non-farrowing swine was:

$$\log(p/1-p) = 1.6E-3 \times (a-b) - 1.9E-9 \times (a^2-b^2) + 4.4E-10 \times (a^3-b^3).$$

Environmental and occupational variables associated with MRSA nasal carriage

Compared to controls, cases had 0.41 ± 0.32 higher log-odds of reporting ever smelling odor from a farm with animals when they were home. This was based on a comparison of reports of ability to smell odor less than once per month or more versus never. Less than half the study population and higher proportions of controls than cases were current members of the workforce (41.2% vs 31.6%). The unadjusted estimate for this relationship was negative and relatively precise, with a Wald statistic of 2.47. After adjustment for education, the estimate remained negative but moved towards the null.

The effect estimate for the relationship between human population density and case status was close to 0. The effect estimates for relationships between case status and the following variables were all negative and their Wald statistics smaller than 2: living within a 1 mile radius of a CAFO, living in a rural area, handling raw meat, having indirect contact at work or direct contact in the community with livestock, and having indirect contact at work or direct contact in the community with horses.

Culture positive cases

Duplicate swabs from 108 of 117 were available to be cultured, and 49 (45.4%) grew colonies. I reran the models, comparing the cases whose MRSA isolates cultured to their matching controls (N=52 controls), and the cases whose isolates did not culture to their matching controls (N=69; Table 5.3).

Among culture-positive cases, there was a positive relationship with reported odor when at home (0.90 ± 0.54). There was also a positive relationship between culture positive MRSA carriage and living in block groups with medium densities of total permitted swine

(1.59 ± 1.1) and medium densities of farrowing permitted swine (0.88 ± 0.55). None of the other variables that I considered demonstrate substantial prediction of culture-positive MRSA carriage. Among non-culture positive cases, the following variables were positively associated with case status: medium densities of total, farrowing, and non-farrowing swine (1.62 ± 0.79 , 0.58 ± 0.48 , and 1.22 ± 0.83 , respectively). In the analysis of non-culture positive cases, there was a negative relationship with living within 1 mile of a swine or poultry CAFO (-0.88 ± 0.43) and with living in block group areas with high densities of permitted farrowing swine (-1.62 ± 0.82).

Molecular typing

None of the 49 cultured MRSA isolates matched the CC398 isolate in the Diversilab® library. Seven of the 49 (14.3%) did not match any MRSA types in the DiversiLab® library. Twenty-one of the MRSA cases were carrying HA strains, and 21 were carrying CA strains. I ran conditional logistic regression models, adjusted for education, to compare CA or HAMRSA carriers to their matched controls.

However, compared to controls, CA MRSA carriers had lower log-odds of being currently employed (-0.86 ± 0.60 , Wald=2.08), of living in a rural block group (-1.46 ± 0.79 , Wald=3.41), of handling raw meat (-2.34 ± 1.12 , Wald=4.40), and of living in a block group area with more than 149 total swine/square mile (-1.19 ± 0.82 , Wald=2.12) or non-farrowing swine/square mile (-0.85 ± 0.72 , Wald=1.38). CA MRSA carriers had higher log-odds of reporting odor from a farm when at home (1.84 ± 1.08 , Wald=2.34). All other Wald statistics, including all of those from the analysis of HA MRSA carriers, were less than 1, indicating poor model fit and weak prediction of the outcome.

Discussion

In recent years, MRSA has gained prominence as a zoonotic pathogen [172]. Identical strains of MRSA have been found on humans and their companion animals [64, 160], and human strains of MRSA have been found on livestock [173]. Furthermore, distinct clones of MRSA, including CC398 and CC9, are believed to have evolved in response to selective pressures from subtherapeutic administration of antibiotics in livestock production [172]. Antibiotic resistant bacteria have been found in bioaerosols [120, 174], soil [174], and air samples [122] collected in or near CAFOS. Thus, MRSA could be transmitted from CAFOs into surrounding communities via airborne pollutants and/or livestock workers.

This is one of the first studies to detect a positive association between MRSA carriage and residence in areas with moderate densities of swine. However, the relationship between case status and density of swine was not linear; it became negative when I compared the highest swine density census blocks to census blocks with zero densities of swine. The absence of a non-linear relationship between case status and swine densities could reflect higher densities of poultry operations in areas with medium densities of swine operations. I could not calculate densities of permitted poultry because, in North Carolina, most poultry operations do not hold non-discharge waste-water permits from the Division of Water Quality and their locations are therefore not publicly available. Reported ability to smell odor from a farm with animals was also positively associated with MRSA nasal carriage; however the effect estimate was small and imprecise. Living within 1 mile of a CAFO was negatively associated with MRSA carriage.

Relationships between carriage of LA strains of MRSA and livestock density have been investigated previously and observed in some but not all studies. In the Netherlands,

van Cleef surveyed adults living in high density pig areas. In their study, only 1 of 534 (0.2%) people without livestock contact was a MRSA nasal carrier, but 13 of 49 people with livestock contact were carrying MRSA [175]. In Germany, 0 of 422 students ages 10-16 years not living on pig farms were carrying MRSA [97]. However, in the Netherlands, van Loo et al. found that a higher proportion of LA MRSA carriers vs. carriers of other MRSA strains lived in rural areas and had contact with swine or cattle [133].

Whereas most studies of MRSA carriage and infection in the United States have been conducted in urban settings [67, 176], over 50% of the participants in this study population lived in rural areas. The proportions of MRSA nasal and controls living in rural areas were not substantially different. However, based on small numbers, a higher proportion of controls than CA MRSA carriers lived in rural areas. In a study of MRSA nasal carriers at a hospital in Hershey, Pennsylvania, Peterson et al. did not detect differences in terms of the genetic strain of carriage when residents of rural counties were compared with residents of urban counties [9]. A population-based study in Pennsylvania reported higher odds of MRSA infection among residents of cities or small towns compared to rural areas [157]. Van Loo et al. reported a higher prevalence of human associated MRSA strains in areas with high human population densities [133]. Although I did not observe a comparable association, none of the residential areas in this population of patients at VMC were as densely populated by humans as the mostly densely human populated areas in the Netherlands. Also, results from a study of MRSA carriage in the United States are not necessarily comparable those from a study in the Netherlands, where MRSA control measures are more rigorous at a national level [177].

In North America and Europe, most research on LA MRSA has focused on CC398 [88, 173] although human clones have been identified on livestock, as well [178]. None of the 49 MRSA colonies from cases in this study were CC398. Recently, MRSA CC398 was found in the nares of 2 of 99 industrial livestock workers in eastern North Carolina; only 4 of the 99 workers reported being hospitalized in the past year (Rinsky and Nadimpalli et al, unpublished data, 2012). The absence of this strain in my study could be attributable to the lack of occupational livestock exposures, especially since research has shown that carriage of this CC is related to direct occupational contact [71, 97, 133, 179].

In this study population, the overall prevalence of employment was low. Despite restricting the study to patients ages 18 - 65, fewer than half were members of the workforce. Higher percentages of controls than cases were currently employed and hospitalized for injury or poisonings, rather than chronic conditions that would indicate poor underlying health. These results are suggestive of a healthy worker effect. Negative relationships between MRSA carriage and other variables that I considered—livestock contact, horse contact, and meat handling, for example—might also reflect controls being healthier than cases, since very sick people would be less inclined or able to engage in such activities. Contact with horses has been associated with human MRSA carriage [68]. Evidence that meat handling might be associated with MRSA carriage is mixed, however. MRSA has been detected in meat products [81], but the prevalence of MRSA among meat handlers has been shown to be relatively low [180].

This work had several limitations. Results are not generalizable to non-hospitalized members of the eastern North Carolina community. Furthermore, participants' knowledge of their MRSA screening results could have influenced their responses to questions. Also, only

49 cases identified by the PCR assay were confirmed to be MRSA by culture, which could indicate false positives. The PCR might have misidentified methicillin susceptible *Staphylococcus aureus* with remnants of SCCmec as MRSA [149, 162]. The assay might also have detected non-viable, non-culturable bacteria [164, 165] or DNA residue from MRSA that was previously there but removed by mupirocin treatments received during a prior hospitalization [149, 164]. Additionally, since the hospital only swabs the anterior nares, patients carrying MRSA at other locations of their bodies would have been classified as controls. Also, since swabs were tested for MRSA but not MSSA, there was a lack of information on *S. aureus* that was susceptible to beta-lactam antibiotics, and/or resistant to non-beta lactam antibiotics. This is important because a recent study found that resistance to tetracycline was the most common type of antibiotic resistance among CAFO workers (Rinsky and Nadimpalli et al, unpublished data, 2012). Another limitation is that only one MRSA colony was molecularly typed; it is possible that carriers were co-colonized by multiple strains of the bacteria. Finally, variables representing swine densities within block groups and swine or poultry CAFOs within 1 mile radii of addresses might have been misclassified. The density calculations were based on a publicly available data set showing permitted CAFOs; however, some of the facilities might not have been operating or producing the maximum number of animals that they were permitted. It is also possible that, in studying satellite images to identify CAFOs within 1 mile radii of addresses, I misidentified buildings as facilities or failed to identify some operations.

Despite these limitations, this work is useful for designing further research. A handful of participants reported environmental or household member contact with livestock, and approximately 45% of the study population lived in a block group with permitted swine.

This suggests that livestock specific strains of MRSA could be introduced into the hospital. Already, an outbreak of MRSA CC398 has been described at a Dutch hospital [135] and nursing home [136]. This is concerning, since under selective pressures within a clinical environment, LA clones could become more drug resistant, more virulent, and/or better adapted to human hosts. Active surveillance for CC398 and other novel strains of MRSA is essential, especially at VMC, the largest hospital in eastern North Carolina, which is a region with dense populations of CAFOs. Similar investigations to this, but with in- and out-patients at smaller regional hospitals in eastern North Carolina would also be useful.

In conclusion, moderate densities of swine in participants' block groups of residence were associated with nasal MRSA carriage detected by PCR at the time of hospital admission; however other measures of livestock exposure showed little relationship. Results also suggested that lower proportions of nasal MRSA carriers than non-carriers were currently employed. The study provides useful information for designing future studies of the ability of antibiotic resistant bacteria to spread from CAFOs into human communities.

Tables

Table 5.1. Characteristics of methicillin resistant *Staphylococcus aureus* nasal carriers and their matched controls

	No. (%)			
	Controls (n = 119)		Cases (n = 117)	
Female	68	57.1	67	57.3
Age, y				
18-29	29	24.4	24	20.5
30-39	15	12.6	16	13.7
40-49	17	14.3	22	18.8
50-59	40	33.6	37	31.6
60-65	18	15.1	18	15.4
Non-white race	61	51.3	63	53.9
< High School degree	13	10.9	24	20.5
Antibiotic use within past 12 mo.	93	78.2	93	79.5
Hospitalized within past 12 mo.	70	58.8	71	60.7
Primary diagnosis for factors influencing health status and contact with health professionals	12	10.1	6	5.1

Abbreviations: month, mo.

Table 5.2. Estimates of associations of methicillin resistant *Staphylococcus aureus* nasal carriage with environmental and occupational exposures, derived from conditional logistic regression models

	No. (%)				Logistic regression models conditioned on age and gender				Logistic regression models conditioned on age and gender, adjusted for education			
	Controls (n = 119)		Cases (n = 117)		B	SE	Wald	OR	β	SE	Wald	OR
Permitted swine per square mile of block group ^a												
>0-149	7	5.9	20	17.1	1.67	0.64	6.88	5.30	1.56	0.64	5.93	4.76
>149	40	33.6	38	32.5	0.03	0.30	0.01	1.03	-0.05	0.30	0.03	0.95
Permitted farrowing swine per square mile of block group ^a												
>0-149	17	14.3	34	29.1	0.80	0.35	5.33	2.24	0.69	0.36	3.67	1.99
>149	15	12.6	6	5.1	-0.78	0.49	2.54	0.46	-0.88	0.51	2.97	0.42
Permitted non-farrowing swine per square mile of block group ^a												
>0-149	5	4.2	10	8.6	0.78	0.61	1.63	2.17	0.71	0.62	1.33	2.04
>149	37	31.1	37	31.6	0.04	0.29	0.02	1.05	-0.05	0.29	0.03	0.95
Live within 1 mile of a swine or poultry CAFO	30	25.2	23	19.7	-0.33	0.32	1.09	0.72	-0.51	0.34	2.30	0.60
Ever smell odor from a farm with animals when at home	22	18.5	31	26.5	0.46	0.32	2.07	1.59	0.41	0.32	1.63	1.51
Contact with pigs, chickens, cows, or turkeys ^b	10	8.4	5	4.3	-0.65	0.62	1.11	0.52	-0.65	0.64	1.04	0.52
Contact with horses ^b	9	7.6	7	6.0	-0.28	0.59	0.23	0.75	-0.36	0.59	0.37	0.70
Handle uncooked meat products at work or at home ^c	79	66.4	73	62.4	-0.18	0.28	0.42	0.84	-0.21	0.29	0.56	0.81
Current member of the work-force ^d	49	41.2	37	31.6	-0.45	0.29	2.47	0.64	-0.30	0.30	1.03	0.74
Human population density in block group of residence ^e	-	-	-	-	-0.03	0.09	0.09	0.97	0.00	0.10	0.00	1.00
Live in a rural area ^f	62	52.1	63	53.9	0.03	0.26	0.02	1.03	-0.08	0.26	0.10	0.92

Abbreviations: odds ratio, OR; standard error, SE

^a Referent category is 0 swine per square mile of block group. 149 was the 25% of the distribution of the non-zero values of total permitted swine.

^b Exposed category includes participants who reported direct contact outside of work and/or indirect contact at work; no participant reported having direct contact at work

^c During interviews participants were asked about handling of meat within 2 weeks of their hospitalization. However, 1 control and 2 cases were screened for MRSA 9 or more days prior to the hospitalization. Data on meat handling represents the 2 weeks prior to hospitalization but not the 2 weeks prior to screening.

^d Defined as working within the 2 weeks prior to the current hospital admission

^e Defined as population/square mile in census block group of residence. Variable was entered into the model as a linear term, and the estimate represents the change in the log-odds of case status for every increase in 1,000 people/square mile

^f Defined based on the home address and using 2010 United States Census Bureau definition of rural and urban areas

Table 5.3. Estimates of association of methicillin resistant *Staphylococcus aureus* nasal carriage with environmental and occupational exposures from conditional logistic models adjusted for education, stratified by culture status

Variable	Culture positive cases					Culture negative cases				
	Controls (n=52)	Cases (n=49)	β	SE	Wald	Controls (n=69)	Cases (n=68)	β	SE	Wald
Permitted swine per square mile of block group ^a										
> 0-149	1.9	10.2	1.59	1.10	2.10	8.7	22.1	1.62	0.79	4.21
> 149	38.5	36.7	-0.12	0.43	0.08	29.0	29.4	0.08	0.42	0.04
Permitted farrowing swine per square mile of block group ^a										
> 0-149	13.5	30.6	0.88	0.55	2.52	14.5	27.9	0.58	0.48	1.48
> 149	11.5	8.2	-0.16	0.69	0.05	13.0	2.9	-1.62	0.82	3.93
Permitted non-farrowing swine per square mile of block group ^a										
> 0-149	3.8	4.1	-0.02	1.02	0.00	4.3	11.8	1.22	0.83	2.19
> 149	34.6	36.7	-0.03	0.42	0.01	27.5	27.9	-0.04	0.41	0.01
Live within 1 mile of a swine or poultry animal production facility	17.3	22.4	0.24	0.60	0.17	30.4	17.6	-0.88	0.43	4.16
Ever smell odor from a farm with animals when at home	13.5	24.5	0.90	0.54	2.72	21.7	27.9	0.11	0.42	0.07
Contact with livestock ^b	7.7	4.1	-0.97	1.23	0.62	8.7	4.4	-0.50	0.75	0.44
Contact with horses ^b	7.7	6.1	-0.43	0.92	0.22	7.2	5.9	-0.29	0.76	0.14
Handle uncooked meat products at work or at home ^c	71.2	65.3	-0.42	0.44	0.91	60.9	60.6	-0.07	0.39	0.03
Current member of the work-force ^d	40.4	32.7	-0.24	0.43	0.33	42.0	30.9	-0.38	0.42	0.84
Human population density in block group of residence, ^e	-	-	-0.03	0.13	0.04	1254.7	1357.0	0.05	0.14	0.10
Live in a rural area ^f	51.9	49.0	-0.21	0.43	0.24	52.2	57.4	0.01	0.33	0.00

^a Referent category is 0 swine per square mile of block group; 149 was the 25% of the distribution of the non-zero values of total permitted swine.

^b Exposed category includes participants who reported direct contact outside of work and/or indirect contact at work; no participant reported having direct contact at work.

^c During interviews participants were asked about handling of meat within 2 weeks of their hospitalization. However, 1 control in the culture positive analysis and 2 cases in the culture negative analysis were screened for MRSA 9 or more days prior to the hospitalization. Data on meat handling represents the 2 weeks prior to hospitalization but not the 2 weeks prior to screening.

^d Defined as working within the 2 weeks prior to the current hospital admission.

^e Defined as population/square mile in census block group of residence. Variable was entered into the model as a linear term, and the estimate represents the change in the log-odds of case status for every increase in 1,000 people/square mile.

^f Defined based on the home address and using 2010 United States Census Bureau definition of rural and urban areas

CHAPTER 6

Conclusion

Background

Since its discovery in the 1960s, MRSA has demonstrated an adaptive resilience. Once confined to healthcare settings, it emerged in the community in the 1990s. In the Netherlands in 2004, a novel strain of MRSA, belonging to clonal complex 398 (CC398), was detected and associated with direct occupational contact with pigs [181]. In 2009, the first paper reporting the presence of MRSA CC398 in the United States, in Iowa, was published [27]. The novel strains of MRSA were believed to have evolved in livestock, as a result of selective pressures from subtherapeutic antibiotic administration, which is common practice in industrial food animal production [88]. The emergence of MRSA in livestock represents the newest phase in the evolution of this bacterium.

North Carolina is an important state for industrial livestock production. As of August of 2009 there were 2,166 swine concentrated animal feeding operations (CAFOs) with active wastewater discharge permits from the North Carolina Division of Water Quality; most of these operations are located in the southeastern region of the state [137]. Despite intensive animal production activities in eastern North Carolina, there have been few investigations of the relationship between human MRSA carriage and environmental and occupational exposures. The overall objectives of my dissertation were to investigate relationships

between MRSA carriage and occupational and environmental exposures in eastern North Carolina.

The largest hospital in eastern North Carolina, Vidant Medical Center (VMC), screens all admitted patients for nasal MRSA carriage [147]. Because of its location in eastern North Carolina and its universal MRSA screening program, VMC seemed an ideal place for the conduct of my dissertation work. Below, I outline the results, lessons learned, research gaps, and public health importance of this research.

Summary of work conducted

From July - December of 2011, I conducted structured interviews with inpatients at VMC. Cases were patients who were identified as having MRSA in their anterior nares, based on a BD GenOhm® rapid polymerase chain reaction (PCR) screen that the hospital administers to all admitted patients. Nasal swabs from patients with a positive PCR screen were cultured. Controls were patients who were not carrying MRSA in their anterior nares at the time of admission; they were age and gender matched to cases.

In total, I interviewed 121 cases and 122 controls. After completing data collection, I excluded 7 participants from the analyses—4 cases and 3 controls. Three cases and three controls reported addresses that were outside the eligible study area and another case reported having been most recently living and working out of North Carolina.

I developed a multivariable model. The following variables were included: education, race, hospitalization and MRSA screening history, visiting a gym or playing sports in the past 2 weeks before the current hospitalization, smoking cigarettes in the past

year, household member prior hospitalization and antibiotic use, and cats or dogs living inside the home. Having been previously hospitalized and screened positive for MRSA carriage in the past year and living with a household member who used antibiotics in the past 4 weeks or was hospitalized in the past year were the most predictive terms in the model; both were positively associated with case status. Being hospitalized but not screening positive for MRSA within the past year was associated with a lower log-odds of MRSA carriage. I also found that higher proportions of controls than cases had at least a high school degree or GED. I reran the multivariable model on only the 49 matched sets with cases whose MRSA status was confirmed by culturing. Interestingly, the relationship between being hospitalized and screening positive for MRSA in the past year moved to the null in this sub-analysis.

The results from the first results chapter suggest that community exposures are important predictors of MRSA carriage. In particular, the finding that previous MRSA carriage was associated with current MRSA carriage supports this conclusion. Patients who screened positive for MRSA on a previous visit to the hospital were treated with the topical antibiotic, mupirocin; I reviewed medical charts and confirmed that all but one of the participants were prescribed the antibiotic following their previous positive screen. Thus, if they were decolonized during a previous hospital visit but found to be carrying MRSA during the current visit, this suggests that they were recolonized in the community. Also supporting the importance of environmental exposures in predicting MRSA carriage were the findings that: a higher proportion of cases than controls had lower levels of education, which could serve as a proxy measure for socioeconomic status and living conditions, and antibiotic use and hospitalizations of a household member predicted current MRSA carriage.

However, this work had certain limitations. In particular, the rapid PCR test could have identified false positives; it might have detected either nonviable bacteria, and/or DNA residue from bacteria that was previously present but removed via antibiotic treatments. Therefore, the results from this chapter must be interpreted cautiously.

In the second results chapter, I reported on relationships between several indicators of environmental exposures to livestock—reported odor from a farm when at home, density of swine (total, farrowing and non-farrowing) in the block group of the participants' residence, and indirect contact at work or direct contact in the community with livestock, among others. I coded the swine density variables such that I was able to estimate the effects of living near medium densities of swine versus none, and of living near the highest densities of swine versus none. I also considered relationships between MRSA carriage and employment, residence in rural areas, density of human populations in the census block of residence, contact with horses, and meat handling. In addition to running univariable conditional models, I ran all of the models adjusted for potential confounding by education level.

Many of the estimates of association from these analyses were negative but close to the null and imprecise. There was a negative relationship between employment status and MRSA carriage. This relationship was especially pronounced in the model that was not adjusted for education. The negative effect estimate suggested a healthy worker effect, since a higher proportion of cases than controls were unemployed. The relationships of ability to smell odor from an animal farm when at home and medium densities of total, farrowing, and non-farrowing swine in the block group of residence were all positive. The estimate representing the effect of medium densities of total swine was the most positive and precise.

The positive relationship between MRSA nasal carriage and medium densities of permitted swine was suggestive of a relationship between MRSA carriage and environmental exposures to pollutants from animal production facilities. However, I did not observe a linear relationship between swine density and MRSA carriage since the strongest and most positive relationships were with medium rather than highest densities of swine. Also, most previous research of MRSA and livestock has focused on livestock-associated strains, especially MRSA CC398. This strain was not identified from among the 49 MRSA colonies that were sequence typed,

While the implications are not clear, the findings are suggestive of potential relationships between livestock and MRSA carriage and point to the need for further research in eastern North Carolina. A handful of participants reported they lived with people who worked with livestock, about a quarter of the participants lived within 1 mile of a swine or poultry CAFO, and nearly 50% of the study population lived in block group areas with any permitted swine. This research indicates that a proportion of hospitalized patients at VMC are exposed to livestock, and that the introduction of LA strains of MRSA into the hospital is possible.

Discussion and implications for future work

The results from this work suggest that community and household factors are predictive of MRSA carriage, and that residence in areas with swine production might be an important environmental exposure. Below, I describe some of the limitations associated

with this work. I then suggest future research that might help improve and expand upon this research.

Potential misidentification of MRSA carriers by the rapid PCR screen

Use of a rapid PCR screen to identify cases allowed me to quickly identify MRSA carriers. Some of the participants might have been discharged from the hospital had I waited for culture results to ascertain their case status.

However, there were important limitations associated with relying on the rapid test to identify cases. Thirty of the 117 PCR-identified cases had screened positive for MRSA on a previous occasion within the same year; 59% of these cases were not confirmed as MRSA positive by culture. This is concerning because the PCR might have misidentified as current MRSA carriers patients who were previously nasally colonized, decolonized, and at the time of the current MRSA screening, had DNA residue in their nares following mupirocin treatments. Other possibilities are that the PCR misidentified as MRSA carriers people who had methicillin susceptible *S. aureus* with remnants of the SCCmec gene in their noses.

This limitation implies that the results from this work must be interpreted tentatively. In the analysis of medical and household exposures associated with MRSA nasal carriage, previous MRSA carriage was strongly predictive. However, it is difficult to know if patients had MRSA removed via mupirocin therapy during a previous hospitalization, subsequently returned to the source of the MRSA in the community and were then recolonized, or if the PCR identified artifacts of bacteria that were previously present. I reran the models on only the 49 cases that were correctly identified via culturing methods; however, these analyses were underpowered. Future research studies that utilize the rapid PCR test to identify MRSA carriers should consider the sample size and power implications of potential false positives.

Potential misidentification of non-carriers as MRSA carriers is also concerning within the context of VMC's search and destroy MRSA screening program. Under hospital procedures, patients who are identified as MRSA carriers are decolonized using a topical antibiotic. If people who are not truly MRSA carriers are being exposed to antibiotics, then this could represent overuse of these drugs. There is a need for further investigations of the relationship between treatment for previous MRSA carriage and current MRSA carriage, and the effects of previous antibiotic treatment on the nasal environment. Future work should be designed to compare the results from the rapid PCR screen to results from cultures to help elucidate the reasons for a lack of concordance between the methods.

Low prevalence of occupational exposure

By conducting this work in a hospital, I was able to conveniently and inexpensively identify asymptomatic MRSA carriers in eastern North Carolina. However, I found that hospitalized inpatients were a poor source population for an occupational study. Approximately 31% of the patients that I approached about participating declined. Many were too physically weak to participate. Others were not entirely lucid, thus making them ineligible to participate in the consent process and interview. Some invited patients declined because they were exhausted—because of being sick, and also because they were constantly woken up by hospital activities. Even those who agreed to participate were, for the most part, chronically sick. Because so many had long-term illnesses, large proportions were also unemployed. Also, most were too sick to have recently engaged in a number of the activities that I asked about—swimming, fishing, hunting, participating in contact sports, and visiting a gym to exercise, for example.

Future research studies on relationships between occupational exposures and MRSA carriage should consider drawing study populations from outpatients and emergency department visitors, since these groups are probably healthier and more likely to be currently employed.

Potential health worker effect

A higher proportion of cases than controls were unemployed; thus, results from this work were suggestive of a healthy worker effect. The potential for a healthy worker effect within the context of research on MRSA carriage should be further investigated, and also considered in planning and interpreting results from occupational studies of the bacteria.

Importance of surveillance in the hospital

This work demonstrated the potential for livestock specific clones of MRSA to enter the hospital. This observation underscores the importance of continued surveillance and monitoring for new clones of MRSA.

Potential negative consequences of MRSA screening and research

While conducting in-hospital interviews with patients at VMC, I observed that for many participants, MRSA carriage was an ambiguous and frightening concept. Patients who screened positive for MRSA were startled by and nervous about the results; not only were they being hospitalized for a medical issue, but they were also told that they had a bacterium living on their bodies and this bacterium needed to be removed via antibiotic treatments. In addition to being concerned about the implications for their own health, MRSA carriers often expressed worries about being contagious. For example, one person who screened positive for MRSA asked me if they should stay away from their grandchildren in order to avoid exposing them to the bacteria. Also unexpected, I found that by asking participants about

numerous environmental variables that could be related to MRSA carriage, I caused them concern about coming into contact with these things. For example, when I asked one control participant about hunting experiences, they expressed concerns about coming into contact with a work colleague who engages in this activity.

These observations raise important questions about the ethical implications of screening people for MRSA carriage—both within a hospital setting and also within the context of a research study. This implies the need for qualitative and quantitative research on the best ways to communicate results about and meaning of MRSA carriage to study participants and patients. Also, the potential for causing fears and concerns among study participants and patient communities should be considered in cost-benefit analyses of research investigations and of hospital based screening programs. More generally, these observations illustrate the importance of carefully considering the internal and external effects of epidemiologic research—both to study participants, and to the general public.

Conclusions

Because MRSA is a clonal organism, molecular biology studies of the bacteria are useful for tracing sources of newly emergent strains. However, MRSA's long history demonstrates that over time, a mixing of clones has occurred. Thus, epidemiologic work is important since it provides information about population level trends in carriage, independent of clonal origins. Such knowledge is especially valuable for the purposes of infection control and prevention.

My dissertation was designed as a traditional epidemiologic case control study; as such, the conclusions that can be drawn from the results are limited to the bounds of the field.

I asked patients questions about their recent exposures, and used their responses to calculate estimates of associations with MRSA carriage. Results from this work suggested that community and home environments might be important predictors of MRSA carriage. More than anything, however, this research demonstrated a number of remaining gaps in knowledge about MRSA carriage. This study also illustrated the benefits and pitfalls of conducting epidemiologic research within a hospital setting. Results from this work should inspire the design of improved studies of the relationship between MRSA carriage and the community environment, and enhanced awareness of the potential connections between them.

APPENDIX

QUESTIONNAIRE USED FOR INTERVIEWS

Q1.1 [Interviewer: Please write in the participant's study identification number].

A or B : _____ Number: _____

Q1.2 Do you work outside of the home?

- Yes
- No
- Refused to answer

Q1.3 What is the name of your current employer(s)? Please list each of your employers if you have multiple employers.

[Example: Harris Teeter Grocery Store, Smithfield foods, East Carolina University, Pitt County Public Schools, Bank of America]

Employer 1. _____ Employer 2. _____

Employer 3. _____ Employer 4. _____

Q1.4 What is the street address for each of your workplaces?

[If the participant doesn't know, then ask:] What is the name of the road and the city/town where your workplace(s) is located?

Employer 1. _____

Employer 2. _____

Employer 3. _____

Employer 4. _____

Q1.5 What kind of business or industry do you currently work in? Please list the business or industry for each of your current jobs. For example: Health care, construction, food manufacturing, farming, auto engine manufacturing, retail (clothing store), retail (grocery store).

Employer one _____ Employer two _____

Employer three _____ Employer four _____

Q1.6 What kind of work do you currently do, or what is your job title? Please list the job title for each of your current jobs. For example: Salesperson at clothing store, farm worker, cashier at grocery store, registered nurse, fire fighter, mechanic

Employer one _____ Employer two _____

Employer three _____ Employer four _____

Q1.7 How many hours per week do you spend working at each of your jobs?

Employer one _____ Employer two _____ Employer three _____ Employer four _____

Q1.8 Do you work on a farm?

- Yes
- No
- Don't know

- Refused to answer

Q1.9 What type of farm do you work on? Your choices are: [Interviewer: read choices the participant and check those that apply].

- A farm that grows crops or plants
- A farm that grows animals
- Don't know
- Refused to answer

Q1.10 Do the animals on the farm where you work spend all of their time in confinement or in a house?

- Yes
- No
- Don't know
- Refused to answer

Q1.11 How often do you have direct contact with live animals at work, if ever? Direct contact means that you touch the live animals with your hands. Your choices are:

- Daily
- Several times each week
- Several times each month
- About once per month
- Less than once per month
- Never
- Don't know
- Refused to answer

Q1.12 Do you generally wear gloves when you have direct contact with live animals at work?

- Yes
- No
- Don't know
- Refused to answer

Q1.13 What types of live animals do you have direct contact with at work? [Select all that apply]

- Pigs
- Chickens
- Turkeys
- Cows
- Horses
- Goats
- Dogs
- Cats
- Other _____
- Refused to answer

Q1.14 Generally, about how many of these live animals do you have direct contact with at work? [Select all that apply]

	0	1-249	250-1,000	1,001-5,000	5,001-10,000	> 10,000	Refused to answer
--	---	-------	-----------	-------------	--------------	----------	-------------------

Pigs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chickens	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Turkeys	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cows	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Horses	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Goats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dogs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other _____	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q1.15 Starting the day before you were admitted to PCMH, when is the last time that you had direct contact with these live animals at work, if ever?

	Pigs	Chickens	Turkeys	Cows	Horses	Goats	Dogs	Cats	Other
Less than one day before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Between one and three days before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Between four and seven days before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
More than a week but less than 2 weeks before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
More than two weeks but less than a month before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
One month or more before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Don't know	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Refused to answer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q1.16 How often do you have indirect contact with live animals at work, if ever? Indirect contact means that you work in the same building as animals, or on a property that houses livestock such as pigs, cows, or chickens, or that you handle animal manure.

Your choices are:

- Daily
- Several times each week
- Several times each month
- About once per month
- Less than once per month
- Never
- Don't know
- Refused to answer

Q1.17 What types of live animals do you have indirect contact with at work?

- Pigs
- Chickens
- Turkeys
- Cows
- Horses
- Goats
- Dogs
- Cats
- Other _____

Q1.18 Generally, about how many of these live animals do you have indirect contact with at work?[Select all that apply]

Pigs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chickens	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Turkeys	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cows	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Horses	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Goats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dogs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Other	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q1.19 When is the last time that you had indirect contact with these live animals at work?

	Pigs	Chickens	Turkeys	Cows	Horses	Goats	Dogs	Cats	Other
Less than one day before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Between one and three days before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Between four and seven days before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
More than a week but less than 2 weeks before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
More than two weeks but less than a month before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
One month or more before I was admitted to PCMH	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Don't know	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Refused to answer	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
-------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------	--------------------------

Q1.20 When is the last time that you had any kind of contact with dead animals at work, if ever?

- Less than one day before I was admitted to PCMH
- Between one and three days before I was admitted to PCMH
- Between four and seven days before I was admitted to PCMH
- More than a week but less than 2 weeks before I was admitted to PCMH
- More than two weeks but less than one month before I was admitted to PCMH
- One month or more before I was admitted to PCMH
- Never
- Don't know
- Refused to answer

Q1.21 How often do you generally have contact with dead animals at work? Your choices are:

- Daily
- Several times each week
- Several times each month
- About once per month
- Less than once per month
- Don't know
- Refused to answer

Q1.22 What type of dead animals do you have contact with at work?

- Pigs
- Chickens
- Turkeys
- Cows
- Horses
- Goats
- Dogs
- Cats
- Other _____
- Don't know
- Refused to answer

Q1.23 Do you handle raw meat products at work? Raw meat products are defined as meat products that have not been cooked.

- Yes
- No
- Don't know
- Refused to answer

Q1.24 What type of raw meat products do you handle for work?

- Pork (pig)
- Poultry (chicken)
- Poultry (turkey)
- Beef (cow)
- Fish
- Other
- Don't know

Refused to answer

PART 2

Now I am going to ask you some questions about the members of your household.

Q2.1 Including yourself how many people are members of your household? A household member is someone who has lived in your house most of the time for the past 3 months. [If answer is 1, meaning the participant lives alone, then skip to part 3]

Number of household members: _____

Q2.2 Does any member of your household, other than yourself, work outside the home?

- Yes
- No
- Don't know
- Refused to answer

Q2.3 Does any member of your household, other than yourself, work on a farm?

- Yes
- No
- Don't know
- Refused to answer

Q2.4 On average, about how many hours each week does your household member work on a farm?

- More than 32 hours each week
- 20-32 hours each week
- Less than 20 hours each week
- Don't know
- Refused to answer

Q2.5 What type of farm does your household member work on? Your choices are: [Interviewer, read choices, select all that apply]

- A farm that grows crops or plants.
- A farm that grows animals
- Don't know
- Refused to answer

Q2.6 Do the animals on the farm where your household member works spend all of their time in confinement or in a house?

- Yes
- No
- Don't know
- Refused to answer

Q2.7 Does the household member who works on the farm :

	Yes	No	Don't know	Refused to answer
Change his or her clothes before coming home from work?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Shower before coming home from work?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q2.8 Does anyone in your household other than yourself work:

In a health care setting such as a clinic, hospital or doctor's office, nursing home or long-term care facility?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
As a butcher?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In a job where they come into contact with live animals?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In a job where they come into contact with dead animals?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
At a waste water treatment plant?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
At a prison or correctional facility?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

PART THREE

Now I'm going to ask you some questions about your community and home.

Q 3.1 For how long have you lived in your current town or city of residence?[Answer should be reported in years, months, weeks and/or days . [Interviewer, please fill in the appropriate line.]

Years _____ Months _____ Weeks _____ Days _____

Q3.2 Do you live on a farm?

- Yes
- No
- Don't know
- Refused to answer

Q3.3 What type of farm do you live on?

Your choices are: [Interviewer, read choices]

- A farm that grows plants or crops
- A farm that grows animals
- Don't know
- Refused to answer

Q3.4 Do the animals on the farm that you live on spend all of their time in confinement or in a house?

- Yes
- No
- Don't know
- Refused to answer

Q3.5 On average, how often do you smell odors from a livestock farm when you are at home, if ever?

- Daily
- Several times each week
- Several time each month
- Less than once a month
- Never
- Don't know
- Refused to answer

Q3.6 How many of the following pets do you have living inside your home?

	0	1-3	4-5	6-10	> 10	Don't know	Refused to answer
Dogs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q3.7 Which of these animals that lives inside your home also goes outside?

- Dogs
- Cats

PART FOUR

Q4.1 Outside of work, how often do you currently have direct contact with any of the following animals, if ever? Direct contact means that you touch the animals with your hands. [Interviewer-ask each animal category and if there is contact check all the boxes that apply.]

	No contact	Less than once/month	About once/month	Several times/month	Several times each week	Daily contact
Pigs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Chickens	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Turkeys	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cows	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Horses	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Goats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Dogs	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Cats	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

PART FIVE

In the next two questions I will ask you about antibiotic use by you and by any member of your household.

Q5.1 Starting the day before you were admitted to Pitt County Memorial Hospital, have you taken antibiotics:

- a.) in the past 4 weeks? b.) in the past 6 months? c.) in the past year?

[Interviewer: If the participant answers yes to 4 weeks, then you do not need to ask about 6 months and the past year. If the participant answers yes to 6 months, then you do not need to ask about the past year.]

	Yes	No	Don't know	Refused to answer
In the past 4 weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In the past 6 months	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In the past year	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q5.2 Starting the day before you were admitted to Pitt County Memorial Hospital, has someone in your household other than yourself taken antibiotics: a.) in the past 4 weeks? b.) in the past 6 months? c.) in the past year?

[Interviewer: If the participant answers yes to 4 weeks, then you do not need to ask about 6 months and past year. If the participant answers yes to 6 months, then you do not need to ask about the past year.]

	Yes	No	Don't know	Refused to answer
In the past 4 weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In the past 6 months	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In the past year	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

In these next questions I am going to ask about recent hospitalizations of you or of any member of your household. Hospitalization means being admitted to or seen by a provider in a hospital for at least 8 hours.

Q5.3 Other than this hospital visit, have you been hospitalized at any point:

a.) in the past 4 weeks? b.) in the past 6 months? c.) in the past year?

[Interviewer-if you the participant answers yes to four weeks, then you do not need to ask about 6 months or past year. If they answer yes to six months then you do not need to ask about the past year.]

	Yes	No	Don't know	Refused to answer
In the past four weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In the past six months	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
In the past year	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q5.4. Has anyone in your household been hospitalized at any point:

a.) in the past 4 weeks? b.) in the past 6 months? c.) in the past year?

Again, a household member is someone who has lived in the same house as you most of the time for at least the last 3 months.

	Yes	No	Don't know	Refused to answer
in the past 4 weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
in the past 6 months	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
in the past year	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q5.5 Starting the day before you were admitted to Pitt County Memorial Hospital, have you been treated for a Methicillin resistant Staphylococcus aureus (or MRSA) infection:

a.) in the past 4 weeks? b.) in the past 6 months? c.) in the past year?

	Yes	No	Don't know	Refused to answer
in the past four weeks	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
in the past six months	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
in the past year	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

PART 6. [Interviewer: Let the participant know that we are almost finished with the interviewer here.]

Q6.1 What is your current age?

AGE: _____

Q6.2 What is your gender?

- Male
- Female
- Other
- Refused to answer

Q6.3 Which of the following best describes your racial background and ethnicity? Choose all that apply.

[Interviewer, read the choices]

- Black/African American
- White
- American Indian or Alaska Native
- Asian
- Native Hawaiian or Pacific Islander
- Hispanic, Latino or of Spanish origin
- Other _____
- Refused to answer

Q6.4 What is the highest degree that you earned in school? *[Interviewer, read choices]:*

- Less than high school
- High School Diploma
- Associate Degree (GED)
- Bachelor's Degree
- More than Bachelor's Degree
- Other _____
- Refused to answer

Q6.5 In the two weeks before the day you were admitted to Pitt County Memorial Hospital, have you:

[Select all that apply]

	Yes	No	Don't know	Refused to answer
Gone hunting?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gone fishing?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Participated in sports such as football, soccer, or basketball?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>
Gone swimming or wading in a recreational body of water such as an ocean, lake, or stream, but not a swimming pool?	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>	<input type="checkbox"/>

Q6.6 In the two weeks before the day you were admitted to Pitt County Memorial Hospital, in what city or town & state did you go:

[Interviewer: ask about the location for each of the recreational activities that the participant indicated having done in the past year]

	City/Town and state	
	City/town	State
Hunting		
Fishing		
Swimming or wading		

Q6.7 In the two weeks before the day you were admitted to PCMH, how often do you estimate that you visited a gym to work-out or exercise in?

- 6-7 days each week
- 2-5 days each week
- About 1 day each week

- Less than one day each week
- Not at all
- Don't know
- Refused to answer

Q6.8 In the two weeks before the day you were admitted to Pitt County Memorial Hospital, about how often do you estimate that you touched or handled raw meat? Raw meat is defined as chicken, pork, beef, or goat that hasn't been cooked.

- 6-7 days each week
- 2-5 days each week
- about 1 day each week
- Less than 1 day each week
- Never
- Don't know
- Refused to answer

Q6.9 Starting the day before you were admitted to PCMH, when is the last time that you smoked tobacco cigarettes?

- Less than one day before I was admitted to PCMH
- More than a day but less than one week before I was admitted to PCMH
- More than a week but less than one month before I was admitted to PCMH
- More than a month but less than a year before I was admitted to PCMH
- More than a year before I was admitted to PCMH
- I have never smoked tobacco cigarettes
- Don't know
- Refused to answer

PART 7.

Q7.1 In this section I am going to ask you for your home address. We will use your address to explore whether there are associations between the location of your home and whether or not you have been exposed to MRSA. We will keep your address confidential, and we will never publish it or share it with anyone.

What is your home address (Street address, town and zip) ?

STREET _____
 CITY _____ STATE _____
 ZIP _____

Q7.2 Are you willing to be contacted in the mail about participating in follow-up studies? If you consent to be contacted about possible follow studies we will mail you a letter using only your study number and we will identify you only as "Dear Participant".

- Yes
- No

Q7.3 Is your mailing address the same as your home address?

- Yes
- No

Q7.4 What is your mailing address?

STREET _____

CITY _____ STATE _____ ZIP _____

Q8.1 This is the end of the survey. Thank you for your time and for your participation. Do you have any questions or comments regarding this survey or our research?

REFERENCES

1. Lowy FD. Staphylococcus aureus infections. The New England journal of medicine **1998** Aug 20;339(8):520-32.
2. Klevens RM, Morrison MA, Nadle J, et al. Invasive methicillin-resistant Staphylococcus aureus infections in the United States. *Jama* **2007** Oct 17;298(15):1763-71.
3. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant Staphylococcus aureus in children with no identified predisposing risk. *JAMA : the journal of the American Medical Association* **1998** Feb 25;279(8):593-8.
4. Loeffler A, Lloyd DH. Companion animals: a reservoir for methicillin-resistant Staphylococcus aureus in the community? *Epidemiol Infect* **2010** May;138(5):595-605.
5. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant Staphylococcus aureus in pig farming. *Emerging infectious diseases* **2005** Dec;11(12):1965-6.
6. Juhasz-Kaszanyitzky E, Janosi S, Somogyi P, et al. MRSA transmission between cows and humans. *Emerg Infect Dis* **2007** Apr;13(4):630-2.
7. Graveland H, Duim B, van Duijkeren E, Heederik D, Wagenaar JA. Livestock-associated methicillin-resistant Staphylococcus aureus in animals and humans. *Int J Med Microbiol* **2011** Dec;301(8):630-4.
8. Seybold U, Kourbatova EV, Johnson JG, et al. Emergence of community-associated methicillin-resistant Staphylococcus aureus USA300 genotype as a major cause of health care-associated blood stream infections. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2006** Mar 1;42(5):647-56.
9. Peterson AE, Davis MF, Julian KG, et al. Molecular and Phenotypic Characteristics of Healthcare- and Community-Associated Methicillin-Resistant Staphylococcus aureus at a Rural Hospital. *PLoS One* **2012**;7(6):e38354.
10. Gorwitz RJ, Kruszon-Moran D, McAllister SK, et al. Changes in the prevalence of nasal colonization with Staphylococcus aureus in the United States, 2001-2004. *J Infect Dis* **2008** May 1;197(9):1226-34.
11. Wulf MW, Verduin CM, van Nes A, Huijsdens X, Voss A. 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** Jan;31(1):61-5.
12. Wright GD. The antibiotic resistome: the nexus of chemical and genetic diversity. *Nat Rev Microbiol* **2007** Mar;5(3):175-86.
 13. Chambers HF, Deleo FR. Waves of resistance: *Staphylococcus aureus* in the antibiotic era. *Nat Rev Microbiol* **2009** Sep;7(9):629-41.
 14. Vanderhaeghen W, Hermans K, Haesebrouck F, Butaye P. Methicillin-resistant *Staphylococcus aureus* (MRSA) in food production animals. *Epidemiology and Infection* **2010** Feb 2:1-20.
 15. Pan A, Battisti A, Zoncada A, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* ST398 infection, Italy. *Emerg Infect Dis* **2009** May;15(5):845-7.
 16. Malachowa N, Deleo FR. Mobile genetic elements of *Staphylococcus aureus*. *Cell Mol Life Sci* **2010** Sep;67(18):3057-71.
 17. Molbak K. Spread of resistant bacteria and resistance genes from animals to humans--the public health consequences. *J Vet Med B Infect Dis Vet Public Health* **2004** Oct-Nov;51(8-9):364-9.
 18. Campbell NA. *Biology*. **1993**;Chapter 17(Third Edition):344-71.
 19. Kohanski MA, Depristo MA, Collins JJ. Sublethal Antibiotic Treatment Leads to Multidrug Resistance via Radical-Induced Mutagenesis. *Mol Cell* **2010** Feb 12;37(3):311-20.
 20. Pantosti A, Sanchini A, Monaco M. Mechanisms of antibiotic resistance in *Staphylococcus aureus*. *Future Microbiol* **2007** Jun;2:323-34.
 21. Lim D, Strynadka NC. Structural basis for the beta lactam resistance of PBP2a from methicillin-resistant *Staphylococcus aureus*. *Nat Struct Biol* **2002** Nov;9(11):870-6.
 22. Peng HL, Novick RP, Kreiswirth B, Kornblum J, Schlievert P. Cloning, characterization, and sequencing of an accessory gene regulator (*agr*) in *Staphylococcus aureus*. *J Bacteriol* **1988** Sep;170(9):4365-72.
 23. Price LB, Stegger M, Hasman H, et al. *Staphylococcus aureus* CC398: host adaptation and emergence of methicillin resistance in livestock. *MBio* **2012**;3(1).
 24. Tenover FC, Arbeit RD, Goering RV, et al. Interpreting chromosomal DNA restriction patterns produced by pulsed-field gel electrophoresis: criteria for bacterial strain typing. *J Clin Microbiol* **1995** Sep;33(9):2233-9.

25. Bens CC, Voss A, Klaassen CH. Presence of a novel DNA methylation enzyme in methicillin-resistant *Staphylococcus aureus* isolates associated with pig farming leads to uninterpretable results in standard pulsed-field gel electrophoresis analysis. *J Clin Microbiol* **2006** May;44(5):1875-6.
26. Fessler AT, Olde Riekerink RG, Rothkamp A, et al. Characterization of methicillin-resistant *Staphylococcus aureus* CC398 obtained from humans and animals on dairy farms. *Vet Microbiol* **2012** May 15.
27. Smith TC, Male MJ, Harper AL, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) strain ST398 is present in midwestern U.S. swine and swine workers. *PLoS One* **2009**;4(1):e4258.
28. McDougal LK, Steward CD, Killgore GE, Chaitram JM, McAllister SK, Tenover FC. Pulsed-field gel electrophoresis typing of oxacillin-resistant *Staphylococcus aureus* isolates from the United States: establishing a national database. *J Clin Microbiol* **2003** Nov;41(11):5113-20.
29. Tenover FC, Gay EA, Frye S, Eells SJ, Healy M, McGowan JE, Jr. Comparison of typing results obtained for methicillin-resistant *Staphylococcus aureus* isolates with the DiversiLab system and pulsed-field gel electrophoresis. *J Clin Microbiol* **2009** Aug;47(8):2452-7.
30. Deurenberg RH, Stobberingh EE. The evolution of *Staphylococcus aureus*. *Infect Genet Evol* **2008** Dec;8(6):747-63.
31. Yamamoto T, Nishiyama A, Takano T, et al. Community-acquired methicillin-resistant *Staphylococcus aureus*: community transmission, pathogenesis, and drug resistance. *J Infect Chemother* **2010** Mar 25.
32. Enright MC, Day NP, Davies CE, Peacock SJ, Spratt BG. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J Clin Microbiol* **2000** Mar;38(3):1008-15.
33. Shopsis B, Gomez M, Montgomery SO, Smith DH, Waddington M, Dodge DE. Evaluation of protein A gene polymorphic region DNA sequencing for typing of *Staphylococcus aureus* strains. *J Clin Microbiol* **1999**;37:3556-63.
34. Pearson T, Okinaka RT, Foster JT, Keim P. Phylogenetic understanding of clonal populations in an era of whole genome sequencing. *Infect Genet Evol* **2009** Sep;9(5):1010-9.
35. Gordon RJ, Lowy FD. Pathogenesis of methicillin-resistant *Staphylococcus aureus* infection. *Clin Infect Dis* **2008** Jun 1;46 Suppl 5:S350-9.

36. Graham PL, 3rd, Lin SX, Larson EL. A U.S. population-based survey of *Staphylococcus aureus* colonization. *Ann Intern Med* **2006** Mar 7;144(5):318-25.
37. Wertheim HF, Melles DC, Vos MC, et al. The role of nasal carriage in *Staphylococcus aureus* infections. *The Lancet infectious diseases* **2005** Dec;5(12):751-62.
38. Lautenbach E, Tolomeo P, Nachamkin I, Hu B, Zaoutis TE. The impact of household transmission on duration of outpatient colonization with methicillin-resistant *Staphylococcus aureus*. *Epidemiol Infect* **2010** May;138(5):683-5.
39. Robicsek A, Beaumont JL, Peterson LR. Duration of colonization with methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* **2009** Apr 1;48(7):910-3.
40. Mattner F, Biertz F, Ziesing S, Gastmeier P, Chaberny IF. Long-term persistence of MRSA in re-admitted patients. *Infection* **2010** Oct;38(5):363-71.
41. Graveland H, Wagenaar JA, Bergs K, Heesterbeek H, Heederik D. Persistence of livestock associated MRSA CC398 in humans is dependent on intensity of animal contact. *PLoS One* **2011**;6(2):e16830.
42. van Cleef BA, Graveland H, Haenen AP, 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** Mar;49(3):1030-3.
43. Boucher HW, Corey GR. Epidemiology of methicillin-resistant *Staphylococcus aureus*. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2008** Jun 1;46 Suppl 5:S344-9.
44. Udo EE, Pearman JW, Grubb WB. Genetic analysis of community isolates of methicillin-resistant *Staphylococcus aureus* in Western Australia. *The Journal of hospital infection* **1993** Oct;25(2):97-108.
45. Moreno F, Crisp C, Jorgensen JH, Patterson JE. Methicillin-resistant *Staphylococcus aureus* as a community organism. *Clin Infect Dis* **1995** Nov;21(5):1308-12.
46. Prevention CfDCa. Four pediatric deaths from community-acquired methicillin-resistant *Staphylococcus aureus*-Minnesota and North Dakota, 1997-1999. *Jama* **1999**;282:1123-5.
47. Klein E, Smith DL, Laxminarayan R. Community-associated methicillin-resistant *Staphylococcus aureus* in outpatients, United States, 1999-2006. *Emerging infectious diseases* **2009** Dec;15(12):1925-30.

48. Otter JA, French GL. Community-associated methicillin-resistant *Staphylococcus aureus* strains as a cause of healthcare-associated infection. *J Hosp Infect* **2011** Nov;79(3):189-93.
49. McDougal LK, Fosheim GE, Nicholson A, et al. Emergence of resistance among USA300 methicillin-resistant *Staphylococcus aureus* isolates causing invasive disease in the United States. *Antimicrob Agents Chemother* **2010** Sep;54(9):3804-11.
50. Cohen AL, Calfee D, Fridkin SK, et al. Recommendations for metrics for multidrug-resistant organisms in healthcare settings: SHEA/HICPAC Position paper. *Infect Control Hosp Epidemiol* **2008** Oct;29(10):901-13.
51. Shapiro A, Raman S, Johnson M, Piehl M. Community-acquired MRSA infections in North Carolina children: prevalence, antibiotic sensitivities, and risk factors. *North Carolina medical journal* **2009** Mar-Apr;70(2):102-7.
52. Bradley SF, Terpenning MS, Ramsey MA, et al. Methicillin-resistant *Staphylococcus aureus*: Colonization and Infection in a Long-term care facility. *Annals of Internal Medicine* **1991**;115(6):417-22.
53. Montgomery K, Ryan TJ, Krause A, Starkey C. Assessment of athletic health care facility surfaces for MRSA in the secondary school setting. *J Environ Health* **2010** Jan-Feb;72(6):8-11; quiz 66.
54. Denis O, Suetens C, Hallin M, et al. Methicillin-resistant *Staphylococcus aureus* ST398 in swine farm personnel, Belgium. *Emerg Infect Dis* **2009** Jul;15(7):1098-101.
55. Bowers AL, Huffman GR, Sennett BJ. Methicillin-resistant *Staphylococcus aureus* infections in collegiate football players. *Med Sci Sports Exerc* **2008** Aug;40(8):1362-7.
56. Baillargeon J, Kelley MF, Leach CT, Baillargeon G, Pollock BH. Methicillin-resistant *Staphylococcus aureus* infection in the Texas prison system. *Clin Infect Dis* **2004** May 1;38(9):e92-5.
57. Sztramko R, Katz K, Antoniou T, et al. Community-associated methicillin-resistant *Staphylococcus aureus* infections in men who have sex with men: A case series. *Can J Infect Dis Med Microbiol* **2007** Jul;18(4):257-61.
58. From the Centers for Disease Control and Prevention. Public health dispatch: outbreaks of community-associated methicillin-resistant *Staphylococcus aureus* skin infections--Los Angeles County, California, 2002-2003. *Jama* **2003** Mar 19;289(11):1377.

59. Morrison-Rodriguez SM, Pacha LA, Patrick JE, Jordan NN. Community-associated methicillin-resistant *Staphylococcus aureus* infections at an Army training installation. *Epidemiol Infect* **2010** May;138(5):721-9.
60. Simor AE, Ofner-Agostini M, Paton S, et al. Clinical and epidemiologic features of methicillin-resistant *Staphylococcus aureus* in elderly hospitalized patients. *Infect Control Hosp Epidemiol* **2005** Oct;26(10):838-41.
61. Charlebois ED, Perdreau-Remington F, Kreiswirth B, et al. Origins of community strains of methicillin-resistant *Staphylococcus aureus*. *Clin Infect Dis* **2004** Jul 1;39(1):47-54.
62. Kuehnert MJ, Kruszon-Moran D, Hill HA, et al. Prevalence of *Staphylococcus aureus* nasal colonization in the United States, 2001-2002. *J Infect Dis* **2006** Jan 15;193(2):172-9.
63. Leung YH, Lai RW, Chan AC, et al. Risk factors for community-associated methicillin-resistant *Staphylococcus aureus* infection in Hong Kong. *J Infect* **2012** Feb 23.
64. Ferreira JP, Anderson KL, Correa MT, et al. Transmission of MRSA between companion animals and infected human patients presenting to outpatient medical care facilities. *PLoS One* **2011**;6(11):e26978.
65. Reilly JS, Stewart S, Christie P, et al. Universal screening for methicillin-resistant *Staphylococcus aureus* in acute care: risk factors and outcome from a multicentre study. *J Hosp Infect* **2012** Jan;80(1):31-5.
66. Wang CY, Wu VC, Wang WJ, et al. Risk factors for nasal carriage of methicillin-resistant *Staphylococcus aureus* among patients with end-stage renal disease in Taiwan. *J Formos Med Assoc* **2012** Jan;111(1):14-8.
67. Hidron AI, Kourbatova EV, Halvosa JS, et al. Risk factors for colonization with methicillin-resistant *Staphylococcus aureus* (MRSA) in patients admitted to an urban hospital: emergence of community-associated MRSA nasal carriage. *Clin Infect Dis* **2005** Jul 15;41(2):159-66.
68. Weese JS, Rousseau J, Traub-Dargatz JL, Willey BM, McGeer AJ, Low DE. Community-associated methicillin-resistant *Staphylococcus aureus* in horses and humans who work with horses. *J Am Vet Med Assoc* **2005** Feb 15;226(4):580-3.
69. Nemati M, Hermans K, Lipinska U, et al. Antimicrobial Resistance of Old and Recent *Staphylococcus aureus* Isolates from Poultry: First Detection of Livestock-Associated Methicillin-Resistant Strain ST398. *Antimicrob Agents Chemother* **2008**;52(10):3817-9.

70. Persoons D, Van Hoorebeke S, Hermans K, et al. Methicillin-resistant *Staphylococcus aureus* in poultry. *Emerg Infect Dis* **2009** Mar;15(3):452-3.
71. Mulders MN, Haenen AP, Geenen PL, et al. Prevalence of livestock-associated MRSA in broiler flocks and risk factors for slaughterhouse personnel in The Netherlands. *Epidemiol Infect* **2010** May;138(5):743-55.
72. Shaw SE. Dogs, cats and methicillin-resistant *Staphylococcus aureus*. *J Small Anim Pract* **2004** Dec;45(12):587-8.
73. Duquette RA, Nuttall TJ. Methicillin-resistant *Staphylococcus aureus* in dogs and cats: an emerging problem? *J Small Anim Pract* **2004** Dec;45(12):591-7.
74. Hanselman BA, Kruth SA, Rousseau J, et al. Methicillin-resistant *Staphylococcus aureus* colonization in veterinary personnel. *Emerg Infect Dis* **2006** Dec;12(12):1933-8.
75. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Veterinary microbiology* **2008** Apr 30;128(3-4):298-303.
76. Huijsdens XW, van Dijke BJ, Spalburg E, et al. Community-acquired MRSA and pig-farming. *Annals of clinical microbiology and antimicrobials* **2006** Nov 10;5(26).
77. de Neeling AJ, van den Broek MJ, Spalburg EC, et al. High prevalence of methicillin resistant *Staphylococcus aureus* in pigs. *Veterinary microbiology* **2007** Jun 21;122(3-4):366-72.
78. Graveland H, Wagenaar JA, Heesterbeek H, Mevius D, van Duijkeren E, Heederik D. Methicillin resistant *Staphylococcus aureus* ST398 in veal calf farming: human MRSA carriage related with animal antimicrobial usage and farm hygiene. *PLoS One* **2010**;5(6):e10990.
79. Normanno G, Corrente M, La Salandra G, et al. Methicillin-resistant *Staphylococcus aureus* (MRSA) in foods of animal origin product in Italy. *Int J Food Microbiol* **2007** Jun 30;117(2):219-22.
80. de Boer E, Zwartkruis-Nahuis JT, Wit B, et al. Prevalence of methicillin-resistant *Staphylococcus aureus* in meat. *Int J Food Microbiol* **2009** Aug 31;134(1-2):52-6.
81. Hanson BM, Dressler AE, Harper AL, et al. Prevalence of *Staphylococcus aureus* and methicillin-resistant *Staphylococcus aureus* (MRSA) on retail meat in Iowa. *J Infect Public Health* **2011** Sep;4(4):169-74.
82. O'Brien AM, Hanson BM, Farina SA, et al. MRSA in conventional and alternative retail pork products. *PLoS One* **2012**;7(1):e30092.

83. Elie-Turenne MC, Fernandes H, Mediavilla JR, et al. Prevalence and characteristics of *Staphylococcus aureus* colonization among healthcare professionals in an urban teaching hospital. *Infect Control Hosp Epidemiol* **2010** Jun;31(6):574-80.
84. Suffoletto BP, Cannon EH, Ilkhanipour K, Yealy DM. Prevalence of *Staphylococcus aureus* nasal colonization in emergency department personnel. *Ann Emerg Med* **2008** Nov;52(5):529-33.
85. Burstiner LC, Faires M, Weese JS. Methicillin-resistant *Staphylococcus aureus* colonization in personnel attending a veterinary surgery conference. *Vet Surg* **2010** Feb;39(2):150-7.
86. Van Cleef BA, Broens EM, Voss A, et al. High prevalence of nasal MRSA carriage in slaughterhouse workers in contact with live pigs in The Netherlands. *Epidemiology and infection* **2010** Feb 9:1-8.
87. Van Den Broek IV, Van Cleef BA, Haenen A, et al. Methicillin-resistant *Staphylococcus aureus* in people living and working in pig farms. *Epidemiology and infection* **2009** May;137(5):700-8.
88. Smith TC, Pearson N. The emergence of *Staphylococcus aureus* ST398. *Vector Borne Zoonotic Dis* **2011** Apr;11(4):327-39.
89. Armand-Lefevre L, Ruimy R, Andremont A. Clonal comparison of *Staphylococcus aureus* isolates from healthy pig farmers, human controls, and pigs. *Emerg Infect Dis* **2005** May;11(5):711-4.
90. van Rijen MM, Van Keulen PH, Kluytmans JA. Increase in a Dutch hospital of methicillin-resistant *Staphylococcus aureus* related to animal farming. *Clinical infectious diseases : an official publication of the Infectious Diseases Society of America* **2008** Jan 15;46(2):261-3.
91. Richter A, Sting R, Popp C, et al. Prevalence of types of methicillin-resistant *Staphylococcus aureus* in turkey flocks and personnel attending the animals. *Epidemiol Infect* **2012** Feb 10:1-10.
92. Cuny C, Strommenger B, Witte W, Stanek C. Clusters of infections in horses with MRSA ST1, ST254, and ST398 in a veterinary hospital. *Microb Drug Resist* **2008** Dec;14(4):307-10.
93. Waters AE, Contente-Cuomo T, Buchhagen J, et al. Multidrug-Resistant *Staphylococcus aureus* in US Meat and Poultry. *Clin Infect Dis* **2011** May;52(10):1227-30.

94. Gomez-Sanz E, Torres C, Lozano C, 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** Aug 2.
95. Grisold AJ, Zarfel G, Hoenigl M, et al. Occurrence and genotyping using automated repetitive-sequence-based PCR of methicillin-resistant *Staphylococcus aureus* ST398 in Southeast Austria. *Diagnostic microbiology and infectious disease* **2010**;66(2):217-21.
96. Lewis HC, Molbak K, Reese C, et al. Pigs as source of methicillin-resistant *Staphylococcus aureus* CC398 infections in humans, Denmark. *Emerg Infect Dis* **2008** Sep;14(9):1383-9.
97. Cuny C, Nathaus R, Layer F, Strommenger B, Altmann D, Witte W. Nasal colonization of humans with methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 with and without exposure to pigs. *PLoS One* **2009**;4(8):e6800.
98. Bashkireva AS, Artamonova VG, Khavinson V. [Productive aging or "healthy worker effect"? Retrospective analysis of morbidity in motor transport drivers]. *Adv Gerontol* **2009**;22(4):539-47.
99. Lim SK, Nam HM, Jang GC, Lee HS, Jung SC, Kwak HS. The first detection of methicillin-resistant *Staphylococcus aureus* ST398 in pigs in Korea. *Vet Microbiol* **2012** Feb 24;155(1):88-92.
100. Leedom Larson KR, Harper AL, Hanson BM, et al. Methicillin-resistant *Staphylococcus aureus* in pork production shower facilities. *Appl Environ Microbiol* **2011** Jan;77(2):696-8.
101. Leedom Larson KR, Smith TC, Donham KJ. Self-reported methicillin-resistant *Staphylococcus aureus* infection in USA pork producers. *Ann Agric Environ Med* **2010** Dec;17(2):331-4.
102. Bos ME, Graveland H, Portengen L, Wagenaar JA, Heederik DJ. Livestock-associated MRSA prevalence in veal calf production is associated with farm hygiene, use of antimicrobials, and age of the calves. *Prev Vet Med* **2012** Jun 1;105(1-2):155-9.
103. Cuny C, Friedrich AW, Witte W. Absence of livestock-associated methicillin-resistant *staphylococcus aureus* clonal complex CC398 as a nasal colonizer of pigs raised in an alternative system. *Appl Environ Microbiol* **2012** Feb;78(4):1296-7.
104. Wassenberg MW, Bootsma MC, Troelstra A, Kluytmans JA, Bonten MJ. Transmissibility of livestock-associated methicillin-resistant *Staphylococcus aureus* (ST398) in Dutch hospitals. *Clin Microbiol Infect* **2011** Feb;17(2):316-9.

105. Moritz ED, Smith TC. Livestock-associated *Staphylococcus aureus* in childcare worker. *Emerg Infect Dis* **2011** Apr;17(4):742-4.
106. Kock R, Siam K, Al-Malat S, et al. Characteristics of hospital patients colonized with livestock-associated methicillin-resistant *Staphylococcus aureus* (MRSA) CC398 versus other MRSA clones. *J Hosp Infect* **2011** Dec;79(4):292-6.
107. Cavaco LM, Hasman H, Aarestrup FM. Zinc resistance of *Staphylococcus aureus* of animal origin is strongly associated with methicillin resistance. *Vet Microbiol* **2011** Jun 2;150(3-4):344-8.
108. Jamrozny DM, Fielder MD, Butaye P, Coldham NG. Comparative Genotypic and Phenotypic Characterisation of Methicillin-Resistant *Staphylococcus aureus* ST398 Isolated from Animals and Humans. *PLoS One* **2012**;7(7):e40458.
109. Uhlemann AC, Porcella SF, Trivedi S, et al. Identification of a highly transmissible animal-independent *Staphylococcus aureus* ST398 clone with distinct genomic and cell adhesion properties. *MBio* **2012**;3(2).
110. Silbergeld EK, Graham JP, Price LB. Industrial food animal production, antimicrobial resistance, and human health. *Annu Rev Public Health* **2008**;29(15):151-69.
111. Mellon M, Benbrook C, Benbrook KL. Hogging it: Estimates of antimicrobial abuse in livestock: Union of Concerned Scientists, **2001**.
112. DuPont HL, Steele JH. Use of antimicrobial agents in animal feeds: Implications for human health. *Reviews of Infectious Diseases* **1987**;9(3):447-60.
113. McDermott PF, Zhao S, Wagner DD, Simjee S, Walker RD, White DG. The food safety perspective of antibiotic resistance. *Anim Biotechnol* **2002** May;13(1):71-84.
114. Food and Drug Administration F. Summary report on antimicrobials sold or distributed for use in food-producing animals. In: services. Dohah, ed. Silver Spring: FDA, **2009**.
115. Love DC, Davis MF, Bassett A, Gunther A, Nachman KE. Dose imprecision and resistance: free-choice medicated feeds in industrial food animal production in the United States. *Environ Health Perspect* **2011** Mar;119(3):279-83.
116. Hayes JR, English LL, Carr LE, Wagner DD, Joseph SW. Multiple-antibiotic resistance of *Enterococcus* spp. isolated from commercial poultry production environments. *Appl Environ Microbiol* **2004** Oct;70(10):6005-11.
117. Skjot-Rasmussen L, Ethelberg S, Emborg HD, et al. Trends in occurrence of antimicrobial resistance in *Campylobacter jejuni* isolates from broiler chickens,

- broiler chicken meat, and human domestically acquired cases and travel associated cases in Denmark. *Int J Food Microbiol* **2009** May 31;131(2-3):277-9.
118. Wulf M, Voss A. MRSA in livestock animals-an epidemic waiting to happen? *Clin Microbiol Infect* **2008** Jun;14(6):519-21.
 119. Nandi S, Maurer JJ, Hofacre C, Summers AO. Gram-positive bacteria are a major reservoir of Class 1 antibiotic resistance integrons in poultry litter. *Proc Natl Acad Sci U S A* **2004** May 4;101(18):7118-22.
 120. Gibbs SG, Green CF, Tarwater PM, Mota LC, Mena KD, Scarpino PV. Isolation of antibiotic-resistant bacteria from the air plume downwind of a swine confined or concentrated animal feeding operation. *Environ Health Perspect* **2006** Jul;114(7):1032-7.
 121. Hamscher G, Pawelzick HT, Sczesny S, Nau H, Hartung J. Antibiotics in dust originating from a pig-fattening farm: a new source of health hazard for farmers? *Environ Health Perspect* **2003** Oct;111(13):1590-4.
 122. Chapin A, Rule A, Gibson K, Buckley T, Schwab K. Airborne multidrug-resistant bacteria isolated from a concentrated swine feeding operation. *Environ Health Perspect* **2005** Feb;113(2):137-42.
 123. Anderson ME, Sobsey MD. 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):211-8.
 124. Sapkota AR, Curriero FC, Gibson KE, Schwab KJ. Antibiotic-resistant enterococci and fecal indicators in surface water and groundwater impacted by a concentrated Swine feeding operation. *Environ Health Perspect* **2007** Jul;115(7):1040-5.
 125. Holzel CS, Schwaiger K, Harms K, et al. Sewage sludge and liquid pig manure as possible sources of antibiotic resistant bacteria. *Environ Res* **2010** May;110(4):318-26.
 126. Borjesson S, Matussek A, Melin S, Lofgren S, Lindgren PE. Methicillin-resistant *Staphylococcus aureus* (MRSA) in municipal wastewater: an uncharted threat? *J Appl Microbiol* **2010** Apr;108(4):1244-51.
 127. Atyah MA, Zamri-Saad M, Siti-Zahrah A. First report of methicillin-resistant *Staphylococcus aureus* from cage-cultured tilapia (*Oreochromis niloticus*). *Vet Microbiol* **2010** Aug 26;144(3-4):502-4.
 128. Graham JP, Price LB, Evans SL, Graczyk TK, Silbergeld EK. Antibiotic resistant enterococci and staphylococci isolated from flies collected near confined poultry feeding operations. *Sci Total Environ* **2009** Apr 1;407(8):2701-10.

129. Cole D, Drum DJ, Stalknecht DE, et al. Free-living Canada geese and antimicrobial resistance. *Emerg Infect Dis* **2005** Jun;11(6):935-8.
130. Zhao C, Liu Y, Zhao M, et al. Characterization of Community Acquired *Staphylococcus aureus* Associated with Skin and Soft Tissue Infection in Beijing: High Prevalence of PVL(+) ST398. *PLoS One* **2012**;7(6):e38577.
131. Ekkelenkamp M, Sekkat M, Carpaij N, Troelstra A, Bonten M. Endocarditis due to methicillin-resistant *Staphylococcus aureus* originating from pigs. *Nied Tijdschr Geneesk* **2006**;150(44):2442-7.
132. Witte W, Strommenger B, Stanek C, Cuny C. Methicillin-resistant *Staphylococcus aureus* ST398 in humans and animals, Central Europe. *Emerg Infect Dis* **2007** Feb;13(2):255-8.
133. van Loo I, Huijsdens X, Tiemersma E, et al. Emergence of methicillin-resistant *Staphylococcus aureus* of animal origin in humans. *Emerg Infect Dis* **2007** Dec;13(12):1834-9.
134. Fanoy E, Helmhout LC, van der Vaart WL, et al. An outbreak of non-typeable MRSA within a residential care facility. *Euro Surveill* **2009** Jan 8;14(1).
135. Wulf MW, Markestein A, van der Linden FT, Voss A, Klaassen C, Verduin CM. First outbreak of methicillin-resistant *Staphylococcus aureus* ST398 in a Dutch hospital, June 2007. *Euro Surveill* **2008** Feb 28;13(9).
136. Verkade E, Bosch T, Hendriks Y, Kluytmans J. Outbreak of methicillin-resistant *Staphylococcus aureus* ST398 in a Dutch nursing home. *Infect Control Hosp Epidemiol* **2012** Jun;33(6):624-6.
137. North Carolina Division of Water Quality Afou. Permit List-Animal Numbers and Locations. Raleigh, North Carolina: North Carolina Department of Environment and Natural Resources, **2009**.
138. North Carolina Department of Agriculture and Consumer Services. Agricultural statistics-North Carolina's rank in US agriculture. Available at: <http://www.ncagr.gov/stats/index.htm>. Accessed April 23, 2010.
139. Florini JD, Denison R, Stiffler T, Fitzgerald T, Goldberg R. Resistant bugs and antibiotic drugs: State and county estimates of antibiotics in agricultural feed and animal waste. Washington, DC: Environmental Defense Fund, **2005** June 2005.
140. Warrick J, Stith P. Money talks: The smell of money. *The News and Observer*. **1995**.

141. O'Laughlin DM, Cook J. Financial analysis of methicillin-resistant *Staphylococcus aureus* in a high school wrestler. *Dermatology nursing / Dermatology Nurses' Association* **2009** Nov-Dec;21(6):337-44.
142. Chambers HF. The changing epidemiology of *Staphylococcus aureus*? *Emerg Infect Dis* **2001** Mar-Apr;7(2):178-82.
143. Costelloe C, Lovering A, Montgomery A, Lewis D, McNulty C, Hay AD. Effect of antibiotic prescribing in primary care on methicillin-resistant *Staphylococcus aureus* carriage in community-resident adults: a controlled observational study. *Int J Antimicrob Agents* **2012** Feb;39(2):135-41.
144. Moran GJ, Krishnadasan A, Gorwitz RJ, et al. Methicillin-resistant *S. aureus* infections among patients in the emergency department. *N Engl J Med* **2006** Aug 17;355(7):666-74.
145. Davis MF, Iverson SA, Baron P, et al. Household transmission of methicillin-resistant *Staphylococcus aureus* and other staphylococci. *Lancet Infect Dis* **2012** Sep;12(9):703-16.
146. Lu D, Holtom P. Community-acquired methicillin-resistant *Staphylococcus aureus*, a new player in sports medicine. *Curr Sports Med Rep* **2005** Oct;4(5):265-70.
147. Pofahl WE, Goettler CE, Ramsey KM, Cochran MK, Nobles DL, Rotondo MF. Active Surveillance Screening of MRSA and Eradication of the Carrier State Decreases Surgical-Site Infections Caused by MRSA. *J Am Coll Surg* **2009**;208:981-8.
148. Boyce JM, Havill NL. Comparison of BD GeneOhm methicillin-resistant *Staphylococcus aureus* (MRSA) PCR versus the CHROMagar MRSA assay for screening patients for the presence of MRSA strains. *J Clin Microbiol* **2008** Jan;46(1):350-1.
149. French GL. Methods for screening for methicillin-resistant *Staphylococcus aureus* carriage. *Clin Microbiol Infect* **2009** Dec;15 Suppl 7:10-6.
150. Augustino K. Molecular epidemiology of MRSA among patients and employees in a surgical intensive care unit. Greenville: East Carolina University, **2011**.
151. United States Census Bureau GD. 2010 Census TIGER/Line Shapefiles. Available at: <http://www.census.gov/geo/www/tiger/tgrshp2010/tgrshp2010.html>. Accessed August 3, 2012.
152. Broens EM, Espinosa-Gongora C, Graat EA, et al. Longitudinal study on transmission of MRSA CC398 within pig herds. *BMC Vet Res* **2012** May 18;8(1):58.

153. Popovich KJ, Weinstein RA, Hota B. Are community-associated methicillin-resistant *Staphylococcus aureus* (MRSA) strains replacing traditional nosocomial MRSA strains? *Clin Infect Dis* **2008** Mar 15;46(6):787-94.
154. Uhlemann AC, Knox J, Miller M, et al. The environment as an unrecognized reservoir for community-associated methicillin resistant *Staphylococcus aureus* USA300: a case-control study. *PLoS One* **2011**;6(7):e22407.
155. Cookson B. Five decades of MRSA: controversy and uncertainty continues. *Lancet* **2011** Oct 8;378(9799):1291-2.
156. Kleinbaum DG, Klein M. *Logistic Regression A Self-Learning Text*. Second Edition. ed. New York, NY: Springer-Verlag New York, Inc., **2002**.
157. Casey JA, Cosgrove SE, Stewart WF, Pollak J, Schwartz BS. A population-based study of the epidemiology and clinical features of methicillin-resistant *Staphylococcus aureus* infection in Pennsylvania, 2001-2010. *Epidemiol Infect* **2012** Aug 29:1-14.
158. Haley CC, Mittal D, Laviolette A, Jannapureddy S, Parvez N, Haley RW. Methicillin-resistant *Staphylococcus aureus* infection or colonization present at hospital admission: multivariable risk factor screening to increase efficiency of surveillance culturing. *J Clin Microbiol* **2007** Sep;45(9):3031-8.
159. Minhas P, Perl TM, Carroll KC, et al. Risk factors for positive admission surveillance cultures for methicillin-resistant *Staphylococcus aureus* and vancomycin-resistant enterococci in a neurocritical care unit. *Crit Care Med* **2011** Oct;39(10):2322-9.
160. Weese JS, Dick H, Willey BM, et al. Suspected transmission of methicillin-resistant *Staphylococcus aureus* between domestic pets and humans in veterinary clinics and in the household. *Vet Microbiol* **2006** Jun 15;115(1-3):148-55.
161. Cohen PR. The skin in the gym: a comprehensive review of the cutaneous manifestations of community-acquired methicillin-resistant *Staphylococcus aureus* infection in athletes. *Clin Dermatol* **2008** Jan-Feb;26(1):16-26.
162. Huletsky A, Giroux R, Rossbach V, et al. New real-time PCR assay for rapid detection of methicillin-resistant *Staphylococcus aureus* directly from specimens containing a mixture of staphylococci. *J Clin Microbiol* **2004** May;42(5):1875-84.
163. Rossney AS, Herra CM, Fitzgibbon MM, Morgan PM, Lawrence MJ, O'Connell B. Evaluation of the IDI-MRSA assay on the SmartCycler real-time PCR platform for rapid detection of MRSA from screening specimens. *Eur J Clin Microbiol Infect Dis* **2007** Jul;26(7):459-66.

164. Lucke K, Hombach M, Hug M, Pfyffer GE. Rapid detection of methicillin-resistant *Staphylococcus aureus* (MRSA) in diverse clinical specimens by the BD GeneOhm MRSA assay and comparison with culture. *J Clin Microbiol* **2010** Mar;48(3):981-4.
165. Sarda V, Molloy A, Kadkol S, Janda WM, Hershov R, McGuinn M. Active surveillance for methicillin-resistant *Staphylococcus aureus* in the neonatal intensive care unit. *Infect Control Hosp Epidemiol* **2009** Sep;30(9):854-60.
166. Malhotra-Kumar S, Van Heirstraeten L, Lee A, et al. Evaluation of molecular assays for rapid detection of methicillin-resistant *Staphylococcus aureus*. *J Clin Microbiol* **2010** Dec;48(12):4598-601.
167. Snyder JW, Munier GK, Johnson CL. Comparison of the BD GeneOhm methicillin-resistant *Staphylococcus aureus* (MRSA) PCR assay to culture by use of BBL CHROMagar MRSA for detection of MRSA in nasal surveillance cultures from intensive care unit patients. *J Clin Microbiol* **2010** Apr;48(4):1305-9.
168. Lowy FD. *Staphylococcus aureus* infections. *N Engl J Med* **1998** Aug 20;339(8):520-32.
169. Gorwitz RJ. A review of community-associated methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections. *Pediatr Infect Dis J* **2008** Jan;27(1):1-7.
170. Herold BC, Immergluck LC, Maranan MC, et al. Community-acquired methicillin-resistant *Staphylococcus aureus* in children with no identified predisposing risk. *Jama* **1998** Feb 25;279(8):593-8.
171. Gorak EJ, Yamada SM, Brown JD. Community-acquired methicillin-resistant *Staphylococcus aureus* in hospitalized adults and children without known risk factors. *Clin Infect Dis* **1999** Oct;29(4):797-800.
172. McCarthy AJ, Lindsay JA, Loeffler A. Are all methicillin-resistant *Staphylococcus aureus* (MRSA) equal in all hosts? Epidemiological and genetic comparison between animal and human MRSA. *Vet Dermatol* **2012** Aug;23(4):267-e54.
173. Pantosti A. Methicillin-Resistant *Staphylococcus aureus* Associated with Animals and Its Relevance to Human Health. *Front Microbiol* **2012**;3:127.
174. Schulz J, Friese A, Klees S, 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** Aug;78(16):5666-71.
175. van Cleef BA, Verkade EJ, Wulf MW, et al. Prevalence of livestock-associated MRSA in communities with high pig-densities in The Netherlands. *PLoS One* **2010**;5(2):e9385.

176. Popovich KJ, Smith KY, Khawcharoenporn T, et al. Community-Associated Methicillin-Resistant *Staphylococcus aureus* Colonization in High-Risk Groups of HIV-Infected Patients. *Clin Infect Dis* **2012** May;54(9):1296-303.
177. Vos MC, Behrendt MD, Melles DC, et al. 5 years of experience implementing a methicillin-resistant *Staphylococcus aureus* search and destroy policy at the largest university medical center in the Netherlands. *Infect Control Hosp Epidemiol* **2009** Oct;30(10):977-84.
178. Khanna T, Friendship R, Dewey C, Weese JS. Methicillin resistant *Staphylococcus aureus* colonization in pigs and pig farmers. *Vet Microbiol* **2008** Apr 30;128(3-4):298-303.
179. Gilbert MJ, Bos ME, Duim B, et al. Livestock-associated MRSA ST398 carriage in pig slaughterhouse workers related to quantitative environmental exposure. *Occup Environ Med* **2012** Apr 27.
180. de Jonge R, Verdier JE, Havelaar AH. Prevalence of methicillin-resistant *Staphylococcus aureus* amongst professional meat handlers in the Netherlands, March-July 2008. *Euro Surveill* **2010** Nov 18;15(46).
181. Voss A, Loeffen F, Bakker J, Klaassen C, Wulf M. Methicillin-resistant *Staphylococcus aureus* in pig farming. *Emerg Infect Dis* **2005** Dec;11(12):1965-6.