2010

Staphylococcus aureus in Iowa child care facilities

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STAPHYLOCOCCUS AUREUS IN IOWA CHILD CARE FACILITIES

by

Erin Denise Moritz

An Abstract

Of a thesis submitted in partial fulfillment
of the requirements for the Doctor of
Philosophy degree in Epidemiology
in the Graduate College of
The University of Iowa

May 2011

Thesis Supervisor: Assistant Professor Tara Smith

ABSTRACT

Staphylococcus aureus (S. aureus) is a ubiquitous bacterium that has the potential to cause severe disease in children and adults. Asymptomatic carriage of *S. aureus* is an important risk factor for developing infection, as well as a key contributor to transmission. Carriage of respiratory bacteria (mainly *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Moraxella catarrhalis*) has been studied extensively in child care attendees. Prevalence of these pathogens and resistance rates among them vary widely, and the introduction of the pneumococcal vaccine has the potential to alter patterns of carriage in this population. Molecular characterization of bacteria in child care facilities, primarily of *S. pneumoniae*, has shown that each facility hosts its own bacteriological profile, but can also serve as a community reservoir for epidemic and internationally-recognized clones. Limitations in current research on this topic exist. The majority of data is cross-sectional with no comparison group, and there has been little research published since 2000 on child care employees, bacterial contamination of environmental surfaces, and bacteria causing diarrheal illness.

Despite the fact that child care workers are at risk of infections, little research has focused on asymptomatic carriage in this occupational group. We collected samples from 110 employees, 81 children, and 214 surfaces at twelve child care facilities, as well as 111 age- and gender-matched adults not employed at child care centers. After adjusting for age, a household contact with a recent influenza-like illness, and a household contact with exposure to cattle, the odds ratio for *S. aureus* carriage in child care employees was 0.68 (95% CI 0.31 – 1.50, p-value 0.34). The odds of MRSA carriage was 3.28 times higher in child care employees than unexposed adults after adjusting for a history of

cigarette smoking (95% CI 0.31 – 169.02, p-value 0.53). Employees were significantly more likely to carry erythromycin-resistant *S. aureus* than unexposed adults (crude OR 3.67, 95% CI 1.06 – 12.68, p-value 0.033). Colonization rates of all *S. aureus* and MRSA in children were 19.8% and 1.23%, respectively. *S. aureus* and MRSA were isolated from 9.80% and 0.90% of surfaces. Washing children's hands upon arrival had a protective effect among employees (adjusted OR 0.17, 95% CI 0.095 – 0.32, $p < 0.0001$). Molecular characterization suggested evidence of transmission of *S. aureus* among children, employees, and environmental surfaces; the bacterial population in each facility was, however, heterogeneous overall.

In the analysis of data collected from individuals with limited exposure to the healthcare setting, a substantial portion (30.0%) of this study population carried *S. aureus* only in the throat. In multivariate analysis, age, season, race, and gender were the most important predictors of colonization location, with age, season, and race significantly predicting exclusive throat carriage. These findings suggest that including a throat swab in addition to a nasal swab could play an important role in the success of active surveillance culture screening programs and population-based surveys, particularly when administered on community members with limited exposure to healthcare.

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CERTIFICATE OF APPROVAL

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PH.D. THESIS

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To Dad, Mom, and Mr. B

ACKNOWLEDGMENTS

This work would not have been possible without the invaluable help and support of many individuals. I would like to thank the members of my committee, Drs. Tara Smith (my advisor), Bill Field, Jody Murph, Jeff Dawson, Jim Torner, and Greg Gray, for their patience and advice. This project was funded through a pilot/small project grant awarded by the Heartland Center for Occupational Health and Safety, a research center funded by the National Institute for Occupational Safety and Health. The Center for Emerging Infectious Diseases at the University of Iowa also provided support from its general funds. Fellowship support was provided by the Occupational Epidemiology Training Program within the NIOSH-funded Heartland Center for Occupational Health and Safety (T42 OH008491-03).

I also thank the facility directors and participants, as well as student volunteers who accompanied me on site visits, including Raj Nair, Anne Dressler, Abby Harper, Shruthi Bhatt, Shylo Wardyn, Brianna Dannen, Hiba Ahmed, and Margaret Chorazy. Laboratory training and support was provided by Jennifer Kroeger, Blake Hanson, Anne Dressler, Shylo Wardyn, Abby Harper, and Dr. Kris Heilman's group at the University of Iowa Hospitals and Clinics. Margaret Chorazy and Spencer Lourens provided advice on statistical analyses. I also thank Dr. M. Bridget Zimmerman and Joan Xie at the University of Iowa College of Public Health Biostatistics Consulting Center for conducting the statistical analysis for the nose vs. throat carriage paper.

Finally, I thank Margaret Chorazy and Greg Thomas, Jennifer Kroeger and Shaun McDanel, and Ron Bedford and Fran Roushar for "residential" and moral support in the last several months of my journey.

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CHAPTER I

BACKGROUND AND SIGNIFICANCE

Staphylococcus aureus (*S. aureus*) is a ubiquitous bacterium that has potential to cause severe disease in children and adults. One method of transmission of this organism is via "carriage" (also known as colonization). The carrier state occurs when a pathogen grows in the normal flora of an individual without causing disease (Casadevall and Pirofski 2000). This is opposed to infection, in which signs and symptoms of disease occur. Although colonization itself does no harm to the host, it is a known risk factor for developing and transmitting clinically relevant infections (Wertheim 2005). Given the rise in the frequency and severity of infections with bacteria resistant to antimicrobials, colonization remains of interest to researchers involved with infectious diseases.

A vast quantity of time and resources has gone into the study of colonization and infection with bacteria among healthcare workers (e.g. Vonberg 2006, Eveillard 2004, Cesur 2004, Devine 2001, Boyce 1993, Sherertz 1996). However, high risk occupations outside of healthcare have received little attention. Employment in the child care industry poses known infectious disease risk (Cordell 2001, Keeffe 2004, Adler 1989, Jackson 1996). Due to fecal contamination of the environment as well as contact with body fluids, working in a child care center is a known risk factor for gastrointestinal and respiratory illnesses. It also provides the potential for exposure to bloodborne pathogens (Cordell 2001). However, there is a relative paucity of evidence focusing on bacterial carriage in child care workers, despite the fact that outbreaks of bacterial infections are known to occur in the child care environment (Cordell 2001).

Because record numbers of parents are entering the workforce, the number of persons employed in the child care industry is expected to increase rapidly in the near future (Bureau of Labor 2007). Given this increase, it would be worthwhile to invest

more resources into researching this burgeoning occupational group. Moreover, the identification and characterization of *S. aureus* in the child care setting will ideally lead to interventions and policies aimed at decreasing spread of infections in the child care environment. Such interventions and policies could play a vital role in avoiding community outbreaks of this potentially severe pathogen.

Public Health Significance of *S. aureus*

Bacterial infections are an important source of disease burden in the United States (CDC 2010). One bacterium that is often implicated in morbidity and mortality of adults and children is *Staphylococcus aureus*.

Staphylococcus aureus (*S. aureus*) are ubiquitous gram-positive cocci that have the potential to cause severe disease in adults and children. Such diseases include impetigo, scalded skin syndrome, folliculitis, food-borne illness, osteomyelitis, septic arthritis, toxic shock syndrome, pneumonia, thrombophlebitis, deep tissue infection, and septicemia (Lowy 1998). Much of the disease burden caused by this pathogen occurs in the form of skin and respiratory tract infections.

Skin and soft tissue infections (SSTIs) are frequent manifestations of *S. aureus*. SSTIs are the most common bacterial infections in humans, and in 2001, nearly 24% of SSTIs requiring hospitalization in the United States were caused by *S. aureus* (Eisenstein 2008, Jones 2003). In patients with co-morbidities (such as diabetes), SSTIs can lead to complications with high mortality rates, including bacteremia, bacterial endocarditis, and necrotizing fasciitis (Jones 2003).

S. aureus is also able to infect the upper respiratory tract. It has recently been implicated as a major cause of bacterial rhinosinusitis and is one of the most common causes of bacterial conjunctivitis in adults (Payne 2007, Singer 1988). There is evidence that, since the introduction of the pneumococcal vaccine, *S. aureus* is an increasing cause

of bacterial rhinosinusitis and acute otitis media in adults as well as children (Benninger 2008).

The epidemiology of *S. aureus* in children is slightly different from that of adults and the general population. It is the most common pathogen isolated from children visiting medical centers in North America (Fedler 2006). As in adults, SSTIs are the most frequent manifestation of *S. aureus* infection in children and recent data suggest *S. aureus* SSTIs are becoming increasingly pervasive in kids (Zaoutis 2006, Kaplan 2005). *S. aureus*, and methicillin-resistant *S. aureus* (MRSA) in particular, are also increasingly being associated with severe cases of pneumonia in children (Wolf 2007, Four Pediatric Deaths 1999, Campbell 2003, Kaplan 2005, Buckingham 2004, Hageman 2006).

It is difficult to estimate the overall incidence of *S. aureus* infections in the community setting because such infections often go untreated, and those that are treated are not nationally reportable (CDC 2010). Over the period 2001 through 2003 in the United States, there were 11.6 million ambulatory care visits made for treatment of skin and soft tissue infections, a common manifestation of *S. aureus* infection (McCaig 2006). Incidence of bloodstream infections has been determined at the community level and national hospital discharge data can provide estimates of serious disease. Such data indicated nearly 300,000 hospital discharges that listed *S. aureus* septicemias, *S. aureus* pneumonias, or other *S. aureus* infections as diagnoses per year (Kuehnert 2005). Examples of data indicating disease burden from *S. aureus* are included in Table 1.

A worrisome trend in *S. aureus* epidemiology is the rise in frequency and severity of strains that are resistant to antimicrobials, particularly methicillin-resistant *S. aureus* (MRSA). While MRSA has been recognized in the healthcare environment since 1968, the deaths of four children in the late 1990s from community-associated MRSA (CA-MRSA) captured attention nationwide (Barrett 1968, Centers for Disease Control (CDC) 1999). CA-MRSA occurs in individuals in the community without established risk factors for hospital-associated MRSA (i.e. little or no exposure to a healthcare setting) (Fridkin

2005). There is evidence that the incidence of CA-MRSA is rapidly increasing and it has been suggested that CA-MRSA is driving the nearly 100%- increase in SSTI visits nationwide (Hersh 2008). Increasing CA-MRSA rates have been reported from several institutions and CA-MRSA annual incidence has even outpaced hospital-associated disease in some locations (Table 2) (Bothwell 2007, Liu 2008). These trends are not specific to adult populations; the rapid increase of CA-MRSA occurs in children as well (Purcell 2005, Buckingham 2004, Seal 2006, Chen 2006). CA-MRSA infections most commonly occur as comparatively mild SSTIs (one study found that 95.6% of CA-MRSA isolates were obtained from SSTIs), but serious complications that lead to death are possible (Kaplan 2005).

The changing epidemiological patterns of *S. aureus*, particularly the rising rates in children, bring to mind the potential for a corresponding change in adults. The proposed study is an efficient way to look at patterns of colonization in an environment where both adults and children are constantly in contact with one another.

Asymptomatic Colonization with *S. aureus*

It is well-recognized that *S. aureus* is transmitted via asymptomatic carriers of the bacteria. In the early 1930s, Danbolt showed evidence that nasal carriage with *S. aureus* was associated with furunculosis. Since that time, numerous studies have demonstrated the same effect and the fact that nasal carriage of *S. aureus* increases risk of infection is generally accepted (Wertheim 2005).

The National Health and Nutrition Examination Survey estimates that approximately 30% of the U.S. population carries *S. aureus* asymptomatically and 1.5% of the population is colonized with MRSA (Gorwitz 2008). While some countries report high rates of carriage of *S. aureus* in children, data reflecting methicillin-susceptible *S. aureus* (MSSA) colonization in the U.S. are variable (Cheng Immergluck 2004).

However, Creech et. al. report that colonization with MRSA is increasing rapidly in children (Table 3) (Creech 2005).

Bacteria in the Child Care Setting

Infectious agents have the potential to thrive in child care settings, where large numbers of children (who are often on antibiotics) and employees are in close contact with one another for long periods of time. Child care settings are associated with many respiratory agents, including *Bordetella pertussis*, *Haemophilus influenzae* type b, *Mycobacterium tuberculosis*, *Neisseria meningitidis*, *Streptococcus pyogenes* and *Streptococcus pneumoniae*. Bacteria that are spread through the fecal-oral route (*Campylobacter* species, *Clostridium difficile*, *Escherichia coli* 0157:H7 and other enterohemorrhagic *E. coli*, *Salmonella*, and *Shigella*) may also be present. Transmission of pathogens spread by skin-to-skin contact, including Group A *Streptococcus* and *S. aureus*, might also occur (Churchill 1997).

Out-of-home child care has been associated with increased risk of bacterial colonization in children. A multitude of studies of colonization rates have been conducted in child care centers, the majority of which were cross-sectional and focused on *Streptococcus pneumoniae* (*S. pneumoniae*), *Moraxella catarrhalis* (*M. catarrhalis*), and *Haemophilus influenzae* (*H. influenzae*). Tables 4 – 6 depict a small portion of studies on these pathogens that have been published in the last decade.

Compared to *S. pneumoniae*, *H. influenzae*, and *M. catarrhalis*, few studies have addressed *S. aureus* and MRSA in children attending child care centers. Table 7 summarizes all studies (to our knowledge) that have been published since 1999. Rates of *S. aureus* colonization in children have ranged from 10.1% to 35.0%, and proportions of children carrying MRSA are between 0% and 6.7% (Velazquez-Guadarrama 2009, Zanelli 2002).

Risk to Child Care Employees

Due to fecal contamination of the environment as well as contact with body fluids, working in a child care center is a known risk factor for gastrointestinal and respiratory illnesses and provides the potential for exposure to bloodborne pathogens (McGrath 2007). While the list of potential organisms to which child care workers might be exposed is long, occupational risk has only been quantified for a few pathogens, including cytomegalovirus, infectious diarrhea, and human parvovirus B19 (Bright 1999).

In the past ten years, very few studies have been published that focus on bacterial colonization in child care workers. Colonizing pathogens that have been studied in this group include *S. pneumoniae*, *H. influenzae*, *M. catarrhalis*, *Kingella kingae* (*K. kingae*), *β-hemolytic Streptococci*, and *S. aureus* and MRSA (Table 8).

The studies that have been published have some important limitations. The number of child care employees that have been studied is not large enough for results to be generalizable to the millions of child care workers employed in the countries where the research took place. Most of the data available are from studies where the primary focus is children (i.e. data from employees are incidental). A substantial portion of the studies were initiated after an index case or case of severe disease was reported. While it is worthwhile to concentrate research efforts on these facilities in order to decrease transmission, the experiences of workers in facilities without an index case may differ. Moreover, very few of the studies included unexposed individuals as a basis for comparison. These comparison groups are crucial in order to evaluate whether employment in a child care facility poses a probability of colonization above and beyond that of unexposed individuals.

The Role of Fomites in *S. aureus* Ecology

Many pathogens can be transmitted by contact with a contaminated object. It is generally accepted that environmental contamination plays an important role in

healthcare-associated infections (Boyce 2007). Kramer et. al. performed a literature review pertaining to nosocomial pathogens and concluded that many gram-positive organisms, including *S. aureus*, could survive on dry surfaces for months (Kramer 2006). The role fomites play in transmission of *S. aureus* outside the healthcare setting has been studied less frequently. A residential study found *S. aureus* in 97% of homes where a child in diapers, a dog, or a cat was present. MRSA was isolated from 26% of these homes and was present on a variety of surfaces (kitchen and bathroom sinks, dish sponge, high chair, etc.) (Scott 2008). In the child care setting, a wide diversity of organisms (including *Staphylococcus*) have been found to contaminate toys, changing tables, and other surfaces (Lee 2007). Hewlett et. al. obtained 195 swabs from environmental surfaces in one facility and found MSSA on 17 (8.7%) (Hewlett 2010). The same study reported MRSA on four surfaces, including two cribs, a cloth toy, and a nap mat (Hewlett 2009).

Anatomical Location of *S. aureus* Carriage

Determining the colonization state of an individual is important in several settings. For example, many research studies rely on swabs to create accurate estimates of carriage prevalence. Also, the rapid increase of health and financial burden of MRSA in the healthcare setting has spawned interest in screening programs at hospitals and clinics worldwide. In both situations, it is important to choose body sites to swab that will maximize sensitivity.

The anterior naris is the most consistent location of *S. aureus* colonization (Wertheim 2005). Recent studies, however, have suggested that this assumption may not hold in all situations. Some studies have shown that a substantial number of individuals are exclusive throat carriers and that including a throat swab increases sensitivity of detection. The proportion of individuals in these studies who were solely throat carriers ranged from 12 – 17% (Meurman 2005, Mertz 2007, Ringberg 2006).

Perhaps even more important, however, are figures on how many carriers would have been missed if throat cultures had not been obtained. Bignardi reported that, in one study, eliminating throat swabs from MRSA screening procedures would have decreased identified carriers by 19%, a figure higher than those if nares (9%) or perineum (7%) swabs were eliminated (Bignardi 2009). Mertz et. al. concluded in a *S. aureus* study published in 2007 that swabbing the throat as well as the nares increased sensitivity by 25.7% (Mertz 2007). Finally, in a study of 259 patients and 87 staff members at a hospital in Sweden, the throat was the most common site of carriage (40% of participants were colonized in the throat compared to 31% that were colonized in the nares). In this group sampling solely from the nares would have identified 64% of *S. aureus* carriers, while sampling solely from the throat would have identified 83% (Nilsson 2006).

While the above findings are certainly compelling and cause for attention, most of the participants in those studies had exposure to a healthcare setting (i.e. were patients, staff, or had family members in healthcare setting). It is unclear whether results from these studies can be generalized to individuals with little or no healthcare exposure. In fact, there is evidence to suggest that colonization site may differ according to level of healthcare exposure. A study of over 3,000 individuals in Switzerland found that probability of exclusive throat carriage increased as exposure to a healthcare environment decreased. Exclusive throat carriage of healthy blood donors with no substantial exposure to healthcare was 30.2%, while exclusive throat carriage in patients and healthcare workers was significantly lower (18.4%, $p<0.001$). The same study found that an age of 30 years or younger significantly predicted exclusive throat colonization (Mertz 2009).

In response to these findings, Mertz et. al. state that “throat carriage may indeed be more common among healthy individuals than among individuals who are exposed to the health care system, but such a hypothesis requires confirmation by other investigators in different, non-health care populations” (Mertz 2007). By combining results of three

cross-sectional studies of individuals with limited exposure to healthcare, the proposed analysis will contribute to this body of evidence.

Summary and Rationale

This chapter has illustrated several important concepts crucial to the development and success of this study.

- Bacteria, including *S. aureus*, are important sources of disease burden in adults and children
- Asymptomatic colonization is a key risk factor for infection and transmission
- Child care centers provide optimal environments for pathogen transmission
- When compared to adults not employed in a child care setting, child care workers are at increased risk of exposure to infectious agents
- Anatomical location of *S. aureus* carriage may vary according to population characteristics

While the primary focus of this project is the prevalence of colonization in child care employees, the results have the potential to serve a broader purpose. The results of this study, in furthering our knowledge of pathogen behavior, would ideally serve as an impetus to evaluate current hygiene practices and policies in child care centers, as well as screening procedures in a number of settings. As the treatment arsenal for these bacteria is reduced by increasing resistance, averting community transmission could play a key role in disease prevention.

Specific Aims

The purpose of this study is to estimate and describe the prevalence of bacterial colonization in child care employees. The proposed project will evaluate the hypotheses that employment in a child care center increases the probability of colonization with *S.*

aureus and that child care centers serve as reservoirs for these organisms. This study, in conjunction with two other colonization studies, will also evaluate the hypothesis that the throat is an important site of *S. aureus* carriage.

To address these hypotheses, the following specific aims are proposed.

1. **Determine the prevalence of *S. aureus* carriage in a cross-sectional sample of child care workers employed in Johnson County, Iowa.** We hypothesize that the prevalence of colonization for these agents will be higher in employees of child care facilities than the prevalence in a comparison sample with no occupational exposure to child care centers.
2. **Evaluate the presence of *S. aureus* in children and in the child care environment.** Child care providers might be colonized at work via their contact with children, either directly (from children's' nasal secretions) or indirectly (via bacteria from children who contaminate environmental surfaces). Molecular type and antibiotic resistance profiles will be obtained for all isolates. We hypothesize that identical or very closely-related bacterial strains will be present in swabs taken from child care workers, children, and environmental surfaces (Figure 1).
3. **Identify risk factors for colonization with *S. aureus* in a child care setting using questionnaire data.** We hypothesize that several variables present in the child care environment (e.g. contact with a high number of children per day) will predict elevated prevalence of colonization in child care employees.
4. **Compare the prevalence of *S. aureus* found in the nose, the throat, or in both locations.** We hypothesize that a substantial number of *S. aureus* isolates would be missed if only swabs from the anterior nares were collected.
5. **Determine molecular and epidemiological predictors of colonization location.** We hypothesize that certain characteristics of the bacteria and the host (e.g. spa

type or a host exposed to the healthcare setting) will be predictive of colonization location.

- **Figure 1.** Diagram of potential pathogen interactions within a child care setting.

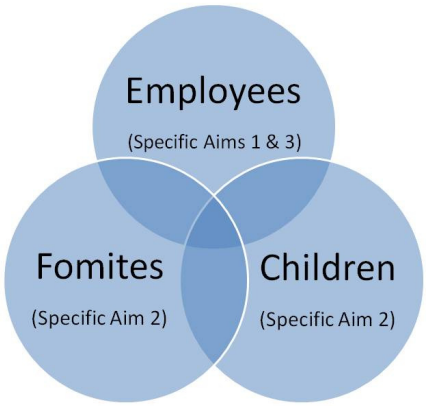


Table 1. Selected citations reflecting the disease burden of *Staphylococcus aureus* (*S. aureus*) in the United States and North America.

Authors	Year Published	Disease Manifestation	Data Source	Location	Population	Sample Size	Results
Morin and Hadler	2001	Blood stream infections (BSI)	Population-based surveillance	Four metropolitan areas in Connecticut (US)	All ages		Incidence of community-onset BSI was 17/100,000 persons
Diekema *et. Al.*	2001	Blood stream infections	SENTRY Antimicrobial Surveillance Program	United States	All	17399	25% of BSIs were caused by *S. aureus*
Diekema *et. al.*	2001	Lower respiratory tract infections (LRTI)	SENTRY Antimicrobial Surveillance Program	United States	All	6711	26% of LRTIs were caused by *S. aureus*
Diekema *et. al.*	2001	Skin and soft tissue infections (SSTI)	SENTRY Antimicrobial Surveillance Program	United States	All	2328	42% of SSTIs were caused by *S. aureus*
Jones *et. al.*	2003	Skin and soft tissue infections	The Surveillance Network Real-Time Database	United States	All ages	26,233	24% of SSTIs were caused by *S. aureus*
Kaplan *et. al.*	2005	Skin and soft tissue infections	Cohort from the Texas Children's Hospital	Houston, TX (US)	Pediatrics	3578	92% of *S. aureus* infections were SSTIs
Kuehnert *et. al.*	2005	All	National Nosocomial Infections Surveillance System	Nationwide	All ages		*S. aureus* infections occurred in 9.13/1,000 hospital discharges
Fedler *et. al.*	2006	All	SENTRY Antimicrobial Surveillance Program	North America	Pediatrics (Birth to 18 years)	3500	*S. aureus* was isolated from 27.4% of clinical samples
Zaoutis *et. al.*	2006	Skin and soft tissue infections	Cohort from the Children's Hospital of Philadelphia	Philadelphia, PA (US)	Pediatrics	446	66% of confirmed *S. aureus* infections were SSTIs
Payne and Benninger	2007	Acute bacterial rhinosinusitis	Meta-analysis of literature (English language)		All ages	5790	*S. aureus* is the offending pathogen in 10% of bacterial rhinosinusitis cases
Laupland *et. al.*	2007	Blood stream infections	Population-based surveillance	Calgary Health Region (Canada)	All ages	4467	Incidence of community-onset BSI was 13.5/100,000 persons

Table 2 Selected citations reflecting the disease burden of methicillin-resistant *Staphylococcus aureus* (MRSA) in the United States.

Authors	Year Published	Disease Manifestation	Data Source	Location	Population	Sample Size	Results
Kaplan *et. al.*	2005	Skin and soft tissue infections	Cohort from the Texas Children's Hospital	Houston, TX (US)	Pediatrics	3578	96% of CA-MRSA isolates were from SSTIs
Bothwell *et. al.*	2007	Head and neck infections	Otolaryngology service at a tertiary care center	Hawaii (US)	All ages	325	The proportion of methicillin resistant *S. aureus* (MRSA) increased from 21% to 64% in a five year period
Liu *et. al.*	2008	All	Laboratory isolates from nine medical centers	San Francisco, CA (US)	All ages	3826	Estimated population-based incidence of community-acquired MRSA was 316/100,000 persons
Liu *et. al.*	2008	All	Laboratory isolates from nine medical centers	San Francisco, CA (US)	All ages	3826	Estimated population-based incidence of hospital-acquired MRSA was 31/100,000 persons

Table 3. Selected citations reflecting prevalence of asymptomatic colonization with *Staphylococcus aureus* (*S. aureus*) in the United States.

Authors	Year Published	Data Source	Location	Population	Sample Size	Results
Cheng Immergluck *et. al.*	2004	Healthy children at an urban pediatric clinic	Chicago, IL (U.S.)	Under age five	291	18.6% of children were colonized with *S. aureus*
Cheng Immergluck *et. al.*	2004	Healthy children at an urban pediatric clinic	Chicago, IL (U.S.)	Under age five	291	9.2% of children were colonized with MRSA
Creech *et. al.*	2005	Children presenting for health maintenance visits to two clinics	Nashville, TN (U.S.)	Two weeks – 21 years	500	Rates of MRSA colonization increased from 0.8% to 9.2% in a three year time span
Gorwitz *et. al.*	2008	National Health and Nutrition Examination Survey 2001-2004	United States	All ages greater than one year	9004	1.5% of individuals were colonized with MRSA
Gorwitz *et. al.*	2008	National Health and Nutrition Examination Survey 2001-2004	United States	All ages greater than one year	9004	28.7% of individuals were colonized with *S. aureus*

Table 4. Selected citations of publications in the last decade showing variation in rates of *S. pneumoniae* colonization in children attending child care centers.

Authors	Year Published	Location	Sample Size	Results
Kellner *et. al*.	1999	Canada	1322	Crude mean carriage rate of 44.3%; after adjusting for clustering among centers, carriage rate was 43.1%
Stratchounski *et. al.*	2000	Russia	733	Carriage rate of 55.9%
Marchisio *et. al.*	2001	Italy	1580	Carriage rate was 3.8% in the fall and 4.7% in the spring
Peerbooms *et. al.*	2002	Netherlands	535	Carriage rate was 58.0%
Nunes *et. al.*	2005	Portugal	942	Carriage rate was 63%
Zemlickova *et. al.*	2006	Czech Republic	425	Carriage rate was 38.1%
Dueger *et. al.*	2008	Guatemala	367	Carriage rate in private CCCs was 63.8%. Mean carriage rate in public CCCs was 84.2%

Subjects were not noted to have been vaccinated. CCC = child care center.

Table 5. Selected citations of publications in the last decade showing variation in rates of colonization with non-type b *H. influenzae* in children attending child care centers.

Authors	Year Published	Location	Sample Size	Results
de Lencastre *et. al.*	1999	Portugal	586	Carriage rate of 72%
Marchisio *et. al.*	2001	Italy	1580	Carriage rate in the fall was 13.0% and 18.2% in the spring
Masuda *et. al.*	2002	Japan	156	Carriage rate of 53.2%
Dabernat *et. al.*	2003	France	1683	Mean carriage was 40.9% (highly vaccinated population)
Farjo *et. al.*	2004	Michigan (U.S.)	198	64% carriage rate
Kontiokari *et. al.*	2005	Finland	341	Carriage ranged from 11 – 16%
Torun *et. al.*	2007	Turkey	195	48.7% carriage rate

Table 6. Selected citations of publications in the last decade showing variation in rates of colonization with *M. catarrhalis* in children attending child care centers.

Authors	Year Published	Location	Sample Size	Results
de Lencastre *et. al.*	1999	Portugal	586	54.0% carriage rate
Peerbooms *et. al.*	2002	Netherlands	259	80% carriage rate
Henriques Normark *et. al.*	2003	Sweden	595	24.0% carriage rate
Quinones *et. al.*	2005	Cuba	150	64.7% carriage rate
Kontiokari *et. al.*	2005	Finland	341	Carriage rates ranged from 7 – 25%
Zemlickova *et. al.*	2006	Czech Republic	425	22.1% carriage rate

Table 7. Studies of prevalence and risk of colonization with *S. aureus* and MRSA published in the last decade in children attending child care centers.

Authors	Year Published	Location	Sample Size	Results
Shahin *et. al.*	1999	Canada	164	24.4% of children were colonized with *S. aureus* and 1.2% were colonized with MRSA
Palanduz *et. al.*	1999	Turkey	135	2.2% of children were colonized with MRSA. Study was conducted in a CCC for children of hospital staff
Principi *et. al.*	1999	Italy	1723*	18.7% of children without another respiratory pathogen were colonized with *S. aureus*. Carriage rate in children testing positive for at least one other pathogen was 14.6%.
Sa-Leao *et. al.*	2001	Portugal	2111	*S. aureus* isolated from 13.0% of children; MRSA isolated from 0.24% of children
Zanelli *et. al.*	2002	Italy	374**	35.0% *S. aureus* carriage rate in children; no MRSA isolated
Masuda *et. al.*	2002	Japan	156	*S. aureus* carriage rate of 17.9%; 42.9% of S. aureus isolates were MRSA.
Cheng Immergluck *et. al.*	2004	Chicago (U.S.)	291 (at clinic)	Children colonized with *S. aureus* were 2.30 times more likely to report some care outside the home than children who weren't colonized
Zemlickova *et. al.*	2006	Czech Republic	425	*S. aureus* colonization rate of 16.0%
Soysal *et. al.*	2006	Turkey	1000 (at clinic)	Child care attendance was not significantly associated with *S. aureus* carriage
Fritz *et. al.*	2008	St. Louis (U.S.)	1300 (at clinic)	Children who attended child care were 0.10 times as likely to be colonized with MRSA than children who did not attend child care
Velazquez-Guadarrama *et. al.*	2009	Mexico	2345	10.1% of children were colonized with *S. aureus*; 0.93% of children were colonized with MRSA
Hewlett *et. al.*	2009	Texas (U.S.)	104	6.7% of children were colonized with MRSA
Hewlett *et. al*	2010	Texas (U.S.)	104	21.2% of children were colonized with MSSA

Table 8. Studies from the last decade providing data on colonization in child care workers.

Authors	Year Published	Pathogen	Location	Sample Size	Results
Shahin *et. al.*	1999	*S. aureus*, MRSA	Canada	98	24% of staff member were colonized with *S. aureus*. None carried MRSA*
Henriques Normark *et. al.*	2003	*S. pneumoniae*	Sweden	123	Carriage was 1.6% in child care employees.
Henriques Normark *et. al.*	2003	*H. influenzae*	Sweden	123	Carriage was 0.8% in child care employees
Henriques Normark *et. al.*	2003	*M. catarrhalis*	Sweden	123	Carriage was 0.8% in child care employees
McVernon *et. al.*	2004	*H. influenzae* type b	England	21	Carriage in staff was 4.8%*
Kiang *et. al.*	2005	*K. kingae*	Minnesota (U.S.)	28	No staff was colonized*
Jensen *et. al.*	2006	MRSA	Denmark	60	1.7% carriage rate*
Rosen *et. al.*	2007	*S. pneumoniae*	Texas (U.S.)	63	4.8% of workers were colonized
Rosen *et. al.*	2007	β-hemolytic *Streptococci*	Texas (U.S.)	63	19.1% of workers were colonized
Rosen *et. al.*	2007	*S. aureus*	Texas (U.S.)	63	14.3% of workers were colonized
Rosen *et. al.*	2007	*M. catarrhalis*	Texas (U.S.)	63	3.2% of workers were colonized
Hewlett *et. al.*	2009	MRSA	Texas (U.S.)	32	3.1% carriage rate in staff
Hewlett et. al.	2010	MSSA	Texas (U.S.)	32	28.13% carriage rate in staff

* Studies conducted after an index case was reported.

CHAPTER II

BACTERIAL CARRIAGE IN CHILD CARE FACILILTIES: A REVIEW OF LITERATURE PUBLISHED SINCE JANUARY 2000

Introduction

Bacterial infections are an important source of disease burden in the United States and worldwide (Lederberg 1993, Scott 2007, Watt 2009, O'Brien 2009). Asymptomatic colonization of a host is an important risk factor for developing infection, as well as a key contributor to transmission of many types of bacteria, some of which include *Haemophilus influenzae*, numerous *Streptococcus* species, *Neisseria meningitidis*, and *Staphylococcus aureus* (Nelson 2007).

Infectious agents have the potential to thrive in the childcare setting, where large numbers of children and employees are in close contact with one another for long periods of time. Behavioral factors of children (e.g. the tendency of children to put hands and objects in their mouths) and adults (e.g. hygiene practices and close physical contact with children), as well as physiologic factors in children (e.g. immature immune systems and incontinence) contribute to the success of pathogen transmission in child care facilities (Thacker 1992, Osterholm 1994, Thompson 1994, Nesti and Goldbaum 2007).

A review by Churchill and Pickering in 1997 found that child care settings are ideal settings for transmission of many respiratory agents, including *Bordetella pertussis, Haemophilus influenzae* type b*, Mycobacterium tuberculosis, Neisseria meningitidis, Streptococcus pyogenes* and *Streptococcus pneumoniae*. Bacteria that are spread through the fecal-oral route (*Campylobacter* species, *Clostridium difficile, Escherichia coli* 0157:H7 and other enterohemorrhagic *E. coli, Salmonella*, and *Shigella*) may also be present. Transmission of pathogens spread by skin-to-skin contact, including *S. pyogenes* and *S. aureus*, might also occur (Churchill 1997). Studies conducted in the 1970s – 1990s found that the risk of acute respiratory tract infections in children attending child care

facilities was up to 1.6 times greater than that in children receiving care at home (Wald 1991, Loda 1972, Fleming 1987, Hurwitz 1991, Bell 1989, and Denny 1986). Similarly, the risk of diarrheal illness among children attending child care facilities was 1.6 – 3.5 times greater than that in children being cared for at home (Alexander 1990, Bartlett 1985, Doyle 1976).

Many researchers, physicians, and authors recognize the importance of bacterial colonization of children and adults in child care centers as a critical factor in disease spread. One study found that 90.4% of children attending child care facilities carried at least one of four respiratory bacterial pathogens tested, illustrating the potential for child care facilities to serve as focal points of bacterial transmission (Masuda 2002). We performed a review of literature published since January 2000 in order to 1) evaluate prevalence and risk of bacterial carriage among individuals at child care facilities; 2) report changes in understanding of carriage due to advances in medical science (namely molecular typing and the introduction of the pneumococcal vaccine); and 3) discuss limitations of available data and identify gaps in knowledge.

Materials and Methods

An initial MEDLINE literature search was conducted using various combinations of the terms “colonization”, “carriage”, “daycare”, “day care”, “childcare”, “child care”, “nursery”, “out of home care”, “preschool”, and “pre school”. English-language article titles and abstracts were scanned, and papers of interest published between January 2000 and September 2010 were marked for further evaluation. Each of the marked papers was perused and basic study data (e.g. year published, location, study population, findings) were entered into a qualitative database (Microsoft Excel 2007). Citations of these articles were then studied to further identify other potential articles of interest.

The vast majority of returned studies focused on four bacterial pathogens, including *Streptococcus pneumoniae*, *Haemophilus influenzae*, *Moraxella catarrhalis*,

and *Staphylococcus aureus*. *Streptococcus pneumoniae* are Gram-positive cocci that cause a wide spectrum of infections, including those that are common in children (acute otitis media, bacterial rhinosinusitis, bacterial lower respiratory tract infections, and conjunctivitis) (Yamanaka 2008, Benninger 2008, Wolf 2007, and Tarabishy 2008). *S. pneumoniae* also is a primary cause of invasive infections, such as meningitis and bacteremia, although incidence of serious infection has generally declined since introduction of the pneumococcal vaccine (Lynch 2009, Whitney 2003, Talbot 2004, Kaplan 2004, Albrich 2007). Despite the fact that *Haemophilus influenzae* type b (also an important cause of invasive disease) is vaccine-preventable as well, non-type-b *Haemophilus influenzae*, a small Gram-negative coccobacillus, remains a leading cause of acute otitis media, rhinosinusitis, and bacterial community-acquired pneumonia (CDC 2008 *Haemophilus*, Ulanova 2009, Liebovitz 2007, Gehanno 2001, Block 2004, Benninger 2008, Pfaller 2001). *Moraxella catarrhalis* are Gram-negative cocci that often cause otitis media and sinusitis in children as well (Hager 1987). Like *S. pneumoniae*, *Staphylococcus aureus* causes a wide spectrum of diseases, but is most often associated with skin and soft tissue infections (Zaoutis 2006, Kaplan 2005).

Results

This review contains information from 81 studies published between January 2000 and September 2010 in English-language peer-reviewed journals. *Streptococcus pneumoniae* (*S. pneumoniae*) was the most studied pathogen, followed by *Haemophilus influenzae* (*H. influenzae*), *Moraxella catarrhalis* (*M. catarrhalis*), and *Staphylococcus aureus* (*S. aureus*). Sporadic articles about several other pathogens (*Streptococcus pyogenes*, *Kingella kingae*, *Clostridium difficile* and *Escherichia coli*) were also uncovered.

The vast majority of articles (72%) published since January 2000 either measured the prevalence of pathogens in children attending child care facilities or assessed child

care attendance as a risk factor in children recruited at other locations (i.e. well-child visits). In addition, many of these studies provided antibiotic resistance data on isolates, allowing for evaluation of prevalence and risk among pathogens with reduced susceptibility to antibiotics.

Findings of each publication were abstracted and grouped according to the following categories: prevalence of bacterial carriage in attendees; child care attendance as a risk factor for bacterial carriage; antibiotic resistance in colonizing bacteria; the effect of pneumococcal on carriage in child care attendees; molecular epidemiology of bacteria in the child care setting; and findings related to child care employees. Most research focused on carriage in child care attendees only.

Streptococcus pneumoniae

Prevalence, seasonal variation, and risk of carriage in child care attendees

Point prevalence of *S. pneumoniae* in child care attendees reported since 2000 ranged from 3.8% to 84.2% (Marchisio 2001, St. Sauver 2000, Stratchounski 2000, Chiu 2001, Masuda 2002, Marchisio 2002, Dagan 2002, Rey 2002, Henriques Normark 2003, Sulikowska 2004, Sandgren 2004, Tomasson 2005, Dagan 2005, Kontiokari 2005, Frazao 2005, Nunes 2005, Zemlickova 2006, Volonakis 2006, Stratchounski 2000, Soysal 2006, Katz 2007, Grivea 2008, Dueger 2008, Abut 2008, Dunais 2008, Leino 2008, Sa-Leao 2008, Katsarolis 2009, Espinosa-de los Monteros 2007, Vasoo 2010, Franco 2010, Vestrheim 2008). The reason for such substantial variation is unclear. These studies consistently used nasopharyngeal swabs for sampling, but *S. pneumoniae* is somewhat fastidious and does not compete well against other microbiota in culture (Murray 2003). It is possible that researchers had varying levels of experience and success at culturing this organism. However, the degree of variability in this review reflects overall variability found in another review. Cardozo et. al. published a literature review in 2006 reporting *S.*

pneumoniae colonization rates in all ages and study populations, and also reported highly variable carriage rates. While conclusions regarding broad geographic areas could be reached (e.g. the highest carriage rates were reported in Africa), a high degree of variability was reported even in areas that were geographically close together. For example, the colonization rate in one Chilean city was 14%, while the rate in a second Chilean city was 42% (Cardozo 2006).

In terms of seasonal variation, studies in Italy and Poland reported negligible differences in carriage rates between spring and fall/winter (Marchisio 2001, Sulikowska 2004). Another study conducted in Norway, however, found that the overall carriage rate of *S. pneumoniae* in four child care centers was significantly higher in August than in February (Sogstad 2006). A study of *S. pneumoniae* in Turkish child care centers found that although the highest rate of strain entry into a facility occurred during winter months, transmission among children was highest in the summer months (Abut 2008). The acquisition rate of *S. pneumoniae* in children attending a Mexican child care facility was highest during the winter (January through March), but carriage rates peaked several times during the year (March, June, September, and December) (Gomez-Barreto 2002). Many studies published since 2000 reported that attending a child care facility increases the risk of *S. pneumoniae* colonization (Table 9).

Antibiotic resistance in *S. pneumoniae*

Infections with antibiotic resistant organisms are associated with higher morbidity, mortality, and economic costs than their counterparts that are susceptible to treatment (Levy 2004). Child care facilities are thought to be ideal reservoirs for the development and spread of resistant organisms because large numbers of children (who are frequently taking a wide variety of antibiotics) have close person-to-person contact with one another (Greenberg 2008). Because children are often prescribed antibiotics for their myriad ailments (e.g. otitis media or upper respiratory tract infections), the carriage

of resistant organisms is particularly of interest to experts studying bacterial ecology in child care facilities.

The percentage of *S. pneumoniae* with reduced susceptibility to penicillin varies widely in child care populations. Some countries, including Italy, the Netherlands, Norway, the Czech Republic, Russia, and the United Kingdom, report that less than 5% of *S. pneumoniae* isolated from child care attendees have reduced susceptibility to penicillin (Marchisio 2001, Peerbooms 2002, Sogstad 2006, Zemlickova 2006, Katz 2007). Studies conducted in Hong Kong, Brazil, Japan, Mexico, and Singapore, however, reported higher rates of 58%, 57.6%, 61%, 63.7%, and 69.5% (Chiu 2001, Velasquez 2009, Masuda 2002, Espinosa-de Los Monteros 2007, Vasoo 2010). Prevalence rates of non-susceptible isolates between 5 and 50% have been reported in Russia, Italy, Sweden (in facilities where PNSP index cases were identified), Poland, Portugal, Iceland, Turkey, Greece, and Brazil (Stratchounski 2000, Marchisio 2002, Peerbooms 2002, Henriques Normark 2003, Sulikowska 2004, Frazao 2005, Nunes 2005, Tomasson 2005, Stratchounski 2006, Abut 2008, Katsarolis 2009, Franco 2010).

Some data are available regarding temporal trends in antibiotic resistance among *S. pneumoniae* isolated from child care attendees. It is important, however, to interpret these trends in the context of vaccination, as changes in resistance have been associated with introduction of the pneumococcal vaccine into pediatric populations (see vaccination section). Trends in penicillin resistance among child care attendees prior to mass vaccination campaigns varied. Volonakis et. al. reported a significant increase in penicillin resistance from 2000 – 2003 (2.07% - 16.2%, $p < 0.001$), while Tomasson et. al. reported a non-significant decrease from 9.7% - 6.0% between 1992 and 1999 ($p = 0.24$) and Sa-Leao et. al. reported fluctuations in resistance rates between 1996 and 1998 (Volonakis 2006, Tomasson 2005, Sa-Leao 2000). Several studies published since 2000 have reported increased risk of colonization with *S. pneumoniae* with reduced susceptibility to penicillin in child care attendees, with statistically significant ($p < 0.05$)

odds ratios ranging from 2.3 – 3.9 (Huang 2009, Dunais 2008, Regev-Yochay 2003, Finkelstein 2003).

Pneumococcal vaccination and child care populations

The development and subsequent use of a 7-valent pneumococcal conjugate vaccine (PCV7) beginning in 2000 has changed the "landscape" of bacterial carriage in child care facilities (CDC 2008 Pneumococcal). While studies of the effects of vaccination on child care attendees do not directly assess any relationship with child care attendance, child care facilities have been the focus of many studies seeking to elucidate trends in carriage after vaccination is introduced.

Serotyping of *S. pneumoniae* isolated from child care attendees prior to widespread pneumococcal vaccine use allowed researchers to predict the proportion of carriers that would be affected by immunization. Studies conducted in Beijing, Hong Kong, the United Kingdom, Norway, and Greece uncovered carriage rates of PCV7 serotypes between 44.6 and 66.1% (McGee 2001, Ho 2004, Roche 2007, Espinosa-de los Monteros 2007, Vasoo 2010, Frazao 2010, Vestrheim 2008). Theoretically, the PCV7 vaccine would have eliminated between 44 and 66% of *S. pneumoniae* colonizing child care attendees. Moreover, the same figures for PCV7 serotypes resistant to antibiotics were 70.6 and 82.8% in studies conducted in Hong Kong and Greece (Ho 2004, Katsarolis 2009). While rates of non-susceptibility among *S. pneumoniae* isolates in Norway were low, those isolates with decreased susceptibility were associated with PCV7 serotypes, suggesting that widespread vaccination would reduce carriage of resistant serotypes as well (Vestrheim 2008).

The predictions of decreased prevalence of vaccine-type *S. pneumoniae* in child care attendees generally proved to be correct, as shown in multiple studies conducted in a variety of geographic areas (Dagan 2002, Frazao 2005, Dueger 2008, Grivea 2008, Sa-Leao 2009, Vestrheim 2010). Overall carriage rates, however, remained the same.

Prospective randomized trials and observational data showed increases in prevalence of non-typeable isolates and serotypes not included in the vaccine, a finding that can be explained by strain replacement or strain "unmasking" (Dagan 2002, Frazao 2005, Nunes 2008, Dunais 2008, Rodrigues 2009, Sa-Leao 2009, Vestrheim 2010, Lipsitch 1999, Lipsitch 2001).

Similar shifts in serotype patterns can be seen in children that have not been vaccinated. A prospective study of Greek child care attendees noted that as vaccination rates increased, PCV7 serotypes decreased among non-vaccinated carriers (Grivea 2008). Sa-Leao et. al. noted serotype replacement in both vaccinated and unvaccinated Portugese children, as did Vestrheim et. al. in Norway (Sa-Leao 2009, Vestrheim 2010).

Data collected prior to vaccination campaigns also suggested that rates of resistant *S. pneumoniae* would be affected due to the fact that many resistant strains were those included in the vaccine (Ho 2004, Espinosa-de los Monteros 2007, Katsarolis 2009, Vasoo 2010). Several studies have shown decreased carriage of resistant vaccine-type *S. pneumoniae* in vaccinated children or as mass vaccination campaigns were implemented (Dagan 2003, Frazao 2005, Dunais 2008, Grivea 2008, Rodrigues 2009). However, there appears to be a parallel increase in either reduced susceptibility among non-vaccine serotypes or intermediate resistance among all isolates, resulting in no overall decrease in resistance among carriers (Frazao 2005, Grivea 2008, Sa-Leao 2009).

Molecular Epidemiology of *S. pneumoniae*

The considerable development of molecular characterization techniques in the past decade has allowed researchers to more thoroughly investigate strain behavior within child care facilities. This review focuses primarily on molecular comparisons made using pulsed field gel electrophoresis (PFGE). However, comparisons of isolates have also been made using antimicrobial resistance profiles; serotyping; the presence, absence, and

type of a gene of interest; multi locus sequence typing (MLST); restriction fragment length polymorphisms (RFLP), etc.

Horizontal spread of *S. pneumoniae* within child care facilities has been well documented, as several studies have uncovered indistinguishable clones among children attending the same facility (Sa-Leao 2000, Bogaert 2001, Henriques Normark 2003, Leino 2008, Abut 2008, Vestrheim 2008, Sa-Leao 2008, Hoti 2009). Moreover, the degree of transmission appears to be greater within a child care facility than would occur in the general population, as shown by Bogaert et. al. Leino et. al. found that children were more likely to acquire *S. pneumoniae* from child care facilities than from family members (Leino 2008).

Many studies provide molecular typing for *S. pneumoniae* and several patterns have emerged. There is evidence that the degree of genetic heterogeneity of *S. pneumoniae* in a facility depends on the strains that are present. Sandgren et. al. identified two general classes of isolates in Swedish child care facilities: one class was genetically homogeneous and comprised mainly of invasive isolates, while the second class was comprised of genetically diverse non-invasive isolates (Sandgren 2004). Frazao et. al. reported similar findings, with most vaccine-type isolates belonging to nine internationally-spread epidemic clones, while non-vaccine-type isolates produced unique PFGE patterns (Frazao 2005).

These results coincide with findings of several other studies indicating that each child care facility has a unique molecular profile that consists of a more clonal *S. pneumoniae* population made up of “epidemic” strains, as well as a more heterogeneous population that is unique to each facility (Sa-Leao 2000, Sa-Leao 2000, Nunes 2005, Abut 2008, Vestrheim 2008). Sa-Leao et. al. described child care facilities “autonomous epidemiological units “ (Sa-Leao 2000). The findings of a longitudinal study by the same group of researchers could help explain this general pattern. Sa-Leao et. al. identified three general varieties of *S. pneumoniae*: one made up of strains that caused prolonged

carriage and underwent high rates of transmission; one clone with prolonged colonization and low capacity for transmission; and one clone with high rates of transmission, but low capacity for persistence in any given child (Sa-Leao 2008;).

Haemophilus influenzae

Prevalence, seasonal variation, and risk of carriage in child care attendees

Percent carriage of non-type b *H. influenzae* at any given time point ranged from 11 – 77% in child care attendees (Marchisio 2001, Fontanals 2000, Oguzkaya-Artan 2007, Akcakaya 2001, Stratchounski 2001, Peerbooms 2002, Masuda 2002, Dabernat 2003, Henriques Normark 2003, Sulikowska 2004, Bricks 2004, Kontiokari 2005, Zemlickova 2006, Torun 2007, Farjo 2004, da Silva and Marin 2001). Reported carriage rates for *H. influenzae* type b (Hib) were between 0 and 3.4% in highly vaccinated populations, and 4.2 and 42.6% in populations of children attending child care prior to widespread Vaccination (McVernon 2004 Long-term, McVernon 2004 Outbreak, Oguzkaya-Artan 2007, Karimi 2009, da Silva 2001 and Marin 2001, Akcakaya 2001, Bricks 2004, Bakir 2002). There is evidence that *H. influenzae* carriage is higher in the spring months than winter or fall (Marchisio 2001, Dabernat 2003, Sulikowska 2004). Child care attendance appears to increase risk of *H. influenzae* carriage (Table 10).

Antibiotic resistance in *H. influenzae*

Many studies providing data on *H. influenzae* supplied information about resistance in isolates. Prevalence of β-lactam-producing organisms ranges from 0 to 44.5% of isolates in any given study conducted in child care attendees (Masuda 2002, Stratchounski 2001, Zemlickova 2006, Henriques Normark 2003, Neto 2003, Sa-Leao 2008, Dabernat 2003). Reduced susceptibility to amoxicillin and ampicillin ranges between 4.6 and 10.1%, and 5.5 and 7.3% of isolates, respectively (Zemlickova 2006,

Peerbooms 2002, Marchisio 2001, Bricks 2004, Sulikowska 2004, Torun 2007). Resistance rates vary in populations of Hib as well. Bakir et. al. reported that 2.3% of Hib isolated from Turkish child care attendees produced β-lactamase (Bakir 2002). In another study conducted in Turkish child care attendees, ampicillin resistance was observed in 7.3% of isolates, the majority of which were Hib (Torun 2007).

H. influenzae isolates may also acquire β-lactam resistance through mechanisms other than β-lactamase production; these organisms are termed β-lactamase negative ampicillin resistant (BLNAR). In most studies in child care attendees, BLNAR isolates make up a minority of resistant strains (Bakir 2002, Sa-Leao 2008, Torun 2007, Dabernat 2003, Henriques Normark 2003). However, Stratchounski et. al. and Masuda et. al. both found more BLNAR than β-lactamase producing strains in their *H. influenzae* isolates (Stratchounski 2001, Masuda 2002). Few studies have addressed risk of resistance among *H. influenzae* carriers. Peerbooms et. al. found no increased risk of carrying resistant strains in child care attendees when compared to levels in children receiving home care (Peerbooms 2002).

While the percentage of *H. influenzae* isolates resistant to β-lactams remains, in general, low, some authors have reported higher levels of resistance to trimethoprim alone or in combination with sulfamethoxazole (TMX). These levels range from 2.9% to 21% of all *H. influenzae* isolates (Sulikowska 2004, Sa-Leao 2008, Bakir 2002, Neto 2003, Stratchounski 2001), while a study in Turkish child care attendees reported a TMX resistance prevalence of 8.5% in Hib isolates (Bakir 2002).

Molecular Epidemiology of *H. influenzae*

While clonality of *H. influenzae* has been observed in child care settings (Fontanals 2000, Mcvernon 2004, Farjo 2004, Sa-Leao 2008), the types of strains in any given facility seem to be heterogeneous overall (St. Sauver 2000, Dabernat 2003, Sa-Leao 2008). For example, St. Sauver et. al. reported 56 distinct PFGE patterns within one

child care facility alone (St. Sauver 2000). Dabernat et. al. also reported a high degree of *H. influenzae* genomic heterogeneity (366 patterns from 663 isolates) in French attendees, leading the authors to conclude that clonal diffusion plays a minor role in *H. influenzae* diversity (Dabernat 2003).

The amount of heterogeneity in bacteria found in child care facilities may be inherent in the organisms' ecology, rather than a direct function of the child care environment, however. Since very few studies include a control group (i.e. children who did not attend group child care), it is difficult to conclude whether a high degree of clonal heterogeneity would occur in any population of children, regardless of child care status. Peerbooms et. al. reported evidence that *H. influenzae* in child care facilities might be more clonal than that in the general population. This article reports that among a group of children attending group care, 38% of *H. influenzae* strains were identical, while that value dropped to 4% in children who did not attend group care (Peerbooms 2002). Reports on molecular characteristics of bacteria in child care facilities are still valuable in determining microorganisms' behavior, regardless of whether that behavior is a result of child care attendance.

Moraxella catarrhalis

Prevalence, seasonal variation, and risk of carriage in child care attendees

Studies of *M. catarrhalis* in children attending child care facilities revealed carriage rates between 4.5% and 80% (Marchisio 2001, Peerbooms 2002, Masuda 2002, Henriques Normark 2003, Sulikowska 2004, Quinones 2005, Kontiokari 2005, Zemlickova 2006). Studies in Italy and Poland found no significant difference in *M. catarrhalis* carriage between spring and winter/fall seasons (Sulikowska 2004, Marchisio 2001).

Two studies have found conflicting results regarding child care attendance as a risk factor for carriage. Peerbooms et. al. found that, in the Netherlands, children attending child care facilities were significantly more likely to carry *M. catarrhalis* than children receiving home care (aOR = 4.55, p-value not given) (Peerbooms 2002). On the other hand, Sulikowska et. al found no statistically significant difference in *M. catarrhalis* carriage rates between children receiving out of home care and children receiving in-home care (36.9% vs. 11.1% in winter, 31.2% vs. 29.0% in spring, $p > 0.05$) (Sulikowska 2004).

Resistance in *M. catarrhalis*

In contrast to *H. influenzae*, resistance to β-lactam antibiotics in *M. catarrhalis* carried by child care attendees is high. Studies conducted since 2000 have reported β-lactamase production in 85.1 – 100% of *M. catarrhalis* isolates (Zemlickova 2006, Marchisio 2001, Henriques Normark 2003, Peerbooms 2002, Masuda 2002, Sulikowska 2004). A study of Cuban child care attendees found that 97.9% of isolates were intermediate or resistant to penicillin, but that all strains (even susceptible strains) produced β-lactamase (Quinones 2005). Despite these high levels of resistance, evidence does not suggest an increased risk of carrying a resistant *M. catarrhalis* strain in child care attendees compared to children receiving home care (Peerbooms 2002). There does not seem to be substantial seasonal variation in resistance levels in *M. catarrhalis* (Marchisio 2001). Also in contrast with *H. influenzae* findings, resistance to TMX seems to be slightly lower, with decreased susceptibility rates of 5.3% and 9.0% reported in the Czech Republic and Cuba, respectively (Zemlickova 2006, Quinones 2005).

Staphylococcus aureus

Prevalence, seasonal variation, and risk of carriage in child care attendees

The prevalence of *S. aureus* in child care attendees has been found to be between 10.1 and 31.1% (Velasquez 2009, Sa-Leao 2001, Masuda 2002, Zemlickova 2006, Lamaro-Cardoso 2009). Table 11 presents the results of studies published since 2000 that assessed child care attendance as a potential risk factor for colonization with *S. aureus*. Interestingly, out-of-home child care has rarely been shown to be a risk factor for *S. aureus* carriage in children, although the number of studies assessing this relationship is relatively small. Only one study reported a positive association between colonization and care outside the home. Cheng Immergluck et. al. reported that out-of-home care was positively associated with carriage of either *S. aureus* or *S. pneumoniae* (OR 2.30, 95% CI 1.17 – 3.51) (Cheng Immergluck 2004).

Antibiotic resistance in *S. aureus*

Prevalence of methicillin-resistant *S. aureus* (MRSA) carriage in children attending child care facilities remains low, and is reported to be between 0 and 7.7% (Sa-Leao 2001, Tavares 2010, Zanelli 2002, Masuda 2002, Hisata 2005, Zemlickova 2006, Oguzkaya-Artan 2008, Hewlett 2009, Lamaro-Cardoso 2009, Velasquez 2009). As a reference, the prevalence in the United States general population is approximately 1.5%. (Gorwitz 2008) A 10% prevalence of MRSA was reported in Denmark after 2 index cases were identified in the facility (Jensen 2006). Relatively few studies published since 2000 have reported on MRSA, so it is difficult to determine if the recent rise of MRSA in children in general is reflected in child care attendees (Kaplan 2005, Creech 2005, Gorwitz 2008, Lo 2010). Hewlett et. al. reported a 6.7% prevalence in children attending a hospital-based child care facility (Hewlett 2009). This finding might be due to the fact that the facility was associated with a healthcare system, although the majority of isolates

were community-associated strains (Hewlett 2009). On the other hand, studies published in Mexico and Portugal found low prevalence of MRSA (0.93% and 0.14%, respectively) in child care attendees (Velasquez 2009, Tavares 2010). These data could have underestimated the prevalence of MRSA due to the fact that nasopharyngeal swabs were collected instead of swabs of the anterior nares (Velasquez 2009, Tavares 2010).

Zanelli et. al. found a greater risk of multi-resistant *S. aureus* in children (from three nurseries and one primary school) and their guardians than in members of the general population (Zanelli 2002). The same study found a notably high rate of rifampin resistance in methicillin-susceptible *S. aureus*. Hisata et. al. found a high prevalence (28.2%) of methicillin-resistance coagulase negative *Staphylococcus* in Japanese children, suggesting these organisms could serve as reservoirs for the SCC*mec* elements that encode methicillin resistance(Hisata 2005).

Molecular epidemiology of *S. aureus*

Most molecular epidemiology data on *S. aureus* is provided in studies of MRSA. Several studies have shown similar or indistinguishable strains colonizing children attending the same facility, both in outbreak and non-outbreak situations, indicating horizontal spread (Sa-Leao 2001, Masuda 2002, Hisata 2005, Jensen 2006, Hewlett 2009, Lamaro-Cardoso 2009, Velasquez 2009). Hisata et. al. 2005 provided data showing that MRSA seemed to continually circulate among child care attendees, rather than consistently colonize any specific individual (Hisata 2005).

Child care facilities could serve as reservoirs for community-associated MRSA (CA-MRSA) (Jensen 2006, Hisata 2005, Hewlett 2009, Tavares 2010). Hewlett et. al. reported that 90% (18/21) of MRSA isolates were classified as community-associated, based on PFGE. Moreover, several studies have identified healthcare-associated MRSA (HA-MRSA) strains at child care facilities, with prevalence reported up to 59% of MRSA

isolated (Sa-Leao 2001, Hisata 2005, Hewlett 2009, Lamaro-Cardoso 2009, Velasquez 2009).

Studies Including Employees

The vast majority of published studies have focused on bacterial carriage in children. While a few studies collected data on child care employees, workers have rarely been the focus of study hypotheses. Table 12 summarizes studies that include data on child care employees published between January 2000 and September 2010. In general, bacterial carriage appears to be low in child care employees. To our knowledge, only one bacterial carriage study has been published in the past decade that included employees as a direct focus. Rosen et. al. found similar rates of colonization with S. pneumoniae, β-hemolytic *Streptococci, S. aureus*, and *M. catarrhalis* in employees and individuals who did not work at a child care facility (Rosen 2007).

Other Bacterial Pathogens

While publications reporting outbreaks in child care facilities are fairly common, very few studies of asymptomatic colonization with other pathogens have been published. Studies conducted in Finland and Brazil reported *S. pyogenes* carriage rates in children attending group care of 2%, 8%, and 24% (Kontiokari 2005, Vieira 2006). *Vieira* et. al. reported substantially higher rates of *S. pyogenes* carriage in children attending group child care than in children who did not attend group care (8% vs. 2%, $p = 0.02$ in one city, 24% vs. 16% in a second city, $p = 0.015$)(Vieira 2006).

After three children were reported to have invasive infections with *Kingella kingae* (a fastidious Gram-negative coccobacillus and colonizer of the oropharynx) at a Minnesota child care facility, it was reported that 13% of children tested at the facility carried the organism, with the highest prevalence occurring in the classroom of the index patients. Similar levels of carriage were reported at a facility not experiencing cases of invasive disease (Yagupsky 2004, Kiang 2005). *Matsuki* et. al. reported a 48.0%

Clostridium difficile (*C. difficile*) carriage rate in children enrolled in group child care in Japan. Upon molecular typing, clustering of strains within children and environmental surfaces was identified (Matsuki 2005). A study in Germany found no statistically significant association between attendance at a child care facility and carriage of antibiotic-resistant *Escherichia coli* (Lietzau 2007). Frequency of *Neisseria meningitidis* carriage in Mexican child care attendees was 1.9% (Espinosa de los Monteros 2009).

Summary of Findings

Prevalence and risk

Prevalence of *S. pneumoniae*, *S. aureus*, *H. influenzae*, and *M. catarrhalis* varies widely among child care attendees, and there is evidence that seasonal variation occurs. Attending a child care facility appears to increase the risk of *S. pneumoniae* and *H. influenzae*, but data are scarce regarding risk of carrying *M. catarrhalis* and *S. aureus*. Carriage in child care employees seems to be low, but this population is rarely the direct subject of study hypotheses and findings should be viewed with caution.

Antibiotic resistance

Antibiotic resistance levels among colonizing bacteria vary widely. Introduction of the pneumococcal vaccine has decreased prevalence of highly resistant vaccine-type *S. pneumoniae*, which appears to be accompanied by an increase in intermediate resistance or resistance in non-vaccine-type strains. Antibiotic resistance in *H. influenzae* can be due to β-lactamase production or through other mechanisms (these organisms are called BLNAR). β-lactamase has been shown to be the mechanism of antibiotic resistance in the majority of *H. influenzae* isolated from child care attendees, but this is not always the case. In contrast, resistance to β-lactam antibiotics is nearly universal in *M. catarrhalis*. Reported rates of methicillin-resistant *S. aureus* are currently low in child care attendees.

Pneumococcal vaccine conclusions

Studies conducted in multiple geographic areas have documented the fact that widespread introduction of the pneumococcal vaccine has altered the make-up of *S. pneumoniae* populations in both vaccinated and unvaccinated child care attendees. Prevalence of vaccine-type *S. pneumoniae* has decreased substantially, only to be replaced by non-vaccine serotypes, a process that can be explained by either strain replacement or strain unmasking (Lipsitch 1999, Lipsitch 2000). Strain replacement occurs when the ecological niche vacated by vaccine-type strains is subsequently inhabited by non-vaccine-type strains. Strain unmasking, on the other hand, occurs as a result of increased frequency of *detection* of non-vaccine-type strains that have been present all along (Lipsitch 2000). Lipsitch observes that strain replacement is a legitimate public health concern, while strain unmasking is an artifact of laboratory assay techniques (Lipsitch 2000). Frazao et. al. have reported data indicating strain replacement occurs when vaccine administration prevents an individual from acquiring new vaccine-type *S. pneumoniae*, while at the same time enhancing detection of non-vaccine-type strains (Frazao 2010). Auranen et. al. provide further support for the notion that "the…mechanism of competition works in acquisition rather than in clearance of carriage" (Auranen 2010).

Molecular epidemiology conclusions

Child care facilities can act as reservoirs for epidemic and internationally-recognized clones. Internationally-epidemic clones of *S. pneumoniae* have frequently been reported in child care facilities. Hospital-associated and community-associated strains of MRSA have also been reported in child care attendees to varying degrees

Populations of organisms found in child care facilities have molecular profiles that are unique to each facility. In addition to previously-recognized clones, child care attendees have also been found to harbor unique and genetically diverse strains of

bacteria. Such evidence suggests that not only do child care centers serve as amplifiers of existing organisms, but they might also contribute to the emergence of previously unrecognized strains. Moreover, there is evidence that these two roles work in tandem to contribute to the spread of virulence factors within and outside child care facilities. Hisata et. al. reported a 28.2% prevalence of methicillin-resistant coagulase-negative *Staphylococcus* (MRC-NS) in child care attendees and suggests that the MRC-NS strains "serve as a reservoir for the SCCmec element carried by MRSA strains disseminated in the community" (Hisata 2005).

Discussion: Limitations and Gaps in Current Knowledge

Limitations in knowledge of bacterial carriage in the child care setting can be classified according to weaknesses in available data and gaps in knowledge. Data that are currently available can be lacking in terms of study design and generalizability, and there also exist gaps in published literature regarding relevant topics.

Prevalence data collected from only child care facilities have limited utility in terms of assessing risk of colonization associated with out-of-home care. The vast majority of the studies published in the past decade include no control group. This is understandable, given that including a control group of children would mean recruiting and sampling from children who receive in-home care, an endeavor that would be costly and time consuming. Numerous studies assessed the risk of child care attendance on probability of carriage among other populations of children (those attending well-child clinics, for example) (see Tables 9 - 11), providing an efficient method of recruiting participants. These findings must also be viewed with caution, however, due to the fact that data available on potential confounders varied widely across studies. Including a

comparison group of participants receiving in-home care (as in Peerbooms 2002 and Sulikowska 2004) is a preferable method of assessing risk of carriage above and beyond that of the general pediatric population.

Conclusions reached from data on child care employees may not be generalizable. Carriage of bacterial pathogens in child care employees generally seems to be low (see Table 12). However, colonization data on child care providers is conspicuously limited in the studies included in this review, likely due to power calculations being based on children (i.e. not enough providers participated, given the limited number of facilities that were sampled in most studies). The total study population presented in Table 12 is comprised of only 426 individuals from 10 studies. These measures might be lacking in terms of external validity, considering there are over 1.3 million child care providers in the United States alone (Bureau of Labor 2007). The number of facilities studied is also fairly small (32 total, 16 of which were included in one study), which might also affect external validity.

Child care employees are worthy of further study for two reasons. First, occupational exposure to pathogens could put child care workers at increased risk of infections, as is evident with some viruses (Adler 1989, Pass 1990, Murph 1991, Jackson 1996, Gilbert 2005). Second, employees could play an important role in the transmission of infectious agents in the child care setting and also in the community as a whole.

Bacterial strains in child care employees that are related or indistinguishable from those found in children and the environment have been reported (Jensen 2006, Hewlett 2009, Sesli Cetin 2010, Hewlett 2010). Dabernat et. al. suggest that some of the genomic diversity of *H. influenzae* in child care attendees could be attributed to “exchanges with

adult subjects" (Dabernat 2003). Including employees in research could be necessary to further understand transmission dynamics of bacterial carriage in child care settings.

There are also little or no recent data on several topics of interest, including contamination of environmental surfaces, asymptomatic carriage of gastrointestinal bacteria, and carriage of MRSA. Fomites play an important role in the transmission of many agents (Rusin 2002, Boone 2007, Boyce 2007). Fingerprint patterns of *S. aureus* isolated from a child care facility in Texas showed indistinguishable clones from children, employees, and surfaces, illustrating the potential for inanimate objects to contribute to transmission (Hewlett 2010). It is important to gather more information about number and types of surfaces that are contaminated in child care facilities, otherwise efforts to eliminate a pathogen from a facility via decolonization of children and workers might be futile. Lee et. al sampled surfaces and toys in four rooms at a child care facility and characterized a wide diversity of bacteria, including several that are pathogenic to humans (Lee 2007). More data on which environmental surfaces are most likely to be contaminated would be helpful to child care providers. Given the little spare time child care workers have along with the myriad opportunities for surfaces to become contaminated, employees could then target problem surfaces with hygiene measures to maximize benefits of cleaning efforts.

While the association between bacteria causing diarrheal illness and out-of-home child care was studied in the 1980s and 1990s, data collected since 2000 are sparse, especially in non-outbreak settings. It would be prudent to continue research regarding these pathogens, particularly in light of the rise of antibiotic resistance in enteric pathogens (Houndt 2000). The finding that nearly 50% of Japanese children under age

five attending group day care carried *C. difficile* is particularly relevant, as *C. difficile* spores have been shown to survive on environmental surfaces and are resistant to traditional disinfection agents (Matsuki 2005, Riggs 2007, Fawley 2007).

The recent increase in rates of MRSA carriage and infections in children raises the question of how this will affect *S. aureus* presence and its behavior in child care facilities (Kaplan 2005, Creech 2005, Gorwitz 2008, Lo 2010). In 2009, Hewlett et. al. report a 6.7% prevalence of MRSA carriage in children in a Texas child care facility (Hewlett 2009). This rate is higher than that reported in two large studies of children conducted elsewhere in the United States (Fritz et. al reported a mean MRSA carriage rate of 2.6%, while Lee et. al. reported a rate of 0.9%) (Fritz 2008, Lee 2009). While the data on child care attendance as a risk factor for MRSA carriage are sparse, the majority of these studies show either no significant association or a protective effect (Table 11). This could be due to the fact that an inverse relationship between carriage of *S. aureus* and *S. pneumoniae* has been documented in this age group (Bogaert 2004, McNally 2006, Regev-Yochay 2004). There is evidence that risk of *S. aureus* carriage is lower in individuals colonized with *S. pneumoniae* and vice versa, although the relationship may be limited to vaccine-type strains of *S. pneumoniae* (McNally 2006, Bogaert 2004, Regev-Yochay 2004).

Conclusions

The amount of data that has been published since 2000 regarding bacterial carriage in the child care setting is extensive and has provided valuable insight into the prevalence and risk of bacterial carriage among individuals at child care facilities, the behavior of bacteria in this setting, and limitations and gaps in

knowledge. Regardless of the effects group care has on risk of acquiring a pathogen, child care facilities, their attendees, and staff are still valuable sources of data in terms of surveillance and behavior of bacterial pathogens. Moreover, as the number of children enrolled in out-of-home care continues to expand, results of studies conducted at child care facilities will become more and more applicable to the general pediatric population.

Table 9. Studies published since January 2000 assessing child care attendance as a potential risk factor for *S. pneumoniae* colonization in children

Authors	Year	Location	Sample	Risk Factor	Findings	p-value
Huang et. al.	2009	Massachusetts (U.S.)	2638 children 3 months to 7 years at primary care practices in multiple Massachusetts communities	Group child care	OR = 2.2 (95% CI 1.6 – 3.2) OR = 2.3 (95% CI 1.4 – 3.9)[a]	≤ 0.05 ≤ 0.05
Dunais et. al.	2008	Guatemala	951 children aged 5 – 60 months recruited from public and private outpatient clinics and child care facilities	Private pre-school Public daycare center	OR = 2.6 (95% CI 1.7 – 4.0) OR = 2.4 (95% CI 1.3 – 4.4)[a] OR = 5.4 (95% CI 3.3 – 8.9) OR = 3.1 (95% CI 1.6 – 6.0)[a]	< 0.001 < 0.004 < 0.001 0.001
Kellner et. al.	2008	Canada	3398 children ages 1 – 6 years recruited from routine vaccination clinics	Child care attendance	OR = 2.27 (95% CI 1.89 – 2.75)	< 0.005
Sleeman et. al.	2005	United Kingdom	213 infants assessed prospectively over 24 weeks	Out of home care	HR = 1.2 (95% CI 0.6 – 2.2)[b]	0.6
Jones et. al.	2005	Louisville (U.S.)	106 children presenting to first health maintenance visit	Child care attendance	OR = 2.10 (95% CI 1.54 – 2.87)[c]	<0.0001
Nilsson and Laurell	2005	Sweden	Data collected from official statistics representing a city of 290,000 people	Proportion of children less than 6 years of age who attended child care centers	Proportion of children attending child care centers was not correlated with PNSP cases per 1000 children under age 6[a]	Not given
Sulikowska et. al.	2004	Poland	226 children between 6 months and five years recruited from three different social groups	Attendance at a child care facility	Carriage rates were significantly higher in the child care facility than in children receiving home care	p<0.05
Bogaert et. al.	2004	Netherlands	3198 healthy children ages 1 – 19 years participating in a national meningococcal vaccine campaign	Child care attendance	OR = 2.14 (95% CI 1.44 – 3.18)	Not given
Moore et. al.	2004	Alaska (United	1275 children ages 3 – 59 months presenting to	Child care attendance	OR = 2.1 (95% CI 1.6 – 2.7)	Not given

Table 9 – continued

		States)	clinics over a three year period			
Cheng Immergluck et. al.	2004	Chicago (United States)	2914 healthy children under the age of five seeking well-child care at a pediatric clinic	Care outside the home	OR = 2.56 (95% CI 1.08 – 6.07)	Not given
Ghaffar et. al.	2004	Texas (United States)	Infants 2 – 18 months presenting to receive primary immunizations	Child care attendance	No significant differences in carriage among child care attendees over three time periods	>0.999, 0.760, 0.548
Huang et. al.	2004	Massachusetts (United States)	710 children under age 7 attending clinic visits in 16 communities	Attending child care ≥ 4 hours per week	OR = 2.9 (95% CI 1.8 – 4.6)[d] OR = 2.7 (95% CI 1.3 – 5.5)[e] OR = 4.5 (95% CI 2.1 – 9.5)[f] OR = 8.8 (95% CI 1.6 – 47.3)[g]	Not given
Regev-Yochay et. al.	2004	Israel	790 children 40 months or younger seen at primary care clinic	Child care attendance	OR = 2.32 (95% CI 1.73 – 3.11)	< 0.001
Neto et. al.	2003	Portugal	466 children ≤ 12 years old presenting to a pediatric emergency department	Child care attendance	Attending child care was risk factor for carrying a respiratory pathogen	p<0.05
Regev-Yochay et. al.	2003	Israel	429 children younger than 6 years presenting to 15 pediatric clinics	Child care attendance	OR = 4.7 (95% CI 2.5 – 8.6) OR = 3.8 (95% CI 1.9 – 7.5)[a]	Not given p<0.0001
Finkelstein et. al.	2003	Massachusetts (United States)	766 children younger than age 7 presenting for clinic visits in 16 Massachusetts communities	Childcare for ≥ 4 hours/week	OR = 2.3 (95% CI 1.6 – 3.4) OR = 3.9 (95% CI 2.3 – 6.5)[a]	p < 0.001 p< 0.001
Peerbooms et. al.	2002	Netherlands	259 children ages 3 – 36 months attending child care 276 children ages 3 – 36 months receiving no form of group child care	Child care at least 3 days per week	aOR = 2.51 (95% CI Not given)[h]	Not given
Rey et. al.	2002	Brazil	482 children ages 2 – 59 months presenting to a hospital with pneumonia	Child care attendance	Carriage rate was higher in healthy children attending the child care facility (72%) than in children with pneumonia (50%)	p<0.001

Table 9 - continued

			215 children ages 2 – 59 months attending a public child care facility			
Ciftci et. al.	2001	Turkey	300 children aged 2 months – 12 years presenting to a pediatric unit	Child care attendance	OR = 2.82 (95% CI 1.40 – 5.68) OR = 2.29 (95% CI 0.58 – 9.10)[a]	p = 0.003
Samore et. al.	2001	Utah (United States)	737 children ≤ 8 years recruited from 2 rural communities	Child care exposure	OR = 2.4 (95% CI 1.6 – 5.0) OR = 0.5 (95% CI 0.1 – 2.1)[a]	p = 0.019 Not given
Li et al.	2001	Beijing (China)	100 cases of S. pneumoniae ages 0 – 5 years presenting to a children's hospital	Child care attendance	RR = 3.2 (95% CI 0.9 – 11.1)[a]	Not given

[a] Outcome of interest was *S. pneumoniae* with reduced or no susceptibility to penicillin

[b] Outcome of interest was time to *S. pneumoniae* acquisition

[c] Outcome of interest was presence of acute otitis media pathogens (including *S. pneumoniae*)

[d] Risk of pneumococcal carriage among children living in communities where median household income ≥ $35,000

[e] Risk of pneumococcal carriage among children living in communities where median household income < $35,000

[f] Risk of penicillin-nonsusceptible *S. pneumoniae* among children living in communities where less than 40% of adults have less than a high school education

[g] Risk of penicillin-nonsusceptible *S. pneumoniae* among children living in communities where less than 40% of adults have less than a high school education

[h] Odds ratio adjusted for age, family size, and history of asthma or chronic bronchitis

Table 10. Studies published since January 2000 assessing child care attendance as a potential risk factor for *H. influenzae* colonization in children

Authors	Year	Location	Sample	Risk Factor	Findings	p-value
Sulikowska et. al.	2004	Poland	60 children between 6 months and 5 years attending one child care facility; 58 children between 6 months and 5 years brought up at home	Attended child care (study included group receiving at-home care)	23.9% carriage in child care facilities vs. 11.1% in children receiving at-home care (winter) 50.0% carriage in child care facilities vs. 3.2% in children receiving at-home care (spring)	p<0.05
Neto et. al.	2003	Portugal	466 children ages 4 – 35 months recruited from emergency room visits	Parent reported child care attendance		p = 0.001[b]
Bakir et. al.	2002	Turkey	1404 healthy infants and children ages 10 days – 10 years from well-child clinic visits and elementary schools	Attendance at child care	aOR = 7.08 (95% CI 3.25 – 15.46)[ac]	p<0.001
Peerbooms et. al.	2002	Netherlands	259 children attending child care facilities; 276 children receiving at-home care (ages 3 – 36 months)	Attended group care ≥ 3 days/week	aOR = 4.30[d]	(Not given)
Akcakaya et. al.	2001	Turkey	190 kids recruited from a hospital-based child care center and 75 kids recruited from a center not affiliated with a hospital (ages 36 – 71 months)	Carriage rates over time among children who attended child care ≥ 40 hours/week	Prevalence rose significantly from 4% at initial screening to 11% at the second screening to 40.7% at the third screening. Hib carriage was affected by duration of child care attendance	Initial → second screening p = 0.007 Second → third screening p < 0.05 Hib carriage p<0.05

[a] Outcome of interest was *H. influenzae* type b

[b] Outcome of interest was carriage of respiratory pathogens, most of which were *H. influenzae*

Table 11. Studies published between since January 2000 assessing child care attendance as a potential risk factor for *S. aureus* colonization in children

Authors	Year	Location	Sample	Risk Factor	Findings	p-value
Regev-Yochay et. al.	2009	Israel	4,648 children 0 to 40 months who visited primary care clinics	DCC attendance	OR = 0.55 (95% CI 0.40 – 0.77)	<0.0001
Lee et. al.	2009	Eight Massachusetts (United States) communities	1,968 children 3 months to < 7 years[a] seen for well child or sick visits in primary care offices	Childcare or school for ≥ 4 hours	OR = 1.24 (95% CI 0.95 – 1.60)	0.11
Fritz	2008	St. Louis (United States)	1,300 participants from birth to 18 years[a]	Attends child care	MRSA vs No MRSA: OR = 0.10 (95% CI 0.02 – 0.59) MSSA vs. No MSSA: Not significant MRSA vs. MSSA: OR = 0.20 (95% CI 0.06 – 0.70)[b]	Not given
Soysal et. al.	2006	Turkey	1,000 children 0 to 16 years[a] who attended a pediatric outpatient clinic	Childcare attendance during last 6 months	OR = 1.10 (95% CI 0.7 – 1.8)	0.5
Regev-Yochay et. al.	2004	Israel	790 children age 5 days to 40 months who visited primary care clinics	Childcare attendance[c] Carrying *S. pneumoniae* while attending child care[d]	OR = 0.39 (95% CI 0.24 – 0.65) OR = 0.27 (95% CI 0.10 – 0.72)	<0.001 0.009
Cheng Immergluck et. al.	2004	Chicago (United States)	291 children less than 5 years who sought routine well-child care at a pediatric clinic	Child care or nursery attendance	OR = 2.30 (95% CI 1.17 – 3.51)[e]	.007

Table 11 - continued

[a] Child care attendance was evaluated as a risk factor only in the appropriate age groups

[b] Child care attendance was excluded as a risk factor during multivariate analysis

[c] Univariate analysis

[d] Multivariate analysis

[e] Outcome of interest was colonization with either *S. aureus* or *S. pneumoniae*

Table 12. Studies published since January 2000 providing data on colonization in child care workers.

Authors	Year Published	Pathogen	Location/Number of Facilities Sampled	Sample Size	Results
Sesli Cetin et. al.	2010	*S. aureus*	Turkey/1	19	31.6% carriage rate in staff
Hewlett et. al.	2010	*S. aureus* (Methicillin-susceptible)	Texas (United States)/1	32	28.1% carriage rate in staff
Hewlett *et. al.*	2009	MRSA	Texas (United States)/1	32	3.1% carriage rate in staff
Leino et. al.	2008	*S. pneumoniae*	Finland/1	37	2.9% frequency of carriage (over 9 months)
Rosen *et. al.*	2007	*M. catarrhalis*	Texas (United States)/6	63	3.2% of workers were colonized
Rosen *et. al.*	2007	*S. aureus*	Texas (United States)/6	63	14.3% of workers were colonized
Rosen *et. al.*	2007	β-hemolytic *Streptococci*	Texas (United States)/6	63	19.1% of workers were colonized
Rosen *et. al.*	2007	*S. pneumoniae*	Texas (United States)/6	63	4.8% of workers were colonized
Jensen *et. al.*	2006	MRSA	Denmark/2	60	1.7% carriage rate frequency of carriage over 26 months*
Kiang *et. al.*	2005	*K. kingae*	Minnesota (United States)/2	28	No staff was colonized*
McVernon *et. al.*	2004	*H. influenzae* type b	England/1	21	Carriage in staff was 4.8%*
Henriques Normark *et. al.*	2003	*M. catarrhalis*	Sweden/16	123	Carriage was 0.8% in child care employees
Henriques Normark *et. al.*	2003	*H. influenzae*	Sweden/16	123	Carriage was 0.8% in child care employees
Henriques Normark *et. al.*	2003	*S. pneumoniae*	Sweden/16	123	Carriage was 1.6% in child care employees*
Gomez-Barreto et. al.	2002	*S. pneumoniae*	Mexico/1	20	35% of adults acquired pneumococci over two year period
da Silva and Marin	2001	*H. influenzae*	Brazil/1	23	Carriage was 4.4% in child care employees

*Studies conducted after an index case was reported.

CHAPTER III

RISK AND TRANSMISSION OF *STAPHYLOCOCCUS AUREUS* IN IOWA CHILD CARE EMPLOYEES

Introduction

Staphylococcus aureus (*S. aureus*) are ubiquitous bacteria that can cause severe disease in children and adults. One method of transmission of this organism is via "carriers" (individuals who are asymptomatically colonized with an organism). Although colonization itself does no harm to the host, it is a known risk factor for developing and transmitting clinically relevant infections (Wertheim 2005).

Increased risk of *S. aureus* carriage and infection with methicillin-resistant *S. aureus* (MRSA) is associated with many occupations, including healthcare workers, military personnel, agricultural workers, and veterinary practitioners (Johnston 2007, Lucas 2007, Smith 2009, Wulf 2008, Aiello 2006). Despite the fact that child care employees are at risk of infections and outbreaks of MRSA in child care centers have been reported, data on *S. aureus* carriage in this occupational group is limited (Adler 1989, Pass 1990, Murph 1991, Jackson 1996, Gilbert 2005, Adcock 1998, Shahin 1999, Jensen 2006). In studies published since January 2000, carriage of all *S. aureus* was reported to be between 14.3% and 31.6% in child care workers (Rosen 2007, Hewlett 2010, Sesli Cetin 2010), while MRSA was found in 3.1% of staff working at a child care facility in Texas (Hewlett 2009). To our knowledge, only one study has provided data on the risk of *S. aureus* carriage in child care workers compared to the general population. Rosen et. al. reported that the *S. aureus* carriage rate in child care employees was 14.29%, while the rate in adults not employed at a child care facility was 10.77% (Rosen 2007). However, this study was relatively small and did not provide data on potential confounders (Rosen 2007).

child care employees, we sought to determine if child care employees are at increased risk of *S. aureus* colonization when compared to individuals not employed in a child care center; identify sources of potential transmission in the child care setting (i.e. children and inanimate objects) using molecular characterization; and identify predictors of *S. aureus* carriage in child care employees.

Materials and Methods

Selection of Child Care Centers

We conducted a cross-sectional study of *S. aureus* in child care facilities located in Iowa. All protocols were approved by the University of Iowa Institutional Review Board. Child care facilities were identified from the Johnson County list of registered and licensed facilities, as well as through personal connections (Iowa Department of 2008). Contact letters were sent out to all facilities on this list. Follow-up calls were conducted, with priority given to larger licensed facilities.

Recruitment of Participants

Adult participants were at least 18 years old (or able to obtain parental consent) and children sampled were older than 6 months. After the director gave permission for the study team to recruit at the center, employees and parents of children at a facility were recruited with fliers one week prior to the site visit. Unexposed adults who were not employed in child care facilities were matched on age and gender frequencies, and were recruited through mass e-mails sent to the University of Iowa College of Public Health and undergraduate populations.

Swabbing of Participants and Surfaces

All samples were collected using cotton-tipped transport swabs (BD BBL Culture Swabs with Stuart Liquid Media, Becton, Dickinson and Company, Sparks, MD and Remel) as previously described (Smith 2009). Nasal and throat swabs were collected

from adults, while only nasal swabs were collected from children in order to minimize discomfort. Environmental samples were collected throughout the facility, concentrating on areas which are frequently touched (doorknobs, popular toys, etc.). After immersing the swab in sterile phosphate-buffered saline, samples were obtained by rotating a sterile cotton swab in 3 directions across the surface. All samples were stored in liquid Stuart's medium following collection, kept at 4ºC during transport, and processed within 24 hours of collection.

S. aureus Isolation and Confirmation

S. aureus and MRSA were isolated and confirmed as previously described (Smith 2009). Briefly, swabs were inoculated into *S. aureus* enrichment broth and subsequently plated onto Columbia colistin-nalidixic agar (CNA) with 5% sheep blood (Columbia CNA, Remel, Lenexa, KS) and selective MRSA agar plates (BBL CHROMagar MRSA, Becton, Dickinson and Company, Sparks, MD) for identification. S. aureus isolates were confirmed using Gram stain, the catalase test, the slide coagulase test and a S. aureus latex agglutination assay (Pastorex Staph-plus, Bio-Rad, France). Methicillin resistance was identified with an MRSA latex agglutination test (Oxoid Ltd., Hants, UK) and confirmed using a *mec*A PCR (Oberdorfer 2006).

Staphylococcal Protein A (*spa*) Typing

Sequencing and assignation of *Staphylococcal* protein A (*spa*) type was performed as described elsewhere (Harmsen 2003). Briefly, polymerase chain reaction (PCR) was performed to amplify the polymorphic X region of the *S. aureus spa* gene. PCR products were sequenced at the University of Iowa DNA Facility using an Applied Biosystems Model 3730 DNA sequencer. *Spa* types were assigned and compared using RidomStaphType software (Ridom GmbH).

Pulsed-Field Gel Electrophoresis

Isolates of *S. aureus* were examined using pulsed-field gel electrophoresis (PFGE). PFGE was employed using the *SmaI* restriction enzyme as previously described (Mulvey 2001). Isolate patterns were entered into a database and data from these isolates were compared using the BioNumerics program (BioNumerics, Applied Maths, Kortrijk, Belgium). PFGE data were also compared to isolates representing common community-associated and hospital-associated MRSA strains circulating in Iowa. Banding patterns were interpreted according to standard definitions (Tenover 1995).

Antibiotic Susceptibility Testing

All *S. aureus* isolates were tested at the University of Iowa Hospitals and Clinics for antimicrobial susceptibility by the broth dilution method described by the Clinical and Laboratory Standards Institute (Clinical and Laboratory 2006). Isolates were tested for susceptibility to oxacillin, tetracycline, erythromycin, clindamycin, linezolid, levofloxacin, vancomycin, trimethoprim/sulfamethoxazole (TMX), daptomycin, and quinupristin/dalfopristin.

Statistical Analysis

Statistical analysis was performed using SAS 9.2 (SAS Institute Inc., Cary, NC). Prevalence of all S. aureus and MRSA in employees, children, and unexposed adults was calculated. For data collected through questionnaires, frequencies were calculated for categorical variables, as well means, standard deviations, minimums, and maximums for continuous variables. Univariate and multivariate analyses were conducted on data from 1) all adults to determine risk of carriage in employees compared to an unexposed population; 2) children to identify variables that would be used as potential predictors for employees; and 3) child care employees to determine if variables associated with their work predict carriage.

Univariate analysis was performed separately using carriage of any *S. aureus* and MRSA as outcomes. Chi-square or Fisher's exact test were used to identify potential predictors for the multivariate analysis. In order to maintain a parsimonious model, only variables that were significantly associated at $p < 0.15$ with both the exposure and outcome were considered for multivariate modeling. Multivariate analysis was performed with unconditional logistic regression due to the fact that age and gender matching was not one-to-one. Models were created using generalized estimating equations to adjust for clustering among facilities. Due to small cell counts, exact logistic regression was used for multivariate modeling of MRSA carriage in employees. Model selection was conducted using manual backwards elimination.

Results

Center Characteristics

Letters explaining the study were sent to approximately 280 licensed and registered facilities in Johnson County listed on the Iowa Department of Health and Human Services website. Study personnel were able to speak with 25 directors, 13 of whom agreed that their facility would participate. Data and samples from one center were discarded due to fungal overgrowth of cultures, and one center did not have to participate because our designated sample size was achieved. This study contains data from 11 facilities that were visited between February 2009 and February 2010. Reported capacities for children ranged from 16 to 168 (mean capacity 53 children, median capacity 33 children), while capacities for employees ranged from three to 60 employees (mean capacity 21 employees, median 18 employees).

Employee Characteristics

One hundred ten employees participated in this study. Employee participation rates at each center ranged from 35.0% to 100.0%, and the average participation rate was

59.3%. Reasons for not participating when approached included: the participant wasn't interested or was afraid of discomfort. The average age of employee participants was 29.7 (median age 24 years, range 16 to 64 years). Females made up 92.6% of the employee population. Overall carriage of *S. aureus* in employees was 35.2%. The carriage rate per facility ranged from 11.8% to 66.7%. Overall carriage of MRSA in employees was 3.70% (range from 0 to 16.7%) (Table 13).

Child Characteristics

Eighty-one children participated in this study (mean child participation rate 22.8%, range 5.0% to 68.8%). Reasons given by parents for non-participation included: did not want to put their child through discomfort, did not have time, and weren't interested. The average age of child participants was 2.97 years (median age three years, range six months to seven years), and females accounted for 57.9% of the child participants. Prevalence of *S. aureus* in children was 19.8%, while 1.23% (one child) carried MRSA. Prevalence in children at each facility ranged from zero to 28.6% for all *S. aureus* (although the only child who participated at one facility was colonized, yielding a carriage rate of 100%) (Table 13).

Unexposed Adult Characteristics

One hundred eleven adults not employed at a child care facility participated in this study. The average age of unexposed adult participants was 31.8 years (median age 27 years, range 18 to 78 years). Females made up 88.3% of the unexposed population. The prevalence of all *S. aureus* in unexposed adults was 33.6%, while MRSA was carried by 0.90% of unexposed adults.

Surface Characteristics

A mean of 20 surfaces was sampled at each facility, and depended on center size (range 10 to 44). A total of 214 surface samples were obtained. Overall, 19/214 (9.8%,

range 3.4% to 30%) of the surface swabs were positive for *S. aureus*, and 0.90% (2/214) (range 0% to 3.7%) were positive for MRSA (Table 13). Methicillin-susceptible *S. aureus* (MSSA) was found on child-size chairs, a high chair, doors, an activity table, restroom sink handles, small toys, "activity centers" for infants and children (e.g. an infant play gym and child-size tool bench), and the floor of an infant room. Two MRSA isolates were identified in different centers, one from an infant high chair and the other from a plastic "learning walker" in an infant room.

Analysis of Exposed and Unexposed Adults

The crude odds ratio (OR) for child care employment as a risk for any *S. aureus* colonization was 1.09 (95% confidence interval 0.62 – 1.90, p-value 0.77), while the crude OR for MRSA colonization was 4.23 (95% CI 0.47 – 38.48, p-value 0.21). Questionnaire variables for all adults are presented in Table 14. After adjusting for age, a household contact who developed an influenza-like illness in the past 12 months, and a household contact with exposure to cattle, the OR for *S. aureus* carriage in child care employees was 0.68 (95% CI 0.31 – 1.50, p-value 0.34) (Table 15). The odds of MRSA carriage was 3.28 times higher in child care employees than unexposed adults after adjusting for a history of cigarette smoking (95% CI 0.31 – 169.02, p-value 0.53) (Table 16).

Analysis of Children

Questionnaire variables for child participants are presented in Table 17. Variables that predicted *S. aureus* carriage in children at $p < 0.05$ included asthma, history of an acute asthma attack, the number of times the parent exercised at a gym in the past month, and recent contact with animals, goats, and sheep. The proportion of child participants reporting these variables in each facility was considered as a potential predictor in the multivariate analysis of risk factors in employees.

Analysis of Child Care Employees

Potential risk factors for carriage of *S. aureus* that were statistically significant during univariate analysis at p-values < 0.15 were considered for multivariate analysis. None of the individual level characteristics of employees (e.g. age of children supervised or type of duties performed) was a significant predictor of *S. aureus* carriage (Table 18). Due to the high number of facility-level variables (such as years the center had been in operation and hand washing practices as reported by the director) that were statistically significant during univariate analysis (Table 19), final variables for entering the multivariate model were chosen according to a lowered p-value of 0.05, biologic plausibility, and presence of co-linearity with another variable (see Appendix for selection methods). The variables "center separates children at least once during the day", "children wash hands upon arrival at the center", and "child participants reporting contact with animals" were chosen for multivariate consideration (Table 20).

Antibiotic Resistance Profiles

Antibiotic resistance rates among *S. aureus* carriers are presented in Table 21. All isolates were classified as either susceptible or resistant to the antibiotics tested, with the exception of one surface isolate displaying intermediate-level resistance to erythromycin. Child care employees were significantly more likely to carry *S. aureus* that was resistant to erythromycin than unexposed adults (OR 3.67, 95% CI 1.06 – 12.68, p-value 0.033). Among employees only, presence of another employee carrying erythromycin-resistant *S. aureus* (OR 5.10, 95% CI 1.11 – 23.27, p-value 0.029) was predictive of carrying erythromycin-resistant *S. aureus* (Tables 22 and 23). Further multivariate analysis with adjustment for clustering is warranted. Resistance to linezolid, vancomycin, daptomycin, and quinupristin/dalfopristin was not observed.

Molecular Characteristics

The distribution of *spa* types among *S. aureus* and MRSA isolates is presented in Table 24. The most frequently occurring *spa* types in employees were t189 (found in five employees); t012, t008, and t216 (found in 3 employees each); and t084 and t002 (found in two employees each). We identified 25 *spa* types among the 38 employees who were colonized with *S. aureus*. In children, t012 was found most often (in four children), followed by t338 and t3214 (each found in two children). The most frequently occurring *spa* types on environmental surfaces were t002, t012, and t164, which were found on four, three, and two surfaces, respectively. The most frequently carried spa type among unexposed adults was t334 (carried by three individuals), followed by t012, t085, t189, t216, and t338, which were each carried by two individuals. In this group, 28 spa types were represented among isolates from 35 adults. Pulsed field gel electrophoresis confirmed that, although there is evidence of transmission among employees, children, and environmental surfaces within individual facilities, in general the *S. aureus* populations found in child care centers are genetically heterogeneous (Figures 2 – 14).

Discussion

We did not find a significant increased risk of overall *S. aureus* colonization in child care employees. The finding that carriage of erythromycin-resistant *S. aureus* was significantly higher in employees than unexposed adults, the possibility of antibiotic resistance should be carefully considered when treating infections in this occupational group, although more in depth analysis of this issue is warranted. Washing children's hands upon arrival at child care facilities may decrease the risk of *S. aureus* carriage in employees.

The results of our study are remarkably similar to findings of a recent study conducted at one child care facility in Texas (Table 25), with the exception of MRSA rates in children. The lower rate of MRSA in children participating in our study could be

due to several reasons. Hewlett et. al. swabbed several body parts, which would increase the sensitivity of their isolation. This is not likely the case, however, because the rates of MSSA were similar between the two studies. Several of the isolates from the Texas child care facility were related or indistinguishable, so clustering among employees, children, and surfaces may have an effect (Hewlett 2009, Hewlett 2010). Regardless of the reason for the discrepancy, the numbers of MRSA isolates in both studies are small, limiting the validity of any conclusions made on these data alone.

We found evidence of transmission among employees, children, and environmental surfaces in several facilities, reflecting findings published in the Texas study. Because this was a cross-sectional study, we were not able to determine the direction of that transmission. The concept of unidirectional transmission, however, is most likely an oversimplification of actual events. It is unlikely that *S. aureus* passes solely from children to workers, from workers to the environment, etc. In reality, transmission is probably multidirectional and can only be studied under a much more comprehensive and costly longitudinal study design.

In addition to being cross-sectional, this study had several other important limitations. Participation rates for employees and children were low. Due to the fact that we visited a center at most twice, we were unable to access all different shifts of employees, which may have contributed to the low rate. Participation rates in children were likewise low, even when the majority of children were present at the center at the time of sampling. Child care facility recruitment visits were conducted during the 2009 H1N1 outbreak and parents may have mistakenly assumed a more invasive sampling procedure was required, contributing to the low participation rate. However, the primary reason for including children in this study was to evaluate transmission within a center. With the exception of the possibility of missing matching isolates, the low participation rates among children should not influence the main conclusions of this study.

Nevertheless, the prevalence *S. aureus* in children and on environmental surfaces in this study should be viewed with caution. The low participation rate in children limits the internal and external validity of those estimates. The purpose of the environmental swabs (as with the children) was to establish evidence of transmission within a facility; our goal was to find *S. aureus* if it was present in a facility. Because we chose environmental surfaces that we considered likely to be contaminated, the prevalence of contamination could overestimate the true frequency of *S. aureus* on fomites. Alternatively, since samples were collected during the H1N1 outbreak, facilities may have increased hygiene practices, leading to underestimates of the presence of *S. aureus* during non-outbreak situations.

The external validity of our results might also be affected by the type of facilities we visited. The majority of facilities participating in this study were licensed facilities. In Iowa, licensed facilities are larger and held to more stringent standards than registered and nonregistered facilities (Iowa Department of 2009). Since we only visited one registered facility and did not have contact information for nonregistered facilities, our results may not be generalizable to smaller or in-home centers. However, we suspect that due to little oversight of sanitation practices and the presence of pets, employees at non-licensed facilities may be at greater risk than the participants of this study.

We did not identify any individual-level predictors of carriage within the child care employee population. Children washing their hands upon arrival at the facility, on the other hand, predicted a decreased risk of *S. aureus* carriage in employees. Given the apparent highly significant protective effect of this variable on risk of carriage in employees, it may be prudent to recommend this practice as standard protocol in child care facilities.

The results of analyses of facility-level risk factors in employees should be viewed with caution. Because many variables were related to the facility and applicable to all employees at that facility (for example, the number of years the center had been in

operation), the number of variables displaying collinear relationships was high. While every effort was made to narrow variables down to those that made biological sense (see Appendix), it is possible that the effects of another variable could be masking the true relationship between hand washing upon arrival and carriage in employees. The potential decrease in risk of *S. aureus* carriage in child care employees as a result of hand washing upon arrival would best be studied using a controlled intervention trial. However, recommending hand washing upon arrival is unlikely to have adverse effects, even if the relationship has not been accurately characterized.

The number of MRSA isolates in this study is small. While the finding that MRSA carriage was higher in employees was statistically significant, it is certainly possible that the small number limited analysis of potential confounders. Further research with larger sample sizes is needed to accurately characterize the risk of MRSA in child care employees.

Conclusion

Data on risk of resistance in *S. aureus* in relation to child care attendance are currently limited in both children and employees. There is some evidence that child care attendees are at increased risk of carrying antibiotic resistant *S. pneumoniae* when compared to children receiving at-home care (Huang 2009, Dunais 2008, Regev-Yochay 2003, Finkelstein 2003). The finding that child care employees may be at increased risk of carrying *S. aureus* resistant to erythromycin, but not all *S. aureus*, implies that carriage of resistant bacteria is not only a concern for children attending group care, but for employees as well.

Table 13. Number and percentage of swabs testing positive for *S. aureus*, MRSA, and *S. aureus* resistant to at least one antibiotic in each child care facility.

Facility ID	Source	Number of Participants/ Swabs	*S. aureus* N (Facility Prevalence)	MRSA N (Facility Prevalence)	Resistant to One* N (Prevalence in *S. aureus* Isolates)
1	Employees	3	1 (33.33%)	0 (0.00%)	0 (0.00%)
	Children	11	2 (18.18%)	0 (0.00%)	0 (0.00%)
	Surfaces	10	3 (30.00%)	0 (0.00%)	0 (0.00%)
2	Employees	7	4 (57.14%)	0 (0.00%)	0 (0.00%)
	Children	11	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Surfaces	11	1 (9.09%)	0 (0.00%)	0 (0.00%)
4	Employees	4	1 (25.00%)	0 (0.00%)	0 (0.00%)
	Children	1	1 (100.00%)	0 (0.00%)	0 (0.00%)
	Surfaces	11	1 (9.09%)	0 (0.00%)	0 (0.00%)
5	Employees	7	4 (57.14%)	1 (14.29%)	2 (50.00%)
	Children	8	1 (12.50%)	1 (12.50%)	1 (100.00%)
	Surfaces	15	2 (13.33%)	0 (0.00%)	1 (50.00%)
6	Employees	14	4 (28.57%)	0 (0.00%)	1 (25.00%)
	Children	8	2 (25.00%)	0 (0.00%)	1 (50.00%)
	Surfaces	29	1 (3.45%)	1 (100.00%)	1 (100.00%)
7	Employees	12	8 (66.67%)	0 (0.00%)	4 (50.00%)
	Children	7	2 (28.57%)	0 (0.00%)	2 (100.00%)
	Surfaces	18	3 (16.67%)	0 (0.00%)	1 (33.33%)
8	Employees	6	2 (33.33%)	1 (16.67%)	1 (50.00%)
	Children	6	0 (0.00%)	0 (0.00%)	0 (0.00%)
	Surfaces	19	1 (5.26%)	0 (0.00%)	0 (0.00%)
9	Employees	9	4 (44.44%)	0 (0.00%)	0 (0.00%)
	Children	7	2 (28.57%)	0 (0.00%)	0 (0.00%)
	Surfaces	18	3 (16.67%)	1 (33.33%)	1 (33.33%)
10	Employees	17	2 (11.76%)	0 (0.00%)	2 (100.00%)
	Children	7	3 (42.86%)	0 (0.00%)	0 (0.00%)
	Surfaces	20	2 (10.00%)	0 (0.00%)	0 (0.00%)
11	Employees	24	5 (20.83%)	1 (4.17%)	3 (60.00%)

Table 13 - continued

	Children	8	2 (25.00%)	0 (0.00%)	1 (50.00%)
	Surfaces	44	2 (4.55%)	0 (0.00%)	1 (50.00%)
12	Employees	7	3 (42.86%)	1 (14.29%)	2 (66.67%)
	Children	6	1 (16.67%)	0 (0.00%)	0 (0.00%)
	Surfaces	20	2 (10.00%)	0 (0.00%)	0 (0.00%)

* Antibiotics tested include oxacillin, tetracycline, erythromycin, clindamycin, linezolid, levofloxacin, vancomycin, trimethoprim/sulfamethoxazole (TMX), daptomycin, and quinupristin/dalfopristin.

Table 14. Questionnaire variables and their association with colonization with any *S. aureus* and MRSA in the adult study population.

Variable	***Staphylococcus aureus***			**MRSA**		
	Crude OR (95% CI)	**p-value**	**Trend p-value‡**	**Crude OR (95% CI)**	**p-value**	**Trend p-value**
Child care employee	1.09 (0.62 – 1.90)	0.77		4.23 (0.47 – 38.48)	0.21	
Season (Fall/Winter as reference)†	1.83 (0.97 – 3.48)	0.062		2.24 (0.36 – 13.82)	0.33	
Age¥		0.013	0.0010		0.73	0.21
Race¥		0.060			0.34	
Family Income¥		0.69	0.14		0.38	0.33
Participant Education¥		0.71	0.69		1.00	0.92
Spouse education¥		0.81	0.69		0.61	0.84
Number of individuals in household¥		0.18	0.11		0.55	0.85

Table 14 - continued

Youngest child in household¥		0.75	0.17		0.24	0.15
Oldest child in household¥		0.17	0.057		0.048	0.050
History of cigarette smoking	0.69 (0.33 – 1.43)	0.32		6.26 (1.01 – 38.66)	0.058	
History of pipe smoking	1.97 (0.12 – 32.99)	1.00		7.73 (0.33 - 180.71)*	1.00	
History of cigar smoking	0.64 (0.025 - 15.95)*	1.00		12.88 (0.47 - 352.92)*	1.00	
History of chewing tobacco	5.82 (0.23 - 144.59)*	0.34		12.93 (0.47 - 354.57)*	1.00	
Chronic medical condition	0.61 (0.21 – 1.76)	0.36		1.04 (0.054 - 19.97)*	1.00	
Asthma diagnosis	1.53 (0.70 – 3.32)	0.28		4.25 (0.68 – 26.55)	0.15	
Recent acute asthma attack	0.91 (0.25 – 3.42)	1.00		0.88 (0.042 - 18.09)*	1.00	
Steroid medication	0.49 (0.053 – 4.46)	0.67		3.45 (0.17 - 70.36)*	1.00	
Recent antibiotic use	0.81 (0.37 – 1.74)	0.58		0.43 (0.023 - 7.86)*	0.59	
Recent influenza-like illness	0.77 (0.41 – 1.44)	0.41		1.50 (0.24 – 9.20)	0.64	
Recent upper respiratory tract infection	0.65 (0.35 – 1.23)	0.18		1.57 (0.26 – 9.60)	0.64	
Recent hospitalization	1.39 (0.51 – 3.81)	0.52		1.02 (0.054 - 19.14)*	1.00	
Recent skin and soft tissue infection	0.64 (0.13 – 3.25)	0.72		2.17 (0.11 - 42.44)*	1.00	
Recent *S. aureus* infection	0.48 (0.052 – 4.34)	0.66		3.43 (0.17 - 70.02)*	1.00	
Recent MRSA infection	1.97 (0.13 – 31.99)	1.00		7.62 (0.33 - 178.17)*	1.00	
Recent *Streptococcus pneumoniae* infection	0.76 (0.14 – 4.02)	1.00		2.49 (0.13 - 49.30)*	1.00	
Recent infection with *S. pneumoniae* that was resistant to antibiotics	5.73 (0.23 - 142.59)*	0.34		12.82 (0.47 - 351.26)*	1.00	
Family member with recent influenza-like illness	0.62 (0.35 – 1.12)	0.14		0.44 (0.045 – 4.26)	0.64	
Family member with recent upper respiratory tract	0.80 (0.42 – 1.50)	0.53		0.57 (0.062 – 5.17)	1.00	

Table 14 - continued

infection						
Family member with recent hospitalization	1.38 (0.56 – 3.42)	0.48		2.17 (0.23 – 20.30)	0.43	
Family member with recent skin and soft tissue infection	0.23 (0.028 – 1.84)	0.17		1.83 (0.094 - 35.63)*	1.00	
Family member with recent *S. aureus* infection	0.38 (0.043 – 3.29)	0.67		2.68 (0.13 - 53.73)*	1.00	
Family member with recent MRSA infection	0.17 (0.0092 – 3.08)*	0.17		3.20 (0.16 - 65.31)*	1.00	
Family member with recent *Streptococcus pneumoniae* infection	0.38 (0.043 – 3.31)	0.67		3.26 (0.16 - 67.03)*	1.00	
Family member with recent infection with *S. pneumoniae* that was resistant to antibiotics	0.21 (0.011 – 3.89)*	0.29		3.89 (0.19 - 81.48)*	1.00	
Eczema	1.64 (0.65 – 4.17)	0.25		0.80 (0.043 - 15.086)*	1.00	
Psoriasis	2.75 (0.60 – 12.64)	0.23		8.13 (0.79 – 84.10)	0.16	
Folliculitis	1.34 (0.37 – 4.93)	0.73		1.66 (0.086 - 32.03)*	1.00	
Pimples	1.51 (0.78 – 2.91)	0.22		0.82 (0.089 - 7.51)	1.00	
Number of times participant exercised at a gym in the past month¥		0.79	0.52		0.81	0.87
Number of times participant's spouse/partner exercised at a gym in the past month¥		0.88	0.53		1.00	0.29
Presence of children involved in team or contact sports in the household	0.98 (0.42 – 2.27)	0.95		0.29 (0.014 - 6.23)*	0.52	
Participant spends time in a hospital as a patient	1.51 (0.50 – 4.53)	0.56		3.87 (0.40 – 37.11)	0.28	
Participant spends time in a hospital as a visitor	0.40 (0.14 – 1.09)	0.066		0.62 (0.033 - 11.52)*	1.00	
Participant works at a hospital and has direct contact with patients	1.68 (0.49 – 5.69)	0.51		1.61 (0.084 - 30.90)*	1.00	
Participant works at a hospital with no direct	1.65 (0.78 – 3.46)	0.19		3.79 (0.61 – 23.59)	0.17	

Table 14 - continued

patient contact						
Participant spends time in a long term care facility as a patient	Not analyzable			Not analyzable		
Participant spends time in a long term care facility as a visitor	0.42 (0.12 – 1.52)	0.18		1.09 (0.058 - 20.55)*	1.00	
Participant works in a long term care facility and has direct cont act with patients	Not analyzable			Not analyzable		
Participant works in a long term care facility with no direct patient contact	1.96 (0.12 – 31.77)	1.00		53.00 (2.80 – 1010.31)	0.045	
Family member spends time in a hospital as a patient	0.17 (0.0092 – 3.08)*	0.17		3.40 (0.17 - 69.35)*	1.00	
Family member spends time in a hospital as a visitor	0.13 (0.016 – 0.97)	0.020		1.15 (0.061 - 21.71)*	1.00	
Family member works at a hospital and has direct contact with patients	1.10 (0.44 – 2.75)	0.84		2.25 (0.24 – 21.08)	0.42	
Family member works at a hospital with no direct patient contact	3.14 (1.07 – 9.18)	0.030		3.57 (0.37 – 34.13)	0.30	
Family member spends time in a long term care facility as a patient	5.82 (0.23 – 144.59)	0.34		12.70 (0.46 - 347.95)*	1.00	
Family member spends time in a long term care facility as a visitor	0.33 (0.071 – 1.52)	0.23		1.33 (0.070 - 25.34)*	1.00	
Family member works in a long term care facility and has direct contact with patients	0.47 (0.052 – 4.31)	0.66		3.40 (0.17 - 69.35)*	1.00	
Family member works in a long term care facility with no direct patient contact	0.62 (0.025 - 15.30)*	1.00		12.70 (0.46 - 347.95)*	1.00	

Table 14 - continued

Antibacterial hand soap or body wash provided in household	1.66 (0.67 – 4.10)	0.30		1.68 (0.091 - 31.22)*	1.00	
Hand soap or body wash that does not contain antibacterial ingredients provided in household	0.74 (0.42 – 1.30)	0.31		0.40 (0.065 – 2.43)	0.37	
Alcohol-based hand sanitizers provided in household	0.91 (0.51 – 1.65)	0.76		2.07 (0.23 – 18.87)	0.66	
Participant works in an occupation that involves close physical contact with animals	1.29 (0.35 – 4.74)	0.74		5.69 (0.57 – 56.27)	0.21	
Participant works in an occupation that involves close physical contact with animal waste products	3.36 (0.78 – 14.47)	0.13		7.39 (0.73 – 75.01)	0.18	
Participant works in a livestock processing plant	5.72 (0.49 – 66.48)*	1.00		12.58 (0.46 - 344.64)*	1.00	
Family member works in an occupation that involves close physical contact with animals	0.68 (0.21 – 2.20)	0.51		1.15 (0.061 - 21.71)*	1.00	
Family member works in an occupation that involves close physical contact with animal waste products	0.81 (0.20 – 3.22)	1.00		1.74 (0.090 - 33.53)*	1.00	
Family member works in a livestock processing plant	0.62 (0.025 - 15.40)*	0.30		4.17 (0.20 - 87.38)*	1.00	
Recent contact with live animals	0.73 (0.38 – 1.41)	0.36		1.81 (0.30 – 11.13)	0.61	
Recent contact with chickens	0.52 (0.17 – 1.65)	0.26		0.95 (0.051 - 17.88)*	1.00	
Recent contact with cattle	0.72 (0.30 – 1.70)	0.45		1.64 (0.18 – 15.15)	0.52	
Recent contact with swine	1.27 (0.47 – 3.43)	0.63		0.95 (0.051 - 17.88)*	1.00	
Recent contact with horses	0.49 (0.21 – 1.15)	0.10		0.44 (0.024 - 8.08)*	0.59	
Recent contact with goats	0.18 (0.023 – 1.44)	0.10		1.59 (0.083 - 30.45)*	1.00	
Recent contact with sheep	0.57 (0.18 – 1.81)	0.33		1.01 (0.054 - 19.04)*	1.00	

Table 14 - continued

Family member who had recent contact with live animals	0.82 (0.40 – 1.71)	0.60		2.90 (0.47 – 17.93)	0.24	
Family member who had recent contact with chickens	0.53 (0.11 – 2.62)	0.72		1.78 (0.092 - 34.70)*	1.00	
Family member who had recent contact with cattle	0.36 (0.10 – 1.28)	0.12		2.62 (0.28 – 24.76)	0.38	
Family member who had recent contact with swine	0.61 (0.16 – 2.35)	0.55		1.33 (0.070 - 25.53)*	1.00	
Family member who had recent contact with horses	0.64 (0.65 – 1.72)	0.38		0.67 (0.036 - 12.46)*	1.00	
Family member who had recent contact with goats	0.31 (0.036 – 2.60)	0.43		2.28 (0.11 - 45.24)*	1.00	
Family member who had recent contact with sheep	0.70 (0.18 – 2.72)	0.75		1.46 (0.076 - 28.03)*	1.00	
Animals on property	0.81 (0.46 – 1.43)	0.47		0.23 (0.025 – 2.09)	0.20	
Chickens on property	0.38 (0.018 - 8.07)*	0.55		7.69 (0.33 - 179.86)*	1.00	
Swine on property	0.64 (0.025 - 15.95)*	1.00		12.88 (0.47 - 352.92)*	1.00	
Cats on property	0.44 (0.23 – 0.86)	0.014		0.56 (0.061 - 5.08)	1.00	
Dogs on property	1.23 (0.69 – 2.20)	0.49		0.17 (0.0092 - 3.06)*	0.17	
Animal on property that has ever been diagnosed with a skin and soft tissue infection	0.29 (0.033 – 2.47)	0.42		2.87 (0.13 - 65.26)*	1.00	
Amount of contact with children in current place of work¥		0.73	0.70		0.59	0.23

†Fall/Winter season included October through March; Spring/Summer included April – September

‡Based on Cochran-Armitage test for trend

¥Categorical/ordinal variable – individual odds ratios not calculated

*Logit estimator using a correction of 0.5 in every cell of those tables that contain a zero

Table 15. Final multivariate model of risk of *S. aureus* using generalized estimating equations to adjust for clustering*

Parameter	OR (95% CI)	p-value
Employed at a child care facility	0.68 (0.31 – 1.50)	0.34
Age		
Less than 25 years	Reference	--
25 – 34 years	0.55 (0.27 – 1.15)	0.11
35 – 44 years	0.40 (0.15 – 1.07)	0.068
45 years or older	0.21 (0.077 – 0.56)	0.0020
Family member with recent influenza-like illness	0.52 (0.27 – 0.98)	0.045
Family member with contact with cattle	0.31 (0.081 – 1.16)	0.082

*Model-based standard error estimates

Table 16. Final multivariate model of risk of MRSA carriage using exact logistic regression

Parameter	OR (95% CI)	p-value
Employed at a child care facility	3.28 (0.31 – 169.02)	0.53
History of cigarette smoking	5.10 (0.55 – 64.07)	0.18

Table 17. Questionnaire variables and their association with colonization with any *S. aureus* and MRSA in children.

Variable	***Staphylococcus aureus***		**MRSA**	
	Crude OR (95% CI)	**p-value**	**Crude OR (95% CI)**	**p-value**
Age¥		0.90		1.00
Gender (Male as reference)	3.63 (0.93 – 14.14)	0.053	2.24 (0.089 – 56.81)*	0.58
Season (Fall/Winter as reference)†	1.26 (0.41 – 3.91)	0.69	3.24 (0.13 - 82.24)*	0.49
Race¥		0.82		0.18
Family Income¥		0.39		0.25
Participant Education¥		0.48		0.55
Spouse education¥		0.10		0.39
Number of individuals in household¥		0.84		1.00
Youngest child in household¥		0.87		1.00
Oldest child in household¥		0.83		0.45
History of cigarette smoking	0.50 (0.10 – 2.49)	0.50	1.11 (0.043 - 28.59)*	1.00
History of pipe smoking	Not analyzable		Not analyzable	
History of cigar smoking	1.30 (0.050 - 33.51)*	1.00	16.55 (0.45 - 597.23)*	1.00
History of chewing tobacco	0.76 (0.035 - 16.82)*	1.00	9.80 (0.31 - 305.59)*	1.00
Chronic medical condition	Not analyzable		Not analyzable	
Asthma diagnosis	7.03 (1.38 – 35.88)	0.025	3.04 (0.11 - 81.57)*	1.00
Recent acute asthma episode	37.85 (1.49 - 961.33)*	0.022	Not analyzable	
Steroid medication	1.30 (0.050 - 33.51)*	1.00	16.55 (0.45 - 597.23)*	1.00
Recent antibiotic use	0.70 (0.17 - 2.81)*	1.00	0.96 (0.037 - 24.70)*	1.00

Table 17 - continued

Recent respiratory illness	1.35 (0.43 – 4.22)	0.77	0.38 (0.015 - 9.63)*	1.00
Recent URTI	1.33 (0.41 – 4.31)	0.77	0.32 (0.012 - 8.22)*	0.49
Recent hospitalization	0.41 (0.021 - 8.07)*	0.58	5.29 (0.18 - 149.36)*	1.00
Recent SSTI	2.19 (0.18 – 26.05)	0.48	6.90 (0.23 - 201.90)*	1.00
Staphylococcus aureus infection	1.30 (0.050 - 33.51)*	1.00	16.55 (0.45 - 597.23)*	1.00
MRSA infection	Not analyzable		Not analyzable	
Streptococcus pneumonia infection	12.72 (0.49 - 328.60)*	0.20	16.55 (0.45 - 597.23)*	1.00
Infection with resistant *Streptococcus pneumoniae*	Not analyzable		Not analyzable	
Family member with recent respiratory illness	2.52 (0.77 – 8.25)	0.15	0.34 (0.013 - 8.66)*	1.00
Family member with recent upper respiratory tract infection	3.21 (0.73 – 7.33)	0.16	0.42 (0.016 - 10.71)*	1.00
Family member recently hospitalized	0.47 (0.055 – 4.11)	0.68	2.33 (0.088 - 61.51)*	1.00
Family member with a recent skin and soft tissue infection	0.76 (0.035 - 16.82)*	1.00	9.80 (0.31 - 305.59)*	1.00
Family member with a recent *Staphylococcus aureus* infection	Not analyzable		Not analyzable	
Family member with a recent MRSA	Not analyzable		Not analyzable	
Family member with a recent *Streptococcus pneumoniae* infection	12.72 (0.49 - 328.60)*	0.20	16.55 (0.45 - 597.23)*	1.00
Family member with a recent infection with resistant *Streptococcus pneumoniae*	Not analyzable		Not analyzable	
Eczema diagnosis	1.43 (0.41 – 5.03)	0.74	0.79 (0.031 - 20.20)*	1.00
Psoriasis diagnosis	Not analyzable		Not analyzable	
Diaper rash diagnosis	2.14 (0.62 – 7.37)	0.32	0.79 (0.031 - 20.20)*	1.00
Number of times parent exercises at a gym¥		0.047		1.00

Table 17 - continued

Number of times parent's spouse/partner exercises at a gym¥		0.64		0.20
Children at home involved with contact sports	2.24 (0.68 – 7.37)	0.20	0.90 (0.035 - 23.05)*	1.00
Spends time at a hospital as a patient	0.53 (0.026 - 11.00)*	1.00	6.90 (0.23 - 201.90)*	1.00
Spends time at a hospital as a visitor	0.20 (0.011 - 3.71)*	0.34	2.64 (0.099 - 70.28)*	1.00
Employed at a hospital and works with patients	2.72 (0.81 – 9.13)	0.17	1.03 (0.040 - 26.53)*	1.00
Employed at a hospital with no patient contact	0.80 (0.086 – 7.41)	1.00	3.56 (0.13 - 96.63)*	1.00
Spends time at a LTCF as a visitor	1.30 (0.050 - 33.51)*	1.00	16.55 (0.45 - 597.23)*	1.00
Employed at a LTCF and works with patients	0.53 (0.026 - 11.00)*	1.00	6.90 (0.23 - 201.90)*	1.00
Employed at a LTCF with no patient contact	Not analyzable		Not analyzable	
Family member who spends time at a hospital as a patient	0.65 (0.073 – 5.89)	1.00	3.04 (0.11 - 81.57)*	1.00
Family member who spends time at a hospital as a visitor	0.27 (0.014 - 5.16)*	0.59	3.56 (0.13 - 96.63)*	1.00
Family member who is employed at a hospital and works with patients	2.89 (0.80 – 10.45)	0.13	1.41 (0.054 - 36.51)*	1.00
Family member who is employed at a hospital with no patient contact	0.55 (0.063 – 4.86)	1.00	2.64 (0.099 - 70.28)*	1.00
Family member who spends time at a long-term care facility (LTCF) as a patient	Not analyzable		Not analyzable	
Family member who spends time at a LTCF as a visitor	1.30 (0.050 - 33.51)*	1.00	16.55 (0.45 - 597.23)*	1.00
Family member who is employed at a LTCF and works with patients	1.30 (0.050 - 33.51)*	1.00	16.55 (0.45 - 597.23)*	1.00
Family member employed at a LTCF with no patient contact	Not analyzable		Not analyzable	
Antibacterial soap provided at home	2.51 (0.51 – 12.32)	0.33	1.03 (0.040 - 26.48)*	1.00
Non-antibacterial soap provided at home	0.69 (0.22 – 2.16)	0.57	2.01 (0.079 - 51.00)*	1.00

Table 17 - continued

Hand sanitizer provided at home	2.84 (0.81 – 9.91)	0.15	2.62 (0.10 - 6.62)*	1.00
Occupation involving close contact with animals	0.76 (0.035 - 16.82)*	1.00	9.80 (0.31 - 305.59)*	1.00
Occupation involving close contact with animal waste products	0.76 (0.035 - 16.82)*	1.00	9.80 (0.31 - 305.59)*	1.00
Occupation in an animal processing facility	12.72 (0.49 - 328.60)*	0.20	16.55 (0.45 - 597.23)*	1.00
Family member with an occupation involving close contact with animals	0.76 (0.035 - 16.82)*	1.00	6.90 (0.23 - 201.90)*	1.00
Family member with an occupation involving close contact with animal waste products	12.72 (0.49 - 328.60)*	1.00	9.80 (0.31 - 305.59)*	1.00
Family member with an occupation in an animal processing facility	4.07 (0.24 – 69.19)	0.37	9.80 (0.31 - 305.59)*	1.00
Contact with animals	5.09 (1.10 – 23.49)	0.046	2.64 (0.099 - 70.28)*	1.00
Contact with chickens	12.72 (0.49 - 328.60)*	0.20	16.55 (0.45 - 597.23)*	1.00
Contact with cattle	2.07 (0.18 – 24.50)	0.49	6.90 (0.23 - 201.90)*	1.00
Contact with swine	4.21 (0.25 – 71.58)	0.37	9.80 (0.31 - 305.59)*	1.00
Contact with horses	2.15 (0.36 – 13.05)	0.59	3.56 (0.13 - 96.63)*	1.00
Contact with goats	14.75 (1.41 – 154.16)	0.024	5.29 (0.18 - 149.36)*	1.00
Contact with sheep	14.75 (1.41 – 154.16)	0.024	5.29 (0.18 - 149.36)*	1.00
Family member who has contact with animals	1.36 (0.25 – 7.52)	0.66	2.64 (0.099 - 70.28)*	1.00
Family member who has contact with chickens	Not analyzable		Not analyzable	
Family member who has contact with cattle	0.53 (0.026 - 11.00)*	1.00	6.90 (0.23 - 201.90)	1.00
Family member who has contact with swine	1.30 (0.050 - 33.51)*	1.00	16.55 (0.45 - 597.23)*	1.00
Family member who has contact with horses	0.41 (0.021 - 8.07)*	0.57	5.29 (0.18 - 149.36)*	1.00
Family member who has contact with goats	8.62 (0.73 – 102.38)	0.11	6.90 (0.23 - 201.90)*	1.00

Table 17 - continued

Family member who has contact with sheep	8.62 (0.73 – 102.38)	0.11	6.90 (0.23 - 201.90)*	1.00
Animals on property	0.82 (0.26 – 2.54)	0.78	0.24 (0.0098 - 6.31)*	0.43
Chickens on property	Not analyzable		Not analyzable	
Swine on property	Not analyzable		Not analyzable	
Cats on property	1.26 (0.38 – 4.25)	0.76	0.79 (0.031 - 20.20)*	1.00
Dogs on property	0.31 (0.078 – 1.19)	0.14	0.49 (0.019 - 12.61)*	1.00
Animal with recent skin and soft tissue infection	Not analyzable		Not analyzable	
Amount of time parent spends working with children¥		0.57		0.29

¥Categorical/ordinal variable – individual odds ratios not calculated

†Fall/Winter season included October through March; Spring/Summer included April – September

*Logit estimator using a correction of 0.5 in every cell of those tables that contain a zero

Table 18. Individual-level questionnaire variables and their association with colonization with any *S. aureus* and MRSA in employees.

Variable	***Staphylococcus aureus***		**MRSA**	
	Crude OR (95% CI)	**p-value**	**Crude OR (95% CI)**	**p-value**
Perform cleaning	1.42 (0.41 – 4.86)	0.77	1.54 (0.078 - 30.41)*	1.00
Clean kitchen	1.22 (0.54 – 2.75)	0.63	0.14 (0.0071 – 2.58)	0.13
Clean restroom	1.65 (0.74 – 3.65)	0.22	1.08 (0.15 – 7.98)	1.00
Clean play area	1.28 (0.53 – 3.09)	0.59	1.43 (0.14 – 14.32)	1.00
Clean diapering area	0.81 (0.36 – 1.86)	0.63	0.21 (0.021 – 2.05)	0.30
Cooking	1.09 (0.44 – 2.70)	0.84	0.36 (0.018 - 7.04)*	0.57
Changing diapers	1.04 (0.44 – 2.44)	0.93	0.52 (0.071 – 3.87)	0.61
Occasional administrative	0.89 (0.28 – 2.81)	0.85	0.50 (0.025 - 9.77)*	1.00
Administrative only	2.06 (0.28 – 15.28)	0.60	2.13 (0.099 - 46.08)*	1.00
Playing indoors	1.25 (0.23 – 6.79)	1.00	0.91 (0.045 - 18.50)*	1.00
Playing outdoors	0.98 (0.28 – 3.52)	1.00	1.41 (0.071 - 27.91)*	1.00
Work schedule (e.g. full time year round)¥		0.27		1.00
Number of children supervised on an average day¥		0.99		0.40
Supervise children under 6 months old	1.03 (0.39 – 2.71)	0.95	1.24 (0.12 – 12.54)	1.00
Supervise children aged 6 – 12 months	1.09 (0.45 – 2.69)	0.85	0.95 (0.095 – 9.53)	1.00
Supervise children aged 13 – 23 months	1.21 (0.52 – 2.80)	0.66	0.69 (0.069 – 6.85)	1.00
Supervise children aged 24 – 47 months	1.07 (0.47 – 2.45)	0.88	0.18 (0.021 – 2.08)	0.30
Supervise children aged 4 – 5 years	0.97 (0.44 – 2.15)	0.94	0.30 (0.030 – 2.95)	0.35

Table 18 - continued

Supervise children aged 6 years or older	1.23 (0.37 – 4.07)	0.76	2.56 (0.25 – 26.58)	0.41
Amount of time employed by current facility¥		0.71		0.25
Amount of time employed in the child care industry ¥		0.94		0.41

*Logit estimator using a correction of 0.5 in every cell of those tables that contain a zero

¥Categorical/ordinal variable – individual odds ratios not calculated

Table 19. Facility-level variables and their association with colonization with any *S. aureus* and MRSA in employees.

Variable	***Staphylococcus aureus***		**MRSA**	
	Crude OR (95% CI)	**p-value**	**Crude OR (95% CI)**	**p-value**
Facility ID¥		0.061		0.30
Center years operated¥		0.045		0.80
Center employee capacity¥		0.33		0.58
Center kid number capacity¥		0.48		0.30
Written sanitation policy	Not analyzable	1.00	Not analyzable	1.00
Written sanitation policy available for review	Not analyzable	1.00	Not analyzable	1.00
Regular review of sanitation policy	Not analyzable	0.51	Not analyzable	1.00
Posted sanitation policy	1.13 (0.50 – 2.56)	0.76	2.03 (0.20 – 20.21)	1.00
Center provide gloves	Not analyzable		Not analyzable	
Center provides soap at sinks	Not analyzable		Not analyzable	
Center provides hand sanitizer	0.64 (0.28 – 1.50)	0.30	0.44 (0.060 – 3.30)	0.59

Table 19 - continued

Periodic surface disinfection	Not analyzable		Not analyzable	
Employees wash hands upon arrival	Not analyzable		Not analyzable	
Employees wash hands before eating	Not analyzable		Not analyzable	
Employees wash hands before food preparation	Not analyzable		Not analyzable	
Employees wash hands after eating	1.63 (0.57 – 4.63)	0.35	1.01 (0.10 – 10.24)	1.00
Employees wash hands after food preparation	4.69 (1.00 – 21.96)	0.035	2.11 (0.10 - 41.11)*	1.00
Employees wash hands after diapering	Not analyzable		Not analyzable	
Employees wash hands after restroom use	Not analyzable		Not analyzable	
Employees wash hands when returning from outdoors	4.69 (1.00 – 21.96)	0.035	2.11 (0.10 - 41.11)*	1.00
Employees wash hands before administering first aid	2.03 (0.81 – 5.07)	0.092	2.06 (0.21 – 20.53)	0.65
Employees wash hands after administering first aid	Not analyzable		Not analyzable	
Employees wash hands after handling animals	0.45 (0.10 – 1.93)	0.43	0.91 (0.045 - 18.50)*	1.00
Children wash hands upon arrival	0.21 (0.082 – 0.55)	0.00090	1.20 (0.12 – 12.07)	1.00
Children wash hands before eating	Not analyzable		Not analyzable	
Children wash hands before food preparation	Not analyzable		Not analyzable	
Children wash hands after eating	4.69 (1.00 – 21.96)	0.047	2.11 (0.10 - 41.11)*	1.00
Children wash hands after food preparation	4.69 (1.00 – 21.96)	0.043	2.11 (0.10 - 41.11)*	1.00
Children wash hands after being diapered	Not analyzable		Not analyzable	
Children wash hands after handling animals	0.45 (0.10 – 1.93)	0.43	0.91 (0.045 - 18.50)*	1.00
Children wash hands when returning from outdoors	1.11 (0.43 – 2.84)	0.83	4.24 (0.22 - 81.51)*	0.31
Children are separated during at least part of the day	4.69 (1.00 – 21.96)	0.047	2.11 (0.10 - 41.11)*	1.00
Age of youngest group of children attending the center¥		0.031		0.73
Age of oldest group of children attending the center¥		0.0029		0.36

Table 19 - continued

Percent of child participants with asthma¥		0.23		0.27
Percentage of child participants reporting an acute asthma event in the past year¥		0.028		1.00
Percent of child participants whose parent reported exercising at a gym¥		0.055		0.41
Percent of child participants reporting contact with animals¥		0.038		0.46
Percent of child participants reporting contact with goats¥		0.052		0.46
Percent of child participants reporting contact with sheep¥		0.052		0.46

¥Categorical/ordinal variable – individual odds ratios not calculated

Table 20. Final multivariate model of risk of *S. aureus* carriage among child care employees using generalized estimating equations to adjust for clustering*

Parameter	**OR (95% CI)**	**p-value**
Children wash hands upon arrival at the center	0.17 (0.095 – 0.32)	< 0.0001
Children are separated into groups for at least part of the day	1.98 (0.87 – 4.48)	0.11

*Model-based standard error estimates

Table 21. Antibiotic resistance in *S. aureus* isolated from child care employees, unexposed adults, children, and surfaces.

Antibiotic	**Employees (n = 38)**	**Unexposed Adults (n = 37)**	**p-value**	**Children (n = 16)**	**Surfaces (n = 22)**
Oxacillin	3 (7.89%)	1 (2.70%)	0.61	1 (6.25%)	2 (9.09%)
Tetracycline	2 (5.26%)	2 (5.41%)	1.00	1 (6.25%)	0 (0.00%)
Erythromycin*	12 (31.6%)	4 (10.8%)	0.033	5 (31.3)	4 (18.2%)
Clindamycin	3 (7.89%)	0 (0.00%)	0.24	1 (6.25%)	1 (4.55%)
Levofloxacin	0 (0.00%)	2 (5.41%)	0.24	1 (6.25%)	1 (4.55%)
TMX	0 (0.00%)	1 (2.70%)	0.49	0 (0.00%)	0 (0.00%)

Frequency of carriage in employees was compared to frequency in unexposed adults using chi square or Fisher's exact test, depending on expected cell counts.

TMX: trimethoprim/sulfamethoxazole

*One surface isolate displayed intermediate resistance to erythromycin

Table 22. Potential univariate predictors of erythromycin-resistant *S. aureus* colonization in the adult study population.

Variable	Crude OR (95% CI)	p-value
Child care employee	3.67 (1.06 - 12.68)	0.033
Antibiotic use in the past 3 months	0.79 (0.15 - 4.10)	1.00
Macrolide use in the past 3 months*	1.20 (0.047 - 30.90)	1.00
Amount of contact with children in current place of work¥		0.13
Facility ID¥		0.036

*Logit estimator using a correction of 0.5 in every cell of those tables that contain a zero

¥Categorical/ordinal variable – individual odds ratios not calculated

Table 23. Potential univariate predictors of erythromycin-resistant *S. aureus* colonization in child care employees.

Variable	**Crude OR (95% CI)**	**p-value**
Antibiotic use in the past 3 months	1.1 (0.17 - 7.03)	1.00
Facility ID¥		0.22
Presence of child carrying erythromycin-resistant *S. aureus* at facility	3.75 (0.83 - 16.99)	0.077
Presence of child reporting antibiotic use in past 3 months at the facility*	1.42 (0.054 - 37.25)	1.00
Presence of child reporting macrolide use in past 3 months at the facility	0.34 (0.062 - 1.87)	0.28
Presence of another employees colonized with erythromycin-resistant *S. aureus* at the facility	5.10 (1.11 - 23.37)	0.029
Presence of another employees reporting antibiotic use in past 3 months at the facility*	1.42 (0.054 - 37.25)	1.00
Presence of another employees reporting macrolide use in past 3 months at the facility	0.93 (0.24 - 3.62)	0.92

*Logit estimator using a correction of 0.5 in every cell of those tables that contain a zero

¥Categorical/ordinal variable – individual odds ratios not calculated

Table 24. *spa* types among all *S. aureus* isolates collected from child care employees, children, environmental surfaces, and unexposed adults.

Location	Employees	Children	Surfaces	Unexposed Adults
Facility 1‡	t164	t012, t012	t164	
Facility 2‡	t012, t189		t012, t164, t2794	
Facility 4§	t084	t012	t012	
Facility 5	*t002*, t008, t189, t216		t067	
Facility 6‡	t008, t012, t122, t1577	t338, t338	t122, *t400*	
Facility 7†‡§¥	t024, t091, t189, t338, t1017, t1793, t3214, t6696	t3214, t3214	t012, t216, t3214	
Facility 8	*t126*, t363		t3097	
Facility 9*†‡	t002, t012, t189, t189, t216, t216	t012, t216	*t002*, t002, t002	
Facility 10§	t021, t1152	t209, t693, t693	t002, t693	
Facility 11	t084, t2228, t4100, t571	t342		
Facility 12†	t008, *t136*, t4856	t008	t085, t645	
CPH Location 1				t012, t012, t065, t085, t085, t091, t1239, t1293, t189, t216, t228, *t334*, t338, t346, t617, t688, t701, t743
CPH Location 2				t334, t179, t2849, t037
Student Union				t008, t021, t084, t094, t156, t1598, t164, t189, t216, t334, t338, t548, t726

CPH: College of Public Health

*Evidence of transmission between employees

†Evidence of transmission between employees and children

Table 24 - continued

‡Evidence of transmission between employees and surfaces

§Evidence of transmission between children and surfaces

¥Evidence of transmission between employees, children, and surfaces

Italics MRSA isolate

Table 25. Prevalence of MSSA and MRSA in this study and a study conducted in Texas by Hewlett *et. al.*

Sample Source	**MSSA**		**MRSA**	
	This Study	Hewlett et. al.	This Study	Hewlett et. al.
Employees	29.6%	28.1%	3.7%	3.1%
Children	19.8%	21.2%	1.2%	6.7%
Environmental surfaces	8.9%	8.7%	0.9%	2.0%

Figure 2. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 1 (E: Employee, C: Child, S: Surface).

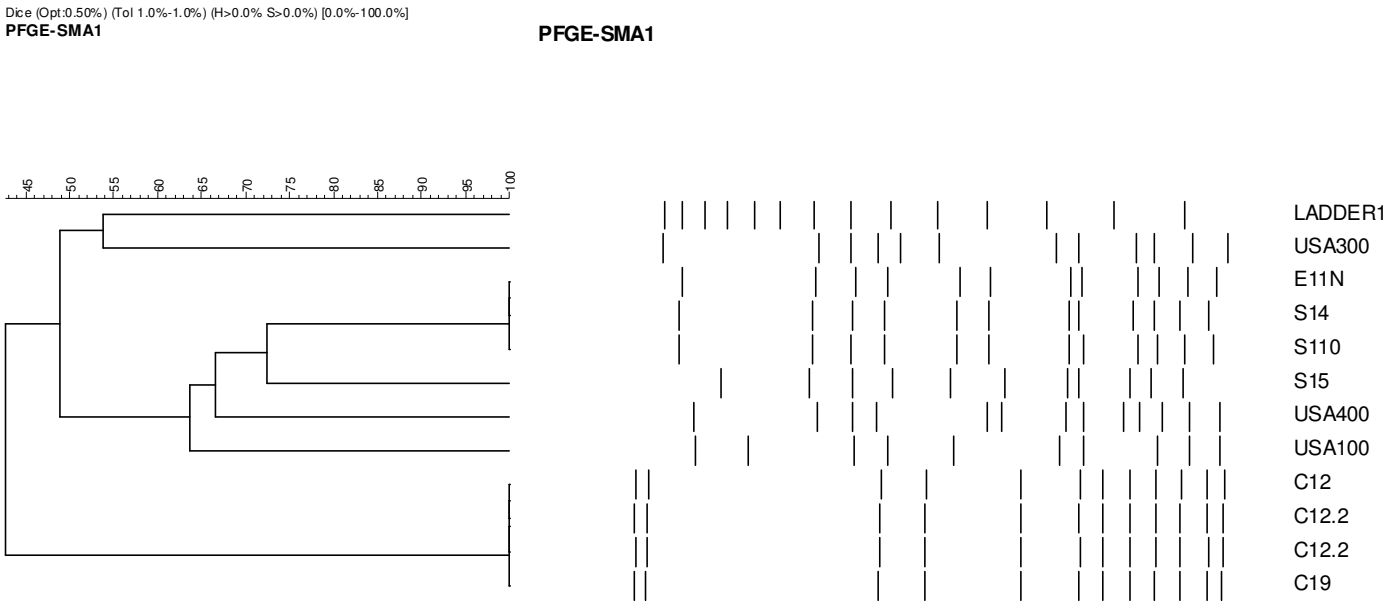


Figure 3. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 2 (E: Employee, C: Child, S: Surface).

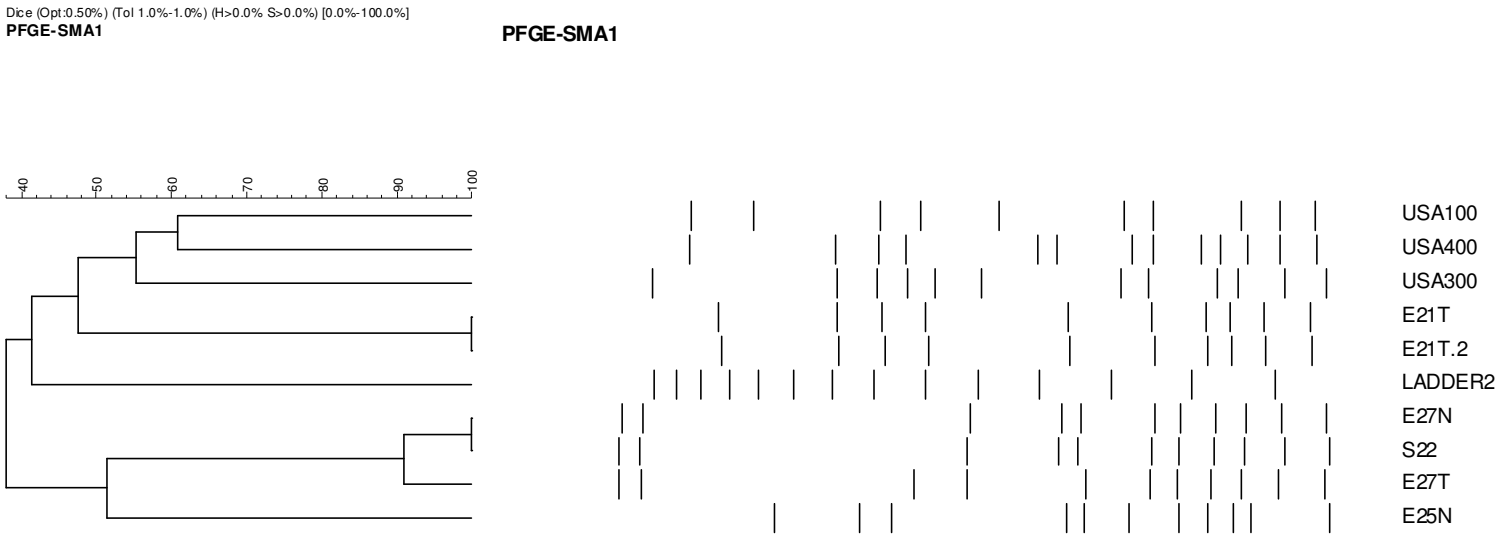


Figure 4. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 4 (E: Employee, C: Child, S: Surface).

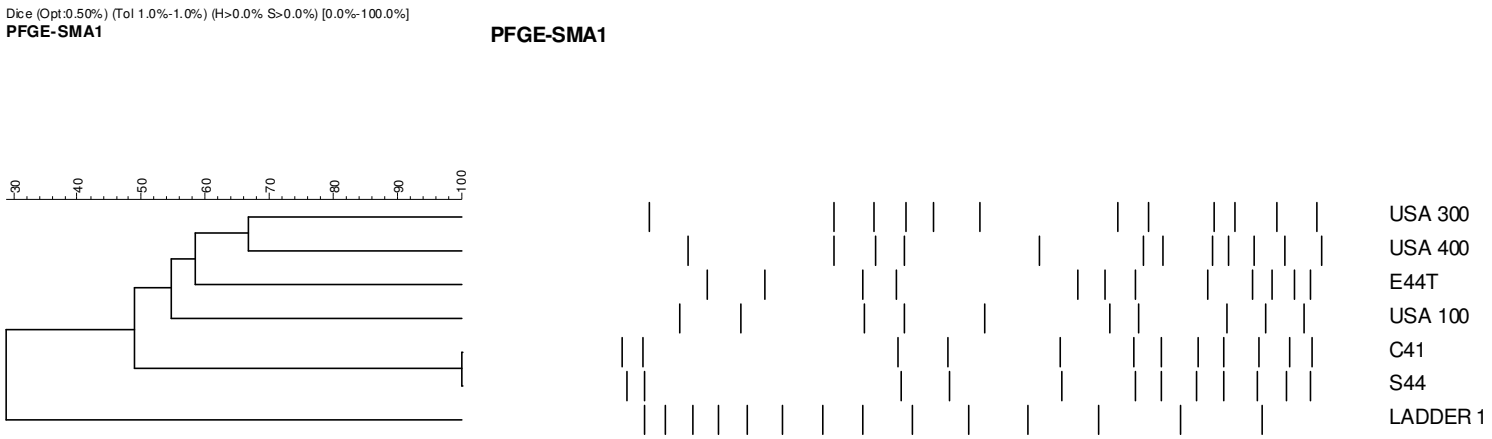


Figure 5. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 5 (E: Employee, C: Child, S: Surface).

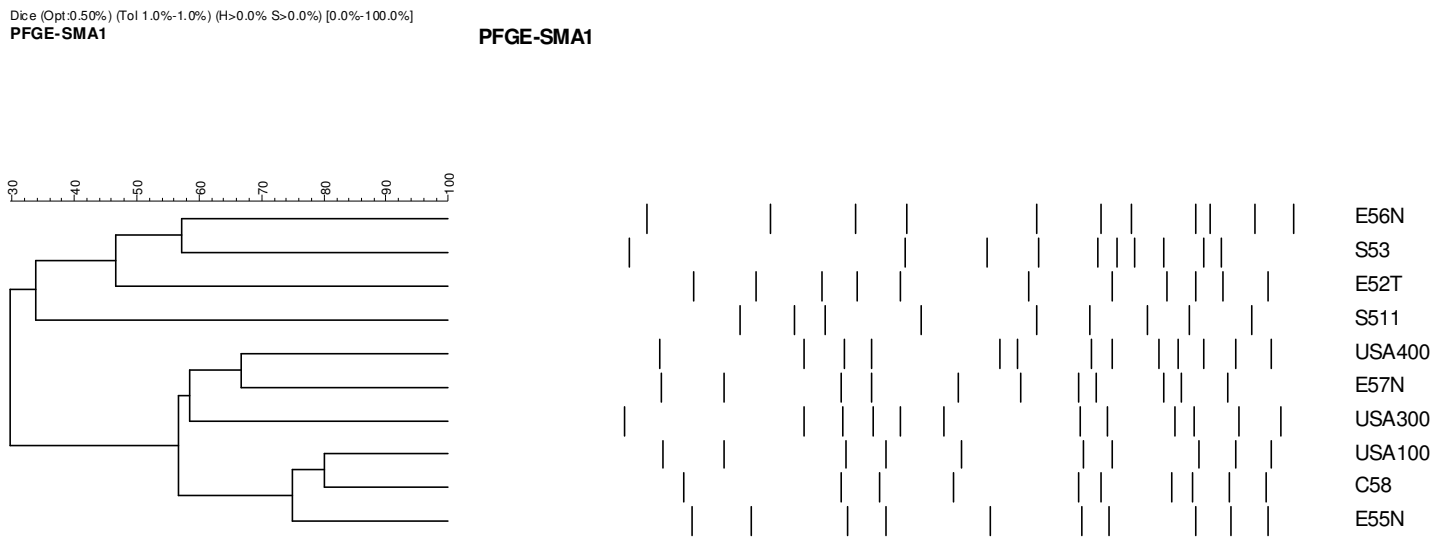


Figure 6. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 6 (E: Employee, C: Child, S: Surface).

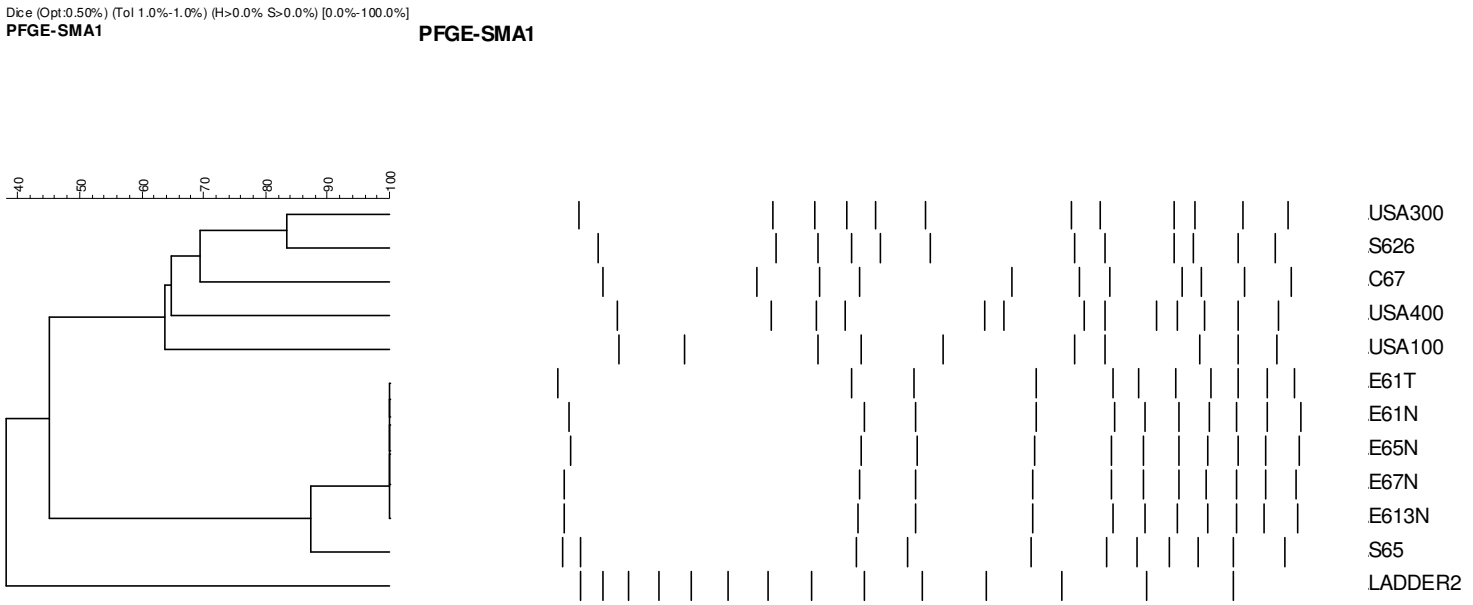


Figure 7. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 7 (E: Employee, C: Child, S: Surface).

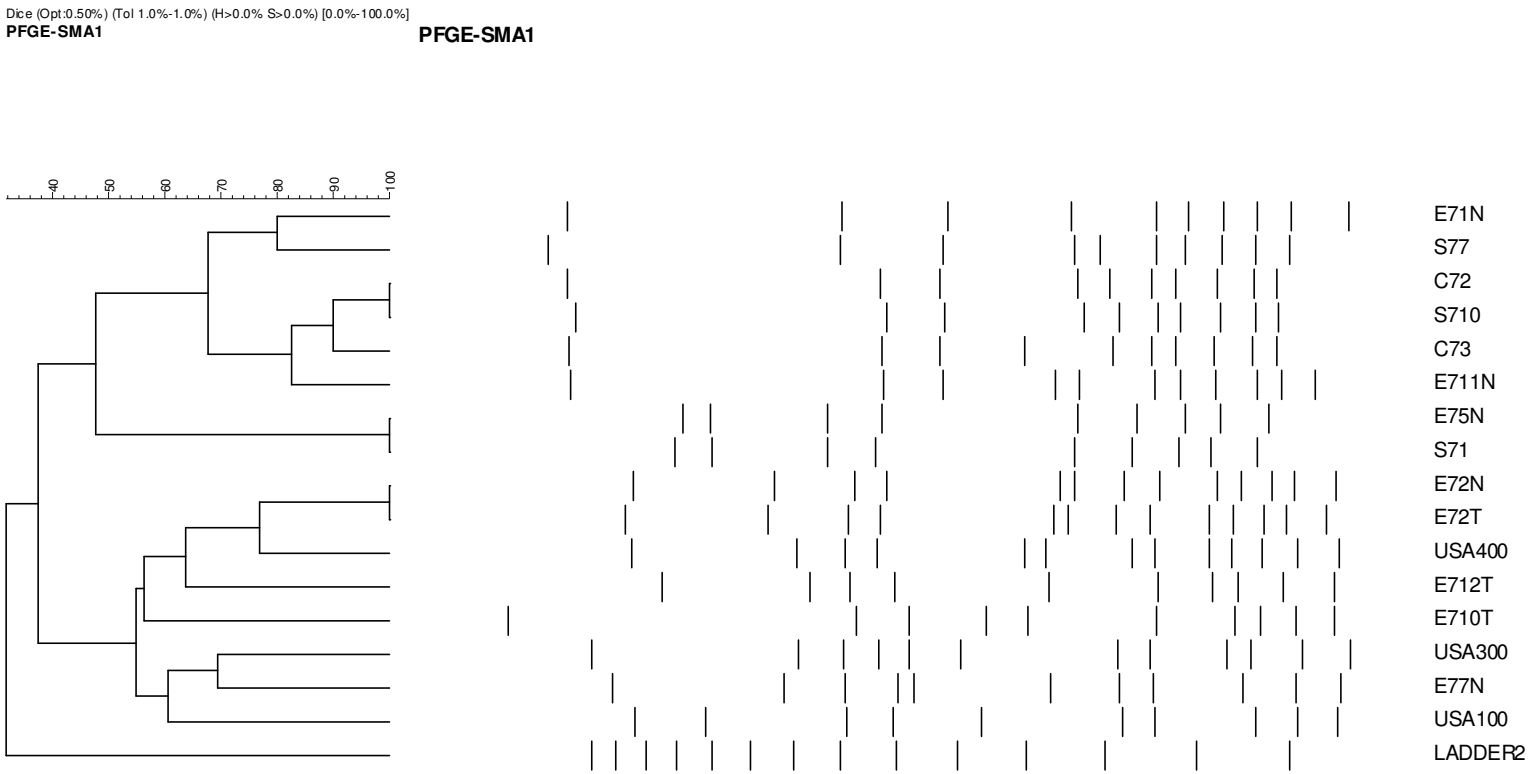


Figure 8. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 8 (E: Employee, C: Child, S: Surface).

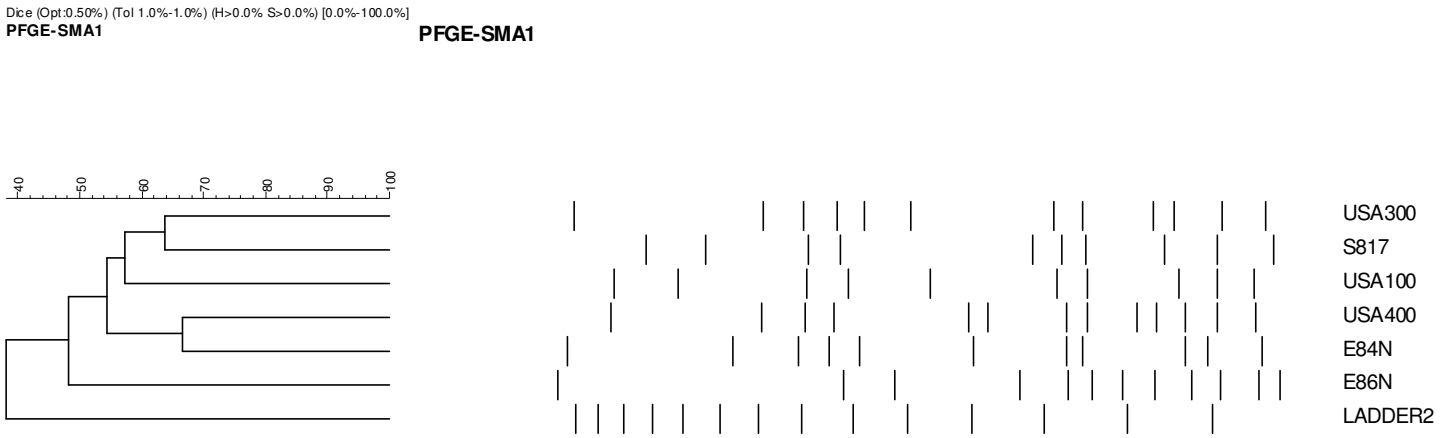


Figure 9. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 9 (E: Employee, C: Child, S: Surface).

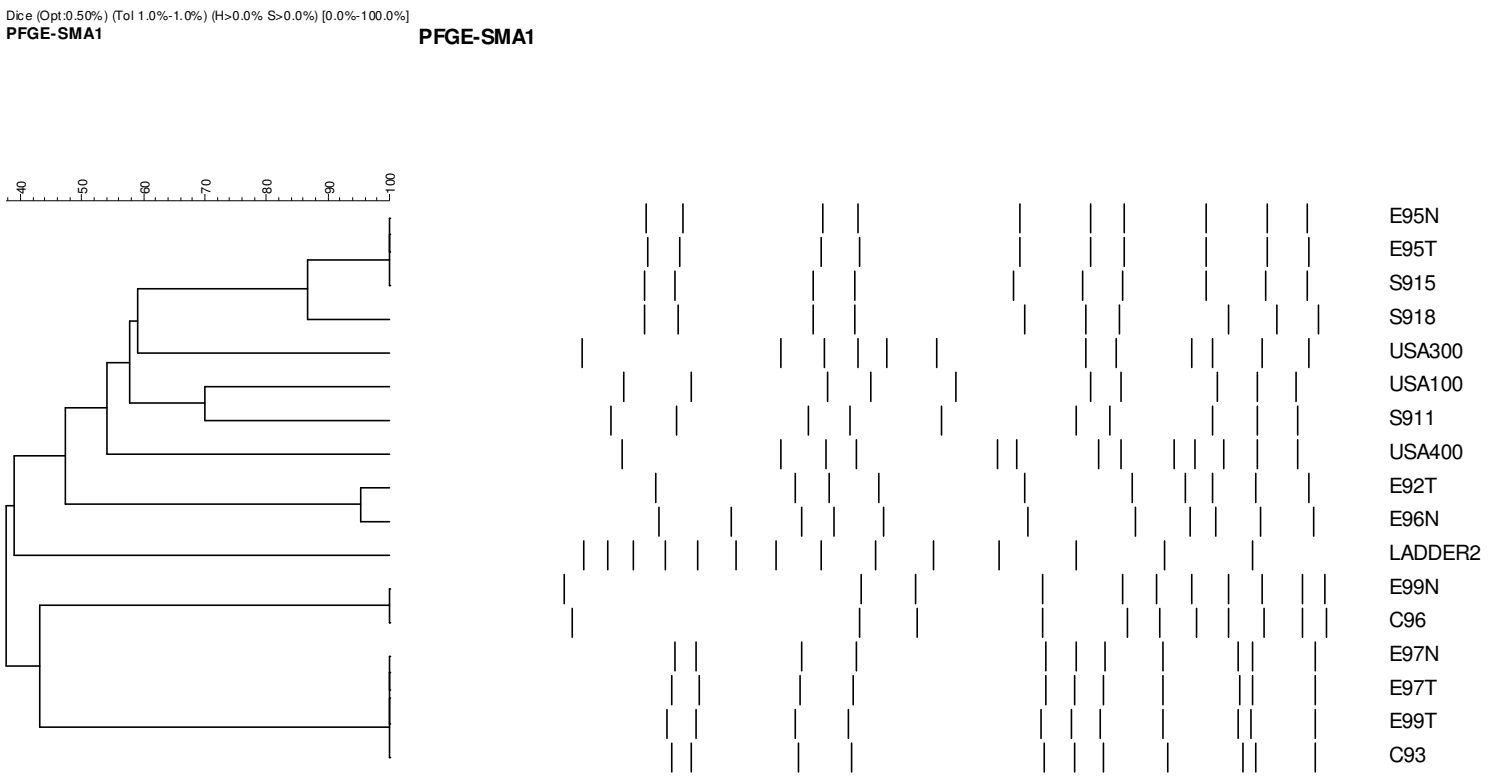


Figure 10. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 10 (E: Employee, C: Child, S: Surface).

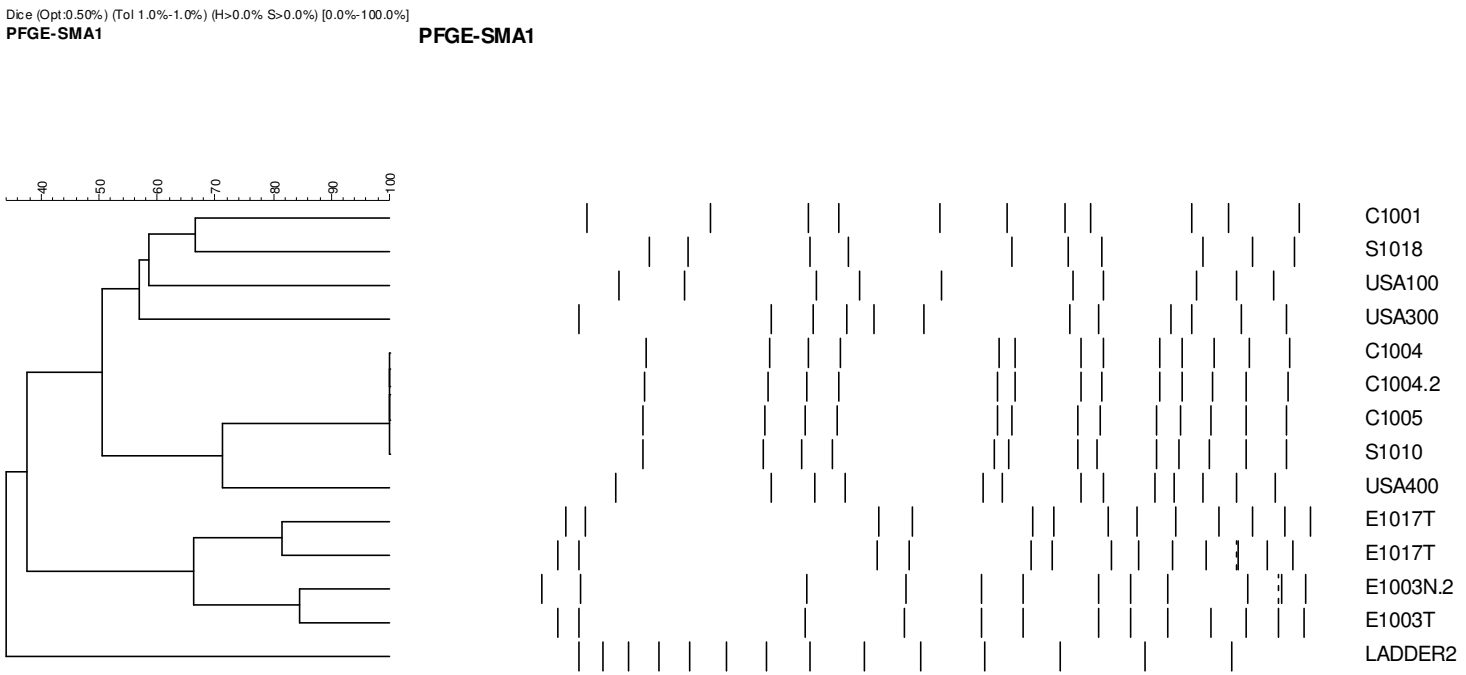


Figure 11. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 11 (E: Employee, C: Child, S: Surface).

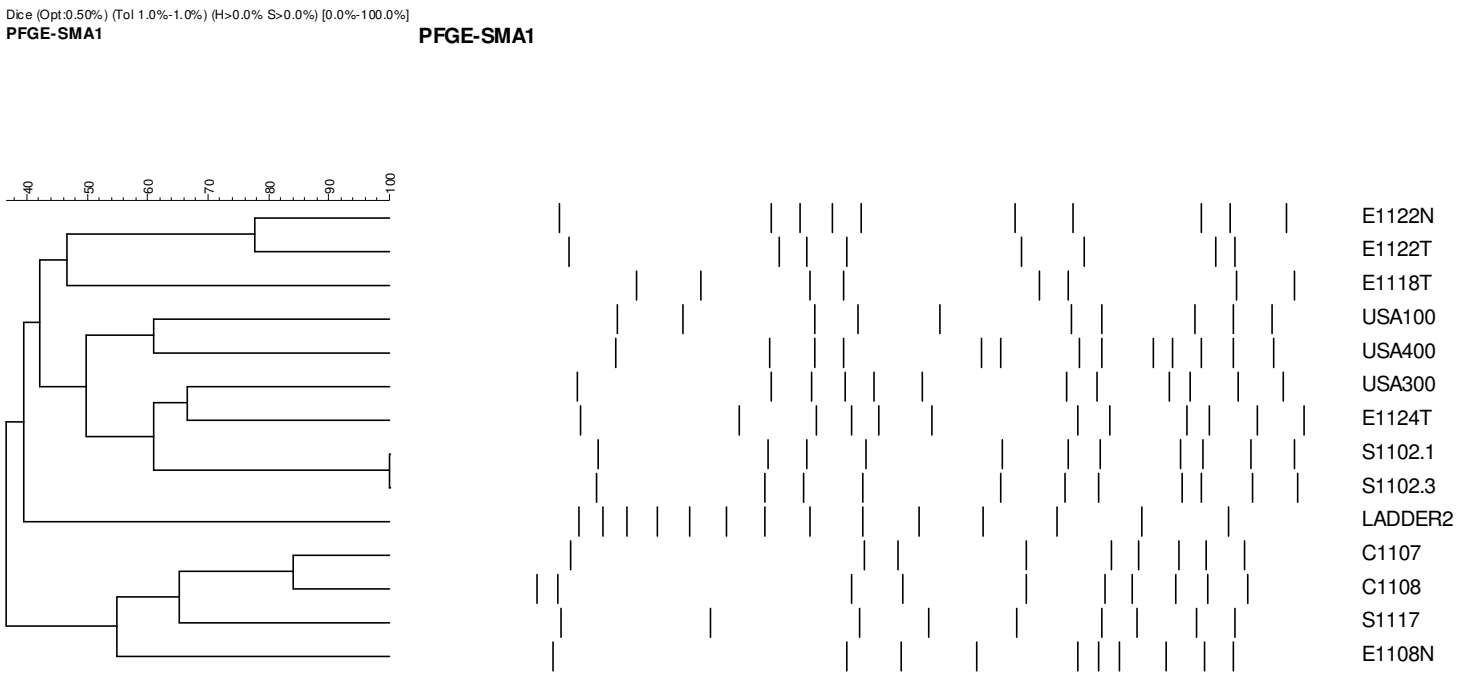


Figure 12. Pulsed field gel electrophoresis banding patterns for *S. aureus* isolates from Facility 12 (E: Employee, C: Child, S: Surface).

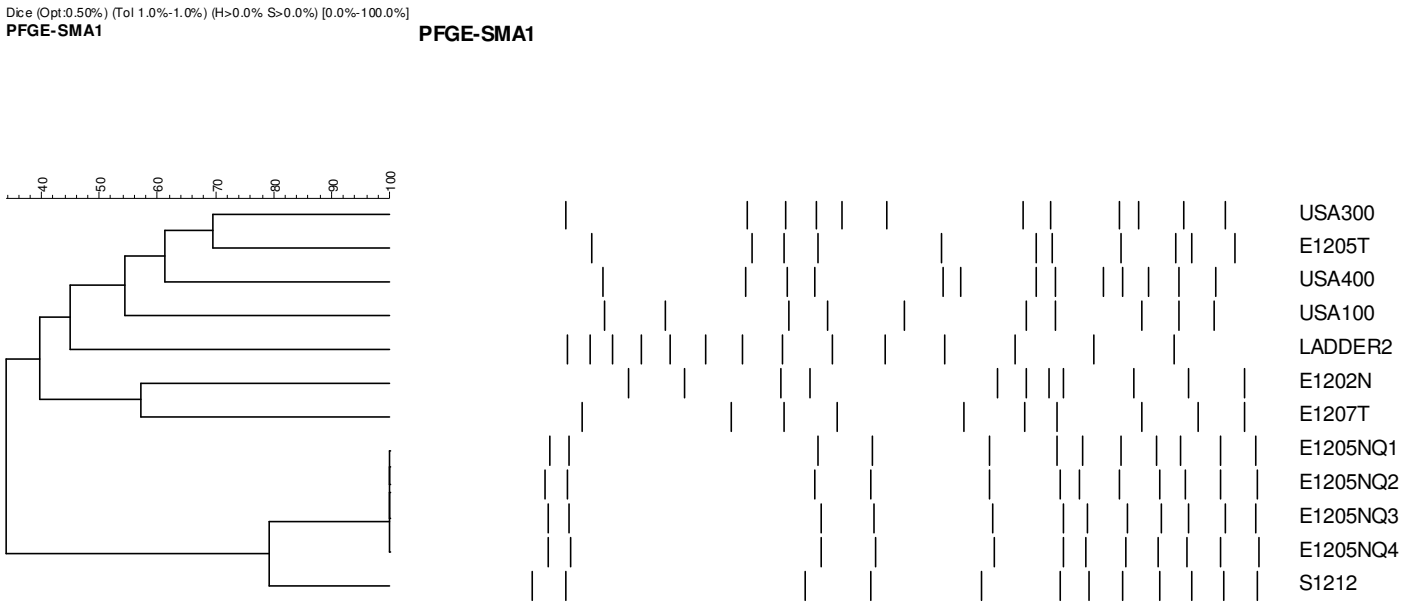


Figure 13. Pulsed field gel electrophoresis banding patterns for MRSA isolates (E: Employee, C: Child, S: Surface, U: Unexposed).

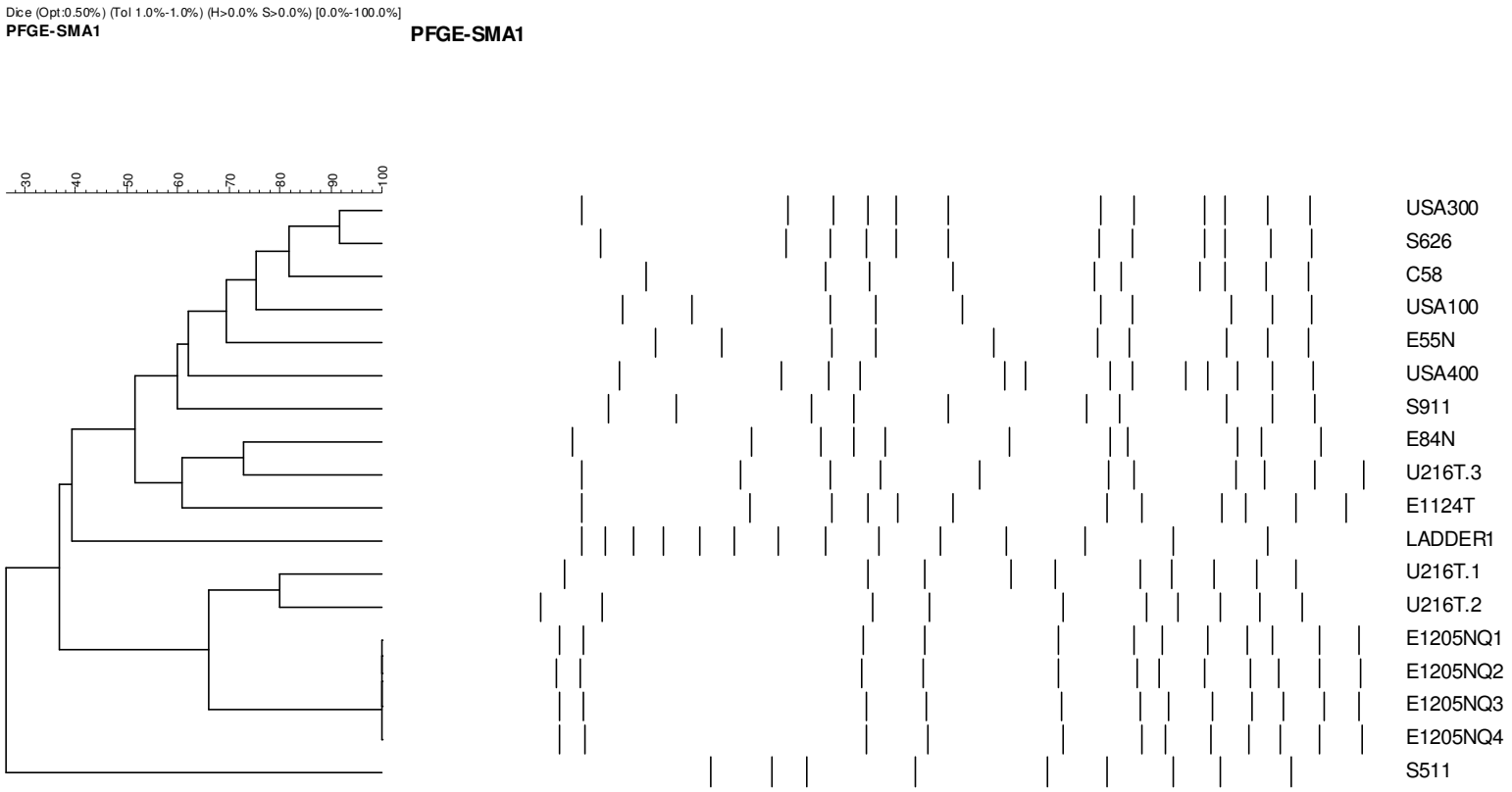


Figure 14. Pulsed field gel electrophoresis banding patterns for all *S. aureus* isolated from child care facilities (E: Employee, C: Child, S: Surface, U: Unexposed).

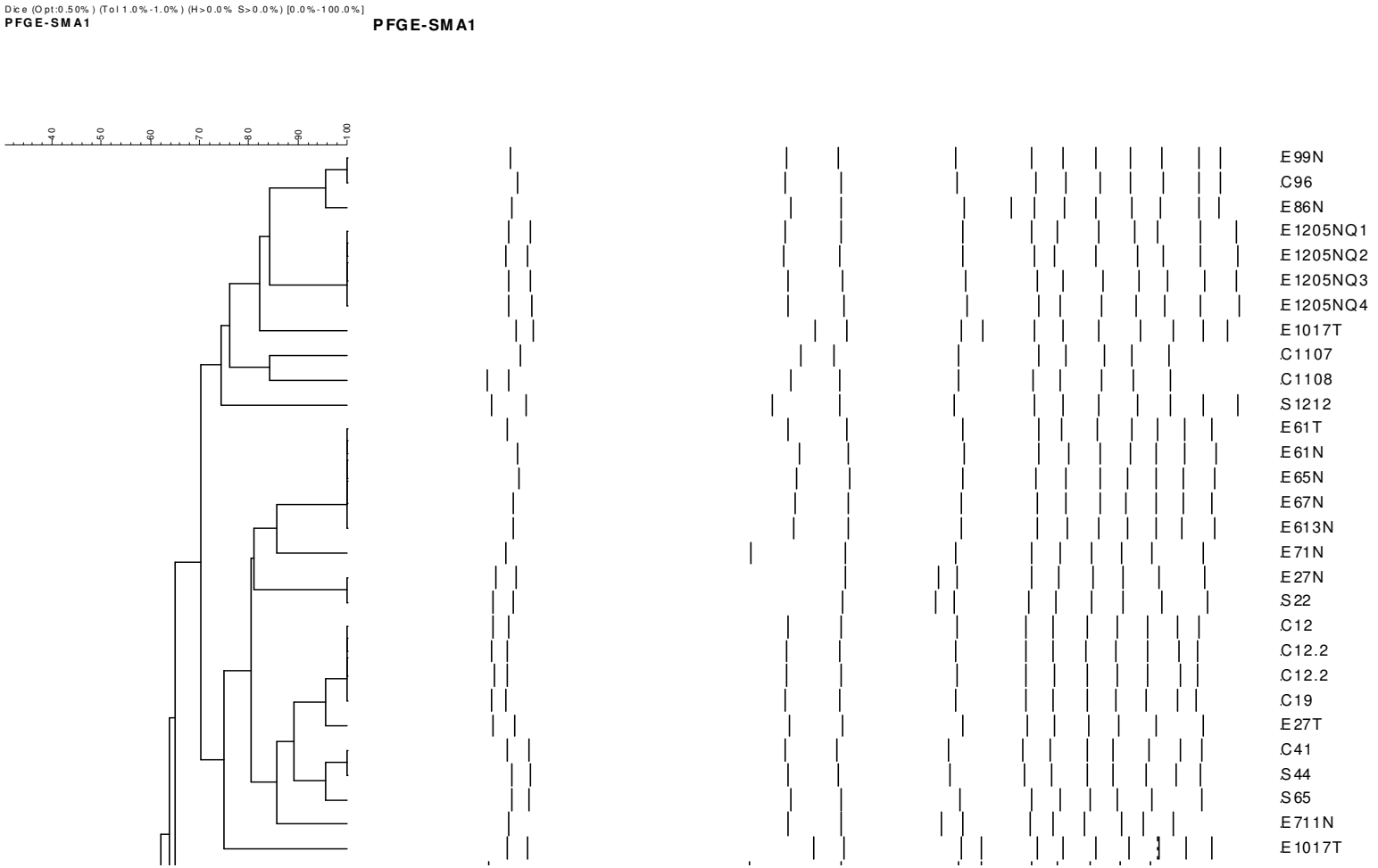


Figure 14 - continued

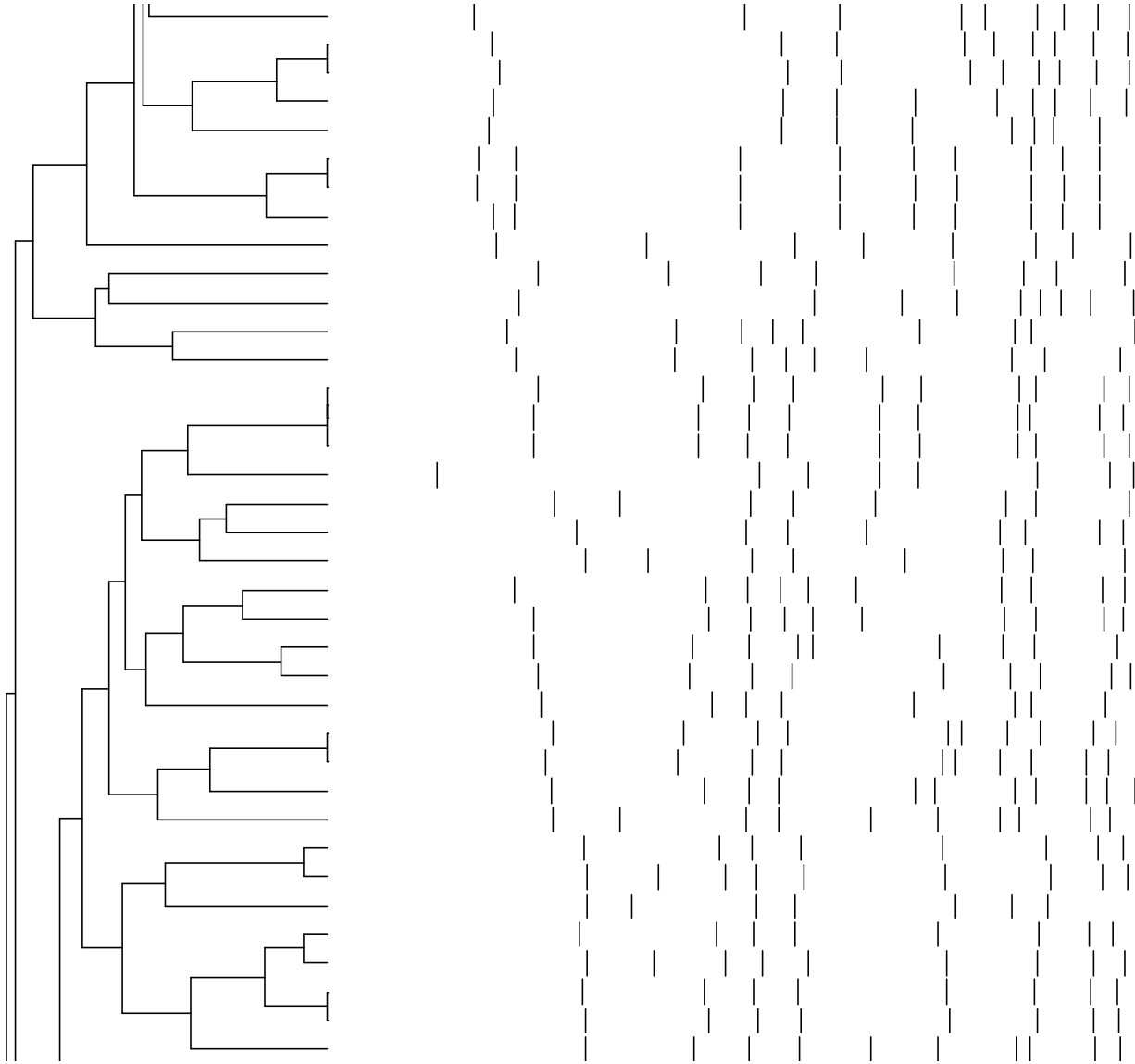
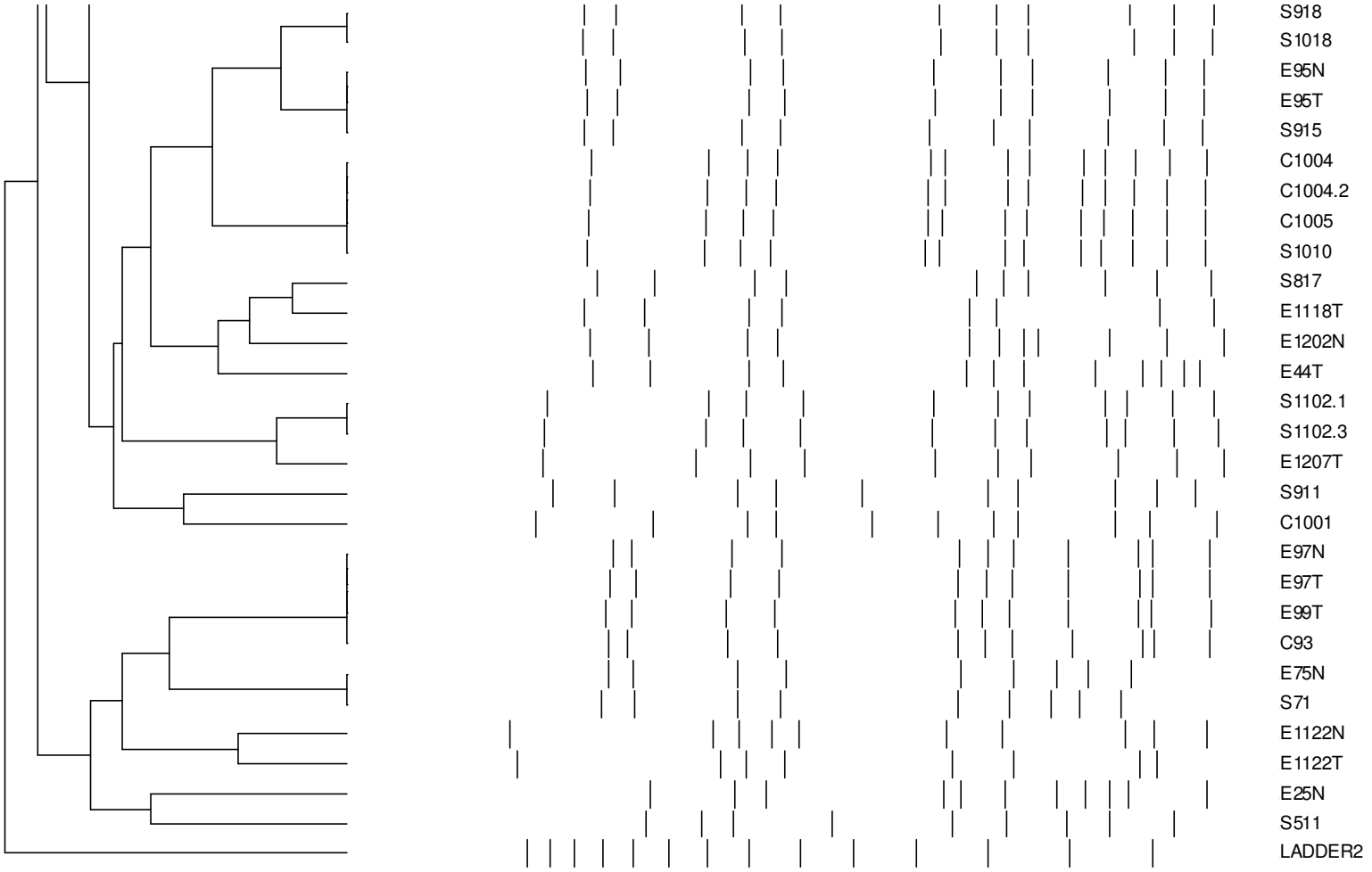


Figure 14 - continued



CHAPTER IV

MOLECULAR AND EPIDEMIOLOGICAL PREDICTORS OF *STAPHYLOCOCCUS AUREUS* COLONIZATION SITE IN A POPULATION WITH LIMITED NOSOCOMIAL EXPOSURE

Introduction

Staphylococcus aureus is a major human pathogen that can be transmitted through asymptomatic colonization (Lowy 1998). The recent adoption of active surveillance cultures (ASC) by the healthcare industry as a method to decrease rates of methicillin-resistant *Staphylococcus aureus* (MRSA) infections has brought to light the importance of anatomical location of carriage (Huskins 2007, Tacconelli 2009, McGinigle 2008). The anterior naris has long been considered the most consistent location of *S. aureus* carriage in humans, and therefore the most appropriate location for screening swabs (Williams 1963, Kluytmans 1997, Wertheim 2005). Recent studies, however, have suggested that this assumption may not hold in all situations. Some studies have shown that a substantial number of individuals are exclusive throat carriers, ranging from 7 – 32% of individuals colonized with *S. aureus* (Lee 2011, Meurman 2005, Mertz 2007, Ringberg 2006, Lauderdale 2010, Jager 2010).

Of direct concern to researchers and practitioners of ACS is the sensitivity of current swabbing practices, many of which involve nasal swabs only. Bignardi et. al. reported that, in one study, eliminating throat swabs from MRSA screening procedures would have decreased identified carriers by 19%, a figure higher than those if nares (9%) or perineum (7%) swabs were eliminated (Bignardi 2009). Mertz et. al. concluded in a *S. aureus* study published in 2007 that swabbing the throat as well as the nares increased sensitivity by 25.7% (Mertz 2007). Finally, in a study of 259 patients and 87 staff

members at a hospital in Sweden, the throat was the most common site of carriage. In this group, sampling solely from the nares would have identified 64% of *S. aureus* carriers, while sampling solely from the throat would have identified 83% (Nilsson 2006).

While the above findings are certainly compelling and cause for attention, most of the participants in those studies had exposure to a healthcare setting. It is unclear whether results from these studies can be generalized to individuals with little or no healthcare exposure. Hamdan-Partida et. al. reported that 38% of healthy community members carrying *S. aureus* had exclusive throat colonization, while only 22% of carriers were colonized solely in the nares (Hamdan-Partida 2010).

Moreover, there is evidence to suggest that colonization site may differ according to level of healthcare exposure. A large study in Switzerland found that probability of exclusive throat carriage increased as exposure to a healthcare environment decreased. The same study found that an age of 30 years or younger was a significant risk factor for exclusive throat colonization ($OR = 1.66$, $p < 0.001$), while exposure to the health care system was protective ($OR = 0.67$, $p < 0.001$) (Mertz 2009).

In response to these findings, Mertz et. al. state that "throat carriage may indeed be more common among healthy individuals than among individuals who are exposed to the health care system, but such a hypothesis requires confirmation by other investigators in different, non-health care populations" (Mertz 2009). By combining results of two cross-sectional studies of individuals with limited exposure to healthcare, this analysis aims to 1) add data to the growing body of knowledge regarding *S. aureus* nose and throat carriage in the general population and 2) determine molecular and epidemiological predictors of colonization location.

Materials and Methods

Data from two studies conducted at the University of Iowa College of Public Health were pooled. One source of data was a child care worker (CCW) study that enrolled child care employees and age- and gender- frequency matched students and personnel from the University of Iowa (these participants will be denoted as "University of Iowa adults", or UIA). The second source of data is a prevalence study of *S. aureus* colonization in a group thought to be representative of the general population in Iowa ("*Staphylococcus* in Rural Iowa" or SIRI).

Each study provided data on nose and throat carriage, questionnaire information, and *Staphylococcal* protein a (*spa*) typing. Detailed recruitment protocols for the three studies are available elsewhere (see Chapter 3 and Smith and Wardyn, unpublished data). Briefly, child care employees were contacted through the Iowa Department of Human Services list of regulated child care providers for the CCW study. University students and personnel (UIA) were contacted through list serves and mass e-mails to serve as a comparison group for the child care worker study. Nasal and throat swabs were collected from all individuals enrolled and cultured as previously described (Smith 2009).

Adults thought to be representative of the general population were recruited for the SIRI study from various locations, including churches and other community gatherings. *spa* typing was performed as described elsewhere (Harmsen 2003). Resulting *spa* types were classified into six categories based on published data (Ruppitsch 2006). Our categories consisted of complexes I – IV and VII as published in Ruppitsch et. al., as well as a sixth category ("None") that consisted of *spa* types that did not belong to any other complexes (Ruppitsch 2006). Three, eight, nine, two, six, and 66 isolates were categorized into complex I, II, III, IV, VII, and "None", respectively. Questionnaires administered to participants were extensive and collected demographic data, medical history, visitations or employment at hospitals or long-term care facilities, and exposure to animals.

Data analysis was conducted using SAS 9.2 (SAS Institute Inc., Cary, NC). The sample was categorized into four groups: carriers with *S. aureus* in both the nose and throat (N+T+), carriers with *S. aureus* the nose only (N+T-), carriers with *S. aureus* in the throat only (N-T+), and participants who did not carry *S. aureus* in either location (N-T-). A second analysis was conducted by collapsing variables to create N+ (any participant who was either an exclusive nose carrier or colonized in both nose and throat) and T+ (any participant who was either an exclusive throat carrier or colonized in both sites). Among *S. aureus* carriers, Fisher's exact test was performed to determine if the distribution of *spa* types differed among nose and throat carriers. Univariate analysis was conducted on the entire study population using ANOVA for age and Chi-square or Fisher's exact test for categorical variables. Multivariate generalized linear models were fit using stepwise model selection.

Results

Descriptive Statistics

This analysis utilizes data collected from 110 child care employees, 111 students and personnel affiliated with the University, and 120 adults recruited from various community locations around Iowa. The total number of participants was 340, 103 of which carried *S. aureus*. Approximately 30% (103/340) of participants carried *S. aureus*: 31 (30.0%) were colonized in the throat only, 44 (42.7%) were exclusive nose carriers, and 28 (27.2%) were colonized in both sites (Table 26).

Analysis among *S. aureus* Carriers

The distribution of *spa* types was not statistically different among the N+T- (i.e. among exclusive nose carriers) group when compared to the N+T+ group ($p = 0.60$), but

approached statistical significance in the group of exclusive throat carriers (N-T+) when compared to the same group ($p = 0.079$).

Univariate Predictors

Variables that predicted category (N-T-, N-T+, N+T-, or N+T+) at $p < 0.05$ included age, season, and race (Table 27 and 28). Significant differences were identified across the categories no carriage (N-T-), exclusive throat carriage (N-T+), and nose carriage (N+) for age, visiting a hospital, the participant or a close family member working in a hospital or long-term care facility, and race (Tables 30 and 31). Significant differences were identified across the categories no carriage (N-T-), exclusive nose carriage (N+T-), and throat carriage (T+) for the following variables: age, season, the participant or a close family member working in a hospital or long-term care facility, owning a cat, and race (Tables 32 and 33).

Multivariate Modeling

Variables that differed across categories at $p < 0.15$ (see tables 27 - 34) were entered into full generalized logit models. Results of multivariate analyses are shown in Tables 35 and 36. Predictors of exclusive throat carriage at $p < 0.05$ include age, season, and race. Predictors of exclusive nose carriage at $p < 0.05$ are season and race. Predictors of any nasal carriage include age and season, while predictors of any throat carriage were age, race, and gender.

Discussion

The prevalence of *S. aureus* in this sample from numerous populations in Iowa is 30.3%. National Health and Nutrition Examination Survey (NHANES) data collected

between 2003 and 2004 suggests that the prevalence of *S. aureus* colonization in the general United States population was 28.6%. However, the NHANES data may underestimate *S. aureus* presence due to the fact that only nasal samples were included (Gorwitz 2008). In this group of Iowa community members with limited exposure to healthcare settings, 30.0% of *S. aureus* carriers were colonized only in the throat, indicating that a substantial portion of positive participants would have tested negative had we not included throat cultures. These findings are similar to several previous studies that suggest throat culture could be an important addition to *S. aureus* screening procedures (Meurman 2005, Mertz 2007, Ringberg 2006, Lauderdale 2010, Jager 2010).

Our sample size of *S. aureus* carriers was small and limited our ability to thoroughly examine effects of molecular type on colonization location. Our ability to detect differences in *spa* type distribution was also reduced due to the large number of *spa* categories (six) used to classify the small number of isolates, and the fact that most of the isolates belonged to the "None" category. This dilemma emphasizes a shortcoming of characterizing isolates based on genetic sequences. *spa* typing is extremely discriminatory and the researcher often ends up with many groups that may not be clinically relevant, as reported in other studies of *S. aureus* (Strommenger 2008). Nevertheless, the finding that the *spa* type distribution among exclusive throat carriers was potentially different than the distribution among nose and throat carriers (the finding approached statistical significance with $p = 0.079$), despite the small numbers, suggests that further research is needed.

Our data confirm that nasal screening will identify the majority of *S. aureus* carriers. However, nearly one third of our *S. aureus* carriers would have been missed had

we not collected throat swabs. Missing 30.0% of carriers could have ramifications for ACS program success, as well as for population-based studies. If universal throat screening (in addition to nose screening) is not feasible for an institution, targeting specific populations for throat swabbing in addition to nasal swabs may be a consideration. We showed that younger age and non-white race were significant predictors of exclusive throat carriage. Institutions may find it necessary to target throat swabbing to specific individuals in order to increase efficacy of screening.

In conclusion, a substantial portion of this study population with limited exposure to healthcare settings carried *S. aureus* only in the throat. In multivariate analysis, age, season, race, and gender were the most important predictors of colonization location, with age and race significantly predicting exclusive throat carriage. These findings suggest that including a throat swab in addition to a nasal swab could play an important role in the success of ASC programs and population-based surveys, particularly when administered on community members with limited exposure to healthcare.

Table 26. Prevalence of *S. aureus* by colonization location in a study population with limited nosocomial exposure.

Colonized	**N+ / T+** **n** **Prevalence** **95% CI**	**N+ / T-**	**N- / T+**	**N- / T-**	**Total**
No	0	0	0	237 69.71% 64.67 – 74.74%	237
Yes	28 8.24% 5.17 – 11.30%	44 12.94% 9.23 – 16.66%	31 9.12% 5.91 – 12.32%	0	103
Total	28	44	31	237	340

N-T-: No carriage, N-T+: Exclusive throat carriage, N+T-: Exclusive nose carriage, N+T+: Nose and throat carriage

Table 27. Sample average and standard deviation of age for each colonization location group.

Groups	n	Mean	Standard Deviation	ANOVA p-value
N+T+	26	25.50	9.73	<0.0001
N+T-	44	38.32	17.80	
N-T+	31	28.97	12.23	
N-T-	236	42.18	18.52	

N-T-: No carriage, N-T+: Exclusive throat carriage, N+T-: Exclusive nose carriage, N+T+: Nose and throat carriage

Table 28. Frequencies and percentages for binary and categorical variables. p values are from Chi-squared tests or Fisher's exact test.

Variable	Level	N-T-		N-T+		N+T-		N+T+		p
		n	Percent	n	Percent	n	Percent	n	Percent	
Season	Spr/Sum	72	30.51	11	35.48	22	50.00	6	22.22	0.047
Gender	Male	56	23.73	8	25.81	8	18.18	9	34.62	0.48
Tobacco	1	51	21.61	3	9.68	12	27.27	6	22.22	0.32
Chronic medical condition	1	52	22.13	6	19.35	8	18.18	6	22.22	0.93
Steroid use	1	7	2.97	0	0	1	2.27	0	0	1.00
Recent use of antibiotics	1	46	19.57	4	12.90	9	20.93	0	0	0.065
Recent influenza-like illness	1	69	29.87	9	30.00	8	18.18	6	22.22	0.38
Recent hospitalization	1	16	6.78	0	0	5	11.36	3	11.11	0.18
Participant or family member who visits hospital	1	41	17.37	1	3.23	6	13.64	1	3.7	0.062
Participant or family member who visits long term care facility	1	24	10.17	1	3.23	1	2.27	1	3.7	0.22
Participant or family member who works in a hospital or long-term care facility	1	54	22.88	13	41.94	15	34.09	9	33.33	0.065
Recent skin and soft tissue infection	1	8	3.39	0	0	3	6.82	1	3.7	0.41
Recent MRSA infection	1	2	0.85	0	0	1	2.27	0	0	0.66
Contact with chickens	1	21	8.90	2	6.45	4	9.09	3	11.11	0.94
Contact with cattle	1	35	14.83	4	12.90	5	11.36	5	18.52	0.85
Contact with swine	1	16	6.78	2	6.45	2	4.55	4	14.81	0.43
Contact with horses	1	46	19.49	3	9.68	5	11.36	6	22.22	0.32

Table 28 - continued

Contact with goats	1	20	8.47	2	6.45	2	4.55	1	3.70	0.83
Contact with sheep	1	18	7.63	1	3.23	2	4.55	2	7.41	0.86
Chickens on property	1	5	2.12	0	0	2	4.55	0	0	0.51
Swine on participant's property	1	1	0.42	0	0	0	0	0	0	1.00
Cats on participant's property	1	88	37.29	7	22.58	17	38.64	4	14.81	0.052
Dogs on participant's property	1	90	38.14	12	38.71	18	40.91	10	37.04	0.98
White race	1	228	96.61	25	80.65	38	86.36	25	96.15	0.0012
Race	American Indian	3		0		0		0		0.0028
	Asian	1		3		3		1		
	Black	1		1		2		0		
	Hispanic	2		1		0		0		
	Other	1		1		1		0		
	White	228		25		38		25		

N-T-: No carriage, N-T+: Exclusive throat carriage, N+T-: Exclusive nose carriage, N+T+: Nose and throat carriage

Table 29. Odds ratios and confidence intervals for exclusive throat carriage, exclusive nose carriage, and nose and throat carriage using no colonization as a reference group.

Variable	N-T+		N+T-		N+T+	
	OR	95% CI	OR	95% CI	OR	95% CI
Age*	0.94	0.91 - 0.97	0.98	0.97 - 1.01	0.91	0.87 - 0.95
Tobacco	0.38	0.11 - 1.33	1.36	0.65 - 2.82	1.03	0.39 - 2.70
Chronic medical condition	0.84	0.32 - 2.16	0.78	0.34 - 1.78	1.01	0.38 - 2.62
Steroid use	NNA	NA	0.76	0.091 - 6.34	NA	NA
Recent use of antibiotics	0.60	0.20 - 1.82	1.08	0.48 - 2.42	NA	NA
Recent influenza-like illness	1.0062	0.43 - 2.31	0.52	0.23 - 1.18	0.67	0.25 - 1.73
Recent hospitalization	NA	NA	1.76	0.61 - 5.08	1.71	0.46 - 6.32
Participant or family member who visits hospital	0.15	0.021 - 1.19	0.75	0.29 - 1.89	0.18	0.024 - 1.38
Participant or family member who visits long term care facility	0.29	0.038 - 2.25	0.20	0.027 - 1.55	0.33	0.044 - 2.61
Participant or family member who works in a hospital or long-term care facility	2.43	1.12 - 5.28	1.74	0.87 - 3.48	1.68	0.71 - 3.96
Recent skin and soft tissue infection	NA	NA	2.08	0.53 - 8.18	1.09	0.13 - 9.11
Recent MRSA infection	NA	NA	2.72	0.24 - 30.67	NA	NA
Contact with chickens	0.71	0.15 - 3.16	1.02	0.33 - 3.14	1.28	0.35 - 4.60
Contact with cattle	0.85	0.28 - 2.58	0.73	0.27 - 1.99	1.31	0.46 - 3.67
Contact with swine	0.94	0.20 - 4.33	0.65	0.14 - 2.95	2.39	0.73 - 7.75
Contact with horses	0.44	0.12 - 1.51	0.52	0.19 - 1.41	1.18	0.45 - 3.09

Table 29 - continued

Contact with goats	0.74	0.16 - 3.35	0.51	0.11 - 2.28	0.41	0.053 - 3.22
Contact with sheep	0.40	0.052 - 3.13	0.57	0.12 - 2.57	0.96	0.21 - 4.42
Chickens on property	NA	NA	2.20	0.41 - 11.71	NA	NA
Swine on participant's property	NA	NA	NA	NA	NA	NA
Cats on participant's property	0.49	0.20 - 1.18	1.05	0.54 - 2.05	0.29	0.098 - 0.87
Dogs on participant's property	1.02	0.47 - 2.21	1.12	0.58 - 2.16	0.95	0.41 - 2.17
White race	0.14	0.047 – 0.45	0.22	0.073 – 0.68	0.87	0.11 – 7.30

*Continuous variable – for each year older the participant is, odds ratio increases by a factor of 0.94.
NA: not applicable due to cell counts of 0

Table 30. Sample mean and standard deviation for age across the categories of no carriage, exclusive throat carriage, and the collapsed variable nose carriage.

Groups	n	Mean	Standard Deviation	ANOVA p-value
N+	70	33.56	16.45	<0.0001
N-T+	31	28.97	12.23	
N-T-	236	42.18	18.52	

N+: Any nose carriage, N-T+: Exclusive throat carriage, N-T-: No carriage

Table 31. Frequency and percentages across the categories of no carriage, exclusive throat carriage, and the collapsed variable nose carriage.

Variable	Level	N-T-		N-T+		N+		p
		n	Percent	n	Percent	n	Percent	
Season	Spr/Sum	72	30.51	11	35.48	28	39.44	0.35
Gender	Male	56	23.73	8	25.81	17	24.29	0.96
Tobacco	1	51	21.61	3	9.68	18	25.35	0.20
Chronic medical condition	1	52	22.13	6	19.35	14	19.72	0.87
Steroid use	1	7	2.97	0	0	1	1.41	0.85
Recent use of antibiotics	1	46	19.57	4	12.90	9	12.86	0.33
Recent influenza-like illness	1	69	29.87	9	30.00	14	19.72	0.23
Recent hospitalization	1	16	6.78	0	0	8	11.27	0.11

Table 31 - continued

Participant or family member who visits hospital	1	41	17.37	1	3.23	7	9.86	0.050
Participant or family member who visits long term care facility	1	24	10.17	1	3.23	2	2.82	0.079
Participant or family member who works in a hospital or long-term care facility	1	54	22.88	13	41.94	24	33.80	0.027
Recent skin and soft tissue infection	1	8	3.39	0	0	4	5.63	0.44
Recent MRSA infection	1	2	0.85	0	0	1	1.41	0.66
Contact with chickens	1	21	8.90	2	6.45	7	9.86	0.85
Contact with cattle	1	35	14.83	4	12.9	10	14.08	0.95
Contact with swine	1	16	6.78	2	6.45	6	8.45	0.88
Contact with horses	1	46	19.49	3	9.68	11	15.49	0.34
Contact with goats	1	20	8.47	2	6.45	3	4.23	0.47
Contact with sheep	1	18	7.63	1	3.23	4	5.63	0.78
Chickens on property	1	5	2.12	0	0	2	2.82	0.82
Swine on participant's property	1	1	0.42	0	0	0	0	1.00
Cats on participant's property	1	88	37.29	7	22.58	21	29.58	0.17
Dogs on participant's property	1	90	38.14	12	38.71	28	39.44	0.98
White race	1	228	96.61	25	80.65	63	90	0.0013

N-T-: No carriage, N-T+: Exclusive throat carriage, N+: Any nose carriage. p-values are from Chi-squared or Fisher's exact test.

Table 32. Sample mean and standard deviation for age across the categories of no carriage, exclusive nose carriage, and the collapsed variable throat carriage.

Groups	n	Mean	Standard Deviation	ANOVA p-value
T+	57	27.39	11.20	<0.0001
N+T-	44	38.32	17.80	
N-T-	236	42.18	18.52	

N-T-: No carriage, N+T-: Exclusive throat carriage, T+: Any throat carriage

Table 33. Frequency and percentages across the categories of no carriage, exclusive nose carriage, and the collapsed variable throat carriage.

Variable	**Level**	**N-T-**		**N+T-**		**T+**		**p**
		n	**Percent**	**n**	**Percent**	**N**	**Percent**	
Season	Spr/Sum	72	30.51	22	50	17	29.31	0.033
Gender	Male	56	23.73	8	18.18	17	29.82	0.38
Tobacco	1	51	21.61	12	27.27	9	15.52	0.34
Chronic medical condition	1	52	22.13	8	18.18	12	20.69	0.83
Steroid use	1	7	2.97	1	2.27	0	0	0.53

Table 33 - continued

Recent use of antibiotics	1	46	19.57	9	20.93	4	6.90	0.062
Recent influenza-like illness	1	69	29.87	8	18.18	15	26.32	0.27
Recent hospitalization	1	16	6.78	5	11.36	3	5.17	0.45
Participant or family member who visits hospital	1	41	17.37	6	13.64	2	3.45	0.025
Participant or family member who visits long term care facility	1	24	10.17	1	2.27	2	3.45	0.099
Participant or family member who works in a hospital or long-term care facility	1	54	22.88	15	34.09	22	37.93	0.035
Recent skin and soft tissue infection	1	8	3.39	3	6.82	1	1.72	0.42
Recent MRSA infection	1	2	0.85	1	2.27	0	0	0.40
Contact with chickens	1	21	8.90	4	9.09	5	8.62	0.99
Contact with cattle	1	35	14.83	5	11.36	9	15.52	0.81
Contact with swine	1	16	6.78	2	4.55	6	10.34	0.53
Contact with horses	1	46	19.49	5	11.36	9	15.52	0.38
Contact with goats	1	20	8.47	2	4.55	3	5.17	0.64
Contact with sheep	1	18	7.63	2	4.55	3	5.17	0.83
Chickens on property	1	5	2.12	2	4.55	0	0	0.24
Swine on participant's property	1	1	0.42	0	0	0	0	1.00
Cats on participant's property	1	88	37.29	17	38.64	11	18.97	0.025
Dogs on participant's property	1	90	38.14	18	40.91	22	37.93	0.93
White race	1	228	96.61	38	86.36	50	87.72	0.0030

N-T-: No carriage, N+T-: Exclusive nose carriage, T+: Any throat carriage. p-values are from Chi-squared or Fisher's exact test.

Table 34. Odds ratios and confidence intervals for the collapsed nose and throat variables using no colonization as a reference group.

Variable	N+		T+	
	OR	**95% CI**	**OR**	**95% CI**
Age*	0.97	0.95 - 0.98	0.93	0.91 - 0.96
Tobacco	1.23	0.66 - 2.28	0.66	0.30 - 1.47
Chronic medical condition	0.86	0.44 - 1.67	0.91	0.45 - 1.86
Steroid use	0.46	0.05 - 3.86	NA	NA
Recent use of antibiotics	0.60	0.28 - 1.31	0.30	0.10 - 0.88
Recent influenza-like illness	0.57	0.30 - 1.10	0.83	0.43 - 1.61
Recent hospitalization	1.74	0.71 - 4.26	0.75	0.21 - 2.66
Participant or family member who visits hospital	0.52	0.22 - 1.21	0.16	0.039 - 0.72
Participant or family member who visits long term care facility	0.25	0.059 - 1.11	0.31	0.072 - 1.37
Participant or family member who works in a hospital or long-term care facility	1.72	0.96 - 3.06	2.05	1.11 - 3.79
Recent skin and soft tissue infection	1.70	0.49 - 5.82	0.50	0.061 - 4.07
Recent MRSA infection	1.67	0.14 - 18.70	NA	NA
Contact with chickens	1.11	0.45 - 2.75	0.96	0.34 - 2.68
Contact with cattle	0.94	0.44 - 2.01	1.05	0.47 - 2.33
Contact with swine	1.26	0.47 - 3.37	1.58	0.59 - 4.25
Contact with horses	0.75	0.36 - 1.55	0.75	0.34 - 1.65
Contact with goats	0.47	0.13 - 1.65	0.58	0.16 - 2.05
Contact with sheep	0.72	0.23 - 2.21	0.66	0.18 - 2.32

Table 34 - continued

Chickens on property	1.33	0.25 - 7.05	NA	NA
Swine on participant's property	NA	NA	NA	NA
Cats on participant's property	0.70	0.39 - 1.25	0.39	0.19 - 0.79
Dogs on participant's property	1.05	0.61 - 1.81	0.99	0.54 - 1.79
White race	0.31	0.11 - 0.90	0.25	0.086 - 0.72

*Continuous variable – for each year older the participant is, odds ratio increases by a factor of 0.94.
NA: not applicable due to cell counts of 0

Table 35. Odds ratio estimates from the generalized linear model models of nose carriage vs. no carriage and exclusive throat carriage vs. no carriage.

Effect	Level	Nasal carriage Vs. No carriage				Exclusive throat carriage Vs. No carriage		
		OR	95% CI		p	OR	95% CI	p
Age	Each year*	0.97	0.95	0.98	0.00060	0.94	0.91 – 0.97	0.0013
Season	Fall/Winter	Ref	--	--		Ref	--	
	Spring/Summer	2.25	1.22	4.12	0.0053	2.31	0.97 - 5.46	0.057
Race	White	Ref	--	--		Ref	--	
	Non white	2.88	0.97	8.51	0.056	4.70	1.37 – 16.10	0.014

*Continuous variable – for each year older the participant is, odds ratio increases by a factor of 0.969.

Table 36. Odds ratio estimates from the generalized linear model models of throat carriage vs. no carriage and exclusive nose carriage vs. no carriage.

Effect	Level	Throat carriage vs. no carriage			Exclusive nasal carriage vs. no carriage		
		OR	**95% CI**	**p**	**OR**	**95% CI**	**p**
Age	Each year*	0.93	0.90 - 0.96	<0.0001	0.98	0.96 – 1.00	0.12
Season	Fall/Winter	Ref	--		Ref	--	
	Spring/Summer	1.73	0.85 - 3.52	0.13	3.13	1.55 – 6.33	0.0015
Race	White	Ref	--		Ref	--	
	Non white	3.20	1.00 - 10.21	0.050	4.67	1.45 – 15.03	0.0098
Gender	Female	Ref	--		Ref	--	
	Male	3.23	6.94 - 1.50	0.0026	0.93	0.39 - 2.21	0.86

*Continuous variable – for each year older the participant is, odds ratio increases by a factor of 0.93.

CHAPTER V

DISCUSSION AND CONCLUSIONS

Summary of Findings

Carriage of respiratory bacteria (mainly *Streptococcus pneumoniae*, *Staphylococcus aureus*, *Haemophilus influenzae*, and *Moraxella catarrhalis*) has been studied extensively in child care attendees. Prevalence of these pathogens and resistance rates among them vary widely, and the introduction of the pneumococcal vaccine has the potential to alter patterns of carriage in this population. Molecular characterization of bacteria in child care facilities, primarily of *S. pneumoniae*, has shown that each facility hosts its own bacteriological profile, but can also serve as a community reservoir for epidemic and internationally-recognized clones. Limitations in current research on this topic exist. The majority of data is cross-sectional with no comparison group, and there has been little research published since 2000 on child care employees, bacterial contamination of environmental surfaces, and bacteria causing diarrheal illness.

Child care employees do not appear to be at increased risk of carrying *S. aureus* or MRSA, but may be at increased risk of carrying *S. aureus* that is resistant to erythromycin when compared to individuals not employed at a child care facility. We found evidence of transmission among employees, children, and environmental surfaces in several facilities; however, the *S. aureus* population in child care facilities remains heterogeneous overall. While the results of predictors of carriage among child care employees should be viewed with caution due to a high degree of collinearity between several variables, it may be prudent to recommend that facilities implement hand washing by children and staff upon arrival at the facility.

In the analysis of data collected from individuals with limited exposure to the healthcare setting (Chapter 4), a substantial proportion of *S. aureus* carriers would have

been missed had we not included throat swabs in our sample collection. Age and race are significant predictors of colonization location in this population.

Child Care Facilities and the "Hygiene Hypothesis"

The first manifestation of the hygiene hypothesis was postulated in 1989 by David Strachan, when he suggested that smaller family size, improved household amenities, and higher levels of personal cleanliness resulted in decreased disease transmission in families, which might in turn cause increased risk of hay fever (Strachan 1989). Since that time, the hygiene hypothesis has been connected with atopy, asthma, type I diabetes, and inflammatory bowel disease (Bjorksten 2009). It is thought that overt infections and exposures to both non-pathogenic microorganisms and non-viable microbial compounds alter the risk of these diseases by influencing an individual's innate and adaptive immune response (Vercelli 2006). Given the number of children who attend group care, the hygiene practices at child care facilities could play an important role in rates of immune-mediated diseases if level of hygiene is in fact a significant predictor of those diseases.

If one is to accept increased hygiene as a risk factor for disease, the next (seemingly) logical conclusion would be to increase exposure to infectious compounds. In a child care facility, this could manifest in several ways, including reduced hand hygiene and sanitation of surfaces. Others have suggested probiotic and utilizing fragments of bacteria to stimulate an immune response without increasing risk of harmful infections (Okada 2010, Petrovsky 2010). These "solutions" remain hypothetical, since the implications of the hygiene hypothesis are unknown. Any recommendation to decrease its effects would be premature at this point, since the hygiene hypothesis has been neither thoroughly accepted nor explained. Given the plethora of evidence supporting the benefits of adequate hygiene, hand washing and surface disinfection should continue to be emphasized in child care facilities (Hadler 1986, Brady 2005, Lee 2008).

Environmental Surfaces

Adequate surface sanitation and disinfection is yet another challenge faced by child care practitioners. The number of surfaces with which children and employees come into contact is substantial and sanitizing all of these surfaces on a regular basis with the goal of decreasing transmission is likely impossible. Lee et. al. found bacterial contamination on every sample collected in four child care rooms and identified different 29 species (Lee 2007). Such overwhelming prevalence of bacteria will lead to sanitation and disinfection failures, no matter how diligent the facility is in maintaining cleanliness.

A more targeted approach to cleaning could be developed if detailed data were available on which surfaces are most likely to be contaminated. Child care facilities currently address this issue in a partial manner (for example, many facilities disinfect toys that have been in a child's mouth, and restrooms and diaper-changing areas are a major focus of disinfection efforts). However, data on bacterial load, types of surfaces (e.g. wood, plastic, or cloth) most likely to harbor bacteria, and any generalizations that can be made about location of those surfaces is scarce. There is evidence that MRSA can survive for over eight days on irregular surfaces. Huang et. al. published data suggesting irregular surfaces, such as a patient chart made from corrugated plastic and cloth curtains, could harbor MRSA in patient hospital rooms (Huang 2006). Given the wide variety of materials and textures in a child care facility, it is likely that some are more likely to harbor bacteria than others. Moreover, ease of disinfection may also play a role in bacterial load on different surfaces. Wiping down a flat plastic table with a bleach solution is easier and more convenient than trying to sanitize an infant activity center (Figure 15). Given the limited time employees have to focus on cleaning, targeted hygiene could be a way of maximizing the benefits of cleaning in terms of reducing risk of infection. Further studies are needed to guide this targeted approach.

Infectious Disease Modeling

A fair number of predictors of *S. aureus* colonization in these data were related to people surrounding the participant, not necessarily the participant him/herself. For example, in multivariate analysis, reporting a family member who had an influenza-like illness in the past 12 months was a significant predictor of *S. aureus* carriage in adults. Moreover, we identified *S. aureus* strain ST-398, a clone that until now has only been associated with animal contact, in a child care employee reporting no contact with animals either herself or in family members. Many of the other participants (both children and employees) at that facility, however, reported contact with animals. This case is a noticeable example of a key trait of infectious disease epidemiology: characteristics and behaviors of those in close contact with an individual can play a role in that individual's risk. This trait gives rise to a number of challenges in infectious disease research. There are practical, monetary, and ethical limitations to how much data can be collected regarding individuals with whom a participant has contact. Data collected on extensive networks of people around a participant becomes more expensive and less accurate the further from the participant information is elicited.

Viewing an individual or even an institute as an isolated, single entity may be misguided. Figure 16 illustrates only some of the potential pathways of *S. aureus* transmission to a child care employee. In theory, the chain of transmission could go on forever, but data collection has to stop somewhere due to limited time and resources (e.g. collecting data about friends of relatives of the participant will lead to high costs for little additional benefit, reflecting a data collection "law of diminishing returns"). Nevertheless, characteristics of these highly-removed friends or family members may still contribute to the risk of the participant. Such complex networks and interactions can be addressed using mathematical models of infectious disease transmission.

A mathematical model is "an explicit mathematical description of the simplified dynamics of a system" (Nelson 2007). In terms of infectious disease transmission, this

involves utilizing known characteristics and relationships of an agent, its hosts, and its environment to approximate and predict epidemiologic behavior (Coburn 2009). Mathematical modeling of infectious diseases can be used to predict size, duration, and geographical spread of an outbreak; to analyze potential effects of different scenarios and interventions, thereby guiding control measures and policy decisions; in retrospective analysis to assess strengths and limitations of courses of action; and in many other situations (Louz 2010).

Transmission modeling has been conducted on MRSA in healthcare settings. Results of these studies have identified important predictors of transmission, as well as suggested the most effective means of control (McBryde 2007, Grundmann 2002, Bootsma 2006). Moreover, child care attendance has been an important component of transmission modeling of *Haemophilus influenzae* in children (Koopman 2005). In the same way that McBryde et. al. concluded that transmission of MRSA was not sustained through secondary MRSA cases within an intensive care unit, but rather through admitting colonized patients, transmission modeling has the potential to provide valuable insight into behavior of *S. aureus* in child care centers. Because several respiratory bacteria (e.g. *Streptococcus pneumoniae* and *H. influenzae*) behave in a similar manner to *S. aureus*, a good *S. aureus* transmission model may even be applicable to multiple pathogens (Wolf 2007 Typical, Wolf 2007 Atypical).

There are limitations to infectious disease modeling. Models can become too complex to provide insight into actual situations. Since the goal of modeling is ultimately to make public health decisions, decision-makers need to be able to understand the model. Many models are built on specific research questions, so existing models may or may not be applicable to different scenarios. One of the biggest criticisms of any type of mathematical model is that assumptions need to be made at some point. Those assumptions are not always reflective of the true state of affairs. Finally, the type of model that would be ideal for representing transmission in child care facilities (a contact

network model) requires detailed empirical data that is not always available, so initial construction can be difficult (Nelson 2007). Despite these limitations, as well-constructed model of transmission of *S. aureus* within child care facilities could prove a valuable tool for future research.

Advantages and Opportunities for Future Child Care Research

Even if spending time in a child care facility does not increase risk of a particular disease, child care centers are still valuable as sources of surveillance and research data. Child care facilities are readily available for recruitment in virtually all populated areas of the United States. In terms of potential study populations, this was a very good group to work with. The directors who participated were knowledgeable of infectious disease concepts and were genuinely concerned about reducing transmission within their facilities. As long as multiple facilities are included, the child population attending group care is likely representative of a large proportion of children residing in a given geographic area.

In addition to research on environmental surfaces and transmission modeling mentioned above, examples of future research concerning child care facilities include more detailed longitudinal transmission studies, antibiotic resistance surveillance, and prevalence of a variety of emerging pathogens. Further research could be conducted to characterize erythromycin resistance in employees, as well as in attendees and on surfaces.

Further analysis of the erythromycin resistance data presented in Chapter 3 is warranted. The crude odds ratio suggests employees are at increased risk of carrying erythromycin-resistant *S. aureus*, but their risk also might be complicated by presence of another carrier in the facility. Moreover, many other predictors were not analyzed. A comprehensive multivariate analysis is necessary to elucidate risk of carrying erythromycin-resistant *S. aureus* in child care employees.

Another important question is whether or not higher carriage rates of erythromycin resistant *S. aureus* actually translate into clinically relevant infections in child care employees. Such a study could be carried out in a retrospective fashion by obtaining medical histories of those carrying resistant organisms as well as a control group. Future studies should also consider collecting data on potential covariates, as this study was not designed to collect data on potential risk factors and confounders for erythromycin-resistant variants of this organism. In particular, more detailed histories of macrolide usage would be helpful. A more time- and resource-intensive option would be a prospective or longitudinal study that involves following child care employees and children over time, establishing carriage status and obtaining information on infections that develop.

As described in Chapter 2, the dynamics of *Streptococcus pneumoniae* transmission have been studied in child care facilities using molecular characterization techniques. Similar studies of *S. aureus* would provide data on transmission between employees, children, and the environment. One particularly thorough method to collect such data is a longitudinal design with periodic sampling over time that included molecular typing of each isolate. Such a study would provide valuable insight into acquisition and turnover rates in carriers and on surfaces, the length of carriage, and frequency of transmission to other participants or surfaces.

In addition to risk of infections, child care providers face a host of additional threats to their health, including physical and psychological ailments (Bright 1999). Considering the wide variety of occupational hazards faced by this group, the amount of current data is likely insufficient to accurately characterize risk, let alone allow for successful remediation of such risks. Large scale prospective studies of child care workers collecting data on numerous health outcomes, including undiagnosed respiratory and diarrheal diseases, injuries, and psychological stressors could provide a good starting point for making child care a less hazardous occupation.

Strengths and Limitations of Molecular Methods

The introduction of molecular characterization of pathogens was a major development in infectious disease epidemiology. Historically, broad characterizations have been made using serotyping and antibiotic resistance profiles Recent methods include pulsed field gel electrophoresis (PFGE); presence, absence, and type of a gene of interest; multi locus sequence typing (MLST); restriction fragment length polymorphisms (RFLP); and many more (Riley 2004). This study used antibiotic resistance profiles, *Staphylococcal* protein a typing, and PFGE to characterize isolates. Each of these methods has strengths and limitations applicable to the study.

Antibiotic resistance profiling methods are well-established and can provide clinically relevant information that guides treatment of infections. When used for epidemiological research, however, this type of characterization usually lacks the discriminatory power necessary to come to meaningful conclusions (Riley 2004).

A second method used to characterize isolates from this study was *spa* typing, a type of single locus sequence characterization based on short-sequence-repeat regions (in this case the polymorphic X region of *Staphylococcal* protein A gene). When this procedure is optimized, it is shorter and less labor-intensive than other molecular techniques (Riley 2004). Sequencing is extremely discriminatory, yielding information at the nucleotide level. Moreover, *spa* typing results can be entered into and compared with an international database (Ridom GmbH). Depending upon information available in the database, this program allows researchers to compare their isolates to *S. aureus* collected from other studies conducted around the globe.

This type of molecular characterization faces limitations as well. During this study, problems were encountered with this technique, leading to added time and cost. Polymerase chain reaction, the method used to amplify the gene of interest, is extremely

sensitive to contamination (Riley 2004). High discriminatory power can also be a drawback. In the nose vs. throat analysis, 63.5% of S. aureus isolates could not be categorized according to published *spa* groups and were placed in the "Other" category. Obtaining too many categories limits statistical power and categories are not always phenotypically or clinically meaningful.

There are also limitations in using the international Ridom software database. The database is an excellent resource, but it is only as strong as the data that is entered into it. It is possible that the Ridom database is more likely to contain more virulent isolates for comparison, since those isolates get studied more often and are more likely to cause severe disease. As noted previously, most of the isolates from the child care study and the rural Iowan study could not be categorized. This is not necessarily because the isolates in these populations are unique, but more likely because they have not caused severe disease in the individuals carrying them.

Pulsed field gel electrophoresis, the third type of characterization used in this project, also has strengths and limitations. Unlike spa typing, PFGE is very robust to errors in the procedure – minor errors will not ruin the entire undertaking. The protocol for *S. aureus* PFGE is well established, and it has a discriminatory power that is similar to *spa* typing (Petersson 2010). On the other hand, PFGE is more time and labor intensive than *spa* typing. Also in contrast to spa typing, we did not have access to a national or global *S. aureus* PFGE database. We could only compare our isolates with common USA MRSA. The lack of a national database is likely due to a lack of standardized results between labs. It has been well documented that consistency in results between laboratories is not guaranteed, so a large national or international database may not be useful (Riley 2004). PFGE faces the same limitations as spa typing regarding discriminatory power – the classifications of *S. aureus* may not be phenotypically or clinically relevant.

A Note on the Zoonotic Connection

Zoonotic agents are defined as microorganisms that can include animal reservoirs in their chain of transmission. It is estimated that a large proportion of emerging infectious diseases are zoonotic in nature (Meslin 2000). Despite this fact, the zoonotic nature of some agents is often overlooked. In recent years *S. aureus* has been acknowledged as a potential zoonotic agent (Cuny 2010). In Iowa, *S. aureus* (namely MRSA) has been found at high levels in swine and employees who have contact with swine (Smith 2009). Despite the seemingly unrelated nature of the child care study to animal exposure, some of our findings serve as a reminder that the animal-human nexus can be an important contributor to risk of *S. aureus* carriage.

Association with animals was a univariate predictor of *S. aureus* carriage in adults (cats on property: p-value = 0.014) and children (contact with animals: p – value = 0.046 and contact with goats or sheep: p-value = 0.024). While this study was not powered to assess contact with animals as the primary predictor of carriage in either group, our results suggest that the zoonotic aspect of *S. aureus* carriage should not be overlooked. This study provides an example of animal contact playing a role in risk of colonization in a seemingly unrelated study. Future studies, particularly in rural areas, should be cognizant of the role animal contact can play in bacterial carriage not only as a primary outcome of interest, but as a potential confounder as well.

Implications of Nose vs. Throat Carriage

Many studies have identified the anterior nares as the ideal source of screening swabs. The vast majority of these studies, however, have been conducted in healthcare situations (Elie-Turenne 2010, Yang 2010, Kaminski 2007). In order to reduce costs associated with MRSA screening programs, many healthcare institutes collect cultures from the nose only (Holzmann-Pazgal 2010). The findings in chapter 4 that over 25% of carriers would not have been identified if we had not collected throat swabs suggests that

this practice might not be appropriate for all screening programs. While swabbing the nose only may be appropriate for patients who have had extended contact with the healthcare system, our data contribute to a growing body of evidence that suggests *S. aureus* may behave differently in members of the general community. A more targeted approach to screening may be appropriate for some healthcare facilities, particularly those that are likely to admit individuals with little prior exposure to healthcare settings.

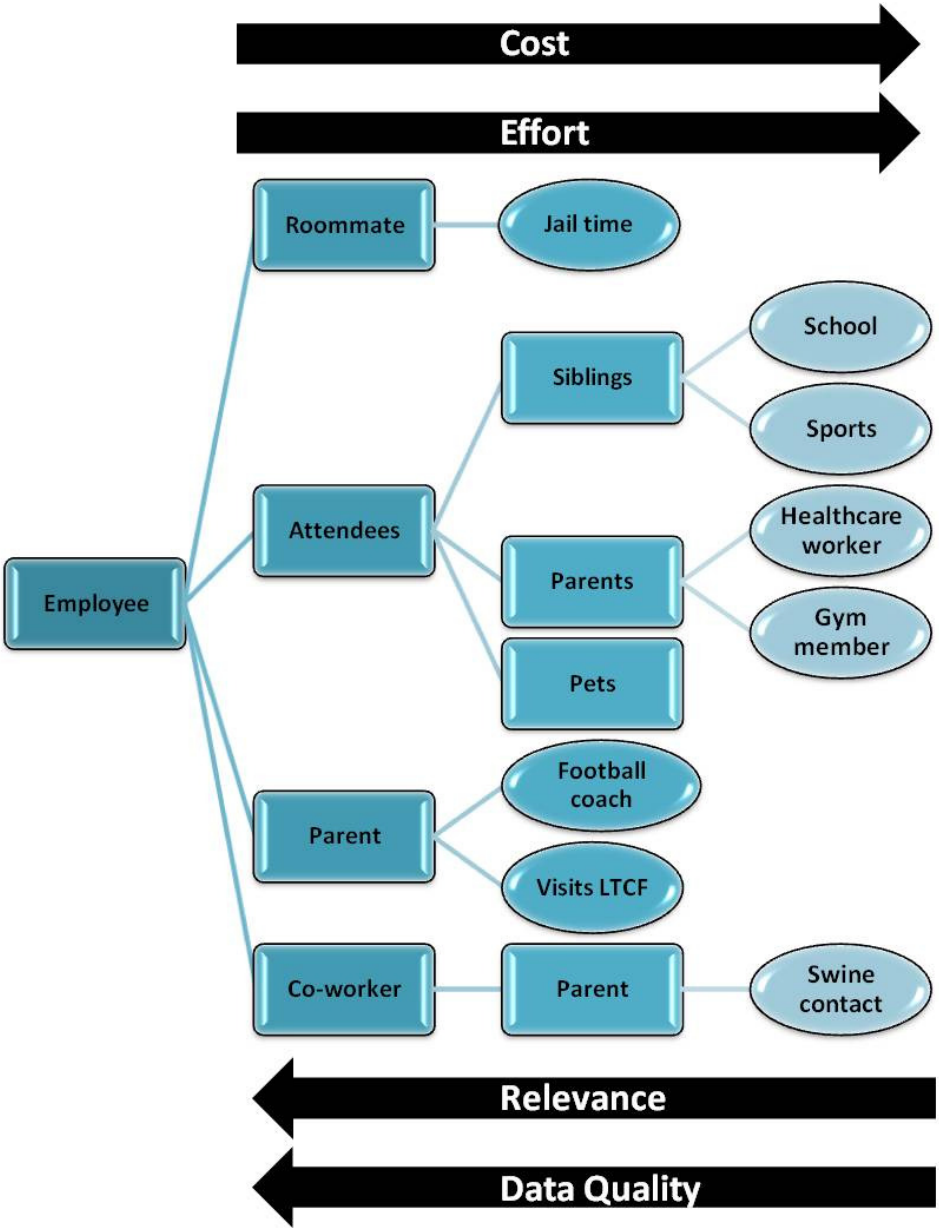
Conclusions

While the prevalence rates of *S. aureus* were not alarmingly high in any of our isolate sources, this project has made some important contributions to the study of child care facilities and *S. aureus* colonization. Bacterial carriage in child care attendees has been studied extensively, and basic patterns of transmission are beginning to be uncovered in this environment. Chapter 3 provides evidence that child care employees may be at increased risk of carrying erythromycin-resistant *S. aureus*, although more research is needed to confirm this finding. Moreover, molecular analysis suggests that employees and environmental surfaces play a role in transmission cycles within a facility, and should not be overlooked in further child care studies. Finally, chapter 4 demonstrates that the throat is an important colonization site in individuals with limited exposure to healthcare, and that age and race are significant predictors of throat carriage. Current MRSA screening practices may need to be re-evaluated in light of these findings.

Figure 15. Examples of different types of surfaces found at child care facilities.

Note: The plastic table, left, takes limited time and effort to sanitize with a bleach solution. The infant activity center (center) contains a mix of porous and nonporous surfaces, and is likely awkward and inconvenient to sanitize. The stuffed toy (right), contains very porous surfaces, but can be machine-washed at high temperatures for sanitization.

Figure 16. Depiction of the hypothetical chain of contact for a child care employee.



Rectangles represent individuals, ovals represent potential sources of *S. aureus* acquisition, and lines represent transmission pathways among entities. As potential carriers (i.e. potential sources of transmission) and their risk factors become more removed from the employee, the cost and effort to obtain data increase, while the relevance and data quality decrease. LTCF: long term care facility.

APPENDIX

Decision-Making Process for Reducing Facility-Level Predictors of Risk in Employees

Much of the potential risk factor data collected on employees was related to the facility in which they worked. At the $p < 0.15$ level, 17 facility-level variables were predictors of *S. aureus* carriage in employees (data available in Table 19), which is too many variables for a data set this size. Since much of this data was collected when a facility director filled out one questionnaire regarding the entire facility, many of the variables are highly collinear. Facility-level univariate predictors significant at $p < 0.15$ of *S. aureus* carriage included: season, center years operated, employees wash hands after food preparation, employees wash hands when returning from outdoors, employees wash hands before administering first aid, children wash hands upon arrival, children wash hands after eating, children wash hands after food preparation, children are separated during at least part of the day, age of youngest group of children attending the center, age of oldest group of children attending the center, percentage of child participants reporting an acute asthma event in the past year, percent of child participants whose parent reported exercising at a gym, percent of child participants reporting contact with animals, percent of child participants reporting contact with goats, and percent of child participants reporting contact with sheep. Facility ID was significant at $p = 0.06$, but since it is the basis for the clustering in GEE analysis, it was eliminated from consideration.

Step one in the process was to reduce p-value for consideration from 0.15 to 0.05. This step eliminated:

Employees wash hands before administering first aid
Percent of child participants whose parent reported exercising at a gym
Percent of child participants reporting contact with goats
Percent of child participants reporting contact with sheep

Step two was to remove collinear variables with odds ratios that don't make biological sense. Five variables had the same odds ratio (4.69) and confidence limits (1.00 – 21.96), and similar p-values (0.035 – 0.047). In terms of biologic plausibility, increased rates of hand washing were unlikely to increase the risk of carriage, so those four variables were eliminated. The effects of separating children during at least part of the day is less clear from a biological perspective, so this variable was kept in consideration. The following variables were eliminated:

Employees wash hands after food preparation
Employees wash hands when returning from outdoors
Children wash hands after eating
Children wash hands after food preparation

Step three was to remove ordinal variables with low evidence of trend. Two variables (center years operated and age of the youngest group of children attending the center) were statistically significant, but did not display a significant trend (trend test p-values 0.85 and 0.70). Age of oldest group of children attending the center was statistically significant, and had a trend test p-value of 0.23, so was kept for consideration. The following variables were eliminated:

Center years operated
Age of the youngest group of children attending the center

Step four was to remove collinear variables based on Pearson correlation coefficients. Season was collinear (correlation coefficient = 0.64) with kids wash hands upon arrival, children are separated at least part of the day was collinear (correlation coefficient = 1.00) with percent of child participants reporting an acute asthma event in the past year, and children wash hands upon arrival was collinear (correlation coefficient = 0.68) with percent of child participants reporting an acute asthma event in the past year.

The final three variables considered for the model were kids wash hands upon arrival, children are separated at least part of the day, and percent of child participants reporting contact with animals.

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