Methicillin-Resistant *Staphylococcus aureus* on Public Transportation Vehicles: Sampler Performance, Prevalence, and Epidemiology

Dissertation

Presented in Partial Fulfillment of the Requirements for the Degree Doctor of Philosophy in the Graduate School of The Ohio State University

By

Jonathan Kyle Lutz, M.P.H.

Graduate Program in Public Health

The Ohio State University

2011

Dissertation Committee:

J. Mac Crawford, Advisor

Armando E.S. Hoet

Jiyoung Lee

John R. Wilkins III
Copyright by
Jonathan Kyle Lutz
2011
Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) is an important public health threat, with significant disease burden. Of particular concern are the growing number of cases that have origins in the community (CA-MRSA); these infections often affect individuals without underlying risk factors for disease. Because MRSA colonizes a large number of people, can be easily spread by fomites, and survives on surfaces for months, the environment is an important element in MRSA transmission. Public transportation vehicles, which have high levels of crowding and significant hand-to-fomite contact, may represent an important site for MRSA transmission. This study determined the background prevalence of MRSA on buses from a large transportation agency.

Prior to vehicle sampling, a laboratory-based study was conducted to evaluate the performance of four sampling methods for recovery of *S. aureus* from a stainless steel surface. Results indicate that overall, the electrostatic wipe resulted in the greatest performance across all inoculating concentrations ([relative sampling efficiency=SE; analytical sensitivity=Sn] \( \text{SE}_{48h} = 17.8\% \); \( \text{Sn}_{48h} = 7 \text{ CFU/100cm}^2 \)). However, the swab sampler performed well when corrected for area actually sampled (\( \text{SE}_{48h} = 24.3\% \); \( \text{Sn}_{48h} = 76 \text{ CFU/100cm}^2 \)), but is greatly limited by the surface area which can be effectively sampled. Among the contact-based methods, the roller sampler outperformed
the contact plate (roller: $SE_{48h}=9.9\%$; $Sn_{48h}=17$ CFU/100cm$^2$; contact plate: $SE_{48h}=0.04\%$; $Sn_{48h}=1,412$ CFU/100cm$^2$), and both contact samplers performed better at higher inoculating concentrations. Statistically significant differences were observed between the samplers only at the lowest two and highest inoculation concentrations ($p_{conc_1}=0.009$; $p_{conc_2}=0.007$; $p_{conc_5}=0.029$). Overall, the wipe resulted in the greatest number of replications that resulted in positive growth, with 73.8% at 24 hours, and 90.5% at 48 hours.

Using this laboratory data, a field-based surveillance study was conducted to determine whether *S. aureus* (both methicillin-susceptible *Staphylococcus aureus* [MSSA] and MRSA) could be isolated from bus surfaces. A total of 237 samples were collected from 40 buses from a major urban transportation agency. Sixty-eight percent of buses were contaminated with *S. aureus* (both MSSA and MRSA), and 63% with MRSA. In total, 17% of the 237 samples were positive for *S. aureus* (both MSSA and MRSA) and 15% for MRSA. Categorical analysis indicated that at the sample level, sampling date ($p_{S. aureus, July}=0.075$) and sample location ($p_{MRSA}=0.000$; $p_{S. aureus}=0.000$) on a vehicle were associated with vehicle contamination. At the bus level, no factors were significantly associated with vehicle contamination, but ridership ($p_{S. aureus}=0.088$) and sampling date ($p_{MRSA, July}=0.074$; $p_{S. aureus, July}=0.074$) indicated potentially important associations.

Finally, the antibiotic resistance profiles and molecular characteristics of positive bus isolates were evaluated. Multiple resistance patterns were observed, with high levels of multi-class resistance. Sixty-five percent (65%) of isolates were of the community-
associated staphylococcal cassette chromosome mec (SCCmec) type IV, and 20% were healthcare-associated SCCmec type II. Multiple pulsed-field gel electrophoresis patterns were noted, with the majority being CA-types USA300/400; the HA-type USA100 was also found. The hospital-associated strain types were resistant to more classes of antibiotics, although multi-class resistance was also observed among the community-associated strains. High levels of clindamycin resistance were noted across all study isolates, for both MSSA and MRSA. Clonality results demonstrated that buses may be effective mixing vessels for MRSA strains, as multiple unique strains were noted in some study vehicles.
Dedication

This document is dedicated to my family; especially my parents, who never discouraged me from unceasingly asking “but why?” to all life’s mysteries. And to my beloved Darlene, who is my anchor, my calming force, and who reminded me to laugh every day. Finally, this process would not have been possible without my brother, my friend, and my mentor – Eric.
Acknowledgments

First and foremost, this project would not have been possible without the tireless efforts of my advisor, Dr. Mac Crawford. Mac provided a rich graduate experience, acting as mentor, counsel, and friend; the value of his academic guidance is only surpassed by the kindness of his heart. Also, I am extremely grateful to the entire esteemed committee: Dr. Armando Hoet, who oversaw all MRSA field activities and first “introduced” me to MRSA; Dr. Jiyoung Lee, who provided expertise for the sampler evaluation, and who always pushed me to examine the practical relevance any research; and Dr. Wilkins, for instilling in me a sense of epidemiological inquisitiveness. I would also like to extend gratitude to the transportation agency for their participation in the study. Special thanks to the employees directly involved in coordinating this work. I am also greatly indebted to the numerous volunteers that helped collect and analyze samples, including Dr. Rocio Hoet, Dr. Joany Van Balen, Jade Braman, Melissa Mikolaj, “View” Wannasawat Ratphitagsanti, Chongtao Ge, Sophia Dailey, Colleen Shockling-Dent, and Debbie Lutz; and the review of Dr. Eric Lutz. Last – but certainly not least – I would like to thank Shasha Bai, whose statistical guidance prevented me from too many gray hairs during the process.
Vita

June 1997 ........................................St. Charles Preparatory School, Columbus, Ohio

1999 – 2000 ....................................Project Manager/Scientist, BioGard Environmental Services, Phoenix, Arizona

2000 – 2002 ....................................Project Scientist, Environmental Management Group, Phoenix, Arizona

June 2001 .......................................B.S. Zoology, The Ohio State University

2003 – 2005 ....................................Project Manager/Staff Industrial Hygienist, PGD Environmental Group, Cleveland, Ohio

2005 – 2006 ....................................Project Manager, Anderson Group International, Bakersfield, California

2006 – 2009 ....................................Operations Manager/Industrial Hygienist, Auburn Environmental, Cleveland, Ohio

May 2007 ...........................................M.P.H., Cleveland State University
2008 to present ................................. Graduate Teaching Associate, Department of Environmental Health Sciences, College of Public Health, The Ohio State University

2011.............................................. President/Industrial Hygienist, Auburn Environmental, Cleveland, Ohio

Publications


Fields of Study

Major Field: Public Health
Table of Contents

Abstract .......................................................................................................................... ii

Dedication ...................................................................................................................... v

Acknowledgments ......................................................................................................... vi

Vita ................................................................................................................................. vii

List of Tables ................................................................................................................ x

List of Figures ............................................................................................................... xiii

Chapter 1: Introduction ................................................................................................. 1

Chapter 2: Background ................................................................................................. 15

Chapter 3: Performance of Surface Sampling Methods for the Recovery of S. aureus ... 48

Chapter 4: Methicillin-Resistant Staphylococcus aureus Contamination on Public
Transportation Vehicle Surfaces ................................................................................... 85

Chapter 5: Molecular Epidemiology of Methicillin-Resistant Staphylococcus aureus
Contamination on Public Transportation Vehicle Surfaces ....................................... 120

Chapter 6: Synthesis and Discussion .......................................................................... 147

References ..................................................................................................................... 167
Appendix A: Efficacy of Modified Cleaning Techniques for the Reduction of *S. aureus* Surface Contamination........................................................................................................... 190

Appendix B: Final Report and Recommendations for Transportation Agency.......... 209

Appendix C: Proposed Field Intervention Protocol......................................................... 236
List of Tables

Table 2.1: Risk Factors for MRSA Colonization and Disease ........................................ 26
Table 2.2: Example S. aureus Fomites in the Hospital .................................................. 38
Table 2.3: Example S. aureus Fomites in the Community .............................................. 39
Table 3.1: Relative Sampling Efficiency, Relative Analytical Sensitivity of the Four Sampling Methods ........................................................................................................... 72
Table 3.2: Percentage of Replications with Positive Growth (Across All Inoculating Concentrations) of the Four Sampling Methods ...................................................... 73
Table 3.3: Comparison of Current Results with Obee et al. Data ..................................... 75
Table 3.4: Comparison of Sampling Devices ..................................................................... 79
Table 4.1: Proportion and Odds Ratios of MRSA-Positive Samples ................................. 105
Table 4.2: Proportion and Odds Ratios of Total S. aureus-Positive Samples ................. 106
Table 4.3: Proportion and Odds Ratios of MRSA-Positive Buses .................................... 107
Table 4.4: Proportion and Odds Ratios of Total S. aureus-Positive Buses ....................... 108
Table 5.1: Antibiotics Utilized for Resistance Profiling .................................................. 125
Table 5.2: PCR Reagents ............................................................................................... 127
Table 5.3: PCR Primer Sequences for SCCmec Typing .................................................. 128
Table 5.4: Criteria for Determining Isolate Clonality using the Tenover Method .......... 129
Table 5.5: MRSA: Percentage of Antibiotic Resistance, Overall and by SCC\textit{mec} Type ................................................................. 133

Table 5.6: Summary of Bus and Isolate Results ................................................................. 137

Table 5.7: Categorical Analysis of SCC\textit{mec} Type and Factors ....................................... 140

Table A.1: Summary of Cleaning Method Performance ...................................................... 201
List of Figures

Figure 1.1: Project Flow Diagram ................................................................. 14

Figure 2.1: Scanning Electron Micrograph of MRSA ........................................ 17

Figure 2.2: MRSA Colonization Sites among the General Population and those Colonized with S. aureus ............................................................ 23

Figure 2.3: Dip slide, contact plate (RODAC), and swab sampling systems .......... 44

Figure 3.1: Roller Sampler Cassette ............................................................... 54

Figure 3.2: Rendering of Two Unsuccessful Flexible Dip Slide Models (the paddle design and the flexible dip slide) ................................................................. 64

Figure 3.3: Roller Sampler Pre-prototype Design (utilized in sampler evaluation) ...... 64

Figure 3.4: Recovered MSSA by Sample Method, “10^2” Concentration ............... 68

Figure 3.5: Recovered MSSA by Sample Method, “10^3” Concentration ............... 68

Figure 3.6: Recovered MSSA by Sample Method, “10^4” Concentration ............... 69

Figure 3.7: Recovered MSSA by Sample Method, “10^5” Concentration ............... 69

Figure 3.8: Recovered MSSA by Sample Method, “10^8” Concentration ............... 70

Figure 3.9: Recovered MSSA by Sample Method, “10^2” and “10^3” Concentrations .... 70

Figure 3.10: Recovered MSSA by Sample Method, “10^4”, “10^5” and “10^8” Concentrations ......................................................................................... 71

Figure 4.1: Microbial Analysis Flow Diagram .................................................... 94
Figure 4.2: MRSA and Total S. aureus by Sampling Date, Sample-Level ...................... 98
Figure 4.3: MRSA and Total S. aureus by Sampling Date, Bus-Level ......................... 99
Figure 4.4: MRSA and Total S. aureus by Sample Location on a Bus ...................... 101
Figure 4.5: Proportion of Positive Samples (MSSA and MRSA) by Sample Location . 102
Figure 5.1: PFGE Band Patterns .................................................................................. 136
Figure 5.2: Visual Representation of Bus 7 and Bus 26 ............................................. 138
Figure A.1: Recovered S. aureus by Cleaning Method, 30 Minutes Post-inoculation Drying ................................................................. 200
Figure A.2: Recovered S. aureus by Cleaning Method, 24 Hours Post-inoculation Drying ......................................................................................... 200
Figure B.1: MRSA and Total Staph. by Sampling Date .............................................. 228
Figure B.2: MRSA and Total Staph. by Sample Location on a Bus ......................... 230
Figure B.3: Percentage of Positive Samples (Staph. and MRSA) by Sample Location 232
Figure C.1: 3M SpongeSicle ......................................................................................... 239
Chapter 1: Introduction

“If an organism can find an appropriate niche in which to survive, it will do so, whatever the origin…” (Dancer, 1999).

1.1 Introduction

The public health impact of methicillin-resistant Staphylococcus aureus (MRSA) infections is significant. MRSA is an important cause of nosocomial infection and is the primary cause of community-acquired skin and soft tissue infection. More severe cases may involve necrotizing pneumonia or sepsis (Maltezou & Giamarellou, 2006; Moran et al., 2006). MRSA infections kill 19,000 hospital patients annually, more than AIDS or tuberculosis (Kleven et al., 2007; CDC, 2005; CDC, 2006). Since first described in 1961, the resistant bacterium has predominately affected immune-compromised patients in healthcare settings (hospital-acquired, or HA-MRSA), leading to surgical site and catheter infections with significantly increased mortality (Cosgrove et al., 2005). However, over the last two decades, the emergence of community-associated strains (CA-MRSA) has become increasingly important. These strains tend to be more virulent and affect people without known health risk factors. Moreover, these community strains are increasing the overall incidence of MRSA infection, as they are adding to, rather than
displacing, hospital-associated strains (Boucher & Corey, 2008; Hota et al., 2007; Miller & Diep, 2008; Popovich et al., 2008).

MRSA is part of the skin and nasal flora for some individuals; these individuals are known as “colonized” (or “carriers”). Approximately 0.8-1.5% of the U.S. population is colonized with MRSA (CDC/Kuehnert et al., 2006; Chambers & DeLeo, 2009; Gorwitz et al. 2008). Subpopulations, particularly health care workers, are colonized to an even greater degree – 4.2% (Albrich & Harbarth, 2008). Those colonized are at greater risk for subsequent MRSA infection (Lowy, 1998; Wenzel & Perl, 1995), are capable of spreading MRSA to susceptible individuals, and may even contaminate their surrounding environment (Rohr et al., 2009). The anterior nares are the most commonly colonized site; however, hands, the pharyngeal area, the groin, and the axilla are all frequently colonized. The ability to asymptptomatically colonize such a large number of healthy individuals allows MRSA to be spread quite easily. Colonized hands are particularly important in spreading MRSA from individuals to fomites, facilitating community transmission via environmental surfaces.

MRSA transmission is primarily through direct, person-to-person contact, with hands acting as the primary disseminators (Ayliffe et al., 1988; Bhalla et al., 2004; Boyce et al., 1997; Chambers & DeLeo, 2009; Gordon & Lowy, 2008; Lowy 1998). There is strong evidence, however, that hand-to-fomite and fomite-to-hand transmission are also important (Miller & Diep, 2008). Given the role that environmental surfaces and fomites play in MRSA transmission, it is not surprising that MRSA has been isolated or linked to a number of surfaces, both following outbreaks and during periods of no known
outbreaks. In the past decade, several professional football players acquired MRSA infections, with epidemiological evidence that fomites facilitated transmission (Begier et al., 2004; Kazakova et al., 2005). Recently, an Ohio teen who was a member of the wrestling team became infected with MRSA, resulting in subsequent closure and disinfection of the wrestling room (Crane, 2010). In Alaska, a community MRSA outbreak was traced to the contaminated wooden benches of a sauna (Baggett et al., 2004). MRSA has also been isolated from prison vans, high chairs, remote control units, sinks, and numerous other community fomites (see Table 2.2 and Table 2.3 for details).

According to the Centers for Disease Control and Prevention (CDC), “MRSA in the community is widespread and therefore, anyone is at risk” (CDC, 2010). Community-acquired MRSA infections, by definition, lack prior healthcare exposure risk factors (CDC, 2005; Klevens et al., 2007; Morrison et al., 2006; Tristan et al., 2007). However, there are several epidemiological risk factors that may indicate an increased risk of MRSA infection. To summarize these, the CDC describes the “5 C’s” of community MRSA transmission: crowding, compromised skin, contaminated items or surfaces, lack of cleanliness, and frequent skin-to-skin contact. Community settings where these factors are frequently present are prisons, military barracks, college dormitories, daycare facilities, households, and contact sport facilities (CDC, 2010).

When reviewing the “5 C’s” in the context of public transportation, one can envision that public transportation vehicles may have a potentially important role in community MRSA transmission. Several factors on vehicles likely contribute to an ideal scenario for possible transmission. First, there is high human density; crowding has been
noted as an important risk factor for MRSA transmission (CDC, 2007). Also, there is significant hand-to-surface contact on public transportation vehicles. People are obligated to touch rails, doors, and other surfaces, particularly as vehicles become crowded. This type of hand-to-fomite contact has been implicated as a means for transmission in CA-MRSA outbreaks (Miller & Diep, 2008). It has also been postulated that fomites, rather than nasal colonization, may play a much larger role during CA-MRSA outbreaks (Baggett et al., 2004; Begier et al., 2004; Kazakova et al., 2005; Miller & Diep, 2008; Rieg et al., 2005). There is also high turnover, with people entering and exiting the vehicle frequently. This increases the likelihood that MRSA carriers (those colonized or carrying MRSA but without active infection and without negative health impacts) will pass through the vehicle at some point during a route. Air drafts, which are common as vehicle doors and/or windows are opened, may suspend or re-suspend skin particles and dusts that may carry MRSA (Dancer, 2008; Hambraeus et al., 1978; Williams et al., 1960). Finally, and perhaps most importantly, there is little to no opportunity for hand hygiene during and immediately after transportation usage. As has been stated by several experts, hand hygiene is the primary method for prevention of CA-MRSA transmission (Boyce & Pittet, 2002; Maltezou & Giamarellou, 2006).

Public transportation may also be used by “cloud” *S. aureus* carriers (colonized individuals who may disperse high concentrations of the bacteria; also called “supershedders”), who can disperse up to a 40-fold increase in airborne MRSA over “non-cloud” carriers (individuals colonized with bacteria, but not enhanced shedders). This could increase the likelihood that MRSA strains, particularly HA-MRSA strains
(because “cloud” carriers are frequently healthcare workers, who are more likely to carry healthcare-associated strains), may be isolated on public transportation vehicles (Belani et al., 1986; Sherertz et al., 1996; Tanner et al., 1980). These “cloud” carriers can transmit MRSA through skin-to-surface contact and the release of skin squames, but may also transmit significant amounts of airborne MRSA, particularly when experiencing an upper respiratory infection (Boyce et al., 1993; Hare & Thomas, 1956). These individuals also transmit higher S. aureus concentrations when moving or where there is skin friction, which is rather unavoidable on public transportation (Cimolai, 2008; Hare & Thomas, 1956).

In a study of hospital intensive care units (ICUs), Hardy found MRSA on environmental surfaces regardless of whether a small or large number of MRSA-colonized patients were present, demonstrating the potential importance of the environment in transmission (Hardy et al., 2006). MRSA is frequently transmitted via the hands of health-care workers who may even carry MRSA on gloves after touching surfaces near colonized patients (Bhalla et al., 2004; Boyce et al., 1997). One study found that some nurses carried a median of 10,000 CFUs of S. aureus on their hands, hands which may then subsequently contaminate vehicle surfaces (Ayliffe et al., 1988). Contaminated footwear may also act to transmit and disperse MRSA, as floors have been found to harbor high concentrations of MRSA (as cited by Cimolai, 2008). Wearing hospital “scrubs,” or even physician neckties outside of the hospital, may provide additional fomites (Dixon, 2000). These “high-concentration carriers” may carry contamination onto transportation vehicles, where “an inoculum of anything from ten to
several million CFUs could potentially cause an infection (in a patient)” (Colbeck, 1960; Dancer, 2008; Noble, 1981).

The impressive survivability of *S. aureus* enhances the potential for environmental transmission. Previous research has demonstrated that *S. aureus* can survive on a variety of surfaces for up to 6 months, and is resistant to desiccation (Dietze et al., 2001; Jawad et al., 1996; Sexton et al., 2006; Wagenvoort & Penders, 1997; Wagenvoort et al., 2000; Williams & Davis, 2009). With such environmental fitness, it is not surprising that staphylococci have been found on vehicles. In a Serbian study, Stepanovic et al. (2008) found 30.1% (422/1400 samples) of samples from public transportation hand rails were positive for antibiotic-resistant coagulase-negative staphylococci (MRCoNS); every vehicle sampled was positive for MRCoNS (55/55). Although MRSA was not specifically isolated, the researchers postulate that their results may be a reflection of the local colonization patterns, where MRSA prevalence may be lower amongst their study population. No data are available for Serbian colonization rates from the European Antimicrobial Resistance Surveillance System, but in a study of healthy pregnant women in Switzerland who emigrated from the former Yugoslavia (which included Serbia), MRSA colonization was 0% (Marschall et al., 2006; Stepanovic et al., 2008). Incidentally, Kassem (2009) has argued that Stepanovic et al.'s microbiological culture methods were inhibitory to MRSA growth (the use of 4.5% NaCl in enrichment broth, which may be inhibitory to some strains of MRSA). Similar results were noted in a study of touch surfaces in a London public transportation system. Methicillin-susceptible *Staphylococcus aureus* (MSSA) was isolated in 8% of samples
(9/118), although no MRSA was detected. However, this study had several limitations (Otter & French, 2009). First, the authors note that London has a low prevalence of CA-MRSA. Although no published colonization rate for London could be identified, the prevalence of community-associated strains is lower in the U.K. than in the U.S., and MRSA bacterimia infections have been declining (Otter et al., 2009; Wall et al., 2009). Also, the use of standard dip slides for sampling prevented pooling of large sampling areas, and the nature of rigid dip slides may have resulted in low analytical sensitivity when sampling grab rails and bars (which are tubular). In addition, there was a low sample number and no enrichment was conducted, which may have affected statistical power and sampling sensitivity (Otter & French, 2009). Finally, in a recently published report from Portugal, a locally endemic hospital MRSA clone (EMRSA-15) was found on public transportation buses, with a total of 26% (22/85) buses positive for MRSA (Simoes et al., 2011). These findings represent the first report of MRSA from public transportation vehicles.

Despite the findings of Stepanovic et al., Otter and French, and Simoes et al., more research is needed to elucidate the role of public transportation in potential MRSA transmission. In this study, the research team hypothesizes that public transportation vehicles are a significant, large-scale fomite which may contribute considerably to the community prevalence and potential transmission of MRSA. This is the first study of its kind in the United States, the results of which will elucidate an important mechanism of transmission – contact with community surfaces (although it should be
noted that the study is measuring surface prevalence/contamination, not transmission. Therefore, any positive finding of *S. aureus* may note potential transmission, but does not demonstrate actual transmission). Despite the clear importance of MRSA as a community pathogen, little research has evaluated MRSA on environmental surfaces in the community. Most investigation has been conducted immediately following an outbreak, or studies have lacked an assessment of factors that may contribute to transmission. Therefore, little is known about the background prevalence of MRSA on community surfaces, including public transportation. These data are critical to increase understanding of potential community transmission of this important pathogen. Further, this work will set the stage for future studies which will evaluate the molecular strain characteristics of MRSA in the community. These research activities will provide valuable data that will permit infection control specialists and epidemiologists to track the pathogen in the community, design and implement more effective education and prevention programs, and help the public health system better respond in the event of community MRSA outbreaks.

### 1.2 Project Overview

Isolating any pathogen from environmental surfaces requires the use of surface sampling. The effectiveness of this sampling is dependent upon a number of factors. A sampling system should be chosen that fulfills the following criteria: 1) the sampling method must be capable of effectively removing the bacteria from the surface; 2) this removal should be done in the most efficient manner possible, without destruction of the
bacteria or rendering the bacteria non-viable; and 3) once collected, the sampling system must be able to fully release the bacteria for subsequent processing (Davidson et al., 1999; Moore & Griffith, 2007).

Public transportation vehicle surfaces represent a number of challenges with respect to choosing an appropriate sampling method. On vehicles, there are a number of surface shapes, including flat surfaces, rounded tubes, and curved edges. Surface materials are equally varied, with vehicles commonly having a combination of stainless steel, rubberized plastic, fabric, roughened hard plastic, and painted metal. In addition, the large area of vehicle surfaces is a challenge to some sampling systems. To effectively sample public transportation vehicles for *S. aureus* and MRSA contamination, the research team desired a sampling method that would balance performance, cost, and field-usability. This need was addressed in Specific Aim 1.

Specific Aim 1 (SA1) sought to achieve two goals. First, there was a desire to develop and construct a pre-prototype novel sampling device for efficient *S. aureus*/MRSA isolation from environmental surfaces. The sampling device was created to overcome the lack of flexibility and the surface size limitation of currently available contact-based sampling methods (dip slides and RODAC contact plates). The second goal was to compare the novel sampling device with three other currently-utilized sampling methods: swabs, RODAC-style contact plates, and electrostatic wipes. To the research team’s knowledge, this is the first evaluation of electrostatic wipes for the isolation of MRSA from environmental surfaces, and the first description of a rolling contact-based sampler.
Using the performance data from SA1 to choose the best sampling method, the research team conducted environmental surveillance sampling for MSSA and MRSA on public transportation vehicles in Specific Aim 2 (SA2). SA2 was the crux of the dissertation project, around which other aims were centered. In SA2, surface samples were collected from the interior of forty buses from a large, metropolitan transportation agency. In addition to providing data on the background prevalence of both MSSA and MRSA on public transportation vehicles, the research team assessed several factors for their potential association with contaminated vehicles. To the research team’s knowledge, this is the first study in the United States to investigate MRSA on public transportation vehicles.

In Specific Aim 3 (SA3), the antibiotic resistance profiles and molecular characteristics of isolates from SA2 were evaluated. These data may provide unique insights into which MRSA strain types are circulating in the community.

_A note to the reader:_ Throughout the document, both the susceptible (MSSA) and resistant (MRSA) forms of *Staphylococcus aureus* will be discussed. Although the distinction between the two is clearly important in clinical treatment and epidemiology, this distinction is somewhat less important in environmental surface contamination. The resistant form of the bacteria is similar to the susceptible form with respect to survivability and transmission; the only difference being molecular characteristics that affect disease outcomes. In other words, MRSA and MSSA are both still *S. aureus*, so what applies to one often applies to another. Therefore, throughout the dissertation there
will be occasions where features of *S. aureus* are described that may apply to both MSSA and MRSA.

### 1.3 Specific Aims

**1.3.1 Specific Aim 1 Hypotheses**

- **Hypothesis 1:** Four surface sampling methods will demonstrate significantly different bacterial recovery (MSSA) from an inoculated stainless steel test surface.
  - **Hypothesis 1.1:** A novel, contact-based roller sampler will demonstrate significantly greater MSSA recovery from a test surface than the swab, wipe, or contact plate.

This specific aim will evaluate the recovery of methicillin-susceptible *Staphylococcus aureus* (MSSA, used as a surrogate for MRSA) from a stainless steel test surface using three common sampling methods – rayon transport swabs, RODAC-style contact plates, and electrostatic wipes – as well as a novel, rolling contact-based sampler that will be specially constructed for the project. Results will indicate recovered colony forming units (CFU), relative sampling efficiency, relative analytical sensitivity, and percentage of positive growth for each sampling method.

**1.3.2 Specific Aim 2 Hypotheses**

- **Hypothesis 2:** MSSA and MRSA can be recovered from public transportation vehicle surfaces. That is, public transportation vehicles are contaminated with MSSA/MRSA.
  - **Hypothesis 2.1:** There will be a significant difference between the prevalence of MSSA and MRSA on vehicles which serve hospitals versus those which do not (prevalence ≠).
o Hypothesis 2.2: There will be a significantly greater prevalence of MSSA and MRSA on vehicles that have high ridership versus low ridership (prevalence >).

o Hypothesis 2.3: There will be a significant difference between the prevalence of MSSA and MRSA on vehicles that run one route in a day versus those that run multiple routes (prevalence ≠).

o Hypothesis 2.4: There will be a significantly greater prevalence of MSSA and MRSA on vehicles sampled at earlier dates than later dates (prevalence >).

o Hypothesis 2.5: There will be no significant difference between the prevalence of MSSA and MRSA on vehicles from two separate service facilities (prevalence =).

o Hypothesis 2.6: There will be a significant difference between the prevalence of MSSA and MRSA at different sample locations (prevalence ≠).

Using a cross-sectional environmental surveillance study design, in this specific aim the research team will determine whether methicillin-susceptible and/or methicillin-resistant *Staphylococcus aureus* can be recovered from public transportation vehicle surfaces. In addition, categorical analysis (Chi-Squared/Fisher’s Exact) and simple logistic regression (to determine odds ratios) will be performed to evaluate whether there is an association between the presence of *S. aureus*/MRSA on a bus and several factors of interest.

1.3.3 Specific Aim 3 Hypotheses

o Hypothesis 3.1: There will be >1 phenotypic profile (a “mix”) among positive isolates (recovered from vehicles).

o Hypothesis 3.2: Both SCCmec Types II and IV will be recovered from vehicles (healthcare-associated, or “HA-” and community-associated, or “CA-”).
Hypothesis 3.3a,b: SCCmec Type I-III (II) (a), USA 100/200 (b) isolates will be resistant to more classes of antibiotics than Type IV-V (IV) (a), USA 300/400 isolates (b) (HA- more resistant).

Hypothesis 3.4a,b: The percentage of SCCmec Type I-III (II) (a), USA 100/200 (b) isolates will be higher on buses that service hospitals than those that do not (HA- related to vehicles that service hospitals).

This specific aim will provide data on the antibiotic resistance profiles of all positive bus isolates, the SCCmec types for all MRSA-positive isolates, and the PFGE types of all isolates from vehicles with multiple positive isolates. In addition, categorical analysis (Chi-Squared/Fisher’s Exact) and simple logistic regression (to determine odds ratios) will be performed to evaluate whether there is an association between SCCmec Type and several factors of interest (particularly multi-class drug resistance and whether a hospital was served by a vehicle or not).
1.5 Conceptual Project Flow Diagram

Figure 1.1: Project Flow Diagram
Chapter 2: Background

*Staphylococcus aureus* (*S. aureus*) is an important nosocomial and community pathogen with a high burden of disease. The bacteria has a number of features which enhance its ability to survive on inanimate surfaces for long periods of time, increasing the chance that viable bacteria will be available for transmission (Dietze et al., 2001; Jawad et al., 1996; Sexton et al., 2006; Wagenvoort & Penders, 1997; Wagenvoort et al., 2000; Williams & Davis, 2009). In addition, a wide range of virulence factors and antimicrobial resistance determinants make *S. aureus* a formidable pathogen. In this chapter, the research team presents an overview of the bacterial features of *S. aureus*, discusses the primary virulence factors and mechanisms of pathogenesis, provides background information regarding antimicrobial resistance, and outlines the important epidemiological characteristics and risk factors for *S. aureus* transmission. Further, the relevant aspects of surface sampling for bacterial isolation from the environment, and cleaning as an intervention strategy for reduction of *S. aureus* contamination, will be introduced. The goal is that the reader obtains the appropriate background understanding of *S. aureus* to place this research project into proper context.
2.1 Bacterial Features

*Staphylococcus aureus* is a Gram-positive, non-motile, non-sporulating coccus. A member of the family Staphylococcaceae, the bacterium is approximately 0.5-1.5µm in diameter and grows in grapelike clusters as a result of cellular division in three planes (Figure 2.1). On growth media, colonies generally appear as beige to yellow in color and may exhibit alpha, beta, gamma, or delta hemolysis on blood agar (typically alpha and beta hemolysis). *S. aureus* is quite halotolerant, able to proliferate in salt concentrations up to 15%. A facultative anaerobe, *S. aureus* is usually catalase and coagulase positive, and is capable of mannitol fermentation (Lowy, 1998; Todar, 2010). Growth range is 15-45°C, with optimal temperature for laboratory isolation 35-37°C (the bacterium grows better at 37°C, but the antimicrobial resistance gene, *mecA*, is more efficiently expressed at 35°C) (R. Hoet, personal correspondence, 2010; Todar, 2010).
There are 36 species of staphylococci, of which 16 are relevant to humans; \textit{S. aureus} is clearly the most important human pathogen of the staphylococci (Que & Moreillon, 2009; Todar, 2010). \textit{S. aureus} has a circular genome \textasciitilde2.8 Mb in length and is believed to share common ancestry with \textit{Bacillus subtilis}; however, \textit{S. aureus} can be distinguished by its low G-C content (30-39\%) (Lowy, 1998; Que & Moreillon, 2009; Todar, 2010).

2.2 Antibiotic Resistance

\textit{Staphylococcus aureus} has been an important pathogen since long before the advent of antibiotic agents. However, the emergence of antimicrobial resistant \textit{S. aureus}
has limited our ability to effectively control the pathogen, particularly in the nosocomial setting. Antibiotic resistant *S. aureus* emerged shortly after the introduction of penicillin, and MRSA soon after the introduction of the β-lactamase stable methicillin (β-lactamase is an enzyme some bacteria produce that confers resistance to β-lactams) (Gordon & Lowy, 2008; Lowy, 1998). According to Chambers and DeLeo (2009), *S. aureus* resistance developed and progressed in four major historic waves. This began with penicillin-resistant *S. aureus* in 1940s, followed by the introduction of methicillin and subsequent resistance to that β-lactam in 1961 (and associated resistance to the entire class of β-lactams). The third wave occurred in the 1970s with the emergence of mecII and mecIII, which are the primary SCCmec (the genetic element that carries the methicillin resistance gene) types in nosocomial settings. The fourth wave has been more recent (1990s), and is marked by the emergence of CA-MRSA (and the highly successful strain, USA300), which may affect people without any healthcare-associated exposure or underlying risk factors (Chambers & DeLeo, 2009).

Penicillin-resistance emerged as a result of a plasmid-encoded penicillinase (Chambers & DeLeo, 2009). Nearly all strains of *S. aureus* are now resistant to penicillin (Gordon & Lowy, 2008; Lowy, 1998). The origins of methicillin resistance seem to be divergent, with some strains arising from a small number of clones, and others from multiple clones (Enright et al., 2002; Lowy, 1998). The emergence of MRSA may have occurred through horizontal gene transfer and/or selective pressure, and in some cases mutation may confer resistance to additional antibiotics (Chambers & DeLeo, 2009; Que & Moreillon, 2009). In some cases, it is believed that horizontal transfer from coagulase-
negative staphylococci (CoNS) lead to the acquisition of meca by S. aureus. In other cases, MRSA may have developed more directly from epidemic MSSA strains (Chambers & DeLeo, 2009; Hanssen et al., 2004; Hanssen & Sollid, 2006; Wu et al., 1996; Lowy, 1998). In studies of the origin of MRSA, Enright et al. (2002) found 5 original lineages of MRSA (from MSSA) from 11 major clones, demonstrating that the resistant strains did not emerge in unison (Enright et al., 2002).

*S. aureus* β-lactam resistance (including methicillin resistance) is conferred by the altered penicillin-binding protein, PBP2a, which is encoded on the meca gene (Chambers & DeLeo, 2009; Gordon & Lowy, 2008; Maltezou & Giamarellou, 2006). An important feature of meca and the altered PBP2 is that, unlike penicillinase-mediated penicillin-resistance, methicillin-resistant strains are usually resistant to all β-lactams, including penicillins, cephalosporins, and carbapenems (Chambers & DeLeo, 2009; Gordon & Lowy, 2008; Lowy, 1998); this has significant clinical treatment implications. In addition to meca, bla (β-lactamase) and fem (“factors essential for methicillin-resistance”) are important accessory genes involved in the expression of resistance to β-lactams and methicillin, respectively (Lowy, 1998). Additional genes confer resistance to other antibiotics, such as vanA and vanH to vancomycin (the “drug of last resort” in MRSA infections); these are also located in the SCCmec complex (Que & Moreillon, 2009).

The meca gene complex that encodes for methicillin resistance is located on the staphylococcal cassette chromosome (SCCmec) (Chambers & DeLeo, 2009; Gordon & Lowy, 2008). There are currently eight known SCCmec types (Zhang et al., 2009).
CoNS (such as *S. epidermis*) also have similar SCC*mec* genetic structures (Chambers & DeLeo, 2009; Ruppe et al., 2009), and may have provided an original source for *S. aureus* to acquire the gene complex (Deurenberg & Stobberingh, 2008; Wisplinghoff et al., 2003). Some of the SCC*mec* types have been the subject of significant research, and have provided epidemiologists a tool to elucidate strain origin and mechanics of strain success. For example, it was discovered that *mecIV* (which is epidemiologically-associated with community infections) is the smallest SCC*mec* structure, and seems to "confer little to no cost in fitness on the organism." *mecIV* also has more rapid growth rates compared to other SCC*mec* types (Chambers & DeLeo, 2009; Okuma, 2002; Robinson & Enright, 2003). These factors may explain, in part, the recent proliferation of this SCC*mec* type.

2.3 Colonization and Disease

*S. aureus* transmission is enhanced by the fact that healthy individuals can become asymptomatic carriers for the bacteria; these individuals are referred to as “colonized” or “carriers” (Chambers & DeLeo, 2009; Gorwitz et al. 2008). Staphylococcal colonization (both MSSA and MRSA) is believed to occur in three states: intermittent colonization, persistent colonization, and non-colonization (Kluytmans et al., 1997). Individuals colonized intermittently may become carriers for a short time period, cease colonization, and either later become colonized again or not. Persistently colonized individuals are always or nearly always colonized. Some individuals are never colonized. There is some evidence that these individuals (those never colonized) may be protected
from *S. aureus* colonization by colonization with a resident commensal bacterium (such as coagulase-negative staphylococci) (Kluytmans et al., 1997). In the case of intermittent colonization, the colonizing strain may vary. Conversely, persistent carriers usually only carry one strain (Kluytmans et al., 1997). In the United States, *S. aureus* nasal colonization occurs at the rate of 20% for chronic carriers and ~30-60% for those intermittently colonized; 20% are rarely or never colonized (Chambers & DeLeo, 2009; Gordon & Lowy, 2008; Gorwitz et al., 2008; Kluytmans et al., 1997). Once colonized, *S. aureus* carriage appears to last from months to over 3 years (David & Daum, 2010; Marschall & Muhlemann, 2006; Sanford et al., 1994; Torell et al., 2005).

The nose, especially the anterior nares, is the major site of *S. aureus* colonization. However, other areas such as the hand, forearm, axilla, perineum, and other body sites can also be colonized (Figure 2.2). Hand and other skin colonization may be particularly important in community-associated *S. aureus* infections, where exposed skin may contact other people or surfaces (David & Daum, 2010; Kluytmans et al., 1997). There is evidence that de-colonization with mupirocin in the anterior nares also results in elimination of *S. aureus* colonization on the hands, which may be an important strategy to reduce both hospital and community transmission (Kluytmans et al., 1997; Reagan et al., 1991). There is strong evidence that colonization often precedes infection, and that the colonizing strain is usually the same strain that causes infection; this indicates that the opportunistic, colonizing *S. aureus* strain may provide a source for endogenous infection (Chambers & DeLeo, 2009; Gordon & Lowy, 2008; Kluytmans et al., 1997; Lowy, 1998). However, this may be less relevant in community transmission, as some have
argued that precursor colonization may be less important than contaminated fomites and surfaces in community transmission (Begier et al., 2004; Kazakova et al., 2005; Miller & Diep, 2008).

The principles of *S. aureus* colonization also apply to the resistant form of the bacterium. Colonization with methicillin-resistant *S. aureus* (MRSA) is quite common, estimated at 1-2% of the U.S. population (Gorwitz et al. 2008). MRSA nasal colonization has increased concurrently as community-associated infections have increased. From 2001 to 2004, MRSA colonization rose from 0.8% to 1.5%, as the proportion of cases from community-associated MRSA (as opposed to healthcare-associated cases) increased from 7% to 24.2% (Chambers & DeLeo, 2009; Gorwitz et al. 2008; Kennedy et al., 2008; Tenover et al. 2008). This increase in MRSA colonization is likely due at least in part to the rise of a particularly successful strain of MRSA – USA300 (Boucher & Corey, 2008; Chambers & DeLeo, 2009; Hota et al., 2007; Kennedy et al., 2008 Miller & Diep, 2008; Popovich et al., 2008). Regardless of whether colonization occurs from MSSA or MRSA, colonization is clearly an important aspect of *S. aureus* transmission and disease.
2.4 Disease and Treatment

*Staphylococcus aureus* infections result in a wide range of clinical diseases, including bacteremia, endocarditis, joint disease, infections of the kidneys and lungs, sepsis, toxic shock syndrome and food poisoning. *S. aureus* is also the primary cause of skin and soft tissue infections (SSTI). In the community, *S. aureus* infection most commonly results in SSTIs, but may also manifest as necrotizing pneumonia (Lowy, 1998; Maltezou & Giamarellou, 2006; Moran et al., 2006). Nosocomial disease from *S.
Staphylococcus aureus usually depends upon the location of the infection or underlying conditions (Naimi et al., 2003).

Less than 5% of *S. aureus* strains are penicillin-susceptible; therefore, penicillin is not a routinely viable treatment option. In the case of penicillin-resistance, oxacillin (which is similar to methicillin but has replaced it in clinical use) or alternately, cefazolin, may be prescribed. However, when the infecting strain is MRSA (oxacillin/methicillin-resistant), vancomycin remains the primary (and often only) treatment option (Chambers & DeLeo, 2009; Lowy, 1998). Treatment remains a challenge, particularly in healthcare settings, where MRSA is often multi-drug resistant (Killic et al., 2006; Robinson & Enright, 2003).

### 2.5 Risk Factors

Risk factors for infection with healthcare-associated MRSA are well-established. Healthcare-associated MRSA infections tend to predominantly affect those older than 65 years old; individuals with underlying health conditions, such as diabetes or HIV; patients with indwelling catheters or other medical devices; people whom have undergone recent surgery; recent antibiotic usage; recent hospitalization; dialysis patients; residents in a long-term care facility; and individuals colonized with MRSA (CDC, 2010; Gorwitz et al., 2008; Graffunder & Venezia, 2002; Klevens et al., 2007; Kluytmans-VanderBergh & Kluytmans, 2006; Lasseter et al., 2010; Naimi et al., 2001).

Less is known about the factors that underlie community-associated infections. By definition, community-associated MRSA infections affect people who lack certain
underlying health risk factors and healthcare exposure (CDC, 2005; Gorak et al., 2010; Klevens et al., 2007; Morrison et al., 2006). Further, according to the CDC, “MRSA in the community is widespread and therefore, anyone is at risk” (CDC, 2010). Despite the absence of health risk factors, CA-MRSA infections have been epidemiologically linked to several groups. High human density settings, such as military barracks, locker rooms, dormitories, day care centers, and prisons, seem to be associated with more infections (CDC, 2010; as cited in David & Daum, 2010; as cited in Kluytmans-VanderBergh & Kluytmans, 2006). Also, those individuals that have more frequent skin-to-skin contact, such as contact sport athletes (football players and wrestlers) and men who have sex with men, may be at higher risk of developing MRSA infection. Overall, CA-MRSA strains tend to affect younger, healthier individuals, and disproportionately affect those who live in urban underserved communities, blacks and Native Americans, and males, (as cited in David & Daum, 2010). The CDC notes five aspects, named the “5 C’s”, which may place individuals at higher risk of CA-MRSA infection. These include crowding, frequent skin-to-skin contact, compromised skin (cuts, wounds, or abrasions), contaminated items or surfaces, and lack of cleanliness (CDC, 2010). Indeed, it is because public transportation serves an urban community and has several elements of the “5 C’s”, that it was chosen for study in this project.
## Risk Factors/Groups for MRSA Colonization and Disease

<table>
<thead>
<tr>
<th>Risk Factor/Group</th>
</tr>
</thead>
<tbody>
<tr>
<td>Neonates</td>
</tr>
<tr>
<td>Children beyond the neonatal period</td>
</tr>
<tr>
<td>Athletes</td>
</tr>
<tr>
<td>Household contacts of MRSA SSTI patients</td>
</tr>
<tr>
<td>Emergency department patients</td>
</tr>
<tr>
<td>Urban undeserved communities</td>
</tr>
<tr>
<td>Indigenous populations</td>
</tr>
<tr>
<td>Detainees in jail or prison</td>
</tr>
<tr>
<td>Cystic fibrosis patients</td>
</tr>
<tr>
<td>Military personnel</td>
</tr>
<tr>
<td>Men who have sex with men</td>
</tr>
<tr>
<td>HIV patients</td>
</tr>
<tr>
<td>Veterinarians, livestock handlers, and pet owners</td>
</tr>
<tr>
<td>Intravenous drug use</td>
</tr>
<tr>
<td>Recent Antibiotic use</td>
</tr>
<tr>
<td>Poor personal hygiene</td>
</tr>
<tr>
<td>Poor cleanliness (individual and household)</td>
</tr>
<tr>
<td>Crowding Activities with frequent skin-skin contact</td>
</tr>
<tr>
<td>Abraded skin/skin damage</td>
</tr>
<tr>
<td>Limited access to healthcare</td>
</tr>
<tr>
<td>Day care attendees</td>
</tr>
<tr>
<td>Prolonged hospital stay</td>
</tr>
<tr>
<td>Long Term Care facility admission</td>
</tr>
<tr>
<td>Indwelling catheters</td>
</tr>
<tr>
<td>Underlying disease: diabetes, respiratory disease</td>
</tr>
<tr>
<td>HIV/AIDS, renal disease</td>
</tr>
<tr>
<td>Family exposure to MRSA</td>
</tr>
</tbody>
</table>

Table 2.1: Risk Factors for MRSA Colonization and Disease (CDC, 2010, David & Daum, 2010; Kluytmans et al., 1997; Landers et al., 2009)
2.6 Epidemiology

*Staphylococcus aureus* is the most frequently isolated human pathogen, resulting in significant disease burden (David & Daum, 2010; Klevens et al., 2007; Lowy, 1998). David and Daum (2010) estimated approximately 1,300,000 MRSA infections occur per year (based on 2005 data from Klevens et al. [2007]). According to the latest data from the CDC’s multicenter Bacterial Core Surveillance system, the incidence rate of invasive MRSA infections is 0.74 per 10,000 person-years (PY) for hospital-onset (HA-MRSA) cases, and 1.84 per 10,000 PY for health care-associated community-onset infections (HACO-MRSA) (Kallen et al., 2010). Although these figures indicate an overall decrease in incidence from 1.02 per 10,000 PY for HA-MRSA invasive infections and 2.18 per 10,000 PY from HACO-MRSA infections, MRSA still accounted for over 21,500 cases of invasive infection from 2005-2008 (Kallen et al., 2010). In addition, these results are inconsistent with other recent findings that found that MRSA infections are increasing both in the hospital and community (Chambers & DeLeo, 2009; Hersh et al. 2008; Kaplan et al. 2005; Klevens et al. 2007). The emergence of CA-MRSA is reason for much of the increase noted in some research (Chambers & DeLeo, 2009; Moran et al., 2006). In the Kallen et al. report, hospital-onset infections accounted for 57% of the cases, healthcare-associated community-onset, 25%, and community-associated, 18% (Kallen et al., 2010). Interestingly, there was not a significant decrease in skin and soft tissue infections during the same time period (Kallen et al., 2010). The annual incidence rate of invasive infections from MRSA abscesses and cellulitis was 32.5/1,000 in 2005, a doubling from 17.3/1,000 in 1997 (David & Daum, 2010; Hersh et
al., 2008). The overall increase in incidence is suspected to be due to the emergence of a highly successful strain, USA300, which causes the majority of community-associated MRSA infections (most of which are skin and soft tissue infections). USA300 is now also “spilling-over” into the nosocomial setting, where it is gaining ground as an important healthcare-associated strain (Boucher & Corey, 2008; Chambers & DeLeo, 2009; Hota et al., 2007; Kennedy et al., 2008; Miller & Diep, 2008; Popovich et al., 2008).

2.7 Healthcare and Community-Associated MRSA

Prior to the 1990s, MRSA was almost entirely a nosocomial pathogen. However, since community-associated strains began to emerge in the early 1990s, a clear divergence in MRSA epidemiology was introduced. HA-MRSA and CA-MRSA strains are generally distinct, both in terms of patient risk factors and outcomes, as well as genotypically. This has greatly aided in epidemiological characterization of patterns of infection and transmission. However, in recent years the clear distinction between healthcare-associated and community-associated strains has been blurring, as community strains cross into the hospital with increasing frequency (Boucher & Corey, 2008; Chambers & DeLeo, 2009; Hota et al., 2007; Kennedy et al., 2008; Miller & Diep, 2008; Popovich et al., 2008).

Well-established molecular epidemiological techniques have proven highly efficient in characterizing MRSA strain variations (David & Daum, 2010). The primary methods used for S. aureus molecular epidemiological investigations are multilocus sequence typing (MLST), pulsed-field gel electrophoresis (PFGE), spa (Protein A)
typing, and SCCmec typing using polymerase chain reaction (David & Daum, 2010; Trindade et al., 2003; van Belkum, 2000). Using these techniques, investigators have been able to effectively characterize MRSA strain origin as either healthcare-associated or community-associated.

Numerous case definitions have been used to assign MRSA infections into HA- and CA- groups. The variety and inconsistency in case definitions has made determining the burden of CA-MRSA difficult (Maltezou & Giamarellou, 2006). According to David and Daum (2010):

“In 2000, the CDC created a case definition for a CA-MRSA infection: any MRSA infection diagnosed for an outpatient or within 48 hours of hospitalization if the patient lacks the following healthcare-associated MRSA risk factors: hemodialysis, surgery, residence in a long-term care facility or hospitalization during the previous year, the presence of an indwelling catheter or a percutaneous device at the time of culture, or previous isolation of MRSA from the patient (CDC, 2005; David & Daum, 2010; Morrison et al., 2006). All other MRSA infections were considered to be of healthcare origin. The case definition has been modified for the purposes of the CDC’s Active Bacterial Core Surveillance Program for invasive MRSA infections
to exclude the previous isolation of MRSA as a criterion for HA-MRSA (Klevens et al., 2007)” (David & Daum, 2010).

Unlike hospital-associated MRSA strains, which are associated with recent admission to a healthcare facility, community-associated MRSA strains are marked by no known medical exposure risk factors (Boucher & Corey, 2008; David & Daum, 2010; Deresinks, 2005; Maltezou & Giamarellou, 2006). Although there is increasing epidemiological cross-over between HA-MRSA and CA-MRSA (that is, CA- strains being found in the hospital and HA- strains in the community), the two are generally distinct genotypically (Boucher & Corey, 2008; Otter & French, 2006; Seybold et al., 2006). HA-MRSA strains are typically SCCmec types I-III, and PFGE types USA100 and USA200 (primarily type II, USA100). They tend to be less virulent, affect older patients, and are multi-drug resistant (David & Daum, 2010; Ito et al., 2001; Oliveira & de Lencastre, 2002; Popovich et al., 2008). CA-MRSA strains are marked by increased virulence due to the presence of the Panton-Valentine leukocidin gene and several other virulence factors, are usually SCCmec types IV-V and PFGE types USA300 and USA400 (primarily type IV, USA300), and affect children more frequently (Chambers & DeLeo, 2009; David & Daum, 2010; Ito et al., 2004; Ma et al., 2002; Maltezou & Giamarellou, 2006; Okuma et al., 2002; Popovich et al., 2008; Robinson & Enright, 2003). Although more virulent, CA-MRSA strains are generally susceptible to non-β-lactam antibiotics (Deresinski, 2005).
2.8 Pathogenesis and Virulence

*Staphylococcus aureus* infections are marked by a number of important structural and secreted elements which dictate pathogenesis and virulence of the bacterium (Gordon & Lowy, 2008). *S. aureus* infection involves adhesion, invasion, and proliferation into host tissue. In the initial stages of invasion, binding proteins are favored to enhance colonization of host tissue. Then, as infection progresses, extracellular proteins are favored to increase bacterial spread (Lowy, 1998). In this section, several of these elements will be briefly outlined.

During the initial phases of infection, *S. aureus* has a number of strategies to resist and evade host defenses, enhancing subsequent adhesion and proliferation. *S. aureus*’s primary defense is the microcapsule, which resists phagocytosis by the host (Gordon & Lowy, 2008; Lowy, 1998). *S. aureus* also produces numerous surface proteins, of which Protein A is most important. Protein A, encoded on the *spa* gene and present in most *S. aureus* strains, imparts resistance to host phagocytosis (David & Daum, 2010; Foster, 2005; Lowy, 1998; Uhle et al., 1984). Microbial surface components recognizing adhesive matrix molecules (MSCRAMMs) are additional surface proteins that enhance bacterial adhesion to host tissue (Lowy, 1998). Biofilm formation may also enhance adhesion, and is particularly important in medical device-mediated infections (Gordon & Lowy, 2008). *S. aureus* may also form small colony variants (SCVs) which reside intercellularly in host cells and are thus partially protected from antibiotics and host defenses, and may induce recurrent infections after the pathogen returns to a more
virulent wild type (Gordon & Lowy, 2008; Kahl et al., 2010; Proctor & Peters, 1998; Proctor et al., 1995).

Once infection has commenced, several virulence factors affect the outcome of disease. At the most basic level is the staphylococcal cell wall, which is 50% peptidoglycan by weight, and has endotoxin-like activity (Lowy, 1998). *S. aureus* also produces numerous toxins which may induce host tissue damage. Some examples are Panton-Valentine leukocidin (PVL) toxin, which is epidemiologically-associated with community-associated or healthcare-associated community-onset (HACO-) necrotizing pneumonia and severe skin disease; enterotoxins and pyrogenic toxic superantigens (proteins that activate T cells and cause a flood of host cytokines that may result in toxic shock syndrome or staphylococcal food poisoning); cytotoxins that destroy cells through pore formation; and exfoliative toxins which cause scalded skin syndrome. Other important proteins and enzymes in the infection process include those that destroy host tissue (protease, lipase, and hyaluronidase); those that impart drug resistance (β-lactamase and penicillinase, which inactivate penicillin); those which are involved in cell wall assembly and thus affect antibiotic activity (PBPs); and binding proteins (such as coagulase) (Chambers & DeLeo, 2009; DeLeo et al., 2009; Gordon & Lowy, 2008; Lowy, 1998; Wang et al., 2007).

Genetic regulation of virulence factors is also critical to the outcome of disease, and no genes are more important than *agr* (Gordon & Lowy, 2008; Lowy, 1998). *Agr* are regulatory genes that induce the expression of extracellular proteins while suppressing expression of surface proteins. They act as regulators of quorum-sensing (bacterial
“signaling” through regulation of gene expression, in response to density), which moderates growth/cellular density. As a result, surface proteins (such as MSCRAMMs) are synthesized during exponential growth phase, favoring cellular adhesion, and secretory proteins (toxins, etc.) are synthesized during stationary phase, enhancing the spread of infection (Gordon & Lowy, 2008; Lowy, 1998; Que & Moreillon, 2009).

With respect to methicillin-resistant S. aureus specifically, it is unclear whether MRSA is more virulent than MSSA (Cosgrove et al., 2003; Engemann et al., 2003; Gastmeier et al., 2005; Gordon & Lowy, 2008; Reed et al., 2005). According to Gordon and Lowy (2008), "To date, there is no compelling evidence that MRSA, in general, is more virulent than MSSA.". However, MRSA does result in higher cost-of-treatment and clearly impacts the availability of effective treatment options (Cosgrove et al., 2005; Engemann et al., 2003; Gordon & Lowy, 2008). In addition, there is strong evidence that community-associated MRSA strains do tend to be more virulent than healthcare-associated strains (and thus, MSSA), resulting in worse clinical outcomes and increased mortality (Baba et al., 2002; Chambers & DeLeo, 2009; Davis et al., 2007; Francis et al., 2005; Gordon & Lowy, 2008; Ito et al., 2004; Okuma et al., 2002; Turner et al., 2007; Zetola et al., 2005). The USA300 strain, which causes most CA-MRSA infections and is becoming the predominant MRSA strain in the United States, is quite virulent (as is USA400, another community-associated MRSA strain). (Baba et al., 2002; Chambers & DeLeo, 2009; Diep et al., 2006; Gordon & Lowy, 2008).

Due to its increased virulence, the virulence factors of CA-MRSA strains have been studied extensively in the past two decades. Some of the more important CA-
MRSA virulence factors include staphylokinase (which results in the cleavage of fibrin); staphylococcal complement inhibitor (which interferes with phagocytosis of *S. aureus* by the host); and *S. aureus* chemotaxis inhibitory proteins (that inhibits the host inflammatory response and neutrophil "recruitment") (Chambers & DeLeo, 2009; Rooijakkers et al., 2006; van Wamel et al., 2006).

Perhaps the most well-researched (and also most frequently debated) MRSA virulence factor is the Panton-Valentine leukocidin protein (PVL). Discovered in 1894 by Van de Velde and linked to skin and soft tissue infections in 1932 by Panton and Valentine, PVL is responsible for pore formation in host leukocytes; which causes subsequent host cell death (Chambers & DeLeo, 2009; Collin et al., 1994; David & Daum, 2010; Meyer et al., 2009; Panton et al., 1932). PVL, encoded for by the lukF-PV and lukS-PV genes, also triggers neutrophils to produce additional reactive oxygen species, which enhances the host inflammatory response and further injures host tissue (Chambers & DeLeo, 2009; Colin & Monteil, 2003). PVL has been epidemiologically-linked to necrotizing pneumonia and severe skin and soft tissue infections, which often manifest in the “spider bite” appearance that is commonly reported in the case report literature (Brown et al., 2008; Bubek et al., 2007; Bubek et al., 2008; Chambers & DeLeo, 2009; David & Daum, 2010; Diep et al., 2008; Dominguez, 2004; Gillet et al., 2007; Labandeira et al., 2008; Montgomery & Daum, 2009; Vetter et al., 2006).

The occurrence of PVL is quite significant, particularly in community-associated MRSA strains. PVL is present in 60-100% of USA300 and USA400 strains (the primary CA-MRSA strains) (David & Daum, 2010; Gordon & Lowy, 2008; Lina et al., 2010;
Said-Salim et al., 2005). Initial research had indicated the presence of PVL in most highly-virulent community infections (Maltezou & Giamarellou, 2006). However, recent data from animal models calls into question the role that PVL plays in increased virulence (as cited in Gordon & Lowy, 2008). There is some evidence that alpha-hemolysin may the cause of increased virulence, not PVL (Wardenburg et al., 2007; Gordon & Lowy, 2008).

There are other potential contributors to the increased virulence observed in CA-MRSA strains. The arginine catabolic mobile element (ACME) is frequently present in USA300 strains and some CoNS (Chambers & DeLeo, 2009; David & Daum, 2010; Diep et al., 2008; Diep et al., 2006). It is believed that ACME allows *S. aureus* to survive at lower pH levels (and thus on human skin) and more readily colonize healthy individuals, better grow in low oxygen environments (such as abscesses, which are an important feature of skin and soft tissue infections [SSTIs]), and inhibit production of nitric oxide and mononuclear cells, which enhances evasion of host defense mechanisms (David & Daum, 2010; Degnan et al., 1998; Diep et al., 2006). Phenol-soluble modulins, which may lyse neutrophils, are yet another potential virulence factor of CA-MRSA strains (Chambers & DeLeo, 2009; David & Daum, 2010; Hongo et al., 2009; Wang et al., 2007; Wardenburg et al., 2008).

Despite the abundance of MRSA virulence factors, most virulence factors described here are not unique to MRSA. However, these elements are very important, as they may increase the severity of infection and have also proven useful for epidemiological investigations. Clearly the body of knowledge regarding MRSA
virulence will only grow, as virulence factors are the subject of considerable study. This will aid physicians and epidemiologists to better deal with future MRSA infections and outbreaks.

2.9 Transmission

*Staphylococcus aureus* is primarily transmitted through direct contact with a colonized or infected individual, or through a fomite intermediate (Chambers & DeLeo, 2009; Kazakova et al. 2005; Lowy 1998; Miller & Diep 2008; Muto et al. 2003). Hands are the critical disseminators, particularly the hands of healthcare workers (Ayliffe et al., 1988; Bhalla et al., 2004; Boyce et al., 1997; Gordon & Lowy, 2008). Healthcare workers are important in transmission for several reasons. First, they care for multiple patients throughout the day, going from room to room, patient to patient. In this process, there are countless occasions to touch infected or colonized patients and contaminated fomites. It has been estimated that an ICU healthcare worker has an average of 43.4 opportunities for hand washing per hour, per patient (Beggs et al., 2008; Pittet et al., 1999). With this high demand for services that require hand washing, it is unfortunate, yet not surprising, that hand hygiene compliance remains low - often less than 50% (Pittet, 2001; Pittet et al., 2000; Randle et al., 2006). In addition, asymptomatic MRSA colonization rates are higher among healthcare workers than the general public (an average of 4.2% for healthcare workers in the U.S. versus 1-2% for the general public) (Albrich & Harbarth, 2008; CDC/Kuehnert et al., 2006; Gorwitz et al. 2008). Therefore, it is not surprising that in hospitals, approximately 50% of those exposed to MRSA will
subsequently develop a MRSA infection (Boyce, 1989; Kowalski et al., 2005). Healthcare workers are not only critical in S. aureus transmission, they are also in a key position to prevent it.

Contaminated objects and surfaces, or fomites, also play an important role in S. aureus transmission. There are countless examples of contaminated fomites from prior research, both in the hospital and in the community (Andrade et al., 2009; Baggett et al., 2004; Begier et al., 2004; Dancer, 2007; Felkner et al., 2009; Giannini et al., 2009; Hardy et al., 2006; Kazakova et al., 2005; Miller & Diep, 2008; Rieg et al., 2005). Fomites become contaminated through hand-to-fomite contact, bioaerosol settling, or splashing/contamination with infectious fluids. Once established on a surface, S. aureus can survive for weeks to months (Dietze et al., 2001; Jawad et al., 1996; Sexton et al., 2006; Wagenvoort & Penders, 1997; Wagenvoort et al., 2000; Williams & Davis, 2009). There is little research regarding the role that airborne transmission plays in spreading S. aureus. However, S. aureus is clearly capable of being aerosolized, and may play an underestimated role in transmission (Dancer, 2008; Gehanno et al., 2009; Hambraeus, 1978; Kagan et al., 2002; Lutz et al., 2010; Motimer et al., 1966; Scott et al., 2009; Williams et al., 1960).

Table 2.2 and Table 2.3 provide a partial list of fomites on which S. aureus contamination has been isolated. For additional information regarding transmission specific to public transportation vehicles, please see Chapter 1 and Chapter 4.
<table>
<thead>
<tr>
<th><strong>Fomite</strong> (Note: MSSA and MRSA only)</th>
<th><strong>Location/Setting</strong></th>
<th><strong>Reference</strong></th>
</tr>
</thead>
<tbody>
<tr>
<td>Toilet Seats</td>
<td>Hospital</td>
<td>Giannini et al., 2009</td>
</tr>
<tr>
<td>Patient Beds (area around, including floor, ledges)</td>
<td>Hospital</td>
<td>Hardy et al., 2006</td>
</tr>
<tr>
<td>Workstations</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Monitors</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control Buttons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Respiratory Therapy Equipment</td>
<td>Hospital</td>
<td>Boyce et al., 2010</td>
</tr>
<tr>
<td>Floor</td>
<td>Hospital</td>
<td>Boyce et al., 1997</td>
</tr>
<tr>
<td>Bed Linens</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Patient Gown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Overbed Tables</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Blood Pressure Cuffs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Nursing Uniform/Gown</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Rails</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pump Buttons</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Door Handles</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Stethoscope</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Window Sill</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilets</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 2.2: Example *S. aureus* Fomites in the Hospital
<table>
<thead>
<tr>
<th>Fomite (Note: MSSA and MRSA only)</th>
<th>Location/Setting</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Chair (from Clinic) Van Seat Faucet Stair Rail Table Toilet Handle Toilet Seat</td>
<td>Community (Correctional Facility)</td>
<td>Felkner et al., 2009</td>
</tr>
<tr>
<td>(Epidemiologically-linked, not sampled) Whirlpool Turf burn (⇒ Turf) Shaving (⇒ Razor)</td>
<td>Community (Football)</td>
<td>Begier et al., 2004</td>
</tr>
<tr>
<td>Whirlpool (MSSA) Taping Gel (MSSA)</td>
<td>Community (Football)</td>
<td>Kazakova et al., 2005</td>
</tr>
<tr>
<td>Wood from a Sauna Bench</td>
<td>Community</td>
<td>Baggett et al., 2004</td>
</tr>
<tr>
<td>(All MSSA) Buttons Toilets Vehicle Grab Rails ATM Keypad Ticket Keypad</td>
<td>Community</td>
<td>Otter &amp; French, 2009</td>
</tr>
<tr>
<td>Office Items Couch Remote Table Kitchen Items Desks Class Tools</td>
<td>Community/Healthcare (Fire/EMS Stations/Offices)</td>
<td>Sexton &amp; Reynolds, 2010</td>
</tr>
</tbody>
</table>

Table 2.3: Example *S. aureus* Fomites in the Community

*Continued...*
Table 2.3 Continued...

<table>
<thead>
<tr>
<th>Kitchen</th>
<th>Community (household)</th>
<th>Scott et al., 2008, 2009</th>
</tr>
</thead>
<tbody>
<tr>
<td>Phone (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sink</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Refrigerator Door Handle (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faucet Handle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sponge/cloth</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Table top (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Countertop</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Garbage Bin</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Drain</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Dish Towel</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Microwave Keypad (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Floor (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Bathroom</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sink</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet Floor (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet Seat (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toilet Bowl (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tub</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Countertop (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Flush Handle (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Faucet Handle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Door Handle</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Light Switch (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Home Office/Other</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Keyboard (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Mouse (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Phone (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>TV Remote Control (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>High Chair</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Changing Mat (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Toy (MSSA)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Pet Food Dish</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

2.10 Surface Sampling

To evaluate the contamination levels of MSSA and MRSA on public transportation vehicle surfaces, an evaluation of four commonly utilized sampling methods was conducted. The results of this research are outlined in Chapter 3. Currently, there are no standard methods for sampling surfaces for bacterial...
contamination, and a wide range of strategies have been used to evaluate MRSA on environmental surfaces (Cimolai, 2008; Felkner et al., 2009; Obee et al., 2007; Otter et al., 2009; Sexton et al., 2006; Sherlock et al., 2009). Countless surface sampling systems are commercially available. Most of these systems can be broadly divided into two major categories: swabs/wipes and contact-based methods.

Swabs samplers are perhaps the most frequently used sampling systems for isolation of bacteria from the environment. Swabs consist of a plastic or wooden base shaft with a rayon, cotton, polyester, or calcium alginate head. Most swab heads are spun around the shaft; however, newer systems using a flocked head design are now available. Most swabs used for surface sampling are not designed for that purpose; rather, they are typically intended for use in clinical specimen collection (although some models are marketed for environmental sampling). Therefore, there are several inherent limitations to the swab-based sampling system. As they are designed to effectively sample a nose, throat, or wound, swabs are not ideal for sampling even moderately sized surfaces. Most environmentally relevant surfaces are beyond the surface area size where a swab is appropriate. Also, previous research has demonstrated that uptake and release may not be ideal (Obee et al., 2007; Lemmen et al., 2001). This may be acceptable in a patient sample where a high bacterial concentration is present. However, this is likely problematic on environmental surfaces with a low bioburden. Despite these limitations, swabs are still widely used because they are often readily available, are low-cost, and most clinical laboratories are accustomed to processing them.
Wipe and sponge systems are another surface sampling option. The principal of operation is similar to that with swabs; that is, the sampler is drawn across the sampled surface and then the sampler is processed for release and analysis of the bacteria. However, sponges and wipes are clearly advantageous over swabs in their ability to effectively sample larger surface areas. Despite this benefit, some wipe sample methods may require the sampling technician to contact the sampler, which introduces additional technical difficulty to properly prevent contamination.

Contact-based sampling systems have been in use for decades. The two most common contact-based methods for bacterial recovery are the contact plate and the dip slide. Contact plates (often called a Replicate Organism Detection and Counting plate, or RODAC) have been used since 1964, when they were developed to sample hospital surfaces (Hall & Harnett, 1964). They consist of a small (~60mm) plastic dish with a convex base. Liquid growth medium is poured into the plate so that when set, the growth medium is raised above the plate surface. Dip slides, whose first iteration were as a crude agar coated spoon utilized for clinical urinalysis, are similar to contact plates (Mackey & Sandys, 1965 from Naylor & Guttmann, 1967). To sample with these systems, the investigator simply applies the contact sampler to the surface being sampled and bacteria become affixed directly to the surface of the growth medium. The inherent benefits of such systems are that no intermediate processing steps are required, and the results can be quantified (or semi-quantified). Therefore, samples can easily be collected and processed without technical expertise, since all that is required is an incubator. However, because there is no manual agitation of the surface during sampling, recovery may be poor.
In addition, because of the design of the support material, contact-based methods are limited to sampling small, flat surfaces. Debris loading creates problems with identifying and counting bacteria, so the surfaces sampled must be relatively clean for accurate analysis; this is clearly problematic in many environmental surface sampling scenarios. Finally, while immediate deposition onto growth medium allows for some selection and differentiation, this may result in low recovery if the medium is overly rich for environmentally stressed bacteria (Reasoner & Geldreich, 1985).

Regardless of the sampling system used, the ideal method will fulfill three important criteria. First, the sampler must be capable of effectively removing bacteria from the surface being sampled. Second, this should be done in such a way that maximizes uptake and minimizes bacterial damage. Third, the sampler must be able to fully release captured bacteria for analysis (Davidson et al., 1999; Moore & Griffith, 2007). In the case of contact-based samplers, the third requirement does not involve release of the bacteria, but rather providing optimal conditions for growth.

Prior research has evaluated the performance of swab and contact-based sampling systems for the isolation of *S. aureus*. Obee et al. (2007) compared common sampling methods under laboratory conditions, and found dip slides to be the most efficient (~percentage recovered) and sensitive (~detection limit). Dip slides required only 9.7 CFU/100cm² MRSA for a positive result (sensitivity; one or more colonies recovered), versus 27.6 CFU/100 cm² for contact plates, 26.0 CFU/ cm² for the most sensitive swab method, and 1,440.1 CFU/ cm² for the least sensitive swab/pour plate method (Obee et al., 2007). Relative sampling recovery efficiency was also much greater for dip slides –
51.33% – versus 0.07% for the least efficient swab method. However, due to their rigid design, dip slides are limited to sampling flat surfaces (Figure 2.3). Overall, swabs were much less efficient and less sensitive than dip slides, and were found to perform better through direct inoculation than dilution/pour plating. The performance of static wipes has not been evaluated for the isolation of *S. aureus*. In a study of *Salmonella* in swine operations, drag swabs were shown to perform slightly better than static wipes (Zewde et al., 2009). However, it is thought that efficiency gains using static wipes may be made due to the large surface area that can be easily sampled; this feature is unique to wipe samples.

In Chapter 3 the research team describes the design of a novel, rolling contact-based sampler that was hypothesized to capture the advantages of both a swab/wipe system (flexibility and large surfaces can be sampled) and a contact-based system (ease
of use, no secondary processing, selective/differential isolation). In addition, the first laboratory analysis of electrostatic wipe samples for collection of \emph{S. aureus} is provided.

\subsection{2.11 \textit{S. aureus} Reduction Strategies}

Since Semmelweis first introduced the notion of hand hygiene in 1846, it has become increasingly relevant in the prevention of disease transmission; particularly for pathogens that are spread easily through direct contact (Boyce et al., 2002; Murphy, 1941 from Lederer et al., 2009). Effective hand hygiene is the primary method for the reduction of MRSA transmission. Numerous studies have shown significant reduction in MRSA and other infection rates with the implementation of comprehensive hand hygiene measures (Bloomfield et al., 2007; Hillburn et al., 2003; Johnson et al., 2005; Lederer et al., 2009; Turabelidze et al., 2006). For example, even nearly fifty years ago, Rammelkamp observed that “newborn infants exposed to nurses who handled colonized infants acquired \emph{S. aureus} 14\% of the time if good hand washing was performed and 43\% of the time in the absence of good hand washing” (Mortimer et al., 1966; Rammelkamp et al., 1964).

\subsubsection{2.11.1 Alcohol-based Hand Sanitizer}

Alcohol-based hand sanitizer has been shown to effectively reduce bacterial bioburden, and when used as part of a comprehensive hand-hygiene program, significant reduction in MRSA infection rates in hospitals have been noted (Lederer et al., 2009). “Waterless hand-hygiene agents reduce the time required to perform hand hygiene and
provide a good alternative to hand washing when access to a sink is not convenient” (quote from Sandora et al., 2005, citing Bischoff et al., 2000; Harbarth et al., 2002; Pettet, 2001). In a randomized control trial, Sandora et al. (2005) demonstrated that hand sanitizer was effective at reducing gastrointestinal infections in the home.

However, public transportation vehicles lack any opportunity for good hand hygiene, and thus may facilitate potential pathogen transmission. Even in the current study vehicles, alcohol-based hand sanitizer dispensers were installed (presumably during the 2009 H1N1 influenza outbreak). However, during limited observational riding, no passengers utilized the hand sanitizer (this may be due in part to the lack of visibility, as all dispensers are gray and black, mounted on a black substrate, without any accompanying signage). In some cases, the dispensers are mounted behind the door, which is nearly impossible to see for a boarding passenger, and completely inaccessible for routine use.

2.11.2 Enhanced Cleaning

Ineffective surface cleaning has been shown to contribute to or cause MRSA outbreaks (Corcoran & Kirkwood, 1999). Suboptimal cleaning may also occur in the home, where MRSA carriers (including healthcare workers, whom have higher colonization rates than the general public) may continually re-colonize themselves or contaminate fomites (Allen et al., 1997; BSACHISICNA, 1998; Haley & Bregman, 1982; Masterson et al., 1995; Reagan et al., 1991; Waldvogel, 1995; Williams, 1963). Conversely, effective terminal cleaning (a highly-detailed cleaning procedure conducted
after an infectious patient has been discharged from a room) has been demonstrated as an effective means to reduce MRSA contamination in healthcare settings (Dancer, 1999; Griffith et al., 2007). Increased and enhanced cleaning (doubling time allotted for cleaning, vacuuming, use of bleach/disinfectant, rinsing, etc.) reduces bacterial bioburden on environmental surfaces, which may act as potential fomites (Dancer, 1999). By substituting current cleaning regimes to one that included a rinse stage and used disposable paper towels over cloths, Griffith et al. (2007) found significantly and more consistently reduced bacterial counts and lower ATP RLU levels (a measure of bacterial contamination). Although cleaning methods likely vary across transportation systems, one bus system indicated that vehicle cleaning is generally focused on “bulk” clean-up, rather than focused sanitation. “Bulk” cleaning consists of picking-up garbage, daily floor sweeping, and washing the windows and hand rails once or twice weekly. In Appendix C the results of a laboratory-based pilot study, demonstrating the efficacy of enhanced cleaning for reduction of S. aureus, are described.
Chapter 3: Performance of Surface Sampling Methods for the Recovery of *S. aureus*

3.1 Introduction

*Staphylococcus aureus* (*S. aureus*) is the most frequently isolated human pathogen, resulting in significant disease burden (David & Daum, 2010; Klevens et al., 2007; Lowy, 1998). Methicillin-resistant *S. aureus* (MRSA) is a major source of nosocomial infection, and the primary cause of skin and soft tissue infections in the community. David and Daum (2010) estimated approximately 1,300,000 MRSA infections occur per year (based on 2005 data from Klevens et al. [2007]). MRSA infections result in 19,000 deaths per year, more than AIDS or tuberculosis (Klevens et al., 2007; CDC, 2005; CDC, 2006). The economic burden of MRSA infections has been demonstrated in several studies, with patient care costs exceeding those of methicillin-susceptible *S. aureus* (MSSA) infections (Cosgrove et al., 2005; Gordon & Lowy, 2008).

*S. aureus* is transmitted primarily through direct contact with a colonized or infected individual. Hands are the critical disseminators, particularly the hands of health care workers (Ayliffe et al., 1988; Bhalla et al., 2004; Boyce et al., 1997). However, environmental surfaces and fomites clearly play an important role in transmission - both in the hospital and community (Chambers & DeLeo, 2009; Kazakova et al. 2005; Lowy,
Effective detection of pathogenic bacteria from environmental surfaces is critical for outbreak prevention and control.

Environmental contamination with *S. aureus* has been demonstrated in numerous studies. Not only is *S. aureus* easily transmitted from skin-to-fomites; once established, it exhibits impressive survivability. *S. aureus* can survive on a variety of surfaces for several months and is highly resistant to desiccation (Dietze et al., 2001; Jawad et al., 1996; Sexton et al., 2006; Wagenvoort & Penders, 1997; Wagenvoort et al., 2000; Williams & Davis, 2009). *S. aureus* contamination has been isolated on numerous fomites, and is believed to be the source, at least in part, of several hospital outbreaks of MRSA. In the health care setting, some MSSA and MRSA fomites include toilet seats, computer keyboards, patient beds and drapes, nursing uniforms and physician neckties, door handles, and stethoscopes (Boyce et al., 2010; Boyce et al., 1997; Giannini et al., 2009; Hardy et al., 2006). In the community, contaminated fomites include van seats, tables, faucets, whirlpools, wooden benches, household items such as phones, remotes, automatic teller machines (ATMs), and toys (Bagget et al., 2004; Begier et al., 2004; Felkner et al., 2009; Otter & French, 2009; Kazakova et al., 2005; Sexton & Reynolds, 2010; Scott et al., 2009).

Despite the numerous examples of environmental surfaces and fomites where MRSA/MSSA has been isolated, there are no widely accepted standards for sampling these surfaces. In order to effectively characterize environmental surface contamination, it is critical to utilize a sampling system that is both sensitive (i.e. the sampler has low detection limits) and efficient (i.e. the sampler captures most of the bacteria present on
Numerous sampling methods have been employed for routine and post-outbreak environmental surveillance (Cimolai, 2008; Felkner et al., 2009; Obee et al., 2007; Otter et al., 2009; Sexton et al., 2006; Sherlock et al., 2009). Most of these systems can be broadly divided into two major categories: swabs/wipes and contact-based methods.

Swab samplers are perhaps the most frequently utilized sampling systems for isolation of bacteria from the environment. Typically swabs used for surface sampling are not designed for that purpose; rather, most are intended for use in clinical specimen collection. Therefore, there are several inherent limitations to the swab-based sampling system in environmental monitoring. Most environmentally relevant surfaces are beyond the size where a swab is appropriate. Also, previous research has demonstrated that bacterial capture and elution may not be ideal using swabs, resulting in low sampling efficiency (Obee et al., 2007; Lemmen et al., 2001). Despite these limitations, swabs are still widely used because they are often readily available, their low-cost, and most clinical laboratories are accustomed to processing them.

Wipe and sponge sampling systems, like swabs, remove surface-bound bacteria through mechanical agitation. However, sponges and wipes are clearly advantageous over swabs in their ability to effectively sample larger surface areas. Compared to the contact-based methods, swab/wipe sampling methods permit pre-enrichment after collection, which may be particularly useful when sampling environmental surfaces with low levels of bacteria. Pre-enrichment has been found to increase the ability to detect bacterial from surface samples (Landers et al., 2010).
Two common contact-based methods for bacterial recover are the contact plate (often called a Replicate Organism Detection and Counting plate, or RODAC) and the dip slide. Some past research has indicated that contact-based methods are more sensitive than swab methods (Griffith et al., 2007; Lemmen et al., 2001; Moore et al., 2001; Moore & Griffith, 2002). Contact plates and dip slides consist of a small plastic dish with a convex base (~20cm$^2$ for dipslides and ~28cm$^2$ for contact plates), to which growth medium is applied. Due to the convexity of the base, the surface of the growth medium is slightly raised to permit direct contact with the sampled surface. To sample with these systems, the investigator simply applies the contact sampler to the surface being sampled and bacteria become affixed directly to the surface of the growth medium. The inherent benefits to such a system are that no intermediate processing steps are required (the plate is incubated directly), and the results can be quantified. Samples also can easily be collected without technical expertise; all that is required is an incubator. However, because there is no manual agitation of the surface during sampling, recovery of well-adhered or biofilm-embedded bacteria may be poor (because adhered bacteria are more difficult to remove) (Bredholt et al., 1999). In addition, because the design of the backing material is hard, rigid plastic, contact-based methods are limited to sampling small, flat surfaces. Debris loading and high surface bacteria concentrations may create problems with identifying and counting bacteria, so the surfaces sampled must be relatively clean for accurate analysis; this is clearly problematic in many environmental surface sampling scenarios (Capita et al., 2004). Finally, although immediate bacterial deposition onto growth media allows for some selection and differentiation, the direct
application of stressed bacteria to media without pre-enrichment may result in low recovery (Reasoner & Geldreich, 1985).

Regardless of the sampling system used, the ideal sampling method should fulfill three important criteria. First, the sampler must be capable of effectively removing bacteria from the surface being sampled. Second, this should be done in such a way that maximizes collection and minimizes bacterial damage. Finally, the sampler must be able to fully release captured bacteria for analysis (Davidson et al., 1999; Moore & Griffith, 2007). In the case of contact-based samplers, the third requirement does not involve release of the bacteria, but rather the sampler needs to provide optimal conditions for growth since no intermediate processing is conducted.

The aim of this laboratory-based study was to evaluate the performance of four sampling methods for the detection of *S. aureus* on environmental surfaces. The research team compared the performance of three currently-used sampling methods (contact plates, swabs, and electrostatic wipes) and one novel sampling method (a lab-created rolling contact-based sampler, or “roller sampler”). Using these results, infection control and environmental health investigators will be equipped to make more informed decisions regarding the performance of sampling systems.

### 3.2 Methods and Materials

Using a series of laboratory-based experiments on stainless steel plates, the research team determined the relative sampling recovery efficiency and analytical sensitivity of a novel roller sampler (in-house design), contact plates (laboratory made,
RODAC-style), rayon swabs (Fisher Transport Swabs), and electrostatic cloths (Swiffer®).

3.2.1 Sampler Preparation

To prepare the novel roller sampler, a custom mold was created from autoclavable polyethylene and polyvinyl chloride. The roller sampler assembly consists of a bottom cap, the mold cylinder, an insert tube, and a top cap (Figure 3.1). The insert was placed into the center of the mold cylinder and sterile mannitol salt agar (MSA, BD BBL, Sparks, MD) was poured into the mold. The medium was air dried in a Class II biosafety cabinet until set, exposed to ultraviolet light for a minimum of 30 minutes (to enhance sterilization), covered, and refrigerated until sampling. The final sampling surface area of the roller is 81cm$^2$.

RODAC-style 60mm contact plates were prepared using 16ml mannitol salt agar per manufacturer’s instructions, to achieve the appropriate convex agar meniscus. Fifty millimeter mannitol salt plates, which are sized for optimal use with membrane filters, were also prepared by addition of mannitol salt agar to the plate. The plates were air dried in a Class II biosafety cabinet to complete solidification, exposed to ultraviolet light for a minimum of 30 minutes, covered, and refrigerated until sampling.

Sterile rayon swabs and electrostatic wipes were used directly from the package and required no preparation. Electrostatic wipes are packaged in boxes of 16 or 24 wipes, and are not sterile. To retain the electrostatic properties of the wipes (which may be diminished through steam autoclaving), wipes were not sterilized prior to sampling.
However, a negative control blank was processed for each run to ensure that no background \textit{S. aureus}-like contamination was present on the wipes.

All media preparation was conducted within a Class II biosafety cabinet with laminar flow HEPA-filtration. Aseptic technique was observed throughout.

Figure 3.1: Roller Sampler Cassette

\subsection*{3.2.2 Inoculation of the Steel Test Plates}

To evaluate sampling method performance on flat surfaces, a stainless steel surface was inoculated with a range of concentrations of MSSA (ATCC 29213). Stock solutions of MSSA were prepared using freshly prepared colonies from blood agar (TSA with 5\% sheep’s blood) after incubation at 37°C for 24 hours. (TSA II, Becton Dickinson, Sparks, MD, USA). The population density of the stock solution culture was determined by the addition of bacteria into saline solution and read using a nephelometer calibrated to a 0.5 McFarland standard. An aliquot of stock solution was inoculated onto
a mannitol salt agar plate in serial dilutions for determining the average starting concentration.

Stainless steel was chosen as the experimental test surface because of the material’s ability to withstand autoclave sterilization, and because public transportation stanchions are primarily stainless steel. The test surfaces consisted of three autoclaved 16 gauge T-304 (#4 annealed) stainless steel plates (40.6cm x 25.4cm), demarcated into 100cm² zones. Within a Class II biosafety cabinet, the plates were visually checked for dryness and each zone was inoculated with 100µl stock solution (prior to inoculation, a blank control sample was obtained to ensure plate sterility). The solution was spread using sterile spreaders (“hockey stick” style spreaders) for a period of one minute per 100cm² zone. The steel plates were allowed to dry inside the biosafety cabinet, and then placed into a sanitized holding container for 24 hours; plates were placed into the container in such a way that the inoculated surface was not contacted by any other surface.

3.2.3 Surface Sampling and Sample Processing

Samples were collected within the biosafety cabinet after 24 hours of drying in the holding container. Using aseptic technique, each 100cm² zone was sampled using the roller sampler, contact plate, swab, or static wipe; the sampling order of zones was random.

To obtain samples using the roller sampler, the roller first was removed from the mold, placed into a sterile glass paragon jar (with Plastisol-lined lid ajar), and allowed to
dry in the biosafety cabinet for approximately 10 minutes; this reduced condensation from the agar surface, which improved rolling performance. Once surface moisture was visibly dissipated, the roller was placed onto a sterile sampling handle (Figure 3.1). The roller was passed along the steel surface four times in each direction, removed from the sampling handle, and returned to the holding jar. The entire 100cm² zone was sampled for each roller sample.

To sample the surface with contact plates, the sampler was placed against the steel surface for 10 seconds at uniform downward pressure. The contact plate was gently placed into the middle of the 100cm² zone and removed without any lateral movement. In order to provide the same pressure throughout the sampling procedure (and across replications), small weights were placed onto the top of the contact plate (total mass ≈ 840 grams). The contact plate permitted sampling of 28.3cm² of the 100cm² zone for each sample.

To collect swab samples, rayon-tipped swabs were placed into the swab holding tube and wetted with Amies solution (which was present in the base of the swab holding tube, as a transport medium). Once saturated, the swab was rotated axially and moved laterally in a zig-zag motion across the steel surface. Each swab was passed along the surface 12 times vertically and horizontally for each 100cm² zone. Although the entire 100cm² zone was passed using each swab, the actual area in contact with the swab surface (and therefore the area sampled) was 36cm² for each sample.

To obtained wipe samples, a new electrostatic wipe was folded once (in addition to the factory folds) and wiped three times each direction on each side; the wipe was then
turned over and wiping was repeated. Once completed, the wipe was placed into a sterile stomacher bag (Nasco, Fort Atkinson, Wisconsin, USA) for processing. To reduce the chance of contamination to the static wipe, a modified “clean hands/dirty hands” method was used, in addition to standard aseptic technique. The sampler’s hands were cleaned using an alcohol-based hand sanitizer, and a new pair of gloves was donned. A second pair of over-gloves was donned, with care taken to avoid touching the outside of the over-gloves. All package manipulation was conducted using one hand (the “dirty hand”), and all sampling/sample-handling was conducted using the other hand (the “clean hand”). A new pair of over-gloves was used for each new sample, and the top and bottom static wipes in each new package were discarded. Wipe sampling covered the full 100cm² zone for each sample.

Roller samplers and contact plates did not require further processing and were placed directly into a 37°C incubator for up to 48 hours. Swabs and static wipes were processed using a membrane filtration procedure for concentrating bacteria (Dietze et al., 2001; Lutz & Lee, 2011). For swab samples, 10ml sterile PBS with Tween 20 was added to the swab reservoir tube. The swab was vortexed for 10 seconds to enhance cell removal from the swab head, and the diluent was passed through a sterile 0.45µm membrane filter (Millipore, Bedford, MA, USA).

A similar procedure was employed to process the static wipes. Fifty milliliters PBS-Tween 20 was added to each stomacher bag, the bag was resealed, and massaged until the PBS solution was absorbed. Each bag was then massaged rigorously for an additional 10 seconds, and the solution passed through a 0.45µm membrane filter.
Membrane filters (both swab and static wipe) were placed directly onto a mannitol salt plate, and incubated at 37°C for up to 48 hours.

3.2.3 Plate Counting

All mannitol salt plates and roller samplers were counted after 24 and 48 hours. To eliminate counting background microorganisms (such as those that may be from static wipes, which are not sterile), only those colonies which were morphologically consistent with *S. aureus* and displayed mannitol fermentation were counted.

In cases where plate counting was difficult due to the pattern of bacterial growth or where growth was “too numerous to count” (TNTC), the data were interpreted in the following manner. Where plates were TNTC, a default value was imputed for analysis that represented the maximum possible colony forming units (CFU) recovered, based-upon inoculated concentration, corrected for actual sampled surface area (in cm$^2$); this default value reflects the CFU value at 100% sampling efficiency. For example, for a swab result TNTC at an inoculation concentration of $6.81 \times 10^2$ CFU/ml (the “$10^3$” spiking concentration, with 100µl inoculated onto the 100cm$^2$ zone), a value would be computed as 24.5 CFU (0.681 CFU/cm$^2$ spiked, with the swab sampling area equal to 36cm$^2$). There is some precedence to interpret plates with growth at or above 200-300 CFU as TNTC. Although this strategy was considered for analysis (that is, assigning all TNTC values as 200 or 300 CFU), there was concern that in the low concentration iterations, this would have far overestimated the bacterial recovery level (since at lower concentrations 200 CFU or 300 CFU would be higher than what was actually spiked onto
the test surface). Using arbitrary 200 CFU and 300 CFU values may have resulted in cases with relative sampling efficiency >100%. By using the default value, resulting colony forming units reported will never exceed 100% efficiency.

3.2.4 Statistical Analysis and Calculations

The arithmetic mean concentration (recovered colony forming units, or CFU), normalized to CFU/cm² sampled, was calculated for each sampling method. To evaluate the difference between the recovered concentration versus sampling method, non-parametric Kruskal-Wallis analyses were conducted, at each inoculating concentration. To further assess where differences between groups existed, pairwise post hoc analysis was performed. Sampling methods were also compared by the percentage of replications that resulted in positive growth (across all inoculating concentrations).
Relative sampling recovery efficiency was determined as the ratio of CFU recovered from the test area to the number of CFU inoculated onto the surface (expressed as a percentage). This can be summarized using the formula:

\[
SE_t = \frac{C_t}{C_i} \times 100
\]

Where \(SE_t\) = relative sampling efficiency at incubation time \(t\) (\(t = 24\text{h}\) or \(t = 48\text{h}\))

\(C_t\) = concentration of MSSA recovered (in CFU/cm\(^2\)) at incubation time \(t = 24\text{h}\) or \(t = 48\text{h}\)

\(C_i\) = concentration of MSSA inoculated (in CFU/cm\(^2\)) at time \(t = 0\text{h}\)

Equation 3.1 - Relative Sampling Efficiency
Analytical sensitivity was calculated for 100 cm$^2$ using the formula:

$$Sn_{100cm^2} = \frac{100cm^2}{(A \times SE_t)}$$

Where $Sn_{100cm^2}$ = analytical sensitivity (CFU required per 100 cm$^2$ to observe a positive result)

$A$ = area sampled (cm$^2$)

$SE_t$ = relative sampling efficiency at incubation time $t$ ($t$ = 24h or $t$ = 48h)

Equation 3.2 - Analytical Sensitivity

(Obee et al., 2007; Whyte et al., 1989).
3.3 Results

3.2.1 Flexible Contact-based Sampler Design

Three pre-experimental contact-based sampler designs were constructed, each using different strategies for gains in flexibility. In the initial design (Figure 3.3), molten agar medium was placed into a dish lined with flexible fiberglass mesh (the “flexible dip slide”). Once the agar set, the mesh-backed agar was removed to evaluate handling performance. A series of qualitative manipulation tests were conducted to determine if handling and flexibility were sufficient for field applications. The flexible dip slide was moved, twisted, bent, and inverted. During these evaluations, it was found that this design suffered from a variety of challenges. First, upon removal from the dish, handling was very difficult, and there was excessive moisture on the surface of the agar. Manipulation tests indicated that upon bending inward or outward, the agar would quickly split, crack, or dislodge from the fiberglass backing. Although the flexible dip slide design shows promise, it was decided that this method was not sufficient for these evaluations.

The second flexible contact-based sampler pre-prototype (Figure 3.3) consisted of a paddle design (“paddle dip slide”). Using a plastic mold, molten agar was placed into the mold and a plastic handle inserted into the middle. Upon setting, the paddle dip slide was removed from the mold, and manipulation tests were conducted. Although this design was considerably more stable and stronger than the flexible dip slide, it was determined that this design was still inadequate for lab evaluation use. The primary detriment of this design was that, although stronger than the flexible dip slide design, the
paddle dip slide lacked sufficient flexibility. Upon even minimal bending and flexing, the agar would crack severely.

The final flexible contact-based sampler pre-prototype (Figure 3.4) utilized a rolling mechanism (the “roller sampler”). By adhering agar to the surface of a cylinder tube, and then attaching this tube to a handle, inherent flexibility could be achieved through a rolling motion. Because the agar itself undergoes very little bending and flexing (which adds considerable stress to the agar), it is much less likely to fracture during use. Through the freely rolling motion, this sampler was found to be able to sample virtually any shaped surface, and moved very effectively on large, flat surfaces as well. It should be noted that in the initial lab design, excess moisture temporarily prevented uniform rolling across very smooth surfaces. However, by exposing the sampler to HEPA-filtered air briefly before sampling (in a biosafety cabinet), the roller sampler performed quite well. In addition, the rolling action was effective during a limited field evaluation. Ultimately, the roller sampler pre-prototype design was chosen as the best novel design to “solve” the problem of rigid dip slides. This sampler was subsequently compared to three other samplers – standard 60mm RODAC-style contact plates, rayon swabs, and electrostatic wipes (Swiffer®). Currently, the roller sampler design is being refined for improved field application and convenience. Although currently a crude prototype, it is thought that the ultimate roller sampler design will demonstrate similar performance as was found in these laboratory studies.
Figure 3.2: Rendering of Two Unsuccessful Flexible Dip Slide Models (the paddle design and the flexible dip slide)

Figure 3.3: Roller Sampler Pre-prototype Design (utilized in sampler evaluation)
3.2.2 Sampler Method Evaluation

Comparison of the four sampling methods indicated that the sampling method greatly affected the degree to which *S. aureus* could be recovered from the steel test surface. Analyzing the sampling methods across five inoculation concentrations indicated a wide range in performance (Figure 3.4-3.10). Overall mean colony forming units recovered were greatest for the wipe and the roller, with the wipe performing better at lower concentrations than the roller. However, when corrected for actual area sampled, swab samples demonstrated similar levels of recovery to the wipe and roller. Wipe and swab methods resulted in higher recovered CFU than contact-based methods at the three lower concentrations. However, at the highest concentration, the roller recovered far more CFU than the other methods, and at the second highest concentration, recovered nearly the same as the wipe. Colony counts were made at both 24 and 48 hours; but because mannitol fermentation may take up to 48 hours, all Kruskal-Wallis data analyses are based upon 48 hour incubation times (with the exception of the “10^8” concentration level, which was only countable at 24 hours).

Relative sampling efficiency (the ratio of CFU recovered over CFU inoculated) varied considerably across sampling methods, ranging from 0.0-25.2% at 24 hours of incubation (Table 3.1). The swab method was the most efficient in nearly all cases (mean: 10.1%, up to 25.2%), followed by the wipe method (mean: 2.3%, up to 8.2%). Again, at the highest inoculating concentration the roller sampler demonstrated the best performance, resulting in the highest sampling efficiency (0.01%). Forty-eight hour
incubation markedly improved efficiency, with efficiency up to 46.1% for the swab (mean: 24.3%) and up to 32.6% for the wipe (mean: 17.8%).

The detection limit, which was expressed as analytical sampling sensitivity (the minimum number of CFU required for a positive result, at a specific inoculation concentration and a given surface area sampled), was determined for all methods (Table 3.1). The wipe and swab methods were far more sensitive than the contact-based methods; that is, they required fewer CFU on the sampling surface to result in a positive result. For the swab, the analytical sensitivity was as low as 4 CFU/100cm² (mean: 4,280 CFU/100cm²); and as low as 12 CFU/100cm² (mean: 5,732 CFU/100 cm²) for the wipe. Among the contact-based methods, the roller sampler was far more sensitive than the contact plate (as low as 55 CFU/100cm² versus 2,114 CFU/100cm²; mean_{roller}: 2,530 CFU/100cm², mean_{contact plate}: 10,061 CFU/100cm²); however, the contact plate sensitivity is based-upon the highest two concentrations only, as the contact plate did not result in any growth at the lowest three concentrations. Analytical sensitivity improved with 48 hour incubation, reducing to as low as 2 CFU/100cm² for the swab (mean: 76 CFU/100cm²), 3 CFU/100cm² for the wipe (mean: 7 CFU/100cm²), 7 CFU/100cm² for the roller (mean: 17 CFU/100cm²), and 1,133 CFU/100cm² for the contact plate (mean: 1,412 CFU/100cm²). Note that these results do not include the “10⁸” concentration, as plates were not readable at 48 hours incubation at that concentration.

Kruskal-Wallis testing indicated a statistically significant difference in median recovered CFU/cm² between the sampling methods at the two lowest inoculating concentrations and the highest inoculating concentration (p_{conc 1} = 0.009; p_{conc 2} = 0.007;
$p_{\text{conc}5} = 0.029$). These results are reported in Figures 3.4-3.10. Pairwise comparison revealed that the median recovered CFU/cm$^2$ was significantly different only between the contact plate and the wipe sampler at the lower concentrations, and between the roller and wipe sampler at the highest concentration.

The number of replications that resulted in positive growth, across all inoculating concentrations, was determined for each sampling method (Table 3.2). This dichotomous outcome of “growth-no growth” was expressed as the percentage of times that at least one colony forming unit was observed among replications of a given sampling method. At 24 hours of incubation, the wipe performed the best, with growth 73.8% of the time, followed by the swab (57.1%) and roller (45.2%). Increased incubation time resulted in more frequent positive results, with the wipe resulting in growth 90.5% of the time, followed by the swab and roller – both at 57.1%. The contact plate resulted in the fewest positive replications – 31.0% at 24 hours, and 35.7% at 48 hours. Chi-squared categorical analysis indicated that there is a significant relationship between growth and sampling method ($p_{24} = 0.001; p_{48} < 0.001$).
Figure 3.4: Recovered MSSA by Sample Method, “$10^2$” Concentration ($p_{KW} = 0.009; p_{contact-wipe} = 0.005$)

Figure 3.5: Recovered MSSA by Sample Method, “$10^3$” Concentration ($p_{KW} = 0.007; p_{contact-wipe} = 0.004$)
Figure 3.6: Recovered MSSA by Sample Method, “10^4” Concentration ($p_{KW} = 0.08$)

Figure 3.7: Recovered MSSA by Sample Method, “10^5” Concentration ($p_{KW} = 0.128$)

*NOTE: Wipe_{48h} and Roller_{48h} values were 608.3 CFU/100cm^2 and 603.0 CFU/100cm^2, respectively*
Figure 3.8: Recovered MSSA by Sample Method, “10^8” Concentration (p_{KW}= 0.029; p_{roller-wipe}=0.022)

Figure 3.9: Recovered MSSA by Sample Method, “10^8” and “10^9” Concentrations
Figure 3.10: Recovered MSSA by Sample Method, “10^4”, “10^5”, and “10^8” Concentrations
<table>
<thead>
<tr>
<th>Sampling Method</th>
<th>Mean Sampling Efficiency @ 24h Incubation (%)</th>
<th>Mean Sampling Efficiency @ 48h Incubation (%)</th>
<th>Mean Sampling Sensitivity @ 24h Incubation (per 100cm²)</th>
<th>Mean Sampling Sensitivity @ 48h Incubation (per 100cm²)</th>
<th>Area Sampled (cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab</td>
<td>10.1%</td>
<td>24.3%</td>
<td>4,280</td>
<td>76</td>
<td>36</td>
</tr>
<tr>
<td></td>
<td>0-25.2%</td>
<td>0-46.1%</td>
<td>4.0-21,028.6</td>
<td>2.2-291.1</td>
<td></td>
</tr>
<tr>
<td>Electrostatic Wipe</td>
<td>2.3%</td>
<td>17.8%</td>
<td>5,732</td>
<td>7</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0-8.2%</td>
<td>0-32.6%</td>
<td>12.3-27,557.6</td>
<td>3.1-9.8</td>
<td></td>
</tr>
<tr>
<td>Contact Plate</td>
<td>0.01%</td>
<td>0.04%</td>
<td>10,061</td>
<td>1,412</td>
<td>28</td>
</tr>
<tr>
<td></td>
<td>0-0.05%</td>
<td>0-0.09%</td>
<td>2,114.2-18,008.5</td>
<td>1,133.3-1,691.4</td>
<td></td>
</tr>
<tr>
<td>Roller</td>
<td>0.7%</td>
<td>9.9%</td>
<td>2,530</td>
<td>17</td>
<td>100</td>
</tr>
<tr>
<td></td>
<td>0-1.8%</td>
<td>0.01-14.7%</td>
<td>55.2-7,741.1</td>
<td>6.8-43.3</td>
<td></td>
</tr>
</tbody>
</table>

Table 3.1: Relative Sampling Efficiency (Mean), Relative Analytical Sensitivity (Mean) of the Four Sampling Methods
<table>
<thead>
<tr>
<th></th>
<th>Mean Positive @ 24h Incubation (%)</th>
<th>Mean Positive @ 48h Incubation (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Chi-Squared:</strong></td>
<td>(p=0.001)</td>
<td>(p&lt;0.001)</td>
</tr>
<tr>
<td>Swab</td>
<td>57.1%</td>
<td>57.1%</td>
</tr>
<tr>
<td>Electrostatic Wipe</td>
<td>73.8%</td>
<td>90.5%</td>
</tr>
<tr>
<td>Contact Plate</td>
<td>31.0%</td>
<td>35.7%</td>
</tr>
<tr>
<td>Roller</td>
<td>45.2%</td>
<td>57.1%</td>
</tr>
</tbody>
</table>

Table 3.2: Percentage of Replications with Positive Growth (Mean, Across All Inoculating Concentrations) of the Four Sampling Methods

3.4 Discussion

This study provides data on the performance of four different sampling methods for isolating *S. aureus* from environmental surfaces. In response to the first objective of this study, the research team was able to design and create a pre-prototype contact-based sampling tool. Due to the novel rolling design of this sampler, it has the potential to overcome two of the major limitations to contact-based sampling methods: lack of flexibility and only being able to sample small surface areas. To address the second objective of this study – sampling method evaluation – four sampling methods were compared for sampling performance, using a series of controlled, laboratory-based experiments. To the research team’s knowledge, this is the first study that evaluated electrostatic wipes for *S. aureus* sampling. The results of this study indicate that overall,
wipe and swab sampling methods are superior to contact-based methods, except at very high concentrations, where the novel roller sampler outperforms all other methods.

Environmental surface sampling for bacterial contamination is an important tool for pathogen detection, cleaning evaluations, and outbreak investigations. Several sampling devices are available for surface sampling, but few evaluations have determined the performance of these devices for the isolation of *S. aureus*. Little prior research is available regarding the best method for the isolation of *S. aureus* from environmental surfaces. In their study of several sampling methods for MRSA isolation, Obee et al. (2007) found similar levels of efficiency and analytical sensitivity (Table 3.3), ranging from 0-72.5% efficiency and sensitivities as low as 9.7 CFU/100cm². Despite the similarities in overall recovery, Obee et al. and other researchers have found that contact based methods were far superior to swabs, contrary to the findings of the current study (Lemmen et al., 2001; Moore & Griffith, 2002; Obee et al., 2007). There are several possible reasons why swabs performed better than contact-based methods in the current work.
First, post-inoculation/pre-sampling drying time may greatly affect the degree to which *S. aureus* can be recovered. In the Obee et al. and Moore and Griffith studies, samples were obtained both immediately after inoculation (“wet” or “unadsorbed” samples) and 30 minutes to 1 hour after sampling (“dry”) (Obee et al., 2007; Moore & Griffith, 2002). A 24 hour drying period was utilized in this study, which may have resulted in poorer recovery. *S. aureus* has been shown to survive well on environmental surfaces for weeks to months; however, die-off may still be considerable after 24 hours. Previous research has demonstrated that sampling performance is negatively affected even after only one hour of drying prior to sampling; in some cases, as much as a 5-log difference in recovery (Obee et al., 2007; Moore & Griffith, 2002). Although sampling

<table>
<thead>
<tr>
<th></th>
<th>Mean Sampling Efficiency @ 24h Incubation</th>
<th>Mean Sampling Efficiency @ 48h Incubation</th>
<th>Mean Sampling Sensitivity @ 24h Incubation (per 100cm²)</th>
<th>Mean Sampling Sensitivity @ 48h Incubation (per 100cm²)</th>
<th>Mean Sampling Sensitivity @ 48h Incubation (per 100cm²)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Swab</td>
<td>10.1%</td>
<td>24.3%</td>
<td>4,280</td>
<td><strong>26.0 – 1,440.1</strong></td>
<td>76</td>
</tr>
<tr>
<td>Electrostatic Wipe</td>
<td>2.3%</td>
<td>NA</td>
<td>5,732</td>
<td>NA</td>
<td>7</td>
</tr>
<tr>
<td>Contact Plate</td>
<td>0.01%</td>
<td><strong>51.33 – 72.5%</strong></td>
<td>0.04%</td>
<td><strong>9.7 – 27.6</strong></td>
<td>1,412</td>
</tr>
<tr>
<td>Roller</td>
<td>0.7%</td>
<td><strong>51.33 – 72.5%</strong></td>
<td>9.9%</td>
<td><strong>9.7 – 27.6</strong></td>
<td>17</td>
</tr>
</tbody>
</table>

Table 3.3: Comparison of Current Results with Obee et al. Data

*Obee et al. utilized five swab iterations (a combination of pour plating and DSI); reported values represent the range of the means for the five swab iterations.

**Obee et al. utilized two contact-based methods (dip slide, contact plate); reported values represent the range of the means for the two contact-based iterations.
immediately after or within an hour of inoculating the steel surfaces would have likely increased recovery in the current study as well, these drying periods are less relevant to actual field conditions. Bacteria deposited on surfaces, such as those found on public transportation vehicles, are unlikely to be removed in a period less than 24 hours, since vehicle cleaning may be infrequent and incomplete. Even in health care settings, where cleaning is routine, it is unlikely that all common touch surfaces (which may harbor *S. aureus*) are cleaned every day. Therefore, it is believed that the bacterial recovery observed here is more likely to represent recovery in real-world settings.

The processing method for swabs and electrostatic wipes may also account for the discrepancy observed between this study and past research. In the Obee et al. and Moore and Griffith studies, swabs were processed using either pour plating or direct swab inoculation (DSI) (Obee et al., 2007; Moore & Griffith, 2002). In their pour plating technique, the swab was vortexed for the same time as in this study. However, pour plating may result in bacterial death due to contact with hot agar (Van Soestbergen & Lee, 1969). Indeed, pour plating resulted in lower recovery in their studies than DSI (Obee et al., 2007; Moore & Griffith, 2002). Using DSI, bacterial loss may result from poor “release” of the bacteria from the swab, since no vortexing is conducted (Lemmen et al., 2001; Moore & Griffith, 2002; Obee et al., 2007). Utilizing both a vortexing and concentrating step (through membrane filtration), current results indicate good recovery (on a per cm² sampled level) using the swab method.

Incubation time may have also resulted in differences between studies. Obee et al. (2007) conducted plate counting after 24 hours of incubation. In the current research,
colonies were counted after both 24 and 48 hours. The additional 24 hours of incubation resulted in much higher recovery than at 24 hours, demonstrating the benefit of extended incubation times.

In both the Obee et al. and Moore and Griffith studies, steel surface preparation was less rigorous than in the current research. In both prior studies, a stainless steel table was cleaned thoroughly with a combination of detergents, sanitizers, and boiled water rinsing (Obee et al., 2007; Moore & Griffith, 2002). However, because the table presumably remained in the laboratory space for the duration of the experiment (due to the large size), recontamination prior to inoculation and sampling is possible. Moore and Griffith’s research evaluated fecal bacteria, which may be less likely to contaminate the experimental test surface given the use of proper laboratory attire and thorough hand hygiene (Moore & Griffith, 2002). However, in the Obee et al. study (which evaluated MRSA), inadvertent contamination is much more likely (Obee et al., 2007). Not only are a significant portion of individuals colonized with MRSA, *S. aureus* has also been found on a variety of personal items and attire (neckties, lab coats, identification badges, etc.), all of which may contaminate experimental work surfaces (Bhusal et al., 2009; Brunk/Butler, 2009; Koh et al., 2009; Kotsanas et al., 2008). By utilizing a series of smaller steel surfaces, the surfaces could be autoclaved and all sampling conducted in a biosafety cabinet with laminar flow HEPA filtration. Therefore, recontamination and cross-contamination is much less likely in the current work.
This study affirms the importance of selecting a sampling method appropriate for the given application. Choosing a method solely on the basis of expense, availability, or convenience may lead to erroneous or biased results. Given that even the best environmental surface sampling methods have moderate to poor recovery in a controlled, laboratory setting, using an inappropriate system is likely to result in a higher occurrence of false-negative results. In the case of an outbreak investigation or the study of a high-priority pathogen (such as MRSA), this scenario may place additional people at unneeded risk for infection. In situations where the accuracy of assigning a sample a negative result is paramount, this could be very costly to human health.

All four sampling methods evaluated in this study have a place in the environmental health and infection control professional’s toolkit (Table 3.4). The strongest method per square centimeter - the swab - is highly convenient, inexpensive, and easy to use. However, although the swab is excellent at recovering bacteria from small surface areas (such as those used in this study) and when processed using a membrane filtration concentrating step, lower recovery may be expected given a smaller sampling area or if different processing methods are applied (such as direct swab inoculation).
<table>
<thead>
<tr>
<th></th>
<th>Swab</th>
<th>Wipe</th>
<th>Roller</th>
<th>Contact Plate</th>
</tr>
</thead>
<tbody>
<tr>
<td>Frequency of Positive Growth*</td>
<td>57.1%</td>
<td>90.5%</td>
<td>57.1%</td>
<td>35.7%</td>
</tr>
<tr>
<td>Efficiency*</td>
<td>24.3%</td>
<td>17.8%</td>
<td>9.9%</td>
<td>0.04%</td>
</tr>
<tr>
<td>Sensitivity*</td>
<td>76 CFU</td>
<td>7 CFU</td>
<td>17 CFU</td>
<td>1,412 CFU</td>
</tr>
<tr>
<td>Low Conc. Performance*^</td>
<td>Good</td>
<td>Good</td>
<td>Moderate</td>
<td>Poor</td>
</tr>
<tr>
<td>High Conc. Performance*^</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Good</td>
<td>Moderate</td>
</tr>
<tr>
<td>Low-Loss Quantification*</td>
<td>No®</td>
<td>No®</td>
<td>Yes</td>
<td>Yes</td>
</tr>
<tr>
<td>Ability to Pool</td>
<td>Not effectively</td>
<td>Yes, effectively</td>
<td>Yes, but effectiveness unknown</td>
<td>Not effectively</td>
</tr>
<tr>
<td>Ease/Time of Processing</td>
<td>Moderate</td>
<td>Moderate</td>
<td>Easy</td>
<td>Easy</td>
</tr>
<tr>
<td>Cost</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
<td>Low</td>
</tr>
<tr>
<td>Ability to Pre-Enrich</td>
<td>Yes</td>
<td>Yes</td>
<td>No</td>
<td>No</td>
</tr>
<tr>
<td>Field Usability</td>
<td>Good</td>
<td>Good, requires strict quality control</td>
<td>Good, but requires additional field study</td>
<td>Good</td>
</tr>
<tr>
<td>Additional Comments</td>
<td>Good for small or delicate surfaces. Poor for large surface areas. May be limited by processing. Vortexing and concentrating or pre-enrichment recommended. DSI or dilution/pour plating may result in poor recovery.</td>
<td>Excellent for large or irregular surfaces. May be limited by processing. Vortexing and concentrating or pre-enrichment recommended. Requires strict quality control measures and two sampling technicians. Not sterile.</td>
<td>Better than contact plate/dip slide for large/irregular surfaces. Current design requires handling of roller. Unproven field performance. Dirty surfaces result in debris overloading.</td>
<td>Good for small, flat surfaces. Recovery may be poor at low surface bioburden. Easy to use with no technical expertise. Dirty surfaces result in debris overloading.</td>
</tr>
</tbody>
</table>

Table 3.4: Comparison of Sampling Devices

*a at 48-hour incubation time

*^ (Poor, Moderate, Good; relative to other tested sampling methods. "Low Conc." reflects concentrations ≤ 501 CFU/100cm²; "High Conc." reflects concentrations > 501 CFU/100cm²).

* Under typical processing. See ®

® Membrane filtration permitted quantification of the swab and wipe in the current project, but is not commonly utilized for surface sample processing. Pour plating, direct swab inoculation, and other processing methods also permit swab/wipe quantification, but are believed to inadequately remove collected bacteria, compared to vortexing and membrane filtration concentration.
The static wipe provides an excellent alternative to the swab. Wipe sampling methods are clearly more efficient than swabs at sampling large and irregular surfaces. This method may be preferable for doors, walls, floors, beds, or other large/irregular surfaces. Despite the nearly endless applications of the static wipe, it is plagued by two significant limitations. First, static wipes are not currently available in sterile form. The wipes can be steam autoclaved, but this may impair the electrostatic properties of the wipe, potentially reducing performance. Gas-phase sterilization or gamma irradiation are also possible, but are expensive and inconvenient. Another limitation in using static wipe samplers is that, by the nature of the sampling method, hand-to-sampler contact is unavoidable. Therefore, strict quality control protocols must be maintained to ensure the integrity of the samples.

Contact-based methods performed poorly in this study, but remain a good option in some sampling situations. If the target environmental surface is small and flat, contact plates and dip slides may be appropriate. Little training is required for use, and the samples require no further processing, so they are very convenient. However, in situations where large or irregular surfaces are to be sampled, the novel roller sampler may be more appropriate. The large surface area of the sampler (~80cm²) covers four times more surface area than a standard dip slide (20cm²). And because of the rolling action, irregular surfaces can be sampled with relative ease. Surface size is only limited by debris overload, so large surface areas may be easily sampled.

Despite the strong potential of the roller sampler, there are certain limitations in the design. Based-upon the results of this study, the roller sampler was less efficient
overall than the swab and wipe methods. However, the research team feels this reduction in efficiency can be countered by the large surface areas which can be sampled. Based upon initial limited field trials, the pre-prototype roller moves well on environmental surfaces, but is limited by overloading with surface debris and by non-specific breakdown of the growth medium (splitting and cracking of medium). In a typical clinical setting, these are less likely to occur. In addition, it is expected that future iterations of the roller sampler will address the problem of medium breakdown.

The final sampling method evaluated in this study - the standard contact plate - performed the worst overall. The reason for this is unclear, and in previous studies similar contact-based methods (dip slides and RODAC plates) performed very well. However, there are a few conditions in this study that may have contributed to the poor recovery using the contact plate. First, unlike the static wipe (and to a lesser extent the swab), the contact plate applies little mechanical agitation to the test surface. Previous research has indicated that stainless steel may trigger polysaccharide production, leading to biofilm attachment; given that increased surface contact time with planktonic bacteria increases adherence, the 24 hour drying time used in this study may have resulted in well-adhered bacteria (as cited in Hood & Zottola, 1997). The contact plate may simply not be able to effectively remove these attached bacteria. Even though the roller sampler is also contact-based, its design allows for multiple passes across a single test zone. In this way, the test surface is moistened during initial passes, possibly enhancing removal in later passes. Despite these limitations, the contact plate may still be a good choice due to convenience and ease of use. No secondary processing is required (only an incubator is
needed), and bacteria are readily countable directly on the surface of the growth medium. Although not evaluated in this study, it is believed that standard dip slides are comparable to standard contact plates, in terms of performance and limitations.

The study results indicate that as bacterial concentration increased, relative recovery efficiency (and sensitivity) decreased. This is likely due to difficulties in counting high concentrations of bacteria on growth medium. As the number of bacteria on the surface of growth medium becomes very high, it is difficult to effectively resolve individual colonies. It should be noted that this is an aspect in which the roller sampler excelled compared to the other sampling methods. Very high numbers of CFU could be counted using the roller sampler (possibly due to the large surface area of the sampler). Another possible reason for performance reductions at higher concentrations is that, given the limited space of the 100cm² test zones, additional bacterial density may amplify the proportion of bacteria which die-off during the 24 hour drying period, given equal nutrient availability. It is not unthinkable that the closer space between the bacterial cells at higher concentrations may have resulted in slower growth triggered by quorum-sensing (bacterial “signaling” through regulation of gene expression in response to density) (Melke et al., 2010; Yarwood et al., 2004).

This study provides important information to the environmental health and infection control specialist about the ability of different sampling methods to effectively recover *S. aureus* from environmental surfaces. These results indicate that commonly used sampling methods perform poorly-to-moderately, with a wide range of recovery, efficiency and sensitivity. However, these results should be viewed in the context of 24
hour post-inoculation drying, which may result in significant bacterial die-off. The results provide data comparable to real-world performance, rather than optimal/ideal performance using short drying times. The poor-to-moderate recovery found in this study confirms that investigators must treat negative sample results with caution and obtain numerous samples, ideally using different sampling methods. Otherwise, costly false negatives may occur.

3.5 Limitations

The results of this research provide data that indicate notable differences in sampler performance. Nevertheless, the study is not without limitations. The membrane filtration method that was used to concentrate *S. aureus* is widely used for water quality investigations, but has not been routinely employed for processing surface samples. Because the aim of the study was to evaluate sampling method performance given maximum recovery, it was felt that the concentrating step may have resulted in higher recovery than without. However, using this method may limit comparisons with previous research.

Another study limitation was the difficulty in counting some medium plates. In some cases, colony forming units were too numerous to count. A logical default CFU value was assigned in these limited cases, but the actual number of CFU is unknown. Similarly, the results indicated high variability with several “no growth” readings (particularly at low concentration). This was particularly true for the contact plate, which did not result in a readable CFU value at the two lowest inoculating concentrations. This
variability and lack of data, although common in microbiology, may have affected the results of the study. The findings indicated notable differences in the mean CFU recovered between each sampling method; however, the high variability among data likely lead to a lack of statistically significant differences in all except the highest inoculating concentration.

3.6 Conclusions

The study demonstrates that sampling method selection is very important, as different methods have varying performance. The wipe and swab performed the best overall for recovery of *S. aureus* from a steel surface, but the contact-based methods (especially the roller sampler) performed well at the highest inoculating concentrations. This is the first study to evaluate static wipe performance for *S. aureus* isolation, as well as the first study to describe a rolling contact-based sampler. Future research efforts should focus on additional sampling methods, and attempt to replicate these results in field conditions.
Chapter 4: Methicillin-Resistant *Staphylococcus aureus* Contamination on Public Transportation Vehicle Surfaces

4.1 Introduction

Once confined almost exclusively to the hospital, methicillin-resistant *Staphylococcus aureus* (MRSA) is increasingly found in the community. Community-associated MRSA (CA-MRSA) infection emerged in the 1990s, and CA-MRSA and healthcare-associated community-onset (HACO-MRSA) infections now account for approximately 15-90% of all MRSA infections (David & Daum, 2010; Liu et al., 2008; Naimi et al., 2003). USA300, a particularly successful CA-MRSA strain, is even overtaking traditional healthcare-associated (HA-) strains in the hospital (Boucher & Corey, 2008; Hota et al., 2007; Miller & Diep, 2008; Popovich et al., 2008). Therefore, characterizing the occurrence of MRSA in the community has important implications for outbreak prevention and infection control.

Methicillin-resistant *Staphylococcus aureus* (*S. aureus*) is one of the most important resistant human pathogens, causing approximately 1,300,000 infections and 19,000 deaths per year (CDC, 2005; CDC, 2006; David & Daum, 2010; Klevens et al., 2007). *S. aureus* is the primary cause of community skin and soft tissue infections, the majority of which are MRSA (David & Daum, 2010; Hersh et al., 2008; Lowy, 1998). In more severe infections, MRSA may cause necrotizing pneumonia (Lowy, 1998; Maltezou
MRSA infections tend to result in higher patient costs and longer hospitalization times (Cosgrove et al., 2005).

*S. aureus* has been found to contaminate a number of community surfaces, including van seats, tables, faucets, whirlpools, wooden benches, household items such as phones, remotes, ATMs, and toys (Bagget et al., 2004; Begier et al., 2004; Felkner et al., 2009; Otter & French, 2009; Kazakova et al., 2005; Sexton & Reynolds, 2010; Scott et al., 2009). Given that *S. aureus* (including MRSA) can survive on surfaces for up to six months and is quite resistant to desiccation, it is not surprising that it has been found in such a wide variety of locations (Dietze et al., 2001; Jawad et al., 1996; Sexton et al., 2006; Wagenvoort & Penders, 1997; Wagenvoort et al., 2000; Williams & Davis, 2009).

Perhaps one of the most important services in most American cities is public transportation. This critical infrastructure is widely used, with approximately 10.5 billion trips per year in the U.S. (APTA, 2010). However, little data exists regarding the prevalence of pathogens on public transportation vehicle surfaces, and no studies have assessed whether MRSA can be isolated from public transportation vehicles in the United States. Previous work has assessed MRSA on public transportation vehicles; however, such research has only been conducted in Europe, where MRSA colonization rates are believed to be lower than in the U.S. (Marschall et al., 2006; Otter et al., 2009; Stepanovic et al., 2008; Wall et al., 2009). In a study of public transportation vehicles in Serbia, no MRSA was isolated, but 30% of samples were positive for methicillin-resistant coagulase negative staphylococci (MRCoNS). One hundred percent of all vehicles sampled were contaminated (positive for at least one sample) (Stepanovic et al., 2008).
Similarly, in a study of public transportation touch surfaces (not only vehicle surfaces), Otter and French (2009) were able to isolate methicillin-susceptible *S. aureus* (MSSA) in 8% of all samples; however, the researchers were unable to isolate MRSA. Finally, in a recently published report from Portugal, a locally endemic hospital MRSA clone (EMRSA-15) was found on public transportation buses, with a total of 26% (22/85) buses positive for MRSA (Simoes et al., 2011). These findings represent the first report of MRSA from public transportation vehicles.

There are several factors that make public transportation vehicles an ideal setting for MRSA isolation. First, there are undoubtedly colonized individuals using public transportation. In the United States, 28-32% of the general population are colonized with MSSA and 0.8-1.5% with MRSA (CDC/Kuehnert et al., 2006; Gorwitz et al., 2008). Public transportation ridership is roughly estimated at 28.8 million trips per day in the United States (APTA, 2010). Using a 1.0% colonization rate, this would equate to an estimated 288,000 trips by MRSA colonized individuals per day. Vehicle use by “cloud” carriers (super shedders who release high concentrations of MRSA via hands and respiratory aerosols) or incidental carriers (those whose hands or clothing may be contaminated with MRSA, but are not colonized) would only increase the likelihood that MRSA may be deposited onto vehicle surfaces (Belani et al., 1986; Sherertz et al., 1996; Tanner et al., 1980). When healthcare workers ride public transportation, the potential for fomite contamination may also be increased, since they are colonized at a much higher rate than the general public (Albrich & Harbarth, 2008; CDC/Kuehnert et al., 2006; Chambers & DeLeo, 2009; Gorwitz et al. 2008).
Once on a vehicle, colonized individuals may spread MRSA through hand-to-fomite contact (and to a lesser extent, airborne transmission) (Cimolai, 2008; Dancer, 2008). Riders routinely touch stanchions, seat rails, doors, seats, and straps, especially as vehicles become crowded. Hand-to-fomite contact of this kind has been previously implicated in CA-MRSA transmission (Miller & Diep, 2008). During high-volume usage times, crowding may increase this hand-to-fomite contact. Indeed, crowding has been noted as an epidemiological risk factor for CA-MRSA transmission (CDC, 2007). Further, the regular opening and closing of vehicle doors and windows may create air drafts, which have been shown to suspend or re-suspend skin particles and dusts that carry MRSA (Dancer, 2008; Hambraeus et al., 1978; Williams et al., 1960). Finally, there is little to no opportunity for hand hygiene during and immediately after transportation usage. Previous research has demonstrated that hand hygiene is the primary method for prevention of MRSA transmission (Boyce & Pittet, 2002; Maltezou & Giamarellou, 2006).

In this research, the aim was to determine the background prevalence of MRSA contamination on public transportation vehicle surfaces in a large, metropolitan setting. Here, the results of a surveillance study conducted on forty buses from a Midwestern United States transportation agency are described. Using categorical analysis, an assessment was conducted to examine whether patterns existed between contaminated and non-contaminated vehicles and several factors of interest (e.g. ridership, whether hospitals were served, etc.).
4.2 Methods and Materials

4.2.1 Transportation Agency

The study transportation agency is a top-50 urban-anchored regional transit authority from a large, metropolitan area with an active daily fleet of approximately 239 buses. The buses are housed off-shift in one of two indoor facilities and serviced throughout the day and evening. An average of 226 buses are active for morning peak hours (6:00am to 9:00am), 145 buses for mid-day service (9:00am to 4:00pm), and 239 during afternoon peak hours (4:00pm to 7:00pm). An attempt was made to collect samples from a variety of routes to facilitate epidemiological analysis. Buses were chosen that served major hospitals and those which did not, those from high ridership and low ridership routes, and those that served one route and multiple routes. Targeted bus routes were established prior to sampling, using the transportation agency’s system map and available buses at the time of sampling. At the time of vehicle sampling, the sampling technician noted the unique bus identifier number (bus number or vehicle number) and the most recent route of service. All sampling was conducted on four Monday evenings (from approximately 7:00pm – 10:30pm) from July, 2010 to October, 2010.

The total sample size (number of buses and number of samples) was based upon a priori estimation and the desire to sample approximately 10% of the bus fleet (daily average) from a daily run from one of the two facilities.
4.2.2 Sampling

Sample locations inside the buses were chosen as having potentially high levels of skin-to-surface contact. Pooled samples were collected from the following vehicle locations: stanchions (overhead hand rails), seats, seat rails, the vehicle operator’s area (steering wheel, arm rests, knobs, and head-rest), back door, and the HVAC return vent. Sampling was always conducted on the same side of a bus, and an attempt was made to sample the same number of seats, seat rails, and similar linear footage of stanchions per bus. All sampling was conducted while vehicles were immediately post-service. Up to six pooled samples were collected from each bus (three buses did not have seat rails, and only five samples were collected), and ten buses were sampled during each sampling event. A total of 237 samples were collected from two facility locations and 40 buses.

Sampling was conducted using electrostatic wipes (“wipe” or “static wipe”). Wipe sampling was performed using two technicians, according to a “clean hands, dirty hands” technique. One technician, the sampler, collected all samples. New gloves were utilized for each sample, and proper donning technique was observed to prevent hand contact with the outer glove surface. To prevent potential cross contamination, the sampler wore an N95 mask during all sampling activities. The second technician, the assistant, opened all bags and handled all non-sterile items. The integrity of static wipe packages was inspected prior to sampling, and a new box was utilized for each sampling event. Upon opening a new package, the first static wipe was discarded; the last wipe in each box was also discarded. Two negative control wipes (field blanks) were held for processing to ensure no contamination occurred during sampling or transport (project
total n=8). Prior to and immediately following all sampling, all equipment (including the transport case) was disinfected in the laboratory.

To sample surfaces with static wipes, the cloth was unfolded and placed over the surface in a manner which maximized surface area contact between the wipe and the sampled surface. For flat surfaces, broad sweeping motions were made across the surface; for tubular items, the wipe was wrapped around the surface and drawn along the length of the tube. Once the sample was collected, the wipe was folded and placed into a sterile, labeled stomacher bag (Nasco, Fort Atkinson, Wisconsin, USA) for transport to The Ohio State University Diagnostic and Research Laboratory in Infectious Diseases (DRLID). All sample transfer was accompanied by a chain of custody.

4.2.3 Isolation, Identification, and Susceptibility Testing

4.2.3.1 Isolation

All samples were transported to the laboratory for processing within one hour, after each sampling event. Samples were pre-enriched for 24 hours at 35°C by the addition of 90ml tryptic soy broth (TSB) with 2.5% sodium chloride. To enhance absorption of TSB into the wipe, samples were manually agitated until the wipe was fully saturated. After enrichment, sample bags were examined for relative turbidity, and a sterile swab moistened with the enriched solution was directly inoculated onto mannitol salt agar plates supplemented with 2µg/ml oxacillin. Plates were incubated for 24-48 hours at 35°C.
After incubation, suspect colonies were selected (based-upon morphology and mannitol fermentation) for further analysis. Up to three cream, cream-white, and/or cream-yellow mannitol-positive colonies were passed to tryptic soy agar with 5% sheep’s blood for purification and isolation; these plates were incubated at 35°C for 24 hours. Once purified, up to three cream, cream-white, or cream-yellow colonies, 0.5-2mm in diameter and displaying alpha hemolysis (and in some cases beta hemolysis) were selected for identification. In the event that negative control samples demonstrated staphylococcal-like growth at any point during this process, all samples were discarded (however, this did not occur during the course of this research).

4.2.3.2 *S. aureus* Identification

After purification, all isolates underwent a series of biochemical tests to identify which isolates were *S. aureus*. First, all suspect isolates were tested for catalase reaction (Becton Dickinson, Sparks, MD, USA). For catalase-positive isolates, coagulase activity was then evaluated using the tube coagulase test with rabbit plasma/EDTA (Becton Dickinson, Sparks, MD, USA). All coagulase-positive isolates were considered “suspected *S. aureus*.” To confirm that coagulase-positive isolates were *S. aureus*, the following tests were conducted: latex agglutination testing to detect the presence of Protein A (Sure-Vue® Color Staph ID, Biokit USA, inc, Lexington, MA); aniline reaction using oxacillin screening agar plates containing 2µg oxacillin with an aniline indicator; polymixin B (Becton Dickinson, Sparks, MD, USA) susceptibility per the Clinical Laboratory Standards Institute protocol; finally, the Vogues-Proskauer (VP A,
Becton Dickinson, Sparks, MD, USA) test to determine acetoin production (CLSI, 2008). Negative and positive controls were utilized for each assay. Coagulase-negative isolates were not further processed in this study.

4.2.3.3 MRSA Phenotyping

All mannitol fermenting, catalase-positive, coagulase-positive, and agglutination-positive isolates (*S. aureus*) underwent susceptibility testing to determine methicillin phenotype. Fresh, 24-hour colonies were prepared to a concentration of a 0.5 McFarland standard, and inoculated onto oxacillin resistance agar plates containing 6µg oxacillin (Oxacillin Screen Agar®, Becton Dickinson Company, Maryland, USA). All media were incubated at 35°C for 24 hours. Positive controls were utilized for all reactions (MRSA: ATCC 43300; MSSA: ATCC 29213; MRSI: in-house isolate).

Isolates were classified as methicillin-resistant *S. aureus* (MRSA) when confirmed as *S. aureus* (as described in 4.3.2.2) and when there was growth on resistance agar. Where confirmed *S. aureus* isolated resulted in no growth on resistance media, the isolate was considered methicillin-susceptible *S. aureus* (MSSA). Isolates were considered to be of intermediate methicillin resistance if there was growth on screening agar, but no growth on resistance agar.

All coagulase positive isolates were archived for future analysis using established storage procedures.
Figure 4.1: Microbial Analysis Flow Diagram
4.2.3.4 Quality Control

To prevent potential cross-contamination from colonized or transient carrier project personnel, strict quality control measures were implemented. Prior to any sampling or laboratory work, new personnel were trained in the principals of sample collection and processing, with emphasis placed on the importance of utilizing proper quality control/contamination control measures (personal protective equipment, field blanks, sample controls, etc.). During the course of the project, all sampling and lab personnel were tested for nasal and nasal-pharyngeal MSSA and MRSA colonization. In the event that an individual was colonized, training was repeated, and the individual wore an N95 mask and covers exposed skin during all sampling and laboratory activities; colonization testing was repeated every six weeks.

4.2.3.5 Statistical Analysis of Data

Statistical analysis was conducted using PASW Statistics 18.0 (SPSS 18.0 SPSS, Chicago, IL, USA) and Stata SE 10.0 (Stata Corp., College Station, TX, USA). Data were analyzed using descriptive statistics, odds ratios and 95% confidence intervals, and regression analysis. Total S. aureus (MSSA and MRSA) and MRSA prevalence on public transportation vehicles were examined on a per-vehicle level and overall (per-sample level). Using multivariable logistic regression modeling, the relationship between total S. aureus/MRSA presence on a bus and potential “predictors” (factors) was assessed (the dependent variable was presence of MRSA on a bus; independent variables included: sampling date, whether major hospitals were served on the route, whether the bus ran the
same or multiple routes in a day, ridership, service facility); similar analyses were conducted for the outcome variable total \textit{S. aureus} (presence of both MSSA and MRSA). Regression analysis was also conducted at the sample-level to determine the effect of sample location. For all statistical analyses, a p-value less than 0.05 was considered statistically significant (and p<0.1 as “notable”).

4.2.3.6 Definition of Factors

Individual vehicles were placed into factor groups based upon pre-determined criteria. For assignment into the multiple route category, a bus (identified using the unique bus number) was assigned as running multiple routes if it serviced two or more routes the day of sampling; single route vehicles only serviced one route the day of sampling. Ridership for a bus was based upon data provided from the transportation agency for the number of riders for the day of sampling (ridership data were dichotomized as 0-199 and ≥200 riders/day, as this was a natural “50%” split in the data). To determine whether a major hospital was served or not, route maps were examined. Facility was based upon which service center a particular bus was assigned to.

4.3 Results

A total of 237 surface samples were collected from 40 buses between July and October of 2010. \textit{S. aureus} (“total \textit{S. aureus}”, which is both MSSA and MRSA) was found in 17% (41/237) of all surface samples, and MRSA was found in 15% (35/237) of all surface samples. On buses, \textit{S. aureus} was isolated in 68% (27/40) of buses (those
buses having at least one *S. aureus*-positive surface sample) and 63% (25/40) of all buses had at least one MRSA-positive surface sample. In total, MRSA accounted for 85% (35/41) of all positive surface samples. Six surface samples (3%, 6/237) were positive for MSSA. Sixty percent (60%, 24/40) of buses had one or two positive surface samples, and 8% (3/40) had three or four positive surface samples; no buses had more than four positive surface samples. See Tables 4.1-4.4 for a summary of *S. aureus* and MRSA results.

Buses were sampled from one of two service facilities (Facility 1: \( n_{\text{surface samples}} = 117 \); Facility 2: \( n_{\text{surface samples}} = 120 \)). Buses served 35 routes, and 39 unique buses were sampled (only one bus was sampled twice). A total of 155 (65%, 155/237) surface samples were from the 26 buses that directly served major hospitals, and 141 (59%, 141/237) surface samples were from the 24 buses that served multiple routes. Approximately 10% of the active fleet from a facility was sampled each sampling day, and approximately 16% of the total active bus fleet was sampled in the course of the study.

### 4.3.1 Sample Date

Surface samples were obtained on four dates: July 12, August 23, October 11, and October 25 of 2010. There was a reduction in overall *S. aureus* contamination levels by advancing date, from 25% (14/57) and 22% (13/60) *S. aureus*-positive surface samples for the first two sampling events, to 12% (7/60) for each of the second two sampling events (Figure 4.2). Similarly, the number of MRSA-positive surface samples
decreased with advancing date, from 23% (13/57) for the July sampling date, to 13% (8/60) for the August date and 12% (7/60) each of the October dates. The number of *S. aureus*-positive buses also decreased, with 90% (9/10) and 80% (8/10) *S. aureus*-positive buses in July and August, versus 50% (5/10 each event) positive buses for the October sampling events (the same results were observed with MRSA-positive buses, with the exception of the August date, which had 60% MRSA-positive buses) (Figure 4.3). The overall proportion of *S. aureus* that were MRSA varied by date, with MRSA accounting for 93% (13/14) and 62% (8/13) of positive surface samples in the first two sampling events, but 100% (7/7 each event) in the final two sampling events.

Figure 4.2: Total *S. aureus* and MRSA by Sampling Date, Sample-Level
4.3.2 Multiple Routes and Facility

A total of 35 of the transportation system’s 68 routes were sampled. Buses frequently serviced multiple routes during the day of sampling, with 60% (24/40) of buses running multiple routes and the remainder only running one route. Multi-route vehicles had a slightly higher proportion of *S. aureus*-positive and MRSA-positive surface samples (*S. aureus* surface samples: 18%, 26/141; MRSA surface samples: 16%, 22/141) than those that serviced the same route (*S. aureus* surface samples: 16%, 15/96; MRSA surface...
samples: 14%, 13/96). Similar results were noted at the bus-level, with multi-route vehicles having a slightly higher proportion of \textit{S. aureus}-positive and MRSA-positive buses (\(\text{S. aureus}_{\text{buses}}: 71\%, 17/24\); \(\text{MRSA}_{\text{buses}}: 67\%, 16/24\)) than those that serviced the same route (\(\text{S. aureus}_{\text{buses}}: 63\%, 10/16\); \(\text{MRSA}_{\text{buses}}: 56\%, 9/16\)). With respect to facility, Facility 1 had a similar number of \textit{S. aureus}-positive and MRSA-positive surface samples (\(\text{S. aureus}_{\text{surface samples}}: 18\%, 21/117\); \(\text{MRSA}_{\text{surface samples}}: 17\%\)) than Facility 2 (\(\text{S. aureus}_{\text{surface samples}}: 17\%, 20/120\); \(\text{MRSA}_{\text{surface samples}}: 17\%\)). Again, similar results were noted at the bus-level, with Facility 1 having a slightly higher number of \textit{S. aureus}-positive and MRSA-positive buses (\(\text{S. aureus}_{\text{buses}}: 70\%, 14/20\); \(\text{MRSA}_{\text{buses}}: 70\%, 14/20\)) than Facility 2 (\(\text{S. aureus}_{\text{buses}}: 65\%, 13/20\); \(\text{MRSA}_{\text{buses}}: 55\%, 11/20\)).

4.3.3 Sample Location on a Bus

The degree to which total \textit{S. aureus} and MRSA could be isolated varied considerably between surface sample locations on a bus. Seat rails (35%, 13/37), seats (33%, 13/40), and the back door (18%, 7/40) had the highest levels of total \textit{S. aureus}. The stanchions (13%, 5/40) and operator’s areas (8%, 3/40) were also contaminated with \textit{S. aureus}. Seats (33%, 13/40), seat rails (30%, 11/37), and back doors (15%, 6/40) demonstrated the highest prevalence of MRSA (Figure 4.4, 4.5). MRSA was also isolated from stanchions (10%, 4/40) and the operator’s area (3%, 1/40); no MRSA was found on the HVAC return.
Figure 4.4: Total *S. aureus* and MRSA by Surface Sample Location on a Bus
Figure 4.5: Proportion of Positive Surface Samples (MSSA and MRSA) by Sample Location

4.3.4 Hospital/Non-Hospital Routes

There was little difference in the number of *S. aureus*-positive and MRSA-positive surface samples between the non-hospital (*S. aureus*<sub>surface samples</sub>: 17%, 14/82;
MRSA\textsubscript{surface samples}:15%, 12/82) and hospital vehicles (\textit{S. aureus}\textsubscript{surface samples}: 17%, 27/155; MRSA\textsubscript{surface samples}:15%, 23/155). However, there was a moderate difference in the number of \textit{S. aureus}-positive and MRSA-positive buses between the non-hospital vehicles (\textit{S. aureus}\textsubscript{buses}:79%, 11/14; MRSA\textsubscript{buses}:71%, 10/14) and the hospital vehicles (\textit{S. aureus}\textsubscript{buses}:62%, 16/26; MRSA\textsubscript{buses}:58%, 15/26). The proportion of MRSA (surface samples) from total \textit{S. aureus} was similar for the non-hospital routes (86%, 12/14) and hospital routes (85%, 23/27).

4.3.5 Ridership

Continuous ridership data were obtained, and dichotomized into two levels (a 50% cut-point): low ridership (0-199 riders/day) and high ridership (200 or more riders/day). The high ridership vehicles had a higher proportion of \textit{S. aureus}-positive and MRSA-positive surface samples (\textit{S. aureus}\textsubscript{surface samples}:20%, 24/120; MRSA\textsubscript{surface samples}:17%, 20/120) than the low ridership vehicles (\textit{S. aureus}\textsubscript{surface samples}:15%, 17/117; MRSA\textsubscript{surface samples}:13%, 15/117). At the bus level, similar results were observed, with high ridership vehicles having a higher proportion of \textit{S. aureus}-positive and MRSA-positive buses (\textit{S. aureus}\textsubscript{buses}:80% ; 16/20; MRSA\textsubscript{buses}:70%, 14/20) than the low ridership buses (\textit{S. aureus}\textsubscript{buses}:55%; 11/20; MRSA\textsubscript{buses}:55%, 11/20).

4.3.6 Regression Analysis

Simple logistic regression was used to estimate unadjusted odds ratios, which indicated that total \textit{S. aureus} was associated with increased odds of being from the seat
rails (OR: 6.68; 95% CI: 1.75, 25.9; p = 0.006) and seats (OR: 5.94; 95% CI: 1.54, 22.9; 
p = 0.010), at the surface sample level. Similarly, at the surface sample level for MRSA, 
MRSA-positive surface samples had increased odds of being from seat rails (OR: 16.5; 
95% CI: 2.01, 135.6; p = 0.009) and seats (OR: 18.8; 95% CI: 2.32, 152.2; p = 0.006). At 
the bus-level analysis, no factors were significantly associated with increased odds of 
either MRSA presence or total *S. aureus*, although the July sampling date was nearly 
significant (OR_{MRSA and S. aureus} = 9.0; 95% CI: 0.809, 100.1; p = 0.074) and high ridership 
approached significance (OR_{S. aureus} = 3.27; 95% CI: 0.802, 13.3; p = 0.098).
<table>
<thead>
<tr>
<th>Variable</th>
<th>MRSA n (%)</th>
<th>No MRSA(^{^*}) n (%)</th>
<th>Unadjusted OR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface Samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sampling Date</strong> (p=0.273)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July, 12</td>
<td>13 (22.8%)</td>
<td>44 (77.2%)</td>
<td>2.23 (0.821, 6.09)</td>
<td>0.115</td>
</tr>
<tr>
<td>August, 23</td>
<td>8 (13.3%)</td>
<td>52 (86.7%)</td>
<td>1.16 (0.394, 3.44)</td>
<td>0.783</td>
</tr>
<tr>
<td>October, 11</td>
<td>7 (11.7%)</td>
<td>53 (88.3%)</td>
<td>1.00 (0.328, 3.05)</td>
<td>1.000</td>
</tr>
<tr>
<td>October, 25</td>
<td>7 (11.7%)</td>
<td>53 (88.3%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td><strong>Facility</strong> (p=0.363)</td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facility 1</td>
<td>20 (17.1%)</td>
<td>97 (82.9%)</td>
<td>referent</td>
<td>0.321</td>
</tr>
<tr>
<td>Facility 2</td>
<td>15 (12.5%)</td>
<td>105 (87.5%)</td>
<td>0.693 (0.336, 1.43)</td>
<td></td>
</tr>
<tr>
<td><strong>Sample Location</strong> <em>(p&lt;0.05)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator’s Area</td>
<td>1 (2.5%)</td>
<td>39 (97.5%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td>Stanchions</td>
<td>4 (10.0%)</td>
<td>36 (90.0%)</td>
<td>4.33 (0.462, 40.6)</td>
<td>0.199</td>
</tr>
<tr>
<td><strong>Seat Rails</strong></td>
<td>11 (29.7%)</td>
<td>26 (70.3%)</td>
<td>16.5 (2.01, 135.6)</td>
<td>0.009</td>
</tr>
<tr>
<td>Seats</td>
<td>13 (32.5%)</td>
<td>27 (67.5%)</td>
<td>18.8 (2.32, 152.2)</td>
<td>0.006</td>
</tr>
<tr>
<td>Back Door</td>
<td>6 (15.0%)</td>
<td>34 (85.0%)</td>
<td>6.88 (0.789, 60.1)</td>
<td>0.081</td>
</tr>
<tr>
<td>HVAC Return</td>
<td>0 (0.0%)</td>
<td>40 (100.0%)</td>
<td>---</td>
<td>---</td>
</tr>
<tr>
<td><strong>Multiple Routes</strong> <em>(p=0.712)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same Route for Day</td>
<td>13 (13.5%)</td>
<td>83 (86.5%)</td>
<td>referent</td>
<td>0.661</td>
</tr>
<tr>
<td>Multiple Routes</td>
<td>22 (15.6%)</td>
<td>119 (84.4%)</td>
<td>1.18 (0.562, 2.48)</td>
<td></td>
</tr>
<tr>
<td><strong>Hospital/Non-Hospital Route</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hospital</td>
<td>12 (14.6%)</td>
<td>70 (85.4%)</td>
<td>referent</td>
<td>0.966</td>
</tr>
<tr>
<td>Hospital</td>
<td>23 (14.8%)</td>
<td>132 (85.2%)</td>
<td>1.02 (0.477, 2.16)</td>
<td></td>
</tr>
<tr>
<td><strong>Ridership</strong> <em>(p=0.258)</em></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-199/day</td>
<td>15 (12.8%)</td>
<td>102 (87.2%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td>200 or more/day</td>
<td>20 (16.7%)</td>
<td>100 (83.3%)</td>
<td>1.36 (0.659, 2.81)</td>
<td>0.405</td>
</tr>
</tbody>
</table>

Table 4.1: Proportion and Odds Ratios of MRSA-Positive Surface Samples

\(^{^*}\)No MRSA includes MSSA and all negative results

*Statistically-significant Chi-Squared/Fisher’s Exact at p<0.05 level
<table>
<thead>
<tr>
<th>Variable</th>
<th>S. aureus n (%)</th>
<th>No S. aureus n (%)</th>
<th>Unadjusted OR (95%CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Surface Samples</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Sampling Date (p=0.134)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July, 12</td>
<td>14 (24.6%)</td>
<td>43 (75.4%)</td>
<td>2.47 (0.914, 6.65)</td>
<td>0.075</td>
</tr>
<tr>
<td>August, 23</td>
<td>13 (21.7%)</td>
<td>47 (78.3%)</td>
<td>2.09 (0.771, 5.69)</td>
<td>0.147</td>
</tr>
<tr>
<td>October, 11</td>
<td>7 (11.7%)</td>
<td>53 (88.3%)</td>
<td>1.00 (0.328, 3.05)</td>
<td>1.000</td>
</tr>
<tr>
<td>October, 25</td>
<td>7 (11.7%)</td>
<td>53 (88.3%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td><strong>Facility (p=0.864)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facility 1</td>
<td>21 (17.9%)</td>
<td>96 (82.1%)</td>
<td>referent</td>
<td>0.794</td>
</tr>
<tr>
<td>Facility 2</td>
<td>20 (16.7%)</td>
<td>100 (83.3%)</td>
<td>0.914 (0.466, 1.79)</td>
<td></td>
</tr>
<tr>
<td><strong>Sample Location <em>(p&lt;0.001)</em></strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Operator’s Area</td>
<td>3 (7.5%)</td>
<td>37 (92.5%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td>Stanchions</td>
<td>5 (12.5%)</td>
<td>35 (87.5%)</td>
<td>1.76 (0.392, 7.93)</td>
<td>0.460</td>
</tr>
<tr>
<td>Seat Rails</td>
<td>13 (35.1%)</td>
<td>24 (64.9%)</td>
<td>6.68 (1.72, 25.9)</td>
<td>0.006</td>
</tr>
<tr>
<td>Seats</td>
<td>13 (32.5%)</td>
<td>27 (67.5%)</td>
<td>5.94 (1.54, 22.9)</td>
<td>0.010</td>
</tr>
<tr>
<td>Back Door</td>
<td>7 (17.5%)</td>
<td>33 (82.5%)</td>
<td>2.62 (0.625, 10.9)</td>
<td>0.188</td>
</tr>
<tr>
<td>HVAC Return</td>
<td>0 (0.0%)</td>
<td>40 (100%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td><strong>Multiple Routes (p=0.605)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same Route for Day</td>
<td>15 (15.6%)</td>
<td>81 (84.4%)</td>
<td>referent</td>
<td>0.574</td>
</tr>
<tr>
<td>Multiple Routes</td>
<td>26 (18.4%)</td>
<td>115 (81.6%)</td>
<td>1.22 (0.609, 2.45)</td>
<td></td>
</tr>
<tr>
<td><strong>Hospital/Non-Hospital Route (p=1.00)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hospital</td>
<td>14 (17.1%)</td>
<td>68 (82.9%)</td>
<td>referent</td>
<td>0.947</td>
</tr>
<tr>
<td>Hospital</td>
<td>27 (17.4%)</td>
<td>128 (82.6%)</td>
<td>1.02 (0.504, 2.08)</td>
<td></td>
</tr>
<tr>
<td><strong>Ridership (p=0.173)</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-199/day</td>
<td>17 (14.5%)</td>
<td>100 (85.5%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td>200 or more/day</td>
<td>24 (20.0%)</td>
<td>96 (80.0%)</td>
<td>1.47 (0.744, 2.91)</td>
<td>0.267</td>
</tr>
</tbody>
</table>

Table 4.2: Proportion and Odds Ratios of Total S. aureus-Positive Surface Samples

*Statistically-significant Chi-Squared/Fisher’s Exact at p<0.05 level
<table>
<thead>
<tr>
<th>Variable</th>
<th>MRSA n (%)</th>
<th>No MRSA n (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Buses</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>July, 12</td>
<td>9 (90%)</td>
<td>1 (10%)</td>
<td>9.0 (0.809, 100.1)</td>
<td>0.074</td>
</tr>
<tr>
<td>August, 23</td>
<td>6 (60%)</td>
<td>4 (40%)</td>
<td>1.5 (0.255, 8.82)</td>
<td>0.654</td>
</tr>
<tr>
<td>October, 11</td>
<td>5 (50%)</td>
<td>5 (50%)</td>
<td>1.0 (0.173, 5.77)</td>
<td>1.00</td>
</tr>
<tr>
<td>October, 25</td>
<td>5 (50%)</td>
<td>5 (50%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td><strong>Sampling Date</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Facility</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facility 1</td>
<td>14 (70%)</td>
<td>6 (30%)</td>
<td>referent</td>
<td>0.330</td>
</tr>
<tr>
<td>Facility 2</td>
<td>11 (55%)</td>
<td>9 (45%)</td>
<td>0.524 (0.14, 1.92)</td>
<td></td>
</tr>
<tr>
<td><strong>Multiple Routes</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Same Route for Day</td>
<td>9 (56.3%)</td>
<td>7 (43.8%)</td>
<td>referent</td>
<td>0.506</td>
</tr>
<tr>
<td>Multiple Routes</td>
<td>16 (66.7%)</td>
<td>8 (33.3%)</td>
<td>1.56 (0.423, 5.72)</td>
<td></td>
</tr>
<tr>
<td><strong>Hospital/Non-Hospital Route</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Non-Hospital</td>
<td>10 (71.4%)</td>
<td>4 (28.6%)</td>
<td>referent</td>
<td>0.395</td>
</tr>
<tr>
<td>Hospital</td>
<td>15 (57.7%)</td>
<td>11 (42.3%)</td>
<td>0.545 (0.135, 2.20)</td>
<td></td>
</tr>
<tr>
<td><strong>Ridership</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0-199/day</td>
<td>11 (55%)</td>
<td>9 (45%)</td>
<td>referent</td>
<td>---</td>
</tr>
<tr>
<td>200 or more/day</td>
<td>14 (70%)</td>
<td>6 (30%)</td>
<td>1.91 (0.520, 2.01)</td>
<td>0.330</td>
</tr>
</tbody>
</table>

Table 4.3: Proportion and Odds Ratios of MRSA-Positive Buses

^No MRSA includes MSSA and all negative results
The results of multivariable logistic regression modeling indicated little statistical association between finding total *S. aureus* contamination (including MRSA) on a bus and factors analyzed. In the bus-level analysis, the full model (independent variables: sampling date, multiple route, facility, hospital/non-hospital, and ridership[dichotomous]) indicated no significant association for total *S. aureus* (p=0.085) or for MRSA (p=0.267). It is likely that there was substantial over parameterization of the model due to the high number of factors and levels to total buses sampled (n=40); this resulted in model instability. In addition, several factor levels were dropped due to perfect prediction of success/failure (full cells and zero cells) or collinearity. Upon removal of multiple route,
hospital/non-hospital, and facility (which is collinear with date), the overall model improved (total *S. aureus*: \( p = 0.0598 \); MRSA: \( p = 0.181 \), and the July sampling date (\( p_{S. aureus} = 0.053 \)) became nearly significant. The total *S. aureus* model with date and ridership indicated good fit and discrimination (Hosmer-Lemeshow Goodness of Fit \( p = 0.53 \); area under ROC = 0.779). The MRSA model with date and ridership, although not significant, also indicated good fit and discrimination (Hosmer-Lemeshow Goodness of Fit \( p = 0.84 \); area under ROC = 0.735).

At the surface sample level, logistic regression modeling resulted in some significant associations between factors analyzed (independent variables: sampling location, sampling date, multiple route, facility, hospital/non-hospital, and ridership [dichotomous]) and total *S. aureus* and MRSA. With respect to total *S. aureus*, the full model was significant (\( p = 0.0115 \)); however, similar to the bus-level, several factor levels were dropped from the analysis. This model indicated that the seat (\( p = 0.008 \)) and seat rail (\( p = 0.004 \)) sample locations were significant factors for finding contamination in surface samples on a bus. A final *S. aureus* model including sample location (\( p_{seats} = 0.008 \); \( p_{seat rails} = 0.004 \)) and date (\( p_{July} = 0.041 \)) was highly significant (\( p = 0.003 \)) and indicated good fit and discrimination (Hosmer-Lemeshow Goodness of Fit \( p = 0.7093 \); area under ROC = 0.732).

The full model assessing factors and MRSA was significant (\( p = 0.004 \)). Again, the seat (\( p = 0.005 \)), seat rail (\( p = 0.007 \)), and back door (\( p = 0.077 \)) sampling locations were important factors. A final MRSA mode including sample location (\( p_{seats} = 0.005 \); \( p_{seat rails} = 0.007 \); \( p_{back door} = 0.078 \)) and date (\( p_{July} = 0.065 \)) was highly significant and indicated
good fit and discrimination (Hosmer-Lemeshow Goodness of Fit p=0.799; area under ROC = 0.750).

Despite finding that sampling location on a bus and sampling date were associated with finding \textit{S. aureus} contamination and MRSA, the results of the surface sample-level analysis should be viewed with caution. The surface samples within a bus are not independent; therefore, bus-level analysis is more appropriate. However, to observe any effect of sample location, surface sample-level analysis was required. Because the independence assumption is violated, the surface sample-level models are provided for illustrative purposes only.

4.4 Discussion

Little is known about the overall occurrence and transmission patterns of MRSA in the community, particularly on environmental surfaces, which have been linked to MRSA outbreaks (Baggett et al., 2004; Begier et al., 2004). In this work, the research team sought to determine if public transportation could harbor MRSA contamination, thus potentially facilitating transmission. Further, several factors were assessed to determine if there was a relationship between finding \textit{S. aureus} and MRSA on a vehicle and sampling date, location, et cetera. The findings indicate that public transportation vehicles are a potential source of both \textit{S. aureus} and MRSA contamination. \textit{S. aureus} was isolated from 17% of all surface samples, and from 68% of buses. MRSA was found in 15% of all surface samples and 63% of all buses. Using this data, infection control professionals and epidemiologists can better understand the dynamics of MRSA in the community.
To the research team’s knowledge, this is the first published study evaluating public transportation vehicles in the United States for *S. aureus* or MRSA contamination. The results from three studies in Europe found notable levels of staphylococci, but only one isolated MRSA (Otter & French, 2009; Simoes et al., 2011; Stepanovic et al., 2008). With respect to the two negative finding studies, there are several reasons why the results of the current study are dissimilar with respect to finding MRSA. First, in Stepanovic et al.’s work in Serbia, swabs were used to sample vehicle surfaces (Stepanovic et al., 2008). Although some environmental studies which resulted in isolation of MRSA from the community were the result of sampling with swabs (and results outlined in Chapter 3 demonstrated that swabs have moderate efficiency), they are poorly suited for sampling large surface areas such as those found in transportation vehicles. The surface area sampled was not disclosed in the Stepanovic et al. study, but it is likely that because swabs were used, only small surface areas were sampled (Stepanovic et al., 2008).

Finally, it has been argued that the salt concentration utilized in the study may have been inhibitory to some strains of MRSA (Kassem, 2009).

Second, in a study of London public transportation surfaces, low sample numbers and sampling technique may have contributed to an inability to isolate MRSA. Of the 118 surface samples obtained, only 47 were from vehicle surfaces (24 on two trains and 23 on two buses) (Otter & French, 2009). The researchers used dip slides (a contact-based method) for surface sampling. Contact-based methods have been found to have poor-to-moderate recovery efficiency; however, they are limited by small size, rigid design (they can only sample flat surfaces), and cannot be pre-enriched (Griffith et al., 2009).
2007; Lemmen et al., 2001; Moore et al., 2001; Moore & Griffith, 2002). In addition, the high nutrient content in the pre-loaded media may be inhibitory to environmentally-stressed bacteria (Reasoner & Geldreich, 1985). The sampling method used in the current study – electrostatic wipes – allow for pooling of large surface areas onto one sample wipe. Also, to increase available bacteria for subsequent analysis, wipes can be placed into a pre-enrichment medium; this likely increases the ability to isolate MRSA, especially where surfaces may have low starting bacterial concentrations (Landers et al., 2010). Because this study was exploratory in nature, it was important to increase the chance of isolating MRSA from the environment; therefore, it was felt that enrichment was an important technique.

In a recently published report from Portugal, Simoes et al. (2011) isolated MRSA in 26% of sampled buses. The authors only obtained one pooled sample per vehicle, from the stanchions. Therefore, although a higher prevalence of MRSA-positive buses was found in the current research (63%), it is possible that additional locations within a given bus (which were not sampled) may have also been contaminated in their study vehicles. The majority of isolated MRSA in their research was an endemic nosocomial strain. The authors point-out that all but one of the positive buses service a hospital (and posit that hospitals therefore are the source for contamination). However, the Portugal study faced similar challenges as the current work, in that control over bus route assignment was limited, and that there were no indications of when and where vehicles became contaminated. Therefore, the research team believes it is a tenuous assumption
by Simoes et al. to state that contamination likely originated from hands of health care
workers, discharged patients, outpatients, and hospital visitors (Simoes et al., 2011).

Finding MRSA in a non-clinical, community setting such as public transportation
vehicles has several important implications. First, these findings increase understanding
about the degree to which MRSA can be found outside of the hospital. Given that 28-
32% of people are colonized with *S. aureus* and 0.8-1.5% with MRSA, it is no surprise
that MRSA has been isolated on a variety of surfaces in the community (CDC/Kuehnert
et al., 2006; Gorwitz et al., 2008). However, many community studies that resulted in
MRSA isolation have been conducted in settings that are health-care related (e.g.,
fire/EMS stations, prison clinics), after an outbreak (sauna), or limited to household
transmission (Baggett et al., 2004; Scott et al., 2009; Sexton & Reynolds, 2010). Not
only is the current study one of a few to find MRSA in the community, but public
transportation represents a potentially important setting for possible transmission. Also,
despite the fact that several studies have isolated MRSA from the community
environment, the bacterium does not appear to be ubiquitous. Several published studies
have failed to isolate MRSA in the community, and one may suspect that many more
unpublished studies have also failed to isolate MRSA (Begier et al., 2004; Kazakova et
al., 2005; Otter & French, 2009; Stepanovic et al., 2008).

Isolating MRSA on public transportation vehicles may have important
implications in the field of bioterrorism preparedness and response. As critical
infrastructure with high human density, public transportation has received considerable
attention as a target for a terrorist attack. The sarin gas release in the Tokyo subway in
1995 and the 2005 London subway bombings provide sobering examples that terrorists see public transportation as a viable target; and obviously commercial airlines have been a continual target for such attacks. Others have noted the potential of public transportation as a terrorist target (ECMT, 2003; Henderson, 1998; Metzger et al., 2006; Nascu, 2005). Henderson (1998) used the theoretical scenario of a smallpox release on a Washington subway as an example to illustrate the high public health impact that such an event would cause. Knowing the degree to which an important pathogen with high survivability (such as MRSA) can be isolated, and on which surfaces, provides important insights for prevention and decontamination.

4.4.1 Sample Location on a Bus

Vehicle surface sample location was an important indicator for whether \textit{S. aureus} and MRSA could be isolated. The seats were the most frequently MRSA-positive surface (33%), followed by the seat rails (30%) and back door (15%). Although there is probably less contact between seats and bare skin (the assumed mechanism of contamination between a carrier/colonized individual and vehicle fomites) than stanchions or seat rails, seats may greatly enhance bacterial survivability due to the porosity of their fabric surface. In addition, the study results are consistent with prior work regarding seat contamination. In a study of fire station surfaces, couches (which are also presumed to have a fabric surface and similar use as a bus seat) were found to harbor the highest concentration of MRSA (Sexton & Reynolds, 2010). In addition, Felkner et al. (2009) found MRSA contamination on a prison van seat and clinic chair, and Baggett et al.
isolated MRSA from a sauna bench. A lack of effective cleaning may also have contributed to the high level of seat contamination. In this study, seats were only cleaned once monthly, as part of a whole-bus cleaning. To limit damage to the porous fabric, the seats were only misted with a disinfectant (Lysol Disinfectant Spray, which is EPA-registered as effective against MRSA). Due to inadequate contact time and lack of penetrating power of the aerosol, it is possible that this method of cleaning may not effectively reduce MRSA bioburden.

Prior to the study, there was some anticipation that stanchions would result in the highest prevalence of MRSA, due to the fact that they are the primary hand hold on vehicles. However, although stanchions were positive in some cases, they were not as frequently contaminated as seat rails, which are similar in basic design and use (a tubular structure which is gripped). The MRSA prevalence on seats rails was 30%, versus 10% for stanchions. It is likely that stanchions were cleaned more frequently, and therefore may have less residual bioburden than other bus components. In addition, in most cases, stanchions are constructed of stainless steel, which may result in less hospitable conditions for S. aureus. In the study buses, seat rails are constructed of plastic, which in most cases has a “roughened” surface for better grip. This surface may enhance the removal of bacteria from hands and increase survivability on the surface. In addition, from observation, these surfaces receive less-thorough cleaning than the stanchions.

Back doors also demonstrated high levels of MRSA contamination (15%). These surfaces are a model of hand-to-fomite and fomite-to-hand transmission (although actual transmission has not been demonstrated in this study). To operate the back door, the
rider must either touch the back door surface “flat palmed”, or grasp two vertical tubes and push on the door. Therefore, throughout a route, there are countless occasions where riders’ hands contact the back door surface. In prior research of community surfaces, *S. aureus* contamination has been found on vehicle rails, stair rails, door and faucet handles, remote controls, key pads, etc. – all surfaces with similar hand-to-fomite dynamics as both the bus the bus doors and seat rails in this study (Felkner et al., 2009; Otter & French, 2009; Scott et al., 2009; Sexton & Reynolds, 2010; Stepanovic et al., 2008).

### 4.4.2 Sampling Date

The four sampling events in this study were all conducted on Monday evenings (in an attempt to reflect business day ridership, as well as to conform with the laboratory sample processing protocol). Sampling date did affect the degree to which *S. aureus* could be isolated, with 90% and 80% positive-buses from the first two dates, but only 50% positive-buses from the second two dates. Little is known about any seasonal trends in community *S. aureus* transmission, but some past research has noted increases in community MRSA infections during summer months (Kaplan et al., 2005). In addition, the current study observations are consistent with one of the primary risk factors for community transmission: skin-to-skin and skin-to-fomite contact. The decreasing prevalence trend in this study may be a reflection of the amount of exposed skin in the days preceding sampling. The average daily temperature of the (ten days prior to sampling) first two sampling events, conducted in July and August, was 78.1°F and 75.9°F. With these warm temperatures, it is more likely that riders will wear clothing
that leaves more skin exposed, such as short sleeves and short pants. In the later two sampling events, conducted in October, the average daily temperature (of the ten days prior to sampling) was 55.9°F and 54.4°F. At this cooler temperature, the potential that riders wear short sleeves and short pants would be low. With increasing exposed skin, there may be greater potential for skin-to-skin (between riders) and skin-to-fomite (between riders and vehicle surfaces) contact. In addition, there may be enhanced skin friction on surfaces, which has been noted as increasing S. aureus shedding (Cimolai, 2008).

4.4.3 Vehicle Conditions

The qualitative condition of the sampled vehicles varied considerably. Buses were visibly soiled in several cases, although a wide range of appearance was noted. The study transportation agency conducts daily cleaning of the bus carriage, which involves a basic wipe-down of stanchions, seat rails, and sweeping the floor. However, based upon observation and discussion with agency cleaners, there is wide variability in how well these surfaces are cleaned, if at all. Once monthly, each bus undergoes a more intensive, “deep” cleaning. This cleaning involves manually wiping all accessible surfaces in the bus with a disinfectant spray (Lysol Disinfectant Spray, EPA-registered as effective against MRSA), wet-cleaning the floor (with Betco Lemon, EPA-registered as effective against MRSA), and misting the seats with disinfectant spray (Lysol). This process is certainly more rigorous than daily cleaning; however, during observation, potential deficiencies were noted (e.g. re-using cleaning towels, not applying enough disinfectant
spray to the cleaning towel, etc.). An attempt was made early in the study to control bus sampling for monthly-cleaning status, but this information was unavailable for vehicle selection and data analysis.

4.5 Limitations

There are several potential limitations in the study. First, despite the efforts in seeking generalizability, the results of this study may not accurately reflect other regions. No information is available regarding the local MSSA/MRSA colonization rates in the study region. Therefore, the results of this study should be viewed in the context of estimated national averages. Colonization rates may vary widely by region (Goff & Dowzicky, 2007). It is possible that the local study population has a greater-than-average colonization rate, leading to potential discrepancies between current results and future studies in other areas. Second, the static wipe sampling method utilized in this study is ideal for pooling large areas and amplifying low levels of bacteria through pre-enrichment, but does not allow for quantification of isolated bacteria. Therefore, it is difficult to make comparisons between surface samples and factors of interest, other than from a categorical standpoint. This research was an exploratory surveillance study, designed to provide baseline data for MRSA prevalence in this important community setting. The isolation of MRSA cannot be equated to a specific exposure assessment, other than in the broadest sense that MRSA has been isolated on vehicle surfaces. Lack of quantitative data (colony forming units or similar) prevents an accurate measurement
of exposure. These findings also cannot be interpreted in the context of risk, as there are no established threshold levels above which MRSA causes infection (no infectious dose).

4.6 Conclusions

The results of this environmental surveillance study demonstrate that MRSA contamination is consistently present on public transportation vehicle surfaces. This is the first study to report such results in the United States, and the first study that has isolated MRSA on vehicle surfaces in the U.S. Using the results of this research, infection control professionals can better understand the dynamics of MRSA in the community, and public transportation agencies can better target cleaning activities.
5.1 Introduction

Methicillin-resistant *Staphylococcus aureus* (MRSA) is a primary human pathogen with significant health and economic burden. MRSA causes approximately 1,300,000 infections and 19,000 deaths per year in the United States – more than HIV or tuberculosis (CDC, 2005; CDC, 2006; David & Daum, 2010; Klevens et al., 2007). Historically confined almost exclusively to the hospital setting, since the 1990s, MRSA has been found increasingly in the community. The increase in community-associated MRSA (CA-MRSA) infections has corresponded with the rise of a particularly successful strain, USA300 (Boucher & Corey, 2008; Hota et al., 2007; Miller & Diep, 2008; Popovich et al., 2008).

Community-associated MRSA strains are especially concerning because, unlike healthcare-associated MRSA (HA-MRSA) strains, they frequently infect individuals without underlying predisposing health risk factors, or previous exposure to healthcare facilities. CA-MRSA strains tend to infect younger, healthier individuals. Despite the lack of healthcare exposure, there are numerous epidemiological risk factors for CA-MRSA infection, such as recent antibiotic use, contact activities (contact sports, men who have sex with men), crowded settings (prisons, dormitories, day cares), working in
healthcare or with animals, and being from an underserved community (Boucher & Corey, 2008; Chambers & DeLeo, 2009; David & Daum, 2010; Deresinksi, 2005; Maltezou & Giamarellou, 2006).

Molecular and antibiotic phenotyping techniques have proven very useful in studying MRSA strain patterns, as HA- and CA- strains are generally quite distinct (David & Daum, 2010). HA-MRSA strains are typically staphylococcal cassette chromosome mec (SCCmec) types I-III, and pulsed-field gel electrophoresis (PFGE) types USA100 and USA200 (primarily type II, USA100). HA-strains tend to be less virulent, affect older patients, and are frequently multi-drug resistant (David & Daum, 2010; Ito et al., 2001; Oliveira & de Lencastre, 2002; Popovich et al., 2008). CA-MRSA strains are marked by increased virulence due to the presence of the Panton-Valentine leukocidin gene and several other virulence factors, are usually SCCmec types IV-V and PFGE types USA300 and USA400 (primarily type IV, USA300), and affect children more frequently (Chambers & DeLeo, 2009; David & Daum, 2010; Ito et al., 2004; Ma et al., 2002; Maltezou & Giamarellou, 2006; Okuma et al., 2002; Popovich et al., 2008; Robinson & Enright, 2003). Although more virulent, CA-MRSA strains are generally susceptible to non-β-lactam antibiotics (Deresinski, 2005).

*S. aureus* is transmitted primarily through direct contact with a colonized or infected individual, or through a fomite or environmental intermediate. Hands are the critical disseminators, particularly the hands of health care workers (Ayliffe et al., 1988; Bhalla et al., 2004; Boyce et al., 1997). However, environmental surfaces and fomites
clearly play an important role in transmission (Chambers & DeLeo, 2009; Kazakova et al. 2005; Lowy 1998; Miller & Diep 2008; Muto et al. 2003).

Although numerous community MRSA outbreaks have been described, little is known about the background prevalence or variation of strains circulating in the community, especially on fomite surfaces. To better understand the prevalence of MRSA in the community, the researchers recently described the results of an environmental surveillance study assessing public transportation vehicle contamination. Public transportation vehicles were chosen because they have high human density, frequent human turnover, and constant hand-to-fomite, fomite-to-hand activity.

In this follow-up report, the antimicrobial resistance profiles and molecular strain characteristics for isolates collected from public transportation vehicle surfaces are described. The results of this work will provide critical data on the potential origins and clonal distribution of MRSA in this important, community setting.

5.2 Methods and Materials

5.2.1 Antibiotic Resistance Phenotyping

All methicillin-susceptible (MSSA, n=8) and methicillin-resistant S. aureus (MRSA, n=76) isolates from the previous study (see Chapter 4) underwent phenotyping analysis for antibiotic resistance to 16 antibiotic agents from 9 antibiotic classes (Table 5.1). A description of vehicle sample collection, bacterial isolation, identification, and oxacillin-resistance (MRSA) testing can be found in section 4.2.3.3.
Antibiotic resistance testing was conducted using the Kirby-Bauer disk diffusion technique, following the Clinical Laboratory Standards Institutes (CLSI) standard methods (CLSI, 2008). Isolates were first “refreshed” (from agar slants or isolate stock, frozen at -80°C) on tryptic soy agar with 5% sheep’s blood, incubated at 35°C for 24 hours. After incubation, a solution was then prepared by placing 1-2 colonies of 24-hour growth into normal saline, to a starting concentration of a 0.5 McFarland Standard. Using sterile cotton swabs, 100mm and 150mm Mueller-Hinton plates were then thoroughly inoculated (streaked) with the bacterial solution. After inoculation, antibiotic disks were deposited onto Mueller-Hinton plates using an automatic disk dispensing system. All disk placement activities were conducted within a Class II biosafety cabinet with laminar flow HEPA-filtered air. To test for vancomycin resistance, vancomycin screening agar plates (Becton Dickinson, Sparks, MD, USA) were inoculated with 10µl of the bacterial solution. All Mueller-Hinton and vancomycin screening plates were incubated at 35°C for 24 hours, at which time readings were obtained. To ensure quality control, the same lot of Mueller-Hinton agar plates was used for all tests.

After incubation, results were obtained by measuring zones-of-inhibition (“halos”) using a precision caliper or metric ruler. All zones were measured at their widest point, and at least two measurements were obtained for each zone. Care was taken when obtaining ruler measurements to prevent measurement errors due to parallax. In the event that there were abnormal growth patterns or difficult-to-interpret zones, the phenotyping process was repeated for the isolate. To read vancomycin screening agar plates, the plate was observed for bacterial growth. In the event of a positive result
(growth) on a vancomycin plate, the process was repeated for the suspected-positive isolate.

Numerous control strains were utilized throughout phenotyping process. To test the quality of Mueller-Hinton agar, Enterococcus faecalis (ATCC #29212) was inoculated onto a fresh plate and observed for growth. For vancomycin plate interpretation, both susceptible E. faecalis (ATCC #29212) and resistant E. faecalis (ATCC #51299) were utilized. For antibiotic disk testing, the following controls were used: S. aureus (ATCC #29253, ATCC #43300, and ATCC #29213), Pseudomonas aeruginosa (ATCC #27853), and Escherichia coli (ATCC #25922). All control plates were read prior to test isolates, and in the event that any control plate resulted in growth outside of established parameters, the entire sample batch was repeated.
<table>
<thead>
<tr>
<th>Antibiotic Agent</th>
<th>Disk Concentration (µg)</th>
<th>Abbreviation</th>
<th>Antibiotic Agent</th>
<th>Disk Concentration (µg)</th>
<th>Abbreviation</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
<td></td>
<td><strong>Macrolide</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>30</td>
<td>Amk</td>
<td>Erythromycin</td>
<td>15</td>
<td>Ery</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>10</td>
<td>Gen</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>β-Lactams (penicillins, cephalosporins)</strong></td>
<td></td>
<td></td>
<td><strong>Quinolones</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/Clavulanate</td>
<td>20/10</td>
<td>Amc</td>
<td>Ciprofloxacin</td>
<td>5</td>
<td>Cip</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>10</td>
<td>Amp</td>
<td>Enrofloxacin</td>
<td>5</td>
<td>Eno</td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>10</td>
<td>Cpd</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>30</td>
<td>Cep</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>1</td>
<td>Oxa</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glycopeptide</strong></td>
<td></td>
<td></td>
<td><strong>Phenicol</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td>6mg (plate)</td>
<td>Van</td>
<td>Chloramphenicol</td>
<td>30</td>
<td>Chl</td>
</tr>
<tr>
<td><strong>Lincosamide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>2</td>
<td>Cli</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td></td>
<td></td>
<td><strong>Potentiated Sulfonamide</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>30</td>
<td>Dox</td>
<td>Sulfamethoxazole/Trimethoprim</td>
<td>1.25/23.75</td>
<td>Sxt</td>
</tr>
<tr>
<td>Tetracycline</td>
<td>30</td>
<td>Tet</td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.1: Antibiotics Utilized for Resistance Profiling

### 5.2.2 SCCmec Typing

Staphylococcal cassette chromosome mec (SCCmec) typing was conducted using a multiplex PCR strategy, as first described by Oliveira and de Lencastre (2002) and updated by Milheirico et al. (2007). Using this protocol, several SCC elements were targeted, including the mec gene complex, the ccr complex, and for sub-typing analysis, the J-regions. Results are reported as mec Types I-VI.

In brief, DNA extraction is first completed through heat shock cell lysis at 95°C for 7 minutes. The PCR mixture was prepared as outlined in Table 5.2, targeting primers listed in Table 5.3, to a final working volume of 25µl. Amplification was conducted...
using a Gradient Thermocycler (Eppendorf, Hamburg, Germany), with the following
cycling conditions: 95°C for 15 minutes; 35 cycles of 94°C for 30 seconds; 57°C for 90
seconds and 72°C for 90 seconds; and a final extension of 72°C for 10 minutes. Ten
microliters (10 µl) of PCR product were resolved using a 3% Seakem LE agarose gel
(Cambrex, Rockland, Maine, USA) in 1x Tris-acetate-EDTA (Promega, Madison,
Wisconsin, USA), at 100V for 2 hours; visualization was facilitated through the use of
ethidium bromide. Band analysis was conducted using the Milheirico et al. (2007)
strategy. All MRSA-positive isolates with unique non-β-lactam antibiotic resistance
patterns from different surfaces on a bus were chosen for SCCmec typing.
<table>
<thead>
<tr>
<th>Reagent</th>
<th>Volume (µl)</th>
<th>Volume (µM)</th>
</tr>
</thead>
<tbody>
<tr>
<td>DNA Template</td>
<td>2</td>
<td></td>
</tr>
<tr>
<td>Multiplex PCR Master Mix (Qiagen, Foster City, CA) containing HotStartTaq Polymerase, 3mM MgCl2 and dNTPs</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Primers</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kdp F1</td>
<td>0.4 µM</td>
<td></td>
</tr>
<tr>
<td>kdp R1</td>
<td>0.4 µM</td>
<td></td>
</tr>
<tr>
<td>CIF F2</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>DIF R2</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>RIF F10</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>RIF R13</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>SCCmec III J1F</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>SCCmec III J1R</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>SCCmec V J1 F</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>SCCmec V J1 R</td>
<td>0.8 µM</td>
<td></td>
</tr>
<tr>
<td>mecl P2</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>mecl P3</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>dcs F2</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>dcs R1</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>mecA P4</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>mecA P7</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>ccrB2 F2</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>ccrB R2</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>ccrC F2</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td>ccrC R2</td>
<td>1.6 µM</td>
<td></td>
</tr>
<tr>
<td><strong>Total Volume</strong></td>
<td><strong>25</strong></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.2: PCR Reagents
### Table 5.3: PCR Primer Sequences for SCCmec Typing (Milheirico et al., 2007; used with permission, License Number 2661951339033)

<table>
<thead>
<tr>
<th>Primer name</th>
<th>Primer sequence (5’→3’)</th>
<th>Primer specificity (SCCmec type, region)</th>
<th>Amplicon size (bp)</th>
</tr>
</thead>
<tbody>
<tr>
<td>CH2 F2</td>
<td>TTCGAGTGGCTGATGAAAGG</td>
<td>I, J1 region</td>
<td>495</td>
</tr>
<tr>
<td>CH2 R2</td>
<td>ATTACCCAGAAGGCTACAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>cerC F2</td>
<td>GTACTCGTTCAAAAGGG</td>
<td>V, cer complex</td>
<td>449</td>
</tr>
<tr>
<td>cerC R2</td>
<td>ATATGCTGCTATGTGACC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>RIF5 F10</td>
<td>TTCTTAAGTAGCAGCTGAA</td>
<td>III, J3 region</td>
<td>414</td>
</tr>
<tr>
<td>RIF5 R13</td>
<td>ATGAAGATGAATACAGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCCmec V J1 F</td>
<td>TTCTCCATTCTGTCCATCC</td>
<td>V, J1 region</td>
<td>377</td>
</tr>
<tr>
<td>SCCmec V J1 R</td>
<td>AGAGACTGAGTCAACATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>dec F2</td>
<td>CATGCTGATAGCTGTC</td>
<td>I, II, IV, and VI, J3 region</td>
<td>342</td>
</tr>
<tr>
<td>dec R1</td>
<td>CTGATCATAGCCATGCGG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>ccrB2 F2</td>
<td>AGTTTCGTAGCAATGCAAGC</td>
<td>II and IV, cer complex</td>
<td>311</td>
</tr>
<tr>
<td>ccrB2 R2</td>
<td>CCGCATAGAAWGGTATACG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>kdp F1</td>
<td>AATCCATCGGCATGTTCTGG</td>
<td>II, J1 region</td>
<td>284</td>
</tr>
<tr>
<td>kdp R1</td>
<td>GGATGAAAGAAGAAGATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>SCCmec III J1 F</td>
<td>CATTGTCGAAGCACTGACG</td>
<td>III, J1 region</td>
<td>243</td>
</tr>
<tr>
<td>SCCmec III J1 R</td>
<td>GGTATAGAAGCTCTAAAGC</td>
<td></td>
<td></td>
</tr>
<tr>
<td>meca P2</td>
<td>ATCAAGCTTCGATCATGAGCC</td>
<td>II and III, mec complex</td>
<td>209</td>
</tr>
<tr>
<td>meca P3</td>
<td>GCCTGTTAAATTACATG</td>
<td></td>
<td></td>
</tr>
<tr>
<td>meca P4</td>
<td>TCCAGATTACAATCTCCACAGG</td>
<td>Internal positive control</td>
<td>162</td>
</tr>
<tr>
<td>meca P7</td>
<td>CCACCTCAATTCTGGAAGG</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

5.2.3 Pulsed-Field Gel Electrophoresis

Pulsed-field gel electrophoresis (PFGE) was conducted to subtype isolates and determine epidemiological origin (HA- or CA-) and clonality. *S. aureus* genomic DNA was digested using a *Sma*I restriction enzyme, and gel plugs were run using a CHEF Manager XA system (BioRad, Hercules, California, USA) using the “auto algorithm” setting (19h run time, 6.0V/cm, 10kb low MW, 700kb high MW, 120° angle, 5.0s initial switch, 40.0s final switch, linear A ramping factor). The molecular size standard, H9812, was used in three wells of each gel. All band patterns were analyzed visually and with
BioNumerics software (Applied Maths, Austin, Texas, USA). Clonal relatedness was assigned for all bands with a DICE coefficient >80%, and per the data interpretation protocol established by Tenover et al. (1995). Table 5.4 provides data interpretation criteria. Isolates were selected for PFGE that were MRSA, underwent SCC\textit{mec} typing, and were from within a bus that had multiple positive surfaces or potentially unique strains on one surface.

<table>
<thead>
<tr>
<th>Category</th>
<th>Number of Genetic Differences Compared with Reference Strain</th>
<th>Typical Number of Fragment Differences Compared with Reference Pattern</th>
<th>*Epidemiologic Interpretation</th>
</tr>
</thead>
<tbody>
<tr>
<td>Indistinguishable</td>
<td>0</td>
<td>0</td>
<td>Isolate is part of the “outbreak”</td>
</tr>
<tr>
<td>Closely Related</td>
<td>1</td>
<td>2-3</td>
<td>Isolate is probably part of the “outbreak”</td>
</tr>
<tr>
<td>Possibly Related</td>
<td>1</td>
<td>4-6</td>
<td>Isolate is possibly part of the “outbreak”</td>
</tr>
<tr>
<td>Different</td>
<td>≥3</td>
<td>≥7</td>
<td>Isolate is not part of the “outbreak”</td>
</tr>
</tbody>
</table>

Table 5.4: Criteria for Determining Isolate Clonality using the Tenover Method (Tenover et al., 1995).

*This study was not conducted during a known outbreak period. However, the Tenover method still applies to clonal relatedness of isolates, but the term “outbreak” may be more accurately viewed here as a “related group.”
5.2.4 Statistical Analysis of Data

Statistical analysis was conducted using PASW Statistics 18.0 (SPSS 18.0 SPSS, Chicago, IL, USA) and Stata SE 10.0 (Stata Corp., College Station, TX). Data were analyzed using descriptive statistics, categorical analysis, and regression analysis for odds ratios. Specifically, Hypotheses 3.3a and 3.4a were analyzed using Fisher’s exact analysis and unadjusted odds ratios were determined using simple logistic regression, comparing SCC$mec$ type II and type IV and the factors described in section 5.2.4.1.

5.2.4.1 Definition of Factors

Individual vehicles were placed into factor groups based-upon predetermined criteria. For assignment into the multiple route category, a bus (identified using the unique bus number) was assigned as running multiple routes if it serviced two or more routes the day of sampling. Ridership for a bus was based-upon data provided by the transportation agency for the number of riders for the day of sampling. To determine whether a major hospital was served or not, route maps were examined. Facility was based-upon which service center a particular bus is assigned to. Sampling date and facility were based on the calendar day that samples were obtained, sample location reflects the surface sampled within a bus, and antibiotic class resistance includes all phenotyped antibiotic classes. Resistance to multiple classes of antibiotics and whether a hospital was served (or not) were the primary independent variables of interest.
5.3 Results

5.3.1 Antibiotic Resistance Profiles

A total of 84 isolates were examined for antibiotic resistance. This included methicillin-susceptible (MSSA) isolates (9.5%, 8/84) and methicillin-resistant (MRSA) isolates (90.5%, 76/84). Among the MSSA isolates, 100% (8/8) were resistant to clindamycin (two of which displayed inducible resistance), and 87.5% (7/8) were resistant to ampicillin. Two MSSA isolates (25.0%, 2/8) were resistant to erythromycin, and only one isolate (12.5%, 1/8) had intermediate resistance to oxacillin. All MSSA isolates were resistant to three or fewer classes of antibiotics (including β-lactams), and 75.0% (6/8) of those were resistant to only two classes (β-lactams and clindamycin).

There was a wide range of resistance patterns amongst the MRSA isolates, with 11 unique, non-β-lactam patterns. All MRSA isolates (100%, 76/76) were resistant to β-lactams (and of those that underwent SCCmec testing [35/76], all were confirmed as carrying the mecA gene); however, one MRSA isolate that carried the mecA gene was susceptible to oxacillin. High levels of resistance were observed to erythromycin (80.3%, 61/76) and clindamycin (97.4% [74/76], with 78.9% [60/76] fully resistant and with 18.4% [14/76] isolates inducible), and moderate resistance was noted to ciprofloxacin (43.4%, n=33) and enrofloxacin (35.5%, n=27). One isolate each (1.3%) was resistant to tetracycline and chloramphenicol, and no resistance was observed to the glycopeptide, potentiated sulfonamide, or aminoglycosides. A total of 10 (13.2%, 10/76) isolates were resistant to two classes of antibiotics (including β-lactams), 33 (43.4%, 33/76) isolates were resistant to three classes, 32 (42.1%, 32/76) isolates were resistant to four classes,
and one (1.3%, 1/76) isolate was resistant to five classes. There was a statistically
significant difference between MSSA and MRSA, with respect to the number of
resistance classes (p ≤ 0.001). Table 5.5 provides a summary of antibiotic resistance
results.

5.3.2 SCCmec Typing

SCCmec typing was conducted on 40 MRSA isolates, obtained from 35 surface
samples collected on 25 buses. Of the typed isolates, 20% (8/40) had SCCmec type II
and 65% (26/40) had SCCmec type IV. Five isolates (12.5%, 5/40) resulted in
ambiguous band patterns (contained elements of both type II and type IV), and one
isolate (2.5%, 1/40) was untypable.

Levels of non-β-lactam class resistance varied between the SCCmec type II (“type
II”) and SCCmec type IV (“type IV”) isolates. Isolates from both types were frequently
resistant to the lincosamide, clindamycin (type II: 87.5%, 7/8; type IV: 88.5%, 23/26).
However, type II isolates were more frequently resistant to the macrolide, erythromycin
(type II: 100%, 8/8; type IV: 73.1%, 19/26) and quinolones (type II: 100%, 8/8; type IV:
26.9%, 7/26). Only one isolate each was resistant to tetracycline (3.8%, 1/26) and
chloramphenicol (3.8%, 1/26), but in both cases this isolate was type IV. Table 5.5 and
Table 5.6 provide a summary of results.
<table>
<thead>
<tr>
<th>Antibiotic Agent</th>
<th>MRSA - Resistant % (*n=76)</th>
<th>Type II - Resistant % (*n=8)</th>
<th>Type IV - Resistant % (*n=26)</th>
<th>Antibiotic Agent</th>
<th>MRSA - Resistant % (*n=76)</th>
<th>Type II - Resistant % (*n=8)</th>
<th>Type IV - Resistant % (*n=26)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Aminoglycosides</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>Macrolide</strong></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Amikacin</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td>Erythromycin</td>
<td>80.3 (61)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Gentamicin</td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>β-Lactams (penicillins, cephalosporins)</strong></td>
<td>100 (8)</td>
<td>100 (26)</td>
<td></td>
<td><strong>Quinolones</strong></td>
<td>100 (8)</td>
<td>26.9 (7)</td>
<td></td>
</tr>
<tr>
<td>Amoxicillin/Clavulan.</td>
<td>73.7 (56)</td>
<td></td>
<td></td>
<td>Ciprofloxacin</td>
<td>43.4 (33)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ampicillin</td>
<td>100 (76)</td>
<td></td>
<td></td>
<td>Enrofloxacin</td>
<td>35.5 (27)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cefpodoxime</td>
<td>98.7 (75)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Cephalothin</td>
<td>15.8 (12)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Oxacillin</td>
<td>98.7 (75)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Glycopeptide</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
<td><strong>Phenicol</strong></td>
<td>0 (0)</td>
<td>3.8 (1)</td>
<td></td>
</tr>
<tr>
<td>Vancomycin</td>
<td></td>
<td></td>
<td></td>
<td>Chloramphenicol</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td></td>
<td>0 (0)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Lincosamide</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Clindamycin</td>
<td>78.9 (60)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Tetracyclines</strong></td>
<td></td>
<td></td>
<td></td>
<td><strong>Potentiated Sulfonamide</strong></td>
<td>0 (0)</td>
<td>0 (0)</td>
<td></td>
</tr>
<tr>
<td>Doxycycline</td>
<td>0 (0)</td>
<td></td>
<td>3.8 (1)</td>
<td>Sulfamethox/Trimethoprim</td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Tetracycline</td>
<td>1.3 (1)</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.5: MRSA: Percentage of Antibiotic Resistance, Overall and by SCCmec Type

*MRSA-resistant isolates represent the 76/84 phenotyped isolates

SCCmec Type II and SCCmec Type IV isolates represent the 34/40 isolates that resulted in an unambiguous mec type

5.3.3 Pulsed-field Gel Electrophoresis and Within-Bus Analysis

Pulsed-field gel electrophoresis was conducted on 22 isolates from 18 surface samples, collected on 9 buses. Of the typed isolates, 54.5% (12/22) were pulsed-field type USA300 and 13.6% (3/22) were USA400 – both community-associated types. All of the USA300 and USA400 isolates were SCCmec type IV. Five isolates (22.7%, 5/22) were of the healthcare-associated type, USA100, and two isolates (9.1%, 2/22) do not fit into established USA types. All but one USA100 isolate were SCCmec type II (one was
type IV). Using both the SCCmec and PFGE data, there are a total of 11 unique strains from the 22 tested isolates (note: this excludes identical patterns across vehicles; utilizing this analysis would result in even fewer unique strains). Band patterns are shown in Figure 5.1.

The isolates that underwent pulsed-field analysis demonstrated similar antibiotic resistance, consistent with the results for SCCmec typing (which is expected, given that USA300 and USA400 isolates were primarily SCCmec type IV, and USA100 isolates were primarily SCCmec type II). All USA100 isolates (100%, 5/5) were resistant to four or five classes of antibiotics, and 86.7% (13/15) of USA300/USA400 isolates were resistant to three or fewer classes.

Within-bus analysis indicated that both the same and multiple strains could be isolated from surfaces within a given vehicle (Table 5.6). Of the profiled vehicles with multiple MRSA-positive surfaces (7/9), 71.4% (5/7) were contaminated on multiple locations with the same strain. Two vehicles (28.6%, 2/7) had multiple strains on multiple surfaces. Figure 5.2 provides a visual representation of strain variation on two example vehicles. On Bus 7, four isolates were positive for MRSA, from three different surfaces (the stanchions, seat rails, and back door). However, the results of the antibiotic resistance, SCCmec, and PFGE testing indicate that there were two probable strains on the vehicle. In Bus 7, it is possible that one individual contaminated the stanchions, and that another individual contaminated seat rails and the back door (or, alternately, two individuals carrying the same strain contaminated the seats rails and the back door).
Bus 26 was also MRSA contaminated on three surfaces (the stanchions, the seat rails, and the seats); however, in this bus, there was only one probable unique strain (determined using antibiotic resistance, SCC\textit{mec}, and PFGE testing). Therefore, on Bus 26, it is possible that either one individual carrying the same strain contaminated the stanchions, seat rails, and seats in the course of a trip (or multiple trips), or that two or three individuals carrying the same strain contaminated the three surfaces.
Figure 5.1: PFGE Band Patterns
Table 5.6: Summary of Bus and Isolate Results (see Figure 5.2 for visual representation of Bus 7 and Bus 26)

<table>
<thead>
<tr>
<th>Bus Number</th>
<th>Isolate</th>
<th>mec Type</th>
<th>USA Type</th>
<th>Antibiotic Resistance Pattern</th>
<th>Vehicle Characteristics</th>
<th>Probable Analysis</th>
</tr>
</thead>
<tbody>
<tr>
<td>3</td>
<td>3-4a</td>
<td>2</td>
<td>100</td>
<td>AmpAmcOxaCpdPsdEnoCprEry Cli</td>
<td>High Ridership, Single Route, Did not Pass a Hospital</td>
<td>Same strain on bus 3, two surfaces</td>
</tr>
<tr>
<td>3</td>
<td>3-6a</td>
<td>2 or 4</td>
<td>300</td>
<td>AmpAmcOxaCpdPsdEry Chlor</td>
<td>Low Ridership, MultiRoute, Passed a Hospital</td>
<td>Same strain on bus 6, two surfaces</td>
</tr>
<tr>
<td>3</td>
<td>4-4c</td>
<td>2 or 4</td>
<td>300</td>
<td>AmpEry Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>6</td>
<td>7-1a</td>
<td>4</td>
<td>300</td>
<td>AmpOxaCpdCml</td>
<td>High Ridership, MultiRoute, Passed a Hospital</td>
<td>Two strains on bus 7, three surfaces</td>
</tr>
<tr>
<td>7</td>
<td>7-1b</td>
<td>4</td>
<td>300</td>
<td>AmpOxaCpdEry Cml</td>
<td>High Ridership, MultiRoute, Passed a Hospital</td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7-2b</td>
<td>2</td>
<td>100</td>
<td>AmpOxaCpdEry Cpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>7-5a</td>
<td>2</td>
<td>100</td>
<td>AmpOxaCpdEry Cpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>7</td>
<td>5-4a</td>
<td>4</td>
<td>300</td>
<td>AmpOxaCpdCml</td>
<td>Low Ridership, MultiRoute, Passed a Hospital</td>
<td>One strain on bus 11, one surface</td>
</tr>
<tr>
<td>7</td>
<td>5-6a</td>
<td>4</td>
<td>100</td>
<td>AmpOxaCpdEry Cpl Ery Cml</td>
<td>Same strain on bus 15, two surfaces</td>
<td></td>
</tr>
<tr>
<td>11</td>
<td>11-6a</td>
<td>4</td>
<td>300</td>
<td>AmpOxaCpdEry Cpl Ery Cml</td>
<td>Low Ridership, MultiRoute, Passed a Hospital</td>
<td>Same strain on bus 18, two surfaces</td>
</tr>
<tr>
<td>18</td>
<td>18-1c</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td>High Ridership, MultiRoute, Passed a Hospital</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>18-1a</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td>Same strain on bus 18, two surfaces</td>
<td></td>
</tr>
<tr>
<td>18</td>
<td>18-1b</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td>High Ridership, MultiRoute, Passed a Hospital</td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>26-1a</td>
<td>4</td>
<td>400</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td>Low Ridership, Single Route, Passed a Hospital</td>
<td>Same strain on bus 26, three surfaces</td>
</tr>
<tr>
<td>26</td>
<td>26-2a</td>
<td>4</td>
<td>400</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>26-3a</td>
<td>4</td>
<td>400</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>26</td>
<td>26-6a</td>
<td>4</td>
<td>400</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>33-6a</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td>Low Ridership, MultiRoute, Passed a Hospital</td>
<td>One strain on bus 33, three surfaces</td>
</tr>
<tr>
<td>33</td>
<td>33-6b</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>33-6c</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>33-6d</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>33-6e</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>33</td>
<td>33-6f</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>35</td>
<td>35-2b</td>
<td>4</td>
<td>100/300</td>
<td>AmpOxaCpdPsd4-4Cml</td>
<td>High Ridership, Single Route, Did not Pass a Hospital</td>
<td>Two strains on bus 35, two surfaces</td>
</tr>
<tr>
<td>35</td>
<td>35-6b</td>
<td>4</td>
<td>300</td>
<td>AmpOxaCpdPsd Eno-3CprEry Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>40-2a</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td>High Ridership, MultiRoute, Passed a Hospital</td>
<td>Same strain on bus 40, two surfaces</td>
</tr>
<tr>
<td>40</td>
<td>40-6a</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml</td>
<td></td>
<td></td>
</tr>
<tr>
<td>40</td>
<td>40-6c</td>
<td>4</td>
<td>300</td>
<td>AmpAmcOxaCpdCpl Ery Cml(Chl)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Others: Low Ridership, MultiRoute, Passed a Hospital
5.3.4 Categorical and Regression Analysis

The results of the categorical analyses indicated that multi-class resistance (p=0.001), sampling date (p=0.002), whether a hospital was passed on the day of sampling (p=0.052), and service facility (p=0.039) were significantly associated with SCCmec type (or nearly significant for “hospital”) (Table 5.3.2). Similarly, results of the simple logistic regression analyses indicated that sampling date, service facility, and
whether a hospital was passed on the day of sampling were significant. The odds of a
July sampling date were significantly elevated for SCC\textit{mec} type II, versus the October,
11 date (OR: 14.0; 95\% CI: 1.25, 156.6; p = 0.032); as was the odds of being from
service Facility 1 versus Facility 2 (OR: 11.2; 95\% CI: 1.19, 105.1; p = 0.034); and for
non-hospital vehicles versus hospital vehicles (OR: 5.56; 95\% CI: 1.02, 30.3; p = 0.048).
There were no statistically significant associations for sample location, ridership, or
“multi-route.” The odds ratios could not be computed for multi-class resistance, as there
were zero cells.
<table>
<thead>
<tr>
<th>Variable</th>
<th>mecc Type II n (%)</th>
<th>mecc Type IV n (%)</th>
<th>Unadjusted OR (95% CI)</th>
<th>OR p-value</th>
<th>Fishers Exact p-value</th>
</tr>
</thead>
<tbody>
<tr>
<td>Multi-class Resistance</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.001</td>
</tr>
<tr>
<td>2 Classes</td>
<td>0 (0%)</td>
<td>6 (100%)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>3 Classes</td>
<td>0 (0%)</td>
<td>13 (100%)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>4 Classes</td>
<td>8 (57.1%)</td>
<td>6 (42.9%)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>5 Classes</td>
<td>0 (0%)</td>
<td>1 (100%)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sampling Date</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td>0.002</td>
</tr>
<tr>
<td>July, 12</td>
<td>7 (63.6%)</td>
<td>4 (36.4%)</td>
<td>referent</td>
<td>0.032</td>
<td></td>
</tr>
<tr>
<td>August, 23</td>
<td>0 (0%)</td>
<td>8 (100%)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October, 11</td>
<td>0 (0%)</td>
<td>6 (100%)</td>
<td>--</td>
<td></td>
<td></td>
</tr>
<tr>
<td>October, 25</td>
<td>1 (11.1%)</td>
<td>8 (88.9%)</td>
<td>0.0714 (0.01, 0.80)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Sample Location</td>
<td></td>
<td></td>
<td></td>
<td>0.883</td>
<td></td>
</tr>
<tr>
<td>Stanchions</td>
<td>1 (25%)</td>
<td>3 (75%)</td>
<td>referent</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seat Rails</td>
<td>2 (20%)</td>
<td>8 (80%)</td>
<td>0.75 (0.048, 11.6)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Back Door</td>
<td>2 (33.3%)</td>
<td>4 (67.7%)</td>
<td>1.5 (0.089, 25.4)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Seats</td>
<td>3 (21.4%)</td>
<td>11 (78.6%)</td>
<td>0.818 (0.061, 11.1)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Facility</td>
<td></td>
<td></td>
<td></td>
<td>0.039</td>
<td></td>
</tr>
<tr>
<td>Facility 1</td>
<td>7 (41.2%)</td>
<td>10 (58.8%)</td>
<td>referent</td>
<td>0.034</td>
<td></td>
</tr>
<tr>
<td>Facility 2</td>
<td>1 (5.9%)</td>
<td>16 (94.1%)</td>
<td>0.089 (0.010, 0.838)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Multiple Routes</td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>Same Route for Day</td>
<td>3 (23.1%)</td>
<td>10 (76.9%)</td>
<td>referent</td>
<td>0.961</td>
<td></td>
</tr>
<tr>
<td>Multiple Routes</td>
<td>5 (23.8%)</td>
<td>16 (76.2%)</td>
<td>1.04 (0.203, 5.34)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Hospital/Non-Hospital Route</td>
<td></td>
<td></td>
<td></td>
<td>0.052</td>
<td></td>
</tr>
<tr>
<td>Non-Hospital</td>
<td>5 (45.5%)</td>
<td>6 (54.5%)</td>
<td>referent</td>
<td>0.048</td>
<td></td>
</tr>
<tr>
<td>Hospital</td>
<td>3 (13.0%)</td>
<td>20 (87.0%)</td>
<td>0.18 (0.033, 0.983)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>Ridership</td>
<td></td>
<td></td>
<td></td>
<td>1.000</td>
<td></td>
</tr>
<tr>
<td>0-199/day</td>
<td>2 (18.2%)</td>
<td>9 (81.8%)</td>
<td>referent</td>
<td>0.613</td>
<td></td>
</tr>
<tr>
<td>200 or more/day</td>
<td>6 (26.1%)</td>
<td>17 (73.9%)</td>
<td>1.59 (0.264, 9.54)</td>
<td></td>
<td></td>
</tr>
</tbody>
</table>

Table 5.7: Categorical Analysis of SCCmecc Type and Factors
*Statistically-significant Chi-Squared/Fisher’s Exact at p<0.05 level
*For sampling date, facility, and hospital/non-hospital, unadjusted odds ratios were reported in two ways. The black text notates the odds ratios with the referent consistent with those in Chapter 4. However, these odds ratios indicate “protective effects” of the non-referent groups. Therefore, for ease of interpretation, the brown text is provided, which has the reverse groups acting as referent. This results in ORs greater than 1.
5.4 Discussion

Very little is known about the underlying phenotypic and molecular characteristics of MRSA circulating in the community. Most published work has either not provided molecular data, or has come as the direct result of a specific outbreak (Bagget et al., 2004; Begier et al., 2004; Otter & French, 2009; Kazakova et al., 2005; Sexton & Reynolds, 2010; Scott et al., 2009). Although these outbreak studies provide considerable insights into risk factors for MRSA infection and transmission, they do not accurately reflect the background occurrence of strains that are circulating in a community. Given that outbreak strains may have increased fitness or occur in scenarios where enhanced transmission may be occurring, they may fail to provide a true reflection of the underlying dynamics of MRSA in the community (Wagenvoort et al., 2000).

This study sought to elucidate the background antibiotic resistance patterns and molecular characteristics of methicillin-resistant S. aureus detected in public transportation vehicles. Results of the antibiotic resistance profiling indicated a wide range of resistance patterns, with both multi-class resistant MSSA and MRSA isolates. The majority of isolates obtained from public transportation vehicle surfaces were SCCmec type IV, which is epidemiologically-linked to community MRSA strains. The remainder of isolates were SCCmec type II, which were more frequently multi-class resistant. Finally, PFGE results indicated a mix of clonal strains, both within and across vehicles, with several closely related community strains, and few healthcare-like strains.

Finding multi-class resistant MRSA is unremarkable, particularly with respect to SCCmec type II, USA100/200 strains. These strains, which are epidemiologically-linked
to healthcare exposure and health risk factors, are frequently multi-class resistant. In contrast, community-associated strains, SCCmec type IV, USA300/400/700, are generally susceptible to many classes of antibiotics other than β-lactams (David & Daum, 2010; Deresinski, 2005; Popovich et al., 2008). However, the results of the current study indicated that although multi-class resistance was significantly associated with SCCmec type II, and 100% (8/8) of healthcare-associated isolates were resistant to four classes, the community-associated strains were also frequently multi-class resistant, with 76.9% (20/26) of isolates resistant to three or more classes. Of note, considerable clindamycin resistance was observed among the community-associated strains. Prior work has demonstrated that most community strains are susceptible to clindamycin, although resistance may be increasing (David & Daum, 2010; Deresinski, 2005). In this study, 88.5% of community-associated isolates were clindamycin-resistant; which was slightly higher than the healthcare-associated isolates. Interestingly, the MSSA strains also demonstrated high levels of clindamycin resistance, with 100% of all isolates resistant (or having inducible resistance). These levels are considerably higher than those noted by Naimi et al. (2003), who found 12% clindamycin resistance among community-associated MRSA isolates.

The factors associated with healthcare- versus community-associated strain types are consistent with prior research, but in some cases difficult to interpret. As described above, multi-class drug resistance was significantly related to SCCmec type. Sampling date was also significantly associated with SCCmec type, with seven of the eight healthcare-associated strains (SCCmec type II) observed in July, while the community-
associated strains (SCCmec type IV) were observed across all sampling events, July to October. Although this study was not designed to assess seasonality across several months, this may potentially indicate differences in strain circulation trends.

The research team hypothesized that healthcare-associated strains would be more associated with vehicles that passed hospitals the day of sampling. Interestingly, although a statistically significant association was observed between these factors, it was in the opposite direction than was hypothesized. The unadjusted odds of being a non-hospital vehicle that resulted in SCCmec type II was 5.56 times higher than for a hospital vehicle; in other words, the hospital vehicle is somewhat “protective” of finding SCCmec type II, and hospital routes were more associated with SCCmec type IV. There are several possible explanations for this observation. First, it is possible that SCCmec type IV is the dominant SCCmec type in the study region. No data is available regarding the background strain types in the study region, but there are some communities where SCCmec type IV has become the dominant strain, both in the community and the hospital (Boucher & Corey, 2008; Hota et al., 2007; Miller & Diep, 2008; Popovich et al., 2008). In addition, because vehicles could not be experimentally assigned to a single route the day of sampling, it is possible that acquisition of SCCmec type IV contamination could have occurred on a route other than one that passed a major hospital. Indeed, of the 20 hospital vehicles that had SCCmec type IV positive isolates, 13 of these ran multiple routes the day of sampling. Finally, the assignment of a vehicle into the “hospital” category reflects the day of sampling only. Under the current study design, there was no method to determine when contamination occurred. Therefore, it is possible that
contamination occurred prior to the day of sampling, and potentially on a route that did not pass a hospital.

Through PFGE strain typing, the results of this study provide key insights into the circulation of MRSA in a public transportation system. In several cases, vehicles were contaminated with the same strain on different surface locations, providing evidence that an infected or colonized individual is capable of contaminating multiple surfaces, which can become potential sources of MRSA in the community. In addition, vehicles can also be contaminated with a mix of strains. This indicates that multiple individuals can contaminate bus surfaces, and vehicles may potentially act as “mixing vessels” for MRSA strains. In this manner, a combination of both healthcare- and community-associated strains may be present on a vehicle. These findings are similar to those noted in a recent study of Portuguese buses, where both multiple isolates of the same strain, and multiple strains, were found within a given bus (Simoes et al., 2011).

5.5 Limitations

Despite the novel findings of the molecular analysis, there are several potential limitations in the study. The SCCmec protocol covers the most frequently isolated types, I-VI. However, there are additional SCCmec types, which may be missed in this protocol. Ambiguous band patterns are not uncommon, as observed with five of the current study isolates. The results of ambiguous patterns are of little value, although an inference may be made into SCCmec type, when combined with PFGE results.
PFGE is a highly effective methodology for analysis of strain clonality; which, in the case of MRSA, provides evidence of possible epidemiological origin and association. However, unlike sequence-based methods, it is very difficult to directly compare PFGE results across long temporal or spatial periods. Therefore, it may be inappropriate to compare the PFGE results from one bus to another, one sampling date to another, or from this study to the results of another.

The categorical analysis provides insights into factors that may be potentially associated with finding a particular strain type in the community. However, this analysis is based-upon a small sample size, and therefore may be limited by power. All categorical results are illustrative, and should not be viewed in the context of risk. In addition, the results of some categories may be difficult to interpret, such as differences between facilities or dates.

Finally, due to resource limitations and underlying study objectives, a limited number of isolates were run at each stage of this study. All positive isolates from the field surveillance work were analyzed for antibiotic resistance; but of these, only those with unique non-β-lactam resistance profiles underwent SCCmec typing. For PFGE, only isolates from vehicles that had multiple contaminated surfaces were analyzed. Therefore, it is possible that unique strains may not have been evaluated.

5.6 Conclusions

The results of this study provide novel information regarding the antimicrobial resistance patterns and genotypic strain variation of MRSA on public transportation –
high-volume community setting. Several resistance patterns were observed, with a substantial degree of multi-class resistance; high levels of clindamycin resistance were noted. SCCmeC typing and pulsed-field gel electrophoresis indicated that both healthcare-associated and community-associated strains were circulating on vehicle surfaces. In addition, some vehicles were contaminated with multiple MRSA strains, while others were contaminated on different surfaces with the same strain. This has important implications for future research, intervention design, and outbreak control.
Chapter 6: Synthesis and Discussion

In this chapter, the research team provides a brief discussion regarding the major findings of the dissertation research, and attempts to synthesize the results and place them into appropriate context. This chapter includes a summary of each project aim, lessons learned, and project limitations. Finally, future research that will benefit from the results of the current study is outlined.

6.1 Major Findings

This dissertation project determined whether methicillin-susceptible (MSSA) and methicillin-resistant Staphylococcus aureus (MRSA) could be isolated from public transportation vehicle surfaces. To achieve this goal, the sampling method best suited for sampling vehicle surfaces was determined. Then, vehicle interiors were sampled to determine if MSSA/MRSA were present on vehicles, and if so, to what degree. Finally, the antibiotic resistance patterns and molecular characteristics of positive vehicle isolates were evaluated to provide data on the epidemiology of MRSA in the community. The project, which consisted of three distinct but interconnected aims (SA1, SA2, and SA3) and seven major objectives, had both a laboratory and field component.

Specific Aim 1 (SA1) was designed with two major goals in mind.
First (Objective 1), based-upon a deficiency observed in contact-based sampling methods (noted through a review of the literature), a sampling device was created that would take advantage of the high sampling efficiency of contact-based sampling methods (as found in prior research), but eliminate the significant limitation of current contact-based methods: lack of flexibility and the inability to sample large surface areas. The second goal (Objective 2) was to evaluate three commonly-used surface sampling methods – swabs, contact plates, and electrostatic wipes – and the novel sampler – for sampling performance on a stainless steel test surface. By inoculating the steel test surface, sampling using these methods, and then conducting standard plate counting, the ability of the samplers to effectively collect \textit{S. aureus} from the test surface was determined; this was achieved through comparison of colony forming units collected (corrected for area sampled), relatively sampling efficiency, relative analytical sensitivity, and percentage of replications with positive growth.

After the performance characteristics and limitations of the four sampling methods were determined, a better bus sampling protocol was designed. Using the data from SA1, a sampling method was chosen that balanced two major criteria – performance and ease of use in a field setting – and several secondary criteria (whether pre-enrichment was possible, the effect that debris loading may have on sampler performance, ability to sample large surface areas and pool samples, whether established protocols for sampling existed, etc.). In SA2, using electrostatic wipes, surface samples were collected from throughout forty bus carriages to determine the background prevalence of both total \textit{S. aureus} (Objective 3a) and MRSA (Objective 3b). During the course of sampling the
research team obtained data on a number of factors to evaluate whether there was any association between the presence of contamination/MRSA on a bus and, sampling date (Objective 4a), whether the vehicle served multiple routes the day of sampling (Objective 4b), service facility (Objective 4c), ridership (Objective 4d), sample location within a bus (Objective 4e), and whether the bus served a major hospital or not (Objective 4f). It was believed that these data would provide valuable information on whether any epidemiological patterns existed regarding bus contamination.

In SA3, phenotypic and genotypic characterization of the positive bus isolates was performed. This was first achieved through antibiotic susceptibility testing (phenotyping) (Objective 5). Then, by comparing resistance profiles, unique isolates could be chosen for molecular analysis. Also, the phenotypic analysis would provide key data on patterns of resistance present in the MRSA strains obtained from the community. Once selected, all potentially unique MRSA isolates were then subjected to SCCmec typing to determine the epidemiologically-related origin of bus strains (healthcare-associated SCCmec types or community-associated SCCmec types) (Objective 6). Finally, using the results of both the phenotyping and the SCCmec typing, pulsed-field gel electrophoresis was conducted on selected isolates to further evaluate origin and establish clonal relatedness of positive bus strains (Objective 7).

6.1.1 Specific Aim 1

The results of the Specific Aim 1 research provide important data on sampler performance. This is not the first project to evaluate sampler efficiency for S. aureus
recovery; however, this study was unique in a few important ways. To the research team’s knowledge, this is the first study that evaluated electrostatic wipe performance under controlled, laboratory conditions for *S. aureus* collection. Also, by using a 24 hour drying time instead of 30 minute or 1 hour drying times, data that are more relevant to real-world conditions are provided. Finally, this is the first description of a rolling contact-based sampler.

The results of the current study show that there was only a statistically significant difference between the sampling methods at three of the five inoculating concentrations, and only between two of the four methods using pairwise analysis. Prior to the study, there was some anticipation (based on the results of prior published research) that the roller and contact plate would result in the greatest recovery, followed by the wipe and swab. However, this was not the case in the current study. Overall, the wipe sampling method resulted in the highest CFU. No prior data are available to compare this finding; however, this result is not entirely surprising, given the manner in which wipe samples are used. More so than the swab, contact plate, or roller, the amount of mechanical pressure applied to the steel surface can be controlled by the technician, allowing more thorough sampling. In addition, the wipe covers a larger surface area than either the contact plate or the swab. This results in more wipe surface in direct contact with the inoculated steel. This benefit translates directly to field settings, where there is often a desire to sample large surface areas or collect pooled samples. Finally, the electrostatic nature of the wipes may enhance bacterial removal from the contaminated surface.
The swab sampler resulted in the good recovery of *S. aureus*, when the results were corrected for surface area sampled. This result came as a surprise, as prior research findings indicated that swabs are quite inefficient for sampling surface contamination (even though they are widely used in field studies). For example, in Obee et al.’s (2007) evaluation of sampler performance for MRSA sampling, swabs performed the worst of all groups. It has been postulated that swabs are limited in their ability to effectively release bacteria collected in the swab head for subsequent cultivation and analysis (Lemmen et al., 2001; Moore & Griffith, 2002; Obee et al., 2007). However, in these prior studies, swabs were processed using direct swab inoculation (DSI), spread plating, or pour plating (Obee et al., 2007; Moore & Griffith, 2002). In the current research, swabs were processed by not only vortexing the swab to enhance removal of bacteria from the swab head, but also through a concentrating step by passing the diluent through a membrane filter. In this manner, it is believed that the current results may more accurately reflect captured bacteria.

The contact-based methods (the novel roller sampler and the contact plate) performed poorly overall, but better than the swab and wipe at the highest inoculating concentration. It is possible that the contact-based methods did not provide enough agitation on the steel surface for adequate removal of adhered or biofilm-embedded bacteria.

In nearly all cases, the four sampling methods resulted in poor to moderate relative sampling efficiency and analytical sensitivity. However, adding an additional 24 hours of incubation time (for plate counting) greatly improved both measures. In some
cases, plate counting at 48 hours resulted in efficiencies more than 10 times higher than at 24 hours, and sensitivities two orders of magnitude (logs) greater. Therefore, one should consider including 48 hour plate counting for all environmental field sampling; this is particularly important where surface bioburden may be low.

There is currently no commercially-available product that resembles the novel, contact-based roller sampler. Even though the roller design evaluated in this research was a pre-prototype (and therefore rather crude), the device did show improvements over the contact plate in terms of sampling performance, due to the flexibility gains from the rolling motion and the large sampler surface area that allows for sampling larger surface areas. Results showed that the roller sampler performance was far superior to the contact plate, with a mean efficiency of 9.9% and sensitivity of 17 CFU, over 0.04% and 1,412 CFU. Despite the promising results of the pre-prototype design, the roller sampler was less efficient than the swab and wipe samples, overall. However, not only did the roller result in recoverable bacteria at all concentrations (which is not the case with the contact plate), it performed as well as the wipe sampler at the “10^4” inoculating concentration, as well as the wipe and better than the swab at the “10^5” concentration, and far better than all methods at the “10^6” concentration (when comparing recovered CFU/100cm^2).

The electrostatic wipe sampler was chosen for field sampling in SA2, but there was an attempt to utilize the roller sampler for a few vehicle surface samples. The goal was not to use the recovered bacteria for the SA2 prevalence study, but rather to better understand the limitations of the current design of the roller sampler pre-prototype, when used in a field application. The roller was able to be successfully used for surface sample
collection, but it was noted that the media surface became so overloaded with debris that colony counting and isolation were impractical. It is likely that this is because bus seats were chosen to test the roller, since they were suspected to be significantly contaminated; these seats were very dirty and loaded with debris. Despite the problems associated with debris overloading, bacterial growth and mannitol fermentation was observed on the roller. Therefore, once refined, it is believed that the roller sampler will be an excellent tool for surface sampling.

6.1.2 Specific Aim 2

Specific Aim 2 – an environmental surveillance study of MRSA on public transportation vehicle surfaces – is the centerpiece of the dissertation. Never before in the United States has any published research assessed whether public transportation vehicle surfaces are fomites for MRSA. These findings indicate that not only could MRSA be isolated from bus surfaces, but that it was present on nearly two-thirds of all buses. Given the importance of public transportation in most major cities, the results of this aim provide valuable insight (on the burden of MRSA contamination) to transportation agencies, epidemiologists, and infection control and environmental health professionals.

Prior research in Europe has been conducted with similar project goals; however, until very recently, these studies have failed to demonstrate that MRSA can be isolated from public transportation vehicles. In Stepanovic et al.’s (2008) work in Serbia, all tested vehicles were positive for methicillin-resistant coagulase negative staphylococci
(MCoNS), but none for *S. aureus* or MRSA. Although MRCoNS may be an indicator for *potential* *S. aureus*/MRSA contamination and demonstrate that resistant commensal bacteria are circulating in the community, MRCoNS represent a less important public health threat than *S. aureus*. Similarly, in Otter and French’s (2009) work in London, MSSA was found on 8% of public transportation surfaces (vehicle and other surfaces); but again, no MRSA was isolated. In a study of Portuguese buses, Simoes et al. (2011) was indeed able to isolate MRSA from 26% of buses (with one sample per bus), but did not sample any surfaces other than stanchions. Therefore, their results may underestimate the true prevalence of MRSA on buses, as the current study indicates that stanchions are less frequently contaminated than seats, seat rails, and the back door.

Several factors (such as sampling date, service facility, sampling location, ridership, etc.) were assessed to determine if there was an association between the factor and finding *S. aureus*/MRSA contamination on a bus. For example, sampling date was important, with considerably less contamination with advancing date. This is possibly due to changes in ambient temperature, with passengers exposing less skin in the later sampling dates. Since colonized or contaminated skin-to-skin and skin-to-fomite contact is the primary mode of *S. aureus* transmission, this would be a logical conclusion (Chambers & DeLeo, 2009; Kazakova et al. 2005; Lowy 1998; Miller & Diep 2008; Muto et al. 2003).

Sampling location within a bus also appeared to be an important indicator of whether *S. aureus*/MRSA contamination could be detected. Seats, seat rails, the back door, and stanchions were the most heavily contaminated surfaces. This is consistent
with the pattern of *S. aureus* transmission, since all of these surfaces receive considerable skin-to-fomite contact. Prior work has found similar surfaces contaminated with *S. aureus* (Table 2.2 and Table 2.3). Further, in the case of seats, the fabric surface makes cleaning difficult, thus enhancing conditions for bacterial survival.

Currently, there is a paucity of data regarding the occurrence of MRSA in the community. Therefore, the findings of this aim are an important contribution to our understanding of the background prevalence of MRSA on community surfaces.

### 6.1.3 Specific Aim 3

Specific Aim 3 built-upon the findings of Specific Aim 2 through molecular analysis of positive bus isolates. Using antibiotic resistance profiling, SCC*mec* typing, and pulsed-field gel electrophoresis, the epidemiologically-linked origins and clonal relatedness of bus strains was determined. These data provide novel insights into what MRSA strains are circulating in the community. There is a scarcity of literature that describes MRSA strains in the community, and all prior work has been associated with an outbreak of infections (or not provided any molecular data). To the research team’s knowledge, this is the first study to provide strain analysis data of MRSA in public transportation in the United States. These results provide key information to infectious disease epidemiologists to better understand the dynamics of MRSA in the community.

The study results demonstrated that several different antibiotic resistance profiles were represented among isolated *S. aureus* (specifically, MRSA isolates had 11 unique, non-β-lactam profiles); this finding is consistent with the current body of knowledge.
Study isolates had a high level of resistance to clindamycin, even among community-associated isolates and MSSA isolates. Prior research has found that community-associated MRSA strains are generally susceptible to clindamycin, although some studies have found an increasing trend in clindamycin resistance (David & Daum, 2010; Deresinski, 2005; Kaplan et al., 2005). Finding such high clindamycin resistance in this work may be indicative of high regional resistance to clindamycin. With respect to vancomycin, no resistance was noted in any of the study isolates. Had vancomycin resistance been observed, this would be potential cause for concern, as the agent is often the only viable treatment option during the course of a MRSA infection. There were very low levels of resistance to tetracycline and chloramphenicol, and no resistance to any aminoglycosides or tri-sul; this too is consistent with prior findings (David & Daum, 2010; Deresinski, 2005; Naimi et al., 2003).

SCCmec typing indicated that the majority of study isolates were SCCmec type IV, which is commonly linked to community-associated MRSA strains (David & Daum, 2010). This finding is logical, given the community setting of public transportation. However, the healthcare-associated SCCmec type II was also observed, providing evidence that these strains are also circulating in the community (and whose origin may or may not be directly linked to healthcare facilities/exposure). Overall, healthcare-associated strains had higher levels of multi-drug resistance; again, consistent with current MRSA epidemiology (Deresinski, 2005; Naimi et al., 2003). The strain analysis is further strengthened by the PFGE analysis, which also demonstrated that the majority (68.1%) of strains tested were community-associated USA300/400. PFGE also provides
strong evidence that buses can be contaminated with the same MRSA strain in several surface locations, or in some cases, by multiple strains in multiple locations. This represents novel data in community MRSA epidemiology.

6.2 Limitations and Lessons Learned

Despite the exciting findings of this dissertation work, the project is not without limitations. The entire project was exploratory in nature, and therefore limited in several notable ways. First, although an attempt was made to collect as much data as possible (both in the laboratory and the field), the small sample size may preclude achieving appropriate statistical power. With respect to the first aim, a priori sample size estimation was utilized to determine the approximate sample size. However, no baseline data were available to utilize in this estimation; therefore, the final estimation may not have been adequate. In addition, it was important to evaluate sampling methods across a range of inoculation concentrations, as this would be more analogous to a real-world scenario than using one artificially high or low inoculation concentration. However, this resulted in five separate, statistically independent sets of experiments, thereby reducing overall study power.

In the second aim, similar a priori estimation was performed. However, the estimation was again limited by the lack of background data regarding the prevalence of S. aureus on vehicle surfaces. The research team utilized a highly conservative estimate of 50% prevalence, but was aware at the project outset that resource limitations may prevent reaching the level of power indicated at this prevalence. When taken at the
surface sample level, the study is likely sufficiently powered. However, at the bus level, this may not be the case. As a secondary criterion for sample size, there was a desire to sample approximately 10% of the active bus fleet from a given facility per sampling day. This level was achieved.

The third project aim – the molecular analysis of positive bus isolates – was conducted to provide origin and clonality data. This type of analysis is very costly, and is routinely conducted on only logically selected isolates. Therefore, \( a \ priori \) estimation was not performed. All positive isolates (MRSA and MSSA) underwent resistance profiling. The results of this analysis provided indications of potential “duplicate strains” (those isolates from the same surface with identical resistance profiles), which were then removed from the analysis pool during SCC\(_{mec}\) or PFGE.

### 6.2.1 Laboratory Study Limitations

An attempt was made to conduct the laboratory study (SA1) in a controlled fashion. However, there were some elements that were not directly measured within the experimental design. These included laboratory temperature, relative humidity, and light exposure. By conducting all iterations on a given experiment day, consistency was achieved with the four sampling methods (that is, if there were different ambient temperature or light levels, it would be equally applied to all test groups). Therefore, it is unlikely that these factors affected one group and not the others.

As with all laboratory-based studies, the work here must be viewed with caution when applying the results to real-world settings. In real environmental settings, there are
numerous factors that may affect sampler performance. Another substantial limitation in
the laboratory-based experiments was the high degree of variability in colony forming
unit results. This is a common problem in studies that involve plate counting, as plate
counts often result in “zero” results (“zero cells”), as well as the opposite – high plate
counts. This phenomenon makes applying statistical analyses to this type of data very
difficult, and introduces complications in achieving statistical significance due to very
high standard deviations. To combat this problem, it is common to only count media
plates which are above a certain minimum CFU threshold and below a maximum CFU
threshold (for example, 300 CFU). However, doing so results in the loss of potentially
important data; therefore, this technique was not utilized in this study, and no data were
eliminated from analysis. Instead, a data imputation strategy was implemented that
applied the maximum possible CFU value (reflecting 100% efficiency) to all results “too
numerous to count”, at a given concentration; the research team believes this resulted in
more accurate values, since it eliminated cases of efficiency >100%. As a comment, the
difficulty of zero-count and high plate counts may be why, very frequently, only
descriptive statistics are reported in published studies similar to the current work.

6.2.2 Field Study Limitations

The field bus sampling was not without limitations; most of which were a result
of the public transportation agency’s operations, and therefore not controllable within the
experimental design. One example is cleaning schedule. An attempt was made to sample
only buses that had not undergone “deep” cleaning in the last month or that had not
undergone “nightly” cleaning at the time of sampling. However, records for “deep” cleaning were not always available, and if known, buses that had not yet been cleaned in the past month were often unavailable at the time of sampling. Similarly, every attempt was made to sample buses prior to “nightly” cleaning, but there may be instances where this did not occur. Given the lack of thoroughness in cleaning activities, this was unlikely to affect the study results.

Prior to the start of the study, the research team desired to assign buses to a route for an extended period of time and then sample. It was thought that this would provide a better reflection of any effects of route (including whether hospitals were served or not and whether the route was a local or express route) and finding contamination. However, this was not possible given the agency’s operations. Instead, bus routes reflect only the day of sampling.

6.3 Future Research

The results of the dissertation work and the lessons learned throughout provide an excellent foundation for future research. There are several relevant studies that may spring directly from each of the specific aims, both the laboratory-based and field studies.
6.3.1 Future Research from Specific Aim 1

In Specific Aim 1, the research team learned that sampling methods resulted in very different recovery of *S. aureus* from a stainless steel surface. There are several follow-up studies that would greatly add to the body of knowledge of sampler performance. In one such study, different substrate materials should be utilized to simulate common materials in the community. These materials should include painted metal, wood, plastic, and fabric. Although this study would extend the results of the current work, a major challenge would be the difficulty in sterilizing the materials before inoculation without changing the material’s surface characteristics.

Another study should assess the effect of post-inoculation drying time on sampler performance. In SA1, a 24 hour drying time was utilized, due to the belief that this time was more relevant to actual field conditions. However, bacteria may be deposited onto a surface at any time, and in the case of *S. aureus*, may remain viable for weeks to months. Therefore, a study that evaluates samplers at several time intervals, from minutes to days post-inoculation drying, would provide excellent information on sampler performance. It should be noted, however, that this study will require a very large sample number, as multiple replications will be required at each time interval for each sampler.

In the current study, common environmental surface sampling techniques were utilized, all of which are processed using standard microbiology. As rapid and molecular methods are becoming more advanced and lower-cost, there is clearly a desire to utilize these for environmental sampling. However, in past work, the performance of these techniques has been limited by high levels of inhibitors and background, non-target
contamination. Despite these limitations, infection control and environmental health professionals would benefit greatly from successful application of molecular and rapid techniques for isolating *S. aureus* from surfaces. A study may be designed which develops a molecular assay using one of many potential techniques, such as IMS-ATP or real-time qPCR.

Three common sampling methods were evaluated in this study. However, there are numerous additional products available for surface sampling, such as sampling sponges and flocked swabs. Similar studies could be conducted that evaluate the performance of these products for the isolation of *S. aureus* from environmental surfaces. In addition, this study or a similar study should evaluate low-cost sterilization methods (such as microwaving and autoclaving) for sterilizing electrostatic wipes.

The environmental health and infection control practitioner would be aided by a cost-benefit analysis study that weighs the advantages and disadvantages of replicate sampling, and/or sampling simultaneously with multiple methods. This type of repeated sampling is anticipated to prevent false negative results, but places additional financial burden on the institution. Determining the ideal number of samples to collect and appropriate combination of sampling methods would be highly beneficial.

Finally, future work should refine the roller contact-based sampler, and extend its scope of use. Once the current design limitations are addressed, additional laboratory and field trials should be conducted. In addition, although the application of the roller was limited to *S. aureus* in this study, the roller may be used for numerous other bacteria and even fungi. It is anticipated that the roller may perform very well for fungal isolation.
from contaminated surfaces. This has significant application in indoor air quality investigations.

6.3.2 Future Research from Specific Aim 2 and Specific Aim 3

Specific Aim 2 provided compelling evidence that both MSSA and MRSA can be isolated from bus surfaces. There are several future directions that will be benefited from this finding. Other transportation settings should be explored, including subway cars, commercial airplanes, passenger rail trains, taxi cabs, and cruise ships. Also, outpatient transit vehicles should be investigated, as there are likely a greater proportion of *S. aureus* carriers and immune compromised passengers using these vehicles (and therefore a high potential health impact from finding MRSA). Finally, there are countless other community settings worthy of assessment for MRSA contamination. As an extension of Specific Aim 3, further insights may be gleaned through sequencing (particularly multi-locus sequent typing, or MLST) of MRSA-positive isolates.
Commentary: MRSA and One Health

The One Health concept - the synergistic, collaborative relationship between human health, animal health, and environmental health professionals - will be critical to effectively combat infectious diseases in the foreseeable future. Just decades ago, scientists thought that infectious diseases would soon be eradicated on a widespread scale, due to the remarkable success of antibiotic therapy. However, this soon proved erroneous, after resistant strains were noted shortly after the introduction of penicillin. The problem has only become worse in recent years, and some suspect we are approaching a “post-antibiotic era.” Resistant pathogens and emerging and re-emerging infectious agents will be primary challenges to Public Health in the coming century. Many of these pathogens are zoonotic (transmitted from animals to humans), and many have an environmental component. Therefore, only through the integrated collaboration of physicians, veterinarians, and environmental health professionals can these infectious agents be effectively managed.

Methicillin-resistant Staphylococcus aureus (MRSA) is a model One Health pathogen. Although the bacterium has been a staple in the hospital for decades, only since the mid-1990s has MRSA emerged as a community pathogen. There are numerous examples of recurrent MRSA infections or colonization that provide a basis for the importance of applying the One Health model to effective treatment of MRSA infections. Case reports describe recurrent colonization or infections that are linked to not only family members, but also family pets (Manian, 2003; Sing et al., 2008;
van Duijkeren et al., 2004). Only through treatment of the infected individuals, colonized household members, and family pets were the patients successfully rid of recurrent infection. Clearly person-to-person transmission is primary in *S. aureus* infections; thus, recurrent infections due to colonized family members are not surprising. However, zoonotic transmission has only recently been described. It seems that because *S. aureus* is a human pathogen, and not a canine or feline pathogen, the direction of transmission is suspected to be from a colonized or infected owner to the pet (“reverse zoonosis” or “humanosis”), then from the pet back to the human through zoonotic transmission; however, more study is required in this area.

Environmental surfaces and fomites also play a role in recurrent MRSA infection and colonization. Masterson et al. (1995) described recurrent MRSA infection in a nurse that was not successfully treated until decolonization and treatment of the nurse, her family, and cleaning the household environment. Similarly, Allen et al. (1997) described a hospital outbreak of MRSA due to a relapse MRSA infection/colonization in a previously-treated healthcare worker. The researchers conducted environmental sampling in the nurse’s home after the nurse had continual recolonization, and found MRSA on numerous household surfaces. Only after commercial cleaning of the home (including discarding the mattress and cleaning linens in the hospital laundry) was the nurse free from future colonization.

As described above, MRSA provides an excellent example of the importance of applying the One Health model to treating infectious diseases. In these cases, only through treatment of the human patient (human medicine), family pets (veterinary
medicine), and the home environment (environmental health) were individuals effectively
rid of recurrent infection, which in the case of healthcare workers, is important in
preventing nosocomial outbreaks.
References


167


Centers for Disease Control and Prevention (CDC). (2006). Table 1: tuberculosis cases, case rates per 100,000 population, deaths, and death rates per 100,000 population, and


173


Appendix A: Efficacy of Modified Cleaning Techniques for the Reduction of S. aureus Surface Contamination

A.1 Introduction

Cleaning is an important strategy to reduce pathogen transmission. However, cleaning has often focused on aesthetic improvement, rather than reduction in surface microorganisms (Dancer, 2008). In recent decades, several nosocomial outbreaks have occurred in which environmental contamination is believed to have been a contributory factor of transmission (Cotterill et al., 1996; de Lassence et al., 2006; Farrington et al., 1990; Kumari et al., 1998; Rampling et al., 2001). Thus, there is increasing interest in developing methods to improve cleaning for bioburden reduction, and evaluating the most effective methods to assess the effectiveness of cleaning.

*Staphylococcus aureus* (*S. aureus*) is the most frequently isolated human pathogen, causing numerous hospital and community outbreaks (David & Daum, 2010; Klevens et al., 2007; Lowy, 1998). Although both susceptible and resistant strains are believed to have similar ability to contaminate environmental surfaces, the emergence of methicillin-resistant *S. aureus* (MRSA) has only increased the importance of effectively controlling transmission. The disease burden of MRSA is significant, causing approximately 1,300,000 infections per year in the United States (David & Daum, 2010;
MRSA is the leading cause of community skin and soft tissue infection, and kills 19,000 people per year (Klevens et al., 2007; CDC, 2005; CDC, 2006).

Direct person-to-person contact is the primary mode of \textit{S. aureus} transmission. However, there is increasing evidence that environmental contamination is a factor in MSSA/MRSA transmission (Chambers & DeLeo, 2009; Kazakova et al. 2005; Lowy 1998; Miller & Diep 2008; Muto et al. 2003). Approximately 28-32\% of individuals in the U.S. are colonized with MSSA, and 0.8-1.5\% with MRSA; the prevalence of colonization is even higher for healthcare workers, who are important disseminators of the pathogen (CDC/Kuehnert et al., 2006; Gorwitz et al., 2008). Whether through colonization or contamination from patients or the patient environment, \textit{S. aureus} is frequently passed from the hands of health care workers to environmental surfaces (Ayliffe et al., 1988; Bhalla et al., 2004; Boyce et al., 1997). Given that \textit{S. aureus} has been found to contaminate a variety of surfaces and fomites in both the hospital (toilet seats, computer keyboards, patient beds and drapes, nursing uniforms and physician neckties, door handles, and stethoscopes) and the community (bus seats/doors/stanchions/seat rails, van seats, tables, faucets, whirlpools, wooden benches, household items such as phones, remotes, ATMs, and toys), and can survive for up to six months on surfaces, environmental bioburden reduction through cleaning seems a prudent strategy to reduce the health and economic impact of \textit{S. aureus} infection (Dietze et al., 2001; Dixon, 2000; Jawad et al., 1996; Sexton et al., 2006; Wagenvoort & Penders, 1997; Wagenvoort et al., 2000; Williams & Davis, 2009; See Table 2.2 and Table 2.3 for
additional references). Indeed, even in hospitals where hand hygiene and patient isolation/barrier precaution interventions have been conducted, MRSA transmission remains problematic (Rampling et al., 2001). This seems to underscore the importance of the environment in MRSA transmission.

Prior research has demonstrated that ineffective cleaning may increase *S. aureus* transmission, both in the hospital and the community (Corcoran & Kirkwood, 1999; Allen et al., 1997; BSACHISICNA, 1998; Haley & Bregman, 1982; Masterson et al., 1995; Reagan et al., 1991; Waldvogel, 1995; Williams, 1963). Conversely, enhanced cleaning has been shown to effectively reduce *S. aureus* contamination in the healthcare setting (Dancer, 1999; Griffith et al., 2007). However, little data exists regarding the effectiveness of cleaning to reduce pathogen load on community surfaces. Given the large number of community surfaces that may be contaminated with *S. aureus*, and because fomite and surface contamination may be more important in community MRSA transmission than precursor colonization, cleaning may be just as important to prevent community transmission as it is for hospital infection control (Baggett et al., 2004; Begier et al., 2004; Kazakova et al., 2005; Miller & Diep, 2008; Rieg et al., 2005). Enhanced cleaning may be a particularly important strategy for pathogen reduction in locations where hand hygiene is difficult or impractical; for example, on public transportation vehicles, where MRSA has recently been isolated (see Chapter 4 for details). On public transportation vehicles, as in many community settings, there is regular hand-to-fomite contact with little opportunity for hand hygiene; thus, there is high potential for pathogen deposition and acquisition.
The aim of this research was to evaluate the efficacy of an enhanced cleaning protocol for more effective *S. aureus* reduction (over a standard cleaning method) on a stainless steel surface – a ubiquitous surface material in community settings. Through a laboratory-based intervention study, the reduction in methicillin-susceptible *S. aureus* (MSSA) bioburden between a routine cleaning method (standard cleaning) and a more intense, deep cleaning method (enhanced cleaning) was compared. The results of this study will provide valuable information to professionals charged with ensuring public surfaces are clean and safe.

*A.2 Methods and Materials*

To evaluate the efficacy of enhanced cleaning methods for the reduction of *S. aureus* on environmental surfaces, the research team conducted a series of controlled, laboratory-based experiments.

*Inoculation of the Steel Test Plates*

To evaluate the ability of different cleaning levels to reduce *S. aureus* surface contamination, a stainless steel test surface was first inoculated with a range of starting concentrations of methicillin-susceptible *S. aureus* (ATCC 29213). Stock solutions of MSSA were prepared using freshly prepared (24 hour incubation at 37°C) colonies from blood agar. Stock solution culture population density was determined by the addition of bacteria into normal saline and read using a nephelometer calibrated to a 0.5 McFarland
An aliquot of stock solution was inoculated onto a mannitol salt agar plate in serial dilution to obtain average starting concentration.

Stainless steel was chosen as the experimental test surface because of the material’s ability to withstand autoclave sterilization, and because public transportation stanchion rails are primarily stainless steel and the material is widely used on community surfaces. The model test surface consisted of three autoclaved 16 gauge T-304 (#4 annealed) stainless steel plates (40.6cm x 25.4cm), demarcated into 100cm² zones. Within a Class II biosafety cabinet, the plates were checked for dryness and each zone was inoculated with 100µL stock solution. The solution was spread using sterile spreaders for a period of one minute per 100cm² zone (prior to inoculation, a blank control sample was obtained to ensure plate sterility). The steel plates were allowed to dry inside the biosafety cabinet, and then placed into a sanitized holding container for approximately 24 hours; plates were placed in such a way that the spiked surface was not contacted by any other surface.

**Cleaning**

The inoculated steel plates were processed in one of three ways: no cleaning, standard cleaning, and enhanced cleaning. The non-cleaning zones underwent no processing and were sampled directly, either 30 minutes or 24 hours after inoculation.
Standard Cleaning

The standard cleaning protocol was designed to mimic actual cleaning techniques utilized by a large transportation agency during their monthly “deep cleaning” of vehicle interiors. Standard cleaning consisted of applying an aerosol disinfectant (Lysol Disinfectant Spray, EPA-registered as effective against MRSA) to a dry, autoclaved white shop towel. All zones on the test surface were wiped in one direction with one pass, using the same cloth for each of eight test zones. Per the disinfectant manufacturer’s labeled instructions for contact time, the test surface was left undisturbed for 10 minutes after cleaning, before any sampling was conducted.

Enhanced Cleaning

An enhanced cleaning protocol was designed utilizing practical changes to the standard cleaning protocol, with the aim of further reducing S. aureus contamination levels. Enhanced cleaning used disposable paper towels instead of re-usable cloth towels, and a liquid-based disinfectant instead of an aerosol. To mimic how the protocol would be applied in the field, clean paper towels were first saturated in a liquid disinfectant (Lysol Concentrate Disinfectant; EPA-registered as effective against MRSA). All zones on the test surface were wiped for five passes, using a new paper towel for each 100cm² zone. Per the manufacturer’s labeled instructions for contact time, the test surface was left undisturbed for 10 minutes after cleaning, before any sampling was conducted.
Surface Sampling and Sample Processing

Samples were collected within the biosafety cabinet after 30 minutes or 24 hours of drying in holding container. Using aseptic technique, each 100 cm² zone was sampled randomly using autoclaved static wipes. To obtained wipe samples, a new, sterile electrostatic wipe was folded once (in addition to the factory folds) and wiped three times each direction on each side; the wipe was then turned over and wiping was repeated. Once complete, the wipe was placed into a sterile stomacher bag. To reduce the chance of contamination to the static wipe, a modified “clean hands/dirty hands” method was used, in addition to aseptic technique. The sampler’s hands were cleaned using an alcohol-based hand sanitizer, and a new pair of gloves was donned. A second pair of over-gloves was donned, with care taken to avoid touching the outside of the over-gloves. All package manipulation was conducted using one hand (the “dirty hand”), and all sampling/handling was conducted using the other hand (the “clean hand”). A new pair of over-gloves was used for each new sample, and the top and bottom static wipes in each new package were discarded. Wipe sampling covered the full 100 cm² zone.

Wipe samples were processed using membrane filtration for concentrating bacteria. Fifty milliliters PBS-Tween 20 was added to each stomacher bag, the bag was resealed, and massaged until the PBS solution was absorbed. Each bag was then massaged rigorously for an additional 10 seconds, and the solution passed through a 0.45 µm membrane filter. Membrane filters were placed directly onto a 50 mm mannitol salt plate, and incubated at 37°C for up to 48 hours.
*Plate Count*

All mannitol salt plates were counted after 24 and 48 hours. Only those colonies which were morphologically consistent with *S. aureus* and displayed mannitol fermentation were counted.

*Statistical Analysis*

To determine whether enhanced cleaning resulted in a reduction in *S. aureus* bioburden, both descriptive statistics and comparison of means was conducted. The arithmetic mean concentration (recovered colony forming units, or CFU) was calculated for each cleaning method. To evaluate the difference in mean concentration versus cleaning method, a one-way analysis of variance (ANOVA) was conducted. To further assess where differences between groups exists, Tukey’s pairwise *post hoc* analysis was performed.

*A.3 Results*

The efficacy of an experimental cleaning intervention to reduce *S. aureus* concentrations was evaluated by comparison of standard and enhanced cleaning methods to a non-cleaning control. Sampling was conducted at both 30 minutes after inoculation (and immediately after cleaning) (n=38), and 24 hours after inoculation (and immediately after cleaning) (n=71). Overall, enhanced cleaning resulted in the lowest recovered CFU – lower than both standard cleaning and the non-cleaning control (Figure A.1, Figure A.2). Standard cleaning also resulted in lower recovered CFU than the non-cleaning
control surfaces. Colony counts were made at both 24 and 48 hours of incubation; however, because mannitol fermentation may take up 48 hours, all analysis of variance data analysis is based-upon 48 hour incubation times.

Post-inoculation drying time affected the amount of recoverable colony forming units after cleaning. At 30 minutes of post-inoculation drying, enhanced cleaning resulted in an average of 95.6% less recovered CFU over the non-cleaning control, and standard cleaning resulted in an average of 92.8% less recovered CFU than the non-cleaning control (Table A.1). Comparing the two levels of cleaning, the enhanced cleaning recovered CFU were 1.5-1.8 times less than for the standard cleaning group.

At 24 hours of post-inoculation drying, the effect of cleaning was less pronounced for both methods, although a greater difference was observed between enhanced and standard cleaning. Enhanced cleaning resulted in an average of 91.5% less recovered CFU than the non-cleaning control, and standard cleaning in 41% less recovered CFU than the non-cleaning control. The recovered CFU in the enhanced cleaning group were 6.8-6.9 times less than for the standard cleaning group.

Drying time resulted in significantly different levels of baseline (non-cleaned) recovered CFU. At 30 minutes of drying, the non-cleaning control resulted in an average of 157.6 CFU, versus 17 CFU at 24 hours (Table A.1). Student’s t-test indicated a statistically significant difference between 30 minute and 24 hour post-inoculation drying times at both 24 and 48 hour plate counting (24h plate counting: p=0.006; 48h plate counting: p=0.0029).
Analysis of variance indicated a statistically significant difference in mean recovered CFU between cleaning methods and the non-cleaning control (24h: \( p=0.012 \); 48h: \( p=0.004 \)) at 30 minutes of post-inoculation drying (Table A.1). However, Tukey’s pairwise post hoc analysis revealed a significant difference between both the cleaning methods and the control (24h: \( p_{\text{standard}}=0.026, p_{\text{enhanced}}=0.025 \); 48h: \( p_{\text{standard}}=0.01, p_{\text{enhanced}}=0.009 \)), but no significant difference between the two cleaning methods (24h: \( p=0.996 \); 48h: \( p=0.996 \)). At 24 hours of post-inoculation drying, ANOVA indicated that there was no significant difference in mean recovered CFU between the groups (24h: \( p=0.296 \); 48h: 0.282). Model diagnostics were conducted and confirmed ANOVA assumptions.
Figure A.1: Recovered *S. aureus* by Cleaning Method, 30 Minutes Post-inoculation Drying

Figure A.2: Recovered *S. aureus* by Cleaning Method, 24 Hours Post-inoculation Drying
### Table A.1: Summary of Cleaning Method Performance

<table>
<thead>
<tr>
<th>Variable</th>
<th>Mean Recovered MSSA (CFU)</th>
<th>Standard Deviation</th>
<th>95% CI</th>
<th>ANOVA p-value</th>
<th>Percent Lower CFU over Control</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>30 Minute Drying</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>24 Hour Plate Incubation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>143.62</td>
<td>213.20</td>
<td>(14.78, 272.45)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Standard Cleaning</td>
<td>9.46</td>
<td>21.94</td>
<td>(-3.08, 22.72)</td>
<td>NA</td>
<td>93%</td>
</tr>
<tr>
<td>Enhanced Cleaning</td>
<td>5.25</td>
<td>8.16</td>
<td>(0.07, 10.43)</td>
<td>NA</td>
<td>96%</td>
</tr>
<tr>
<td><strong>48 Hour Plate Incubation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>171.31</td>
<td>217.66</td>
<td>(39.78, 302.84)</td>
<td>0.004</td>
<td>NA</td>
</tr>
<tr>
<td>Standard Cleaning</td>
<td>13.31</td>
<td>29.30</td>
<td>(-4.40, 31.01)</td>
<td>92%</td>
<td></td>
</tr>
<tr>
<td>Enhanced Cleaning</td>
<td>8.83</td>
<td>17.37</td>
<td>(-2.02, 19.87)</td>
<td>95%</td>
<td></td>
</tr>
<tr>
<td><strong>24 Hour Drying</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>24 Hour Plate Incubation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>13.83</td>
<td>41.71</td>
<td>(-3.78, 31.45)</td>
<td>NA</td>
<td>NA</td>
</tr>
<tr>
<td>Standard Cleaning</td>
<td>8.63</td>
<td>22.03</td>
<td>(-0.68, 17.93)</td>
<td>38%</td>
<td></td>
</tr>
<tr>
<td>Enhanced Cleaning</td>
<td>1.26</td>
<td>2.30</td>
<td>(0.27, 2.26)</td>
<td>91%</td>
<td></td>
</tr>
<tr>
<td><strong>48 Hour Plate Incubation</strong></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Control</td>
<td>20.00</td>
<td>62.41</td>
<td>(-6.35, 46.35)</td>
<td>0.282</td>
<td>NA</td>
</tr>
<tr>
<td>Standard Cleaning</td>
<td>11.13</td>
<td>25.61</td>
<td>(0.31, 21.94)</td>
<td>44%</td>
<td></td>
</tr>
<tr>
<td>Enhanced Cleaning</td>
<td>1.61</td>
<td>2.89</td>
<td>(0.36, 2.86)</td>
<td>92%</td>
<td></td>
</tr>
</tbody>
</table>

### A.4 Discussion

In this research, the research team sought to evaluate whether a modified, enhanced cleaning regimen could result in lower *S. aureus* surface bioburden than both non-cleaning and a standard cleaning method. The findings of the laboratory based-study indicate that manual cleaning resulted in significantly less recovered *S. aureus* on a stainless steel surface than not cleaning at all. Enhanced cleaning, which included using disposable towels and a liquid-based disinfectant, resulted in up to seven times less *S. aureus* on the test surface than standard cleaning, which utilized reusable cloth towels.
and an aerosol-based disinfectant. However, although this difference is notable, statistical significance was not achieved when comparing the enhanced cleaning method to the standard cleaning method. Despite the lack of statistical significance between the cleaning methods, the findings of the study support the need for rigorous cleaning for bioburden reduction.

There are several possible mechanisms by which cleaning reduced *S. aureus* levels in this study. First, the use of a disinfectant agent may have resulted in direct cidal effects to bacteria on the steel surface. Both disinfectant agents used in the study (the aerosol and the liquid-based disinfectant, used in the standard cleaning and enhanced cleaning, respectively) are EPA-registered for use against MRSA (EPA, 2009). According to the Environmental Protection Agency (EPA), these products must be capable of being “used on hard inanimate surfaces and objects to destroy or irreversibly inactivate infectious fungi and bacteria, but not necessarily their spores” (EPA, 2009). The experimental protocol in this project followed product label instructions for contact time, which according to both the aerosol and liquid-based disinfectant product labels, should result in a 99.9% reduction in *S. aureus*. However, this level of reduction was not quite achieved in the current research. The calculated percent reduction$^1$ in this research was only 38% for the standard cleaning method at 24 hours of post-inoculation drying, but was 93% when the surface was only dried for 30 minutes. For the enhanced cleaning method, calculated reduction was 92% at 24 hours of post-inoculation drying and 95% at 30 minutes of drying. This suggests that the liquid-based disinfectant may be more

---

$^1$ Calculated Percent Reduction: since no pair sampling (before/after) was conducted for each cleaning method, comparison of recovered CFU from the cleaning methods to the non-cleaning control was used, as a surrogate measure of percent reduction.
effective than the aerosol, as used in the study. However, it should be noted that both disinfectant types were applied to the wiping cloth, as opposed to applied directly to the test surface (as was observed of the public transportation cleaning staff). In the case of the aerosol, there may not be sufficient disinfectant applied to the test surface using this method, since it is first placed onto the cleaning towel. Indeed, the enhanced cleaning surface appeared wetter than the standard cleaning surface, supporting this notion. Therefore, when using an aerosol-based disinfectant, it may be more effective to spray the surface with the aerosol first, then wipe it.

Wiping is also an important component in reducing bacterial surface contamination. Through the wiping motion, bacteria are removed from the surface and deposited onto the cleaning cloth. In addition, cellular damage is possible during the course of wiping. This mechanical damage effect may be amplified when cleaning a rough surface such as stainless steel. Although wiping may remove bacteria from a surface, it may also redistribute bacteria from one area to another – even from a contaminated area to a previously clean one. Therefore, it may be useful to utilize disposable towels, or replace cleaning cloths frequently. Griffith et al. (2007) found consistently lower bacterial counts when using a method that replaced reusable cloth towels with disposable paper towels. This is consistent with the results of the current study, which indicated that enhanced cleaning (which utilized a disposable paper towel, replaced after each test zone) resulted in less recovered CFU than standard cleaning (which utilized the same cloth towel for several test zones).
Although not a specific objective of the study, it was observed that post-inoculation drying time greatly affected the concentration of recovered *S. aureus*. There was roughly 10 times greater CFU recovered in the control group at 30 minutes of post-inoculation drying compared to the 24 hour drying time. Bacterial die-off is likely even after 24 hours of drying, where a one to two log reduction may occur; this is consistent with the current findings (Jawad, 1996; Wagenvoort et al., 2000). In addition, there may be less bacterial adherence to the surface after 30 minutes versus 24 hours. Bacterial attachment may begin in as little as 20-60 minutes; but, there appears to be a linear increase in adherent bacteria for at least the first 8 hours (Mafu et al., 1990; Escher, 1986). Further, there is evidence that nutrient limitation enhances biofilm formation (as cited in Hood & Zottola, 1997). Therefore, there may not only be less viable bacteria remaining on the steel surface after 24 hours, a greater proportion remaining may be better adhered to the steel surface and thus may not be as efficiently removed through sampling.

Cleaning seems to be more effective after 30 minutes of post-inoculation drying than after 24 hours of drying. In addition, there is a greater difference between the efficacies of the two cleaning methods at 24 hours of drying than 30 minutes of drying, with standard cleaning resulting in far less CFU reduction at 24 hours drying than 30 minutes drying. Despite the lack of statistical significance between cleaning groups at 24 hours drying, it was felt that this drying time was more relevant to a real-world scenario. Hence, the majority of replications were conducted at this drying time.
The results of this study are consistent with previous work, which has supported the hypothesis that improvements to a cleaning method can result in more effective reduction of surface bacteria. In a hospital-based intervention study, Griffith et al. (2007) demonstrated that by introducing a rinse stage to cleaning, replacing cloth towels with reusable paper towels, and cleaning for a longer period of time, *S. aureus* isolations reduced to 94 (using modified cleaning) from 1,690 (using standard cleaning). Staphylococcal CFU went from an average of <2.43 CFU/cm² using the standard cleaning method to <0.88 CFU/cm² using the modified cleaning regimen; aerobic colony counts decreased from <4.08 CFU/cm² to <0.88 CFU/cm². The modified cleaning protocol (enhanced cleaning) used here was similar to the methods used in their study. However, no rinsing stage was included in this research because it was believed that the additional time required (10-12% in the Griffith et al. work) would make their modified cleaning method prohibitive to use in the community, where low value is placed on cleaning. Despite the subtle difference, the current results support that modified cleaning results in lower bacterial levels (at 30 minutes of drying, 8.83 CFU/cm² for enhanced cleaning versus 13.31 CFU/cm² with standard cleaning, and at 24 hours drying, 1.16 CFU/cm² for enhanced cleaning versus 11.13 CFU/cm² for standard cleaning).

Surface cleaning has long been utilized in the nosocomial setting; however, the focus is generally on aesthetic improvement, rather than microbial reduction (Dancer, 2008). Despite the regularity of cleaning in hospitals, the effectiveness of cleaning is rarely assessed through objective measures. Recently, Dancer (2004) proposed standards for assessing hospital cleaning, which use both indicator pathogens and total aerobic
colony counts. According to the author, there should be $< 1$ CFU/cm$^2$ MRSA (along with other indicator organisms) and $< 2.5$ CFU/cm$^2$ or $< 5$ CFU/cm$^2$ (depending-upon the standard) total bacteria for a surface to be considered adequately clean. Hand contact surfaces outside of these criteria should receive additional cleaning, be subjected to repeat sampling, and trigger a risk assessment (Dancer, 2004).

Clearly there is a need for cleaning standards in healthcare settings, where there is a direct link between contaminated surfaces and nosocomial outbreaks. However, the link between surface/fomite contamination and subsequent outbreaks is less compelling in the community. One may argue that the overall risk of *S. aureus* transmission (and subsequent infection) is less in the community than in the hospital, due to the lower proportion of infected and immune compromised individuals. However, every day there are countless immune suppressed individuals circulating throughout the community. Thus, surface contamination in the community cannot be wholly ignored. In addition, there are several high-risk settings that warrant special consideration. These include prisons, military barracks, college dormitories, daycare facilities, households and contact sport facilities, and public transportation vehicles (CDC, 2010; as cited in David & Daum, 2010; as cited in Kluytmans-VanderBergh & Kluytmans, 2006). Indeed, the Centers for Disease Control and Prevention (CDC) has stated, “MRSA in the community is widespread and therefore, anyone is at risk” (CDC, 2010). Therefore, in all of these settings, regular, effective cleaning may reduce the chance of MRSA and other pathogen transmission.
A.5 Limitations

The results of this research should be viewed in the context of the study limitations. As in Chapter 3, the membrane filtration method that was used to concentrate S. aureus is widely used for water quality investigations, but has not been routinely employed for processing surface samples. However, because the same sampling method was utilized for both cleaning methods and the non-cleaning control, any variation as a result of membrane filtration should be equally valid for each group. In addition, as a lab-based study, there are countless factors which may affect the performance of cleaning methods in a field environment, thereby limiting generalizability. Of particular importance is disinfectant efficacy, which in the presence of high levels of surface debris in soiling, may have compromised effectiveness. Finally, as a pilot-scale study, the results may not be adequately powered for application to other scenarios. However, despite these limitations, the results demonstrate that when conducted properly, cleaning is quite effective at reducing surface bioburden.

A.6 Conclusions

The results of this study provide novel information on the ability of a modified cleaning regimen to reduce S. aureus bioburden on steel surfaces. To the research team’s knowledge, this is the first study that has applied cleaning methods in a controlled, laboratory framework for the reduction of S. aureus. This study demonstrates that surface cleaning, when done in a methodical manner, with appropriate disinfectant
contact time, can result in considerably less recovered \textit{S. aureus}. This has important implications for infection control in both in the hospital and the community.
Appendix B: Final Report and Recommendations for Transportation Agency
Staphylococcus aureus Bus Surface Surveillance Study – Summary Report

Conducted at:

Conducted On:
July 12, August 23, October 11, and October 25, 2010

Prepared for:

Prepared By:
Mr. Jonathan Lutz, MPH

Reviewed By:
J. Mac Crawford, PhD, MS, RN
The Ohio State University
Division of Environmental Health Sciences
College of Public Health

Armando Hoet, PhD, DVM
The Ohio State University
Veterinary Preventative Medicine
College of Veterinary Medicine/College of Public Health
February 28, 2011

Re: Results and Recommendations of the 2010 *Staphylococcus aureus* Bus Surface Surveillance Study

Executive Summary

Under authorization from [redacted], Mr. Jonathan Lutz, MPH from The Ohio State University (OSU), College of Public Health, conducted bacterial surface sampling on forty [redacted] buses at the [redacted] facilities. This sampling occurred on July 12, August 23, October 11, and October 25, 2010. The purpose of the study was to determine whether public transportation vehicles may play a potential role in community transmission of *Staphylococcus aureus*. We evaluated susceptible *Staphylococcus aureus* (Staph.), a common human pathogen, and methicillin-resistant *Staphylococcus aureus* (MRSA), the form of the bacterium that can survive commonly-prescribed antibiotics (antibiotic resistant). All work was conducted following established sampling protocols, and all field and laboratory research activities were supervised by four OSU faculty members.

Results from the bus sampling indicated that both antibiotic-susceptible *Staphylococcus aureus* (MSSA) and MRSA were routinely isolated from bus surfaces. The most frequently Staph.-positive surfaces were the seats, the seat rails, the back door, and the stanchions. Staph. was also found on the operator’s area, and minimal Staph. was noted on an HVAC return vent (one positive sample). The results of this study may have important public health implications, since MRSA is an emerging bacterial threat in the community.

The attached report summarizes the findings of the study. First, we provide an overview of the most significant findings, and then outline a set of results-based recommendations. Also attached are supplementary materials that provide background information regarding MRSA, the detailed results of the study, and study limitations. Of note, recommendations were formulated to both reduce MRSA contamination and enhance the rider experience.
Should you have any questions or concerns about this report, please do not hesitate to contact me.

Sincerely,

Jonathan Lutz, MPH, Doctoral Candidate
Graduate Research Associate
Division of Environmental Health Sciences
College of Public Health
The Ohio State University
216.337.8959, jlutz@cph.osu.edu
B.1 Summary of Major Findings

The following list highlights the major findings of the study. These findings are described in greater detail in subsequent sections of the report. Please note that data analysis is ongoing; therefore, this list will be updated in future versions of the Report.

Major Results and Findings

- A total of 237 surface samples were collected from 40 buses (20 at McKinley, 20 at Fields);
- Six samples were collected from each bus, one each from the stanchions, seat rails, operator’s area, the back door, the HVAC return vent, and the seats;
- Total Staph. aureus (both the form of the bacteria that responds to common antibiotics [susceptible, or MSSA], and the form that does not [resistant, or MRSA]) was found in 17% of all samples, and in 68% of all buses;
- MRSA (resistant) was found in 15% of all samples, and 63% of all buses;
- The majority of positive samples were made-up of MRSA (83%);
- The facility had only a slightly higher percentage of positive results than McKinley;
- Sampling date may be an important factor. There were more positive samples and buses (nearly double) in July and August than in October;
- Sample location on a bus also may be an important factor. The most frequently positive surfaces were the seat rails and the seats (~33%), followed by the back door and stanchions (~15%). The operator’s area was rarely positive (8%), and the HVAC return had no Staph. aureus;
- Whether a hospital was passed on a route (based only on the sampling day’s data), and whether a bus was on one route or multiple routes in a day, seem to be less important factors;
- High ridership buses (>200 riders per bus per day, the day of sampling only) seem to be more frequently Staph.-positive (15-25% more) than low ridership buses (0-199 riders per bus per day, the day of sampling only);
A mix of different bacterial strains has been identified (Staph.-positive only), including a majority that have characteristics associated with community infections, and some that have characteristics of hospital infections (this is based-upon preliminary strain-typing analysis, which is ongoing).
B.2 Recommendations

Cleaning

Prior to sampling buses, the research technician conducted brief observation of cleaning, and conducted an interview with a cleaning staff member. The cleaning staff outlined the process utilized for the monthly “premium” cleaning, which consisted of the following (note: this applies to interior bus cleaning only): hot water is applied/sprayed onto all surfaces at the seat level and below (the hard plastic surfaces), followed by removing excess water with a squeegee; stanchions and seat rails are wiped with a cloth towel (moistened with Lysol aerosol disinfectant), then followed-up with a light spray of Lysol aerosol; the tech. then mists seats with Lysol aerosol; cleans windows with Davey-Finch GlassKleen; cleans the back door and HVAC return vent with Lysol/cloth. New cloth towels are used for each bus, and approximately 6-10 towels are used for each. Betco Lemon disinfectant is available, but is only used for the floors, wheel wells, and the back of the seats (plastic areas).

Nightly bus cleaning is much less rigorous (presumably due to time constraints). This cleaning consists of picking up trash, sweeping the floor, wiping the operator’s area, and sometimes wiping the stanchions and seat rails (depending on the cleaning technician). Cloth towels were used, sometimes dry, sometimes with Lysol. Observation during sampling indicated that it was very difficult to tell the difference between buses before and after this process.
Recommendations:

General

- Standardize cleaning methods (including written documentation).
- Retrain cleaning technicians on cleaning methods, and conduct cleaning audits and refresher training at scheduled intervals.
- Focus additional time and cleaning effort on “hot spots”: seats, seat rails, stanchions, and the back door.
- During vehicle operation: if possible, have the vehicle operator open and close the back door for the rider, to reduce hand-to-door contact.

“Premium” Cleaning

- Implement the following changes to the cloth towel cleaning process (especially for “hot spots”): first spray the surface to be cleaned with Lysol aerosol (apply Lysol directly to surface), **10 minutes or more prior to cleaning**. Next, saturate a new towel in Betco Lemon disinfectant (diluted per label instructions in a clean bucket using clean water); the towel should be wrung-out slightly, but remain quite wet. Clean the surface, making sure that the surface remains slightly wet (to be completely effective, **the disinfectant must remain on the surface for 10 minutes or more**). When any towel becomes visibly soiled, discard and repeat the process with a new towel. **Do not place soiled towel back into clean bucket.** In the event that a residue remains, implement a rinse stage (however, this should be unnecessary as Betco Lemon is labeled as a rinse-free product).
- For “hot spots” (seats, seat rails, stanchions, and back door), use additional cloths and/or disposable paper towels. Based-upon the level of soiling observed during sampling, we recommend at least 1-2 towels for stanchions, 2 or more for seat rails, and 2 or more for the back door.
• For “hot spots”, conduct cleaning from back-to-front, top-to-bottom. For example, when cleaning stanchions, start at the rear of the bus and work forward, cleaning and entire side; repeat for the other side. For back doors, clean from the top of the door to the bottom, since the bottom is more heavily soiled.

• Conduct seat cleaning. To more-effectively clean seats, mix fresh Betco Lemon disinfectant (diluted per label instructions) into a hand sprayer. Set the hand sprayer to the mist setting, and spray the seats ~8-12” from the seat surface, ensuring thorough coverage. The seats should be wetted to a point that will leave the surface damp for at least 10 minutes, but dry by the following service. In the event that hand sprayers cannot effectively produce a fine mist (and result in oversaturation), utilize the same method in a pump sprayer, pressurized and set to the fine mist setting.

• For all wiping activities, ensure technicians wipe with moderate pressure and are thorough and methodical.

Nightly Cleaning

• Ensure that “hot spots” are cleaned routinely (consistently in every bus, every day).

• Stanchions, seat rails, and the back door should be cleaned using the wet-cloth method outlined above. Clearly during the nightly cleaning this process will be conducted quicker and be less comprehensive. However, by using a cloth wetted with Betco Lemon disinfectant (instead of a dry cloth or one misted with Lysol aerosol), much greater results should be achieved.

• Mist seats with Lysol aerosol (or mist using Betco Lemon as described above), applying the disinfectant from ~6-8 inches from the seat surface.

• Check and refill alcohol-based hand sanitizer gel.
Alcohol-based Hand Sanitizer

Alcohol-based hand sanitizer (ABHS) has been demonstrated to be quite effective at reducing bacterial levels on hands (and particularly useful for MRSA reduction). In addition, ABHS is a good strategy for hand hygiene when no opportunity for hand washing exists (such as on a bus). We compliment [X] for installing ABHS dispensers. However, we noted several improvements that could be made that would increase the utility of the ABHS dispensers.

Recommendations:

- Move all ABHS dispensers to visible, accessible locations. During sampling, we noted that in several buses, the dispensers were behind the front door, making them nearly impossible to see or access for a boarding passenger.
- Place a sign adjacent to the ABHS dispensers that call attention to the dispenser. Currently, dispensers are black and gray plastic, and often mounted onto a black panel. Therefore, it is highly unlikely that riders are aware that the dispensers are available, since they “blend in” with other bus components. Several existing signs are available for free that are designed to draw attention to dispensers.
- Implement an ABHS refill schedule, so that dispensers are promptly refilled when empty. To effectively determine the optimal time interval for refill, it may be helpful to initially check the gel levels frequently (perhaps weekly) and document the amount remaining. Then (using this information), [X] can determine whether dispensers need to be replenished weekly or monthly.
Methicillin-resistant *Staphylococcus aureus* has been the subject of considerable media attention over the past decade. Often described as a “superbug” (though the accuracy of this moniker is questionable), the media has arguably misrepresented the risk that the pathogen poses to the general public. However, due to the public nature of [redacted] and the results of the study, we recommend the following strategies to prevent any mischaracterization of the study results that may negatively impact [redacted].

**Recommendations:**

- Establish a dialogue between [redacted], the OSU research team (and the College of Public Health Communications Director), and the public health agencies in [redacted]’s coverage zone ([Columbus Public Health and Franklin County Board of Health](https://www.franklinhealth.org/)). The public health agencies would be benefited by prior knowledge of study results and limitations before any results become public.
- With assistance from OSU, compose a risk communication plan (outline) for dissemination of appropriate study result summaries and methodology for managing questions or concerns that may be raised from [redacted] employees, passengers, and/or the general public.
Supplementary Materials
B.3 Project Background

*Staphylococcus aureus* (“Staph.”) is a common bacterial pathogen of significant public health impact. The bacterium is the most frequently isolated human pathogen, and causes diseases ranging from skin and soft tissue infection to necrotizing pneumonia or bacterimia. Some strains of the bacterium have acquired specialized genes that make the bacterium resistant to commonly-prescribed antibiotics; these strains are known as methicillin-resistant *Staph. aureus* (MRSA). The majority of Staph. infections are now caused by MRSA strains, leading to worse health outcomes, longer hospital stays, and increased cost of treatment. In the U.S., the disease burden of MRSA has grown considerably over the past two decades, with MRSA causing 19,000 deaths per year – more than tuberculosis or AIDS. It has been estimated that MRSA results in over 1.3 million infections per year, with an economic burden of $3.2-4.2 billion to hospitals.

Traditionally, MRSA infections have been confined primarily to the hospital setting, where infected patients have underlying risk factors (such as diabetes or recent surgery). However, since the 1990’s, MRSA infections have emerged in the community, affecting individuals without established health risk factors. Frequently, these individuals are young and healthy, and lack any health care exposure that may place them at risk for MRSA infections. Clearly, this emergence of community-associated MRSA infections has caused the health community to focus more effort on identification and control.
efforts. However, these community-associated infections are now endemic, accounting for approximately 15% of all MRSA infections. Indeed, community-associated MRSA is now so significant that the Centers for Disease Control and Prevention (CDC) have stated that, “MRSA in the community is widespread and therefore, anyone is at risk”. Despite the lack of health risk factors, there are several scenarios that have been associated with increased risk of community infection. To summarize these, the CDC has described the “5 C’s” of community MRSA transmission: crowding, compromised skin, contaminated items or surfaces, lack of cleanliness, and frequent skin-to-skin contact. Community settings where these factors are frequently met are prisons, military barracks, college dormitories, daycare facilities, households, and contact sport facilities.

Staph. (including MRSA) is somewhat unique among human pathogens in that healthy individuals can become asymptomatic carriers for the bacteria; these individuals are referred to as “colonized” or “carriers”. Under this condition, the bacteria may live and remain in the nose and throat, and on the hands, groin, and arm pits, of an individual for weeks to months. Although this condition does not result in immediate disease in the carrier (although the individual may be at higher risk for later disease), it provides a mechanism by which the pathogen can be easily transmitted from person-to-person and person-to-environment/surface. In the U.S., colonization rates are significant – roughly 30% of the population carries Staph., and 1-2% carries MRSA. Some subpopulations, such as health care workers, may have MRSA colonization rates of 5% or more. In addition, there seems to be considerable regional variation in colonization rates; however,
only a few metropolitan areas in the U.S. have been evaluated, and little is known about
the colonization rate of the study region.

Staph. is primarily transmitted through direct contact with a colonized or infected
individual, or through an environmental/surface intermediate. Hands are the critical
disseminators (the primary way the pathogen is spread), particularly the hands of
healthcare workers. In healthcare settings, this model of transmission is clearly the
fundamental means to pass MRSA from one person to another. However, in community-
associated MRSA infections, there is evidence that colonization may be less important
than environmental contamination or sharing of contaminated personal items. There are
countless examples of MRSA-contaminated surfaces and objects in the community.
Chairs, van seats, ATM keypads, toilet seats, couches, remote controls, door handles, and
numerous other items have been found to harbor Staph. and MRSA. These contaminated
items may potentially play a role in transmission.

Perhaps one of the most important civic services in most American cities is public
transportation. This critical infrastructure is widely used, with approximately 10.5 billion
trips per year in the U.S. (per APTA 2010 data). However, little data exists regarding the
prevalence of pathogens on public transportation vehicle surfaces, and no studies have
assessed whether MRSA can be isolated from public transportation vehicles in the United
States. Currently, the only published work that has assessed MRSA on public
transportation vehicles has been conducted in Europe, where MRSA colonization rates
are believed to be lower than in the U.S. For example, in a study of public transportation
vehicles in Serbia, no MRSA was isolated, but 30% of samples were positive for other staphylococci (less-pathogenic bacteria of the same genus as Staph. aureus). One hundred percent of all sampled vehicles were contaminated. Similarly, in a study of public transportation touch surfaces (not only vehicle surfaces) in London, researchers were able to isolate methicillin-susceptible Staph. (Staph. aureus that is susceptible to commonly-prescribed antibiotics); however, the researchers were unable to isolate MRSA. Finally, in a recently published report from Portugal, a locally endemic hospital MRSA clone (EMRSA-15) was found on public transportation buses, with a total of 26% (22/85) buses positive for MRSA (Simoes et al., 2011). These findings represent the first report of MRSA from public transportation vehicles.

There are several factors that make public transportation vehicles an ideal setting for MRSA isolation. First, there are undoubtedly colonized individuals (those carrying the bacterium but not infected) using public transportation. Public transportation ridership is roughly estimated at 28.8 million trips per day in the United States (per APTA 2010 data). Assuming a 2% colonization rate, this would equate to 576,000 trips by MRSA colonized individuals per day. When healthcare workers ride public transportation, the potential for environmental/surface contamination may also be increased, since they are colonized at a much higher rate than the general public.

Once on a vehicle, colonized individuals may spread MRSA through hand-to-environment/surface contact (and to a lesser extent, airborne transmission). Riders routinely touch stanchions, seat rails, doors, seats, and straps, especially as vehicles become crowded. Hand-to-environment/surface contact of this kind has been previously
implicated in community-associated MRSA transmission. During high-volume usage times (such as the peak morning and evening hours), crowding may increase this hand-to-environment/surface contact. Further, the regular opening and closing of vehicle doors and windows may create air drafts, which have been shown to suspend or re-suspend MRSA-infected skin particles and contaminated dust. Finally, there is little to no opportunity for hand hygiene during and immediately after transportation usage. Previous research has demonstrated that hand hygiene is the primary method for prevention of MRSA transmission.

In this research project, the aim was to determine the background prevalence of Staph. and MRSA contamination on bus surfaces. Here, the results of the surveillance study conducted on forty buses are described.
B.4 Detailed Findings

A total of 237 surface samples were collected from 40 buses between July and October of 2010. The results of the study indicate that public transportation bus surfaces routinely harbor both susceptible and resistant *Staph. aureus*\(^2\) (MSSA or “Staph.”, and MRSA). Staph. was found in 17% (n=41) of all samples, and in 68% (n=27) of buses (those buses having at least one Staph.-positive sample)\(^3\). MRSA was found in 15% (n=35) of all samples, and 63% of all buses had at least one MRSA-positive sample. In total, MRSA accounted for 83% of all positive samples. Six samples (3%) samples were positive for susceptible Staph., and one sample (0.4%) was positive for an unidentified methicillin-susceptible coagulase-positive staphylococci. Sixty percent (60%) of buses had one or two positive samples, and 8% had three or four positive samples; no buses had more than four positive samples.

A general representation of in-service buses were sampled from one of two service facilities (Fields Ave.: n=117; McKinley Ave.: n=120). Six samples were obtained from each bus, with the exception of three buses that did not have seat rails. Buses served one of 35 routes (although in several cases, a bus ran multiple routes in a day), and 39 unique buses were sampled. A total of 155 (65%) samples were from the 26

---

\(^2\) Where “Staph.” or “total Staph.” are used, this indicates a finding of any Staph. aureus, both antibiotic resistant and susceptible. “MRSA” is used only to indicate a finding of antibiotic resistant Staph. aureus.

\(^3\) Analysis was conducted on two levels (by sample and by bus), and for two indicators of contamination (all Staph. aureus, and resistant Staph. aureus [MRSA]).
buses that directly served major hospitals, and 141 (59%) samples were from the 24 buses that served multiple routes. Approximately 10% of the active fleet from a facility was sampled each sampling day, and approximately 16% of the total active bus fleet was sampled in the course of the study.

Sample Date

Samples were obtained on four dates: July 12, August, 23, October 11, and October 25 of 2010. There was a reduction in overall Staph. levels by advancing date, with the first two sampling events resulting in 90% and 80% Staph.-positive buses, versus 50% positive buses for the second two sampling events (Figure B.1). The number of Staph.-positive samples also decreased, from 26% and 22% for the first two sampling events, to 12% for each of the second two sampling events. However, the percentage of MRSA increased with advancing date, with MRSA accounting for 86% and 62% of positive samples in the first two sampling events, but 100% in the final two sampling events.
Multiple Routes and Facility

A total of 35 of the transportation system’s 68 routes were sampled. Buses frequently serviced multiple routes during the day of sampling, with 60% of buses running multiple routes and the remainder only running one route. The percentage of both samples and buses with MRSA and total Staph. was slightly higher for buses that serviced multiple routes (MRSA\textsubscript{samples}: 16%; MRSA\textsubscript{buses}: 67%; total Staph\textsubscript{samples}: 19%; total Staph\textsubscript{buses}: 71%), compared to those that serviced the same route in a day (MRSA\textsubscript{samples}: 14%; MRSA\textsubscript{buses}: 56%; total Staph\textsubscript{samples}: 16%; total Staph\textsubscript{buses}: 63%). With respect to facility, [facility name] had a slightly higher number of positive samples.
(19\% versus 17\%) and buses (70\% versus 65\%), and a greater percentage of MRSA-positive samples (91\% versus 75\%) and buses (70\% versus 55\%) than McKinley Ave.

Sample Location on a Bus

The degree to which total Staph. and MRSA could be isolated varied considerably between sample locations on a bus. Seats (33\%), seat rails (30\%), and back doors (15\%) demonstrated the highest prevalence of MRSA (Figure B.2, B.3). MRSA was also isolated from stanchions (10\%) and the operator’s area (3\%); no MRSA was found on the HVAC return. Similarly, seat rails (35\%), seats (33\%), and the back door (18\%) had the highest levels of total Staph. The stanchions (13\%), operator’s areas (8\%), and HVAC (3\%) were also Staph.-positive. Only one sample from the HVAC returns was positive, but the isolate was an unidentifiable methicillin-susceptible coagulase-positive staphylococcus (not Staph. aureus).
Figure B.2: MRSA and Total Staph. by Sample Location on a Bus

**Hospital/Non-Hospital Routes**

There was no difference in the number of MRSA-positive samples between the non-hospital (15%) and hospital routes (15%), or in the number of samples positive for total Staph. (non-hospital samples: 18%; hospital samples: 17%); however, there is a moderate difference in the number of positive buses for MRSA (non-hospital buses: 71%; hospital buses: 58%) and total Staph. (non-hospital buses: 79%; hospital buses: 62%). In addition, the percentage of total Staph. that was from MRSA (samples) is five percent higher for the hospital routes (85%) versus non-hospital routes (80%).

230
Ridership

Ridership data were categorized into four levels: low ridership (0-99 riders/day), medium-low ridership (100-199 riders/day), medium-high ridership (200-299 riders/day), and high ridership (300 or more riders/day). The medium-high ridership level resulted in the highest level of MRSA-positive buses (81.8%). The high ridership level was next highest (62.5% MRSA-positive buses), which was only slightly higher than the low ridership level (58.3% MRSA-positive buses); the medium-low ridership level had the lowest percentage of MRSA-positive buses (50%). Results are similar for total Staph., with the exception that the medium-high ridership indicates an even higher percentage of Staph.-positive buses (90.9%). At the sample level, the same trend is observed – the medium-high ridership level has the highest percentage of MRSA- and Staph.-positive samples, and the other three levels are similar.
Figure B.3: Percentage of Positive Samples (Staph. and MRSA) by Sample Location

*Bus Conditions*

During sampling, the sampling technician observed the overall condition of the bus interiors. The bus surfaces varied considerably with respect to debris loading and cleanliness. Some locations within a bus were much dirtier than others, although this did
not necessarily correspond to Staph. levels. For example, the HVAC return was extremely dirty in nearly all samples, but resulted in low levels of Staph. The stanchions sampled were moderately dirty, but ranged from very clean to very dirty. The operator’s area was relatively clean in most cases, resulting in little debris loading of the sampling wipe. Conversely, the back doors were routinely very dirty, soiling the sampling wipe with both dirt and an oily film. The seat rails were generally not dirty, but very oily. Overall, the seats were the most consistently dirty surface; even after wiping just one seat the sampling wipe would become visibly soiled.
B.5 Miscellaneous

Study Limitations

There are several potential limitations in the study. First, the static wipe sampling method utilized in this study is ideal for pooling large areas and amplifying low levels of bacteria, but does not allow for quantification of isolated bacteria. Therefore, it is difficult to make comparisons between samples and factors of interest, other than from a categorical standpoint. This research was an exploratory surveillance study, designed to provide baseline data for MRSA prevalence in this important community setting. The isolation of MRSA cannot be equated to a specific exposure assessment, other than in the broadest sense that MRSA has been isolated on vehicle surfaces. Lack of quantitative data prevents an accurate measurement of exposure. These findings also cannot be interpreted in the context of risk, as there are no established threshold levels above which MRSA causes infection. Finally, molecular analysis is pending and currently, no molecular epidemiology data is available. Once available, this data will provide information regarding the potential origins and mixing of S. aureus strains.
B.6 Acknowledgements

The research team would like to extend gratitude to [Redacted] for their participation in the study. Special thanks to the [Redacted] employees directly involved in coordinating this work, including [Redacted]. We are also greatly indebted to the numerous volunteers that helped collect and analyze samples, including Dr. Rocio Hoet, Dr. Joany Van Balen, Jade Braman, Melissa Mikolaj, “View” Wannasawat Ratphitagsanti, Chongtao Ge, Sophia Dailey, Colleen Shockling-Dent, and Debbie Lutz; and the review of Dr. Eric Lutz. Finally, this study project would not be possible without the diligent oversight and contribution of the primary research team, Dr. John “Mac” Crawford, Dr. Armando Hoet, Dr. Jiyoung Lee, and Dr. Jay Wilkins.

B.7 References

Study references (primary literature) available as requested...
Appendix C: Proposed Field Intervention Protocol
**PROPOSED INTERVENTION BUS SAMPLING METHODOLOGY**

Currently-used Sampling Systems:

<table>
<thead>
<tr>
<th>Positives</th>
<th>Negatives</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Swabs:</strong></td>
<td></td>
</tr>
<tr>
<td>- Easy, inexpensive, quick</td>
<td>- Impractical for large surface areas</td>
</tr>
<tr>
<td>- Quantification possible with dilutions</td>
<td></td>
</tr>
<tr>
<td>- Use with any/prepared media</td>
<td></td>
</tr>
</tbody>
</table>

→ **POOR CHOICE FOR SA3b**

**Contact Plates:**

- Easy, relatively inexpensive when lab-made, quick  
- Prone to overload on dirty surface
- Allows direct quantification  
- No dilutions possible
- Requires custom/powder media for our project

→ **POTENTIAL CHOICE FOR SA3b**
Wipe Samples:

- Easy pooling/coverage of large surface areas
- Requires two people to sample
- Relatively inexpensive
- Requires glove change with each sample
- Use with any/prepared media
- Not sterile, so quantification may be more difficult

→ POOR CHOICE FOR SA3b

Sponge Sticks/Spongesicles (~$100-$150/pack of 100): Can use almost exactly like the Swiffer but only requires one person to sample and sponge is sterile

- Easy, relatively inexpensive, quick
- Requires only one person (has a handle so you don’t touch the sample)
- Can quantify using dilution
- Can use prepared and any media

→ MY PROPOSED CHOICE FOR SA3b
**Available Media:**

**OXOID BRILLIANCE** – PREPARED

**OXOID ORSAB OXACILLIN SCREENING BASE** – ANTIBIOTIC POWDER WITH MSA

**BD – CHROMAGAR MRSA** – PREPARED (100pk/$300 or 20pk/$60)

➤ My proposed choice due

**REMEL – SPECTRA** – PREPARED (10pk/$82.49 or 100pk/$816.39)

**BioRAD – MRSA SELECT** – PREPARED

**BIOMERRIEUX – MRSA ID** – PREPARED
**Proposed Sampling/Processing:**

**Field Sampling**

1) Using gloved hands, obtain sterile SpongeStick. Collect sample from back door, seat rails, and seats. **Use one sampler to make a pooled sample of entire back door half, all seat rails (half), and seats (half).** This will result in 3 pre-cleaning samples per bus, and 3 post-cleaning samples per bus.

**NOTE:** A small, initial pilot (~10-20 samples) will determine if pooled samples are possible. If not, multiple samples of each bus component can be obtained (for example, multiple seats).

**NOTE:** SpongeSticks come either dry, or pre-moistened with 10ml Letheen Broth, Peptone Buffered Broth, or Neutralizing Buffer. We may want to use the Letheen Broth model to enhance uptake.

2) Place sample directly into a labeled 50ml centrifuge tube or similar container

3) To prevent re-sampling locations sampled during pre-cleaning, place a piece of masking tape or something to identify areas already sampled (on back door side sampled, seat handles sampled, or seats sampled; place tape in a location where the cleaners cannot see or remove it, to avoid bias). Other methods of identifying samples from the pre-cleaning sampling can be explored

4) Take samples to laboratory for analysis
**Laboratory Processing:**

1) Agitate/vortex container for 20 seconds. Spread plate 200µl (100-500µl) diluent to selective and differential CHROMagar MRSA plate; spread with sterile spreader.

**NOTE:** during the pilot, we can determine whether 200µl is appropriate. If overgrowth occurs, either less solution can be spread onto the plate or serial dilutions can be prepared (conduct dilutions only if required, as they will result in use of significantly more resources and time).

2) Count plates at 24 and 48 hours. The CFU reflected here will be reported as

   *CFU/sample of suspected* Methicillin-resistant Staphylococcus aureus *(we cannot call these MRSA)*

3) →*IF REQUIRED* - (Add ___ml TSB with NaCl to holding vessel for enrichment)

4) Process selected, suspected Staph. aureus colonies through typical MRSA processing protocol (beta-hemolysis on blood agar, coagulase, catalase, latex agglutination, and susceptibility).
INTERVENTION Bus Sampling – Field Protocol/Notes

**Bus Selection**

-Where possible, buses will be assigned that are (in order of importance): 1) same bus model; 2) same route; 3) same approximate age of bus

-Buses will be assigned to either Standard Cleaning or Enhanced Cleaning

**Pre-Cleaning Sampling**

*Sample one side of the bus only, noting the approximate linear footage of stanchions, the number of seat rails, and the number of seats. Only half the driver's area, back door, and HVAC will be sampled.*
1) Before you sample, check to make sure Swiffer box seal is not broken/box is not damaged

2) Dirty hands puts on gloves, prepares for sampling

3) Clean hands puts on gloves when Dirty hands is ready;

4) Dirty hands opens Swiffer box without touching inside, without breathing/talking

5) Clean hands takes first Swiffer, discards

6) Clean hands changes gloves, takes second Swiffer and places into Negative Control

7) Clean hands changes gloves, obtains next series of Swiffers (until the bottom in the box) and samples. CHANGE GLOVES BEFORE EACH NEW SAMPLE

8) Dirty hands obtains Whirlpak bag, peels top, opens by the outside tabs, and the Clean hands puts folded Swiffer (contact surface facing out) into the bag without touching the inside of the bag; Dirty hands seals bag, and places into the bottom container.

NO BREATING/TALKING WHEN THE BAG IS OPEN

Cleaning

Standard Cleaning

-Clean according to normal “nightly” cleaning (clean only those surfaces that would normally be cleaned, in the exact manner they’re normally cleaned)
Enhanced Cleaning

- Using disposable towels saturated in Betco Lemon disinfectant, clean as normally conducted in the monthly “Deep Clean” (4 passes (back-forth back-forth wiping) of stanchions, seat rails, driver’s area, back door, HVAC return, and seats). To clean seats, spray the seats with Betco Lemon from a spray bottle on mist setting. Seats should be lightly damp to the touch, but not wet (using the fan for cleaning and overnight drying should be sufficient to adequately dry the seats by the next morning). Additional areas cleaned should include windows, floors, etc.

Cleaning Principals

- Wipe from back of the bus forward
- Wipe high to low, furthest to closest
- Change disposable towel at signs of soiling, several times throughout the cleaning process

Post-Cleaning Sampling

Sample the other side of the bus only, equaling the linear footage of stanchions, the number of seat rails, and the number of seats. Sample the other half of the driver’s area, back door, and HVAC.
1) Before you sample, check to make sure Swiffer box seal is not broken/box is not damaged
2) Dirty hands puts on gloves, prepares for sampling
3) Clean hands puts on gloves when Dirty hands is ready;
4) Dirty hands opens Swiffer box without touching inside, without breathing/talking
5) Clean hands takes first Swiffer, discards
6) Clean hands changes gloves, takes second Swiffer and places into Negative Control
7) Clean hands changes gloves, obtains next series of Swiffers (until the bottom in the box) and samples. **CHANGE GLOVES BEFORE EACH NEW SAMPLE**
8) Dirty hands obtains Whirlpak bag, peels top, opens by the outside tabs, and the Clean hands puts folded Swiffer (contact surface facing out) into the bag **without touching the inside of the bag**; Dirty hands seals bag, and places into the bottom container.

**NO BREATING/TALKING WHEN THE BAG IS OPEN**

**Sampling Case**
- Top is for clean items; never place samples on/in top
- Bottom is for samples that have already been collected
**How to Don Gloves**

- With clean hands, take first glove from box (start with a new box) by the cuff area; try to avoid touching the outside. **Handle the first glove from the inside.**

- Take the second glove using the first gloved hand, by the cuff, and **secure the second glove by only touching the outside.**