Multidrug-Resistant Organisms: Preventing Acquisition and Transmission

by

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Dedication

To my family, my friends and my partner, Alison. Thank you for your support. I love you all.

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Abstract

Multidrug-resistant organisms (MDRO) are a global problem, causing treatment challenges which result in worse health outcomes and high healthcare costs. As resistance to antibiotics continues to develop causing treatment failures, infection prevention strategies have become necessary. This dissertation offers research into discovering potential targets for intervention of MDRO acquisition through prevention of multiple MDRO colonization and infection and prevention of infection and transmission through the environment. The introduction for these topics will be covered in chapter one.

In chapter two, we analyzed a four-year, prospective study to identify risk factors among hospitalized patients for co-colonization and coinfection (CCCI) with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE), a predecessor to vancomycin-resistant *S. aureus*. Using conditional logistic regression, we identified admission from another healthcare facility as a risk factor when comparing CCCI patients to patients with MRSA only and patients without MRSA or VRE, indicating healthcare exposure increases the risk of CCCI. Molecular analysis indicated CCCI may be more likely to occur in patients colonized or infected with MRSA strains typically associated with hospital-associated infections. We recognized patients who may benefit from additional resources for infection prevention and control in the hospital to prevent acquisition of an emerging MDRO through multiple organism colonization or infection.

MDRO environmental contamination facilitates MDRO transmission. In chapter three, we identified a hospitalized patient type colonized with MDRO more likely to contaminate their

environment. Using latent class analysis, we categorized patients into two classes based on mechanical ventilation status, consciousness status at admission and requiring assistance with activities of daily living prior to admission. Low functional status patients were more likely to contaminate their environment. Infection prevention practices, such as hygiene assistance, enhanced environmental disinfection and contact precautions may contribute to reducing the burden of environmental contamination by these patients.

Chapter four includes a surveillance study of environmental contamination in a child care center with the goal of guiding environmental cleaning and disinfection practices. We demonstrated the feasibility of longitudinal surveillance of a variety of fomites to detect overall contamination and the frequency of contamination with MDRO and viral pathogens. Water-associated sites were identified as harboring a higher bioburden and being contaminated more frequently with pathogens, demonstrating the potential of water to act as a reservoir for microorganisms and distribute them in the environment. We detected a higher bioburden on objects with irregular surfaces or which were cleaned less frequently. Child care providers should consider the ability to decontaminate a surface balanced against the development of children when including items in the classroom.

Chapter five examines the knowledge added from this research and the public health implications related to our findings. Support for current infection prevention and control recommendations are addressed and additional strategies for interventions are considered. Potential future research directions informed by findings in this dissertation, including investigations into mechanisms of acquisition and transmission and potential interventions to interrupt these processes are discussed.

In the appendix, I present a proposal for a future research project to develop a self-screening method for VRE. While this may have multiple applications, my goal is to use this in community-based studies, where VRE is prevalent but under-investigated. Better surveillance of VRE in the community will inform best practices for prevention of transmission and acquisition in this setting, while also guiding future research regarding VRE in community.

Chapter 1: Introduction

1.1: Antibiotic Resistance

The United Nations' (UN) Interagency Coordination Group on Antimicrobial Resistance describes antimicrobial resistance as a global crisis that jeopardizes the worldwide effort to achieve the UN Sustainable Development Goals.[1] A report commissioned by the United Kingdom Government and Wellcome Trust estimated 700,000 global deaths per year from antimicrobial resistance disease and predicts a continued rise in resistance will result in 10 million deaths annually and cost up to $100 trillion by 2050.[2] The Infectious Disease Society of America estimated infections from antimicrobial-resistant organisms cause $21-$34 billion in healthcare costs and 8 million additional hospital days annually in the U.S.[3]

Antimicrobial resistance is prevalent among all groups of infectious organisms. Antifungal resistance is an emerging problem worldwide. Four classes of antifungals are primarily used to treat fungal infections, azoles, polyenes, pyrimidine analogs and echinocandins. Resistance to any of these drugs would reduce an already limited pool of treatment options. Clinical laboratories often do not have the capabilities to test for antifungal resistance, which can result in underdiagnosis and treatment failures.[4] Resistance to antifungals has been observed among numerous genera, with antifungal resistance among *Candida* and *Aspergillus* presenting a major challenge due to their prevalence.[4–7]

Antifungal-resistant *Candida* spp. are of particular concern. Drug-resistant *C. auris* has been labeled an urgent threat by the Centers for Disease Control and Prevention (CDC); a 318%

increase in drug-resistant *C. auris* cases in the U.S. was observed in 2018 compared to the average number of cases from 2015-2017.[4] Pan-resistant *C. auris* isolates have emerged worldwide.[8,9] Resistance to antifungals may result in worse health outcomes. Investigators identified 51 cases of *C. auris* infections in New York City, including 50 resistant to at least one antifungal, during a retrospective review of cases from May 2013 to April 2017.[10] Of these 51 patients, 23 (45%) died within 90 days, although deaths attributable to *C. auris* were unknown. Other species of drug-resistant *Candida* are also a concern, causing an estimated 34,800 infections and 1,700 deaths in the U.S. in 2017.[4] Approximately 7% of bloodstream infections with *Candida* spp. are resistant to antifungals, with resistance to fluconazole in 8.6% of *C. glabrata* isolates and 7.7% of *C. parapsilosis* isolates.[11]

Aspergillius spp. are the other major emerging drug-resistant threat among fungi.[4] *Aspergillus* infections are treated primarily with azoles and resistance to these antifungals is developing which can worsen health outcomes. Investigators detected 37/196 (19%) aspergillus isolates from invasive infections were resistant to azoles in a five-year retrospective study at three tertiary care facilities in the Netherlands.[12] Patients with resistant strains had significantly higher mortality than patients with susceptible strains on day 42 (49% vs 28%; P = .017) and on day 90 (62% vs 37%; P = .0038). Although less common in the U.S., drug-resistant *Aspergillus* has been observed, with 10/274 (3.6%) resistant to azoles and 18/274 (6.6%) resistant to amphotericin B in isolates collected from the Transplant-Associated Infection Surveillance Network from 2001 to 2006.[13] The emergence of antifungal resistance among *Candida and Aspergillus* and the presence of resistance among other genera of fungi demonstrate the potential for antifungal resistance to become a problem for healthcare providers and worsen health outcomes for patients.Antimicrobial resistance is present among viruses as well, causing treatment challenges for infections caused by

these pathogens. Identifying antiviral resistance can be difficult. Phenotypic testing can determine antiviral susceptibility but is time consuming and costly. Genotypic testing can be performed quickly but may rely on known mutations causing resistance; novel mutations or a lack of known resistance mutations may limit the detection of antiviral resistance, especially without phenotypic information.

As antiviral resistance increases among viruses causing both acute and chronic infections, identifying resistant pathogens is important for determining treatment options. Influenza, a common cause of acute respiratory infections, has demonstrated resistance to anvirials. Adamantanes, which had been previously used to treat influenza A infections, are no longer recommended for treatment due to high levels of resistance (>99% for influenza A(H3N2) and influenza A(H1N1)pdm09 in the U.S.).[14] Neuraminidase and endonuclease inhibitors are recommended by the CDC for treatment of both influenza A and B infections; resistance to these antivirals is present in less than 1% of isolates tested by the CDC in the 2019-2020 influenza season.[14] With one class of antiviral no longer used to treat influenza and resistance observed to the other antiviral class primarily used to treat this virus, the potential exists for continued antiviral-resistance to emerge among influenza, further reducing treatment options of these infections.

Antiviral resistance is present in among viruses causing chronic infections, posing a different challenge as antivirals are often used for long-term management of chronic infections. Human immunodeficiency viruses (HIV) have a propensity to be error prone during replication and are highly variable..[15,16] These variations result in treatment failures with single agents, necessitating the use of combination antiretroviral therapy. For hepatitis B infections, antiviral resistance develops in >70% of patients receiving lamivudine.[17] Hepatitis B resistance to other antivirals is low (less than 2% in patients with long term treatment using entecavir), but resistance

is detected in higher proportions of patients treated with entecavir following lamivudine treatment failure (>50% after five years of treatment), indicating cross-resistance can occur at a high rate.[18] Resistance to nucleoside analogues has been observed among herpesviruses. For herpes simplex viruses, resistance is low for immunocompetent patients (<1.0%) but is more common for immunocompromised patients (3.5%-10%).[19–26] Prolonged use of nucleoside analogues results in increased risk of antiviral resistance in herpesvirus infections.[27] The prolonged use of antivirals in chronic infections allows for the development of resistance over time, complicating the treatment of these pathogens.

Antibiotic-resistant bacteria also contribute substantially to the burden of antimicrobial resistance disease. Based on national reports, the World Health Organization (WHO) noted antibiotic resistance in ≥50% of isolates among bacteria commonly causing infections in hospitalized patientsand the community including 1) *Klebsiella pneumoniae* resistant 3rd generation cephalosporins in all six WHO regions, 2) *Escherichia coli* resistant to 3rd generation cephalosporins, *E. coli* resistant to fluoroquinolones and methicillin-resistant *Staphylococcus aureus* (MRSA) in five of six WHO regions and 3) *Klebsiella pneumoniae* resistant to carbapenems in two out of six WHO regions.[28] Antibiotic resistance was reported in ≥25% of isolates commonly causing infections in the community including 1) *Streptococcus pneumoniae* non-susceptible or resistant to penicillin in all six WHO regions, 2) nontyphoidal *Salmonella* resistant to fluoroquinolones and *Neisseria gonorrhoeae* resistant to 3rd generation cephalosporins in three out of six WHO regions and 3) *Shigella* species resistant to fluoroquinolones in two out of six regions.[28] In the U.S. more than 2.8 million antibiotic-resistant infections occur annually resulting in 35,000 deaths.[4] The prevalence of infections with antibiotic-resistant bacteria is especially problematic due to difficulties in treating these infections, resulting in higher morbidity

and mortality compared to susceptible bacteria.[29–31] Antibiotic resistant infections also increase healthcare costs through longer lengths of hospital stays, the necessity of treating morbidity resulting from infections and use of expensive antibiotics.[32,33]

Much of the focus of antibiotic-resistant infections lies in healthcare settings due to their prevalence and impact on patient health outcomes. Among eight of the most common bacterial pathogens that caused healthcare-associated infections (HAI) in the U.S. from 2015-2017, seven demonstrated greater than 20% non-susceptibility to at least one class of antibiotics in device-associated infections, including *S. aureus*, *P. aeruginosa* and *Acinetobacter* spp., and four in surgical-site infections, including *E. coli* and *Enterococcus faecium*.[34] Risk factors for antibiotic-resistant bacteria colonization and infection, such as antibiotic use, presence of indwelling devices and increased duration of hospital stay, are commonly found among hospitalized patients, especially critically ill patients.[35–37] Furthermore, shared environments and healthcare workers (HCW) can become contaminated and provide indirect transmission routes for pathogens between patients.[38–42] The combination of these factors create an environment where the prevalence of multidrug-resistant organisms (MDRO), defined as non-susceptibility to at least one antimicrobial agent in at least three different antimicrobial classes, in hospitals is high and mechanisms to transmit MDRO between at risk patients exist, including routes without direct contact between patients.[43] A cycle forms of patients acquiring MDRO and contaminating both the environment and HCW resulting in transmission of MDRO to other patients. Patients with newly acquired MDRO then begin the cycle again. Due to the morbidity and mortality associated with MDRO and their limited treatment options because of antibiotic-resistance, these patterns of transmission and acquisition in hospitals require interventions to improve health outcomes for patients.

Antibiotic-resistant bacteria in community settings receive less attention but likewise contribute to the overall burden of adverse health outcomes resulting from these organisms. Some pathogens, such as methicillin-resistant *Staphylococcus aureus* (MRSA) and extended-spectrum β-lactamase (ESBL)-producing Enterobacteriaceae, transitioned from being primarily nosocomial pathogens to being prevalent in the community as well.[44,45] Emergence in the community may occur for multiple reasons. In the case of MRSA, a novel staphylococcal cassette chromosome was identified in community strains, suggesting resistance was newly acquired in the community and not directly related to strains causing HAI.[46,47] The potential also exists for MDRO carriers in healthcare settings to introduce MDRO into the community. Calfee et al. conducted an investigation of MRSA transmission between 88 discharged patients with hospital-acquired MRSA and both 130 of their close contacts (spouse, parent, child or caregiver) and 42 of their casual contacts (friends, siblings or roommates).[48] Screening of the personal contacts revealed 24 (18.5%) close contacts and 1 (2.4%) casual contact were positive for MRSA. Pulsed-field gel electrophoresis (PFGE) was performed on MRSA isolates from 9 index patients and 11 contacts. Isolates from contacts for eight out the nine index patients were identical to those recovered from the index patient. This study demonstrates the potential for MDRO introduction from healthcare facilities into the community, leading to further spread outside healthcare facilities.

Some resistant bacteria are still considered to be primarily hospital-acquired pathogens and receive little attention in the community, such as vancomycin-resistant *enterococcus* (VRE). However, some small, community based studies have observed VRE colonization prevalence to range from 5-38%, indicating their presence in the community may be greater than previously thought.[49–54] Carriers of MDRO in the community may introduce these pathogens during healthcare admissions. Denkinger et al. conducted a 12-year study at a single tertiary care facility

including both infection and surveillance samples.[55] During that time, there were an average of 39,197 admissions per year (range 36,733–42,262) with an average of 7534 positive bacterial cultures per year (range 5541–8726). The researchers observed a significant increase in the prevalence of VRE, multidrug-resistant gram-negative (MDRGN) and MRSA at admission in patients <65 years old and a significant increase in VRE and MDRGN in patients at admission ≥65 years old.[55] The proportion of *Enterococcus*, gram-negative and *S. aureus* isolates detected at admission with resistance to antibiotics increased significantly as well for patients ≥65 years old. This study suggests MDRO prevalence in the community could be increasing and that community carriers of MDRO may provide a means of introducing MDRO into healthcare facilities. The potential for a cycle of transmission of antibiotic-resistant bacteria between community and hospital settings exists and addressing these problems could provide substantial relief to the burden posed by antimicrobial-resistant organisms.

Combatting the global antibiotic-resistance crisis first requires an understanding of the development of antibiotic resistance. Bacteria acquire resistance genes through mutations during replication or horizontal gene transfer from other bacteria, both of which have been demonstrated *in vitro*.[56,57] Antibiotics kill susceptible organisms, leaving resistant bacteria to grow and spread. With each newly developed antibiotic, resistant organisms arise, limiting the utility of these drugs.[58] This cycle of drug development and resistance has been exacerbated in recent decades by the dearth of development of new antibiotic classes.[28] Additionally, the combination of resistance acquisition methods and selective pressures from antibiotic use have resulted in the emergence of MDRO, which have few or no treatment options.[28,43,59,60] These treatment challenges force the development of other strategies to prevent infections with antibiotic-resistant bacteria. Determining patients at risk for MDRO acquisition guides infection prevention and control

policies. The role of multiple organism colonization and infection in MDRO acquisition will be investigated in this context.

1.2: Multiple Organism Colonization and Infection

Multiple organism infections present a variety of challenges to clinicians and researchers. Surveillance of these infections demonstrates that multiple organism infections are present with considerable prevalence in hospital settings. Royo-Cebrecos et al. observed 194 (10.2%) of all bloodstream infections in cancer patients at a university hospital in Spain over ten years to be caused by multiple organisms.[61] *Escherichia coli* (33.8%) and *Enterococcus spp.* (30.6%) were the most common organisms identified in the multiple organism infections. MDRO were significantly more likely (20.6% vs 12.9%; p = 0.003) to be cultured in multiple organism infections. During a six-year study of military personnel with deployment-related traumatic injuries at Walter Reed National Military Medical Center, Heitkamp et al. reported 259 (66.1%) of 392 patients with a at least one wound infection and a positive culture had multiple organism infections.[62] Patients with *Enterococcus spp.* in their wounds were significantly more likely to have multiple organism infections (n=141; 91.0% vs. n=118; 49.8%; p<0.001). Ferrer et al. investigated pneumonia acquired in the intensive care unit (ICU) over nine years at a university hospital in Spain, reporting 41/215 (16.0%) were caused by multiple organism.[63] *S. aureus* (41.4%) was the most commonly identified organism in multiple organism pneumonia. In addition to their propensity to be detected in multiple organism infections, *S. aureus* and *Enterococcus* have been frequently identified together in hospitalized patients. In an eight-month prospective study by investigators at Cleveland Veterans Affairs Medical Center, 23 (62%) of 37 patients with VRE intestinal colonization were also colonized with intestinal *S. aureus*, including 20 (54%) with MRSA.[64] The prevalence of multiple organism colonization and infection has prompted further

research regarding the health outcomes of these infections. While this research may focus on outcomes such as morbidity and mortality, multiple organism colonization and infection may result in other outcomes, such as the development of additional resistance phenotypes.

1.3: Multiple Organism Infection and Vancomycin-Resistant *Staphylococcus aureus*

One unique situation regarding multiple organism colonization and infection is the development of vancomycin-resistant *S. aureus* (VRSA) during cases of co-colonization or coinfection (CCCI) with MRSA and VRE. VRSA is an emerging pathogen worldwide.[65–74] The acquisition of vancomycin resistance has been hypothesized to occur during CCCI with MRSA and VRE through horizontal gene transfer.[57] Preventing MRSA and VRE CCCI may prevent the future emergence of VRSA. MRSA and VRE are two of the most common pathogens causing HAI and are also commonly found contaminating the hospital environment and the hands of patients and HCW.[34,75,76] The prevalence of MRSA and VRE in hospitals suggests hospitalized patients may have an increased risk of exposure to these organisms.[34] Identifying patients at risk for MRSA and VRE CCCI can aid in developing infection prevention strategies to prevent CCCI and, potentially, VRSA acquisition. Previous research on MRSA and VRE CCCI has been limited by sample size, exclusion of patient types, restriction of *Enterococcus* species and a lack of molecular analysis of pathogens. The objective of aim 1 was to identify risk factors for MRSA and VRE CCCI to prevent future co-acquisition in hospitalized patients. Furthermore, a molecular analysis of pathogens was performed to detect associations between characteristics of MRSA and VRE and their likelihood of co-existing in a host. Molecular markers and strain types may provide information such as identifying isolates associated with HAI which could aid in determining how CCCI occurs.

1.4: MDRO Environmental Contamination in Hospitals

Infection prevention and control strategies can be directed toward individuals at risk for MDRO acquisition. Interrupting pathogen transmission is a primary area of focus of infection prevention in hospitals. Bacteria can be transmitted through a variety of routes, providing many potential points of intervention. Of these transmission modes, MDRO contamination of fomites, both in hospital and community settings, will be explored here.

MDRO can be carried by asymptomatic and symptomatic individuals and are shed into the environment by both host types from multiple body sites, including in respiratory secretions and fecal matter, contributing to the transmission of these pathogens.[40,77–79] Shedding can result in environmental contamination and subsequent MDRO transmission through direct environmental contact or contact with individuals contaminated by the environment.[80–82] Carriers can shed MDRO for months or years, creating a long-term risk of pathogen transmission by unwitting hosts.[83–85] MDRO viability on surfaces adds to the risk of transmission through the environment. Wagenvoort et al. used MRSA clinical isolates to inoculate glass bottles. These isolates survived between 225 and 318 at temperatures between 20°C and 22°C and humidity ranging from 24-47% during the study period.[86] Wendt et al. inoculated polyvinyl chloride with VRE. Strains survived for up to four months.[87] Temperature during the study was 22°C±2 and humidity was 50%±5. Long survival times of MDRO in the environment can create reservoirs facilitating transmission.

Environmental contamination has been implicated in the transmission of MDRO in hospitals. MDRO have been isolated from environmental surfaces repeatedly in patient rooms.[79,88,89] For transmission of MDRO to occur from one patient to another through the environment, several steps are required. First, an MDRO carrier must shed the organism into the environment, such as through direct contact with the environment by colonized skin or by

excreting contaminated bodily fluids. Wilson et al. identified 52 patients colonized with MRSA in ICUs of two London, England hospitals.[90] In 34 cases, the patient room environment was subsequently contaminated by a strain similar to that in the colonized patient, identified by phage typing, which was not present prior to the patient occupying the room. This study demonstrates the potential for a patient to directly contaminate the hospital environment.

Once in the environment, MDRO must be transferred to other patients. One mechanism of transmission may be through a vector, such as a HCW. Wilson et al. observed 120/585 (20.5%) patient and HCW interactions resulted in MDRO contamination of gloves and gowns.[38] PFGE was performed for isolates from 22 of these interactions; 18 HCW had strains related to patients and 20 had strains related to the environment, displaying the ability for MDRO transfer to HCW to occur. HCW workers who are carriers of MDRO can then transmit these pathogens to patients. Bertin et al. documented a MRSA outbreak among 37 neonates in a neonatal ICU caused by direct care of a HCW infected with the outbreak strain, confirmed by repetitive sequence PCR.[91] The outbreak began two months after the HCW started in the unit and no new cases were detected in the year following the HCW leaving the unit. Direct care from HCW can facilitate inoculation of the MDRO in a patient, which is required for colonization or infection.

Patients can also have direct contact with contaminated environments. Transfer of the MDRO through this contact and subsequent self-inoculation would be required for MDRO colonization or infection. Prior residence in a hospital room by an MDRO carrier can result in environmental contamination which can lead to transmission of MDRO through patient contact. Creamer et al. identified 92/929 (10%) of patients screened in two medical and two surgical wards at a tertiary hospital during two six-week periods were positive for MRSA.[42] Using epidemiological investigations and PFGE for isolate typing, they determined the source of eleven

MRSA transmission events among patients residing in the same ward bay within three weeks of each other. In six events, only patients were the source, in three cases, patients and the environment were the source, and in two cases, the environment was the source.[42] This study illustrates the potential of MDRO transmission to occur through the environment.

Other studies have identified prior room occupancy with an MDRO carrier increases the risk MDRO acquisition for subsequent inpatients residing in the same room, indicating MDRO contamination of the environment could affect patient outcomes.[92–94] While demonstrating direct transmission and acquisition of MDRO through the environment is not feasible experimentally, these studies reveal the mechanisms required for transmission to occur are possible. The variety of MDRO transmission routes in the environment demonstrate its viability as a focus for infection prevention and control actions such as environmental decontamination.

Several points of intervention have been targeted to reduce MDRO transmission and acquisition in healthcare facilities. Hand hygiene and contact precautions are important tools to prevent MDRO transmission but HCW are not always compliant and these methods of prevention do not address contamination in the patient's room from prior occupants.[92–95] Environmental decontamination has been shown to be effective at reducing the burden of MDRO infections in hospitals, especially when part of a broader effort.[96–98] High compliance with these practices can be effective at reducing the impact of environmental contamination, but compliance is time-consuming and logistically difficult and can often be sub-optimal.[99–101] Automated cleaning options are designed to cover all surfaces in a room, but are more expensive and are not always more effective than manual cleaning methods.[102] Further complicating disinfection practices, evidence suggests MDRO are developing resistance to commonly used cleaning agents.[103,104] These challenges influence decision making regarding environmental disinfection and require

efficient use of limited resources directed toward interventions which would have the greatest impact.

1.5: Risk Factors for Environmental Contamination by MDRO Carriers

Identification of patients at risk of contaminating their environment can aid in guiding resource allocation for environmental disinfection. Patients with MDRO infections may be identified through the course of clinical care. However, asymptomatic MDRO carriers may go unnoticed without screening. Numerous studies have identified risk factors of MDRO colonization in hospitalized patients who could all potentially shed MDRO into the environment..[105–108] However, determining if specific patient characteristics increase the risk of contaminating the environment has received less attention.[82,109–111]

Research determining who contaminates their surrounding environment in hospitals has been approached from several perspectives. Investigators have quantified the burden of shedding, by determining the bacterial load at the screening site or in bodily fluids, with the goal of detecting associations with contamination.[109,111] These studies aim to understand the role the amount of bacteria present in or on a host may have in environmental contamination. Other research have examined if the presence of MDRO on the body, including hand contamination and number of body sites colonized, is associated with contamination.[82,110] This research demonstrates MDRO presence on the skin, which interacts with the environment through physical contact, provides a mechanism for contamination by patients. Investigators have also explored other routes for MDRO to contaminate the environment. MDRO detected in bodily fluids, including diarrhea and urine, could result in MDRO excreted from the body into the environment without skin contact and have been examined in relation to contamination.[109,111,112] Wounds may present another route of

contamination, either transmitting MDRO to the environment through direct contact or by fluid secretions, and have been implicated in environmental transmission.[112]

Prior investigations have been limited regarding patient characteristics which may influence behaviors, such as how they physically interact with the environment, their ability to maintain personal hygiene or coughing during respiratory illness, which could facilitate environmental contamination by a patient. Other factors such as the presence of indwelling devices or incontinence could provide routes for MDRO excretion from the body. Factors influencing the bacterial load during shedding, including antibiotic use, may influence contamination as well.[113,114] With the potential for many variables to impact environmental contamination by a patient, an investigation of the association between patient characteristics and contamination would be worthwhile. Characteristics that can be determined during standard clinical care may aid in detecting patients more likely to contaminate their environment without additional testing. Identifying these factors may also elucidate mechanisms for contamination, allowing for the development of infection prevention strategies. The objective of aim 2 was to use patient characteristics to identify a patient type more likely to contaminate their environment with the goal of aiding resource allocation for environmental decontamination and infection prevention in hospitals.

1.6: MDRO in Young Children

Children are an understudied population with regards to MDRO, especially in community settings. However, evidence suggests this is a growing problem, as exemplified with MRSA. Investigators in Argentina reported 45% (99/221) of pediatric, community-associated (CA) *S. aureus* pneumonia from three children's hospitals during 2007-2008 were caused by MRSA, a significant increase over the prior two years.[115] The median age for CA-MRSA infections was 6.1

years old. The rate and prevalence of these infections increased significantly compared to the prior two years. Purcell and Fergie conducted fourteen-year study at Driscoll Children's hospital and identified 1002 pediatric infections with MRSA, 92.6% being CA-MRSA.[116] The proportion of all *S. aureus* isolates that were methicillin-resistant increased from 2.9%-10.6% in the first 10 years of the study and dramatically increased in the final four years, ranging from 19.0%-62.4%. These studies coincided with the emergence of CA-MRSA, which began in the late 1990's in the U.S., with the USA300 strain becoming the dominant strain in the community.[117] The increase in CA-MRSA among children in these studies demonstrates that young children were not exempt from the overall emergence of CA-MRSA.

Extended spectrum β-lactamase (ESBL)-producing Enterobacteriaceae present another developing problem in the community. Dayan et al. conducted a six-year study at a secondary-care Israeli medical center and identified 37 pediatric cases of CA-urinary tract infections (UTI) caused by ESBL producers (3.2% of all CA-UTI).[118] A significant increase in the proportion of ESBL-producing isolates was observed during the study (1.2% in year one vs. 5.8% in year six; p for trend=0.28). Zhu et al. identified 111 cases of ESBL-producers causing community acquired-UTI at Children's Hospital of Michigan over a five-year period, with a median age of four years old.[119] These studies demonstrate the potential burden of infections caused by ESBL-producers in the community, including for young children. Finding targets for intervention to disrupt MDRO acquisition by children in the community will help alleviate MDRO emergence in this population.

1.7: MDRO Environmental Contamination in Child Care

Child care centers provide a setting for pathogen transmission between children, where attendees are in close contact and share environments. Young children also display behaviors which may facilitate pathogen transmission, such as interacting with the environment

with their hands and mouthing of fomites. Child care attendees who carry MDRO may transmit these pathogens through direct contact with other children or through contamination of their environment. Stoesser et al. detected ESBL-producers in 92 (23%) screened children from 12 child care centers in Laos.[120] In several recent screening studies of child care attendees, MRSA was detected in 0.53%-7.4% of children.[121–124] In one of these studies, seven children (6.7%), one employee (3.1%), six household members of attendees and employees (35%) and four environmental samples (2.0%) screened positive for MRSA in a North Carolina child care center.[123] Ten isolates, one from an employee, three from the employee's child, two from attendees, and four from environmental sites were indistinguishable or highly similar by DiversiLab microbial genotyping system for strain typing. Three other isolates from two children were similar to each other and were different from all other isolates. These highly related isolates demonstrate the potential for transmission within the child care center, including through the environment, and within households of attendees and employees. Infection control programs including environmental cleaning and disinfection have reduced illnesses among child care attendees.[125,126] National recommendations for environmental decontamination, provided through a joint effort by the American Academy of Pediatrics, the American Public Health Association, and the National Resource Center for Health and Safety in Child Care and Early Education, are comprehensive.[127] However, local regulatory agencies may be the primary source for cleaning and disinfection regulations. In Michigan, the Licensing and Regulatory Affairs (LARA) branch of the Michigan state government regulates CCC's and provides these guidelines, which are less comprehensive than the national recommendations.[128] LARA does not provide guidance for decontamination of many items such as toys, floors, toilets, cabinets or water tables. Inconsistent guidelines from these agencies can result in suboptimal compliance with best practices for

environmental decontamiantion.[129] Staff of child care centers have other responsibilities including supervision, education, food preparation and child hygiene, which may limit time dedicated to cleaning and disinfection, impacting compliance as well.

Surveillance of environmental contamination in CCC's can guide environmental cleaning and disinfection policies when time and resources are limited. Much of the previously conducted research of environmental contamination in CCC's assesses viral contamination.[130–133] Other research has focused on fecal coliforms, although this may be of less relevance for centers with children aged out of diapers or when no outbreaks of diarrheal disease are present.[134–141] Food preparation areas have also received attention from researchers, but these surfaces likely have limited contact with attendees.[142,143] MDRO contamination surveillance in child care centers has been sparse.[144] Longitudinal surveillance of CCC environments, sampling from a large variety of fomites and targeting a range of pathogens, including viral and antibiotic-resistant, would provide the best guidance for environmental cleaning and disinfection strategies in these settings. The objective of aim 3 was to characterize environmental sites with high bioburden and high frequency of contamination with antibiotic-resistant and viral pathogens in a CCC with preschool age children to direct infection prevention policies. We also aimed to demonstrate the feasibility of such a study for application in larger scale studies in the future.

1.8: Specific Aims and Hypotheses

The overarching objective of this dissertation is to investigate MDRO acquisition and transmission. First, the risk factors of MDRO acquisition in the context of multiple organism infections were investigated in aim 1. Secondly, mechanisms of MDRO transmission in the environment were explored in aims 2 and 3. We investigated who is at risk for contaminating the

environment in hospitals in aim 2. Finally, we examined patterns of MDRO contamination in the environment in child care.

Aim 1: Characterize the epidemiology of co-colonization or coinfection (CCCI) with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) among hospitalized patients.

Sub-Aim 1: Identify clinical and demographic characteristics at the individual level that are risk factors of MRSA and VRE CCCI among hospitalized patients

Hypothesis: Individual characteristics which were identified in previous MRSA and VRE CCCI studies, including prior hospitalizations, previous antibiotic use and the presence of indwelling medical devices, and those observed in prior VRSA infections, including diagnosis of diabetes, will be associated with MRSA and VRE CCCI.

Sub-Aim 2: Identify the molecular characteristics of MRSA among hospitalized patients colonized or infected with MRSA that are risk factors of MRSA and VRE CCCI

Hypothesis: Clonal complex 5 MRSA isolates and Panton-Valentine leukocidin-negative MRSA isolates will also be associated with MRSA and VRE CCCI, reflecting the characteristics seen in previous VRSA infection isolates.

Main Findings: Healthcare exposure was a risk factor for MRSA and VRE CCCI. Other previously identified MDRO risk factors were also risk factors for MRSA and VRE CCCI including antibiotic use, indwelling devices, diabetes and chronic skin wounds.

Aim 2: Identify characteristics among hospitalized MDRO colonized patients which allow for the classification of a type of MDRO carrier at increased risk for contaminating their environment.

Hypothesis: Risk factors for environmental contamination by MDRO carriers will be similar to those for prolonged shedding including the presence of severe comorbidities, previous antibiotic use, the presence of indwelling medical devices and prior residence in long-term care facility.

Main Findings: Patients with low functional status contaminated their environment more frequently than high functional status patients.

Aim 3: Use longitudinal surveillance of the environment of a CCC for preschool aged children to characterize the bioburden on fomites and the frequency of contamination with MDRO and viral pathogens to identify points intervention for cleaning and disinfection practices.

Hypothesis: Our study methodology will prove to be feasible for sampling the environment for overall bioburden and bacterial and viral pathogens. Fomites cleaned less frequently or thoroughly will be contaminated with a higher bioburden. These fomites will also be contaminated with viral and bacterial pathogens more frequently. Irregular surfaces or surfaces which may be damaged during cleaning or disinfection are fomite characteristics which limit the thoroughness of cleaning and disinfection.

Main Findings: Sites where children washed and played with water were among the most contaminated. Additionally, sites that were difficult to clean or were cleaned less frequently were also among the most contaminated.

Chapter 2: Epidemiology of Methicillin-Resistant *Staphylococcus aureus* and Vancomycin-Resistant Enterococci Co-colonization and Coinfection

2.1: Author Summary

Vancomycin-resistant *Staphylococcus aureus* (VRSA) is likely preceded by co-colonization or coinfection (CCCI) with methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). We identified admission from another healthcare facility to be a risk factor for MRSA and VRE CCCI in two of our three primary analyses, suggesting healthcare exposure is a risk factor for the acquisition pathogens of concurrently. We also observed patients with Panton-Valentine leukocidin-negative MRSA isolates and clonal complex 5 MRSA isolates to have an increased risk of MRSA and VRE CCCI. These isolates are typically associated with healthcare-associated infections, providing further evidence healthcare exposure is a risk factor for CCCI.

2.2: Abstract

Methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE) are common in healthcare settings. MRSA resistance to vancomycin, a first-line treatment, can independently arise through horizontal gene transfer with VRE and may arise independently in individuals co-colonized or coinfected with both organisms. The objective of this study was to identify risk factors for MRSA and VRE co-colonization or coinfection (CCCI) among hospitalized adults.

A 1:1 matched case control study was conducted at the five Detroit Medical Center inpatient hospitals from January 2012 to April 2016. Cases were prospectively identified and

obtained from hospitalized CCCI patients and compared to three separate inpatient control groups: 1) MRSA infection only 2) VRE infection only and 3) inpatients without MRSA or VRE. We used multivariable logistic regression to evaluate risk factors for CCCI.

A total of 134 CCCI cases, 109 MRSA controls, 88 VRE controls and 99 controls without MRSA or VRE were included in the analysis. Admission from another healthcare facility was significantly associated with CCCI in comparisons with MRSA controls [OR = 3.09 (1.45, 6.61)] and controls without MRSA or VRE [OR = 3.80 (1.62, 8.94)]. When restricting to cases and MRSA-only controls, carriers of either Panton-Valentine leukocidin-negative MRSA isolates [OR = 0.09 (0.01, 0.74)] or clonal complex 5 isolates [OR = 5.61 (1.49, 21.15)] were more likely to be co-colonized or co-infected with VRE.

Admission from either a long-term care facility or another hospital was found to be a risk factor for CCCI, suggesting that healthcare exposures, a known risk factor of antibiotic-resistant bacteria acquisition, may increase the risk of CCCI. Targeted interventions aimed at this high-risk population may aid infection prevention and control efforts surrounding CCCI and VRSA.

2.3: Introduction

Vancomycin-resistant enterococci (VRE) and methicillin-resistant *Staphylococcus aureus* (MRSA) are among the most common healthcare-associated antibiotic resistant pathogens [145]. Vancomycin is a first-line treatment for MRSA infections and the development of vancomycin resistance in MRSA further complicates treatment for this pathogen. The first case of vancomycin-resistant *S. aureus* (VRSA) infection occurred in southeast Michigan in 2002, and eight of the fourteen U.S. cases have been identified in this region.[65,146,147] VRSA has also been identified worldwide in clinical isolates from Brazil, Portugal, India, Iran.[67–69,72]

One proposed mechanism for the acquisition of vancomycin resistance by *S. aureus*, which has been demonstrated *in vitro* using *E. faecalis*, involves the conjugative transfer of a vancomycin resistance gene complex from a VRE donor to a MRSA recipient, requiring carriage of both organisms in the same host.[147–149] In the U.S., no documented person-to-person transmission events of VRSA have occurred.[65,147,148] The first seven VRSA cases occurred in individuals with a history of MRSA and *Enterococcus* (including four VRE) infection or colonization and VRE was recovered from ten of the fourteen U.S. VRSA cases.[65,147,148] Sequencing of the twelve isolates from the first eleven VRSA cases indicated these isolates were independently acquired.[150] Sequencing demonstrated the first thirteen U.S. VRSA cases arose from MRSA parent lineages.[65,147,150] The totality of this evidence supports the hypothesis that co-colonization or coinfection (CCCI) with MRSA and VRE is likely to precede VRSA infections. Knowledge of the epidemiology of CCCI would provide insight into key populations for interventions to prevent the further emergence and dissemination of VRSA.

Several studies have investigated risk factors for CCCI but are hampered by small sample sizes and inconsistencies in study populations which limit generalizability and comparability.[151–158] In this study, we compare a single case group to multiple comparison groups to investigate the various unique pathways through which CCCI may arise. An investigation of MRSA and VRE molecular characteristics as potential CCCI risk factors, including those identified in patients with VRSA infections, was conducted to further enhance knowledge of the relationship between CCCI and VRSA infection.[150,159] Using these strategies, this study aims to identify CCCI risk factors in hospitalized patients.

2.4: Methods

2.4.1: Study Design and Participants

Data were obtained from a prospective, case control study which included individuals, at least 18 years of age, who received clinical care at one of the five Detroit Medical Center (DMC) inpatient facilities from January 2012 to April 2016. Eligible cases were prospectively identified and defined as hospitalized patients with clinical identification of MRSA and VRE isolated from infected body sites within seven days of one another. Individuals may become co-colonized or coinfected through three pathways, by acquiring either organism first with a subsequent acquisition of the second organism or by acquiring both organisms simultaneously. With this process in mind, cases were matched 1:1 with a control from each of three groups. Control group 1 included admitted individuals with MRSA infection only. Control group 2 included admitted patients with VRE infection only. These controls were matched to cases by admitting hospital, hospital unit at time of culture, index infection site and hospital length of stay at time of culture (</≥ 72 hours); the last criterion was subsequently relaxed during the study period to increase the number of eligible VRE controls. Control group 3 included admitted patients without MRSA or VRE infection, matched to cases by admitting hospital, unit at time of culture and hospital length of stay (control length of stay must have been no less than 48 hours shorter than the case length of stay at the time of the index culture). Additionally, admission for uninfected controls must have occurred within one month of the index culture date for the case. All control groups could include individuals with methicillin-sensitive *S. aureus* or vancomycin-sensitive *Enterococcus* infections.

2.4.2: Data Collection

Data were abstracted from participants' electronic medical records and included: demographic information (age, gender, race), medical history (comorbidities, antibiotic use,

procedure history, medication history, infection history, presence of indwelling devices, prior hospitalizations), admission information (length of stay, location of patient, level of consciousness at admission, residence prior to hospitalization), pathogen-related information (species identification, antibiotic susceptibility, date of culture), and variables necessary for the calculation of the Charlson comorbidity index score.[160]

2.4.3: Culture Collection and Processing

Cultures were obtained from all patients with a clinical suspicion of infection as assessed by the treating physician. All cultures were processed by the DMC clinical microbiology lab for organism identification and antibiotic susceptibility testing prior to delivery to the investigators for further analysis. Organisms were delivered on agar slants and were cultured onto tryptic soy agar (Neogen, Lansing, MI) at 37°C for 18-24 hours. Isolated colonies were used to create 1 McFarland inoculums in at least 2 mL of normal saline (0.9%). 800 µL of the McFarland inoculum was stored with 200 µL of 50% glycerol (Thermo Fisher Scientific, Waltham, MA) at -80°C.

2.4.4: Panton-Valentine Leukocidin (PVL) Identification

PVL genes were identified by PCR for MRSA isolates utilizing the Luk-PV-1 and Luk-PV-2 primers as previously described.[161] PCR was performed using the ProFlex PCR system (Applied Biosystems, Foster City, CA). A PVL-positive MRSA control, AIS 2006061, was used to ensure PCR success.

2.4.5: MRSA Multilocus Sequence Typing (MLST)

PCR of all seven housekeeping genes (*arcC*, *aroE*, *glp*, *gmk*, *pta*, *tpi* and *yqiL*) used for MLST was performed for MRSA isolates using the protocol and primers as previously described by Enright et al. except for the *tpi* forward primer (tpif 5′-GCATTAGCAGATTTAGGCGT-3′)

described in a separate study from Witte et al.[162,163] Sequencing was performed using Sanger sequencing at the University of Michigan Advanced Genomics Core.

2.4.6: VRE Virulence Genes

The presence of five VRE virulence genes, *asa1*, *gelE*, *hyl*, *esp*, and *cylA*, was investigated using a multiplex PCR.[164] Three VRE isolates with known status of the genes of interest were used as controls. All amplicons were visualized with a 1.5% agarose electrophoresis gel made with agarose (Invitrogen, Carlsbad, CA) 1X Tris-acetate-EDTA (Promega, Madison WI) and SYBR Safe DNA gel stain (Invitrogen, Carlsbad, CA).

2.4.7: Statistical Analysis

Covariates included in the analysis were selected *a priori* based on previous studies.[65,148,151–157,165] Unadjusted analysis comparing the case group with each control group was performed using conditional logistic regression. To identify risk factors of CCCI, three separate multivariable analyses, one with each control group, were implemented. All adjusted analyses were performed using conditional logistic regression with a stepwise, forward selection process ($\alpha \leq 0.05$ for inclusion in the model). Two secondary multivariable risk factor analyses comparing cases and MRSA infected controls were performed to assess any association between CCCI and either MRSA isolate sequence type or PVL status. The distribution of VRE virulence genes was assessed among cases and VRE controls. For individuals with multiple isolates of a specific genus, only the earliest identified isolate was considered for analyses including molecular characteristics of the microorganisms. As physical contact is likely required for the transfer of vancomycin resistance genes from VRE to MRSA, two unadjusted sensitivity analyses using conditional logistic regression were performed. First, cases with MRSA and VRE identified on the same day were compared to matched controls. Secondly, cases with MRSA and VRE identified in the same

specimen were compared to matched controls. All statistical analyses were carried out using SAS version 9.4 (SAS Institute, Cary, NC) except for the construction of the forest plot which was carried out using the forestplot package in R version 3.5.1 (R Foundation for Statistical Computing, Vienna, Austria).

2.5: Results

Of the 152 CCCI patients identified during the study period, 134 had no missing data and were matched to at least one patient from one of the control groups eligible for matching. These cases were included in the analysis (Figure 2-1). For matching to these 134 cases, 120 eligible MRSA controls, 96 eligible VRE controls and 109 eligible controls without MRSA and VRE. Were identified. Controls from each group were later excluded resulting in 109 cases matched to MRSA controls, 88 cases matched to VRE controls and 99 cases matched to controls without MRSA or VRE (Figure 2-1). Overall, the study population was predominantly black (82.6%) and male (56.1%) with a median age of 60 (Table 2-1).

2.5.1: Cases and MRSA-Only Controls

Compared to MRSA controls, cases were more often admitted from another healthcare facility and more likely to have reduced consciousness on admission, at least one prior hospitalization in the past year, recent antibiotic exposure, an indwelling medical device and a history of chronic skin wounds (Table 2-1). In the adjusted analysis, admission from another healthcare facility, having a prior hospitalization in the past year, having an indwelling medical device at the time of organism isolation and a history of chronic skin wounds significantly increased the odds of CCCI (Figure 2-2).

PVL status of 57 pairs of cases and controls was determined. Among these pairs, 19.3% of cases and 43.9% of controls were PVL-positive. A restricted, multivariable analysis revealed that

the presence of PVL genes in MRSA isolates was significantly associated with reduced odds of CCCI (Table 2-2).

Among the cases and MRSA controls included in the main analysis, the sequence types (ST) for 157 MRSA isolates were determined (Figure 2-3). Four sequence types (ST8, ST450, ST683 and ST770) belonged to clonal complex (CC) 8, four were members of CC5 (ST5, ST100, ST105 and ST1863) and the remaining two, ST30 and ST278, were singletons. Analysis of the relationship between MRSA isolate ST and CCCI was restricted to cases and controls carrying CC5 or CC8 isolates, resulting in 58 matched pairs (Figure 2-3). In a matched multivariable analysis restricted to these 58 pairs, CC5 MRSA isolates were associated with CCCI (Table 2-4). Of the 82 cases with MRSA and VRE isolated on the same day, 68 were matched to MRSA controls. All significant associations between patient characteristics and CCCI status in the main unadjusted analysis were also significant in this sensitivity analysis (Table 2-5). Fifty of 63 cases with MRSA and VRE isolated from the same specimen were matched to MRSA controls. Three of the six significant associations observed in the main unadjusted analysis were also identified in this sensitivity analysis. (Table 2-6).

2.5.2: Cases and VRE-Only Controls

Cases were more likely than VRE controls to have a prior hospitalization in the past year and a previous MRSA infection in the past year (Table 2-2). A previous MRSA infection in the past year was significantly associated with CCCI in the multivariable analysis (Figure 2-2).

The status of *asa1*, *gelE*, and *hyl* was determined for 22 pairs of cases and VRE controls; *esp*, and *cylA* status was determined for 19 pairs (Table 2-7). No isolates were positive for *hyl* while *cylA* was rare. A majority of isolates were positive for *asa1* and *gelE* while *esp* was identified

in approximately one third of isolates. Due to small sample sizes, differences in the frequency of these genes between cases and controls was not assessed.

Fifty-four of the 82 cases with MRSA and VRE identified on the same day were matched to VRE controls. No significant associations between the patient characteristics and CCCI were identified (Table 2-5). Among the 63 cases with MRSA and VRE isolated in the same specimen, 42 were matched to VRE controls. No difference was identified between cases and controls across all other covariates (Table 2-6).

2.5.3: Cases and Controls Without MRSA or VRE

Cases were more likely than controls without MRSA or VRE to be admitted from another healthcare facility and to have at least one hospitalization in the prior year, recent antibiotic exposure, a MRSA infection in the past year, an indwelling medical device, renal insufficiency, a history of chronic skin wounds, steroid exposure in the past three months and a Charlson comorbidity index score of at least five (Table 2-2). In the adjusted model, admission from another healthcare facility, recent antibiotic exposure and diabetes significantly increased the odds of CCCI (Figure 2-2).

Sixty-one of the 82 cases with MRSA and VRE isolated on the same day were matched to controls without MRSA or VRE. All significant associations observed in the main unadjusted analysis were also identified in this sensitivity analysis except for the association between Charlson comorbidity index score and CCCI status (Table 2-5). An additional significant association between race and CCCI status was also detected in this sensitivity analysis. Of the 63 cases with MRSA and VRE isolated in the same specimen, 45 were matched to controls without MRSA or VRE. All significant associations found in the main unadjusted analysis between the participant

characteristics and CCCI status were also observed in this sensitivity analysis excluding the association of renal insufficiency and CCCI status (Table 2-6).

2.6: Discussion

Our findings indicate healthcare exposure is a risk factor for MRSA and VRE CCCI. One potential risk factor for CCCI, admission from another healthcare facility, was identified among hospitalized patients when comparing CCCI patients to patients with MRSA only or those without MRSA or VRE. Furthermore, among persons colonized or infected with MRSA, those with PVL-negative MRSA isolates and those with CC5 MRSA isolates are more likely to be co-colonized or coinfected with VRE, providing more support healthcare exposure is a CCCI risk factor. We also identified several other risk for CCCI which have been observed in previous CCCI studies as well, including skin wounds, antibiotic use, indwelling devices, previous MRSA infection and diabetes.[151–158]

CCCI may develop through multiple pathways, including sequential or simultaneous pathogen acquisition, which have largely not been compared in prior research but was addressed in our study by comparing our cases to three different control groups.[151–157] Similar risk factors were observed when comparing cases with both MRSA controls and controls without MRSA and VRE. Admission from another healthcare facility was a risk factor in both groups. Diabetes was a risk factor for CCCI when comparing cases with controls without MRSA and VRE. Chronic skin wounds are common in diabetes patients and was a risk factor for CCCI when comparing cases with MRSA controls.[166] When comparing cases to VRE controls, a previous MRSA infection in the past year was the only risk factor for CCCI. These patients were likely to receive antibiotics to treat their infection. Antibiotic use was a CCCI risk factor when comparing to controls without MRSA and VRE, demonstrating some similarity of risk factors between these groups.

Individuals admitted to hospitals from other healthcare facilities may have exposure to MRSA and VRE as both organisms are among the most common healthcare associated pathogens.[58] In their study of long-term care facility (LTCF) residents, Flannery et al. similarly compared CCCI individuals to three control groups.[157] While they did not explore any association between prior residence and CCCI status, they found no association between long duration of LTCF stays (admissions longer than 90 days) and CCCI, although their investigation included just 17 CCCI individuals.[157] The presence of skin wounds and antibiotic exposure were identified as risk factors in two of their three analyses, supporting our findings.[157] Both MRSA and VRE are commonly isolated from chronic skin wounds, providing a rationale for an association between these wounds and CCCI.[167]

LTCF may also serve as a reservoir for CCCI individuals. Flannery et al. observed a MRSA and VRE co-colonization incidence rate of 2.4/100 resident months in one LTCF.[157] Researchers in a second LTCF study found the prevalence of MRSA and VRE co-colonization to be 8.7% among LTCF residents.[158] Once admitted to the hospital, LTCF residents may be contributing to the burden of CCCI in hospitals, partially explaining the association with CCCI and admission from another healthcare facility.

Our findings garner mixed support from previous literature. Similar to our findings, Warren et al. identified prior admission from an LTCF and prior hospitalizations as CCCI risk factors.[151] Han et al. also observed prior hospitalizations, as well as urinary catheterization, to be CCCI risk factors.[154] Other studies also identified invasive medical devices as risk factors.[153,156,158] Multiple studies found antibiotic use to be associated with CCCI, further supporting our results.[152,155,158] Heinze et al identified diabetes and wounds as risk factors for CCCI, providing additional support

for our findings. Diabetes was also present in 10 of the 14 U.S. VRSA cases, indicating a potential association with VRSA.[65,165]

Our prospective study had other advantages over previous CCCI research: 1) a larger sample of CCCI participants 2) no restrictions on patient types 3) no restrictions on *Enterococcus* species and 4) pathogen molecular analysis. Inconsistencies between the CCCI risk factors identified in our study and those observed by other researchers, may be due to differing study populations. Variations in the types of patients (hospital-wide vs. ICU only) and the carrier status of MRSA and VRE among study participants may limit comparability across studies.[151–156] Additionally, our study included patients from five inpatient facilities and accounts for the multiple pathways by which a person might become co-colonized or coinfected with VRE and MRSA through the inclusion of analyses with multiple comparison groups.

The majority of CCCI risk factor investigations have lacked molecular analyses. In our study, PVL-negative isolates were associated with CCCI. PVL-positive MRSA isolates have been associated with skin and soft tissue MRSA infections and community-associated MRSA strains.[168,169] PVL genes have been markedly absent from VRSA clinical isolates including all 13 U.S. isolates tested by Saravolatz et al. in 2012 and four of five isolates in two Iranian studies.[71,159,170] Boan et al. have previously reported PVL-positive MRSA isolates to be associated with mono-microbial infections, providing further support that these isolates are less likely to be found cohabitating with VRE.[171] In contrast, Shettigar et al. identified an association between PVL positivity and multiple organism infections in diabetic foot ulcers, although this association was only observed when other virulence factors were absent in the MRSA isolate.[172] Further research is needed to determine the impact of PVL and other virulence factors regarding the risk for CCCI and VRSA.

Our analysis also identified an association between CC5 MRSA isolates and CCCI. Twelve isolates (including two from one individual) from the first eleven U.S. VRSA cases belonged to CC5, although CC8 *S. aureus* isolates with a fully vancomycin-resistant phenotype have been observed.[67,147,150] CC5 MRSA isolates are associated with healthcare-associated infections while CC8 isolates are more often identified as community isolates.[173] CC5 isolates are also associated with more severe disease but have not been observed to cause more persistent infections.[174,175] Coupled with the PVL analysis, these findings suggest CCCI may be more likely to occur among individuals carrying healthcare-associated MRSA strains.

An additional consideration for MRSA and VRE CCCI is biofilm formation. Biofilms are polymicrobial communities which are a common cause of infections.[176] Bacteria in biofilms engage in synergistic activities, through extracellular excretion of molecules or gene transfer (including antibiotic resistance genes), which could affect health outcomes, including affecting response to treatment.[177–180] Resistance gene transfer in biofilms may have implications for VRSA development. Both MRSA and VRE are able to exist in biofilms and were both isolated from a nephrostomy tube biofilm along with VRSA in a U.S. VRSA case.[181–183] In our study, 63 cases had MRSA and VRE isolated in the same specimen, indicating the potential for biofilm formation with both isolates. Biofilms are common in infections involving indwelling devices and chronic skin wounds.[184,185] In our unadjusted analyses restricted to cases with MRSA and VRE isolated in the same specimen, indwelling devices and a history of chronic skin wounds were significantly associated with CCCI when comparing to MRSA controls and controls without MRSA or VRE. Both factors were also significantly associated with CCCI in the main adjusted analysis when comparing all cases to MRSA controls. Biofilms may be influencing the development of MRSA and VRE CCCI and could provide a setting for gene transfer to occur for the development of

VRSA.Our study is limited by our inability to evaluate other molecular elements purported to drive VRSA emergence, including the Inc18-like plasmid found in enterococci and pSK41-like plasmid of *S. aureus* which have been hypothesized to facilitate conjugation between the two pathogens.[149,186] VRSA isolates have been noted to either maintain the enterococcal Inc18-like plasmid or to insert the vancomycin resistance genes into a host plasmid, mediated by the transposon Tn1546.[149,186] Previous research using the same source population during similar time periods as our study have found unexpectedly low prevalence of these markers, which may explain the decrease in VRSA emergence in the region since the last identified isolate from Michigan in 2009.[187,188]

Other limitations were present in our study as well. Our use of stepwise regression can result in inappropriate inclusion or exclusion of covariates from our regression model which can bias the estimate of regression coefficients.[189,190] We attempted to mitigate this problem by using prior knowledge of risk factors from previous CCCI and MDRO research to determine variable inclusion in the stepwise regression process.[44,65,148,151–157,165,191] Our use of multiple comparison groups comes with potential limitations as well. We cannot statistically compare findings between groups, but can do a qualitative assessment, such as identifying variables associated with CCCI in multiple groups. Restrictions among the controls may result in comparisons with control groups that do not completely reflect the source population (i.e., all patients without CCCI), which could result in selection bias. However, these exclusions are likely a small percentage of the source population which lessens their effect on our findings.

The potential risk factors of MRSA and VRE CCCI identified in this study demonstrate a connection between both healthcare exposure and the presence of other risk factors for multidrug-resistant organism acquisition with CCCI. Interventions such as timely and effective treatment of

wounds, appropriate treatment of previous infections, avoiding unnecessary antimicrobial therapy and improved management of health-related issues could reduce the time individuals are at risk for co-colonization or co-infection with MRSA and VRE. Using the best practices for infection prevention in hospitals including surveillance, contact precautions, limiting time of hospital admissions and avoiding inappropriate antibiotic therapy including for patients colonized with MRSA and VRE can facilitate control of MRSA and VRE CCCI acquisitions preventing further VRSA emergence. Coupling of infection control practices with VRSA surveillance among patients with risk factors MRSA and VRE CCCI are important to mitigate the continued emergence of VRSA.

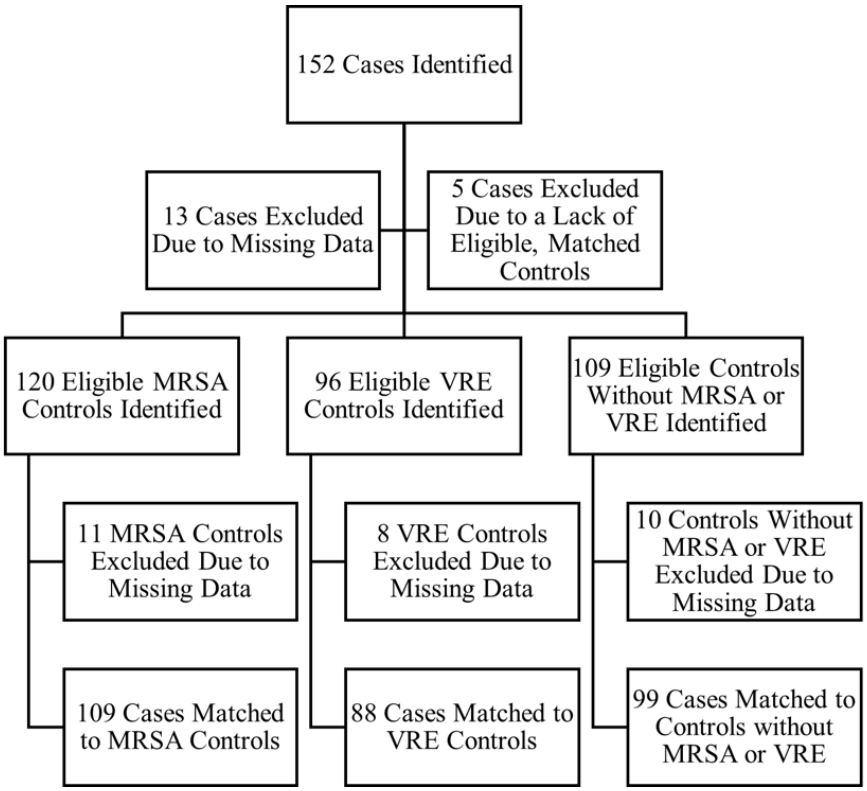

Figure 2-1: Participant Inclusion Workflow

Table 2-1: Distribution of characteristics by case and control group.

Characteristics	MRSA/VRE CCCI (N = 134)	MRSA Only (N = 109)	VRE Only (N = 88)	No MRSA or VRE (N = 99)
Age	61 (52, 74)	57 (48, 70)	61.5 (50, 70.5)	60 (51, 69)
Female	55 (41.0)	44 (40.4)	47 (53.4)	43 (43.4)
Black	107 (79.9)	94 (86.2)	67 (76.1)	87 (87.9)
Admission from Other Healthcare Facility	73 (54.5)	30 (27.5)	43 (48.9)	21 (21.2)
Altered Consciousness at Admission	53 (39.6)	27 (24.8)	31 (35.2)	25 (25.3)
>0 Prior Hospitalizations (Past Year)	119 (88.8)	69 (63.3)	70 (79.6)	55 (55.6)
Surgeries (Past 30 Days)	28 (20.9)	18 (16.5)	24 (27.3)	13 (13.1)
Antibiotics (Past 30 Days)	96 (71.6)	58 (53.2)	55 (62.5)	30 (30.3)
Previous MRSA Infection (Past Year)	31 (23.1)	21 (19.3)	10 (11.4)	3 (3.0)
Current Medical Devices	87 (64.9)	40 (36.7)	50 (56.8)	30 (30.3)
Diagnosis of Diabetes	64 (47.8)	54 (49.5)	44 (50.0)	33 (33.3)
Renal Insufficiency	37 (27.6)	30 (27.5)	30 (34.1)	14 (14.1)
History of Chronic Skin Wounds	76 (56.7)	30 (27.5)	49 (55.7)	9 (9.1)
Steroid Use (Past 3 Months)	32 (23.9)	25 (22.9)	22 (25.0)	14 (14.1)
Charlson Comorbidity Index Score				
0-2	55 (41.0)	58 (52.3)	31 (35.2)	54 (54.6)
3-4	45 (33.6)	27 (24.8)	29 (33.0)	31 (31.3)
≥5	34 (25.4)	25 (22.9)	28 (31.8)	14 (14.1)
***Enterococcus* Species**				
E. faecalis	76 (58.0)	NA	51 (60.0)	NA
E. faecium	54 (41.2)	NA	36 (40.9)	NA
E. raffinosus	1 (0.8)	NA	1 (1.1)	NA

Continuous data includes median (Q1, Q3).
Categorical data includes total (proportion).
All variables included in the table except for *Enterococcus* species were included in subsequent stepwise models for adjusted analyses unless otherwise noted.
Abbreviations: CCCI = Co-colonization or Coinfection; MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus*; NA = Not Applicable

Table 2-2: Unadjusted Analysis of Patient Characteristics and Co-Colonization or Coinfection Status

Characteristic	MRSA Only		VRE Only		No MRSA or VRE	
	Odds ratio (95% CI)[a]	p[a]	Odds ratio (95% CI)[a]	p[a]	Odds ratio (95% CI)[a]	p[a]
Age	1.02 (1.00, 1.03)	0.07	1.00 (0.99, 1.02)	0.62	1.00 (0.99, 1.02)	0.87
Female	1.08 (0.62, 1.89)	0.78	0.56 (0.29, 1.08)	0.08	0.93 (0.54, 1.60)	0.78
Black	0.72 (0.35, 1.47)	0.37	1.44 (0.62, 3.38)	0.40	0.44 (0.18, 1.06)	0.07
Admission from Other Healthcare Facility	3.46 (1.87, 6.42)	<0.0001	1.00 (0.51, 1.96)	1.00	4.56 (2.21, 9.37)	<0.0001
Altered Consciousness at Admission	2.39 (1.25, 4.56)	0.009	1.00 (0.50, 2.00)	1.00	1.75 (0.95, 3.23)	0.07
>0 Prior Hospitalizations (Past Year)	5.00 (2.22, 11.26)	0.0001	2.43 (1.01, 5.86)	0.05	4.88 (2.28, 10.43)	<0.0001
Surgeries (Past 30 Days)	1.31 (0.64, 2.69)	0.47	0.77 (0.37, 1.57)	0.47	1.60 (0.73, 3.53)	0.24
Antibiotics (Past 30 Days)	2.83 (1.47, 5.47)	0.002	1.29 (0.69, 2.44)	0.42	7.43 (3.38, 16.35)	<0.0001
Previous MRSA Infection (Past Year)	1.38 (0.72, 2.62)	0.33	2.83 (1.12, 7.19)	0.03	7.33 (2.20, 24.50)	0.001
Current Medical Devices	5.00 (2.34, 10.68)	<0.0001	1.69 (0.85, 3.36)	0.13	3.67 (1.94, 6.94)	<0.0001
Diagnosis of Diabetes	0.83 (0.45, 1.52)	0.54	1.00 (0.53, 1.89)	1.00	1.85 (1.07, 3.19)	0.03
Renal Insufficiency	1.00 (0.49, 2.05)	1.00	0.90 (0.47, 1.72)	0.74	2.17 (1.09, 4.29)	0.03
History of Chronic Skin Wounds	4.67 (2.27, 9.59)	<0.0001	1.27 (0.64, 2.49)	0.49	17.33 (5.41, 55.50)	<0.0001
Steroid Use (Past 3 Months)	1.13 (0.57, 2.21)	0.73	0.87 (0.41, 1.82)	0.71	2.09 (1.02, 4.29)	0.04
Charlson Comorbidity Index Score						
3-4	1.34 (0.76, 2.38)	0.31	0.99 (0.49, 2.00)	0.98	1.31 (0.68, 2.52)	0.41
≥5	1.53 (0.74, 3.17)	0.26	0.80 (0.36, 1.78)	0.58	2.38 (1.05, 5.39)	0.04

a. Odds ratios and p-values calculated using conditional logistic regression
Abbreviations: MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus*; CI = confidence interval

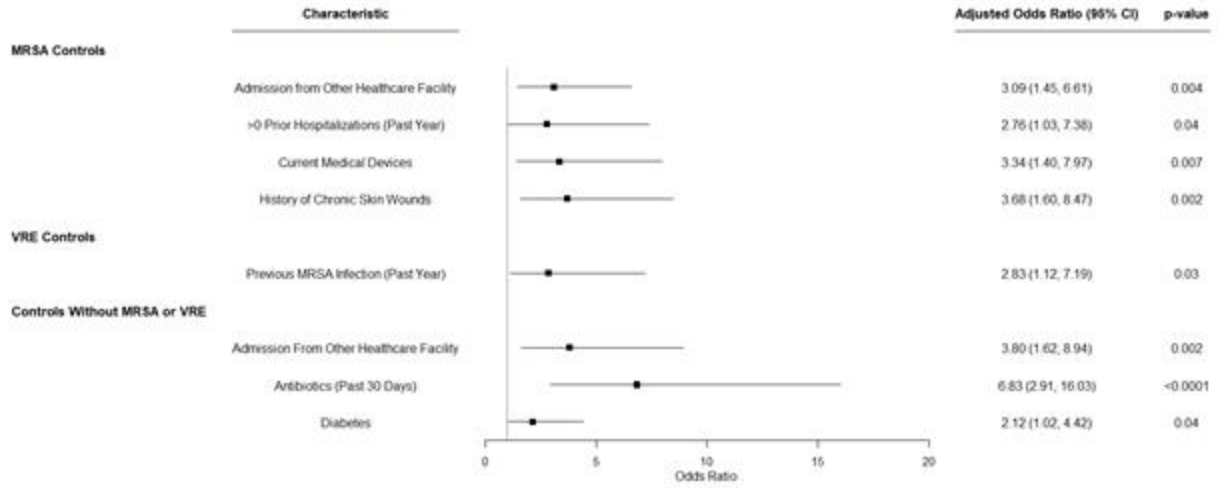

Figure 2-2: Forest Plot of Adjusted Analysis to Identify Risk Factors of Co-colonization or Coinfection.

Odds ratios and p-values calculated using conditional logistic regression. All variables included in the models are present in the figure.
Abbreviations: MRSA = methicillin-resistant *Staphylococcus aureus*; VRE = vancomycin-resistant *Enterococcus*; CI = confidence interval

Table 2-3: Adjusted analysis for the Association Between PVL Gene Presence and Co-colonization or Coinfection

Characteristic	Odds Ratio (95% CI)[a]	p[a]
PVL Gene Present	0.087 (0.01, 0.74)	0.03
Admission from Other Healthcare Facility	7.40 (1.55, 35.27)	0.01
Surgeries (Past 30 Days)	0.18 (0.03, 1.02)	0.053
Current Medical Devices	8.53 (1.41, 51.46)	0.02
History of Chronic Skin Wounds	8.79 (1.31, 58.95)	0.03

a. Odds ratios and p-values calculated using conditional logistic regression
Restricted to cases and matched methicillin-resistant *S. aureus* controls (**N=57**)
Race not included due to small cell sizes
Abbreviations: PVL = Panton-Valentine leukocidin; CI = confidence interval

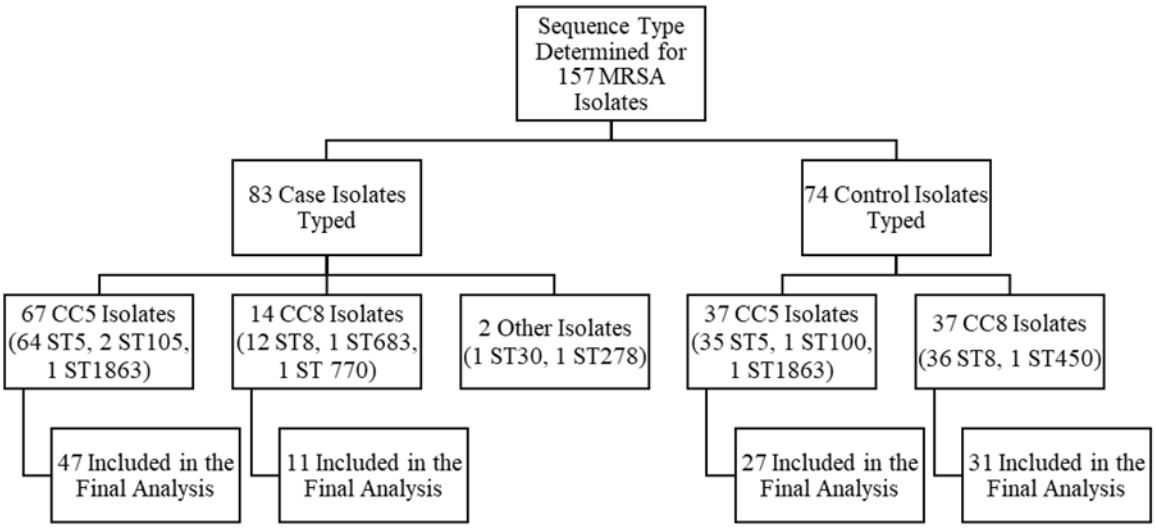

Figure 2-3: MRSA Isolate Sequence Type Flow Chart

Abbreviations: MRSA = Methicillin-Resistant *S. aureus*; CC = Clonal Complex; ST = Sequence Type

Table 2-4: Adjusted Analysis for the Association Between Clonal Complex and Co-colonization or Coinfection.

Characteristic	Odds Ratio (95% CI)[a]	p[a]
CC5	5.61 (1.49, 21.15)	0.01
Altered Consciousness at Admission	4.39 (1.30, 14.79)	0.02
History of Chronic Skin Wounds	8.80 (1.88, 41.14)	0.006

a. Odds Ratios and p-values calculated using conditional logistic regression
Restricted to cases and matched MRSA controls (N=58)
Race, prior hospitalizations and surgeries in the past 30 days not included due to small cell sizes
Abbreviations: CC = Clonal Complex; CI = Confidence Interval

Table 2-5: Unadjusted Analysis for Cases with MRSA and VRE Identified on the Same Day

Characteristic	MRSA Only (N=68)[a]		VRE Only (N=54)[a]		No MRSA or VRE (N=61)[a]	
	Odds ratio (95% CI)	p[a]	Odds ratio (95% CI)	p[a]	Odds ratio (95% CI)	p[a]
Age	1.01 (0.99, 1.04)	0.24	1.01 (0.99, 1.03)	0.41	1.01 (0.99, 1.03)	0.46
Female	1.33 (0.63, 2.82)	0.45	0.71 (0.32, 1.61)	0.42	1.14 (0.56, 2.34)	0.72
Black	0.60 (0.22, 1.65)	0.32	1.60 (0.52, 4.89)	0.41	0.25 (0.07, 0.89)	0.03
Admission from Other Healthcare Facility	2.36 (1.17, 4.78)	0.02	0.63 (0.25, 1.64)	0.35	3.83 (1.56, 9.41)	0.003
Altered Consciousness at Admission	5.25 (1.80, 15.29)	0.002	0.85 (0.38, 1.89)	0.68	2.00 (0.94, 4.27)	0.07
>0 Prior Hospitalizations (Past Year)	4.75 (1.62, 13.96)	0.005	1.80 (0.60, 5.37)	0.29	5.40 (2.08, 14.02)	0.0005
Surgeries (Past 30 Days)	0.82 (0.34, 1.97)	0.66	0.78 (0.29, 2.09)	0.62	1.80 (0.60, 5.37)	0.29
Antibiotics (Past 30 Days)	3.17 (1.27, 7.93)	0.01	1.44 (0.62, 3.38)	0.40	10.33 (3.16, 33.80)	0.0001
Previous MRSA Infection (Past Year)	0.85 (0.38, 1.89)	0.68	2.00 (0.69, 6.64)	0.26	7.00 (1.59, 30.80)	0.01
Current Medical Devices	3.29 (1.41, 7.66)	0.006	1.38 (0.55, 3.42)	0.49	4.00 (1.75, 9.16)	0.001
Diagnosis of Diabetes	0.50 (0.23, 1.11)	0.09	1.27 (0.58, 2.80)	0.55	2.18 (1.07, 4.54)	0.03
Renal Insufficiency	0.70 (2.67, 1.84)	0.47	0.75 (0.32, 1.78)	0.51	2.67 (1.04, 6.82)	0.04
History of Chronic Skin Wounds	4.80 (1.83, 12.58)	0.001	1.11 (0.45, 2.73)	0.82	17.50 (4.21, 72.76)	<0.001
Steroid Use (Past 3 Months)	1.08 (0.49, 2.37)	0.84	0.91 (0.39, 2.14)	0.83	3.20 (1.17, 8.74)	0.02
Charlson Comorbidity Index Score						
3-4	0.88 (0.43, 1.79)	0.72	0.86 (0.35, 2.14)	0.75	1.48 (0.66, 3.35)	0.34
≥5	0.94 (0.38, 2.32)	0.89	0.62 (0.20, 1.90)	0.41	2.40 (0.83, 6.91)	0.11

a. Odds ratios and p-values calculated using conditional logistic regression
Abbreviations: MRSA = Methicillin-Resistant *S. aureus*; VRE = Vancomycin-Resistant *Enterococcus*; CI = Confidence Interval

Table 2-6: Unadjusted Analysis for Cases with MRSA and VRE Identified in the Same Specimen

Characteristic	MRSA Only (N=50)		VRE Only (N=42)		No MRSA or VRE (N=45)	
	Odds ratio (95% CI)[a]	p[a]	Odds ratio (95% CI)[a]	p[a]	Odds ratio (95% CI)[a]	p[a]
Age	1.02 (0.99, 1.06)	0.14	1.02 (0.99, 1.05)	0.32	1.01 (0.98, 1.04)	0.45
Female	1.00 (0.40, 2.52)	1.00	1.00 (0.38, 2.67)	1.00	1.09 (0.48, 2.47)	0.84
Black	0.50 (0.15, 1.67)	0.26	1.25 (0.34, 4.66)	0.74	0.63 (0.20, 1.91)	0.41
Admission from Other Healthcare Facility	2.25 (0.98, 5.18)	0.06	0.60 (0.22, 1.65)	0.32	2.80 (1.01, 7.77)	0.048
Altered Consciousness at Admission	2.60 (0.93, 7.29)	0.07	0.63 (0.20, 1.91)	0.41	1.57 (0.61, 4.05)	0.35
>0 Prior Hospitalizations (Past Year)	16.00 (2.12, 120.65)	0.007	NA[b]	NA	12.50 (2.96, 52.77)	0.0006
Surgeries (Past 30 Days)	1.60 (0.52, 4.89)	0.41	0.70 (0.27, 1.84)	0.47	2.00 (0.60, 6.64)	0.26
Antibiotics (Past 30 Days)	3.50 (1.15, 10.63)	0.28	1.14 (0.41, 3.15)	0.80	12.50 (2.96, 52.77)	0.0006
Previous MRSA Infection (Past Year)	0.50 (0.19, 1.33)	0.17	2.50 (0.49, 12.89)	0.27	8.00 (1.00, 63.96)	0.0499
Current Medical Devices	2.67 (1.04, 6.82)	0.04	1.00 (0.35, 2.85)	1.00	2.43 (1.01, 5.86)	0.048
Diagnosis of Diabetes	0.42 (0.15, 1.18)	0.10	1.67 (0.61, 4.59)	0.32	4.00 (1.50, 10.66)	0.006
Renal Insufficiency	0.70 (0.27, 1.84)	0.47	0.67 (0.24, 1.87)	0.44	2.60 (0.93, 7.29)	0.07
History of Chronic Skin Wounds	4.50 (1.52, 13.30)	0.007	1.50 (0.53, 4.21)	0.44	25.00 (3.39, 184.50)	0.002
Steroid Use (Past 3 Months)	1.00 (0.40, 2.52)	1.00	1.00 (0.35, 2.85)	1.00	4.33 (1.24, 15.21)	0.02
Charlson Comorbidity Index Score						
3-4	1.05 (0.43, 2.60)	0.91	0.86 (0.31, 2.42)	0.77	1.17 (0.47, 2.93)	0.74
≥5	1.17 (0.39, 3.51)	0.78	0.54 (0.15, 1.96)	0.35	3.93 (1.03, 15.04)	0.046

a. Odds ratios and p-values calculated using conditional logistic regression
b. Analysis not performed due to small cell sizes
Abbreviations: MRSA = Methicillin-Resistant *S. aureus*; VRE = Vancomycin-Resistant *Enterococcus*; CI = Confidence Interval; NA = Not Applicable

Table 2-7: Prevalence of VRE Virulence Genes Among Cases and VRE Controls

Gene	MRSA/VRE CCCI (%)	VRE Only (%)
***asa1* (N=22)**	20 (90.9)	15 (68.2)
***gelE* (N=22)**	20 (90.9)	16 (72.7)
***hyl* (N=22)**	0 (0)	0 (0)
***esp* (N=19)**	7 (36.8)	7 (36.8)
***cylA* (N=19)**	1 (5.3)	2 (10.5)

Abbreviations: MRSA = Methicillin-Resistant *S. aureus*; VRE = vancomycin-resistant *Enterococcus*; CCCI = Co-colonization or Coinfection

Chapter 3: The Role of Patient Functional Status in Environmental Contamination in the Hospital

3.1: Author Summary

Identifying patients at risk of contaminating their environment with multidrug-resistant organisms (MDRO) can guide infection prevention and control programs. We input patient characteristics into latent class analysis to classify patients based on functional status. We observed low functional status (LFS) patients contaminated their environment more frequently than high functional status patients. LFS patients may benefit from increased MDRO screening and more resources dedicated to patient hygiene and environmental cleaning and disinfection.

3.2: Abstract

Carriers of multidrug-resistant organisms (MDRO) can shed these organisms into the environment which can lead to pathogen transmission, especially in hospitals. Research assessing who contaminates their environment has focused on the measurement of bacterial load or presence at body sites but has been limited with regards to other characteristics of MDRO carriers which can affect bacterial load or a patients ability to interact with their environment, such as patient functioning, use of indwelling devices and antibiotic exposure. Detecting patients at increased risk of contaminating their environment may benefit decision-making for infection prevention and environmental decontamination policies.

We used data collected during a prospective cleaning intervention study at Shamir Medical Center in Israel from patients admitted between October 2016 and January 2018. Patients were screened at admission and during admission to identify patients positive for MDRO colonization.

Environmental samples were collected at one time point from five high-touch surfaces in the patient room for any organism detected during screening. Patient characteristics were used for latent class analysis (LCA) to categorize patients and determine associations between patient type and both any environmental contamination and contamination of all sites.

During the study, environmental samples were collected at 262 sampling points from the rooms of 211 MDRO carriers. Contamination of at least one site was detected in 163 (62.2%) of the sampling points and of all five sites in 33 (12.6%) sampling points. Requiring assistance with activities of daily living, altered consciousness at admission, and mechanical ventilation during admission were used for LCA to classify patients based on functional status. Low functional status (LFS) patients had higher odds of contaminating at least one site for all organisms (69.8% vs. 52.2%; OR=2.2, 95% CI=[1.3, 3.7]; p=0.003), a composite of gram-positive bacteria (39% vs. 18%; OR=2.4; 95% CI=(1.1, 5.4); p=0.03), MRSA (OR=2.3; 95% CI=[1.0, 5.2]; p=0.046) and a composite of gram-negative bacteria (74.7% vs. 59.4%; OR=2.0; 95% CI=[1.0, 3.9]; p=0.04). LFS patients also contaminated all sites more frequently for all organisms (OR=2.5; 95% CI=[1.1, 5.8]; p=0.03) and gram-positive bacteria (OR=8.7; 95% CI=[1.0, 73.6]; p=0.048).

LFS patients colonized with MDRO contaminated their environment more frequently than high functional status patients. These patients may be a target for infection prevention strategies including MDRO screening, isolation precautions, patient hygiene and more frequent environmental cleaning of high-touch surfaces.

3.3: Introduction

Antibiotic-resistant organisms are responsible for over 2,800,000 infections and over 35,000 deaths each year in the United States.[4] Treatment options are limited for multi-drug resistant organisms (MDRO), necessitating strategies for infection prevention and control, such as

environmental decontamination, to prevent MDRO transmission through the environment. In healthcare settings, carriers of MDRO can contaminate the environment and healthcare workers (HCW) with MDRO.[38,40,192,193] This contamination has the potential to lead to MDRO transmission to other patients who subsequently occupy the same room or to patients in other areas through HCW and mobile equipment.[42,80,194] Strategies to reduce MDRO environmental contamination, including staff education, enhanced cleaning, use of markers to ensure adequacy of cleaning and patient isolation precautions, have been effective in reducing the burden of these pathogens.[97,195,196] Infection prevention control is costly and implementation of new programs redirects valuable time and resources from existing procedures. However, targeting these interventions toward programs which will be most beneficial for health outcomes of patients has been demonstrated to be cost-effective.[196,197]

To apply infection prevention and control programs toward MDRO carriers, they must first be identified. While carriers with symptomatic infections would be identified through the standard clinical course among hospitalized patients, asymptomatic patients may go unnoticed without screening. Screening for MDRO carriers is expensive but targeted screening can produce a cost-savings for hospitals.[198] Determining patient factors which could result in environmental contamination can guide screening practices. Research on these characteristics has suggested some potential factors, including MDRO in bodily fluids, patient hand colonization, the number of body sites colonized and microbial concentration at the colonization site.[82,109–111,199] These studies provide insight into the routes transmission of MDRO to the environment, such as through skin contact or from excreted bodily fluids. However, other factors may also contribute to environmental contamination. Physical ability to interact with the environment may influence the ability to contaminate one's surroundings. The presence of indwelling devices may provide routes

for contaminated bodily fluids to contaminate the environment. Antibiotics may alter bacterial load at the colonization site.[113] Studies analyzing a variety of patient traits which could be assessed at bedside or through the standard clinical care could allow for the identification of a type of patient more likely to contaminate the environment toward whom interventions could be directed.

Latent class analysis (LCA) allows the grouping of individuals into classes of unobserved (latent) characteristics based on the relationships of observed characteristics. We can classify patients using observed characteristics into categories which are not observed or cannot be determined with a single measurement. These categorizations can be used to identify a patient type at risk for contaminating the environment more frequently. LCA provides several advantages for the analysis. With limited prior knowledge regarding associations between individual characteristics and environmental contamination, LCA can be used to build a model of patient types including multiple characteristics without requiring any additional knowledge. Traditional regression models require prior knowledge for the inclusion of variables. Following the assignment of class membership, associations between class membership and dependent outcomes can be determined. Class membership can be included in traditional regression models, allowing for the adjustment of confounders or investigation of interaction and effect modification if necessary.

Previous research identified factors resulting in increased contamination which require additional testing (hand or body contamination, MDRO in bodily fluids and MDRO load at the colonization site). Characteristics which are observed without additional testing can provide information quickly and without additional cost to identify patients at increased risk of contaminating their environment. Infection prevention practices such as patient hygiene could then be directed toward these patients. We conducted a prospective cohort study using observable

patient characteristics to identify a hospitalized patient type among MDRO carriers who are more likely to contaminate their environment.

3.4: Methods

3.4.1: Study Setting and Data Collection

Data for this study were collected as part of a larger prospective investigation regarding the efficacy of Clinell Universal Wipes (intervention) vs. bleach at a concentration of 1,000 ppm (standard hospital practice) for environmental cleaning in a hospital setting. Data were collected for patients admitted from October 20, 2016 through January 22, 2018 in four internal medicine units at the Shamir Medical Center in Israel. The study was divided into five periods 1) a one-month pre-study period with bleach as the only cleaning agent used, 2) a six-month intervention period during which rooms in units A and D were cleaned with Clinell Universal Wipes and rooms in units B and C were cleaned with bleach, 3) a one-month washout period during which bleach was the only cleaning agent used, 4) a second six-month intervention period during which rooms in units B and C were cleaned with Clinell Universal Wipes and rooms in units A and D were cleaned with bleach ad 5) a one-month post-study periods during which bleach was the only cleaning agent used.

Data regarding patient demographics, medical history and comorbidities were collected. Admission surveillance screening (within 48 hours of admission) for MDRO was performed for patients transferred from another hospital, patients transferred from another department within the hospital, functionally dependent patients, residents of long-term care facilities, patients hospitalized in an acute care hospital in the prior six months and prisoners. Additionally, as part of the infection prevention program at the hospital, weekly MDRO screening was performed for ten patients at high-risk for infection as identified in previous research, including antibiotic

exposure, presence of indwelling devices, intensive care unit admission, and being bedridden.[200,201] Weekly screening of sputum was performed for all mechanically ventilated patients. Based on risk factors for organism acquisition, patients were screened for at least one, but not necessarily all, of the following multidrug-resistant organisms: 1) nasal screening for methicillin-resistant *Staphylococcus aureus*, (MRSA) 2) rectal screening for carbapenemase-producing carbapenem-resistant Enterobacteriaceae (CP-CRE), non-carbapenemase-producing carbapenem-resistant Enterobacteriaceae (non-CP-CRE) and vancomycin-resistant *Enterococcus* (VRE) and 3) sputum screening of mechanically ventilated patients for multi-drug resistant *Acinetobacter baumannii* and *Pseudomonas aeruginosa*. MDRO-positive patients were placed in contact precautions and moved, when possible, to a single-patient room, or more commonly, to a multi-patient room with physical barriers placed between patients.

Following a positive test, the environment was sampled at a single timepoint for any MDRO detected during patient screening; multiple samples were collected for patients positive for more than one MDRO meaning a patient could screen positive for different MDRO on different days and environmental sampling for those organisms could occur on different days as well. Five high-touch surfaces within the patient room were sampled (the right bedrail, the bedside table, underneath a binder with patient medical information hanging at the foot of the bed, the nurse's call button and the lamp switch; Figure 3-1). These surfaces were subject to enhanced cleaning with bleach at a concentration of 1,000 ppm or Clinell® Universal Wipes and were cleaned daily when an MDRO carrier was identified.

3.4.2: Statistical Analysis

In LCA, individuals are classified into two or more classes based on responses to items included in the analysis.[202] Classes can represent categories or characteristics of individuals not

otherwise measured. Two sets of class-level parameters are determined. Gamma is the probability that any individual will be a member of a class calculated as functions of logistic regression coefficients for inputted items. Rho is the response probability to each item given class membership. Parameters are estimated by maximum likelihood using expectation-maximization. Based on item responses for each covariate included in the analysis and the model parameters, posterior probabilities are generated, providing the probability an individual belonged to each class.

To determine which variables would be included in the LCA analysis, we first investigated the relationship between these variables and environmental contamination. In our study, patients with any surface contamination for an organism and patients who contaminated all environmental sites with an organism were identified. Unadjusted analyses were performed using generalized estimating equations (GEE) with exchangeable correlation and clustering on individuals to identify relationships between individual characteristics and two separate outcomes when compared to individuals with no contaminated sites in the environment: either contaminating any environmental site with an organism or contaminating all sites with an organism. Clustering was used as more than one set of environmental samples could be collected from a patient room at different times if the patient screened positive for more than one organism. All GEE models used throughout the analysis included an exchangeable correlation structure and clustering on individuals.

Any significant ($p<0.05$) variables in the unadjusted analyses were included as items in LCA to categorize patients into groups based on their potential to contaminate their environment. Remaining variables were added individually and various class numbers were tested. Up to four variables and four classes were tested at a time. The maximum class size equaled the number of items in the LCA model. Models were compared using Akaike Information Criterion (AIC) and

Bayesian Information Criterion (BIC) and the model with the best fit was chosen. Individuals were assigned to the class with the highest posterior probability for that individual.

Following the assignment of participants to latent classes, GEE models were used to identify associations between latent class membership and two outcomes 1) any environmental contamination with an organism vs. no contamination and 2) contamination all environmental sites with an organism vs. no contamination. These two analyses included all organisms. Because this data was collected during a cleaning intervention study, GEE models were used to compare any association between cleaning products used and latent class membership. Cleaning products were also included in separate models with latent class membership for both contamination outcomes to check for changes in effect measures and effect modification. No significant difference in environmental contamination was observed when between cleaning products in the prior research (unpublished data).

GEE models were used to identify associations between latent class membership and contamination at each individual site for both contamination outcomes. The same model was used to identify associations between latent class membership and both contamination of any environmental site and contamination of all environmental sites with a composite of gram-negative organisms. Logistic regression was used to calculate associations between latent class membership and both contamination outcomes for each organism separately and for a composite of gram-positive organisms.

Inconsistencies were present in the time between patient screening and environmental sampling. Decolonization may occur for patients with longer times between screening and environmental sampling which could affect our ability to detect environmental contamination. GEE models were used for a sensitivity analysis to determine if time between either first positive

screen date or latest positive screen date and environmental sample collection (≤5 vs. >5 days) affected the detection of contamination at any site with an organism or contamination of all sites with an organism when all organisms were included. All statistical analyses were performed using SAS version 9.4 (SAS Institute, Cary, NC) including the PROC LCA package (The Methodology Center, Penn State University College of Health and Human Development).[202]

3.5: Results

Data were collected for 9,362 patients during the study period; 211 patients screened positive for an MDRO and were in rooms where environmental samples were collected with no missing data for patient characteristics. The median age of the study population was 80 (interquartile range 71-86) and 123 (58.3%) were male. Out of the total population, 98, 8, 7, 13, 100 and 36 patients were positive for MRSA, VRE, CP-CRE, non-CP-CRE, *A. baumannii* and *P. aeruginosa* respectively; environmental samples were collected at 262 sampling points. Environmental samples were collected at two sampling points for 39 (18.5%) patients and three sampling points for six (2.8%) patients. One individual who was included in the analysis and screened positive for MRSA did not have environmental contamination data from the lamp switch or call button. The frequency of positive admission screens and the time between positive screening dates and environmental sampling dates is in Table 3-1. Time between environmental sampling points and latest positive screen collection date was greater than ten days for five (1.9%) sampling points. At least one environmental site was contaminated in 163 (62.2%) sampling points and all environmental sites were contaminated in 33 (12.6%) sampling points.

Three variables were significantly associated with environmental contamination at any site, requiring assistance with activities of daily living prior to admission, altered consciousness at admission and requiring mechanical ventilation during the admission (Table 3-2). Altered

consciousness at admission was also associated with contamination of all sites (Table 3-3). These variables were included in the latent class analysis. No other models with additional variables or classes were identified as a better fit. A two-class analysis was performed, resulting in 141 (52.8%) patients in class one (low functional status [LFS] class) and 126 (47.2%) patients in class two (high functional status [HFS] class) based on the highest posterior probability for each patient. The probability of being positive for each variable included in the LCA was higher for the LFS class (Figure 3-2). The odds of any MDRO contamination was significantly higher for LFS patients (69.8% vs. 52.2%; OR=2.2, 95% CI=[1.3, 3.7]; p=0.003; Table 3-4). The odds of contaminating all sites vs. no sites was significantly higher for LFS patients (32.8% vs. 16.9%; OR=2.5; 95% CI=[1.1, 5.8]; p=0.03; Table 3-4).

The bedrail was the most frequently contaminated site for all organisms (Table 3-5). The odds of contamination for LFS patients at each individual environmental site was significantly higher for all sites except for the call button (Table 3-4).

Odds of contaminating at least one site was significantly higher for LFS patients when evaluating a composite of gram-positive organisms (39% vs. 18%; OR=2.4; 95% CI=[1.1, 5.4]; p=0.03; Table 3-4) and when evaluating MRSA (34% vs 16%; OR=2.3; 95% CI=[1.0, 5.3]; p=0.046;). The odds of contaminating all sites with gram positive organisms was significantly higher for LFS patients (13.5% vs. 1.8%; OR=8.7; 95% CI=[1.0, 73.6]; p=0.0048; Table 3-4). Odds of contamination of all sites with MRSA was higher, but not significantly, for LFS patients (20.7% vs. 3.9%; OR=6.5; 95% CI=[0.7, 58.4]; p=0.1). These analyses were not performed for VRE alone due to small sample sizes.

When restricting to all gram-negative organisms, odds of contaminating any environmental site (74.7% vs. 59.4%; OR=2.0; 95% CI=[1.0, 3.9]; p=0.04) was significantly higher for LFS

patients (Table 3-4). A similar direction of effect, but not significant, was observed for *A. baumannii* and *P. aeruginosa*. The odds of contaminating all sites with all gram-negative organisms and *A. baumannii* were higher, but not significantly, for LFS patients (Table 3-4). Analyses of contamination at any site were not performed for CP-CRE and non-CP-CRE and analyses of contamination of all sites were not performed for CP-CRE, non-CP-CRE and *P. aeruginosa* due to small sample sizes.

Sensitivity analyses were performed for all organisms (Table 3-6) to evaluate the effect of time from positive screen date to environmental sample collection date. Longer times between screening and sampling can result in a greater chance of patient decolonization, potentially causing missed contamination events. Stratified analyses were performed to determine whether time between screening and sampling confounded or modified the relationship between latent class membership and the contamination outcomes. The first analysis examined differences in contamination outcomes between the first positive screen collection date and the environmental sampling collection date (≤5 days vs. > 5 days). Both groups had a median of 5 days between first the screening date and the environmental sampling date (interquartile range of 3-6). The odds of contamination was greater for patients with more than five days between these dates for any contamination (OR=4.8; p=0.002 vs. OR=1.8; p=0.04) and contamination of all sites (OR=4.1; p=0.4 vs. OR=2.1; p=0.1). This is explained by the distribution of outcomes between LFS and HFS patients. The proportion of LFS patients was higher, but comparable, among those with greater than five days between the first positive screen collection date and environmental sample collection date for any contamination (72.0% vs. 68.7%) and lower, but comparable, for contamination of all sites (30.0% vs 34.0%). The proportions of HFS patients were lower for patients with five days between the first positive screening collection date and environmental

sample collection date for both any contamination (36.4% vs 58.8%) and all contamination (8.7% vs 21.4%). We did not observe a large decrease in the frequency of either contamination outcome among either of the patient groups for patients with >5 days between the first screening date and the environmental sampling date. This finding suggests environmental contamination events were not missed due to patient decolonization.

The second sensitivity analysis examined differences in contamination outcomes between the latest positive screen collection date and the environmental sampling collection date (≤5 days vs. > 5 days). Both groups had a median of 4 days between latest screening date and the environmental sampling date (interquartile range of 2-5). The odds ratio was higher for patients with more than five days between the latest positive screen collection date and environmental sample date for any contamination (OR=6.7; p=0.003 vs. OR=1.9; p=0.04) but similar for contamination of all sites (OR=2.6; p=0.3 vs. OR=2.5; p=0.6; Table 3-6). The proportion of LFS patients was higher, but comparable, for patients with more than five days between the latest positive screen collection date and environmental sample date for any contamination (71.4% vs. 69.4%) and lower, but comparable, for contamination of all sites (27.3% vs. 33.9%). The proportion of HFS patients was lower for patients with more than five days between the latest positive screening collection date and environmental sample date for any contamination (29.2% vs. 58.4%) and contamination of all sites (10.5% vs. 19.6%). We did not detect a large decrease in the frequency of environmental contamination for patients with >5 days between the latest screening date and the environmental sampling date. This finding indicates environmental contamination events were not missed due to patient decolonization.

We determined if cleaning products affected the findings of the relationship between functional status and environmental contamination. In order to confound the association between

functional status and contamination, intervention arm would need to be associated with patient functional status. No direct or indirect causal pathway exists between cleaning products and patient functional status. Cleaning product may be associated with patient functional status by chance. After assessing any statistical relationship between cleaning products and patient functional status, no significant association was observed (OR=1.1; 95% CI=[0.6, 1.9]; p=0.7). A lack of association and no hypothesized relationship between cleaning products and patient functional status indicates cleaning products are not a confounder. When cleaning products were included in the models with patient functional status, the odds ratio and confidence interval of patient functional status were comparable to the main analysis for contamination of any site (OR=2.4; 95% CI=[1.4, 4.2]; p=0.046 vs. OR=2.2, 95% CI=[1.3, 3.7]; p=0.003) and for contamination of all sites (OR=3.6; 95% CI=[1.4, 9.0]; p=0.008 vs. OR=2.5; 95% CI=[1.1, 5.8]; p=0.03). Cleaning product was associated with both any contamination (OR=3.9; 95% CI=[1.3, 3.8]; p=0.005) and contamination at all sites (OR=12.7; 95% CI=[3.3, 48.5]; p=0.0002). Because of this association, models were run to determine if cleaning product is an effect modifier. When including cleaning products and an interaction term between cleaning product and patient functional status in the model, the odds ratio of LFS patients were comparable to the main analysis for both contamination of any site (OR=2.4, 95%; CI=[1.4, 4.1]; p=0.002 vs. OR=2.2; 95% CI=[1.3, 3.7]; p=0.003) and contamination of all sites (OR=2.6; 95% CI=[0.7, 9.7]; p=0.2 vs. OR=2.5; 95% CI=[1.1, 5.8]; p=0.03). The interaction between cleaning product and patient functional status was not significant for any contamination (p=0.3) or contamination of all sites (p=0.5). Cleaning product is likely associated with contamination, but not functional status and is not a confounder or an effect modifier of the relationship between functional status and contamination. Cleaning product was not included in any other models.

3.6: Discussion

We identified three factors associated with greater frequency of environmental contamination, altered consciousness at admission, requiring assistance with activities of daily living and requiring mechanical ventilation. Patients with a higher probability of these three traits were classified together as LFS patients and we found that this classification of patients contaminated at least one environmental site more frequently and all sites more frequently. The same result was observed when restricting to all gram-positive organisms. LFS patient contaminated at least one site with MRSA and all gram-negative organisms more frequently. LFS patients also contaminated all but one environmental site more frequently. These findings suggest a LFS patient type who has reduced mobility and physical capabilities and requires more interaction with HCW, which may facilitate environmental contamination, is more likely to contaminate the surrounding environment. Furthermore, they indicate the way which a patient interacts with their environment may play a role in environmental contamination. In our study, individuals' functional status was assigned using probabilities determined during LCA, based on data collected easily collected from medical records or during clinical care. Research using a direct assessment of functional status for patients may provide greater insight unto its association with environmental contamination, but the findings presented here provide evidence that an association is present.

Our findings may be due to multiple factors. Patients with limited mobility and altered consciousness may be unable attend to their personal hygiene, including hand hygiene. Colonization of patients' hands in post-acute care facilities was observed to be a risk factor for environmental contamination in a previous study of 650 patients and rooms at six post-acute care facilities ($p<0.001$ for MRSA and VRE and $p=0.01$ for resistant gram-negative bacilli).[110]

Associations between disabilities with activities of daily living and hand contamination were also identified in the study. We detected associations between environmental contamination for both MRSA and gram-negative bacteria in our research but could not analyze VRE alone due to the small number of VRE-positive patients. During an investigation of mechanically ventilated patients, Bonten et al. identified an association between the number of body sites colonized with VRE and environmental contamination.[82] We did not explore relationships between body site and environmental contamination but patient hygiene may affect body site contamination, which could explain their findings. In another study, Cheng et al. observed a significant reduction of environmental contamination with MDRO in communal spaces following a resident hand washing intervention during a multi-site study of residential care homes.[203] While most research regarding hand hygiene focuses on HCW, investigating the impact of patient hand hygiene, especially for those who cannot independently perform this task, may aid in infection control and prevention.

The findings in our study may also result from low functioning patients who require more assistance from HCW. MDRO can be transmitted from patients to HCW, which could result in the contamination of the patient environment.[39] This mode of contamination is supported in our study by the frequency of contamination in all patient rooms under the medical binder at the foot of the bed (between 14.3%-55.0% for all organisms except non-CP CRE). The binder, presumably, is primarily handled by HCW and contamination underneath it would likely be caused by HCW and not patients. Additional resources and precautions may be required for these patients to prevent contamination of fomites in the room through HCW.

Mechanical ventilation was one of the factors used to determine functional status in our study and may contribute to environmental contamination. Lerner et al. found two patient factors associated with CRE contamination of the environment for patients from two internal medicine

wards in a tertiary care hospital: rectal CRE concentration and respiratory illness.[111] The investigators posited that the association between respiratory illness and contamination was possibly due to immobility, physical limitation or antibiotic use. This may be indicative of a group of patients more likely to have respiratory illness with restricted mobility and physical limitations. These patients may require more HCW attention than others, providing a mechanism for environmental contamination.

The link to between environmental contamination and contaminated bodily fluids from patients has been observed in other studies. Boyce et al. observed patients that with diarrhea and higher concentrations of MRSA in their stool contaminated their environment more frequently, in a study of eight patients with high MRSA levels in their stool and six patients negative for MRSA in the stool but positive at other body sites.[109] Researchers in another study observed among 37 consecutive patients admitted to a teaching hospital, patients with MRSA in wounds or urine contaminated their environment more than patients with MRSA at other body sites.[199] Urine and wound secretions may provide routes for MDRO to contaminate the environment other than direct contact. These studies demonstrate release of contaminated bodily fluids may be a potential cause for increased environmental contamination, indicating secretions from other sites, such as respiratory secretions during mechanical ventilation, could contribute to contamination.

Our results potentially contradict a previous study. Pilmis et al. conducted a prospective, hospital-wide study during which environmental samples were collected from five high-touch surfaces, twice in one day before and after cleaning, in 107 randomly selected patient rooms to detect contamination with any bacteria by total colony counts on brain-heart infusion agar.[204] In multivariate analysis, three patient variables were associated with environmental contamination, including being a known MDRO carrier (OR=0.25; 95% CI=[0.09, 0.72]; p=0.01), being in a

single room (OR=0.3; 95% CI=[0.15, 0.6]; p=0.0005) and having a urinary catheter (OR=0.19; 95% CI=[0.04-0.89]; p=0.03). A lower frequency of dependent patients was in contaminated rooms (25.9% vs 32%) and no significant association was observed between dependent status and contamination by univariate analysis (p=0.52). No effect measure was provided. Two comatose patients were included in the study, both contaminated the environment, but no significant association was observed during univariate analysis (p=0.49) and no effect measure was provided. This study had several limitations. Only 18% of patients in their analysis were known carriers of an MDRO through a medical record review. Time between identification of MDRO carriers and environmental sampling was not provided. Contamination with specific organisms was not investigated so potential relationships between carriers of specific organisms and contamination with those organisms could not be determined. These differences from our study may explain our contradictory findings.

Our study did have some limitations. The long time from positive screen to environmental sampling for a small number of our patients may result in decolonization occurring prior to environmental sampling. However, our sensitivity analysis did not demonstrate a consistent reduction in the frequency of detecting contamination events for patients whose time between screening and sampling dates were long. Another potential limitation was the use of a maximum-probability assignment method to classify patients during LCA, which can lead to an attenuation of results.[205] However, we were still able to identify significant associations, even if attenuation was present. The lack of molecular analysis to confirm the organism identified from the patient was related to the organism in the environment is another limitation. This concern is somewhat mitigated by enhanced cleaning of the high-touch surfaces sampled in the study prior to movement of the colonized patients to the room, which may have reduced the likelihood that the organisms

detected in the environment were from other sources. We also sampled only one time point for each organism in each room, allowing for the potential to miss transient contamination episodes, potentially limiting our findings. However, our large sample size and sampling of five high-touch surfaces in the room provided us the opportunity to capture a large enough number of contamination events to support the analysis. A lack of repeat sampling would likely not impact our findings. Our screening criteria may limit our generalizability as patients screened were not from a hospital-wide population. Lastly, our study population median age was 80, possibly limiting the generalizability of our findings.

LFS patients may contaminate their surrounding environment in hospitals with greater frequency than HFS patients. These patients may be candidates for MDRO screening during admission when they might not be otherwise. For LFS patients who are known MDRO carriers, they may be targets for other infection prevention practices, such as improved patient hygiene, staff education on cleaning and hygiene practices, isolation precautions and increased environmental cleaning and disinfection. Investigations into the mechanisms of contamination by LFS patients and the benefits of infection prevention practices targeted toward them is warranted.

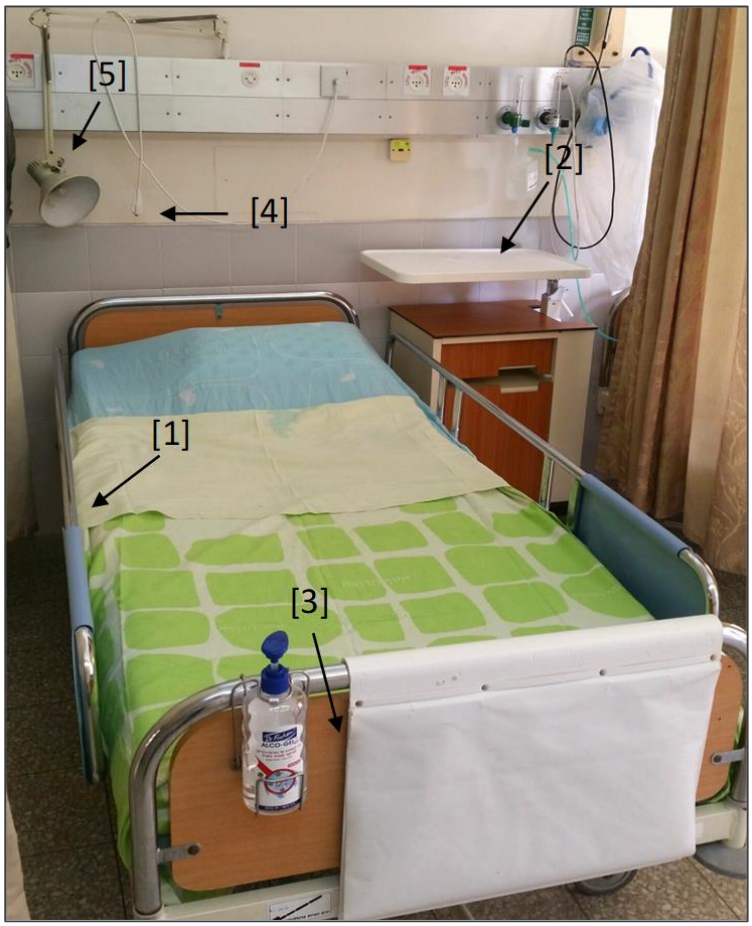

Figure 3-1: Locations of Sampling Sites in the Patient Room.

1) Right bedrail
2) Bedside table
3) Underneath a binder with patient medical information hanging at the foot of the bed
4) Nurse's call button
5) Lamp switch

Table 3-1: Frequency of Positive Admission Screens and Time Between Positive Screen Collection Dates and Environmental Sampling Dates

Organism	Positive Admission Screen[a]	Time from Admission to First PS[b]	Time from First PS to ES[b]	Time from Latest PS to ES
MRSA	59 (60.0)	1 (0, 61)	5 (1, 64)	4 (0, 18)
VRE	6 (75.0)	0.5 (0, 14)	5 (5, 21)	5 (5, 7)
CP-CRE	4 (57.1)	1 (0, 21)	2 (0, 13)	2 (0, 13)
Non-CP-CRE	3 (23.1)	10 (0, 41)	6 (4, 7)	6 (0, 7)
A. baumannii	19 (23.0)	10 (0, 56)	3 (0, 81)	3 (0, 28)
P. aeruginosa	8 (22.2)	12 (0, 126)	5 (1, 35)	3.5 (0, 25)

a. Screened within 48 hours of admission; frequency (percent)

b. Time in days; median (interquartile range)

Abbreviations: MRSA=Methicillin-Resistant *S. aureus*; VRE=Vancomycin-Resistant *Enterococcus*; CP-CRE=Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae; Non-CP-CRE=Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae; PS=Positive Screen Collection Date; ES=Environmental Sample Collection Date

Table 3-2: Unadjusted Analysis of the Association Between Patient Characteristics with Any Environmental Contamination.

Characteristics	Any Contamination (N=163)[a]	No Contamination (N=99)[a]	OR (95% CI)[b]	p[b]
Age	79 (71, 86)	78 (70, 84)	1.0 (1.0, 1.0)	1.0
Male	94 (57.7)	60 (60.6)	0.9 (0.5, 1.5)	0.7
Residence Prior to Admission				
Home (ref)	79 (48.5)	54 (54.6)		
LTCF	57 (21.8)	27 (27.3)	0.7 (0.4, 1.3)	0.2
Other Hospital	3 (1.2)	2 (2.0)	1.0 (0.2, 4.2)	1.0
Other Location	3 (1.2)	2 (2.0)	1.0 (0.2, 6.1)	1.0
Other Hospital Unit	21 (12.9)	14 (14.1)	1.0 (0.4, 2.1)	1.0
LTCF Stay in Prior 3 Months	91 (55.8)	50 (50.5)	1.2 (0.7, 2.1)	0.4
Hospitalization in Prior 3 Months	81 (49.7)	42 (42.4)	1.3 (0.8, 2.2)	0.3
≥3 Outpatient Visits in Prior 3 Months	11 (6.8)	6 (6.1)	1.1 (0.4, 3.2)	0.9
Hemodialysis	7 (4.3)	3 (3.0)	1.4 (0.4, 5.2)	0.6
Requires Assistance in any ADL Prior to Admission	132 (80.1)	69 (69.7)	1.9 (1.0, 3.4)	0.03
Altered Consciousness at Admission	106 (65.0)	47 (47.5)	2.1 (1.3, 3.6)	0.004
Steroid	14 (8.6)	9 (9.1)	0.9 (0.4, 2.4)	0.9
Immunosuppression	16 (9.8)	8 (8.1)	1.2 (0.5, 3.3)	0.7
Diabetes	68 (41.7)	46 (46.5)	0.8 (0.5, 1.4)	0.5
Permanent Device	53 (32.5)	27 (27.3)	1.3 (0.7, 2.3)	0.4
Invasive Procedure in Past 6 Months	41 (25.2)	21 (21.2)	1.3 (0.7, 2.3)	0.5
MDRO in Past 2 Years	46 (28.2)	34 (34.3)	0.8 (0.4, 1.3)	0.3
Antibiotics in Past 3 Months	67 (41.1)	35 (35.4)	1.3 (0.8, 2.2)	0.4
Mechanically Ventilated	60 (36.8)	24 (24.2)	1.8 (1.0, 3.3)	0.03

a. Frequency (percent) for categorical variables and median (quartile 1, quartile 3) for continuous variables

b. Odds ratios and P-values calculated with generalized estimating equations clustering on individuals.

Abbreviations; PC=Positive Contamination; NC=Negative Contamination; LTCF=Long Term Care Facility; ADL=Activities of Daily Living; MDRO=Multidrug-resistant Organism

Table 3-3: Unadjusted Analysis of the Association Between Patient Characteristics with Contamination at All Sites.

Characteristic	Contamination of All Sites (N=33)[a]	No Contamination (N=99)	Odds Ratio (95% CI)[b]	p[b]
Age	78 (70, 86)	78 (70, 84)	1.0 (1.0, 1.1)	0.2
Male	20 (60.6)	60 (60.6)	1.0 (0.5, 2.3)	1.0
Residence Prior to Admission				
Home (ref)	17 (51.5)	54 (54.6)		
LTCF	12 (36.4)	27 (27.3)	0.7 (0.3, 1.7)	0.4
Other Hospital	1 (3.0)	2 (2.0)	0.6 (0.1, 7.4)	0.7
Other Location	1 (3.0)	2 (2.0)	0.6 (0.1, 7.4)	0.7
Other Hospital Unit	2 (6.1)	14 (14.1)	2.2 (0.4, 10.9)	0.3
LTCF Stay in Prior 3 Months	18 (54.6)	50 (50.5)	1.2 (0.5, 2.6)	0.7
Hospitalization in Prior 3 Months	12 (36.4)	42 (42.4)	0.8 (0.3, 1.8)	0.5
≥3 Outpatient Visits in Prior 3 Months	1 (3.0)	6 (6.1)	0.5 (0.1, 4.1)	0.5
Hemodialysis	0 (0)	3 (3.0)	-[c]	-
Requires Assistance in any ADL Prior to Admission	28 (84.9)	69 (69.7)	2.6 (0.9, 6.9)	0.06
Altered Consciousness at Admission	22 (66.7)	47 (47.5)	2.3 (1.0, 5.3)	0.046
Steroid	4 (12.1)	9 (9.1)	1.3 (0.4, 5.0)	0.6
Immunosuppression	5 (15.2)	8 (8.1)	2.0 (0.6, 7.1)	0.3
Diabetes	15 (45.5)	46 (46.5)	1.0 (0.4, 2.2)	1.0
Permanent Device	10 (30.3)	27 (27.3)	1.2 (0.5, 2.9)	0.7
Invasive Procedure in Past 6 Months	6 (18.2)	21 (21.2)	0.8 (0.3, 2.3)	0.7
MDRO in Past 2 Years	11 (33.3)	34 (34.3)	1.0 (0.4, 2.2)	0.9
Antibiotics in Past 3 Months	11 (33.3)	35 (35.4)	0.9 (0.4, 2.1)	0.8
Mechanically Ventilated	10 (30.3)	24 (24.2)	1.4 (0.6, 3.3)	0.5

a. Frequency (percent) for categorical variables and median (quartile 1, quartile 3) for continuous variables

b. Odds ratios and P-values calculated with generalized estimating equations clustering on individuals.

c. Not performed due to small sample size

Abbreviations; LTCF=Long Term Care Facility; ADL=Activities of Daily Living; MDRO=Multidrug-Resistant Organism

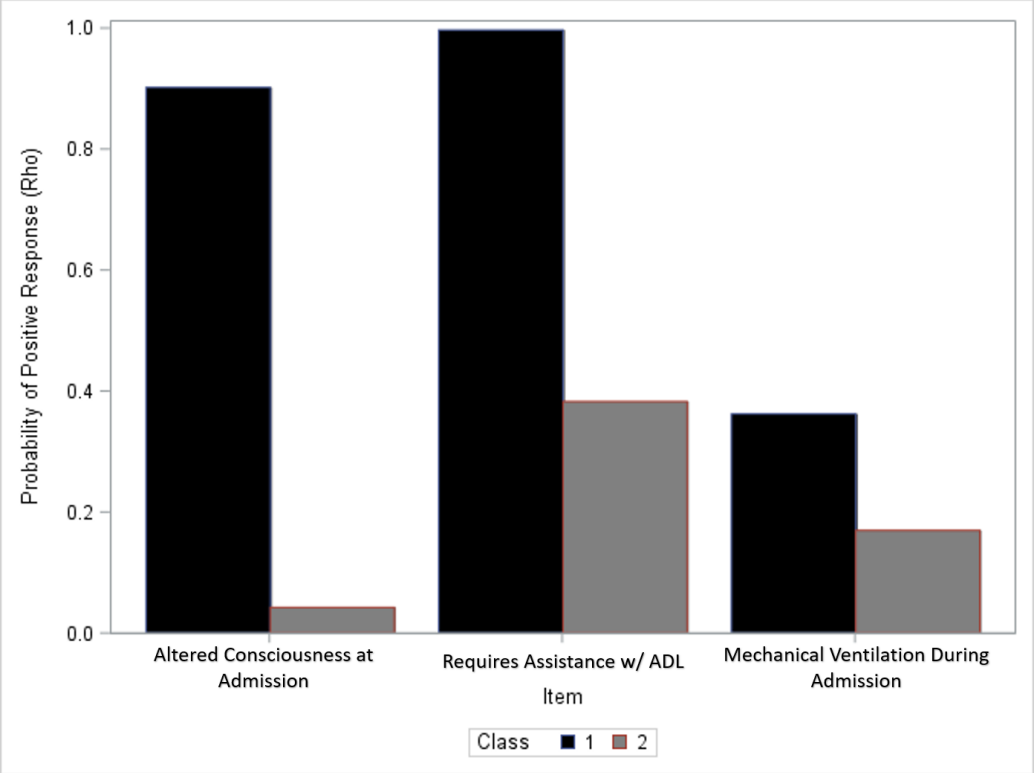

Figure 3-2: Probability of Positive Item Response in Latent Class Analysis by Class Membership

Abbreviations: ADL=Activities of Daily Living; Class 1= Low Functional Status Patients; Class 2=High Functional Status Patients

Table 3-4: Association of Functional Status with Environmental Contamination for All Environmental Sites and by Site or Organism.

Site or Organism	Any Contamination			Contamination of All Sites		
	Frequency (%)	OR (95% CI)[a]	p[a]	Frequency (%)	OR (95% CI)[a]	p[a]
All Sites		2.2 (1.3, 3.7)	0.003		2.5 (1.1, 5.8)	0.03
LFS	104 (69.8)			22 (32.8)		
HFS	59 (52.2)			11 (16.9)		
Bedrail		1.7 (1.1, 2.8)	0.03			
LFS	77 (51.7)			-[b]	-[b]	-[b]
HFS	43 (38.1)					
Bedside Table		2.6 (1.4, 4.6)	0.001			
LFS	56 (37.6)			-[b]	-[b]	-[b]
HFS	22 (19.5)					
Binder		2.2 (1.3, 3.8)	0.004			
LFS	62 (41.6)			-[b]	-[b]	-[b]
HFS	28 (24.8)					
Call Button		1.6 (0.9, 2.8)	0.1			
LFS	43 (28.9)			-[b]	-[b]	-[b]
HFS	23 (20.5)					
Lamp Switch		2.5 (1.4, 4.5)	0.002			
LFS	51 (34.2)			-[b]	-[b]	-[b]
HFS	19 (17.0)					
GPB		2.4 (1.1, 5.4)	0.03		8.7 (1.0, 73.6)	0.048
LFS	39 (62.9)			7 (13.5)		
HFS	18 (40.9)			1 (1.8)		
MRSA		2.3 (1.0, 5.2)	0.046		6.5 (0.7, 58.4)	0.1
LFS	34 (59.7)			6 (20.7)		
HFS	16 (39.0)			1 (3.9)		
VRE						
LFS	5 (100.0)	-[c]	-[c]	1 (100)	-[c]	-[c]
HFS	2 (66.7)			0 (0)		
GNB		2.0 (1.0, 3.9)	0.04		2.0 (0.8, 5.4)	0.2
LFS	65 (74.7)			15 (40.5)		
HFS	41 (59.4)			10 (26.3)		
CP-CRE						
LFS	2 (33.3)	-[c]	-[c]	0 (0)	-[c]	-[c]
HFS	0 (0.0)			0 (0)		
Non-CP CRE						
LFS	1 (20.0)	-[c]	-[c]	0 (0)	-[c]	-[c]
HFS	0 (0)			0 (0)		
A. baumannii		2.9 (0.9, 9.4)	0.08		3.0 (0.8, 11.8)	0.6
LFS	53 (91.4)			15 (75.0)		
HFS	33 (78.6)			9 (50.0)		
P. aeruginosa		1.3 (0.3, 4.6)	0.6			
LFS	9 (50.0)			0 (0)	-[c]	-[c]
HFS	8 (44.4)			1 (9.1)		

a. Odds ratios, 95% confidence intervals and p-values calculated for all sites, site specific, and gram-negative analyses using generalized estimating equations clustering for the individual and gram-positive and organism specific analyses using logistic regression.
b. Site specific analyses for contamination of all sites not performed.
c. Not calculated due to small sample size

Abbreviations: LFS=Low Functional Status Patients; HFS=High Functional Status Patients; GP=Gram-Positive Bacteria; MRSA=Methicillin-Resistant *S. aureus*; VRE=Vancomycin-Resistant *Enterococcus*; GN=Gram-Negative Bacteria; CP-CRE=Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae; Non-CP-CRE=Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae

Table 3-5: Frequency of Contamination by Site and Organism

Contaminating Organism	Bedrail	Bedside Table	Binder	Call Button	Lamp Switch
MRSA	33 (33.7)	17 (17.4)	23 (23.5)	22 (22.7)	20 (20.6)
CP-CRE	1 (14.3)	1 (14.3)	1 (14.3)	0 (0)	1 (14.3)
Non-CP-CRE	1 (7.7)	0 (0)	0 (0)	0 (0)	0 (0)
VRE	6 (75.0)	3 (37.5)	3 (37.5)	2 (25.0)	4 (50.0)
A. baumannii	68 (68.0)	50 (50.0)	55 (55.0)	39 (39.0)	42 (42.0)
P. aeruginosa	11 (30.6)	7 (19.4)	1 (22.2)	3 (8.3)	3 (8.3)

Frequency includes total number (%)

Abbreviations: MRSA=Methicillin-Resistant *S. aureus*; CP-CRE=Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae; Non-CP-CRE=Non-Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae; VRE=Vancomycin-Resistant *Enterococcus*

Table 3-6: Association of Functional Status with Environmental Contamination for All Organisms Accounting for Number of Days Between Patient MDRO Screen and Environmental Sample Collection

Organism	Any Environmental Contamination			Contamination of All Sites		
	Frequency (%)	OR (95% CI)[a]	p[a]	Frequency (%)	OR (95% CI) [a]	p[a]
ES ≤6 days from first MDRO PS (N=210)		1.8 (1.0, 3.4)	0.07		2.1 (0.8, 5.7)	0.1
LFS	68 (68.7)			16 (34.0)		
HFS	47 (58.8)			9 (21.4)		
ES >6 days from first MDRO PS (N=52)		4.8 (1.8, 12.7)	0.002		4.1 (0.7, 22.7)	0.4
LFS	36 (72.0)			6 (30.0)		
HFS	12 (36.4)			2 (8.7)		
ES ≤6 days from latest MDRO PS (N=232)		1.9 (1.0, 3.4)	0.04		2.5 (1.0, 6.2)	0.6
LFS	84 (69.4)			19 (33.9)		
HFS	52 (58.4)			9 (19.6)		
ES >6 days from latest MDRO PS (N=30)		6.7 (1.9, 22.9)	0.003		2.6 (0.4, 17.8)	0.3
LFS	20 (71.4)			3 (27.3)		
HFS	7 (29.2)			2 (10.5)		

a. Odds ratios and P-values calculated with generalized estimating equations clustering on individuals.

Abbreviations: MDRO=Multidrug Resistant Organism; LFS=Low Functional Status Patients; HFS=High Functional Status Patients; ES = Environmental Sample Collection Date; PS=Positive Screen Collection Date

Chapter 4: Antimicrobial-Resistant Bacteria and Viruses Detected Through Systematic Sampling in the Child Care Environment

4.1: Author Summary

We conducted a study at a single-classroom child care center to detect overall bioburden and frequency of contamination with viruses and antibiotic-resistant bacteria from nineteen environmental sites over seventeen sampling days during four months. Sites where children wash and play with water had higher median colony forming unit counts and were amongst the most frequently contaminated with pathogens. These sites would most benefit from enhanced cleaning and disinfection practices, such as cleaning basins and changing still water between play activities, to reduce the potential pathogen transmission through the environment.

4.2: Abstract

Approximately two-thirds of children under five years of age in the United States receive out-of-home child care. Child care attendees have an increased risk of infections compared to children not in child care settings, possibly due to their close contact in a shared environment and unique physiologic and developmental characteristics. As multidrug-resistant organisms (MDROs) increasingly move from healthcare to community settings, child care centers can provide a venue for further transmission of these pathogens. Our objective was to evaluate the bioburden of pathogens present on fomites in child care centers and to detect patterns of surface contamination over time.

The study was conducted in a single classroom of an Ypsilanti, Michigan, child care center. Samples were collected from 19 sites (furniture, toys, room fixtures) for seventeen sampling days

from September 2019 to December 2019. Overall bioburden and frequency of contamination with viruses (adenovirus and norovirus), antibiotic-resistant bacteria (methicillin-resistant *Staphylococcus aureus* [MRSA], extended-spectrum beta-lactamase [ESBL]-producing Enterobacteriaceae, and vancomycin-resistant *Enterococcus* [VRE]), and fecal coliforms were detected.

A total of 276 samples were collected. Sites where children washed or played with water had the highest median colony-forming unit counts, including the sink (3.0 $\log_{10}$ CFU/100 cm^2; IQR 2.3-3.3), water table basin (2.7 $\log_{10}$ CFU/100 cm^2; IQR 1.0-4.4) and water from the water table (3.4 $\log_{10}$ CFU/mL; IQR 3.0-4.0). The median CFU count for water-associated sites, excluding water samples, was significantly higher than all other sites (2.3 $\log_{10}$ CFU/100 cm^2; IQR 1.9-3.2 vs. 1.79; IQR 1.5-2.2; $p<0.0001$). The most frequently contaminated sites for MRSA were the building block table (29.4%), wood blocks (29.4%), the water table basin (25%), and the imitation kitchen (17.7%). For extended-spectrum beta-lactamase-producing Enterobacteriaceae, the most frequently contaminated sites were the sink (35.3%), water from the water table (25%) and glue bottles (17.7%). The most frequently contaminated sites for adenovirus were the building block table (35.3%) and the water table tabletop (23.5%).

The presence of MDRO and viruses on childcare center fomites raised concern for exposure to these pathogens among vulnerable populations. We found the highest bioburdens and most frequent contamination with pathogens on sites where children played or washed with water, identifying targets for environmental cleaning and disinfection practices to mitigate the potential spread of infections among children attending child care centers.

4.3: Introduction

In the U.S., approximately 60% of children under five years-of-age receive child care in a regular arrangement with a non-parental provider.[206] Infections are a major concern in child care centers (CCC's). Child care attendees have an increased risk for respiratory and gastrointestinal infections and use more healthcare resources at a greater cost compared to children cared for at home.[207,208] Pathogen transmission may occur between child care attendees and employees and their family members.[123] Additionally, parents of attendees with child care-related illnesses face a direct socioeconomic burden due to the need to seek care for children as part of return-to-care requirements for ill children.[209]

Multidrug-resistant organisms (MDRO) are a growing problem among young children of child care age. During a four-year, multicenter study including isolates recovered during routine clinical care for outpatients and hospitalized patients ≤21 years old, 201 extended-spectrum beta-lactamase (ESBL)-producing Enterobacteriaceae isolates were recovered from patients with a median age of 4.3 years old and 91 AmpC-producing isolates were recovered from patients with a median age of 7.7 years old.[210] Purcell and Fergie conducted a 14-year study at Texas children's hospital, observing the proportion of *S. aureus* isolates resistant to methicillin increased from 2.9%-10.6% in the first 10 years of the study and from 19.0%-62.4% final four years.[116] The mean age of children with 749 MRSA infections from 2002-2003, the only time during the study with age data collected, was 7.9 years old. CCC's provide a setting where MDRO transmission may occur. MRSA colonization has been detected in 0.14%-7.4% of child care attendees in several studies.[121–124] In Laos, 92 (23%) screened children from 12 child care centers were positive for ESBL-producers.[120] Investigations of MDRO transmission in child care centers could identify targets of intervention to prevent MDRO acquisition among young children.

Children may be more predisposed to infections due to immature immune systems and displaying behaviors that may facilitate pathogen transmission through the environment, such as mouthing of toys and exploring their environment through physical contact. The American Academy of Pediatrics (AAP) recommends comprehensive infection control and prevention practices, including environmental cleaning, sanitation and decontamination practices as one of the three major components of a healthy CCC setting.[211] Children who attend CCC's implementing infection control and prevention practices including environmental cleaning and decontamination practices have a reduced risk of infections while requiring less medical care and reducing parental absence from work.[125] Inconsistent environmental decontamination recommendations between national organizations and local regulators result in reduced compliance with these guidelines.[129] Structured pathogen surveillance in CCC's identifies patterns of environmental contamination which can guide cleaning and decontamination recommendations.

Longitudinal investigations of environmental contamination at CCC's can better inform the role of contamination in pathogen transmission and the effectiveness of environmental decontamination strategies for reducing transmission. Our study aim was to demonstrate the feasibility of longitudinal environmental sampling for microorganisms in the CCC classroom to aid in cleaning and disinfection practices. Our objectives include determining the bioburden on fomites in a CCC and identifying the frequency of fomite contamination with bacterial and viral pathogens.

4.4: Methods

4.4.1: Study Setting

The study was conducted at HighScope Demonstration Preschool, a CCC in Ypsilanti, MI. The school typically enrolls sixteen students, aged 3-5 years, for the academic year, and employs

two adult preschool instructors and a teacher's aide. Students spend their time in a single room, except for a 30-minute recess (two times a day) at an adjacent outdoor playground, five days a week. We separated the classroom into five zones based on activity types (Figure 4-1). A single restroom and a second room, where food preparation and other staff activities occur, are adjacent to the classroom.

Environmental samples were collected from September 2019 to December 2019, including 17 sampling days. Samples were collected twice weekly, every Tuesday and Thursday for the first four weeks. Unexpected interruptions such as school closures for snow days and researcher illnesses disrupted the once weekly sampling plan for the following ten weeks. Samples were collected on nine days during those ten weeks, either on Tuesdays or Thursdays. The preschool staff maintained standard cleaning protocol throughout the study period. Staff regularly cleaned countertops and tables with 50-200 ppm bleach. Toys were cleaned in a dishwasher, approximately once every two months (some items excepted such as wooden blocks). Blankets and pillows were washed weekly, dress-up clothes were washed monthly or if soiled, and the carpet was cleaned twice a year. An outside cleaning crew cleaned the classroom daily using an Environmental Protection Agency-approved disinfectant with claims against emerging viral pathogens. The crew cleaned the bathroom, mopped the floors, vacuumed, and wiped down tables and hard surfaces.

4.4.2: Environmental Sampling

Sampling sites were selected to represent the assortment of furniture and toys with regular use by preschool students (Table 4-1) and found throughout the room (Figure 4-1). These fomites were sampled to capture the variety of sizes (large furniture to small toys), material types (wood, metals, plastic, water and laminate), and functionality (toys, furniture and other room features) of items found in the classroom. Toy samples were collected by randomly selecting individual toys

at each time point. Samples were collected at midday during the 30-minute recess following small group activities. Tables used for these activities were routinely cleaned by child care personnel prior to recess and before the sampling time point; no other items were cleaned during this time.

Two samples were collected simultaneously using paired individual swabs for viral and bacterial samples. Viral samples were collected using PurFlock Ultra 6" Sterile Standard Flock Swab w/Plastic Handle (Puritan Medical Products, Guilford, ME) and swabs were moistened with Universal Transport Media (Copan Diagnostics, Murrieta, CA) and placed in the same media for transport following swabbing. Bacterial samples were collected with a regular flocked ESwab moistened with liquid Amies media (Copan Diagnostics, Murrieta, CA) and stored in the same media for transport. For larger items, an area of 700 cm^2 was swabbed using a premade template; multiples of smaller items with less than 700 cm^2 were swabbed to approximate the same surface area. Following sample collection, samples were transported to the lab and processed within an hour of collection.

4.4.3: Standard Bacterial Plate Counts

Following a ten-second vortex of the liquid amies samples with swabs, 100 µL of undiluted, 10^{-1}, 10^{-2}, and 10^{-3} concentrations of media were plated separately on tryptic soy agar (Neogen, Lansing, MI) and incubated at 37°C for 24 hours. Plates with colony counts between 20 and 300 were used to calculate $\log_{10}$ colony-forming units (CFU)/100 cm^2 for surfaces and $\log_{10}$ CFU/mL for water. If no plate from a single sample site had more than 20 colonies, then the undiluted sample was used to count CFU.

4.4.4: Resistance and Fecal Coliform Testing

Identification of methicillin-resistant Staphylococcus aureus (MRSA), extended-spectrum beta-lactamase (ESBL) producing Enterobacteriaceae, and vancomycin-resistant Enterococcus

(VRE) were performed using CHROMagar MRSA II (BD, Franklin Lakes, New Jersey), HardyCHROM ESBL (Hardy Diagnostics, Santa Maria, CA) and Spectra VRE (Thermo Fisher Scientific, Waltham, MA), respectively. Fecal coliform testing was performed using m-FC Agar with 1% Rosolic Acid (Hardy Diagnostics, Santa Maria, CA). At least one isolated colony from each selective media culture indicative of a positive result was cultured in tryptic soy broth (Neogen, Lansing MI) and stored in a 50% glycerol solution (Thermo Fisher Scientific, Waltham, MA) at -80°C.Viral Genomic Extraction

UTM samples were vortexed for ten seconds with the swab; a volume of 1 mL of media was used for genomic extraction. Extraction was performed using High Pure Viral Nucleic Acid Large Volume Kit (Roche, Basel, Switzerland) with elution into 100 µL of elution buffer. Samples were further purified using RNA Clean & Concentrator-5 Kit (Zymo Research, Irvine, CA) with elution into 50 µL of elution buffer. Samples were stored at -80°C following elution with each kit.

4.4.5: Viral PCR

Singleplex PCR was used to identify the viral targets, adenovirus and norovirus (GI and GII). Adenovirus detection was performed using previously published primers, probes and protocols.[212] PCR was performed using the AgPath-ID One Step RT-PCR Kit (Life Technologies, Carlsbad, CA) as previously described.[212] FTD Respiratory pathogens 21 (Fast Track Diagnostics, Luxembourg) was included as a positive control for adenovirus during PCR.

Norovirus GI and GII primers and probe sequences and thermocycler conditions have been previously described.[213] A modification from previous methods to use two singleplex PCR assays for norovirus GI and GII was performed in this study. The same concentrations of primers, probes and buffers (AgPath-ID One Step RT-PCR Kit [Life Technologies, Carlsbad, CA]) were used as previously published.[213] Two norovirus positive controls were included during PCR (norovirus GI

- ATCC VR-3234SD; norovirus GII - ATCC VR-3235SD). All PCR assays were performed using the Applied Biosystems 7500 (Thermo Fisher Scientific, Waltham, MA).

4.4.6: Statistical Analysis

Median CFU counts and frequency of contamination with antibiotic-resistant bacteria were calculated. The Wilcoxon rank-sum test was used to compare median CFU counts between sites. All statistical analysis was performed using SAS version 9.4 (SAS Institute, Cary, NC).

4.5: Results

A total of 276 samples were collected from 19 sites in the classroom on 17 different days; 13 of the 19 sites had samples collected on all 17 days (Table 4-2). Sampling of the side and bottom of Large Table 2 started on the second day of sample collection. Water sampling from the water table was began on the third day of sampling. On the four days water was not present, samples were collected from the water table basin. Sampling of the toy trains began on the third sampling day. Sampling of chairs began on the seventh sampling day. A camera mounted on a wall out of the reach of the children was sampled on three days. The median time between sampling days was 5 days, with a range of 2-16 days.

Twelve sites were positive for bacterial growth from all samples collected (Table 4-2). The range of the frequency of positive samples was 83%-100% for all sites. The $\log_{10}$ CFU/100 cm^2 (or $\log_{10}$ CFU/mL for water) was determined for all samples with bacterial growth. The median CFU count for all sites excluding water samples was 1.8 $\log_{10}$ CFU/100 cm^2 (interquartile range [IQR] 1.5-2.3). The sites with the highest median CFU counts were sites where children washed or played with water including the sink (3.0 $\log_{10}$ CFU/100 cm^2; IQR 2.3-3.3), water table basin (2.7 $\log_{10}$ CFU/100 cm^2; IQR 1.0-4.4) and water from the water table (3.4 $\log_{10}$ CFU/mL; IQR 3.0-4.0). Among the classroom furniture, samples collected from the building block table (2.4 $\log_{10}$

CFU/100 cm^2; IQR 2.3-3.6) and the imitation kitchen (2.2 log_{10} CFU/100 cm^2; 1.8-2.3) had the highest median CFU counts. The small wood blocks were observed to have the highest median bioburden among the toys (2.4 log_{10} CFU/100 cm^2; IQR 2.0-2.9). CFU counts ranged from 0.63-4.68 log_{10} CFU/100 cm^2 among all water-associated sites, excluding water samples, and 0.15-3.67 log_{10} CFU/100 cm^2 for all other sites (Figure 4-2).

The median bioburden of all water-associated sites, excluding water collected from the water table, was significantly higher than the bioburden at all other sites (2.3 log_{10} CFU/100 cm^2; IQR 1.9-3.2 vs. 1.8 log_{10} CFU/100 cm^2; IQR 1.5-2.2; $p<0.0001$; Figure 4-2). Among the tables, the median CFU count for the building block table, which has an irregular surface, was significantly higher than the two large tabletops (2.4 log_{10} CFU/100 cm^2; IQR 2.3-2.6 vs. 1.5 log_{10} CFU/100 cm^2; IQR 1.1-1.8; $p<0.0001$). The median CFU count for the imitation kitchen, which has a large smooth surface, was significantly higher than for the other two large tabletops (2.2 log_{10} CFU/100 cm^2; IQR 1.8-2.3 vs. 1.5 log_{10} CFU/100 cm^2; IQR 1.1-1.8; $p = 0.0002$). Among the toys, the wood blocks had a higher median CFU count than all other toys (2.4 log_{10} CFU/100 cm^2; IQR 2.0-2.9 vs. 1.7 log_{10} CFU/100 cm^2; IQR 1.5-2.0; $p<0.0001$).

Twenty-two out of 276 total samples (8.0%) were positive for MRSA. Eleven sampling sites were positive on at least one occasion (Table 4-2). The sites most frequently contaminated with MRSA included the building block table (n=5; 29.4%), the small wood blocks (n=5; 29.4%), the water table basin (n=1; 25%) and the imitation kitchen (n=3; 17.7%).

Seventeen samples out of 276 total samples (6.2%) were positive for ESBL-producing Enterobacteriaceae; seven sampling sites were positive on at least one occasion (Table 4-2). The sink (n=6; 35.3%), water from the water table (n=3; 25%) and the glue bottles (n=2; 17.7%) were

contaminated most frequently with ESBL-producers. No samples were positive for VRE or fecal coliforms from any site.

Twenty-one total samples (7.6%) were positive for adenovirus. Samples from nine sites were positive for adenovirus on at least one occasion (Table 4-2). The most frequent sites of adenovirus contamination were the building block table (n=6; 35.3%) and the water tabletop (n=4; 23.5%). Norovirus was not detected in any sample.

4.6: Discussion

Our study demonstrates the feasibility of a longitudinal sampling investigation in a child care center to detect environmental contamination with bacterial and viral pathogens on various fomites. We identified adenovirus and antibiotic-resistant bacteria in the environment, indicating the potential for fomites to facilitate transmission of these pathogens. The bioburden was generally low and contamination with pathogens was absent or infrequent at most sites. However, sites where children washed and played with water and fomites with irregular surfaces or cleaned less frequently harbored higher bioburdens and were contaminated more frequently with pathogens.

The median bioburden detected for sites in our study is in line with other previous research. Li et al. detected a median of 2.04 $\log_{10}$ CFU/100 cm^2 for smooth surfaces from 40 CCC's with a mean of eight samples collected per site.[214] Our findings are similar to their results. During twice monthly sampling from six CCC's over eight months, Cosby et al. detected mean counts of 1.64 $\log_{10}$ CFU/50 cm^2, 1.58 $\log_{10}$ CFU/50 cm^2, and 1.53 $\log_{10}$ CFU/50 cm^2 for food serving, diaper changing and food preparation areas.[143] We did not sample from surfaces used for these activities, but we did find similar bioburden on other large, smooth furniture.

Our surveillance identified several locations with higher levels of contamination. Most notably, sites were children wash and play with water, the sink and water table (including the

tabletop, basin and water from the basin), were among the highest bioburden sites and most frequently contaminated with pathogens. Water can act as a reservoir for microbes and spreads these organisms to surrounding areas during hand hygiene and play activities. These water-associated sites may benefit from more frequent cleaning. The AAP, in conjunction with American Public Health Association, and the National Resource Center for Health and Safety in Child Care and Early Education recommend water tables should be filled before their use and the basin should be cleaned after each use.[127] They also recommend children should wash their hands before and after using the water table to limit the spread of pathogens. Our findings support these recommendations.

Some fomite surfaces may be irregular and difficult to clean. The building block table in our study, which had the highest median bioburden of the furniture, is an example. The tabletop for the building block table contains many ridges, making this surface more difficult to clean than a flat tabletop, potentially explaining the increased contamination. Li et al. observed significant differences in aerobic plate counts and coliform counts when contamination on regular and irregular surfaces were compared in 40 CCC's, demonstrating surface type can play a role in contamination.[214] Irregular surfaces may require special attention and cleaning instruments other than cloth like scrubbing brushes.

Materials which are difficult to clean may result in higher contamination due to less frequent cleaning. The small wood blocks were identified as having a higher median bioburden and were more frequently contaminated among the toys. The blocks are not typically cleaned like the other toys as the wood may be damaged during cleaning, potentially explaining their higher and more frequent contamination. Contamination of toys that cannot be cleaned regularly could

be managed by rotating toys out of circulation to allow organisms to die off before being used again.

Frequency and timing of cleaning may also affect the detection of contamination among other furniture. The imitation kitchen has a similar surface to the large tables in the room. However, the large tables were cleaned by the staff following small group activities which immediately preceded environmental sampling while the imitation kitchen was not cleaned. The large tables had a low median bioburden and limited pathogen contamination, potentially demonstrating the impact of frequent environmental cleaning. Both the furniture and toy results demonstrate frequent cleaning can effectively reduce bioburden in the environment.

Material type may also play a role in the recovery of organisms from fomites. Several studies have demonstrated bacterial transfer and survivability is reduced for wood compared to other materials, including plastics and metals.[215–219] Wood is porous, providing space for bacteria to inhabit making recovery difficult and causing the surface to dry more quickly than non-porous materials which can reduce the survival of organisms.[215] Furthermore, surfaces such as plastics, which appear smooth macroscopically, may have microscopic crevices which improve adherence of organisms to the surface and can protect organisms from desiccation. Recovery and survivability of bacteria on smooth surfaces without microscopic crevices, such as glass, is reduced, as these surfaces do not protect against desiccation.[215] In our study, we observed a high median bioburden on the wood blocks. As viability and recovery from wood is expected to be reduced, the higher level of contamination suggests the contamination is likely more recent or occurring with a larger inoculum than on other materials as we would expect a lower bioburden if the timing or amount of contamination was similar between sites. For plastic and metal fomites, recovery of bacteria is expected to be similar when accounting for time from contamination and

bacterial load during contamination.[215–219] If this is the case for the materials sampled in this study, differences among these materials may therefore be related to frequency and quality of cleaning.

We detected adenovirus on a variety of surfaces. Adenovirus is a common cause of respiratory and gastrointestinal disease among children, and has caused illness outbreaks in CCC's.[220–222] Transfer of adenovirus from the environment to human skin has been demonstrated experimentally, indicating its potential for environmental transmission. Adenovirus environmental contamination has been implicated in illness outbreaks. Sammons et al. identified contaminated ophthalmologic equipment as the source of an adenovirus outbreak in a neonatal intensive care unit.[223] Detection of adenovirus in our study could signify transmission of adenovirus through the environment is possible. Adenovirus has been detected in the environment of CCC's previously. Lyman et al. identified environmental contamination from 13 of 22 CCC's with acute gastroenteritis outbreaks in North Carolina, including contamination with adenovirus at 10%-100% of sampled sites during three outbreaks.[132] The frequency of contamination with adenovirus in this study was higher than ours, which could be due to the presence of illness outbreaks during their study.

MDRO in child care requires more investigation. In our study, contamination with antibiotic-resistant bacteria was infrequent, including limited contamination with ESBL-producers and no contamination with VRE. Research regarding environmental contamination in CCC's with these pathogens is sparse. Moritz et al. detected MRSA in 1.4% of cross-sectional samples from eleven CCC's in Iowa.[144] Our study detected a higher frequency of contamination, which may be due to differences in susceptibility testing (selective media vs. broth microdilution). In two California CCC's, 6% of fomites were contaminated with any *Enterococcus* spp. and none were contaminated with *E. coli*. However, antibiotic resistance was not investigated.[224]

Our lack of fecal coliform contamination does not correspond with previous research. Researchers in Tennessee detected total coliforms in 24.7% of environmental samples at six CCC's. However, they did not distinguish between total and fecal coliforms. Also, the sites sampled were food contact surfaces and diaper changing stations, which may explain the difference in findings. Researchers from three previous studies found low proportions of fecal coliform contamination of fomites (3.0%, 4.3% and 9.5%), differing from our findings of no contamination, but still infrequent.[136,138,224] In a longitudinal study including ten rooms from six CCC's, fecal contamination was detected on 26 fomites, ranging 2-52% for each item.[135] The ages of the attendees were 24 months and younger. These attendees are younger than those in our study and would still require diapering, potentially accounting for the higher contamination rates.

The small class size and single classroom may limit the amount and variety of environmental contamination found in our study and could reduce the generalizability of our findings. However, the repeated sampling and number of sampling sites would help mitigate these limitations. We also used selective media to identify MDRO in the environment. Susceptibility testing using selective media may result in reduced sensitivity and specificity when compared to traditional culture methods but are still highly effective at detecting antibiotic resistant organisms.[225–227] If the prevalence of MDRO in the environment is low in CCC's then our findings will overestimate the frequency of contamination, even with a high sensitivity and specificity for the selective media.

For environmental pathogen transmission to occur, viable organisms must be present. We demonstrated the viability of the bacteria detected through culturing, but our viral detection was PCR-based only, limiting our study. Ganime et al. detected viable adenovirus from 50% of environmental samples that were PCR-positive.[228] Viable organisms need to inoculate the host

directly, as may occur with mouthing of fomites among young children, or mediated through the host's hands. The efficiency of organism transfer from the environment to hands and then from hands to inoculation sites is low for viruses and bacteria, including multi-drug resistant organisms (MDRO).[229–232] However, low doses of inoculation can still result in infection.[233,234] Studies regarding transfer of organisms to hands and inoculation sites were performed on adults and may not be generalizable to children. Children use their hands to explore the environment and their own bodies which may provide more opportunities for bacterial transfer and self-inoculation.

Developmental, behavioral and environmental factors present unique challenges to controlling infections in child care centers. We have demonstrated the feasibility and importance of longitudinal surveillance of key child care center environmental sites for various organisms. Our research identified problem areas within the classroom, which can guide targeted infection control practices when time and resources are limited. With this uncertainty and the threat of environmental transmission of other pathogens, vigilance with best practices for environmental decontamination should be maintained.

Table 4-1: Items Sampled, Material Type, Sampling Instructions Corresponding Sampling Numbers

Item	Sampling Instructions	Sample No.
Large Table 1 (Laminate)	Use template on the table edge and tabletop	1
Sink (Stainless Steel)	Swab the upper edge around the entirety of the basin	2
Waste basket (Plastic Bag)	Swab the entire upper edge covered by the trash bag	3
Glue Bottles (Plastic)	Holding the bottle top, swab the entire bottle	4
Water Table Tabletop (Laminate)	Use a template on the center of the tabletop	5
Large Table 2 - Top (Laminate)	Use template on tabletop	6
Teflon Toy Pots (Teflon/Metal)	Swab the entire pot	7
Imitation Kitchen (Laminate)	Use a template on the center of the countertop	8
Doll (Plastic)	Holding the clothed area, swab the unclothed area	9
Building Block Table (Plastic)	Use a template on the center of the tabletop	10
Building Blocks (Plastic)	Swab the entire block	11
Crib (Wood)	Swab the upper and lower wood board on one long edge of the crib	12
Large Table 2 – Edge and Bottom (Laminate/Wood)	Swab the edge and bottom using curved template	13
Small Wood Blocks (Wood)	Swab the entire surface of the block	14
Water from Water Table (Water)	Collect at least 10 mL by submerging a sterile container in the water	15
Toy Train (Mixed – Wood, Plastic, Metal)	Swab the entire small train piece	16
Chair (Wood)	Swab the outer edge of the seat and back (both sides of the back	17
Camera Stand (Plastic)	Swab the base of the camera (done monthly)	18
Water Table Basin (Plastic)	Use template on basin (only to be performed if no water is present in the water Table)	19

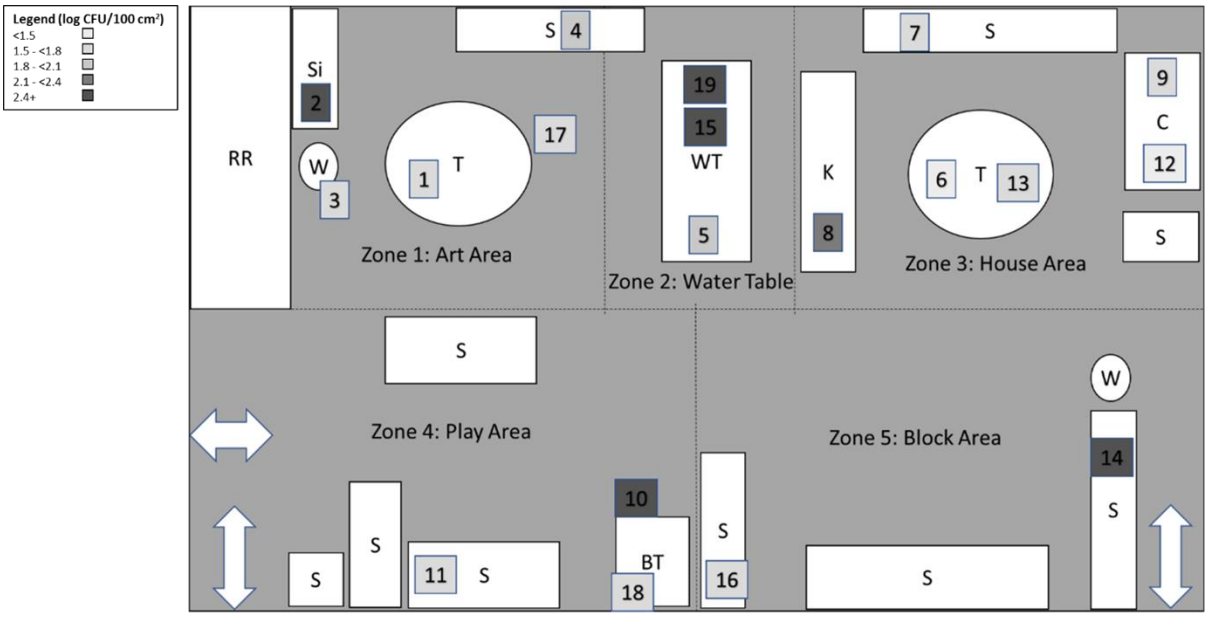

Figure 4-1: Room Layout and Location of Sampling Sites.

Numbers correspond to sample numbers in table 1. Double headed arrows indicate room entrance/exit.
Abbreviations: BT = Building Block Table; C = Crib; RR = Restroom; S = Shelves; Si = Sink; T = Table; WT = Water Table

Table 4-2: Colony-forming Unit Counts and the Frequency of Antibiotic-Resistant Bacteria and Virus Detection at Each Sampling Site.

Sample No.[a]	Total Samples	Positive Growth (%)	Median log_{10} CFU/100 cm^2 (IQR)	MRSA-Positive Samples (%)	ESBL Producer-Positive Samples (%)	Adenovirus-Positive Samples (%)
1	17	15 (88)	1.6 (1.2, 2.1)	0 (0)	0 (0)	0 (0)
2	17	17 (100)	3.0 (2.3, 3.3)	1 (5.9)	6 (35.3)	1 (5.9)
3	17	17 (100)	1.8 (1.5, 2.0)	1 (5.9)	0 (0)	0 (0)
4	17	17 (100)	2.0 (1.9, 2.2)	1 (5.9)	2 (17.7)	1 (5.9)
5	17	17 (100)	2.0 (1.8, 2.3)	1 (5.9)	2 (11.8)	4 (23.5)
6	17	16 (94)	1.4 (1.0, 1.7)	0(0)	0 (0)	0 (0)
7	17	17 (100)	1.8 (1.6, 1.9)	1 (5.9)	0 (0)	2 (11.8)
8	17	17 (100)	2.2 (1.8, 2.3)	3 (17.7)	1 (5.9)	0 (0)
9	17	16 (94)	1.7 (1.5, 2.0)	1 (5.9)	0 (0)	2 (11.8)
10	17	17 (100)	2.4 (2.3, 2.6)	5 (29.4)	2 (11.8)	6 (35.3)
11	17	16 (94)	1.5 (1.2, 2.0)	0 (0)	0 (0)	0 (0)
12	17	14 (88)[c]	1.2 (0.8, 1.7)	2 (11.8)	0 (0)	0 (0)
13	16	15 (94)	1.6 (1.1, 2.3)	0 (0)	1 (6.3)	2 (12.5)
14	17	16 (100)[c]	2.4 (2.0, 2.9)	5 (29.4)	0 (0)	1 (11.8)
15	12	10 (83)	3.4 (3.0, 4.0)[b]	0 (0)	3 (25)	0 (0)
16	15	13 (100)[d]	1.7 (1.7, 1.9)	0 (0)	0 (0)	2 (13.3)
17	11	11 (100)	1.5 (1.3, 1.8)	0 (0)	0 (0)	0 (0)
18	3	3 (100)	1.8 (1.6, 1.9)	0 (0)	0 (0)	0 (0)
19	4	4 (100)	2.7 (1.0, 4.4)	1 (25)	0 (0)	0 (0)

a. Sample no. corresponds to the sample no. in Table 4-1

b. Log_{10} CFU/mL for water

c. One sample not processed for standard bacterial plate count due to contamination

d. Two samples not processed for standard bacterial plate count due to contamination

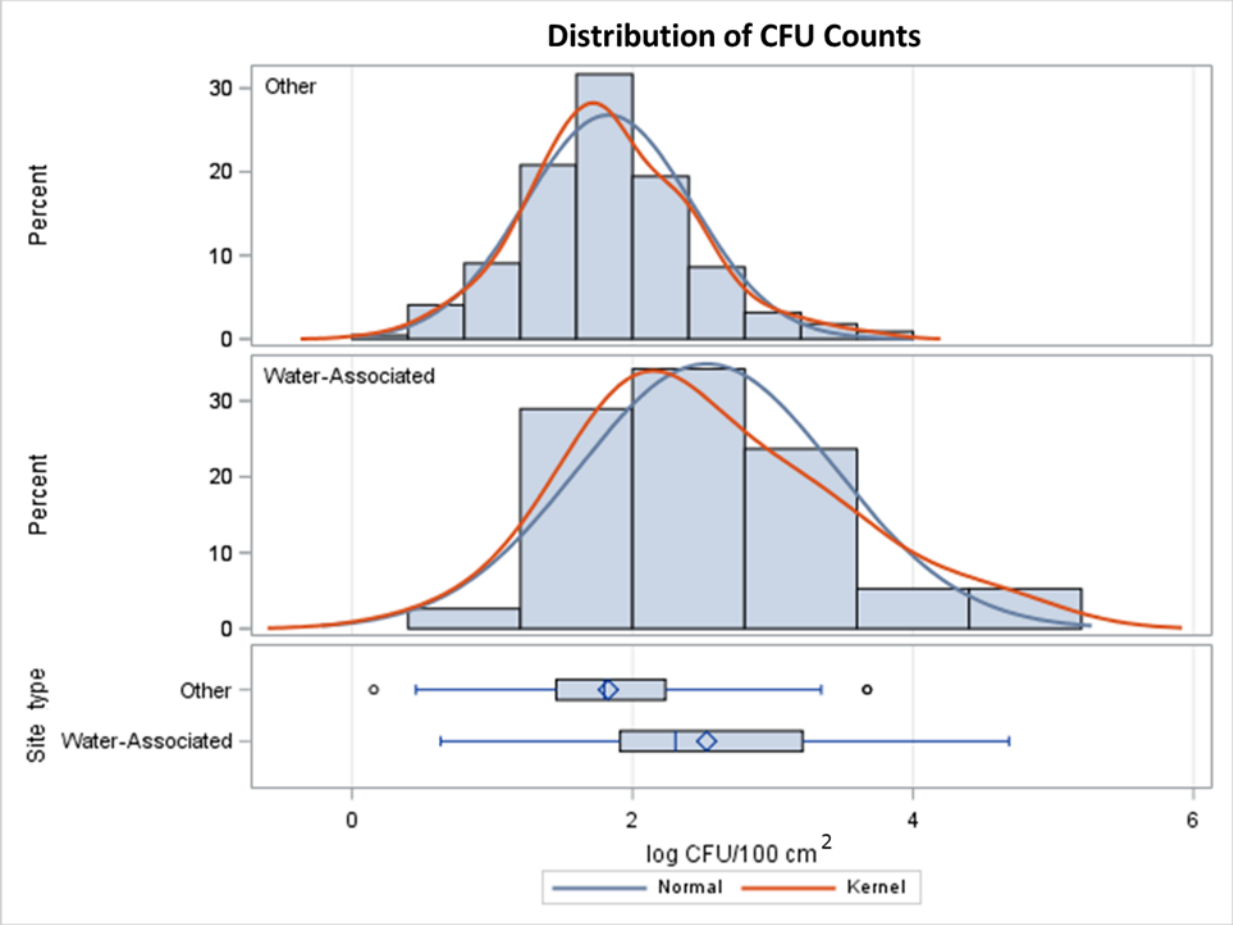

Figure 4-2: Distribution of CFU counts for Water-Associated Sites and Other Sites

The histogram (top) and box plot (bottom) show the distribution of colony-forming unit (CFU) counts transformed to $\log_{10}$ CFU/100 cm^2 for all samples collected where children wash and play with water (excluding water samples) compared to all samples collected from other sites. A normal curve and fitted curve are overlayed over the histogram. The median CFU count for water-associated sites was significantly higher than for all other sites by the Wilcoxon rank-sum test.

Chapter 5: Conclusions

The overall goal of this work was to identify targets for infection prevention and control measures to interrupt multidrug-resistant organism (MDRO) transmission and acquisition. This dissertation investigated two topics, ascertaining the risk of multiple organism colonization and infection, a potential preceding event to MDRO acquisition, and environmental contamination with MDRO, which contributes to pathogen transmission. In chapter 2, risk factors for methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant *Enterococcus* (VRE) co-colonization or coinfection (CCCI), a likely predecessor to vancomycin-resistant *S. aureus* (VRSA) acquisition, were determined for hospitalized patients in the region with highest number of VRSA cases detected in the U.S. In chapter 3, we used patient characteristics to identify a type of MDRO carrier among hospitalized patients that contaminates the surrounding environment. Finally, surveillance of a CCC classroom in chapter 4 revealed multiple locations in the environment harboring a higher bioburden and more frequently contaminated with potential pathogens. A summary of these findings is discussed here, focusing on the knowledge added, public health implications, strengths, weakness and future considerations.

5.1: Aim 1

5.1.1: Aim 1 Knowledge Added and Implications

The emergence of VRSA demonstrates the potential for *de novo* MDRO emergence through horizontal gene transfer during multiple organism colonization or infection. However, the dearth of cases and the lack of person-to-person transmission provides an opportunity to intervene

on VRSA emergence and contain its dissemination.[148] Molecular and epidemiological evidence suggest MRSA acquires vancomycin resistance from VRE during CCCI resulting in VRSA acquisition, a serious concern considering vancomycin is a first-line treatment option for MRSA.[65,148–150,235] Since the majority of all documented U.S. VRSA cases occurred in southeast Michigan, determining risk factors for MRSA and VRE CCCI in this population can be of enhanced importance to prevent the continued emergence of VRSA.

We observed that healthcare exposure was a risk factor for MRSA and VRE CCCI. MDRO infections are a common problem in healthcare facilities, including MRSA and VRE, so this finding is not surprising.[34] Warren et al. identified prior hospitalization and admission from another healthcare facility and a Han et al. identified prior hospitalizations as risk factors for CCCI. [151,154] We build on this evidence using molecular characteristics of pathogens. Admission from another healthcare facility was identified as a risk factor when comparing CCCI patients with MRSA controls and controls without MRSA or VRE in our study. Having at least one hospitalization in the past year was a risk factor when comparing to MRSA controls, further implicating healthcare exposure as a CCCI risk factor. Our molecular analysis revealed patients harboring PVL-negative MRSA isolates and CC5 MRSA isolates were at greater risk of CCCI with VRE. PVL-positive MRSA isolates have been associated with community-associated MRSA strains and CC5 isolates are associated with healthcare-associated MRSA, providing additional evidence that healthcare exposure may be a risk factor for CCCI.[169,173] Molecular analysis is sparse in prior CCCI research and is a strength our study. The totality of our findings strengthens the observation that healthcare exposure is a risk factor for CCCI.

Our findings can inform infection prevention and control practices. Transfer of patients between healthcare facilities presents an opportunity for introduction and transmission of MDRO

between settings. Communication of MDRO carrier status between facilities can aid in identification of MDRO carriers. The Centers for Disease Control and Prevention (CDC) recommends screening of patients transferred from healthcare facilities known to have a high prevalence of MDRO.[236] Our findings add more support for this recommendation. The CDC also recommends cohorting patients with the same MDRO when single-patient rooms are not available.[236] In this case, screening can be beneficial as to prevent cohorting of patients with MRSA together when one is an unknown VRE carrier, or vice versa, particularly for patients with CCCI risk factors. Screening can aid antibiotic stewardship, another infection control strategy recommended by the CDC.[236] In the case of multiple organism colonization or infection, using vancomycin on a patient with MRSA who unknowingly harbors VRE, may result in killing susceptible bacteria while allowing resistant organisms, such as VRSA, to grow with reduced competition. Recent antibiotic exposure was also a risk factor for CCCI when comparing to controls without MRSA or VRE, providing further support for antibiotic stewardship.

The CDC recommends enhanced barrier precautions when caring for residents in long-term care facilities with indwelling devices to prevent the transmission of MDRO, which is supported by our findings.[236] Our research and other CCCI research identified indwelling devices as a risk factor for CCCI and barrier precautions may reduce MDRO acquisition among hospitalized patients.[153,154,156] Device-associated infections were responsible for 179,639 (57.6%) healthcare associated infections reported to the National Healthcare Safety Network from 2015-2017, including antibiotic-resistant infections in 0.7-82.1% of cases depending on the organism.[34] The Society for Healthcare Epidemiology of America (SHEA) recommends judicious use of indwelling devices and implementing appropriate sterile techniques for the placement, management and removal of these devices, which our findings support.[237] Judicious use of

indwelling devices can improve patient outcomes. For example, Burnham et al. observed failure to remove a central venous catheter was a risk factor for all-cause 30-day mortality (HR=13.5; 95% CI=[6.8–26.7], p<0.001) for patients with MDRO central-line bloodstream infections during a seven-year retrospective study at a 1250 bed academic medical center.[238] Following these recommendations when caring for patients with indwelling devices would potentially mitigate MRSA and VRE CCCI.

5.1.2: Aim 1 Strengths and Weaknesses

We conducted a multi-year, multi-site, prospective, hospital-wide investigation including all species of VRE, comparisons of CCCI to multiple control groups, molecular analysis of pathogens and a separate analysis of patients with pathogens isolated on the same day and in the same specimen to determine the epidemiology of MRSA and VRE CCCI. While previous research has been conducted to determine risk factors for CCCI with these pathogens, none have been conducted with the totality of parameters included in our study, indicating the strength of our research.[151–157]

Our use of three control groups is a major strength of our study design. MRSA and VRE CCCI may occur through three different pathways: simultaneous acquisition of both organisms or acquisition of VRE or MRSA with subsequent acquisition of the other at a later time. Using three control groups of MRSA only, VRE only and no MRSA or VRE allows for an investigation of CCCI risk through all three pathways. One drawback of this design is the inability to statistically compare findings of associations between analyses, but this can be offset, in part, through qualitative comparisons such as observing the effect of the same variable in multiple models.[239] The restrictions on pathogen carriage among control groups also creates control groups which may not completely reflect the source population (all patients without MRSA and VRE CCCI), a

possible source of selection bias. These excluded patients likely make up a small proportion of control groups, limiting the effect of their exclusion.[239] Furthermore, multiple comparisons would not be possible without their exclusions.[239]

Analyses including patients with MRSA and VRE isolates collected on the same day and in the same specimen is relevant to VRSA acquisition and is another strength of our study. For resistance gene transfer to occur, MRSA and VRE must be in close contact. Detecting both pathogens on the same day or in the same sample demonstrates their cohabitation and potential for gene transfer. The concordance of these analyses with our main findings provides some evidence for potential VRSA risk factors to be investigated among MRSA and VRE CCCI risk factors.

Other strengths are present in our study. Our source population was from healthcare facilities in southeastern Michigan, the region of the U.S. with the most VRSA cases. Determining risk factors of MRSA and VRE CCCI in this population adds to the understanding of why VRSA may be occurring in this region. A comparison of these factors to hospitalized patients outside of southeast Michigan may reveal a patient population more at risk for VRSA than elsewhere. Our molecular analysis was another strength. The molecular characteristics provided more evidence of healthcare exposure as a risk factor and could provide information as to why VRSA cases occurred more frequently in southeast Michigan versus the rest of the U.S. A multi-faceted approach to CCCI epidemiology provided us with additional evidence of healthcare exposure as a risk factor.

Stepwise regression is a limitation of our study. This method of variable selection can lead to multiple problems including adjustment of inappropriate covariates and bias in the estimate of regression coefficients, especially with smaller samples, which can invalidate findings.[189,190] We attempted to mitigate these potential sources of bias through our selection of variables included in the stepwise process. We used a prospective study design to identify new cases of CCCI. We

included variables in our analysis with a temporal relationship to CCCI which allows for the plausibility of causality. Use of *a priori* knowledge is another strategy for reducing bias with model seletion.[240] As research into risk factors for CCCI is still limited, prior knowledge of characteristics for use in models is difficult. However, we included risk factors from prior research, as well as potential risk factors for VRSA and other MDRO risk factors to limit the potential for inappropriate inclusion of covariates.[44,65,148,151–157,165,191]

5.1.3: Aim 1 Future Considerations

Our MRSA and VRE CCCI investigation included a molecular analysis of MRSA isolates using multi-locus sequencing typing (MLST) to determine associations between sequence type and CCCI. Other sequencing methods may provide advantages over MLST, particularly whole genome sequencing (WGS). WGS provides the sequence for the entirety of the genome, including the core genome, which is present in all strains of a clade, and the accessory genome, which may be present in a subset of strains. Sequences produced during WGS can be used for traditional sequencing methods such as MLST but can also be used for more discriminatory methods, such as core-genome MLST and whole-genome MLST, to determine relationships between isolates.[241,242] As WGS is used more frequently, relationships between strain types identified using these more discriminatory typing methods and other factors such as location of isolate acquisition (healthcare facilities vs. community), infection site or associations with comorbidities can be determined. These associations could provide additional evidence of risk factors for CCCI in our study. While of limited utility in our CCCI study, determining relationships between organisms can be of benefit in outbreak analysis or used in our hospital environmental contamination aim to determine the relationship between organisms isolated from patients and the environment.

WGS sequencing can provide additional information as well. In our CCCI study, we detected the presence of PVL genes, supplying additional evidence of healthcare exposure as a CCCI risk factor. Using WGS, the presence of other virulence genes can be identified, which can provide additional information regarding how an organism was acquired.[243] WGS also allows for the identification of other genes including resistance genes.[244] In our study, we could potentially identify vancomycin resistance genes in our MRSA isolates, providing information regarding the development of VRSA. As WGS costs have decreased, its application has become more practical.[245] WGS provides the information garnered from older typing methods but also offers more discrimination in determining relationships between isolates and additional information regarding the presence of genes, which can inform mechanisms of organism acquisition and antibiotic susceptibility.

Other avenues of research can be pursued relating to MRSA and VRE CCCI. While we investigated risk factors of MRSA and VRE CCCI, outcome analysis could also be useful in providing more support for implementing the recommendations discussed previously. Outcome measures such as mortality and length of stay have been collected with our data and can be evaluated for their relationship to CCCI. Outcome analysis for MRSA and VRE CCCI has received less attention than risk factor analysis and this additional work would add to this knowledge gap of MRSA and VRE CCCI.[151,156]

Our findings could also guide investigations into preventing CCCI. Healthcare exposure as a risk factor for CCCI may inform infection prevention and control strategies such as screening practices, cohorting and environmental cleaning practices for patients with other CCCI risk factors aside from healthcare exposure. Decolonization of MRSA carriers has demonstrated a reduced risk of MRSA infection.[246] The CDC recommends decolonization on a case-by-case basis, including

for VRSA prevention.[247,248] SHEA recommends decolonization targeted toward MRSA-colonized patients and patients at high risk of infection.[249] Decolonization benefits for patients with risk factors for CCCI may mitigate the occurrence of CCCI and VRSA and is another potential avenue of investigation.

Broadly, the findings from this and other CCCI risk factor research can guide investigations regarding VRSA emergence. Characteristics increasing the likelihood of MRSA and VRE CCCI may be potential risk factors for VRSA acquisition as well. For example, eleven of the fourteen U.S. VRSA cases were in patients with diabetes.[65,165] We also identified diabetes as a risk factor for CCCI in comparison with controls without MRSA or VRE. The mechanism through which diabetes may facilitate VRSA acquisition, perhaps through immune modification, chronic skin wounds, or causes vascular disease, could be of clinical interest. Although VRSA cases are still sparse, identifying patients at risk for VRSA would aid in mitigating its emergence.

5.1.4: Aim 1 Summary

We have demonstrated the role of healthcare exposure in MRSA and VRE CCCI. This knowledge can aid in prevention of MRSA and VRE CCCI acquisition and potentially alleviate the emergence of VRSA. Additional research on health outcomes related to CCCI and the relationship of risk factors to VRSA development can inform infection prevention policies and the direction of scarce resources.

5.2: Aim 2

5.2.1: Aim 2 Knowledge Added and Implications

Healthcare-associated infections caused by MDRO are a common problem in U.S. hospitals.[34] Preventing transmission of MDRO through the hospital environment remains a

challenge, but has proven to be successful and cost-effective.[97,195,196] Limited time and resources demand an efficient use of prevention practices. Determination of patient populations for whom targeted practices which would be most beneficial for reduction of environmental pathogen transmission would guide the application of these strategies. In chapter 3, we used latent class analysis, inputting observed patient characteristics, to identify a low functional status (LFS) patient type who contaminates their surrounding environment more frequently than high functional status (HFS) patients.

Our identification of the role patient functional status plays in MDRO environmental contamination has significant public health implications. Patient functional status can be easily assessed by clinicians and can provide a means for identifying patients to whom additional infection prevention practices should be directed. Our findings build off previous research into patient characteristics associated with environmental contamination and shed light on potential mechanisms which contribute to this problem. We provide three potential mechanisms explaining increased contamination by LFS patients.

First, hand and body site contamination have both been implicated in increased environmental contamination, demonstrating the importance of patient hygiene.[82,110] Functionally limited patients may be unable to maintain their hygiene, potentially explaining our findings. The CDC recommends hospitalized patients maintain proper hand hygiene, which our findings support.[250] [236] Haverstick et al. observed a significant decrease in MRSA and VRE infections in a post-surgical step-down unit following the implementation of a patient hand hygiene education program, demonstrating this practice can improve patient outcomes.[251] While hand hygiene for HFS patients could be addressed primarily through education, LFS patients may need additional assistance with hygiene practices which could be a low-cost prevention strategy. SHEA

recommends daily chlorhexidine bathing of ICU patients.[237] Our findings support patient hygiene strategies such as daily chlorhexidine bathing for patients unable to maintain their personal hygiene.

Second, bodily fluids may also play a role in increasing environmental contamination as well. MDRO in diarrhea, urine and wounds have been implicated in environmental contamination.[109,199] In our study, mechanical ventilation was a marker for functional status. Mechanical ventilation provides a mechanism through which contaminated bodily fluids can contaminate the environment. Suction of respiratory secretions during mechanical ventilation may contribute to contamination. Yu et al. observed significantly more bacterial contamination within 100 cm of the endotracheal intubation site of ventilated patients when open suctioning was used compared to closed suctioning.[252] Although the potential for environmental contamination by respiratory secretions exists, no specific recommendations are available for the prevention of environmental contamination from mechanical ventilation. This highlights the importance of other infection prevention strategies including hand hygiene, standard precautions and environmental decontamination for preventing environmental contamination when caring for mechanically ventilated patients. Guidance regarding measures to limit environmental contamination during mechanical ventilation could mitigate contamination with MDRO by LFS patients.

Another mechanism of environmental contamination by LFS patients may be mediated through healthcare worker (HCW) interactions. LFS patients may require additional care from HCW compared to their HFS counterparts, such as for the maintenance of mechanical ventilation. HCW have been implicated in environmental transmission of pathogens with patients and the environment.[38,192] Currently, the CDC recommends contact precautions for patients colonized with target MDRO and our findings support that recommendations, particularly for LFS patients.[236] Our

findings also suggest that rooms with LFS MDRO carriers may benefit from more frequent cleaning targeting the immediate environment of the patient. The CDC does recommend prioritizing cleaning high-touch surfaces, especially those close to the patient, for any patient in contact precautions, which our findings support as well.[236] To ensure these precautions are appropriately used, LFS patients may benefit from MDRO screening. No current screening recommendations are targeted toward this group.

5.2.2: Aim 2 Strengths and Weaknesses

Our use of LCA to determine functional status in this aim comes with strengths and weaknesses. As no one specific measurement can assess functional status, LCA allowed us to use several characteristics to categorize patients based on this unobserved trait. LCA also allowed us to identify associations between functional status and environmental contamination. Variable selection and class number determination for LCA can be done in a variety of often complicated methods. While our methodology was likely simplified, our use and agreement of both AIC and BIC to determine model fitness has been demonstrated to be appropriate.[253] Our item choices also make logical sense for the identification of LFS patients. LCA also provides posterior probabilities for the likelihood of class membership for each individual. We used maximum-probability assignment, assigning each member to the class with the higher posterior probability. This method can cause attenuation of associations between latent classes and other variables due to potential misclassification of individuals in latent classes, although it does perform better in this regard than some other methods.[205,254] However, we still detected significant findings with strong effect measures. The possibility of attenuation indicates a stronger association than we observed.

Our study population was derived from a larger cleaning intervention trial which comes with strengths and weaknesses as it was not specifically designed for our purposes. One weakness

is the single environmental sampling time point for each MDRO identified during patient screening. Repeat sampling may provide the opportunity to determine the role of length of stay on environmental contamination, which we could not do. Our study did include a large sample of patients and sampling of five high-touch sites, allowing us to capture a large number of contamination events.

The trial included an investigation of six organisms, which allowed us to assess total contamination as well as contamination with individual organisms to identify differences between species. However, another weakness was the lack of molecular analysis of isolates, allowing for the potential misidentification of relationships between isolates from patients and the environment which can cause misclassification bias of our results. Daily cleaning of the five high-touch surfaces used for environmental sampling reduced the possibility of misclassification. Fluorescent markers were used to determine the frequency of cleaning for a selected sample of these patients. Additional analysis of this data would aid in determining the thoroughness of cleaning for patients in this study. Ideally, the room would be cleaned and sampled prior to occupation by a new resident to ensure a lack of contamination, but this would be impractical in the clinical setting.

Another weakness was the inconsistency of sampling times following detection of MDRO colonized patients, resulting in delays of environmental sampling for a small number of patients. We performed a sensitivity analysis to ensure patients with delays in sampling were not attenuating results through decolonization resulting in a lack of environmental contamination. Furthermore, MDRO colonization can be prolonged and result in shedding for months, indicating decolonization is unlikely for most patients over a period of days.[83–85]

Lastly, the study was not designed to quantify MDRO at screening sites or in bodily fluids, detect MDRO on hands or other body sites, or include information on diarrhea among patients, all

factors with limited evidence indicating they could be risk factors for environmental contamination. These exclusions could result in confounding and bias our results. However, patient functionality may explain the occurrence of these factors, such as hand contamination, and adjustment would be inappropriate as they may act as intermediate variables. More research may be needed to understand these relationships.

5.2.3: Aim 2 Future Considerations

Our findings provide evidence to support future investigations regarding the impact of patient functional status on environmental contamination. Future research would benefit from a well-defined measurement of functional status which could assess mechanisms of contamination, such as hand contamination or contamination due to HCW interactions. Molecular testing to confirm relatedness of isolates and repeat sampling to assess time to contamination and capture contamination events would strengthen this research. Sampling from environmental sites further from the patient may provide additional information regarding the mechanisms through which the association of functional status and contamination occur.

Other research exploring the mechanisms of this association may provide guidance for infection prevention and control. Patient hygiene is an area that is understudied. SHEA recommends daily bathing of ICU patients with chlorhexidine. The implementation of such a strategy toward all LFS patients may mitigate MDRO environmental contamination and transmission. Determining the burden of pathogen contamination or colonization on the hands and bodies of LFS patients would inform whether patient hygiene influences environmental contamination by these patients. Investigations into possible benefits of hand or body hygiene for low functioning patients with regards to reducing environmental contamination would further

provide evidence for this mode of transmission. LFS patient hygiene can also be examined as a potential cost-effective strategy to prevent MDRO transmission and improve patient outcomes.

Interactions between HCW and LFS patients is another potential mechanism of environmental contamination to be evaluated. Comparisons between contamination of HCW by LFS and HFS patients is one way to gauge the impact of this interaction. Environmental sampling of sites distant from LFS patients but frequently contacted by HCW can demonstrate transmission of MDRO through HCW. Contamination of HCW during interactions with LFS patients may occur as well. These contamination events could result in transmission to other patients cared for by the same HCW, another potential target of investigation and intervention.

The role of bodily secretions among LFS patients, particularly those mechanically ventilated, can be explored as well to assess the contribution of these fluids to environmental contamination. Strategies to reduce excreted secretions during suctioning of ventilated patients can be investigated. Research on hygiene practices with regards to urine, stool and wounds for LFS patients could provide other targets of intervention. The inability of LFS patients to address these potential sources of contamination demands HCW vigilance and best practices should be determined.

Research regarding other infection prevention strategies geared toward LFS patients should be initiated. Strategies to improve cleaning of high-touch surfaces near LFS patients can be explored. The benefits of screening these patients for MDRO when not otherwise indicated can be examined to determine if identifying LFS MDRO carriers can allow for better prevention of MDRO transmission. These patients may also benefit from decolonization, which could also be investigated. The variety of potential mechanisms which may contribute to environmental contamination by LFS patients provides many potential directions for future research.

5.2.4: Aim 2 Summary

We have detected the impact of patient functional status on environmental contamination. Our findings can aid in the development of strategies to disrupt transmission of MDRO through the environment due to their excessive contamination. Research into understanding the exact mechanisms of this association can further inform best practices to mitigate MDRO contamination and transmission.

5.3: Aim 3

5.3.1: Aim 3 Knowledge Added and Implications

Children are an understudied population with regards to MDRO, but evidence suggests MDRO infections in this population may be increasing.[115,116,118] MDRO carriage among child care attendees has been observed to be as high as 23% for ESBL-producers and 7% for MRSA.[120–124] Child care presents a setting where MDRO transmission can occur due to shared environments and behaviors of attendees such as mouthing of toys and poor hygiene. In aim 3, we surveilled the environment of a child care center to determine the bioburden of fomites and the frequency of contamination with MDRO and viral pathogens.

We demonstrated a methodology that allows for surveillance of overall bioburden and viral and bacterial pathogens in child care centers (CCC). Our surveillance design provides a means to detect patterns of contamination for multiple pathogens and, for bacteria, confirmation of viability indicating infectious potential, informing environmental decontamination practices. Previous contamination investigations in CCC's have targeted either bacterial or viral contamination but surveilling for both enhances knowledge regarding pathogen transmission through the environment in this setting.[130–135,137–139,143] In particular, MDRO sampling has been lacking in this area of research and our study demonstrates a feasible method for surveilling these pathogens.

Sampling was performed within thirty minutes, allowing for data collection with minimal disruption for the CCC. The collection process was simple and requires minimal training for investigators. The sampling method permits sampling from a variety of sites within centers, allowing for comparisons of multiple materials and sites with different size, functionality and mobility. Future investigations with this study design can be applied to improve the understanding of environmental contamination in CCC's.

We identified sites where children play and wash with water as harboring the highest bioburden and being most frequently contaminated with MDRO and viral pathogens, a significant finding in CCC research. Previous investigations of CCC environmental contamination have neglected sampling from areas where children wash or play with water.[130–135,137–139,143] The American Academy of Pediatrics, the American Public Health Association, and the National Resource Center for Health and Safety in Child Care and Early Education have published national recommendations regarding CCC environmental decontamination practices.[127] For water tables, if flowing, potable, fresh water is not available, they recommend filling the table with fresh, potable water immediately before play begins and changing water if a new group of children plays at the table, even if the new group comes from the same room. The basin and toys used at the water table should be washed and sanitized at the end of the day. Children should wash their hands prior to water table play and hands should be free from cuts, scratches or sores. Our findings support these recommendations. They also recommend cleaning and disinfecting handwashing sinks at the end of the day. Our findings support this recommendation as sinks were another site with high bioburden. In Michigan, where the CCC studied is located, the Licensing and Regulatory Affairs (LARA) branch of the Michigan state government publishes licensing guidelines for Michigan

CCC's.[128] They provide no guidance for decontamination of water tables or sinks, which should be reconsidered.

We identified other sites with excessive contamination as well which were not cleaned as frequently as other sites. Wood blocks harbored the highest bioburden among the toys. National recommendations include cleaning of toys which are mouthed or contaminated with other bodily secretions to be washed and sanitized by hand or cleaned in a dishwasher.[127] However, they recommend not using toys which cannot be cleaned in this manner. Wood blocks may be damaged from this cleaning process and were not cleaned in the dishwasher with other toys in the CCC in our study. A potential alternative for cleaning may be to remove toys from circulation for a period of time to allow for a natural death of microorganisms. LARA provides no regulations for CCC's regarding cleaning toys which should be reevaluated.[128]

Frequent cleaning may also be responsible for the lower bioburden observed at the large tables compared to the imitation kitchen, even though the kitchen had a similar smooth surface to the tables. The other large tables were cleaned immediately prior to our sampling due to their use during group activities. This difference could demonstrate the benefit of cleaning these surfaces. National recommendations for decontamination of tables includes cleaning and sanitizing before and after each use, which our findings support.[127] LARA recommends washing tables before and after use for eating only.[128]

Sites which may be difficult to clean can also result in more contamination. The building block table, which had an irregular surface, harbored a higher median bioburden than the other smooth tables at the CCC. This site poses a unique challenge due to the irregularity of its surface. Recommendations for cleaning of tables do not address irregular surfaces. These surfaces may

need additional guidelines regarding decontamination or centers should consider removing them if disinfection is not feasible.

With regard to all sites of high contamination identified in our study, child care providers may need to determine the benefit of these items and weigh them against their potential to harbor and transmit pathogens. These considerations should be given to items where children play with water, items with large irregular surfaces and items difficult to clean due to material type. Providers may consider items which are easier to disinfect but still provide educational and developmental benefits for children.

5.3.2: Strengths and Weaknesses

Our methodology for sample collection is a major strength for this study. We detected overall bioburden and the presence of both viral and bacterial pathogens, allowing for the assessment of contamination with multiple organisms of interest. However, we only investigated three MDRO and two viruses, but have samples stored from the study which could be used in future investigations. We sampled from fomites including a variety of material types, surface types, sizes and functionality, which aids in determining the types of fomite which could be responsible for pathogen transmission. Other sites could have been included in our analysis, such as floors (carpets, vinyl, etc.), cloth toys and play clothes to provide additional information regarding patterns of pathogen contamination. Repeat sampling from environmental sites allowed for the detection of patterns of contamination within the CCC's.

Several limitations exist for this study as well. We did not perform confirmatory identification of MDRO detected using selective media. We have stored isolates and this confirmation can be performed when laboratory access improves following the COVID-19 pandemic. However, the sensitivity and specificity of the media is high, indicating the possibility

of misidentifying pathogens was low.[225–227] We did not culture viral samples to confirm the viability of detected viruses. Ganime et al. observed 50% viability of adenovirus from sites with PCR-detected adenovirus collected from hospital fomites, suggesting the presence of viral DNA detected in the environment may indicate viable adenovirus contamination to a certain degree.[228] Furthermore, sites with more frequent adenovirus contamination were also sites with higher bioburden, suggesting the DNA could be a marker for contamination.

Our study setting may also be a limitation, particularly with regards to generalizability. We collected samples from one room with a small class size of limited age range and a small number of staff. Variability may exist between classes, especially with those including children still using diapers. Other classes may have different cleaning protocols which could affect environmental contamination. No illness outbreaks occurred during the study period, which could alter the presence of pathogens in the environment. Including more classes with more students would allow for the ability to capture a larger variety of settings to investigate.

5.3.3: Future Considerations

The research presented demonstrates a strategy for environmental sampling in CCC's. The next step would be to expand this research with a larger study. This extension would include multiple centers and classrooms with larger class sizes of wider age ranges and increased staff members. Additional fomites can be examined, including food preparation surfaces, diaper changing areas and flooring. Sampling from hands or screening attendees and staff for pathogens would provide additional information regarding the patterns and mechanisms of environmental contamination. The expansion could also add other pathogens of interest, such as target organisms implicated in illness outbreaks within specific centers. This methodology could potentially be applied for environmental surveillance in settings other than CCC's as well.

Other potential investigations could be guided by our specific findings. Sites where children wash and play with water have been ignored in previous research. Water tables would be of particular interest. The impact of changing water and cleaning basins and the immediate surroundings could be examined and would aid in the guidance of policies directed toward minimizing contamination at these sites. Irregular surfaces would be another target for future studies. Li et al. detected differences in contamination between smooth and irregular surfaces.[214] however, their focus was small objects like toys and utensils. Investigating larger surfaces which should be cleaned daily, such as the building block table in our study, would determine whether additional cleaning resources should be applied for these fomites. Removal of objects from circulation would be another research avenue which our findings provide support for exploring. Currently, no recommendations are provided for such actions for the purpose of decontamination.[127,128] This practice could provide CCC's with a strategy to prevent pathogen transmission that would be cheap and require limited time commitments from staff. The limited research on environmental contamination in CCC's provides ample opportunity for gaps in knowledge to be filled and the research presented here can be built upon toward that goal.

5.3.4: Aim 3 Summary

We demonstrated a method of CCC environmental sampling which can guide infection prevention and control practices in these settings. The association of contamination with sites where children wash and play with water is a significant finding which adds to the public health knowledge regarding CCC's. We also identified other potential areas of intervention, including irregular surfaces and objects which may be difficult to decontaminate using traditional methods. Our study design can be used to investigate these areas and other research objectives in child care settings.

5.4: Overall Implications

MDRO are a global problem in healthcare. Preventing adverse outcomes from these organisms requires intervening on targets where MDRO transmission and acquisition can occur. In this dissertation, topics related to preventing MDRO acquisition and transmission were explored. In chapter 2, we identified risk factors for MRSA and VRE CCCI in hospitalized patients, a predecessor to VRSA acquisition, and determined healthcare exposure is a risk factor. In chapters 3 and 4, we investigated environmental contamination with MDRO. In chapter3, LFS patients were determined to be contaminating their surrounding environment more frequently among MDRO colonized inpatients. In chapter 4, we moved to the community setting to investigate MDRO environmental contamination in a CCC. We validated a methodology for detecting environmental contamination, including both viral and bacterial pathogens, which can be applied to large scale studies. We determined sites where children wash and play with water, sites with irregular surfaces and fomites difficult to clean with traditional methods were more contaminated than other sites, including with MDRO, informing decontamination practices in these facilities. The research presented here adds to the public health knowledge regarding MDRO acquisition and transmission and presents new paths of investigation to continue to combat the burden of these pathogens.

The worldwide prevalence of MDRO, the difficulty to treat infections they cause and the risk of adverse health outcomes from these organisms necessitate ways to prevent MDRO acquisition and transmission. Interrupting MDRO transmission and acquisition requires interventions at multiple levels of settings and populations, which this dissertation presents. Although strategies may be targeted toward specific groups or settings, MDRO interventions in one group or setting may alleviate the burden in others. For example, we observed LFS patients

contaminated their environment more frequently in chapter 3. A patient hygiene intervention directed toward LFS patients may reduce the burden of contamination and provide indirect benefits to other patients not in this group. Prior room occupancy by an MDRO carrier is a risk factor for MDRO acquisition.[92–94] Reducing contamination in the environment may reduce the risk of MDRO acquisition for the next occupant, particularly if that occupant has risk factors for MDRO acquisition as identified in chapter 2, such as having an indwelling medical device or recent antibiotic exposure.

These interventions may have other ramifications beyond the specific setting where they are implemented. Hospital floors are not isolated environments. Infection prevention in one unit of the hospital may alleviate MDRO prevalence in others. Patients moving between settings, such as general floors and critical care units may provide opportunities for MDRO transmission. During a 24-month, prospective study at three ICU in two Greek hospitals, Papadimitriou-Olivgeris et al. detected VRE in 71/497 (14.3%) patients screened.[36] Of the 71, only 12 (16.9%) were admitted directly to the ICU without hospitalization, demonstrating the potential for MDRO introduction to the ICU from other hospital units. Referring back to the patient hygiene example, environmental contamination may be reduced through such an intervention. Preventing MDRO acquisition, such as through the previous patient hygiene example, may reduce MDRO contamination. A patient at high risk for MRSA and VRE co-colonization may subsequently reside in that room. A reduction in environmental contamination could prevent that patient from acquiring an MDRO and potentially transferring it elsewhere, such as a critical care unit.

Stepping back further, hospitals themselves are not isolated as well. In chapter 2, we identified admission from another healthcare facility as a CCCI risk factor when comparing CCCI patients to MRSA controls. The high-risk patient in the patient hygiene example may be spared

MRSA acquisition due to the intervention. If that patient is then transferred to another facility, the risk of acquiring VRE resulting in CCCI could be mitigated. The CDC recommendation to screen admitted patients transferred from facilities with known high MDRO prevalence is another example of an intervention which can aid in preventing transmission between facilities.[236] A patient from a high MDRO prevalence facility with risk factors for MRSA and VRE CCCI might be an ideal candidate for MDRO screening. Interactions between and within healthcare centers demonstrate the importance of preventing MDRO acquisition and transmission for a single hospital or unit, but more broadly within the healthcare system.

In the community, interactions between settings occur as well. Hewlett et al. performed molecular analysis of MRSA isolates collected from attendees and employees of a CCC and their household members.[123] Indistinguishable MRSA isolates were collected from an employee, the employee's child who did not attend the CCC, and two children who did attend the CCC. The findings indicate the potential for MDRO to be transmitted between settings in the community. In chapter 4, we identified environmental sites with higher organism contamination which can inform cleaning and disinfection practices in CCC's, such proper water table maintenance, reducing MDRO environmental contamination in these centers. Reduced contamination could result in reduced transmission, not only in the CCC, but to household members of CCC attendees and employees. Our findings may have broader implications than reduction of MDRO transmission within CCC's.

Healthcare and community settings interact as well. Riccio et al. followed 71 patients colonized with intestinal ESBL-producing Enterobacteriaceae discharged from five European university hospitals for up to four months.[255] They identified 19 household transmission events of clonally related isolates, 13 from index patients to household members and six from household

members to index patients, with 1.18 transmissions/100 participant-weeks. These findings show transmission of MDRO from the hospital into the community is possible. Interventions in one setting, such as patient hygiene to reduce MDRO contamination in the hospital, may not only reduce transmission in that setting, but also between facilities or into the community. The findings presented in this dissertation can advise infection prevention practices resulting in a direct effect in the setting where they are implemented but may also have a far-reaching impact.

Considering the intertwined nature of populations and settings where MDRO are transmitted, research aimed at characterizing the movement of MDRO between settings will aid in mitigating MDRO emergence. In Appendix A, a proposal is presented for developing a self-collection method for VRE screening. Self-collection of these samples can make surveilling VRE in the community more practical. If valid, this method of screening could be applied to longitudinal surveillance of discharged hospitalized patients to determine the duration of colonization and potential for VRE transmission in the community. VRE surveillance within households and CCC's could be conducted to discover patterns of transmission within and between these settings. Self-screening can be another tool to better understand MDRO transmission and acquisition to aid in infection prevention and control.

5.5: Concluding Remarks

Antibiotic-resistant bacteria are common worldwide. These organisms are difficult to treat and cause substantial morbidity and mortality. A multi-faceted approach to prevention of MDRO transmission and acquisition is required across settings and populations. This dissertation identified targets for intervention , providing new paths for MDRO infection prevention and control as part of a larger approach to help mitigate the burden of MDRO.

Appendix: Development of a Self-Collection Screening Method to Identify Vancomycin-Resistant *Enterococcus* Colonization

A.1: Introduction, Motivation and Objectives

Vancomycin-resistant enterococci (VRE) are among the most common healthcare-associated pathogens in the U.S.[145] Limited treatment options are available for VRE, resulting in poorer outcomes compared to their susceptible counterparts, including increased mortality, longer length of hospital stay and increased risk of infection recurrence.[29,256,257] While the burden of VRE in healthcare facilities has encouraged extensive research in this setting, VRE in the community has received less attention.

Most community-based, VRE studies are cross-sectional and limited by small sample sizes, restricting the analysis which can be performed using this data.[49–52,258–266] However, these studies have demonstrated the presence of VRE in community settings, estimating the prevalence of asymptomatic colonization to be as high as 39%.[49–52,258–266]

The presence of VRE in the community may be, in part, due to the movement of VRE carriers between healthcare and community settings. Baran et al. observed the prevalence of VRE among members of households of healthcare workers with direct patient contact from one Detroit, MI hospital was higher (7.3%) compared to the households of healthcare workers without patient contact (2.2%).[267] This finding supports the hypothesis that VRE carriers in healthcare settings may introduce VRE into the community. Conversely, VRE carriers in the community may serve as a reservoir for VRE reintroduction into healthcare facilities. The prevalence of VRE colonization among newly admitted ICU patients from home was 10% in one Maryland hospital.[268]

However, these studies demonstrating the interaction between healthcare and community settings included fewer than 25 participants colonized with VRE, indicating the need for continued VRE research to validate this connection.

One barrier to prospective, longitudinal studies of asymptomatic individuals in the community are the current methods of VRE colonization screening. VRE reside in the digestive tract of colonized individuals and are shed in fecal matter. The CDC recommends rectal or perirectal swabs or stool samples as the standard for VRE colonization screening.[236] However, stool samples cannot always be conveniently returned to investigators from study participants in the community. Additionally, rectal and perirectal swabs require administration by a healthcare professional, limiting their utility in community-based research. An alternative to the current screening methods needs to be developed to better facilitate VRE research in the community.

Rectal/perirectal self-collection is a possible alternative to current VRE screening methods. Although this method has not been validated for VRE colonization screening, rectal self-swabbing has been utilized for sexually transmitted infections research. Rectal self-collection was observed to be comparable to clinician-collected rectal screening for anal cytology and a variety of sexually transmitted pathogens.[269–273] Rectal self-collection was feasible and acceptable to perform for study participants.[271,274–276] In addition to the feasibility and acceptability, some participants may be more amenable to self-collection compared to clinician-collection for screening. Among women who preferred self over provider anal HPV sampling in one Puerto Rican study, 28% felt less embarrassed and 69% felt more comfortable with self-sampling compared to clinician-sampling.[272] The successful implementation of rectal self-screening in sexually transmitted infection research demonstrates the potential utility of this method for VRE screening.

Other sampling sites may provide convenient targets for self-screening as well. In a 2010 study sampling at six different body sites, inguinal sampling was demonstrated to be as effective as perirectal swabbing for multidrug resistant organism (MDRO) screening.[277] Investigators observed similar results in a 2016 study comparing rectal and inguinal swabbing for MDRO screening, including VRE.[278] The inguinal region is easily accessible for self-sampling and may be an additional viable alternative to rectal/perirectal swabbing.

Current VRE screening methods may be impractical to conduct on a large scale in the community. The proposed study aims to develop a new sampling method using self-collected perirectal wipes to overcome the limitations of traditional VRE screening methods.

A.2: Specific Aims and Research Activities

Specific Aim 1: Compare the performance of a clinician-collected perirectal swab versus a self-collected perirectal wipe for the detection of VRE colonization

Specific Aim 2: Identify the acceptability and feasibility of self-collecting perirectal wipes for VRE colonization screening among participants.

Research Activities

Eligible inpatients at University Hospital in Ann Arbor, MI will be identified, consented and enrolled and immediately instructed on how to self-collect a perirectal wipe for specimen collection immediately following enrollment. Specimens from other body and environmental sites will be collected by researchers and will be processed along with the self-collected specimen for the identification of VRE. These findings will be compared to clinician-collected rectal/perirectal surveillance swabs obtained during the same admission to validate the self-collection method. Participants will also be surveyed regarding the acceptability and feasibility to perform the self-collection. This work will be performed by myself or other investigators whom I will train.

A.3: Research Materials and Methods

Specific Aim 1

Study Setting and Subjects

Fifty subjects will be recruited from the University Hospital in Ann Arbor, MI, part of the University of Michigan Medicine System. As part of the hospital's active surveillance for VRE, rectal/perirectal swabs are used to screen high-risk inpatients for VRE upon admission and subsequent weekly screens given an initial negative screen. Criteria for eligibility include: positive VRE screen, 18 years of age and older, alert mental status, and provision of informed consent to participate in the study. Individuals with perirectal skin disruption or reduced immune function, indicated by neutropenia, will be excluded. Patients with a positive VRE screen will be identified using electronic medical records (MiChart).

Data Collection

Patient data will be abstracted from patient medical records and will include demographics (age, race, gender), patient clinical information (reason for admission, comorbidities, indwelling devices) and surveillance culture information (surveillance organism identification, antibiotic susceptibility profile).

Clinician-collected rectal/perirectal swabs

The sample collection process is outlined in Figure A-1. The clinician-collected rectal/perirectal swabs will be obtained as part of the standard clinical course by hospital staff and processed in the hospital clinical microbiology laboratory per hospital protocol prior to enrollment of the patient in the study. This swab will be considered the gold standard for VRE detection.

Perirectal wipe

Immediately following consent into the study of a VRE positive patient, a member of the study team will instruct the patient on how to collect a sample using a perirectal wipe. Participants will be provided verbal, written and diagrammatic instructions for self-collection. The participant will wipe a moistened square of filter paper over the participant's perirectal region. Following sample collection, each perirectal wipe will be placed in a medium for short-term storage. Specimens will be agitated and cultured on VRESelect within 72 hours of collection. All wipes will be refrigerated at 4°C prior to culturing and frozen at -80°C for long-term storage.

Investigator collected swabs

All swabs to be collected by the investigative team are listed in Table A-1. These swabs will be collected following the participant self-collection of the perirectal wipe. Inguinal swabs may provide another alternative VRE screening method and will be collected. Hand and environmental swabs will be collected to quantify the frequency of their contamination among VRE colonized individuals as both play a role in VRE transmission. Specimens will be placed in a medium for short-term storage and will be cultured on VRESelect within 72 hours of collection. All investigator-collected swabs will be stored in the same manner as the perirectal wipes.

Statistical Considerations

Sensitivity of the perirectal wipe, inguinal swab and hand swab will be determined, using the clinician-collected rectal/perirectal swab for comparison. Patients with a positive VRE screen as identified by the clinician-collected swab will be eligible for the study. However, the results of this screen will not be known for 24-48 hours following the sample collection. This processing time will result in a delay between the collection of this screening swab and the identification and subsequent enrollment into the study of eligible patients. This delay may result in a loss of colonization with VRE in the study participants. However, the conversion rate of positive to

negative VRE screens for inpatients at University Hospital is less than 1% daily (unpublished data), indicating the delay between clinician and self-sampling will have a minimal impact on sensitivity. The positive VRE screen requirement will also limit the statistical analysis to sensitivity only; specificity, positive and negative prediction values and measures of agreement will not be calculated. For an expected sensitivity of 80%, 50 subjects will be required to obtain a confidence interval of ±11.1% with α=0.05 (Figure A-2).[279] A sample size of 50 will be feasible to obtain within six months using the entire hospital population (personal communication, unpublished data). The frequency of hand and environmental contamination will be assessed individually. Environmental contamination data will also be stratified by hand contamination status.

Specific Aim 2

Sample and Data Collection

All subjects included in specific aim 1 will be eligible for specific aim 2. Surveys will occur in University Hospital. Following the collection of all samples, participants will complete five-item Likert scale questions regarding the acceptability and comfort for undergoing self-collection of specimens and the feasibility of the procedure. Each participant's preference of collection method, comparing self- and clinician-collected techniques, will be recorded. Finally, the willingness of participants to participate in a household study, which would include home visits by investigators and swabs of household members and the environment, will be assessed to inform potential future research.

Statistical Considerations

The proportion of participants who identify self-collection as acceptable and feasible to perform will be assessed along with overall screening method preference.

Study Safety

Participant Safety

Risk to the participants will be minimal. There will be a low risk of perirectal abrasions due to the perirectal wipes.

Laboratory Safety

All laboratory analysis will be performed in the laboratory of Dr. Emily Martin at the University of Michigan School of Public Health, a Biosafety Level 2 laboratory specifically designed for the safe analysis of pathogens which pose a moderate hazard to personnel (e.g. VRE). Laboratory work with the pathogen will be conducted in a biological safety cabinet and personnel will wear appropriate personal protective equipment (gloves, goggles, laboratory coats) to minimize the risk of pathogen acquisition.

Figure A-1: Flow Chart of Enrollment and Data Collection for the Study

Table A-1: Investigator-Collected Swabs.

Swab Type	Swab Description
Inguinal	A moistened swab will be moved over the participant's left and right inguinal crease.
Hand	A moistened swab will be moved over the participant's palms, finger webs and fingertips on both hands
Call Button	A moistened swab will be moved over the call button in the participant's room.
Bedrail	A moistened swab will be moved over a predefined 50 cm^2 area of the participant's bedrail.
Phone	A moistened swab will be moved over the phone keypad, speaker and microphone in the participant's room.

$$\frac{1.96^2 * 0.8 * (1 - 0.8)}{0.111^2} = 50$$

Figure A-2: Power Calculation for Sensitivity of Self-Collected Perianal Wipes

Expected Sensitivity: 0.8
Critical Score for Significance Threshold of 0.05: 1.96
Target Sample Size: 50
Confidence Interval Width = 11.1%
Assumes All Patients Have VRE

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