Assessment of Environmental Contamination with Three Pathogens in a Hospital Laundry Facility

Karen E Michael

A dissertation

submitted in partial fulfillment of the

requirements for the degree of

Doctor of Philosophy

University of Washington

2016

Reading Committee:

Marilyn C. Roberts, Chair
Michael Dodd
Noah Seixas

Program Authorized to Offer Degree:

Environmental and Occupational Health Sciences

University of Washington

Abstract

Assessment of Environmental Contamination with Three Pathogens in a Hospital Laundry Facility

Karen E Michael

Chair of the Supervisory Committee:
Marilyn C. Roberts, Professor
DEOHS

Very little is known about the environmental contamination, due to soiled clinical linens, of hospital laundry facilities. To study this environment, an exposure assessment was performed at an industrial clinical laundry facility in Seattle, WA, USA. Surface swab samples (n=240) from the environment were collected from the facility over four time periods in 2015. These samples were cultured for three pathogens; *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Isolates were characterized by various methods including antibiotic resistance, presence of toxin producing genes, Multilocus sequence typing, 16S sequencing and/or SCC*mec* typing. Voluntary participation among the employees consisted of nasal swabs for detection of MRSA, observations during their work and questionnaires. Odds of finding surface contamination with ≥ 1 pathogen were calculated.

Contamination with all three pathogens was observed in the facility in both dirty and clean areas. The dirty area had a higher odds ratio than the clean area for overall contamination (≥1 pathogen) (OR=18.0). The odds ratios of individual pathogen presence varied for *C. difficile* (OR=15.5), MRSA (OR=14.8) and VRE (OR=12.6). The highest odds of finding surface contamination occurred in the primary and secondary sort areas where dirty linens are manually sorted by employees (OR=63.0, $p<0.001$). MRSA nasal contamination was identified in 5/23 (22%) employees, with four out of five working in the dirty area. Improved protocols for prevention and reduction of environmental contamination as well as occupational exposure were implemented as a result of this study.

TABLE OF CONTENTS

Chapter 1. Introduction 8

1.1 *Clostridium difficile* 12

1.2 Methicillin-resistant Staphylococcus aureus (MRSA) 14

1.3 Vancomycin-resistant Enterococcus spp. (VRE) 16

1.4 Hypothesis for the thesis work 18

Chapter 2. *Clostridium difficile* Environmental Contamination within a Clinical Laundry Facility in the USA 19

2.1 Abstract 19

2.2 Introduction 19

2.3 Materials and Methods 21

2.3.1 Laundry Facility Setting 21

2.3.2 Surface Sample Collection 21

2.3.3 Isolation of *C. difficile* 22

2.3.4 PCR for toxin A, toxin B and Multilocus sequence typing (MLST) 22

2.3.5 Statistical Methods 23

2.4 Results and Discussion 23

2.4.1 Isolation of *C. difficile* from environmental surfaces 23

2.4.2 Genetic Characterization of *C. difficile* 24

2.5 Tables 28

2.5.1 Table 2-1 Prevalence of *C. difficile* by location 28

2.5.2 Table 2-2 Prevalence of *C. difficile* by date 29
2.5.3 Table 2-3 Molecular characterization of *C. difficile* isolates 30
2.6 Supplementary Figure 31
2.6.1 Figure S2-1 eBURST 31
2.7 Supplementary Tables 33
2.7.1 Table S2-1 Surface Sample Details 33
2.7.2 Table S2-2. MLST data with associated ribotypes and locations isolated 35
Chapter 3. Methicillin-Resistant *Staphylococcus aureus* (MRSA) Isolates from Surfaces and Personnel at a Hospital Laundry Facility 37
3.1 Abstract 37
3.2 Introduction 38
3.3 Materials and Methods 39
3.3.1 Laundry Facility Setting 39
3.3.2 Surface Sample Collection 40
3.3.3 Personnel Sample Collection 40
3.3.4 Isolation of MRSA 41
3.3.5 Genetic Characterization of MRSA Isolates 42
3.4 Results 43
3.4.1 Isolation of MRSA from environmental surfaces of laundry facility 43
3.4.2 Isolation of MRSA from personnel 44
3.4.3 Characterization of MRSA 44
3.5 Discussion 46
3.6 Figures 49

3.6.1 Figure 3-1 MRSA Antibiotic Resistance Profiles 49
3.7 Tables 50
3.7.1 Table 3-1 Prevalence of MRSA by location (environmental) 50
3.7.2 Table 3-2 Prevalence of MRSA Positive Nasal Isolates Among Laundry Personnel 51
3.7.3 Table 3-3 Molecular characterization of environmental and personnel isolates resistance profiles 52
3.7.4 Table 3-4 Antibiotic susceptibility for environmental isolates 55
3.8 Supplemental Figures 56
3.8.1 Table S 3-1 Surface sample details 56
3.8.2 Table S 3-2 Nasal swab sample results 58
3.8.3 Table S 3-3 Comparison of antibiotic resistance between Laundry and Hospital isolates60
Chapter 4. *van*A Positive Multidrug-Resistant *Enterococcus* spp. Isolated from Surfaces of a US Hospital Laundry Facility 61
4.1 Abstract 61
4.2 Introduction 62
4.3 Methods 63
4.3.1 Laundry Facility Setting 63
4.3.2 Surface Sample Collection 63
4.3.3 Isolation of VRE 64
4.3.4 Genetic Characterization of VRE Isolates 64
4.3.5 Statistical Methods 66

4.4 Results 66
4.4.1 Isolation of VRE from environmental surfaces within the laundry facility 66
4.4.2 Characterization of VRE 67
4.5 Discussion 70
4.6 Conclusions 70
4.7 Tables 72
4.7.1 Table 4-1 Isolation of VRE by location 72
4.7.2 Table 4-2 Molecular characterization of environmental isolates and their resistance profiles 73
4.8 Supplementary Tables 78
4.8.1 Table S 4-1 Surface sample details 78
4.8.3 Table S 4-2 Antibiogram, percentage of resistant isolates collected at the Laundry Facility and UW Hospitals 80
4.9 Supplementary Figures 81
4.9.1 Figure S 4-1 Antibiotic resistance profiles for environmental surface samples 81
4.9.2 Figure S 4-2 eBURST of E. faecium isolates 82
Chapter 5. Assessment of Environmental Contamination with Pathogenic Bacteria at a University Laundry Facility 83
5.1 ABSTRACT 83
5.2 INTRODUCTION 84
5.3 MATERIALS AND METHODS 86
5.3.1 Surface Samples 86
5.3.2 Subject recruitment 87

5.3.3 Observation, Survey and Nasal Samples 88
5.3.4 Laundry Facility 88
5.3.5 Pathogen Isolation 89
5.3.6 Statistical Methods 91
5.4 RESULTS 91
5.4.1 Participant Characteristics 91
5.4.2 Pathogen Isolation from Surfaces 94
5.5 DISCUSSION 95
5.6 CONCLUSIONS 98
5.7 Tables 99
5.7.1 Table 5-1 Observed self-touch data 99
5.7.2 Table 5-2 Prevalence of Surface Contamination by Laundry area and Date of Sample Collection 100
5.7.3 Table 5-3 Probability (Odds Ratio) of Contamination in Dirty Area Compared to the Clean Area 101
5.8 Figures 102
5.8.1 Figure 5-1 Prevalence of surface contamination by location within the laundry facility 102
5.9 Supplementary Tables 103
5.9.1 Table S 5-1 Surface sample details 103
5.9.2 Table S 5-2 Self-reported characteristics of laundry employees by dirty and clean area 105
Chapter 6. Dissertation Discussion 106

6.1 Limitations 109

6.2 Dissertation Conclusion 110

Chapter 7. References 111

Chapter 8. Appendices 128

8.1 Appendix 1 Map of Consolidated Laundry 129

8.2 Appendix 2 Surface Sample Size Calculation 131

8.2.1 Equation 1 131

ACKNOWLEDGEMENTS

The UW Consolidated Laundry Facility provided funds for this research. The management and personnel were instrumental in access to the facility as well as providing additional information about the running of the facility. We would like to thank Ms. Carol O'Hara Manager of Program Operations and Mr. Emmanuel Okoye Director, both from the Consolidated Laundry. Three undergraduates, Jennifer Dankoff, Kyoyi Lee and Elisabeth Lara, were instrumental in assisting with collection and laboratory methods

Chapter 1. INTRODUCTION

In the last 30 years it has become clear that the environment is an important reservoir and transmitter of pathogens ranging from viruses like influenza to antibiotic resistant bacteria like methicillin-resistant *Staphylococcus aureus* (MRSA) [1–10]. There is now evidence of a link between the natural and human built environments, including humans, animals, plants, microbes and their varying ecosystems, and that they should be considered together (One Health concept) [11]. These environments range from the world, as a whole, and specific geographic regions ranging from countries down to city blocks to individual buildings or farms. Both the natural environment, with little to no impact from human activity, and the human built environment impact the health of the eukaryotes (man, animals, plants, etc.), prokaryotes (bacteria and viruses) and archaea living there [12]. The human built environments are where humans and domesticated animals live, work and play [13]. These spaces are important to man because we spend so much of our time there [13]. We now know that the environment in general and changes to the environment specifically impact human, animal and plant health in both the short and long term [14]. In a recent report, The Centers for Disease Control and Prevention (CDC) defined indoor environmental quality as the "quality of a building's environment in relation to the health and wellbeing of those who occupy space within it" [15]. Current studies have suggested that the environment plays a critical role as a source or reservoir of microbes including pathogens in a variety of ecosystems from *Legionella* in air conditioning cooling towers to finding human pathogens amongst soil bacteria to an increased risk of Zika virus in certain parts of the world [16–18]. Even more critical to human health are the built environments associated with health care settings where nosocomial pathogens are common and able to spread among patients [19]. Many of the nosocomial pathogens are able to survive for extended time in the dust and on surfaces [4,20].

One good example is the outbreak of carbapenem-resistant *Klebsiella pneumoniae* (KPC) due to contamination in the air ducts of the National Institutes of Health (NIH) hospital [21].

The health care environment consists of everything from the floors, the air, as well as, equipment used for patient care and electronics used for data storage and point of service medical care. It also includes personnel and their clothes, personal electronics and even small items such as scissors [22]. Every person, whether they are sick or not, has a microbiome made up of commensal and opportunistic bacteria that surrounds them [23,24]. Therefore, it is not surprising that studies have shown that objects in the immediate vicinity of patients are most likely to be contaminated with patients' microbes including microbial pathogens [25–28]. Objects potentially contaminated range from doorknobs, computer keyboards, furniture, medical equipment, pens, cell phones, ties, uniforms, all types of linens, reusable personal protective equipment (PPE) including lab coats, scrubs and masks [6,29,30]. Objects and materials, which are likely to be contaminated with potential pathogens, are called fomites. Contact with these contaminated fomites may promote the transmission and spread of microbes and result in human disease [25,31–35].

Studies have documented that contaminated surfaces in healthcare environments are important source points for transmission of *C. difficile* [36–39], MRSA [4,40,41] and VRE [42,43]. It is also clear that transmission can occur when healthcare workers' hands become contaminated with these pathogens after contact with contaminated surfaces [31,32,25,33–35]. In some cases, touching a contaminated surface has the same risk of hand contamination as touching a patient who is infected with *C. difficile* [44], MRSA [45] and/or VRE [25]. These pathogens have also been isolated from contaminated uniforms and medical devices leading to environmental contamination and infections [46–49].

The level of contamination of the health care environments depends on a number of factors; the severity of patient illness, level of shedding of pathogens, type of microbe, viability of the organism(s) over time in the environment, specific environmental conditions including moisture, temperature and type and level of organic contamination and the frequency of decontamination [20,50]. To measure the contamination levels of specific fomites other factors must be considered including how one detects the microbes (viable vs nonviable methods), frequency of sample collection and the sampling method used to collect the data [50]. This may vary with the microbe of interest because infection dose needed to cause disease can vary from <10 to $>10^8$ CFU [33,51]. Patients with active infections, as well as, asymptotic carriers can shed anywhere from 10^3-10^9 CFUs/ grams of stool for *Clostridium difficile* and vancomycin-resistant *Enterococcus* spp. (VRE) while methicillin-resistant *Staphylococcus aureus* (MRSA) can be shed from the skin [30,52,53].

In 2013, in an effort to outline the threat of antibiotic resistant bacteria in the US, the Centers for Disease Control (CDC) developed three classifications of pathogens; urgent, serious and concerning [54]. Three major pathogens, responsible for human disease; *C. difficile*, MRSA and VRE were included along with 15 other pathogens. *C. difficile* has been classified as an "immediate public health threat that requires urgent and aggressive action" even though it does not have clinically relevant antibiotic resistance [55]. MRSA and VRE have been classified as serious threats which "require prompt and sustained action to ensure that the problems do not grow" [54]. All three pathogens are common in hospital settings and have been increasingly recognized as important community acquired infections [54]. They have the ability to survive on fomites for extended periods of time and may be difficult to remove from the environment by standard cleaning and disinfection protocols [4,20]. To help address this issue healthcare and

veterinary services have begun to add ultraviolet light (UV) irradiation to their cleaning practices [56,57].

Healthcare workers including nurses, doctors, dentists, technicians and others which are in contact with patients, as well as, animal care workers including veterinarians, and other personnel including farm workers who handle sick animals are exposed to pathogens as part of their work [4]. This routine exposure can lead to potentially a higher risk of developing illness and/or colonization as compared to the general public [48,58]. For example, 20.9% of swine farm workers became colonized with livestock-associated MRSA after working with the pigs while primate center researchers have been shown to carry the same strain isolated from both the environment and primates [59,60].

Studies have examined how PPE, standard blood-borne infection control, extensive cleaning procedures and yearly education have been important in reducing the risk of healthcare workers acquiring infections at work [19,61–64]. There are a number of studies that have studied fire fighters and paramedics, who are normally the first to treat patients that end up in the hospital. Studies have found that fire station environments including the fire station surfaces, trucks, bunk gear, and ambulances, equipment, and living area are often contaminated with MRSA [35,65–67]. These studies have shown that environmental contamination in fire stations and ambulances range from 2-10% [35,65–69]. These studies support the idea that environments that have contact with patients could be potential reservoirs for pathogens.

In hospitals, patients colonized or infected with *C. difficile*, MRSA or VRE are identified as "high precaution" patients. This means that barrier precautions including the use of gloves, gowns and additional hand-hygiene practices are required for everyone who enters the room and interacts with these patients [70]. Posted signs make visitors and hospital staff aware of the

additional health hazards associated with these patients. In addition to these mandatory procedures, enhanced terminal room disinfection is now being performed in an increasing numbers of hospitals [2,71]. This involves a thorough scrub down and disinfection of all surfaces with disinfectants and/or UV light in some facilities in the Seattle metropolitan area to reduce the risk of new patients acquiring these pathogens (Mr. J. Dale, Personal communication). In contrast, the soiled linens from these high precautious patients' rooms do not receive special treatment ((Mr. J. Dale, Personal communication, Ms. C. O'Hara, Personal communication). They are placed into dirty laundry bags with other soiled linens from the same ward and sent off to the laundry facility without any identification stating that they may be contaminated with high precaution pathogens. Laundry facilities that process hospital and clinic linens should be considered an extension of the healthcare environment even when the facility is not physically located on site [58,72–75]. Limited studies have been done to assess the potential risk of laundry workers who handle dirty hospital linens. There have been very few documented (12 cases of hepatitis and eight cases of *Salmonella* poisoning) cases of illness related to exposures to soiled linens [48,72,73]. Reports of infections among laundry workers included *Staphylococcus aureus* infections and viral gastroenteritis that was potentially Norovirus [75,76]. In Taiwan, a laundry worker was suspected to be the index case in a SARS viral epidemic within the community [77]. Laundry workers are also at physical risk of cuts and abrasions due to sharps and medical devices left in and among the linens [78]. These medical devices may also be contaminated with infectious body fluids which can cause blood infections [78,79].

1.1 *CLOSTRIDIUM DIFFICILE*

C. difficile is a Gram-positive spore-forming anaerobic rod that was first identified in the feces of healthy infants in 1935 [80]. *C. difficile* can exist in either the vegetative actively growing

state or as a spore which is not actively growing and is used to survive in unfavorable conditions. The spores of *C. difficile* have a tough outer cellular structure [81]. This allows the spores to maintain viability for months to years and to resist acidic conditions, high temperature environments (≥ 71°C), radiation, drying, chemicals and oxygen. In the presence of oxygen the infectious, vegetative cells can remain viable for 24 hours [38,9,82,83]. *C. difficile* spores are far more resistant than vegetative bacteria to common disinfecting chemicals and treatments [84–86]. This environmental hardiness increases the chance of environmental spread [87]. Once environmental conditions improve, the spore will grow back into a vegetative form. *C. difficile* spores are transmitted by fecal-oral route [88] *C. difficile* is an especially important pathogen for immunocompromised patients, the very young and the very old [81]. Changes in the gastrointestinal tract (GIT) flora due to recent use of antibiotics, proton pump inhibitors, antacid medications, hospitalizations and extremes of age all provide the opportunity for *C. difficile* to cause disease [3,89–94].

In the US, *C. difficile* has been identified in 2-3% of healthy adult colons and in 10-25% of hospitalized adults [3,10]. *C. difficile* was originally thought to be a nosocomial pathogen and only in the last decade has it been identified as a contaminant in community settings accounting for 25% of infections [95,96]. *C. difficile* has been identified in the community from fresh and marine water sources, soil, animals used for food and subsequent process meats bought at stores, pets as well as from vegetables [97,98]. *C. difficile* is the most common etiologic agent of hospital acquired diarrhea [99,100]. Disease with *C. difficile* was estimated to cost an excess $1 billion in US health costs in 2011 [54]. A 2012 report determined that the per patient cost of *C. difficile* infection was ~$9,350 [101]. Deaths attributed to *C. difficile* infection increased 400% between 2000 and 2008 [95,54]. Infections can range from asymptomatic carriage to life threatening septicemia [101]. *C.*

difficile can contaminate the patients' skin and bedding and continue to be shed in feces after there are no longer symptoms [102]. Patients are 1.6 to 2.4 times more likely to acquire *C. difficile* if the previous room occupant had *C. difficile* [3,71]. Hence the need for extra cleaning between patients of these rooms.

1.2 METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA)

S. aureus is a Gram-positive catalase-positive cocci that does not form spores and is non-motile [103]. *S. aureus* produces a variety of toxins including TSS toxin-1, and enterotoxins B and C [103,104]. *S. aureus* causes illnesses ranging from pneumonia, toxic shock syndrome (TSS), blood stream infections, skin and wound infections, septicemia and in some cases death [103,105]. *S. aureus* is associated with both clinical and community settings. Over the past 20 years, *S. aureus* has been isolated from community environment including beaches, marine and fresh water as well as sewage treatment fluids, prepared foods, nursing homes, daycares and on money [106–111]. Methicillin-resistant *Staphylococcus aureus* (MRSA) are *S. aureus* that have acquired the *mecA* gene. This gene codes for an alternative penicillin binding protein (PBP2') that confers resistance to all penicillins that are commonly used in *S. aureus* therapy [82]. The *mecA* gene is located on the Staphylococcal Cassette Chromosome *mec* (SCC*mec*) element [113]. Typing of MRSA strains relies on a variety of different traits including the type of SCC*mec* element carried as well as the Multilocus sequence type (MLST) which is a method that relies on partial sequencing of seven housekeeping genes using the Multilocus Sequence Typing databases (www.mlst.net), spa typing (http://spa.ridom.de/index.shtml) which is determined from the order of specific repeats, presence of various toxins, as well as, the characterization of the antibiotic susceptibility pattern and specific antibiotic resistance genes [60,108,114–116]. More recently, comparisons of whole genome sequences of different MRSA strains have been used effectively

to determine relatedness between strains of MRSA[60,66,114–117]. Twenty years ago, specific MLST types were found either in the hospital or the community but rarely both. However, over the last 10 years, the community USA300 strain has replaced both methicillin-susceptible *S. aureus* (MSSA) and other MRSA strains to predominate North American hospital infections [118,119]. USA300 is a common strain in North America that was traditionally associated with community disease that did not carry resistance to other antibiotics, had a different SCCmec type and was rare outside of hospital [119]. In Europe, the EMRSA-15 strain is most common in nosocomial infections and has been reported in community illness [120]. This makes it more difficult to determine the source of the MRSA infection. In the US, MRSA infections are considered community acquired if the patient comes into the hospital with an infection or acquires it within 48 h of admission [109]. If the infection develops > 48 h after admission it is considered hospital acquired [109].

MRSA was first identified in the 1960s where initially it was not a significant issues outside of Australia [121]. However, by 2015, hospital MRSA infections were estimated to cost ~$60,000 per patient in the US [122]. In 2013, there were 72,444 MRSA infections reported in the US [123]. MRSA has overtaken MSSA as the major nosocomial pathogen around the world [120]. In some locations such as Australia, MRSA is also a major community pathogen [121]. This change has been illustrated by a report by the National Healthcare Safety Network in the US that found that MRSA accounted for 54.6% of hospital isolates between 2009 and 2010 [124].

Transfer from a contaminated fomite to and from people's skin is possible in three seconds under laboratory conditions [34,35]. A 1997 study reported that 40% of gloves became contaminated even if there was no direct contact between the HCW and the patient leading investigators to suggest that contact with contaminated surfaces in the room were the source of

contamination [8]. Among patients with MRSA, 40% of bed linens, 22% of bedside tables, 21% of bed railings, 3% of inner door knob to hospital room, 4% of outer door knobs and 30% of hands were identified as contaminated [125].

MRSA can survive in the environment for weeks to months on dry surfaces or in marine and fresh water [35,126]. The rate of survival depends on the bacterial load in the original sample and environmental conditions including temperature and sunlight, etc. [127,128]. In one laboratory study, MRSA was found to survive up to 41 days on glass, 45 days on tile and 60 days on a nonporous countertop [128].

1.3 VANCOMYCIN-RESISTANT *ENTEROCOCCUS* SPP. (VRE)

Enterococcus spp. are catalase negative Gram-positive cocci that are non-spore forming [129]. They are able to live in the presence or absence of oxygen and are salt tolerant [129]. *Enterococcus* spp. are abundant in the gastrointestinal tract of most animals. *Enterococcus* spp. are ubiquitous in the environment and have caused both community and hospital acquired infections [124]. The majority (60%) of hospital associated *Enterococcus* spp. infections in the US are due to *E. faecalis* [124,130–132]. Transfer of contamination occurs via contact with contaminated fomites, environmental surfaces, linens and/or directly with the patient. HCWs' hands have been documented as contaminated even when using gloves while dealing with infected patients [133]. The contamination was detected on fomites and surfaces that were not in direct contact with infected patients such as roommates' bedding [32,129,133]. People who are asymptomatic or presymptomatic can shed VRE thus contaminating their environment [52]. In the US, by 2011, there were an estimated 66,000 infections due to *Enterococcus* spp. annually, with 22% of those infections being fatal [134,135] [105].

The drugs used to treat enterococcal infections have included vancomycin, linezolid, daptomycin, tigecycline, teicoplanin, telavancin and quinupristin/dalfopristin [136–138]. The first vancomycin resistant strain of *Enterococcus faecium* was identified in France in 1986 followed by the first report of VRE infection two years later in US [139]. Isolates that express high level resistance to vancomycin (MIC 32 μg/ml) were considered VRE [141]. Between 2007 and 2010, 60% of hospital acquired *Enterococcus* spp. infections were due to *E. faecalis* and 40% were due to *E. faecium* [132]. Of these *E. faecalis* infections, 14% were vancomycin resistant (VRE) and 87% of the *E. faecium* infections were VRE [132].

The genes that code for vancomycin resistance are the *vanA*, *vanB*, *vanC*, *vanD*, *vanE*, *vanG* *vanL*, *vanM* and *vanN* genes [142,143]. Both *vanA* and *vanB* genes are normally associated with mobile elements and have been reported in both community and hospital acquired *E. faecalis* and *E. faecium* strains [144]. The *vanA* and *vanB* genes are important in clinical disease with *vanA* responsible for the majority of human VRE disease worldwide [143]. However, there are some geographic locations such as Australia and Europe where *vanB* predominates [132,145–147]. The other *van* genes are not normally associated with mobile elements, but are found on the chromosome of specific enterococcal species [143]. *Enterococcus* spp. generally have chromosomal genes which confer innate, low level resistance to aminoglycosides (minimum inhibitory concentrations (MIC) ≤ 500 μg/ml), cephalosporins (MIC ≤ 32 μg/ml) and penicillins (MIC ≤ 16 μg/ml) [148–150]. These genes have rarely been isolated in infections and are more commonly identified in the environment [144].

Treatment of VRE infections lead to higher costs (an additional $81,000) and mortality (17-25% higher) than infections with vancomycin-susceptible *Enterococcus* (VSE) [151,152]. Patients infected with VRE had increased hospitals stays ranging from 17-25 days longer (median length

of stay) than VSE infected patients [151,152]. Risk factors for VRE infection include exposure to antibiotics including, cephalosporins, metronidazole and/or oral glycopeptides, history of HIV/AIDS, drug use, liver transplant and colonization with *E. faecium* [151]. VRE remains stable in the environment ≤ four months and is resistant to bleach cleaning solutions and high temperatures (up to 78°C) [4,153–155]. They are so environmentally stable that enterococci are used as indicators of fecal contamination in tropical marine to temperate fresh water [156,157].

1.4 HYPOTHESIS FOR THE THESIS WORK

The hypothesis of this dissertation was that laundry facility workers, who receive and sort soiled linens, are more likely to come into contact with pathogens due to contamination of the environment than laundry workers who do not work with soiled linens. The pathogens included were *C. difficile*, MRSA and VRE. The study measured surface contamination in both the dirty and clean areas of the laundry facility via surface swab samples, collecting personnel samples for nasal carriage of MRSA, observation of work practices and self-report questionnaires about work practices and demographics. This hypothesis is supported by a few studies which have shown that exposure to soiled linens is associated with illness among laundry workers [48,72,73].

Chapter 2. *CLOSTRIDIUM DIFFICILE* ENVIRONMENTAL CONTAMINATION WITHIN A CLINICAL LAUNDRY FACILITY IN THE USA

2.1 ABSTRACT

Clostridium difficile is both a hospital and community acquired pathogen. The current study determined if *C. difficile* could be cultured from clinical laundry facility surfaces. A total of 240 surface samples were collected from dirty areas (n=120), which handle soiled clinical linens, and from clean areas (n=120), which process and fold the clean linens, within the University of Washington Consolidated Laundry facility in 2015. Sampling was done four times over the course of one year. The dirty area was significantly more contaminated than the clean area (21% vs 2%, $p<0.001$). *C. difficile* isolates were genetically characterized using multilocus sequence typing and PCR for the detection of genes encoding toxin A and toxin B. The MLST types 1, 2, 3, 15, 26, 34, 35, 39, 42, 43, 44, 53, 63 and 284 were identified and have previously been found in both clinical and community settings. Toxin positive isolates were identified in both the dirty (n=16/25) and clean areas (n=2/2). Seasonal variation was observed with 40% of the 27 isolates cultured in April 2015. The study suggests that soiled clinical linens may be a source of *C. difficile* surface contamination.

2.2 INTRODUCTION

Clostridium difficile are spore forming anaerobic bacteria that have been identified in 2-3% of healthy, non-hospitalized adults and in 10-25% of hospitalized adults [3,158]. Toxin producing *C. difficile* is the most common etiologic agent of hospital acquired diarrhea [159]. The genes encoding toxins A (*tcdA*) and B (*tbdB*) are important for the pathogenicity of the bacteria.

Carrying either toxin allows the strain increased virulence and disease potential while *C. difficile* without these toxins are considered nonpathogenic [95,160]. Changes in the gastrointestinal tract flora, due to recent use of antibiotics, proton pump inhibitor, antacids or hospitalizations, may increase the risk of overgrowth and infection with *C. difficile* [92]. *C. difficile* disease has been classified as an "immediate public health threat that requires urgent and aggressive action" by the Centers for Disease Control and Prevention (CDC) [54]. Infections can range from asymptomatic carriage to life threatening septicemia [101]. *C. difficile* infections have been estimated to cost between $1-3 billion in US health care costs [54].

C. difficile was originally thought to be a nosocomial pathogen, but more recently data indicate that it is a community contaminant and a cause of community-acquired disease [95]. It is now estimated that 25% of all *C. difficile* infections occur from exposures in the community with potential sources including water, soil, livestock, meats, vegetables and pets [96–98]. Common community strains in North America include Ribotype 002, 027, 078 and 106 [161] which are associated with Multilocus Sequence Types (MLST) ST8, ST1, ST11 and ST42 respectively [162,163].

C. difficile contamination of linens and room surfaces are of concern since shedding continues even after symptoms of infection are gone [164]. The spores of *C. difficile* are far more resistant than vegetative *C. difficile* to common cleaning agents [84–86]. This makes disinfection more difficult in rooms that have *C. difficile* infected patients, or recently vacated rooms that housed infected patients. Shaughnessy *et al.* (2011) reported that patients were 2.35 X more likely to acquire *C. difficile* if the previous room occupant had a *C. difficile* infection [3].

Very limited information has been published on *C. difficile* contamination of environmental surfaces not directly related to hospitals and clinics. The aim of the current study was to

determine if *C. difficile* could be cultured from surfaces within the University of Washington Consolidated Laundry Facility (UWCLF) and whether the environmental *C. difficile* MLST types were the same as those commonly found in humans.

2.3 MATERIALS AND METHODS

2.3.1 *Laundry Facility Setting*

All samples were collected at the University of Washington Consolidated Laundry (UWCLF) in Seattle, WA. This facility processes linens from six Seattle area hospitals, 30 local outpatient clinics and the Washington National Primate Research Center (WaNPRC). Each week ~300,000 lbs of laundry are processed. The facility is separated into two floors with the majority of the soiled linen handled on the 2nd floor and the clean linen handled exclusively on the 1st floor.

2.3.2 *Surface Sample Collection*

Surface samples were collected from the same surfaces four times (January, April, July and October of 2015), over the course of 12 months. Twenty-five of the 60 samples were composite surface samples which included 2-4 surfaces and 35 samples were collected from a single surface. Samples were collected with Sanicult™ Sterile Neutralizing Buffer Swabs with 5mL of buffer solution (Starplex® Scientific, Etobicoke, Ontario, Canada) and 3M™ Sponge-Sticks with 10mL of buffer solution (3M Co. St. Paul, MN) [35]. Areas sampled included ~10 cm^2 areas for swabs, while the Sponge-Sticks covered an area of ~30 cm^2. The dirty area surface sample sites included; receiving area (n=8), primary sort area (n=8), secondary sort area (n=8) and customer owned goods (COG) area (n=6) for a total of 30 samples at each visit. The clean area sampling sites included; COG washers (n=6), folding area (n=8), processing area (n=8) and break area (n=8). Thirty samples per sampling time with 120 samples total were collected each from the

dirty and clean areas. Table S2-1 illustrates the breakdown of locations in each area and where each sample was collected during each sampling period.

2.3.3 *Isolation of C. difficile*

All samples were transported at room temperature to the laboratory within 4h of collection. Swab samples were vortexed for 5 sec and Sponge-Sticks samples were massaged in their bags for 10 sec to ensure release of the bacteria into the liquid buffer as per manufacturer's directions [35,65]. One mL of liquid from each sample was transferred to anaerobic tubes containing 5ml of Cycloserine Cefoxitin Mannitol Broth with taurocholate and lysozyme (CCMBTAL) (Anaerobe Systems, Morgan Hill, CA USA) media and incubated for 48h at 36.5ºC under anaerobic conditions according to manufacturer's instructions. The CCMBTAL media was chosen because it is formulated for environmental samples, which normally have fewer organisms than clinical samples, and contains cysteine which is an anaerobic culture enhancer [165]. Tubes with a color change from red to yellow/orange were vortexed and 0.1ml were inoculated onto a selective CHROMagar™ *C. difficile* media, (CHROMagar™, Sparks, Maryland, USA) and incubated for 48h at 36.5°C under anaerobic conditions according to the manufacturer's directions. A UV light (360 nm) was used to determine the presence of fluorescence as described by manufacturer. White colonies that fluoresced were Gram stained to verify the typical Gram-positive rods. One *C. difficile* isolate from each positive sample at each time point was stored at -80°C in sterile milk for future characterization.

2.3.4 *PCR for toxin A, toxin B and Multilocus sequence typing (MLST)*

The presence of the genes *tcdA* and *tbdB* was determined by separate PCR assays as described elsewhere [166]. *C. difficile* ATCC 9689, which carries both toxin genes, was used as a

positive control with water as the negative control. The MLST typing used PCR assays for the seven housekeeping genes: *adk, atpA, dxr, glyA, recA, soda* and *tpi* [166]. Amplicons were sequenced as previously described [35]. Alleles were assigned using the *C. difficile* **Multi**Locus Sequence Typing databases (www.mlst.net).

2.3.5 *Statistical Methods*

The Fisher exact test was used to test for significant differences ($P < 0.05$) between all four sampling times and between dirty vs clean areas sampled in the laundry facility.

2.4 RESULTS AND DISCUSSION

2.4.1 *Isolation of C. difficile from environmental surfaces*

In the clean areas, two (2%) of the 120 samples were positive for *C. difficile*. The two positive samples were isolated in January (n=1) and October (n=1) and both were from the COG washer area (Tables 1 and 2). In the remaining samples from the clean areas, *C. difficile* was not detected and listed as negative. Because of the low number of *C. difficile* positive samples obtained there was no statistical difference between levels of *C. difficile* contamination throughout the clean area of the laundry.

In the dirty areas, 25 (21%) of the 120 samples were *C. difficile* positive. Contaminated surfaces including: 1) receiving area; 2) COG area; 3) primary sort area and 4) secondary sort area. The prevalence of *C. difficile* in these areas was 9% (n=3), 17% (n=4), 28% (n=9) and 28% (n=9) respectively (Table 2-1). There was no significant difference in isolation of *C. difficile* within the four locations within the dirty area (p=0.17). The lack of statistical significance may be because the number of positive samples on the dirty side (n=25) did not have the resolution to capture potential statistical differences. Prevalence of *C. difficile* over the

four time periods ranged from 10% (n=3) in January, 40% (n=12) in April, 13% (n=4) in July and 20% (n=6) in October (Table 2-2). April was statistically more likely to have *C. difficile* positive surfaces than either January (p=0.01) or July (p=0.03). There was no significant difference between April and October (p>0.05) levels of contamination. The dirty area of the laundry facility was significantly more contaminated than the clean area (21% vs 2%, p<0.001) and April was the month with the highest *C. difficile* contamination levels (Table 2-2).

There is some evidence that *C. difficile* infections are seasonal. They are correlated with respiratory illness in the winter, following the use of antibiotics [167]. This may explain in part why the levels of contamination were higher in April as compared to January or July. It was also a possibility that the apparent seasonality was due to overall low numbers of *C. difficile* contaminated surfaces and that the increased prevalence of surface contamination in April was a chance occurrence. The samples that consisted of composite surfaces were not statistically different than the samples that were from only 1 surface (p=0.61). Data on *C. difficile* incidence at the hospitals whose linens were processed at UWCLF were not available. However, the incidence of healthcare associated *C difficile* infection was reported to be between 50-160 cases per 100,000 people in 10 different areas within the US [168].

2.4.2 *Genetic Characterization of C. difficile*

Toxin A and/or B genes were present in 64% of isolates from the dirty area (n=16/25), of these 10 isolates carried both genes. In the clean areas both isolates carried both toxin genes. Fourteen different MLST types were identified in the study: 1, 2, 3, 15, 26, 34, 35, 39, 42, 43, 44, 53, 63 and 284 (Table 2-3). The ST types have been associated with various ribotypes which can be found in Table S2-2. However, these relationships are associations and some ribotypes are associated with multiple MLST types [162,169] and were used to help compare MLST types isolated

in this study (Figure S2-1). The two isolates from the clean area were ST42 and ST43. Among the 25 isolates from the dirty areas, ST1, ST2, ST3, ST15, ST26, ST34, ST35, ST39, ST42, ST44, ST53, ST63 and ST284 were identified. The most common MLST types were ST26 (24%, n=6), ST2 (16%, n=4) and ST15 (12%, n=3) (Table 2-3). A clustering analysis was performed using eBURST, which uses a mathematical model to assess relatedness of sequence types submitted to the MLST database, of which the majority are human isolates [166,170] (Figure S2-1). All but ST284 are associated with ≥1 ribotype(s) (Table S2-2).

The *C. difficile* ST26 (n=6) was the most common ST type identified with isolation in April, July and October and from different locations in the primary sort area (n=2), the secondary sort area (n=2) in the receiving area (n=1) and the COG area (n=1). This ST type has been found to cause disease in clinical settings in Europe and the Middle East [171,172]. Three *C. difficile* ST2 were isolated in April, July and October from the primary sort area and one ST2 was isolated in April from the COG area and all four carried both toxin genes. The two isolates cultured in July from the primary sort may represent a single clone. However further genetic characterization would be needed to verify that they represent a single clone. ST2 is a common clinical strain found around the world and one of three ST types from the current study, previously identified in North America [162].

The three ST15 isolates were isolated in January and April from three different areas and differed in carriage of toxin genes. The two isolates in April, one each from the primary and secondary sort areas, carried neither toxin gene, and could be genetically related but more testing would be required and was beyond the scope of the study. The ST15 isolates have been linked to infections in hospitalized adults and in diseased animals in Europe [162,172,173]. ST42 was isolated in July and October in different areas of the dirty laundry area with different toxin gene profiles

(Table 2-3). ST42 is commonly found in clinical and community settings in the UK [162,174,175]. ST44 was isolated in July and October from two different areas of the dirty area and both carried toxin A and B genes [A+, B+]. These ST types are commonly found in UK clinical settings [162] (Table 2-3).

One *C. difficile* ST1 A+B+ was identified in this study. This ST type has been associated with ribotype 027, was described in the early 2000's in Canada, but is now a common clinical strain in the US and Europe [159,162,172,174,176]. C. *difficile* ST3 B+ was isolated from the primary sort in April and has been associated with clinical settings and animals in Japan, Italy and France [162,172]. The ST34 A+ was isolated from the secondary sort in April and has been isolated from human adults in clinical setting in France and the UK [172,175] (Table 2-3). ST35 A+B+ was isolated from the COG area in April and has been reported in the US [174]. ST39 toxin negative was also isolated from the COG area in April and ST53 B+ was isolated from the secondary sort area in April and has been found in the UK [169]. Isolated from the primary sort area in January, ST63 A+ has been identified in the UK [162]. *C. difficile* ST284 B+ was isolated from the receiving are in April. This ST type was originally isolated and typed in 2014 at the University of Oxford, Cambridge UK [177] (www.mlst.net).

This study has a number of limitations including the inherently poor recovery of microbes from environmental surfaces, difficulty in culturing *C. difficile* spores, differences in incubation times and media used [178,179]. All of which could have led to underestimation of true prevalence. The characterization of a single colony from each sample most likely led to an underestimation of the diversity of *C. difficile* present since we most likely characterized the most abundant type from each sample.

It is unlikely that *C. difficile* could have come contaminated linens from the WaNPRC because there have been no outbreaks of *C. difficile* in the primate center (personal communication Dr. J Lane 2016). As expected, the dirty area was much more likely to be contaminated than the clean areas due to the handling of soiled linens. The COG washers are physically located on the clean floor but could actually be considered as part of the dirty area because they wash dirty linens. Additional disinfection measures have now been implemented in the COG washers because of this work (personnel communication Mr. E Okoye, 2016). We did not determine personnel contamination, if employees had diarrhea during the study period or collect fecal samples for culture. However, in a pilot project we found *C. difficile* on the outside cabin door of a facility truck that transports both clean and dirty linens to and from the laundry. This suggests that a potential reservoir of *C. difficile* could include trucks that carry laundry (data not shown) and should be studied in the future. All 14 ST types have been reported among human isolates, but only three of the ST types have previously been identified in the US. Very little data on environmental contamination by *C. difficile*, outside of clinical settings, exists in the literature. This is the first study to examine *C. difficile* environmental contamination in an industrial laundry facility. The study suggests that soiled clinical linens are a potential source of *C. difficile* surface contamination and more studies need to be done to determine if this type of contamination is a risk to laundry workers.

2.5 TABLES

2.5.1 *Table 2-1 Prevalence of C. difficile by location*

Location	Samples collected N	*C. difficile* isolates n (%)
Clean Area		
COG washers	24	2 (8)
Folding	32	0 (0)
Processing	32	0 (0)
Break areas	32	0 (0)
Total	**120**	**2 (2)**
Dirty Area		
Receiving	32	3 (9)
Primary sort	32	9 (28)
Secondary sort	32	9 (28)
COG	24	4 (17)
Total	**120**	**25 (21)**

Table 2-1. Shows the prevalence of *C. difficile* by location isolated from environmental surface samples within the laundry facility.

2.5.2 *Table 2-2 Prevalence of C. difficile by date*

Date	Samples collected N	*C. difficile* isolates n (%)
Clean Area		
January	30	1 (3)
April	30	0 (0)
July	30	0 (0)
October	30	1 (3)
Total	**120**	**2 (2)**
Dirty Area		
January	30	3 (10)
April	30	12 (40)
July	30	4 (13)
October	30	6 (20)
Total	**120**	**25 (21)**

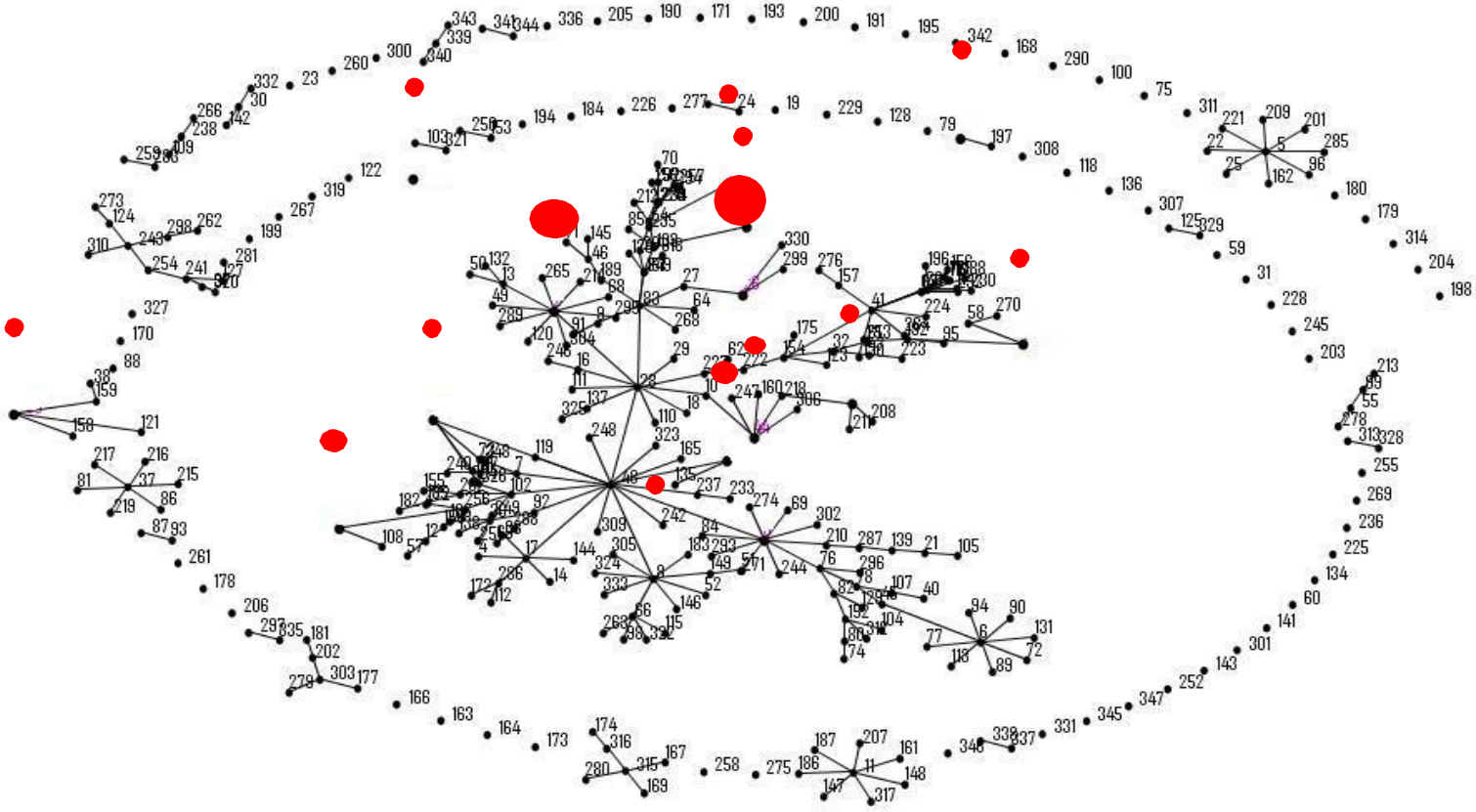
Table 2-2. Shows the prevalence of *C. difficile* by date from environmental surface samples within the laundry facility.

2.5.3 *Table 2-3 Molecular characterization of C. difficile isolates*

Sampling Date	Location	MLST	Clade	Toxin Profile
Clean Area Surfaces				
January	COG Washers	42	1	$A^+ B^+$
October	COG Washers	43	1	$A^+ B^+$
Dirty Area Surfaces				
January	Secondary Sort	1	2	$A^+ B^+$
April	COG	2	1	$A^+ B^+$
July	Primary Sort	2	1	$A^+ B^+$
July	Primary Sort	2	1	$A^+ B^+$
October	Primary Sort	2	1	$A^+ B^+$
April	Primary Sort	3	1	B^+
January	Receiving	15	1	B^+
April	Primary Sort	15	1	neither
April	Secondary Sort	15	1	neither
April	Primary Sort	26	1	neither
July	Secondary Sort	26	1	neither
October	Receiving	26	1	$A^+ B^+$
October	Primary Sort	26	1	neither
October	Secondary Sort	26	1	neither
October	COG	26	1	neither
April	Secondary Sort	34	1	A^+
April	COG	35	1	$A^+ B^+$
April	COG	39	4	neither
July	Secondary Sort	42	1	$A^+ B^+$
October	Primary Sort	42	1	neither
April	Secondary Sort	44	1	$A^+ B^+$
April	Secondary Sort	44	1	$A^+ B^+$
April	Secondary Sort	53	1	B^+
January	Primary Sort	63	1	A^+
April	Receiving	284	NA	B^+

Table 2-3 shows the characterization of the isolates from surface samples. The isolates are broken down by when they were isolated, where they were isolated from, sequence type, clade and presence of toxins A and B.

2.6 SUPPLEMENTARY FIGURE



2.6.1 *Figure S2-1 eBURST*

Figure S2-1 An eBURST map illustrating the relationship between the isolates in the current study with previously identified *C. difficile* isolates by MLST type. Isolated in the current study are red circles with the size of the circle correlating with the number of isolates identified. These include: ST26 (n=6), ST2 (n=4), ST15 (n=3), ST42 (n=2), ST44 (n=2) and one each of ST1, ST3, ST34, ST35, ST39, ST43, ST53, ST63 and ST284.

2.7 SUPPLEMENTARY TABLES

2.7.1 *Table S2-1 Surface Sample Details*

Sample Location	**n**	**Details**	**ST isolated**
Receiving n=8			
Loading Dock	1-2	Ladder handle, door handle of delivery truck, phone handle and face, light switch, and wall dust.	--
Weigh in area	3-4	Phone, computer keyboard, mouse, glove boxes, and operational buttons on scale,	26
Incoming Carts	5-6	Full plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.	--
Glove/gown area	7-8	Surface of table with gloves	15, 284
Primary Sort n=8			
Conveyor Belt	9-16	Safety pins to hold open bags, cart of soiled linens, conveyor belt, trash can at end of belt, and the handle to the stairs.	2, 3, 15, 26, 42, 63
Secondary Sort n=8			
Sorted Dirty Cart	17-18	Empty plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.	26, 34, 44
Wash Deck	19-20	Chute of wash deck, soiled linens waiting to be placed in chute, empty used carts, and bags.	42, 53
Chute 1	21-22	Front, back, and line leading up to chute.	1, 44
Chute 2	23-24	Front, back, and line leading up to chute.	15
COG n=6			

COG	25-30	Sorted and unsorted COG, lost and found items.	2, 26, 35, 39
COG Washers n=6			
COG Washers	31-36	High touch areas (buttons), floor in front of washers, and surfaces of washers.	42, 43
Folding n=8			
Small piece folders	37-38	Scrubs front, on and around folding areas, and carts holding clean unfolded linens.	--
Hand folding	39-41	Linens waiting to be folded, on and around folding areas, conveyor belt bringing clean linens to be folded.	--
Machine Ironers	42-44	On and around folding machine, operation buttons, conveyor belt bringing clean linens to be ironed.	--
Processing n=8			
Sorted Clean Carts	45	Clean lab coats.	--
Clean cart ready to leave	46-47	Cart on way out, top, middle and outside of cart covered in plastic, just washed carts, and open cart with clean linens.	--
Steam tunnel	48-49	High touch areas (buttons).	--
Garment repair	50-52	Sewing machine, surface of table, linens waiting to be repaired, inside, outside and middle of carts holding linens.	--
Break Area n=8			
Locker Room	53-55	Locker handles front, middle and back, and ladies bathroom surfaces.	--
Break Area	56-58	Tables, chairs, phone, water fountain, extinguisher, and kitchen surfaces.	--
Office	59-60	Door handles, duplicating machine buttons, seats, phones, computer keyboards, mice, and waiting room chair.	--

Table S2-1. Characterization of Surface Sample details. Details about where each of the samples were collected in the areas discussed in the text including the receiving, primary sort, secondary sort, COG, COG washers, folding, processing and break areas. --: Denotes that no positive samples, and therefore no ST's, were isolated in these locations.

2.7.2 *Table S2-2. MLST data with associated ribotypes and locations isolated*

Clean Areas

ST	n (%)	Associated Ribotype *	Locations isolated
42	1 (50)	106, 174	COG Washers
43	1 (50)	54	COG Washers

Dirty Areas

ST	n (%)	Associated Ribotype *	Location isolated
1	1 (4)	27	Secondary Sort
2	4 (16)	020, 014, 076, 220	Primary Sort, COG
3	1 (4)	001, 009, 072, 115, 262, 305	Primary Sort
15	3 (12)	70	Receiving, Primary Sort, Secondary Sort
26	6 (24)	140	Receiving, Primary Sort, Secondary Sort, COG
34	1 (4)	56	Secondary Sort
35	1 (4)	46	COG
39	1 (4)	11	COG
42	2 (8)	106, 174	Primary Sort, Secondary Sort
44	2 (8)	15	Secondary Sort
53	1 (4)	103	Secondary Sort
63	1 (4)	53	Primary Sort
284	1 (4)	--	Receiving

Table S2-2 Displays the sequence types, associated ribotypes and locations isolated for all positive samples

*: MLSTs were assigned an associated ribotype as described elsewhere[162,163]

--: There is no known ribotype associated with ST284.

Chapter 3. METHICILLIN-RESISTANT *STAPHYLOCOCCUS AUREUS* (MRSA) ISOLATES FROM SURFACES AND PERSONNEL AT A HOSPITAL LAUNDRY FACILITY

3.1 ABSTRACT

Aim: Examine a clinical laundry facility for the presence of methicillin-resistant *Staphylococcus aureus* (MRSA) on environmental surfaces and among personnel.

Methods: Nasal and face samples along with surface samples were collected four times in 2015. MRSA isolates were confirmed using standardized biochemical assays and molecular characterization.

Results: MRSA was identified in 33/120 (28%) samples from the dirty and 3/120 (3%) samples from the clean environmental areas. MRSA isolates included: (dirty) ST5 SCC*mec* type II, ST8 SCC*mec* type IV, ST 231 SCC*mec* type II, ST239 SCC*mec* type III, ST239 SCC*mec* type IV, ST256 SCC*mec* type IV and (clean) ST5 SCC*mec* type II and ST8 SCC*mec* type IV. Five different employees were MRSA positive, 4/9 (44%) from the dirty: and 1/14 (7%) from the clean but there was a ten-fold higher MRSA carriage 6/22 (27%) dirty vs 1/38 (2.6%) clean when all 50 human samples were combined.

Conclusion: MRSA prevalence was significantly higher (28% vs 3%) in dirty vs clean areas within the laundry facility suggesting a greater risk for personnel on the dirty side.

Significance & Impact of the Study: This is the first report of isolation and characterization of MRSA from surfaces and personnel from a clinical laundry facility.

3.2 INTRODUCTION

Staphylococcus aureus is a common opportunistic bacteria found in people, the community and healthcare settings [106]. *S. aureus* can cause skin, internal infections and death [103,105]. Methicillin-resistant *S. aureus* (MRSA) has acquired the *mecA* gene which confers resistance to all β-lactam antibiotics [113]. *S. aureus* is ubiquitous and can spread easily from person to person, or from the environment [6]. One study reported that 65% of uniforms and 58% of gloves were contaminated with MRSA after healthcare workers treated MRSA positive patients [31].

In hospital rooms with MRSA infected or colonized patients, contamination of sheets, towels and drapes are common [180,181]. Sexton et al. (2006) determined that 54% of the surfaces in hospital rooms with MRSA positive patients were contaminated with MRSA and these included the bed linens [182,183]. There has also been evidence linking environmental MRSA contamination to re-infections in community settings [31,184]. In the community, people who became re-infected with MRSA were more likely (56% vs 38%, p=0.02) to have contamination on surfaces in their home with the same strain as the one they are infected with than people who had no environmental contamination [184].

The aim of the current study was to examine the environment and personnel in a laundry facility that processes linens from six hospitals, 30 clinics, and the WA National Primate Center for the presence of MRSA in both the dirty and clean areas of the facility. A subset of laundry personnel were sampled using nasal and face swabs and the MRSA isolates were compared to those of the laundry environment to assess putative occupational microbial transmission exposure.

3.3 MATERIALS AND METHODS

3.3.1 *Laundry Facility Setting*

Samples were collected at the University of Washington Consolidated Laundry Facility in Seattle, WA. The linens processed include: blankets, cleaning rags, lab coats, mops, patient slings, pillows, pillow cases, scrubs, sheets and wash cloths. Linens came from six Seattle area hospitals, 30 outpatient clinics and the Washington National Primate Research Center (WaNPRC). Approximately 300,000 lbs of laundry are processed each week. Soiled linens are dropped off at a loading dock on the 2nd floor of the facility which is in the "dirty area" of the laundry (Appendix 1). Soiled linen bags are opened and dumped onto a conveyor belt where the linens are then sorted by hand. There is one process line for the laundry owned linens and a 2nd line for customer owned linens (COG). The laundry owns the majority of the textiles which once sorted from the carts are sent down chutes to the 1st floor to be laundered. COG linens are sorted in a separate area and washed in smaller batches in an independent process in a separate area of the facility. The COG linens are manually placed into washers on the 1st floor. Clean linens are dried, sorted, folded, and packaged for delivery exclusively on the first floor "clean area" (Appendix 1).

The laundry facility follows a voluntary mask program with mandatory glove and gown programs on the dirty side. In the dirty area, the floors are swept multiple times a day and mopped once a day. The conveyor belt is also wiped down at least once a day. There are two janitors working 10-12 h a day in the dirty area depending on the workload of the laundry facility. The clean area is also swept and mopped at least once daily. The break area and locker rooms are cleaned once a day after lunch (Ms. O'Hara personal communication).

3.3.2 *Surface Sample Collection*

Composite surface samples from 1 to 4 surfaces were collected, depending on the size of the surface area, as previously described [35]. A total of 35 samples at each time point were collected from a single surface and 25 sample sites included ≥ 2 surfaces [66]. All samples were collected over four times over the course of 12 months (January, April, July and October) of 2015. Samples were collected with Sanicult™ Sterile Neutralizing Buffer Swabs with 5 mL of buffer solution (Starplex® Scientific, Etobicoke, Ontario, Canada) and 3M™ Sponge-Sticks with 10 mL of buffer solution (3M Co. St. Paul, MN) [35]. The dirty area surface sample sites included the receiving area (n=8; sample # 1-8), the primary sort area (n=8; sample # 9-16), the secondary sort area (n=8; sample #17-24) and the COG area (n=6; sample # 25-30). There were a total of 30 samples at each visit for a total of 120 samples. The clean area sampling sites included the COG washers (n=6; sample # 31-36), the folding area (n=8; sample # 37-44), the processing area (n=8; sample # 45-52) and the break area (n=8; sample # 53-60) for each time periods (n=30 per time) with 120 samples overall (Appendix 1). One MRSA isolate per sample was characterized for each time point. Table S 3-1 illustrates the sampling locations within the laundry facility.

3.3.3 *Personnel Sample Collection*

All personnel from both sides of the laundry facility were asked to volunteer for the study. Workers from the dirty side do not work on the clean side and vice versa, but the workers do share the same break room and locker rooms. Each subject signed a consent form. Face and nasal swabs were collected using Sanicult™ Sterile Neutralizing Buffer Swabs with 1 mL of buffer solution (Starplex® Scientific). Both sides of the face, on the outer cheek, were sampled by gently applying swabs to the skin and rubbing in two different directions along the cheeks for no more than five seconds. The rationale for the face samples was the potential of contamination of the face by

contaminated gloves of the workers touching their own face during the work day. Nasal samples were collected as previously described [35]. One MRSA isolate per sample per time point per person was characterized. This study was approved by the University of Washington Institutional Review Board as study #47732.

3.3.4 *Isolation of MRSA*

Samples were transported at room temperature to the laboratory within 4 h of collection. Swab samples were vortexed in their tubes for 5 s, while the bags containing the environmental sponge samples were manually massaged in their bags for 10 s to ensure release of the bacteria into the liquid buffer as per manufacturer's directions [65]. One mL of liquid from all the samples was placed into a new tube containing 1.5 mL of Bacto® m Staphylococcus Broth at 1.5 X concentration (Difco Laboratories, Division BD Sparks, MD), supplemented with 75 μg mL^{-1} polymyxin B and 0.01% potassium tellurite (Sigma-Aldrich, St. Louis, MO) and incubated at 36.5°C with 5% CO_2 [35]. At 48 h, all tubes with growth and black precipitate were streaked for isolation on oxacillin-resistant *S. aureus* Base® media (ORSAB; Oxoid Limited, Basingstoke, England) and incubated at 36.5°C for 48 h [66]. Dark blue colonies were selected and transferred to Brucella agar supplemented with 5% sterile sheep blood (Difco Laboratories, Division BD Sparks, MD) and incubated at 36.5°C in 5% CO_2 for 24 h to test for β- hemolysis [108]. Colonies with β- hemolysis were verified as *S. aureus* with the Staphaurex® test (Remel, Lenexa, Kansas) [35]. All presumptive isolates were verified as MRSA using the Thermo Scientific PBP2' latex agglutination test kit® according to the manufacturer's instructions (Thermo Fisher Scientific Remel Products, Lenexa, KS) [35]. MRSA isolates from each positive sample at each time point were stored in sterile milk at -80°C.

3.3.5 *Genetic Characterization of MRSA Isolates*

3.3.5.1 SSC*mec* typing

SSC*mec* types were determined for all MRSA isolates using SCC*mec* I-V multiplex PCR, as previously described [35]. The SSC*mec* types were confirmed with appropriate single DNA primer sets. Positive and negative controls were used as described elsewhere [66].

3.3.5.2 Multilocus sequence typing (MLST)

MLST typing was performed on all isolates using PCR assays with amplicons sequenced bi-directionally at the University of Washington's Genome Sciences High-Throughput Sequencing facility as previously described [185]. Alleles were assigned by a comparison of their DNA sequences to the corresponding loci in the *S. aureus* Multi Locus Sequence Typing database (www.mlst.net) [185]. A clinical USA300 isolate from Stanford University was used as the positive control, and sterile water was used as a negative control for the PCR assays.

3.3.5.3 Antibiotic Susceptibility

Antibiotic resistance profiles were tested using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (Difco Laboratories, Division BD Sparks, MD) according to Clinical and Laboratory Standards Institute (CLSI) guidelines [141]. Isolates were streaked onto the Mueller-Hinton agar to form a lawn and antibiotic disks were aseptically placed on to the plate for each strain. Plates were incubated for 24 hours at 36.5°C. Isolates were streaked for isolation on Mannitol Salt Agar (EMD Chemical, Gibbstown, NJ, USA) to verify purity. Methicillin-susceptible *S. aureus* ATCC 25923 was used as a negative control. The following antibiotics were examined: GEN: gentamicin, KAN: kanamycin, RIF: rifampin, CIP: ciprofloxacin, NIT: nitrofurantoin, CLI: clindamycin, ERY: erythromycin, CHL: chloramphenicol, Q-D: quinupristin-

dalfopristin, SXT: trimethoprim-sulfamethoxazole, MINO: minocycline and TET: tetracycline. Antibiotic disks were supplied by Becton Dickinson Microbiology Systems (Franklin Lakes, N.J.).

3.3.5.4 Statistical Methods

The Fisher exact test was used to test for significant differences ($P < 0.05$) in environmental contamination between sampling times and between areas sampled within the laundry facility. It was also used to determine differences between personnel colonization from each side of the laundry facility.

3.4 RESULTS

3.4.1 *Isolation of MRSA from environmental surfaces of laundry facility*

In the dirty area, 33/120 (28%) of the environmental samples were MRSA positive. Prevalence of environmental contamination for January, April, July and October were 20% (n=6), 20% (n=6), 37% (n=11) and 30% (n=10) respectively (Table 3-1). No significant difference in MRSA environmental contamination was observed between sampling times (p=0.16). The receiving, COG, primary sort and secondary sort areas had a MRSA prevalence of 6% (n=2), 29% (n=7), 34% (n=11) and 41% (n=13) respectively (Table 3-1). The receiving area was statistically less contaminated than the secondary sort area (p=0.004), the primary sort area (p=0.01) and the COG area (p=0.03).

In the clean area, 3/120 environmental samples (3%) were MRSA positive. This included two samples from the COG washers and one sample from the break area. The folding area and processing area were negative for MRSA. No significant difference in the number of MRSA positive samples was observed in the clean area over the four time periods. In the clean area, The MRSA prevalence for January, April, July and October was 0% (n=0), 3% (n=1), 3% (n=1) and 3%

(n=1) respectively (Table 3-1). No significant difference in the level of MRSA detection was observed (p=1.00).

3.4.2 *Isolation of MRSA from personnel*

Twenty-three different personnel volunteered to participate including; nine people working in the dirty area and 14 people working in the clean area (Table 3-2). Sixty nasal and sixty face samples were taken from subjects who were sampled between one to four times over the course of study at their request (Table S 3-2). None of the face swabs were positive for MRSA. For the nasal swabs, five different employees were MRSA positive; four of the nine (44%) dirty area workers and one of the 14 (7%) clean area workers for a total of seven MRSA isolates. One employee was positive 3 times for nasal MRSA carriage, the others were positive only once or were only sampled once (Table 3-2).

3.4.3 *Characterization of MRSA*

3.4.3.1 MLST and SCC*mec* typing

Environmental MRSA from the dirty area included fourteen ST5 SCC*mec* type II, fourteen ST8 SCC*mec* type IV, two ST231 SCC*mec* type II, one each of ST239 SCC*mec* type III, ST239 SCC*mec* type IV and ST256 SCC*mec* type IV isolates. Environmental MRSA from the clean area included one ST5 SCC*mec* type II and two ST8 SCC*mec* type IV isolates.

Among environmental MRSA isolated from the dirty area, 16 isolates (48.5%) were SCC*mec* type II, 16 isolates (48.4%) were SCC*mec* type IV and one (3%) isolate carried SCC*mec* type III. In the dirty area, ST5 SCC*mec* type II was reported in January and July, ST8 SCC*mec* type IV was isolated in all four time periods, ST231 SCC*mec* type II was isolated in October, both ST239 SCC*mec* type III and ST239 SCC*mec* type IV was isolated in April and ST256 SCC*mec* type IV

was cultured in October. The MRSA isolated from the clean area included two isolates (67%) that were SCC*mec* type IV and one (33%) isolate that carried SCC*mec* type II. ST5 SSC*mec* type II was isolated in July and ST8 SSC*mec* IV was isolated in April and October samples (Table 3-3).

The isolates from personnel in the dirty area were ST5 SCC*mec* type II (n=4, 57%), ST8 SCC*mec* type IV (n=2, 29%) and ST231 SCC*mec* type II (n=1, 14%). ST5 was isolated in January, July and April. The ST8 was isolated in October while the ST231 was isolated in January. From the clean area, one employee carried ST188 SCC*mec* type II in October (Table 3-3).

3.4.3.2 Antibiotic Susceptibility

Antibiotic resistance profiles were determined for 12 antibiotics for all the MRSA isolates (Table 3-4). All MRSA isolates were resistant to at least one other antibiotic class besides penicillins (Table 3-3). There was reduced variation between resistance of environmental (n=36) vs human (n=7) MRSA isolates (Figure 3-1). There were four unique groups among ST5 isolates; A, B, C and D (Table 3-3). Group C consisted of 11 isolates from the dirty area environment area, one environmental isolate from the clean area and one personnel isolate from the dirty area. All ST5 (n=18) isolates were resistant to ciprofloxacin and erythromycin with variable resistance to 89% (n=16) clindamycin, 83% (n=15) kanamycin, 6% (n=1) gentamicin, 6% (n=1) quinupristin-dalfopristin. None of the isolates were resistant to chloramphenicol, minocycline, nitrofurantoin, rifampin, tetracycline or trimethoprim-sulfamethoxazole. Table S 3-3 shows the antibiograms of the surface and personnel samples along with those of two local hospitals that send their linens to the laundry facility.

Among the ST8 isolates, there were 11 unique groups; E through O (Table 3-3). All of the ST8 isolates were from the dirty area environment with the exception of two personnel isolates from the dirty area. There was not a core resistance pattern among the 18 ST8 isolates and resistance levels

varied from 89% (n=16) ciprofloxacin, 72% (n=13) erythromycin, 56% (n=10) clindamycin, 33% (n=6) kanamycin, 22% (n=4) tetracycline, 11% (n=2) gentamicin, 11% (n=2) trimethoprim-sulfamethoxazole, 6% (n=1) nitrofurantoin, 6% (n=1). There was no resistance to chloramphenicol, minocycline, rifampin or quinupristin-dalfopristin. The ST188 isolate from an employee was resistant to ciprofloxacin, erythromycin, kanamycin and tetracycline. There was a core resistance to ciprofloxacin, erythromycin and kanamycin among the three ST231 isolates, while two (67%) were also resistant to clindamycin. No isolates were resistant to chloramphenicol, gentamicin, minocycline, nitrofurantoin, quinupristin-dalfopristin, rifampin, tetracycline and trimethoprim-sulfamethoxazole. There were two different ST239 isolates, one isolate was SCC*mec* III and resistant to ciprofloxacin, clindamycin, erythromycin, kanamycin, tetracycline and trimethoprim-sulfamethoxazole. The second was ST239 SCC*mec* IV and was resistant to erythromycin and kanamycin. The ST256 isolate was resistant to ciprofloxacin, erythromycin, kanamycin and tetracycline (Table 3-4).

3.5 DISCUSSION

This is the first study to examine environmental contamination in an industrial laundry facility for the presence of MRSA or to assess occupational exposure of laundry personnel to MRSA. MRSA was isolated from the environment and personnel from both the dirty and the clean areas of the laundry facility. Environmental MRSA contamination was significantly more likely to be found in the dirty area of the laundry ($p<0.001$) and personnel working on the dirty side were more likely to be colonized with MRSA ($p=0.04$). There was no seasonal difference in MRSA environmental or personnel contamination. The ST5 (n=18) and ST8 (n=18) isolates were the most common MRSA types identified in both environmental and human samples. The ST8 isolates were isolated in all four time periods, however 56% (n=10) of them were isolated in October. In contrast, the ST5

isolates were isolated in January and June (Table 3-3). It is not clear if these results are due to the limited number of isolates or actually represents seasonal differences of particular ST types.

The ST8 isolates had 11 unique resistance patterns compared to the four found among ST5 isolates. Antibiotic resistance genes are normally associated with mobile elements and the *S. aureus* genome can readily acquire and delete its resistance genes [186]. Therefore, there may be fewer than 11 different clones of ST8 and four of ST5 [186]. Even isolates with the same SSC*mec* type and antibiotic susceptibility patterns may represent unique clones.

The three ST231 isolates were cultured in January (n=1) from a personnel sample and in October (n=2) among environmental samples. All isolates had the same core antibiotic resistant pattern and may represent the spread of a single clone. The ST231 are not as common among humans as ST5 or ST8 but have been identified in 1-8% of strains typed in the US [187–189]. The two ST239 isolates were cultured in April, but had different SCC*mec* types and different resistance profiles. Therefore, it is unlikely that they represent a clone. The ST239 strain is pandemic outside of North America, especially in Asia [190].

A single ST188 SSC*mec* type II was cultured from a person working in the clean area. ST188 is almost exclusively found in Pan-Asia and very rarely in North America [191,192]. MRSA ST188 SSC*mec* type IV isolates have been identified from WaNPRC [60]. As a result, there was a concern that this person had acquired the ST188 from working in the laundry, even though there was limited environmental contamination in the clean area and no ST188 in the environmental samples. To determine if the laundry ST188 was closely related to the WaNPRC strains, the isolate was whole genome sequenced and compared to both WaNPRC strains (NCBI Sequence Read Archive, SRA; http://www.ncbi.nlm.nih.gov/sra under accession no. SRP067697) and a previously characterized ST188 SSC*mec* V from Hong Kong (GenBank#JFFV00000000). The laundry personnel ST188

was more closely related to the Hong Kong strain than the WaNPRC strains (unpublished observations; [60]). Based on self-reported surveys (data not shown), the majority of the laundry personnel identify as Pan-Asian immigrants so this in addition to absence of ST188 in the environmental samples reduces the potential that the human ST188 isolate was acquired on the job. The ST256 isolate was cultured in October from an environmental sample. Previously ST256 has been found in local dental settings and fire stations but is not commonly associated with hospital settings [35,68].

The current study suggests that soiled clinical linens are an unrecognized source of hospital bacterial contamination in the laundry facility environment. The dirty area of the facility was more likely to be contaminated (28% vs 3%, $p<0.001$) than the clean areas. The dirty areas could be a potential reservoir for carriage in laundry workers from the dirty side of the facility. Future studies comparing the genomes of the human and environmental MRSA isolates would help to elucidate the relationship between the MRSA from the contaminated laundry environment and those isolated from laundry personnel.

3.6 FIGURES

3.6.1 *Figure 3-1 MRSA Antibiotic Resistance Profiles*

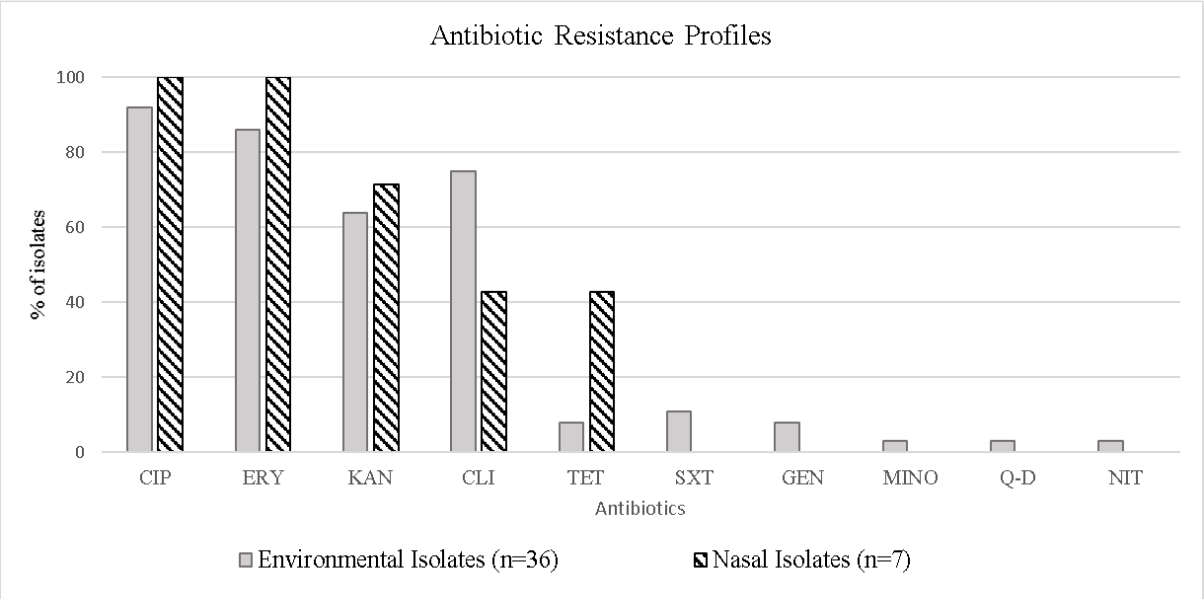


Figure 3-1. The proportion of resistant strains for environmental and nasal isolates. Resistance profiles of environmental and personnel isolates. GEN: gentamicin, KAN: kanamycin, RIF: rifampin, CIP: ciprofloxacin, NIT: nitrofurantoin, CLI: clindamycin, ERY: erythromycin, CHL: chloramphenicol, Q-D: quinupristin-dalfopristin, SXT: trimethoprim-sulfamethoxazole, MINO: minocycline, TET: tetracycline. CHL and RIF were not included because there was no resistance to either antibiotic among any of the environmental or nasal MRSA isolates.

3.7 TABLES

3.7.1 *Table 3-1 Prevalence of MRSA by location (environmental)*

Location	Samples collected N	MRSA isolates n (%)
Dirty Area		
Receiving	32	2 (6)
Primary sort	32	11 (34)
Secondary sort	32	13 (41)
COG	24	7 (29)
Total	**120**	**33 (28)**
Clean Area		
COG washers	24	2 (8)
Folding	32	0 (0)
Processing	32	0 (0)
Break areas	32	1 (3)
Total	**120**	**3 (3)**

Table 3-1 Prevalence of MRSA by location (environmental)Table 3-1. Shows the prevalence of MRSA by location isolated from the environmental samples within the laundry facility.

3.7.2 *Table 3-2 Prevalence of MRSA Positive Nasal Isolates Among Laundry Personnel*

	Sampling Date				
	January	**April**	**July**	**October**	**Total**
Dirty Area					
Total # of MRSA Isolates	2*	1*	1*	2	6
Total # of Employees sampled	9	4	5	4	22
Clean Area					
Total # of MRSA Isolates	0	0	0	1	1
Total # of Employees sampled	12	11	9	6	38

Table 3-2. Reports the prevalence of MRSA positive nasal isolates among laundry personnel who volunteered to participate in the study. Some of the employees were sampled more than once which is why the isolate numbers are higher than the number of employees sampled.

* The same person was positive for MRSA in January, April and July. The other isolates came from separate individuals. A total of 7 positive isolates from 5 people were collected.

3.7.3 *Table 3-3 Molecular characterization of environmental and personnel isolates resistance profiles*

Isolate #	Sampling Date	Location	MLST	SCC*mec* Type	Resistance Profile *	Unique Group
Dirty Area Surfaces						
721	January	Secondary Sort	5	II	CIP, CLI, ERY	A
703	January	Primary Sort	5	II	GEN, CIP, CLI, ERY	B
708	January	Primary Sort	5	II	KAN, CIP, CLI, ERY	C
901	July	Primary Sort	5	II	KAN, CIP, CLI, ERY	C
902	July	Primary Sort	5	II	KAN, CIP, CLI, ERY	C
904	July	Primary Sort	5	II	KAN, CIP, CLI, ERY	C
905	July	Primary Sort	5	II	KAN, CIP, CLI, ERY	C
908	July	Primary Sort	5	II	KAN, CIP, CLI, ERY	C
909	July	Secondary Sort	5	II	KAN, CIP, CLI, ERY	C
911	July	Secondary Sort	5	II	KAN, CIP, CLI, ERY	C
915	July	COG	5	II	KAN, CIP, CLI, ERY	C
918	July	COG	5	II	KAN, CIP, CLI, ERY	C
929	July	Receiving	5	II	KAN, CIP, CLI, ERY	C
715	January	COG	5	II	CIP, CLI, ERY, Q-D	D
1022	October	Secondary Sort	8	IV	No resistance	E
722	January	Secondary Sort	8	IV	CIP	F
811	April	Secondary Sort	8	IV	CIP	F
1018	October	COG	8	IV	ERY	G

822	April	Secondary Sort	8	IV	CIP, ERY	H
812	April	Secondary Sort	8	IV	CIP, SXT	I
1004	October	Primary Sort	8	IV	GEN, KAN, CIP	J
921	July	Secondary Sort	8	IV	KAN, CIP, CLI, ERY	K
1008	October	Primary Sort	8	IV	KAN, CIP, CLI, ERY	K
1029	October	Receiving	8	IV	KAN, CIP, CLI, ERY	K
709	January	Secondary Sort	8	IV	CIP, CLI, ERY, SXT	L
1012	October	Secondary Sort	8	IV	CIP, CLI, ERY, TET	M
920	July	COG	8	IV	GEN, KAN, CIP, CLI, ERY	N
1010	October	Secondary Sort	8	IV	CIP, CLI, ERY, MINO, TET	O
1006	October	Primary Sort	231	II	KAN, CIP, CLI, ERY	P
1007	October	Primary Sort	231	II	KAN, CIP, CLI, ERY	P
813	April	Secondary Sort	239	III	KAN, CIP, CLI, ERY, SXT, TET	Q
819	April	COG	239	IV	KAN, ERY	R
1020	October	COG	256	IV	KAN, CIP, CLI, ERY	S
Clean Area Surfaces						
957	July	Break Area	5	II	KAN, CIP, CLI, ERY	C
1033	October	COG Washers	8	IV	CIP, CLI, ERY	T
832	April	COG Washers	8	IV	KAN, CIP, NIT, ERY	U
Dirty Area Personnel						
1[†]	April	Primary Sort	5	II	KAN, CIP, ERY	V

2	January	Primary Sort	5	II	KAN, CIP, ERY	V
3†	July	Primary Sort	5	II	KAN, CIP, CLI, ERY	C
4	October	Primary Sort	8	IV	CIP, CLI, ERY, TET	M
5	October	Primary Sort	8	IV	CIP, CLI, ERY, TET	M
6†	January	Primary Sort	231	II	KAN, CIP, ERY	W
Clean Area Personnel						
7	October	Clean Room	188	II	KAN, CIP, ERY, TET	X

One MRSA isolate/sample listed in the table

Table 3-3 shows the characterization of the isolates from both the environment and the personnel. The isolates are broken down by when they were isolated, sequence type, SCC*mec* type and what antibiotics they are resistant to.

* GEN: gentamicin, KAN: kanamycin, RIF: rifampin, CIP: ciprofloxacin, NIT: nitrofurantoin, CLI: clindamycin, ERY: erythromycin, CHL: chloramphenicol, Q-D: quinupristin-dalfopristin, SXT: trimethoprim-sulfamethoxazole, MINO: minocycline,
TET: tetracycline

† All from same person

3.7.4 *Table 3-4 Antibiotic susceptibility for environmental isolates*

	Antibiotics*											
	GEN n (%)	**KAN n (%)**	**RIF n (%)**	**CIP n (%)**	**NIT n (%)**	**CLI n (%)**	**ERY n (%)**	**CHL n (%)**	**Q-D n (%)**	**SXT n (%)**	**MINO n (%)**	**TET n (%)**
Dirty Area Surfaces												
# Susceptible	30 (91)	8 (24)	32 (97)	1 (3)	30 (91)	6 (18)	1 (3)	22 (67)	28 (85)	24 (73)	30 (91)	30 (91)
# Intermediate	0 (0)	4 (12)	1 (3)	2 (6)	3 (9)	2 (6)	4 (12)	11 (33)	4 (12)	6 (18)	2 (6)	0 (0)
# Resistant	3 (9)	21 (64)	0 (0)	30 (91)	0 (0)	25 (76)	28 (85)	0 (0)	1 (3)	3 (9)	1 (3)	3 (9)
Clean Area Surfaces												
# Susceptible	3 (100)	0 (0)	3 (100)	0 (0)	2 (67)	1 (33)	0 (0)	1 (33)	2 (67)	3 (100)	3 (100)	3 (100)
# Intermediate	0 (0)	1 (33)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	2 (67)	1 (33)	0 (0)	0 (0)	0 (0)
# Resistant	0 (0)	2 (67)	0 (0)	3 (100)	1 (33)	2 (67)	3 (100)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
Dirty Area Personnel (Nasal Isolates)												
# Susceptible	6 (100)	1 (17)	6 (100)	0 (0)	4 (67)	3 (50)	0 (0)	4 (67)	5 (83)	5 (83)	5 (83)	3 (50)
# Intermediate	0 (0)	1 (17)	0 (0)	0 (0)	2 (33)	0 (0)	0 (0)	2 (33)	1 (17)	1 (17)	1 (17)	0 (0)
# Resistant	0 (0)	4 (67)	0 (0)	6 (100)	0 (0)	3 (50)	6 (100)	0 (0)	0 (0)	0 (0)	0 (0)	3 (50)
Clean Area Personnel (Nasal Isolates)												
# Susceptible	1 (100)	0 (0)	1 (100)	0 (0)	1 (100)	1 (100)	0 (0)	1 (100)	1 (100)	1 (100)	1 (100)	0 (0)
# Intermediate	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)	0 (0)
# Resistant	0 (0)	1 (100)	0 (0)	1 (100)	0 (0)	0 (0)	1 (100)	0 (0)	0 (0)	0 (0)	0 (0)	1 (100)

Table 3-4 displays the number and percentage of the isolates from both the environment and the personnel that are susceptible, intermediate and resistant to the antibiotics tested in this study.

* GEN: gentamicin, KAN: kanamycin, RIF: rifampin, CIP: ciprofloxacin, NIT: nitrofurantoin, CLI: clindamycin, ERY: erythromycin, CHL: chloramphenicol, Q-D: quinupristin-dalfopristin, SXT: trimethoprim-sulfamethoxazole, MINO: minocycline, TET: tetracycline.

3.8 SUPPLEMENTAL FIGURES

3.8.1 *Table S 3-1 Surface sample details*

Sample Location	Sample ID	Details
		Receiving n=8
Loading Dock	1-2	Ladder handle, door handle of delivery truck, phone handle and face, light switch, and wall dust.
Weigh in area	3-4	Phone, computer keyboard, mouse, glove boxes, and operational buttons on scale
Incoming Carts	5-6	Full plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.
Glove/gown area	7-8	Surface of table with gloves
		Primary Sort n=8
Conveyor Belt	9-16	Safety pins to hold open bags, cart of soiled linens, conveyor belt, trash can at end of belt, and the handle to the stairs.
		Secondary Sort n=8
Sorted Dirty Cart	17-18	Empty plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.
Wash Deck	19-20	Chute of wash deck, soiled linens waiting to be placed in chute, empty used carts, and bags.
Chute 1	21-22	Front, back, and line leading up to chute.
Chute 2	23-24	Front, back, and line leading up to chute.
		COG n=6
COG	25-30	Sorted and unsorted COG, lost and found items.
		COG Washers n=6
COG Washers	31-36	High touch areas (buttons), floor in front of washers, and surfaces of washers.
		Folding n=8
Small piece folders	37-38	Scrubs front, on and around folding areas, and carts holding clean unfolded linens.
Hand folding	39-41	Linens waiting to be folded, on and around folding areas, conveyor belt bringing clean linens to be folded.
Machine Ironers	42-44	On and around folding machine, operation buttons, conveyor belt bringing clean linens to be ironed.
		Processing n=8
Sorted Clean Carts	45	Clean lab coats.
Clean cart ready to leave	46-47	Cart on way out, top, middle and outside of cart covered in plastic, just washed carts, and open cart with clean linens.
Steam tunnel	48-49	High touch areas (buttons).
Garment repair	50-52	Sewing machine, surface of table, linens waiting to be repaired, inside, outside and middle of carts holding linens.
		Break Area n=8
Locker Room	53-55	Locker handles front, middle and back, and ladies bathroom surfaces.

Break Area	56-58	Tables, chairs, phone, water fountain, extinguisher, and kitchen surfaces.
Office	59-60	Door handles, duplicating machine buttons, seats, phones, computer keyboards, mice, and waiting room chair.

Table S 3-1. Characterization of Surface Sample details. Details about where each of the composite samples were collected in the areas discussed in the text including the receiving, primary sort, secondary sort, COG, COG washers, folding, processing and break areas.

3.8.2 *Table S 3-2 Nasal swab sample results*

EMPLOYEE	WORK AREA	JANUARY	APRIL	JULY	OCTOBER
1	Primary Sort	Neg	--	--	--
2	Primary Sort	Neg	--	--	--
3	Primary Sort	**MRSA**	--	--	Neg
4	Primary Sort	Neg	Neg	Neg	**MRSA**
5	Primary Sort	Neg	--	--	--
6	Primary Sort	Neg	Neg	Neg	**MRSA**
7	Secondary Sort	Neg	Neg	Neg	--
8	Secondary Sort	**MRSA**	**MRSA**	**MRSA**	--
9	COG	Neg	--	Neg	Neg
10	COG Washers	Neg	Neg	--	--
11	Folding	Neg	Neg	Neg	--
12	Folding	Neg	Neg	Neg	--
13	Processing	Neg	Neg	--	--
14	Processing	Neg	Neg	--	--
15	Clean Room	--	--	Neg	**MRSA**
16	Clean Room	Neg	Neg	Neg	Neg
17	Clean Room	Neg	Neg	--	Neg
18	Clean Room	--	--	Neg	--
19	Clean Room	Neg	Neg	Neg	Neg
20	Clean Room	Neg	Neg	--	Neg
21	Management	Neg	Neg	Neg	Neg
22	Management	Neg	--	Neg	--
23	Maintenance	Neg	Neg	Neg	--

MRSA: Nasal swab was positive for MRSA, Neg: nasal swab was negative, --: employee declined sampling

Table S 3-2. Nasal swab sample results. Details about when the subjects volunteered to be sampled and the result of the sample. MRSA: Nasal swab was positive for MRSA, Neg: nasal swab was negative, --: employee declined sampling

3.8.3 *Table S 3-3 Comparison of antibiotic resistance between Laundry and Hospital isolates*

Location	**Antibiotics***				
	NIT (%)	**CLI (%)**	**ERY (%)**	**SXT (%)**	**TET (%)**
HMC†	4	37	90	14	10
UWMC‡	13	52	89	11	9
UWCL§	0	43	100	0	43
Environmental Surfaces	3	75	86	8	8

* NIT: nitrofurantoin, CLI: clindamycin, ERY: erythromycin, SXT: trimethoprim-sulfamethoxazole and TET: tetracycline.

† HMC: Harborview Medical Center

‡ UWMC: University of Washington Medical Center

§ UWCL: University of Washington Consolidated Laundry Personnel

Table S 3-3. Comparison of antibiotic resistance between laundry and hospital isolates. Details about the resistance of five antibiotics tested among surface and personnel samples and two hospitals that send their linens to the laundry facility [193].

Chapter 4. *VANA* POSITIVE MULTIDRUG-RESISTANT *ENTEROCOCCUS* SPP. ISOLATED FROM SURFACES OF A US HOSPITAL LAUNDRY FACILITY

4.1 ABSTRACT

Background: *Enterococcus* spp. are a normal part of the gastrointestinal tract of humans and animals. *Enterococcus* spp. are important pathogens and were responsible for 14% of US nosocomial infections between 2007-10.

Aim: Examine a laundry facility that processes clinical linens for the presence and seasonality of vancomycin resistant *Enterococcus* spp.

Methods: Surface samples were collected four times in 2015 from the dirty and clean areas of the laundry facility. Isolates were confirmed using biochemical assays and molecular characterization including multilocus sequence typing (MLST), detection of acquired *vanA* and *vanB,* and/or intrinsic *vanC1* genes by PCR, determination of antibiotic susceptibility and eBURST analysis.

Findings: Seventy-four *vanA* positive multidrug-resistant *Enterococcus* spp. were identified including 64/120 (53%) in the dirty areas and 10/120 (8%) in the clean areas. *E. faecium* isolates identified included 14 ST types (ST16, 17, 18, 117, 186, 280, 324, 412, 584, 664, 665, 736, 750 and 1038). Both *E. faecalis* isolates were ST109.

Conclusion: Multidrug resistant VRE isolates was significantly higher (53% vs 8%) in dirty vs clean areas within the facility.

Significance & Impact of the Study: This is the first study to examine environmental contamination in an industrial laundry facility for the presence of VRE and may be an unrecognized reservoir.

4.2 INTRODUCTION

Enterococcus spp. are part of the normal flora of human, other mammals, birds, reptiles and insects' gastrointestinal tracts [132,144]. They are ubiquitous in the environment with the ability to survive for weeks [42]. *Enterococcus* spp. accounted for 14% of hospital acquired infections (HAI) in 2009-10. In 2011, the US had ~66,000 hospital *Enterococcus* spp. infections with 22% fatality rate [105,124]. *Enterococcus* spp. can be difficult to treat because they carry innate low to moderate level resistance to aminoglycosides (minimum inhibitory concentrations (MIC) $\leq$ 500 μg/ml), cephalosporins (MIC $\leq$ 32 μg/ml) and penicillins (MIC $\leq$ 16 μg/ml) [148,150,149].

In the US, between 2007-10, 60% of hospital acquired *Enterococcus* spp. infections were due to *E. faecalis* and 40% were due to *E. faecium* [132]. Of the *E. faecalis* infections 14% were vancomycin resistant (VRE) and 87% of the *E. faecium* infections were VRE with MIC levels of vancomycin $\geq$ 32 μg/ml [132,141]. Vancomycin resistant infections leads to higher costs and mortality than infections with vancomycin susceptible *Enterococcus* spp. (37-52% vs. 16-27%) [151,152]. Patients infected with VRE have increased hospital stays ranging from 20-25 days longer [151,152]. Treatment of VRE infections requires the use of newer antibiotics costing $2,500-$6,400/treatment vs vancomycin treatment of ~$115/treatment in the USA [136]. The Centers for Disease Control and Prevention (CDC) has classified VRE as a serious threat which "requires prompt and sustained action to ensure that the problems do not grow" [54]. VRE positive patients shed VRE and contaminate their environment, and may lead to the transfer of VRE from contaminated surfaces to hands of workers or to the next patient to occupy the room [4,194].

The aim of this study was to determine if VRE could be isolated from surfaces within the laundry facility that processes clinical linens and if the level of VRE contamination varied over the year.

4.3 METHODS

4.3.1 *Laundry Facility Setting*

Samples were collected in Seattle, WA at the University of Washington owned and operated Consolidated Laundry. Laundry comes from six Seattle area hospitals, 30 outpatient clinics and the Washington National Primate Research Center (WaNPRC). The dirty linens enter the loading dock on the 2nd floor of the building which is considered the dirty area of the laundry. There they are hand sorted before heading down to the clean 1st floor area. The majority of the linens are sorted into a standard laundry-owned line, while other linens go into a second processing line for customer-owned goods and linens (COG). The later are manually placed into washers on the 1st floor, while the standard linens are dropped into automated tunnel washers. All clean linens are dried, folded, and packaged for delivery in clean area (Appendix 1 Map of Consolidated Laundry). The laundry facility uses universal contact precautions with all soiled linens regardless of their source or contamination level.

4.3.2 *Surface Sample Collection*

Twenty-five of the 60 samples were composite surface samples, which included 2-4 surfaces, and 35 single-surface samples were collected as previously described (Table S 4-1) [35,115]. Samples of the same surfaces were collected in January, April, July and October of 2015 using either 5 ml Sanicult™ Sterile Neutralizing Buffer Swabs (Starplex® Scientific, Etobicoke, Ontario, Canada) or a 10 ml 3M™ Sponge-Sticks (3M Co. St. Paul, MN) [35]. There were 240

samples collected in the study, with 30 samples each collected in the dirty and clean areas each time. Dirty area samples from the 2nd floor were collected from the receiving area, the primary sort area, the secondary sort area and the customer owned goods (COG) area (Appendix 1). Clean area samples, primarily from the 1st floor, included the COG washers, the folding area, the processing area and the break area as previously described [115].

4.3.3 *Isolation of VRE*

All samples were transported at room temperature to the laboratory ≤ 4 h after collection and processed as previously described [115]. An enrichment culture used one mL of liquid from the environmental sample into 1 mL of Difco™ Brain Heart Infusion broth (BHI) (Difco Laboratories, Sparks, MD) with 20 µg/mL of aztreonam and 6 µg/mL of vancomycin then incubated at 41°C with 5% CO_2 until turbidity was visible (24-72 h). Turbid samples were transferred onto m-Enterococcus™ agar (Difco Laboratories, Division BD Sparks, MD) supplemented with 6 µg/mL vancomycin, and incubated at 41°C for 24-48 h [195]. Pink or maroon colonies were counted, isolated and transferred to BHI plates supplemented with 18 µg/mL vancomycin and incubated at 41°C for 24-48 h [149]. White colonies were Gram stained for verification. One VRE isolate from each positive sample at each time point was stored at -80°C in sterile milk. In this study, vancomycin resistance was defined by growth on media supplemented with 18 µg/mL vancomycin (BHI_{van18}) as detailed elsewhere [144].

4.3.4 *Genetic Characterization of VRE Isolates*

4.3.4.1 PCR

All isolates that grew on the BHI_{van18} plates were screened for *vanA*, *vanB* and *vanC1* genes as previously described using characterized PCR primers and conditions [149]. 16S ribosomal

sequencing species identification was performed on isolates that were positive for *vanA*, *vanB* and/or *vanC1* genes as previously described [149]. The 16S sequence comparisons were performed using the National Center for Biotechnology (NCBI) Blast algorithm (http://blast.ncbi.nlm.nih.gov/Blast.cgi). *E. faecalis* T32605, collected by the Roberts Lab previously, was used as a control for *vanA*, *E. faecium* ATCC 512699 was used as a control for *vanB* and an environmental *E. gallinarum* was used as a *vanC1* controls for all assays as previously described[149]. Sterile water was used as negative control.

4.3.4.2 Antibiotic Susceptibility

Antibiotic resistance profiles were determined using the Kirby–Bauer disk diffusion method on Mueller–Hinton agar (Difco Laboratories, Division BD Sparks, MD) as previously described by the Clinical and Laboratory Standards Institute [141]. *S. aureus* strain ATCC 25923 was used as a negative control. The following antibiotics were tested: ciprofloxacin (CIP), chloramphenicol (CHL), clindamycin (CLI), erythromycin (ERY), linezolid (LIN), quinupristin-dalfopristin (Q-D), minocycline (MINO), tetracycline (TET), and nitrofurantoin (NIT). Antibiotic disks were supplied by Becton Dickinson Microbiology Systems (Franklin Lakes, N.J.). Antibiotic susceptibility was compared to publicly available antibiograms from Harborview Medical Center (HMC) and the University of Washington Medical Center (UWMC) (https://depts.washington.edu/labweb/Divisions/Micro/antibiograms/2015_antibiogram.pdf). HMC is a level 1 trauma and burn center that services Washington, Alaska, Idaho and Montana with 413 beds and 17,000 admissions and 247,350 clinic visits in 2014 [196]. UWMC is known for its teaching and surgery and had 450 beds, 17,915 admissions and 300,487 clinic visits in 2012 [197].

4.3.4.3 Multilocus sequence typing (MLST)

MLST typing was preformed using PCR assays with amplicons sequenced bi-directionally at the University of Washington's Genome Sciences High-Throughput Sequencing facility and at Operon (Eurofins MWG Operon; Huntsville, Alabama) for *E. faecium* and *E. faecalis* as previously described [149]. Alleles were assigned as described by the Multilocus Sequence Typing databases (www.mlst.net). *E. faecalis* T32605 and *E. faecium* ATCC 512699 were used as positive controls, and sterile water was used as a negative control for all PCR assays. An eBURST analysis was performed on all ***E.*** *faecium* isolates to compare them with previously characterized strains. The eBURST analysis uses a mathematical model to assess relatedness of sequence types submitted to the MLST database and provide a clustering analysis using previously characterized isolates [170].

4.3.5 *Statistical Methods*

The Fisher exact test was used to test for significant differences ($P < 0.05$) between all four sampling times and between areas sampled in the laundry facility.

4.4 RESULTS

4.4.1 *Isolation of VRE from environmental surfaces within the laundry facility*

VRE was isolated in 64 out of 120 (53%) samples from the dirty area, with isolation of 53% (n=16), 57% (n=17), 53% (n=16) and 50% (n=15) respectively for January, April, July and October (Table 4-1). There was no seasonal difference in isolation (p=0.97). The prevalence of VRE varied from the receiving area 19% (n=6), COG 50% (n=12), secondary sort 69% (n=22) and primary sort 75% (n=24) (Table 4-1). The primary and secondary sort areas were more contaminated than the receiving area ($p<0.001$). The COG area was more contaminated than the

receiving area (p=0.02). No statistical difference was observed between the secondary and primary sort areas (p=0.58).

In the clean area, 10/120 (8%) environmental samples were VRE positive. All positive samples were from the COG washer area. The VRE isolation rates ranged from 10% for January, April and October and 3% in July. No significant difference in the VRE detection was observed (p=0.97). This observation could be due to low numbers of VRE positive samples identified in the study. The dirty area was statistically more contaminated than the clean area (64/120 vs 10/120, p<0.001).

4.4.2 *Characterization of VRE*

4.4.2.1 Enterococcus Speciation

Three species were identified in this study; 71 *E. faecium*, two *E. faecalis* and one *E. hirae*. Sixty-one (86%) *E. faecium* isolates were isolated from the dirty area and 10 isolates (14%) were from the clean area. Both *E. faecalis* isolates were isolated from the dirty area as was the *E. hirae* isolate (Table 4-2). All isolates carried only the *vanA* gene. The locations of the *vanA* genes were not determined, however most are located on conjugative plasmids [198]

4.4.2.2 Antibiotic Susceptibility

All *E. faecium* and *E. faecalis* isolates to the nine antibiotics were multi-drug resistant (Figure S 4-1). Of the 61 *E. faecium* isolates from the dirty area 89% (n=54) were resistant to CIP, 25% (n=15) to CHL, 90% (n=55) to CLI, 79% (n=48) to ERY, 26% (n=16) to LIN, 33% (n=20) to Q-D, 26% (n=16) to MINO, 77% (n=47) to TET and 13% (n=8) to NIT. The ten *E. faecium* from the clean area were resistant to CIP (70%, n=7), CHL (30%, n=3), CLI (90%, n=9), ERY (60%, n=6), LIN (40%, n=4), Q-D (50%, n=5), MINO (50%, n=5), TET (80%, n=8)

and NIT (70%, n=7). There were no significant differences in resistance levels between the clean and dirty areas (all p-values >0.05).

The resistance levels for *E. faecium* isolates were compared to the 2015 antibiograms from two local hospitals that send their linens to the laundry facility: Harborview Medical Center (HMC) and UW Medical Center (UWMC). Resistance to ERY and TET was > 74% for all locations. LIN was lower than 30% for all three locations, while NIT resistance was higher (73%) at HMC versus UWMC (51%) and the laundry facility (21%).

One *E. faecalis* isolate was resistant to CIP, CLI, ERY, Q-D and TET. The second isolate was resistant to CIP, LIN and TET. The *E. hirae* isolate was resistant to CIP, CLI, ERY, LIN and Q-D. It was difficult to compare the resistance patterns for the *E. faecalis* isolates found in the study and those from HMC and UWMV due to the low numbers (n=2) found in the current study. These data are presented as a reference and not as a side by side comparison. The samples included in the antibiograms are clinical samples and the samples in the current study are surface swabs (Table S 4-2). No hospital resistance data was available for *E. hirae* isolates.

4.4.2.3 MLST

VRE from the dirty area identified 15 different MLST types; ST18 (n=23), ST736 (n=13), ST664 (n=4), three each of ST17, ST117, ST186, ST280 and ST412, one each of ST16, ST324, ST584, ST665, ST750 and ST1038. VRE from the clean area included five different MLST types; ST736 (n=5), ST18 (n=2), and one each of ST117, ST412 and ST664. The two *E. faecalis* isolates were ST109. Isolates with the same ST and antibiotic resistance profile were assigned a unique group letter (Table 4-2). The three most common sequence types isolated were ST18, ST736 and ST664. All three ST types were observed in both the clean and dirty areas of the laundry (Table 4-2).

There were 42 unique *E. faecium* strains (A-PP) based on MLST and antibiotic resistance profile (Table 4-2). All of the ST18 isolates were resistant to CLI and isolated during all four sampling times but consisted of 10 different antibiotic susceptibility groups (E-N). For ST736, there were eight groups (DD-II and OO-PP). The majority (n=11, 61%) of the isolates were isolated in October (Table 4-2). The ST736 isolates accounted for half (n=5) of the isolates from the clean area. ST664 isolates contained four groups (Z, AA, BB and NN), were isolated in January and all were resistant to CIP, CLI, ERY and TET (Table 4-2).

Four isolates each of ST117 and ST412 were isolated from both areas of the laundry. ST117 isolates were had different antibiotic susceptibility patterns but had a core resistance to CIP and were isolated exclusively in April. The ST412 isolates shared a core resistance to CIP and CLI but differed in resistance to ERY, LIN, Q-D, MINO and TET (Table 4-2). Three of the isolates were isolated in April and the other in October. ST186 (n=3) was isolated in the dirty area in April and represented a single group (R). ST280 was isolated from the dirty area in January. The three isolates shared a core resistance to CIP, CLI, ERY and TET with differences in resistance to LIN, MINO and NIT. There were two ST17 isolates, both from January with a core resistance to CIP, CLI, ERY and TET. The remaining ST types (ST16, ST324, ST584, ST665, ST750 and ST1038) were isolated once each (Table 4-2).

The COG washers were the only area on the clean side with contamination. This was not unexpected due to the process of manual loading of the soiled linens. Of the ten isolates, two of them were in the same unique group (DD). Five of the isolates from the clean area shared the same antibiotic resistance profile and were isolated in the same month as isolates from the dirty side and were assigned the same unique group letter (F, K, AA, DD and GG) (Table 4-2). No analysis was performed on the ST109 *E. faecalis* strains.

4.5 DISCUSSION

The current study suggests that soiled clinical linens could be a source of bacterial contamination in the laundry facility environment with the dirty areas much more likely to be contaminated than the clean areas. Because no personnel contamination was identified, further study would need to be done in order to characterize the risk of infection due to potential occupational exposure in the laundry.

ST18 (n=25) and ST736 (n=18) were the two most common VRE identified and found in both the clean and dirty areas. These two ST types have previously been isolated from the environment in Seattle area and have been commonly found in humans [137,149,199]. We used ST type, antibiotic susceptibility which is easily changed due to the gain or loss of mobile genetic elements, date and location to identify the isolates. Thus, two or more isolates that shared all of these properties in common were assigned a unique letter code. However, if other molecular tests were done, such as whole genome sequencing, the various isolates may not be ancestrally related. This additional characterization was beyond the scope of the current project.

4.6 CONCLUSIONS

This is the first study to examine environmental contamination in an industrial laundry facility for the presence of VRE. VRE isolation was significantly higher (53% vs 8%, $p<0.001$) in dirty vs clean areas within the laundry facility. Further studies are needed to determine if there is a potential risk to facility workers. As a result of this study, particularly the contamination of the COG washer area, the laundry facility implemented new protocols in an effort to reduce the level of contamination and potential for occupational exposure. These protocols include the use of EPA registered disinfectants on high touch surfaces, guard rails to

physically block clean carts from getting underneath soiled linen chutes, color coding of carts (red carts are used only for soiled linen), providing additional PPE, such as gloves and face shields, available at the point of use and clearly posted PPE donning and doffing guidelines (Mr. C. Curtis, personal communication, 2016).

4.7 TABLES

4.7.1 *Table 4-1 Isolation of VRE by location*

Location	Samples collected N	VRE isolates n (%)
Dirty Area		
Receiving	32	6 (19)
Primary sort	32	24 (75)
Secondary sort	32	22 (69)
COG	24	12 (50)
Total	**120**	**64 (53)**
Clean Area		
COG washers	24	10 (42)
Folding	32	0 (0)
Processing	32	0 (0)
Break areas	32	0 (0)
Total	**120**	10 (8)

4.7.2 *Table 4-2 Molecular characterization of environmental isolates and their resistance profiles*

Isolate #	Sampling Date	Location[+]	MLST	Resistance Profile [*]	Unique Group[α]
E. faecium					
Dirty Area Surfaces					
708	January	Receiving	16	CIP, CLI, ERY, TET	A
703	January	Receiving	17	CIP, CLI, ERY, LIN, Q-D, TET	B
709	January	Primary Sort	17	CIP, CLI, ERY, MINO, TET	C
721	January	Secondary Sort	17	CIP, CLI, ERY, TET	D
1004	October	Receiving	18	CHL, CLI, Q-D	E
1018	October	Secondary Sort	18	CHL, CLI, Q-D	E
1028	October	COG	18	CHL, CLI, Q-D	E
1022	October	Secondary Sort	18	CHL, CLI, Q-D, TET	**F**
913	July	Primary Sort	18	CIP, CLI, ERY	G
915	July	Primary Sort	18	CIP, CLI, ERY	G
929	July	COG	18	CIP, CLI, ERY	G
908	July	Receiving	18	CIP, CLI, ERY, LIN	H
912	July	Primary Sort	18	CIP, CLI, ERY, LIN	H
810	April	Primary Sort	18	CIP, CLI, ERY, LIN, Q-D	I
909	July	Primary Sort	18	CIP, CLI, ERY, LIN, Q-D	I
819	April	Secondary Sort	18	CIP, CLI, ERY, LIN, TET	J
927	July	COG	18	CIP, CLI, ERY, MINO, TET	**K**

930	July	COG	18	CIP, CLI, ERY, MINO, TET	**K**
914	July	Primary Sort	18	CIP, CLI, ERY, MINO, TET, NIT	L
720	January	Secondary Sort	18	CIP, CLI, ERY, TET	M
807	April	Receiving	18	CIP, CLI, ERY, TET	M
821	April	Secondary Sort	18	CIP, CLI, ERY, TET	M
902	July	Receiving	18	CIP, CLI, ERY, TET	M
907	July	Receiving	18	CIP, CLI, ERY, TET	M
918	July	Secondary Sort	18	CIP, CLI, ERY, TET	M
922	July	Secondary Sort	18	CIP, CLI, ERY, TET	M
827	April	COG	18	CIP, CLI, ERY, TET, NIT	N
805	April	Receiving	117	CIP, ERY, LIN, NIT	O
806	April	Receiving	117	CIP, ERY	P
804	April	Receiving	117	CIP, ERY, LIN	Q
801	April	Receiving	186	CIP, ERY, MINO, TET	R
802	April	Receiving	186	CIP, ERY, MINO, TET	R
822	April	Secondary Sort	186	CIP, ERY, MINO, TET	R
702	January	Receiving	280	CIP, CLI, NIT, ERY, TET, MINO, LIN	S
704	January	Receiving	280	CIP, CLI, ERY, TET	T
710	January	Primary Sort	280	CIP, CLI, ERY, LIN, TET	U
719	January	Secondary Sort	324	CIP, CLI, ERY, LIN, Q-D, MINO, TET	V
811	April	Primary Sort	412	CIP, CLI, ERY, TET	W
816	April	Primary Sort	412	CIP, CLI, ERY	X

1019	October	Secondary Sort	412	CIP, CHL, CLI, Q-D, TET	Y
1007	October	Receiving	584	CIP, CHL, CLI, Q-D, TET	Z
730	January	COG	664	CIP, CLI, ERY, LIN, MINO, TET	Z
712	January	Primary Sort	664	CIP, CLI, ERY, MINO, TET, NIT	AA
718	January	Secondary Sort	664	CIP, CLI, ERY, MINO, TET, NIT	AA
722	January	Secondary Sort	664	CIP, CLI, ERY, TET	BB
713	January	Primary Sort	665	CIP, CLI, ERY, LIN, TET	CC
1015	October	Primary Sort	736	CHL, CLI, Q-D, TET	**DD**
1016	October	Primary Sort	736	CHL, CLI, Q-D, TET	**DD**
1009	October	Primary Sort	736	CIP, CHL, CLI, ERY, Q-D, TET	EE
1008	October	Receiving	736	CIP, CHL, CLI, ERY, Q-D, TET	EE
1001	October	Receiving	736	CIP, CHL, CLI, Q-D, TET	FF
1002	October	Receiving	736	CIP, CHL, CLI, Q-D, TET	FF
1005	October	Receiving	736	CIP, CHL, CLI, Q-D, TET	FF
1010	October	Primary Sort	736	CIP, CHL, CLI, Q-D, TET	FF
1021	October	Secondary Sort	736	CIP, CHL, CLI, Q-D, TET	FF
812	April	Primary Sort	736	CIP, CLI, ERY, LIN, Q-D, MINO, TET	**GG**
921	July	Secondary Sort	736	CIP, CLI, ERY, MINO, TET	HH
803	April	Receiving	736	CIP, CLI, ERY, MINO, TET, NIT	II
920	July	Secondary Sort	736	CIP, CLI, ERY, MINO, TET, NIT	II
808	April	Receiving	750	CIP, CLI, ERY, LIN, TET	JJ
707	January	Receiving	1038	CIP, CLI, ERY, LIN, TET	KK

Clean Area Surfaces					
Isolate #	Sampling Date	Location	MLST	Resistance Profile *	Unique Group[α]
1032	October	COG Washers	18	CHL, CLI, Q-D, TET	**F**
733	January	COG Washers	18	CIP, CLI, ERY, MINO, TET	**K**
832	April	COG Washers	117	CIP, LIN, Q-D	LL
834	April	COG Washers	412	CIP, CLI, ERY, LIN, MINO, TET	MM
734	January	COG Washers	664	CIP, CLI, ERY, MINO, TET	NN
1034	October	COG Washers	736	CHL, CLI, Q-D, TET	**DD**
1033	October	COG Washers	736	CHL, CLI, Q-D, TET	**DD**
735	January	COG Washers	736	CIP, CLI, ERY, LIN	OO
835	April	COG Washers	736	CIP, CLI, ERY, LIN, Q-D, MINO, TET	**GG**
931	July	COG Washers	736	CIP, CLI, ERY, LIN, MINO, TET	PP
E. faecalis					
706	January	Receiving	109	CIP, LIN, TET	
813	April	Primary Sort	109	CIP, CLI, ERY, Q-D, TET	
E. hirae					
904	July	Receiving	-	CIP, CLI, ERY, LIN, Q-D	

One VRE isolate/sample listed in the table

[+] Locations represent the areas inside the laundry facility where contamination was present. The receiving, primary sort, secondary sort and customer owned goods (COG) areas were on the dirty side of the laundry facility. The COG washers were the only area where contamination was observed on the clean side. More information and a map of facility are published elsewhere and available in Appendix 8.1 [115].

[*] ciprofloxacin (CIP), chloramphenicol (CHL), clindamycin (CLI), erythromycin (ERY), linezolid (LIN), quinupristin-dalfopristin (Q-D), minocycline (MINO), tetracycline (TET), and nitrofurantoin (NIT).

[α] A unique group letter was assigned to isolates that shared ST and resistance profiles for *E. faecium*. Codes underlined and in bold were found in both the dirty and clean areas

4.8 SUPPLEMENTARY TABLES

4.8.1 *Table S 4-1 Surface sample details*

Sample Location	N	Details
		Receiving n=8
Loading Dock	1-2	Ladder handle, door handle of delivery truck, phone handle and face, light switch, and wall dust.
Weigh in area	3-4	Phone, computer keyboard, mouse, glove boxes, and operational buttons on scale,
Incoming Carts	5-6	Full plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.
Glove/gown area	7-8	Surface of table with gloves
		Primary Sort n=8
Conveyor Belt	9-16	Safety pins to hold open bags, cart of soiled linens, conveyor belt, trash can at end of belt, and the handle to the stairs.
		Secondary Sort n=8
Sorted Dirty Cart	17-18	Empty plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.
Wash Deck	19-20	Chute of wash deck, soiled linens waiting to be placed in chute, empty used carts, and bags.
Chute 1	21-22	Front, back, and line leading up to chute.
Chute 2	23-24	Front, back, and line leading up to chute.
		COG n=6
COG	25-30	Sorted and unsorted COG, lost and found items.
		COG Washers n=6
COG Washers	31-36	High touch areas (buttons), floor in front of washers, and surfaces of washers.
		Folding n=8
Small piece folders	37-38	Scrubs front, on and around folding areas, and carts holding clean unfolded linens.
Hand folding	39-41	Linens waiting to be folded, on and around folding areas, conveyor belt bringing clean linens to be folded.
Machine Ironers	42-44	On and around folding machine, operation buttons, conveyor belt bringing clean linens to be ironed.
		Processing n=8
Sorted Clean Carts	45	Clean lab coats.
Clean cart ready to leave	46-47	Cart on way out, top, middle and outside of cart covered in plastic, just washed carts, and open cart with clean linens.
Steam tunnel	48-49	High touch areas (buttons).
Garment repair	50-52	Sewing machine, surface of table, linens waiting to be repaired, inside, outside and middle of carts holding linens.
		Break Area n=8

Locker Room	53-55	Locker handles front, middle and back, and ladies bathroom surfaces.
Break Area	56-58	Tables, chairs, phone, water fountain, extinguisher, and kitchen surfaces.
Office	59-60	Door handles, duplicating machine buttons, seats, phones, computer keyboards, mice, and waiting room chair.

4.8.3 *Table S 4-2 Antibiogram, percentage of resistant isolates collected at the Laundry Facility and UW Hospitals*

	ERY	**LIN**	**TET**	**NIT**
Location				
E. faecium				
HMC[§]	92	4	74	73
UWMC[±]	92	10	90	51
UWCL[€]	76	28	77	21
E. faecalis				
HMC[§]	83	-[α]	80	0
UWMC[±]	92	-[α]	78	1
UWCL[€]	50	50	100	0

Erythromycin: ERY, linezolid: LIN, tetracycline: TET and nitrofurantoin: NIT.

[α]: No data for *E. faecalis* linezolid resistance

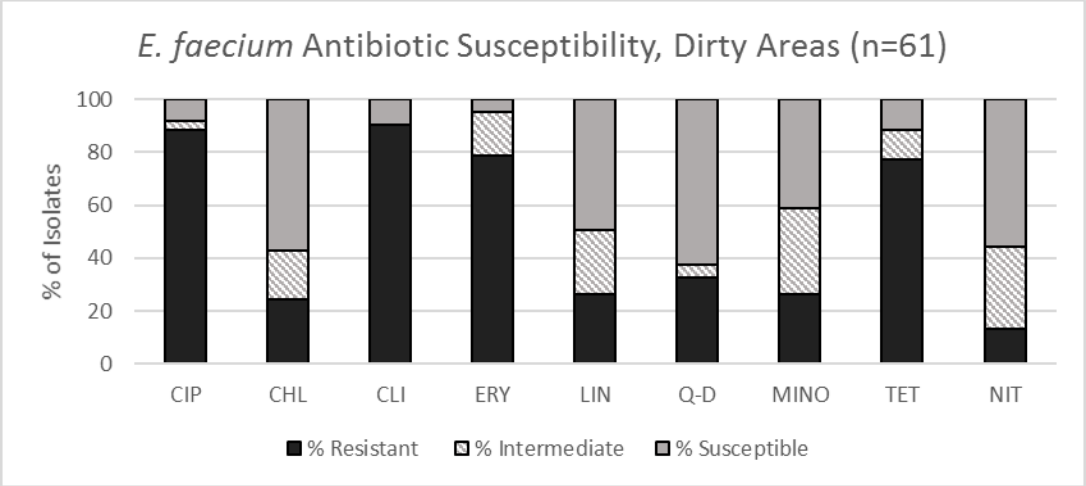
[§]: Harborview Medical Center

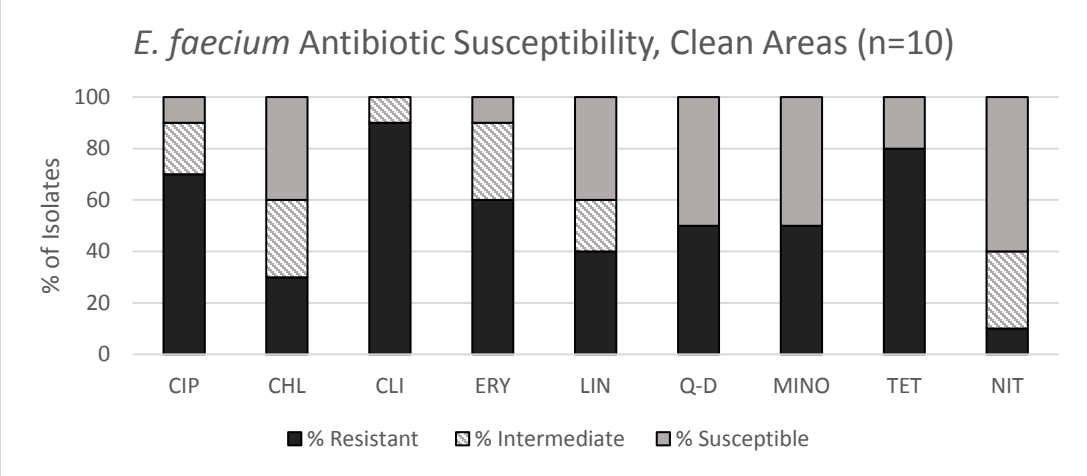
[±]: University of Washington Medical Center

[€]: UWCL: University of Washington Consolidated Laundry (*E. faecium*=71 isolates, *E. faecalis*=2 isolates)

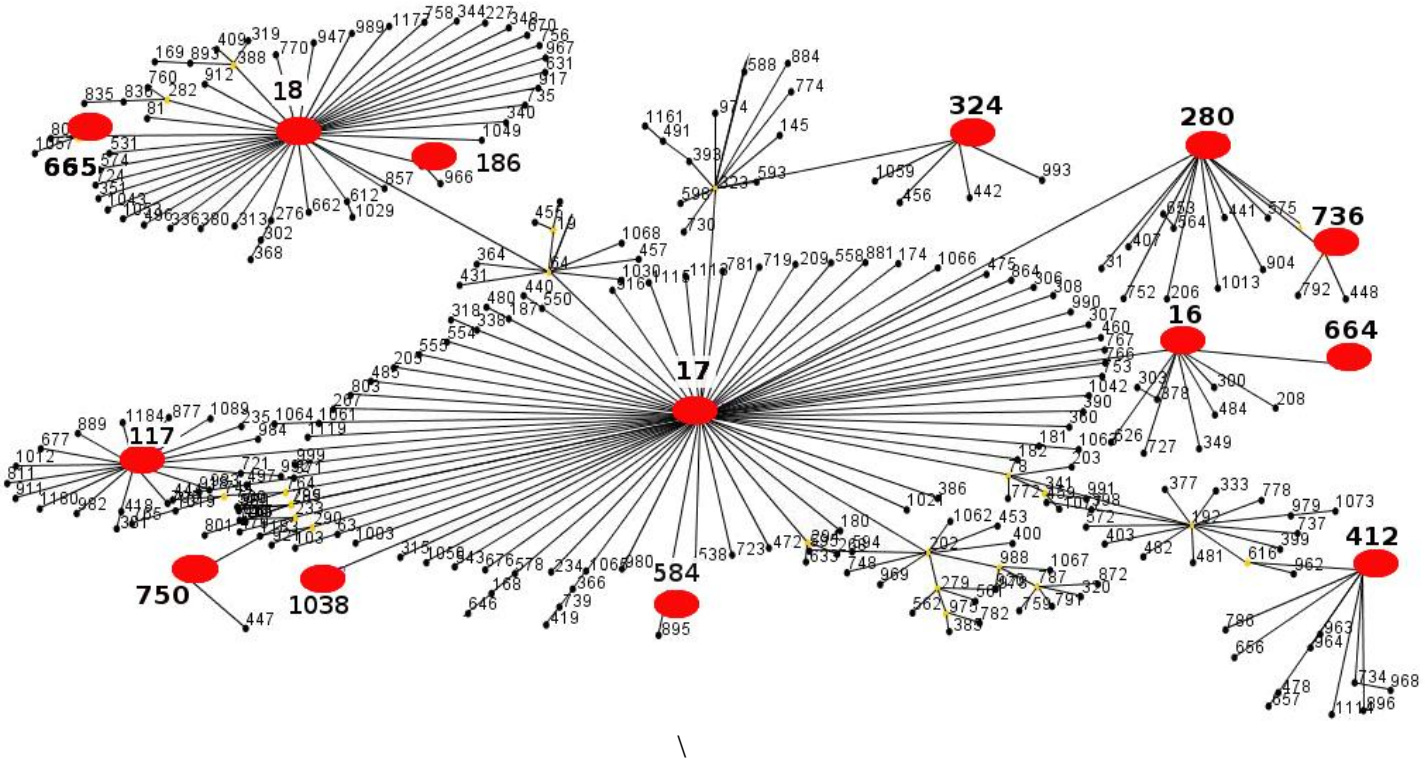
4.9 SUPPLEMENTARY FIGURES

4.9.1 *Figure S 4-1 Antibiotic resistance profiles for environmental surface samples*





4.9.2 *Figure S 4-2 eBURST of E. faecium isolates*



\

Chapter 5. ASSESSMENT OF ENVIRONMENTAL CONTAMINATION WITH PATHOGENIC BACTERIA AT A UNIVERSITY LAUNDRY FACILITY

5.1 ABSTRACT

Laundry workers have similar exposure routes as many healthcare workers, but are not considered to be healthcare workers. Very little is known about exposure to pathogenic bacteria among industrial laundry workers who work with soiled clinical linen. In order to study their exposures, an exposure assessment was performed at an industrial clinical laundry facility in Seattle, WA, USA. Surface swab samples (n=240) from the environment were collected from the facility during four site visits at three-month intervals. These samples were cultured for three pathogens; *Clostridium difficile*, methicillin-resistant *Staphylococcus aureus* (MRSA) and vancomycin-resistant enterococci (VRE). Voluntary participation among 23 employees consisted of nasal swabs for detection of MRSA, observations during their work and questionnaires. Contamination with all three pathogens was observed in the facility in both dirty and clean areas. However, the dirty area had a higher probability of overall contamination (≥1 pathogen) than the clean area (odds ratio, OR=18.0, 95% confidence interval 8.9-36.5, $p<0.001$). The probability of contamination was high for each individual pathogen: *C. difficile*, OR=15.5; MRSA, OR=14.8; and VRE, OR=12.6 (each, $p<0.001$). The highest probability of finding surface contamination occurred in the primary and secondary sort areas where dirty linens are manually sorted by employees (OR=63.0, $p<0.001$). MRSA nasal contamination was identified in 5/23 (22%) employees, with four out of five working in the dirty area. Improved protocols for

prevention and reduction of environmental contamination were implemented as a result of this study. The study suggests that the laundry facility environment can become contaminated by soiled linens and that workers who handle soiled linen may have a higher risk of exposure to *C. difficile*, MRSA and VRE than those who handle clean linens.

5.2 INTRODUCTION

In the last 30 years it has become clear that the environment is an important reservoir and transmitter of pathogens ranging from viruses such as influenza to antibiotic resistant bacteria such as methicillin-resistant *Staphylococcus aureus* (MRSA) [4–6]. Pathogens like *Clostridium difficile*, MRSA and vancomycin-resistant enterococci (VRE) that cause nosocomial infections are able to survive for extended time in dust and on surfaces [4,20]. The Centers for Disease Control and Prevention (CDC) defines healthcare-associated, or nosocomial, infections as "infections patients can get while receiving medical treatment" [200]. Studies have suggested that the clinical environment plays a role as a source or reservoir of these microbes [19]. An example of this was the extended outbreak of carbapenem-resistant *Klebsiella pneumoniae* (KPC) due to contamination in the air ducts of the National Institutes of Health (NIH) hospital [21].

Studies have shown that surfaces and objects in the immediate vicinity of patients are most likely to be contaminated with patients' microbes, including pathogens, in which case these surfaces and objects are called fomites [25–28]. Fomites are important reservoirs for *C. difficile*, MRSA [41]and VRE [4,39,43] in healthcare environments. Human contact with fomites promotes the spread of microbes and human disease especially in healthcare settings [5]. These organisms can survive on fomites from weeks to months and may be difficult to remove from the environment by standard cleaning and disinfection protocols [4,20].

In hospitals, patients colonized or infected with *C. difficile*, MRSA or VRE are identified as "high precaution" patients. This means that barrier precautions including the use of gloves, gowns and additional hand-hygiene practices are required for everyone who enters the room and interacts with these patients [70]. Posted signs make visitors and hospital staff aware of the additional health hazards associated with these patients. In addition to these mandatory procedures, enhanced terminal room disinfection is now being performed in an increasing numbers of hospitals [2]. Terminal room disinfection consists of a thorough cleaning of the whole room after a patient vacates the room [201]. In contrast, the soiled linens from these high precautious patients' rooms do not routinely receive special treatment (Mr. J. Dale, personal communication, 2013, Ms. C. O'Hara, personal communication, 2013).

For a 2013 report the CDC developed three "threat" classifications for 18 antibiotic resistant and related pathogens; urgent, serious and concerning, based on the severity of the disease, cost and difficulty of treatment [54]. That list includes three pathogens addressed in this study. *C. difficile* was classified as an "immediate public health threat that requires urgent and aggressive action". MRSA and VRE were classified as serious threats which "require prompt and sustained action to ensure that the problems do not grow" [54].

Healthcare workers including nurses, doctors, dentists, technicians and other personnel who are in contact with patients are routinely exposed to pathogens during the course of their work [4]. This routine exposure can lead to a higher risk of colonization and illness compared to the general public [48,58]. For example, between 1-15% of healthcare workers are colonized with MRSA compared to 0.2-7.4% of the general population with no known risk factors [202,203]. Multiple studies have examined how personal protective equipment (PPE), standard blood-borne

infection controls, extensive cleaning procedures and yearly education have been important in reducing the risk of healthcare workers acquiring infections at work [19,61–64].

Laundry facilities that process soiled clinical linens have been considered an extension of the healthcare environment even when the facility is not physically located on site [58,74]. Very few studies have assessed the potential risk of laundry workers who handle soiled hospital linens. There are documented case reports of illness (12 cases of hepatitis A and eight cases of *Salmonella* gastroenteritis) related to exposures to soiled linens [73,72,48]. Other reports of infections among laundry workers also included *S. aureus* skin infections and viral gastroenteritis that was potentially norovirus [75,76]. In Taiwan, a hospital laundry worker was suspected of being the index case in a SARS viral epidemic within the community [77]. Laundry workers are also at physical risk of cuts and abrasions due to sharps and medical devices left in and among the linens potentially increasing the infection risk [78,79.]

The aim of this study was to assess contamination with three clinical pathogens, *C. difficile*, MRSA and VRE, in an industrial laundry facility that handles soiled linens from hospitals and clinics.

5.3 MATERIALS AND METHODS

5.3.1 *Surface Samples*

In order to determine the level of surface contamination, surface sampling sites were chosen by identifying high touch areas throughout the laundry facility. This was done by completing a walk-through of the facility with a Certified Industrial Hygienist. The definition used to determine high versus low touch areas came from a report used by the CDC which differentiates between surfaces with minimal hand-contact, such as floors and ceilings, and surfaces with frequent hand-contact such as door knobs, phones and tools used on the job [204]. We determined

areas within the laundry that were "worst case" scenarios which were areas with the highest exposure levels. By including the worst and best case locations we will be able to determine the range of exposures that the employees face. It has been shown that sampling for "worst case" scenarios increases the chances of correctly identifying overexposures when they are occurring in an occupational setting [205].

Samples from the same surfaces were collected in January, April, July and October of 2015 using either 5 ml Sanicult™ Sterile Neutralizing Buffer Swabs (Starplex® Scientific, Etobicoke, Ontario, Canada) or 10 ml 3M™ Sponge-Sticks (3M Co. St. Paul, MN) [16]. The swabs were used to sample smaller surfaces (~10cm^2), and the Sponge-Sticks were used to sample larger surface areas (~30cm^2). There were a total of 240 samples collected over the course of the study, with 30 samples each collected in the dirty and clean laundry areas at every sampling time as previously described [115]. Within 4 h of collection the samples were processed in three parallel enrichment processes that independently targeted one of the three pathogens (*C. difficile*, MRSA and VRE) from each surface sample.

5.3.2 *Subject recruitment*

All personnel (~110 employees), from both the clean and dirty areas of the laundry facility, were invited to volunteer for the study on each of the 4 visits. Participants were asked to sign a consent form at each sampling. The procedures and objectives of the research study were then outlined and explained. Participation throughout the study was entirely voluntary. Subject recruitment, study design, and data handling procedures were all approved by the Institutional Review Board of University of Washington (Human Subjects Application #47732).

5.3.3 *Observation, Survey and Nasal Samples*

Employees who consented to inclusion into the study received a temporary identification number that was taped to their gown for the remainder of their shift. If their gown was taken off or replaced, the number was applied to their new gown. This number was used to identify and link their survey data and observations upon return to the laboratory for that particular observational and sampling date only. Each subject was observed for 20 consecutive min during each site visit. Observations were done one subject at a time. Touches of the nose, eye and face were recorded. The face was defined as everything except the nose and eye. Subjects knew that observations occurred during the shift, but not necessarily when they were being observed individually.

A survey was given to the subjects at the beginning of the first and third sampling visit. Names were needed to link each visit because subjects may have had different numbers for each observation time point. The survey included age, smoking status, where they normally work in the laundry, job title, job task, length of employment, frequency and type of PPE used, six-month antibiotic history and rashes or other skin disorders. Nasal samples were collected and characterized as described elsewhere [115].

5.3.4 *Laundry Facility*

All samples were collected at an industrial laundry facility as described elsewhere [115]. The facility processes ~300,000 pounds of linen per week from six Seattle area hospitals, 30 outpatient clinics and a primate facility. Soiled linens are kept on the 2nd floor, separate from the clean linens on the 1st floor of the facility. There are two main processing lines. The first is the standard laundry owned linens which represents ~98% of all the linens. These are sorted into carts and then sent down chutes to the 1st floor to be laundered. Customer owned goods (COG),

which represent ~2% of the linens, are sorted in a separate area and washed in smaller batches in an independent process. The COG are manually placed into washers on the 1st floor. All clean linens are dried, sorted, folded and packaged for delivery exclusively in the 1st floor "clean area". Gowns and gloves are mandatory in all locations within the dirty area but voluntary in the clean area with the exception of the clean room and the COG washer area.

Surfaces in the same eight locations within the laundry facility were sampled at each sampling visit. The receiving area included the loading dock, the weigh in area, incoming carts and the glove/gown area. The primary sort area was the conveyor belt where linens are manually sorted. The secondary sort area consisted of carts with sorted linens, the wash deck where linens were dropped down to the COG washer area and the two chutes where linens were dropped into tunnel washers. The COG area consisted of surfaces where COG linens were sorted. On the clean side of the laundry, the COG washer area was sampled. The folding area consisted of areas where hand folding, machine-assisted folding and ironing occurred. The processing area consisted of carts with clean linens, steam tunnels and the garment repair areas. The break area consisted of the locker rooms, the eating area and the front office area (Appendix 1).

5.3.5 *Pathogen Isolation*

To determine if samples contained *C. difficile,* 1 mL aliquots were anaerobically incubated in 5 ml of Cycloserine Cefoxitin Mannitol Broth with Taurocholate and Lysozyme (Anaerobe Systems, Morgan Hill, CA) media and incubated for 48 h at 36.5ºC. Tubes with a color change from red to yellow/orange vortexed and 0.1 ml of the solution was inoculated onto a selective CHROMagar *C. difficile* media, (CHROMagar™, Paris, France) and incubated for 24 h at 36.5°C under anaerobic conditions according to the manufacturer's directions. Colonies that

fluoresced under UV light (360 nm) were Gram stained and isolates that were Gram-positive rods were saved for further processing.

Culturing of MRSA used 1 mL of the original sample which was aliquoted into 1.5 mL of Bacto® m Staphylococcus Broth at 1.5 X concentration (Difco Laboratories, Sparks, MD) with 75 µg/mL polymyxin B and 0.01% potassium tellurite (Sigma-Aldrich, St. Louis, MO) and incubated at 36.5°C with 5% carbon dioxide. Tubes were examined for growth at 48 h, and samples that were turbid with black precipitate were further processed. These samples were plated onto oxacillin-resistant *S. aureus* base® media (ORSAB; Oxoid Limited, Basingstoke, England) and incubated at 35°C for 48 h. Dark blue colonies that grew on ORSAB were streaked onto Brucella agar (Difco Laboratories, Sparks, MD) supplemented with 5% sterile sheep blood and incubated at 36.5°C in 5% CO_2 for 24 h to determine if β- hemolysis was present. *S. aureus* and presumptive MRSA isolates were verified using the Staphaurex® (Remel, Lenexa, Kansas) and Oxoid penicillin binding protein latex agglutination test® (Oxoid Microbiology P, Basingstoke, UK) according to manufacturer's directions.

Isolation of VRE consisted of transferring 1 mL of the original surface sample into 1 mL of Brain Heart Infusion broth (BHI; Difco™ Laboratories, Sparks, MD) supplemented with 20 µg/mL of aztreonam and 6 µg/mL of vancomycin at 41°C for 24-72 h. Turbid samples were transferred onto m-Enterococcus™ agar (mE; Difco Laboratories, Sparks, MD), containing 6 µg/mL vancomycin (mE_{Van6}), and incubated at 41°C for 24-48 h [195]. Pink or maroon colonies were isolated and transferred to BHI_{Van18} plates (Difco Laboratories, Sparks, MD) containing 18 µg/mL vancomycin and incubated at 41°C for 24-48 h. Vancomycin resistance was defined as growth on BHI_{Van18} media. For all three pathogens, only a single colony was taken from each positive sample.

5.3.6 *Statistical Methods*

Averages of prevalence of contamination and touching behavior were calculated. STATA 13.1 (StataCorp. College Station, TX) was used for all analyses. Except for tests of possible seasonality, the surface swabs collected in each site visit were assumed to be independent measurements; even though measurements were repeated at the same approximate location, the three-month sampling interval difference was sufficient to justify this assumption. Logistic regression was used to determine the crude odds ratios of detecting surface contamination. Tetrachoric correlation between the pathogens was also assessed. Other between-variable comparisons used the Student t-test for continuous variables, chi-square test for categorical variables, pairwise comparisons to assess seasonal differences and a Wilcoxon rank-sum test to compare touching behaviors. All four areas of the clean area were combined, because of the low number of the three bacteria isolated, into the reference area against which the dirty areas were compared. Odds ratios were determined for finding each pathogen and for ≥1 pathogen in the dirty vs clean area. The odds of finding contamination were also stratified by specific areas and sampling times within the dirty side compared to the reference clean area. Sample size for environmental samples was calculated to have the power to detect the difference between surface contamination between the dirty and clean sides based on pilot data, but not necessarily for comparisons adjusting for other variables (Appendix 2 Surface Sample Size Calculation).

5.4 RESULTS

5.4.1 *Participant Characteristics*

Of the ~110 employees in the facility, 23 volunteered to participate in the study; 14 employees from the clean area and nine employees from the dirty area. The remaining

employees declined to participate. Employees from both the clean and dirty areas participated between one and four times with the employees from the clean area participating on average 2.7 times while employees from the dirty side participated on average 2.4 times.

The employees from the clean and dirty areas, were statistically different for three factors; time spent in US, gender and prevalence of MRSA nasal colonization. The employees from the clean areas lived an average of 31.1 years in the US, while the employees from the dirty areas have lived in the US an average of 17.1 years (p=0.03, t-test). The subjects from the clean area were 78.6% (11/14) female while the dirty area subjects consisted of 22.2% (2/9) females (p=0.006, t-test). The prevalence of MRSA nasal colonization was significantly higher among study participants from the dirty area (44.4%, 4/9) than from the clean area (7.1%, 1/14; p=0.04, chi-square test) as described in detail elsewhere [115]. Employees who worked on the dirty side were more likely to have a positive nasal swab than employees who worked on the clean side, side, although this was only marginally significant (OR 10.4; CI 0.9-117.2, p=0.06). The sample size was not sufficient to evaluate whether these differences were influenced by other variables known to differ between clean and dirty areas (gender and years in the US). Age, household size, smoking behavior, antibiotic use and current work at a hospital were not significantly different between the two groups (p>0.05). Self-reported hand washing behavior was the same between the employees on the clean and dirty areas with the exception of one employee from the dirty area who reported not washing their hands after removing their gloves (Table S 5-1).

The total time spent observing the employees was 1,200 min (20 h) over the course of the entire study period. There were 38 observations of employees from the clean area and 22 observations from employees in the dirty area. Each observation was 20 min long. The average number of total touches, including touches of the nose, eye and face, observed among employees

from both areas was 1.7 (SD=2.4). Employees from the clean area showed more total touching behavior on average with 2.1 touches (SD=2.7) than employees from the dirty area with 1.1 touches (SD=1.7) but the difference was not statistically different (p=0.14, t-test). The only measure that approached statistical significance (p=0.08, t-test) was touching of the face with an average of 0.9 touches (range 0-5) among the clean area employees and 0.3 touches (range 0-3) among the dirty area employees per 20-minute observation period (Table 5-1). All other observed touching behaviors were not significantly different using the Wilcoxon rank-sum test, between employees from the clean and dirty areas. Similar results were observed with a chi-square test after dichotomizing the number of touches ($\geq 60^{th}$ percentile as "high") and with a non-parametric t-test.

To determine if multiple covariates were associated with nasal colonization a mixed model was run, initially adjusting for area (dirty versus clean) and touch frequency. The data for this model consisted of 60 observations clustered into 23 groups, or subjects. Due to the small amount of data, inferences could not be made as the covariate-adjusted mixed model had trouble with convergence (as opposed to the unadjusted model which converged with issue). Even when we altered the type of mixed model, to improve model convergence for small data samples, we still saw evidence of issues with estimation when covariates were included in the statistical model. Much of the issue likely stemmed from only one subject from the clean area having had a MRSA positive nasal sample. While we could model only touch (and not area) or touch only in dirty area, these results were non-significant. This led us to conclude that, while we could do a valid unadjusted mixed model for clean versus non-clean area, adding covariates was incompatible with the available data.

5.4.2 *Pathogen Isolation from Surfaces*

Contamination with all three pathogens was observed in both the dirty and the clean areas of the laundry facility (Table 5-2). The primary and secondary sort areas were the most contaminated areas within the dirty side for all three pathogens and for ≥1 pathogen. The COG washers were the most contaminated area on the clean side. The dirty area had statistically higher contamination than the clean area for contamination with ≥1 pathogen (67% dirty area vs 10% clean area, $p<0.001$, chi-square test) and when stratified by specific pathogen.

There were significant correlations among pathogens. The correlation between MRSA and VRE was 0.64 ($p<0.001$), between *C. difficile* and VRE it was 0.61 ($p<0.001$) and between *C. difficile* and MRSA it was 0.49 ($p=<0.001$). Ten of the samples contained all three pathogens: four were in the primary sort area, four were in the secondary sort area and the COG and COG washers each had one sample. One sample had MRSA and *C. difficile* in the primary sort area. Ten samples had VRE and *C. difficile*: four in the primary sort area, three in the secondary sort area, two in the COG area and one in the receiving area. Seventeen samples contained both MRSA and VRE: seven in the secondary sort, four in the primary sort area, four in the COG area, one in the receiving area and one in the COG washer area.

Prevalence of surface contamination from different locations within the facility is shown by Figure 5-1. The primary and secondary sort areas had the highest levels of contamination for all measures, both overall (>1 pathogen) and for individual pathogens. On the clean side the COG washer area had the highest contamination for all three pathogens (Figure 5-1).

The four sampling times were distant enough in time that they were not considered to be repeated measures. However, to determine if there was a difference in the prevalence of positive surface samples between sampling times a Cochran's Q test was run. Surface contamination

with ≥1 pathogen did not change over time. When the data were examined for the individual pathogens, significant seasonal variation was observed in the dirty area for *C. difficile* only. This was then followed by comparing the sampling times using the McNemar test to determine the difference. Contamination with *C. difficile* in the dirty area was the highest in April with a prevalence of 40% (n=12) and was statistically higher than both January and July, but not October (Table 5-2). MRSA contamination was highest in July (40%, n=12) and VRE had the highest levels of contamination in April (57%, n=17) but no statistical difference by sample date was observed for either pathogen. In the clean areas, the number of positive samples were very low and had very little seasonal difference in prevalence (Table 5-2).

The dirty area had a higher probability of contamination with ≥1 pathogen than the clean area (odds ratio, OR=18.0, 95% confidence interval 8.9-36.5, p<0.001; Table 5-3). The probability of contamination was high for each individual pathogen: *C. difficile*, OR=15.5; MRSA, OR=14.8; and VRE, OR=12.6 (each, p<0.001; Table 5-3). When stratified by specific location, the odds of finding ≥1 pathogen were higher in all locations within the dirty area compared with the clean area including the receiving area (OR=3.3, p=0.02), the primary sort area (OR=69.4, p<0.001), the secondary sort area (OR=53.0, p<0.001) and the COG area (16.5, p<0.001). As with the prevalence, the highest odds ratios were observed in the primary and secondary sort areas (Table 5-3).

5.5 DISCUSSION

This is the first study to assess contamination with *C. difficile*, MRSA and VRE in a dedicated clinical laundry facility servicing bulk hospital linen. The study used self-report, observation and surface swab collection culture methods. The dirty area of the laundry facility was significantly more contaminated than the clean area for ≥1 pathogen (67% dirty area vs 10%

clean area, $p<0.001$, t-test). This remained the case when the pathogens were examined separately for dirty vs clean areas. The results are not unexpected given that soiled hospital linens have previously been shown to be contaminated with a variety of pathogens [206,207]. The results correspond to our original hypothesis that the dirty area would have higher contamination levels than the clean area. The results are also consistent with other studies that assessed peripherally-associated healthcare settings such as dental school clinics, fire stations housing first responders and veterinarian clinics [35,65,66,68,69].

One unexpected finding in this study was the level of surface contamination in the COG washer area which is located within the clean, 1st floor area of the laundry facility. This area had higher contamination levels than the receiving area (45.8% vs 28.3% for contamination with ≥1 pathogen) (Figure 5-1). As a result of this study, the laundry facility has implemented new protocols which aim to reduce the levels of surface contamination in the COG washer area. These protocols included the use of EPA registered disinfectant on the high touch surfaces of the COG washers, guard rails to physically block clean carts from getting underneath soiled linen chutes, color coding of carts (red carts are for soiled linen use only) and providing additional PPE, such as gloves and face shields, available at the point of use and posted PPE donning and doffing guidelines (Mr. C. Curtis, personal communication, 2016).

Finding more than one pathogen in the same location is not unexpected. It makes sense that the presence of one pathogen was correlated with presence of another pathogen for a number of reasons. One reason is that dirty surfaces contain more total bacteria; thus it is more likely to have contamination with multiple pathogens. The other potential reason is that VRE and *C. difficile* are both found in the gastrointestinal tract and if contamination with feces is present, there is a higher chance of finding one or both pathogens. In addition, there is evidence of co-

infections with *C. difficile* and VRE [208]. This is not surprising because colonization or infection with these pathogens have some of the same risk factors: antibiotic use, recent surgery, living in close quarters, etc. [54]. The reason that there were more samples that contained both VRE and MRSA, compared to both VRE and *C. difficile*, may be because so few isolates were positive with *C. difficile*.

C. difficile and MRSA have been shown to be seasonal. *C. difficile* rates in general increase in late winter/early spring and are thought to be due to an increase in antibiotic use for respiratory infections which are also seasonal [167]. MRSA rates also appear to change based on the season, with July-September being the months with the highest prevalence of skin and soft tissue infections [209]. On the dirty side of the laundry, *C. difficile* had a significantly higher prevalence in April while MRSA had a higher, although not significantly different, prevalence in July, which correlates with what has been previously reported in the literature. In contrast, seasonality in VRE infections have not been as well established, and in this study VRE rates did not vary by season. No seasonal differences were observed in the clean side, but due to the low number of positive samples from this area (10/120), we may not have had the resolution to detect seasonality.

This study had several limitations. The first was the low sample size of subjects (n=23). There were few differences between the two groups of subjects based on both observations and self-reported behaviors and demographics. It is not clear if this was due to the small number of participants or if there is no difference. More robust comparisons of behavior and demographics between the two groups of workers may have been possible with a larger sample size of subjects. The small number of positive surface swab samples from the clean areas reduced the resolution of some comparisons as mentioned above. This study assessed exposure risk and not risk of

illness. Determining the risk of illness based on exposure in the laundry was beyond the scope of the study. A known methodological issue in environmental sampling for bacteria is low recovery rates. When sampling environmental surfaces recovery can be less than 1% and is dependent on a number of factors including sampling method, environmental surface, environmental conditions, the original concentration and the target bacteria sampled [210]. Thus it is likely that our recovery rates underestimated the actual level of contamination for each of the bacteria. The lower prevalence of *C. difficile* in this study, compared to MRSA and VRE, may be due in part to the fact that it is difficult to regrow spores from the environment [179]. Additionally, this study only assessed contamination based on presence or absence of each organism. Determining the actual concentration of organisms may have provided additional insights. The assumption that the same surfaces at different sampling times were independent allowed us to pool the data and have a larger sample size, but could have been wrong. The lack of significant difference between the seasons, other than *C. difficile* contamination in April, supports this assumption.

5.6 CONCLUSIONS

This study suggests that laundry facility workers who handle soiled linen may have a higher risk of exposure to *C. difficile*, MRSA and VRE than those who handle the linens once they are clean. Environmental microbiology collection standards, as well as, defined occupational exposure limits have not been developed and thus it is difficult to assess microbiological risk in this occupational setting.

5.7 TABLES

5.7.1 *Table 5-1 Observed self-touch data*

	Mean ± SD, by area of laundry			
	Overall (n=23)	**Dirty (n=9)**	**Clean (n=14)**	**p-value**
Person hours	20.0	7.4	12.7	
Average Touch	0.4 ± 0.7	0.3 ± 0.1	0.5 ± 0.1	0.16
Nose	0.5 ± 1.0	0.3 ± 0.8	0.6 ± 1.1	0.25
Eye	0.1 ± 0.4	0.1 ± 0.2	0.1 ± 0.5	0.59
Face	0.7 ± 1.1	0.3 ± 0.8	0.9 ± 1.0	0.04
Hair	0.4 ± 0.8	0.4 ± 0.7	0.4 ± 0.9	0.74
Total Touch	1.7 ± 2.4	1.1 ± 1.7	2.1 ± 2.7	0.16

5.7.2 *Table 5-2 Prevalence of Surface Contamination by Laundry area and Date of Sample Collection*

	C. difficile **n (%)**		**MRSA n (%)**		**VRE n (%)**		**Any Pathogen n (%)**	
	Dirty	Clean	Dirty	Clean	Dirty	Clean	Dirty	Clean
Jan 2015	3 (10)	1 (3)	6 (20)	0 (0)	16 (53)	3 (10)	20 (67)	4 (13)
April 2015	12 (40)	0 (0)	5 (17)	1 (3)	17 (57)	3 (10)	20 (67)	3 (10)
July 2015	4 (13)	0 (0)	12 (40)	1 (3)	11 (37)	1 (0)	20 (67)	2 (7)
Oct 2015	6 (20)	1 (3)	10 (33)	1 (3)	15 (50)	3 (10)	20 (67)	3 (10)
Total	**25 (21)**	**2 (2)**	**33 (28)**	**3 (3)**	**64 (53)**	**10 (8)**	**80 (67)**	**12 (10)**

5.7.3 *Table 5-3 Probability (Odds Ratio) of Contamination in Dirty Area Compared to the Clean Area*

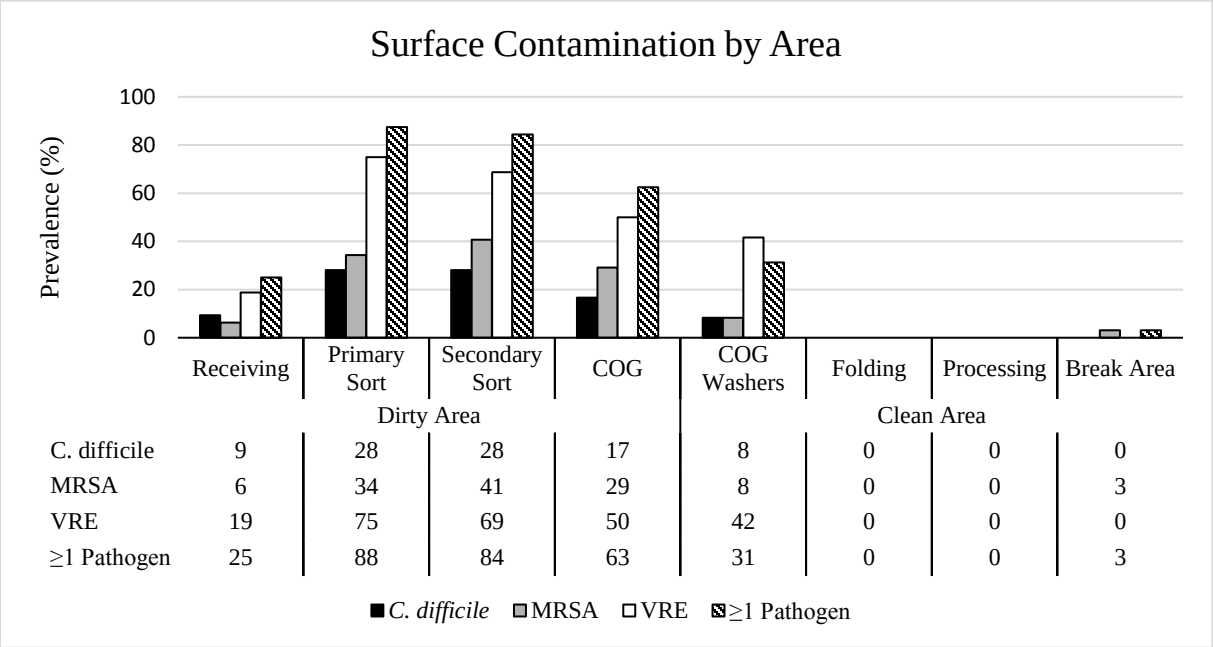
Location	**Any Pathogen**		***C. difficile***		**MRSA**		**VRE**	
	OR	CI	OR	CI	OR	CI	OR	CI
Clean Area	1	--	1	--	1	--	1	--
All Dirty Areas	18.0*	8.9-36.5	15.5*	3.6-67.2	14.8*	4.4-49.8	12.6*	6.0-26.3
Receiving	3.3**	1.2-9.1	6.1	1.0-38.2	2.6	0.4-16.3	2.5	0.8-7.6
Primary Sort	69.4*	20.5-234.3	23.1*	4.7-113.9	20.4*	5.3-79.5	33.0*	11.8-92.4
Secondary Sort	53.0*	17.1-166.9	23.1*	4.7-113.9	26.7*	6.9-102.5	24.2*	9.0-65.1
COG	16.5*	5.9-46.4	11.8**	2.0-68.7	16.1*	3.8-68.1	11.0*	3.9-30.8

* $p<0.001$, ** $p<0.05$

OR=crude odds ratio, calculated by logistic regression, using clean area as reference category, and with no adjustment for other variables. CI= 95% confidence interval. COG = Customer owned goods area

5.8 FIGURES

5.8.1 *Figure 5-1 Prevalence of surface contamination by location within the laundry facility*



COG=Customer owned goods, MRSA= Methicillin resistant *S. aureus*, VRE=Vancomycin resistant Entercocci

5.9 SUPPLEMENTARY TABLES

5.9.1 *Table S 5-2 Surface sample details*

Sample Location	n	Details
Receiving n=8		
Loading Dock	2	Ladder handle, door handle of delivery truck, phone handle and face, light switch, and wall dust.
Weigh in area	2	Phone, computer keyboard, mouse, glove boxes, and operational buttons on scale,
Incoming Carts	2	Full plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.
Glove/gown area	2	Surface of table with gloves
Primary Sort n=8		
Conveyor Belt	8	Safety pins to hold open bags, cart of soiled linens, conveyor belt, trash can at end of belt, and the handle to the stairs.
Secondary Sort n=8		
Sorted Dirty Cart	2	Empty plastic laundry bags in trash, cart on way to chutes, top, middle, outside and inside of bin.
Wash Deck	2	Chute of wash deck, soiled linens waiting to be placed in chute, empty used carts, and bags.
Chute 1	2	Front, back, and line leading up to chute.
Chute 2	2	Front, back, and line leading up to chute.
COG n=6		
COG	6	Sorted and unsorted COG, lost and found items.
COG Washers n=6		
COG Washers	6	High touch areas (buttons), floor in front of washers, and surfaces of washers.
Folding n=8		
Small piece folders	2	Scrubs front, on and around folding areas, and carts holding clean unfolded linens.
Hand folding	3	Linens waiting to be folded, on and around folding areas, conveyor belt bringing clean linens to be folded.
Machine Ironers	3	On and around folding machine, operation buttons, conveyor belt bringing clean linens to be ironed.
Processing n=8		
Sorted Clean Carts	1	Clean lab coats.

Clean cart ready to leave	2	Cart on way out, top, middle and outside of cart covered in plastic, just washed carts, and open cart with clean linens.
Steam tunnel	2	High touch areas (buttons).
Garment repair	3	Sewing machine, surface of table, linens waiting to be repaired, inside, outside and middle of carts holding linens.
Break Area n=8		
Locker Room	3	Locker handles front, middle and back, and ladies bathroom surfaces.
Break Area	3	Tables, chairs, phone, water fountain, extinguisher, and kitchen surfaces.
Office	2	Door handles, duplicating machine buttons, seats, phones, computer keyboards, mice, and waiting room chair.

5.9.2 *Table S 5-3 Self-reported characteristics of laundry employees by dirty and clean area*

Category	**Dirty Area (n=9)**	**Clean Area (n=14)**	**p-value**
Age (y)	54.4	47.7	0.15
Time in US (y)	17.1	31	0.02
Female (%)	22.2	78.6	0.006
Household size			
Adults (mean)	2.1	2	0.86
Children (mean)	1	0.9	0.90
Children in Daycare, n (%)	0 (0)	2 (14.3)	0.26
Own pets, n (%)	1 (11.1)	6 (42.9)	0.12
Smoking			
Current smoker, n (%)	0 (0)	1 (7.1)	0.44
Recent Smoker (last 6 mo), n (%)	0 (0)	0 (0)	--
Antibiotic Use	n (%)	n (%)	n (%)
Self (Current)	1 (11.1)	0 (0)	0.22
Self (Last 6 mo)	1 (11.1)	1 (7.1)	0.76
Household contact (Current)*	1 (11.1)	1 (7.1)	--
Household contact (Last 6 mo)*	1 (11.1)	1 (7.1)	--
Hospital work	1 (11.1)	1 (7.1)	0.76
Hospital work (family)	0 (0)	0 (0)	--
MRSA in nose n (%)	4 (44.4)	1 (7.1)	0.04
Hand washing behavior n (%)			
Before work	9 (100)	14 (100)	--
After work	9 (100)	14 (100)	--
After removing gloves	8 (88.8)	14 (100)	0.22
Before break	9 (100)	14 (100)	--
Before eating	9 (100)	14 (100)	--
After using bathroom	9 (100)	14 (100)	--

* 4 different respondents did not know

Chapter 6. DISSERTATION DISCUSSION

In this study, environmental sampling was used to isolate three pathogens: *C. difficile*, MRSA and VRE. These bacterial were biochemically and genetically characterized to determine relatedness within a single sampling time period, between time periods and to compare isolates from the dirty vs the clean areas. Using all of this data, odds of contamination with all three pathogens as well as nasal colonization with MRSA were determined. The dirty area of the laundry facility was more likely to be contaminated with all three of the pathogens and that employees from the dirty side were more likely to be positive for nasal MRSA. Questionnaires and observation were done to assess behavior of employees in both the dirty and clean areas of the facility.

Chapter one presents a broad overview of the three pathogens covered in this study. In chapter two, contamination of surfaces within the laundry facility with *C. difficile* was determined. Dirty areas of the laundry were more contaminated than clean areas (21% vs 2%, $p<0.001$). *C. difficile* isolates were assessed for carriage of the genes *tcdA* and *tcdB* which code for toxins A and B respectively [95]. *C. difficile* toxins A and/or B were present in 64% of all isolates from the dirty area. Of these, 10 isolates carried both genes. Both isolates from the clean area carried both toxin genes *tcdA* and *tcdB*. The presence of the toxins is important because the presence of either toxin increases the virulence and disease potential of *C. difficile* [95]. April was the month with the highest prevalence of *C. difficile* contamination which may have been due to antibiotic use following respiratory infections in the winter months [167]. The MLST types identified in this study have all previously been found in both clinical and community settings and included 1, 2, 3, 15, 26, 34, 35, 39, 42, 43, 44, 53, 63 and 284. Toxin presence by

ST type was not uniformly distributed and did not appear to be associated with a particular ST with the exception of ST2, ST42 and ST44 which all had both toxins.

Chapter three describes MRSA environmental contamination of the facility as well as nasal carriage among the laundry personnel. MRSA was found at higher rates on the dirty side of the laundry than the clean side (28% vs 3%, $p<0.001$). All of the MRSA isolates were screened for antibiotic resistance, genetically verified to carry the *mecA* gene and the type of *mecA* element carried. In addition, the MLST type was determined. All 36 of the isolates were resistant to methicillin and all other □-lactams as expected due to the carriage of the *mecA* gene [113]. Thirty-five (97%) of the isolates were resistant to two or more antibiotics including gentamicin, kanamycin, rifampin, ciprofloxacin, nitrofurantoin, clindamycin, erythromycin, chloramphenicol, quinupristin-dalfopristin, trimethoprim-sulfamethoxazole, minocycline and tetracycline. Isolates from the dirty side had a core resistance to gentamicin, kanamycin, ciprofloxacin, clindamycin, erythromycin, quinupristin-dalfopristin, trimethoprim-sulfamethoxazole, minocycline and tetracycline. All isolates from the clean side were resistant to fewer antibiotics and included kanamycin, ciprofloxacin, nitrofurantoin, clindamycin and erythromycin.

Subject employees were also assessed for nasal carriage of MRSA. Nasal colonization rates were 44% (4/9) on the dirty side and 7% (1/14) on the clean side. Both of these colonization rates are at or above those of the general population (0.2-7.4%) but this could be due to the relatively small sample size of only 23 subjects [202]. One of the employees from the dirty side was positive for nasal colonization three times. The isolates from this employee's nose were different in January (ST231 SCC*mec* II) vs in April and July (ST5 SCC*mec* II) so it is unclear if this represents the same strain. The one employee from the clean side that carried

MRSA in their nose carried ST188. This was the first time that ST188 was found in humans in Washington State as it is a common strain in Asia [191,192]. This strain was whole genome sequenced and found to be very similar to previously characterized ST188 SSC*mec* V from Hong Kong and is unlikely to have come from the laundry environment. This was the only strain that had whole genome sequencing so it is unknown what, if any, known strains from the dirty side were similar to.

Chapter four describes surface contamination with VRE. VRE was found at much higher rates (74/240 isolates) than *C. difficile* (27/240 isolates) and MRSA (36/240 isolates). As with the other pathogens, VRE was present at higher rates in the dirty area vs the clean area (53% vs 8%, $p<0.001$). VRE isolates were speciated using 16S sequencing and we isolated 71 *E. faecium*, two *E. faecalis* and one *E. hirae*. This distribution correlates with what is normally found in hospital settings for infections with VRE [132]. All isolates were shown to carry the *vanA* gene but not the *vanB* or *vanC1* gene. The *van* genes are important in clinical disease with *vanA* being responsible for the majority of human VRE disease worldwide [143]. However, there are some geographic locations such as Australia and Europe where *vanB* predominates [132,145–147]. The majority of the VRE isolates (98.6%, 73/74) were resistant to three or more antibiotics which included ciprofloxacin, chloramphenicol, clindamycin, erythromycin, linezolid, quinupristin-dalfopristin, minocycline, tetracycline, and nitrofurantoin. 14 different MLST types were identified among the *E. faecium* isolates: 16, 17, 18, 117, 186, 280, 324, 412, 584, 664, 665, 736, 750 and 1038. Both *E. faecalis* isolates were ST109 and the *E. hirae* was not sequenced via MLST.

The fifth chapter examined the odds of exposure to environmental contamination. The locations with a higher odds of surface contamination may represent an increased exposure risk

to workers. The results of this work support the hypothesis that laundry facility workers who handle soiled linens are at higher risk of exposure to bacterial pathogens then workers who do not handle soiled linens. Survey data showed that employees from the dirty and clean side were not statistically different with the exception of time spent in US, gender and MRSA nasal colonization. Age, household size, smoking behavior, antibiotic use and current work at a hospital were not significantly different between the two groups. There was no difference in self-reported hand washing behavior between the two groups of employees with the exception of one employee from the dirty area that reported not washing their hands after removing their gloves. Employee observation also showed some difference in self-touching behavior but it was not significant (Table 5.1).

6.1 LIMITATIONS

This study has a number of limitations including the inherently poor recovery of microbes from environmental surfaces, difficulty in culturing specific bacteria such as with *C. difficile* spores, differences in incubation times and media used [178,179]. All of which could have led to underestimation of true prevalence for each of the pathogens. The characterization of a single colony from each sample most likely led to an underestimation of diversity in strains for each pathogen because we most likely characterized the most abundant type from each sample. Future studies comparing the whole genomes of both the human and environmental isolates would help to elucidate the relationship between the strains from the contaminated laundry environment and those isolated from laundry personnel. In addition, whole genome analysis would allow one to determine if isolates from different areas and different times within the facility were genetically related as some of our data suggests. Further studies involving collection of health records of employees including immunological function and other exposures

would need to be done in order to characterize the risk of infection due to exposure in the laundry. Ideally an exposure limit to each of the three pathogens would be developed. This would help determine if the risk of exposure is high enough to warrant changes in the handling and transportation of soiled clinical linens.

6.2 DISSERTATION CONCLUSION

Despite these limitations, environmental contamination at the UW Consolidated Laundry Facility for the presence of *C. difficile*, MRSA and VRE was assessed for the first time. As hypothesized, the dirty area was much more contaminated than the clean areas of the laundry facility overall and for each specific pathogen. The dirtiest areas were the primary and secondary sort areas. This was not surprising due to the handling of clinically soiled linens in these areas. This indicates that soiled clinical linens are a source of hospital bacterial contamination in the laundry facility. These linens could also be a potential reservoir for prolonged environmental contamination and subsequent occupational exposure.

The COG washers, which are physically located on the clean floor, had contamination at levels much higher than other areas on the clean floor at 45.8%. These levels were between those found in the receiving (28.3%) and COG (62.5%) areas, both on the dirty side (Figure 5.1). Due to the findings of this study, additional disinfection measures, PPE guidelines and hygiene practices were implemented in the COG washer area. These practices should reduce potential exposure and persistence of pathogens.

Chapter 7. REFERENCES

1. Sitzlar, B. *et al.* An environmental disinfection odyssey: evaluation of sequential interventions to improve disinfection of *Clostridium difficile* isolation rooms. *Infect. Control Hosp. Epidemiol.* **34,** 459–465 (2013).

2. Boyce, J. M., Havill, N. L. & Moore, B. Terminal decontamination of patient rooms using an automated mobile UV light unit. *Infect. Control Hosp. Epidemiol.* **32,** 737–742 (2011).

3. Shaughnessy, M. *et al.* Evaluation of hospital room assignment and acquisition of *Clostridium difficile* infection. *Infect. Control Hosp. Epidemiol.* **32,** 201–206 (2011).

4. Dancer, S. The role of environmental cleaning in the control of hospital-acquired infection. *J. Hosp. Infect.* **73,** 378–385 (2009).

5. Boyce, J. M. Environmental contamination makes an important contribution to hospital infection. *J. Hosp. Infect.* **65, Supplement 2,** 50–54 (2007).

6. Boone, S. & Gerba, C. Significance of fomites in the spread of respiratory and enteric viral disease. *Appl. Environ. Microbiol.* **73,** 1687–1696 (2007).

7. Dancer, S. Mopping up hospital infection. *J. Hosp. Infect.* **43,** 85–100 (1999).

8. Boyce, J. M., Potter-Bynoe, G., Chenevert, C. & King, T. Environmental contamination due to methicillin-resistant Staphylococcus aureus: possible infection control implications. *Infect. Control Hosp. Epidemiol. Off. J. Soc. Hosp. Epidemiol. Am.* **18,** 622–627 (1997).

9. Kim, K. H. *et al.* Isolation of *Clostridium difficile* from the environment and contacts of patients with antibiotic-associated colitis. *J. Infect. Dis.* **143,** 42–50 (1981).

10. Fekety, R. *et al.* Epidemiology of antibiotic-associated colitis; isolation of *Clostridium difficile* from the hospital environment. *Am. J. Med.* **70,** 906–908 (1981).

11. Rabinowitz, P. M. *et al.* Toward proof of concept of a One Health approach to disease prediction and control. *Emerg. Infect. Dis.* **19,** (2013).

12. Bijlsma, R. & Loeschcke, V. Environmental stress, adaptation and evolution: an overview. *J. Evol. Biol.* **18,** 744–749 (2005).

13. Samet, J. M. & Spengler, J. D. Indoor environments and health: moving into the 21st century. *Am. J. Public Health* **93,** 1489–1493 (2003).

14. Villanueva, K. *et al.* The impact of the built environment on health across the life course: design of a cross-sectional data linkage study. *BMJ Open* **3,** e002482 (2013).

15. CDC. Indoor Environmental Quality. *NIOSH Workplace Safety and Health Topic* (2015). Available at: https://www.cdc.gov/niosh/topics/indoorenv/. (Accessed: 26th July 2016)

16. Fraser, D. W. The challenges were legion. *Lancet Infect. Dis.* **5,** 237–241 (2005).

17. Forsberg, K. J. *et al.* The Shared Antibiotic Resistome of Soil Bacteria and Human Pathogens. *Science* **337,** 1107–1111 (2012).

18. Zika Virus. *CDC* (2014). Available at: http://www.cdc.gov/zika/geo/active-countries.html. (Accessed: 5th August 2016)

19. Manian, F. A. & Ponzillo, J. J. Compliance with routine use of gowns by healthcare workers (HCWs) and non-HCW visitors on entry into the rooms of patients under contact precautions. *Infect. Control Hosp. Epidemiol.* **28,** 337–340 (2007).

20. Otter, J., Yezli, S. & French, G. The role played by contaminated surfaces in the transmission of nosocomial pathogens. *Infect. Control Hosp. Epidemiol.* **32,** 687–699 (2011).

21. Snitkin, E. S. *et al.* Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci. Transl. Med.* **4,** 148ra116 (2012).

22. Weinstein, R. A. & Hota, B. Contamination, disinfection, and cross-colonization: are hospital surfaces reservoirs for nosocomial infection? *Clin. Infect. Dis.* **39,** 1182–1189 (2004).

23. Belani, A., Sherertz, R. J., Sullivan, M. L., Russell, B. A. & Reumen, P. D. Outbreak of staphylococcal infection in two hospital nurseries traced to a single nasal carrier. *Infect. Control* **7,** 487–490 (1986).

24. Sherertz, R. J. *et al.* A cloud adult: The *Staphylococcus aureus*-virus interaction revisited. *Ann. Intern. Med.* **124,** 539–547 (1996).

25. Hayden, M. K., Blom, D. W., Lyle, E. A., Moore, C. A. & Weinstein, R. A. Risk of hand or glove contamination after contact with patients colonized with vancomycin-resistant *Enterococcus* or the colonized patients' environment. *Infect. Control Hosp. Epidemiol.* **29,** 149–154 (2008).

26. Huslage, K., Rutala, W. A., Sickbert-Bennett, E. & Weber, D. J. A quantitative approach to defining 'high-touch' surfaces in hospitals. *Infect. Control Hosp. Epidemiol.* **31,** 850–853 (2010).

27. Rutala, W. A. & Weber, D. J. in *A guide to infection control in the hospital* 14–21 (International Society for Infectious Diseases, 2008).

28. Dancer, S. J. Controlling hospital-acquired infection: focus on the role of the environment and new technologies for decontamination. *Clin. Microbiol. Rev.* **27,** 665–690 (2014).

29. Trick, W. E. *et al.* Patient colonization and environmental contamination by vancomycin-resistant enterococci in a rehabilitation facility. *Arch. Phys. Med. Rehabil.* **83,** 899–902 (2002).

30. Boyce, J. M., Havill, N. L., Otter, J. A. & Adams, N. M. T. Widespread environmental contamination associated with patients with diarrhea and methicillin-resistant *Staphylococcus aureus* colonization of the gastrointestinal tract. *Infect. Control Hosp. Epidemiol.* **28,** 1142–1147 (2007).

31. Boyce, J., Potter-Bynoe, G., Chenevert, C. & King, T. Environmental contamination due to methicillin-resistant *Staphylococcus aureus* possible infection control implications. *Infect. Control Hosp. Epidemiol.* **18,** 622–627 (1997).

32. Ray, A., Hoyen, C. & Taub, T. Nosocomial transmission of vancomycin-resistant enterococci from surfaces. *J. Am. Med. Assoc.* **287,** 1400–1401 (2002).

33. Lawley, T. D. *et al.* Use of purified *Clostridium difficile* spores to facilitate evaluation of health care disinfection regimens. *Appl. Environ. Microbiol.* **76,** 6895–6900 (2010).

34. Desai, R. *et al.* Survival and transmission of community-associated methicillin-resistant *Staphylococcus aureus* from fomites. *Am. J. Infect. Control* **39,** 219–225 (2011).

35. Roberts, M. C., Soge, O. O., No, D., Beck, N. & Meschke, J. S. Isolation and characterization of methicillin-resistant *Staphylococcus aureus* from fire stations in two northwest fire districts. *Am. J. Infect. Control* **39,** 382–389 (2011).

36. Samore, M. H., Venkataraman, L., DeGirolami, P. C., Arbeit, R. D. & Karchmer, A. W. Clinical and molecular epidemiology of sporadic and clustered cases of nosocomial *Clostridium difficile* diarrhea. *Am. J. Med.* **100,** 32–40 (1996).

37. Mayfield, J. L., Leet, T., Miller, J. & Mundy, L. M. Environmental control to reduce transmission of *Clostridium difficile*. *Clin. Infect. Dis.* **31,** 995–1000 (2000).

38. Wilcox, M. H. *et al.* Comparison of the effect of detergent versus hypochlorite cleaning on environmental contamination and incidence of *Clostridium difficile* infection. *J. Hosp. Infect.* **54,** 109–114 (2003).

39. Boyce, J. M. *et al.* Impact of hydrogen peroxide vapor room decontamination on *Clostridium difficile* environmental contamination and transmission in a healthcare setting. *Infect. Control Hosp. Epidemiol.* **29,** 723–729 (2008).

40. Hardy, K. J., Oppenheim, B. A., Gossain, S., Gao, F. & Hawkey, P. M. A study of the relationship between environmental contamination with methicillin-resistant *Staphylococcus aureus* (MRSA) and patients' acquisition of MRSA. *Infect. Control Hosp. Epidemiol.* **27,** 127–132 (2006).

41. Mahamat, A. *et al.* Impact of infection control interventions and antibiotic use on hospital MRSA: a multivariate interrupted time-series analysis. *Int. J. Antimicrob. Agents* **30,** 169–176 (2007).

42. Bonten, M. J. *et al.* Epidemiology of colonisation of patients and environment with vancomycin-resistant enterococci. *The Lancet* **348,** 1615–1619 (1996).

43. Hayden, M. K. *et al.* Reduction in acquisition of vancomycin-resistant *Enterococcus* after enforcement of routine environmental cleaning measures. *Clin. Infect. Dis.* **42,** 1552–1560 (2006).

44. Guerrero, D. M. *et al.* Acquisition of spores on gloved hands after contact with the skin of patients with *Clostridium difficile* infection and with environmental surfaces in their rooms. *Am. J. Infect. Control* **40,** 556–558 (2012).

45. Stiefel, U. *et al.* Contamination of hands with methicillin-resistant *Staphylococcus aureus* after contact with environmental surfaces and after contact with the skin of colonized patients. *Infect. Control Hosp. Epidemiol.* **32,** 185–187 (2011).

46. Gaspard, P. *et al.* Meticillin-resistant *Staphylococcus aureus* contamination of healthcare workers' uniforms in long-term care facilities. *J. Hosp. Infect.* **71,** 170–175 (2009).

47. Perry, C., Marshall, R. & Jones, E. Bacterial contamination of uniforms. *J. Hosp. Infect.* **48,** 238–241 (2001).

48. Mitchell, A., Spencer, M. & Edmiston Jr., C. Role of healthcare apparel and other healthcare textiles in the transmission of pathogens: a review of the literature. *J. Hosp. Infect.* **90,** 285–292 (2015).

49. Saliou, P. *et al.* Measures to improve microbial quality surveillance of gastrointestinal endoscopes. *Endoscopy* (2016). doi:10.1055/s-0042-107591

50. Otter, J. A., Yezli, S., Salkeld, J. A. G. & French, G. L. Evidence that contaminated surfaces contribute to the transmission of hospital pathogens and an overview of strategies to address contaminated surfaces in hospital settings. *Am. J. Infect. Control* **41,** S6–S11 (2013).

51. Leggett, H. C., Cornwallis, C. K. & West, S. A. Mechanisms of pathogenesis, infective dose and virulence in human parasites. *PLOS Pathog* **8,** e1002512 (2012).

52. Mayer, R. A. *et al.* Role of fecal incontinence in contamination of the environment with vancomycin-resistant enterococci. *Am. J. Infect. Control* **31,** 221–225 (2003).

53. Al-Nassir, W. N. *et al.* Comparison of clinical and microbiological response to treatment of *Clostridium difficile*-associated disease with metronidazole and vancomycin. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **47,** 56–62 (2008).

54. CDC. *Antibiotic resistance threats in the United States, 2013*. (US Department of Health and Human Services, CDC, 2013).

55. Anderson, D. J., MD, MPH *et al.* Decontamination of Targeted Pathogens from Patient Rooms Using an Automated Ultraviolet-C-Emitting Device. *Infect. Control Hosp. Epidemiol.* **34,** 466–471 (2013).

56. Srejic, E. *Seeing the light: evidence mounts for the use of UV technology to reduce surface microbial loads, boost manual cleaning and disinfection*. 1–12 (Infection Control Today, 2015).

57. Weese, J. S., Lowe, T. & Walker, M. Use of fluorescent tagging for assessment of environmental cleaning and disinfection in a veterinary hospital. *Vet. Rec.* **171,** 217 (2012).

58. Sepkowitz, K. A. Occupationally acquired infections in health care workers: part I. *Ann. Intern. Med.* **125,** 826–834 (1996).

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

60. Soge, O. *et al.* Transmission of multidrug resistant methicillin-resistant *Staphylococcus aureus* (MRSA) between primates, their environment and personnel at a United States primate center. *J. Antimicrob. Chemother.* (2016). doi:10.1093/jac/dkw236

61. Akduman, D. *et al.* Use of personal protective equipment and operating room behaviors in four surgical subspecialties: personal protective equipment and behaviors in surgery. *Infect. Control Hosp. Epidemiol.* **20,** 110–114 (1999).

62. Stanton, M. & Rutherford, M. *Hospital nurse staffing and quality of care*. (Agency for Healthcare Research and Quality, US Department of Health and Human Services, 2004).

63. Gammon, J., Morgan-Samuel, H. & Gould, D. A review of the evidence for suboptimal compliance of healthcare practitioners to standard/universal infection control precautions. *J. Clin. Nurs.* **17,** 157–167 (2008).

64. Institute of Medicine. *Preventing transmission of pandemic influenza and other viral respiratory diseases: personal protective equipment for healthcare personnel*. (Committee on Personal Protective Equipment for Healthcare Personnel to Prevent the Transmission of Pandemic Influenza and Other Viral Respiratory Infections, 2010).

65. Sexton, J. & Reynolds, K. Exposure of emergency medical responders to methicillin-resistant *Staphylococcus aureus*. *Am. J. Infect. Control* **38,** 368–373 (2010).

66. Roberts, M. C. & No, D. Environment surface sampling in 33 Washington State fire stations for methicillin-resistant and methicillin-susceptible *Staphylococcus aureus*. *Am. J. Infect. Control* **42,** 591–596 (2014).

67. Vikke, H. S. & Giebner, M. POSAiDA: presence of *Staphylococcus aureus*/MRSA and *Enterococcus*/VRE in Danish ambulances. A cross-sectional study. *BMC Res. Notes* **9,** (2016).

68. Roberts, M. C., Soge, O. O., Horst, J., Ly, K. & Milgrom, P. Methicillin-resistant *Staphylococcus aureus* from dental school clinic surfaces and students. *Am. J. Infect. Control* **39,** 628–632 (2011).

69. Wulf, M. *et al.* Methicillin-resistant *Staphylococcus aureus* in veterinary doctors and students, the Netherlands. *Emerg. Infect. Dis.* **12,** 1939–1941 (2006).

70. Chang, S., Sethi, A., Stiefel, U., Cadnum, J. & Donskey, C. Occurrence of skin and environmental contamination with methicillin-resistant *Staphylococcus aureus* before results of polymerase chain reaction at hospital admission become available. *Infect. Control Hosp. Epidemiol.* **31,** 607–612 (2010).

71. Dale, J. Personal communication. (2013).

72. Borg, M. A. & Portelli, A. Hospital laundry workers — an at-risk group for hepatitis A? *Occup. Med.* **49,** 448–450 (1999).

73. Standaert, S. M., Hutcheson, R. H. & Schaffner, W. Nosocomial transmission of *Salmonella gastroenteritis* to laundry workers in a nursing home. *Infect. Control Hosp. Epidemiol.* **15,** 22–26 (1994).

74. Belkin, N. in *APIC text of infection control and epidemiology* **3,** 103.1-103.8 (APIC, 2009).

75. Fijan, S. *et al.* Rotaviral RNA found on various surfaces in a hospital laundry. *J. Virol. Methods* **148,** 66–73 (2008).

76. Gellert, G. A. *et al.* An outbreak of acute gastroenteritis caused by a small round structured virus in a geriatric convalescent facility. *Infect. Control Hosp. Epidemiol.* **11,** 459–464 (1990).

77. McNeil, D. G. The SARS epidemic: the virus; most Taiwan SARS cases spread by one misdiagnosis. *The New York Times* (2003).

78. O'hara, C. Personal communication. (2012).

79. Cohen, M. C. *Do comparisons between hospital support workers and hospitality workers make sense?* (Simon Fraser University, 2001).

80. Samore, M. H. Epidemiology of nosocomial *Clostridium difficile* diarrhoea. *J. Hosp. Infect.* **43 Suppl,** S183-190 (1999).

81. Pereira, F. C. *et al.* The spore differentiation pathway in the enteric pathogen *Clostridium difficile*. *PLoS Genet* **9,** e1003782 (2013).

82. Donaldson, L., Beasley, C. & McCracken, J. Clostridium difficile *infection: how to deal with the problem*. (Public Health England, Department of Health, 2009).

83. Rodriguez-Palacios, A. & LeJeune, J. T. Moist-heat resistance, spore aging, and superdormancy in *Clostridium difficile*. *Appl. Environ. Microbiol.* **77,** 3085–3091 (2011).

84. Wilcox, M. H. & Fawley, W. N. Hospital disinfectants and spore formation by *Clostridium difficile*. *The Lancet* **356,** 1324 (2000).

85. Fawley, W. N. *et al.* Efficacy of hospital cleaning agents and germicides against epidemic *Clostridium difficile* strains. *Infect. Control Hosp. Epidemiol.* **28,** 920–925 (2007).

86. Davies, A., Pottage, T., Bennett, A. & Walker, J. Gaseous and air decontamination technologies for *Clostridium difficile* in the healthcare environment. *J. Hosp. Infect.* **77,** 199–203 (2011).

87. Bobulsky, G. S., Al-Nassir, W. N., Riggs, M. M., Sethi, A. K. & Donskey, C. J. *Clostridium difficile* skin contamination in patients with *C. difficile*–associated disease. *Clin. Infect. Dis.* **46,** 447–450 (2008).

88. Jump, R. L. P., Pultz, M. J. & Donskey, C. J. Vegetative *Clostridium difficile* survives in room air on moist surfaces and in gastric contents with reduced acidity: a potential mechanism to explain the association between proton pump inhibitors and *C. difficile*-associated diarrhea? *Antimicrob. Agents Chemother.* **51,** 2883–2887 (2007).

89. Bartlett, J. G. Historical perspectives on studies of *Clostridium difficile* and *C. difficile* infection. *Clin. Infect. Dis. Off. Publ. Infect. Dis. Soc. Am.* **46 Suppl 1,** S4-11 (2008).

90. Chitnis AS, Holzbauer SM, Belflower RM & et al. Epidemiology of community-associated *Clostridium difficile* infection, 2009 through 2011. *JAMA Intern. Med.* **173,** 1359–1367 (2013).

91. FDA & Center for Drug Evaluation and. Drug safety and availability - FDA drug safety communication: *Clostridium difficile*-associated diarrhea can be associated with stomach acid drugs known as proton pump inhibitors (PPIs). (2016). Available at: http://www.fda.gov/drugs/drugsafety/ucm290510.htm. (Accessed: 26th February 2014)

92. Lee, L. & Cohen, S. Community-acquired *Clostridium difficile* infection: an emerging problem. *Curr. Emerg. Hosp. Med. Rep.* **1,** 149–153 (2013).

93. Rupnik, M., Wilcox, M. H. & Gerding, D. N. *Clostridium difficile* infection: new developments in epidemiology and pathogenesis. *Nat. Rev. Microbiol.* **7,** 526–536 (2009).

94. Drekonja, D. M. *et al.* Comparative effectiveness of *Clostridium difficile* treatments a systematic review. *Ann. Intern. Med.* **155,** 839–847 (2011).

95. Lessa, F., Gould, C. & McDonald, L. Current status of *Clostridium difficile* infection epidemiology. *Clin. Infect. Dis.* **55,** S65–S70 (2012).

96. Hensgens, M. *et al. Clostridium difficile* infection in the community: a zoonotic disease? *Clin. Microbiol. Infect.* **18,** 635–645 (2012).

97. Saif, N. & Brazier, J. The distribution of *Clostridium difficile* in the environment of South Wales. *J. Med. Microbiol.* **45,** 133–137 (1996).

98. Lund, B. & Peck, M. A possible route for foodborne transmission of *Clostridium difficile*? *Foodborne Pathog. Dis.* **12,** 177–182 (2015).

99. Jabbar, U. *et al.* Effectiveness of alcohol-based hand rubs for removal of *Clostridium difficile* spores from hands. *Infect. Control Hosp. Epidemiol.* **31,** 565–570 (2010).

100. Johnson, E., Summanen, P. & Finegold, S. in *Manual of clinical microbiology* (eds. Murray, P., Baron, E., Jorgensen, J., Landry, M. & Pfaller, M.) **1,** 889–910 (ASM Press, 2007).

101. Doan, L. *et al.* Clinical and cost effectiveness of eight disinfection methods for terminal disinfection of hospital isolation rooms contaminated with *Clostridium difficile* 027. *J. Hosp. Infect.* **82,** 114–121 (2012).

102. CDC. Frequently asked questions about *Clostridium difficile* for healthcare providers. (2012). Available at: http://www.cdc.gov/hai/organisms/cdiff/cdiff_faqs_hcp.html. (Accessed: 8th December 2013)

103. Bannerman, T. & Peacock, S. in *Manual of clinical microbiology* (eds. Murray, P., Baron, E., Jorgensen, J., Landry, M. & Pfaller, M.) **1,** 390–411 (ASM Press, 2007).

104. Dinges, M. M., Orwin, P. M. & Schlievert, P. M. Exotoxins of *Staphylococcus aureus*. *Clin. Microbiol. Rev.* **13,** 16–34 (2000).

105. ABCs: 2011 MRSA surveillance report - active bacterial core surveillance. *Active bacterial core surveillance (ABCs)* (2012). Available at:

http://www.cdc.gov/abcs/reports-findings/survreports/mrsa11.html. (Accessed: 31st January 2014)

106. David, M. & Daum, R. Community-associated methicillin-resistant *Staphylococcus aureus*: epidemiology and clinical consequences of an emerging epidemic. *Clin. Microbiol. Rev.* **23,** 616–687 (2010).

107. Miller, M. *et al. Staphylococcus aureus* in the community: colonization versus infection. *PLoS ONE* **4,** e6708 (2009).

108. Soge, O. O., Meschke, J. S., No, D. & Roberts, M. C. Characterization of methicillin-resistant *Staphylococcus aureus* and methicillin-resistant coagulase-negative *Staphylococcus* spp. isolated from US West Coast public marine beaches. *J. Antimicrob. Chemother.* **64,** 1148–1155 (2009).

109. David, M. Z. *et al.* What is community-associated methicillin-resistant *Staphylococcus aureus*? *J. Infect. Dis.* **197,** 1235–1243 (2008).

110. Gedik, H., Voss, T. A. & Voss, A. Money and transmission of bacteria. *Antimicrob. Resist. Infect. Control* **2,** 22 (2013).

111. Roghmann, M. C. *et al.* Transmission of methicillin-resistant *Staphylococcus aureus* (MRSA) to healthcare worker gowns and gloves during care of nursing home residents. *Infect. Control Hosp. Epidemiol.* **FirstView,** 1–8 (2015).

112. Denyer, S. P., Hodges, D. N. A. & Gorman, S. P. *Hugo and Russell's Pharmaceutical Microbiology*. (John Wiley & Sons, 2004).

113. Peacock, S. & Paterson, G. Mechanisms of methicillin resistance in *Staphylococcus aureus*. *Annu. Rev. Biochem.* **84,** 577–601 (2015).

114. Schmid, D. *et al.* Limited value of routine spa typing: a cross-sectional study of methicillin-resistant *Staphylococcus aureus*-positive patients in an Austrian hospital. *Am. J. Infect. Control* **41,** 617–624 (2013).

115. Michael, K. E., No, D. & Roberts, M. C. Methicillin-resistant *Staphylococcus aureus* (MRSA) isolates from surfaces and personnel at a hospital laundry facility. *J. Appl. Microbiol.* **121,** 846–854 (2016).

116. Salipante, S. J. *et al.* Application of whole-genome sequencing for bacterial strain typing in molecular epidemiology. *J. Clin. Microbiol.* **53,** 1072–1079 (2015).

117. Ito, T. Classification of staphylococcal cassette chromosome *mec* (SCC*mec*): guidelines for reporting novel SCC*mec* elements. *Antimicrob. Agents Chemother.* **53,** 4961–4967 (2009).

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

119. Carrel, M., Perencevich, E. & David, M. USA300 methicillin-resistant *Staphylococcus aureus*, United States, 2000–2013. *Emerg Infect Dis* **21,** (2015).

120. Holden, M. T. G. *et al.* A genomic portrait of the emergence, evolution, and global spread of a methicillin-resistant *Staphylococcus aureus* pandemic. *Genome Res.* **23,** 653–664 (2013).

121. Coombs, G. W. *et al.* Community-onset *Staphylococcus aureus* Surveillance Programme annual report, 2012. *Commun. Dis. Intell. Q. Rep.* **38,** E59-69 (2014).

122. Sannazzaro, A. MRSA: the superbug poised to cost hospitals super sums. *Infection Control Today* (2015).

123. CDC. MRSA Tracking. *Methicillin-resistant* Staphylococcus aureus (2016). Available at: https://www.cdc.gov/mrsa/tracking/index.html. (Accessed: 12th July 2016)

124. Sievert, D. *et al.* Antimicrobial-resistant pathogens associated with healthcare-associated infections: summary of data reported to the national healthcare safety network at the centers for disease control and prevention, 2009–2010. *Infect. Control Hosp. Epidemiol.* **34,** 1–14 (2013).

125. Oie, S., Suenaga, S., Sawa, A. & Kamiya, A. Association between isolation sites of methicillin-resistant *Staphylococcus aureus* (MRSA) in patients with MRSA-positive body sites and MRSA contamination in their surrounding environmental surfaces. *Jpn. J. Infect. Dis.* **60,** 367–369 (2007).

126. Pyrek, K. *The benefits of UV-C light*. 10 (Infection Control Today, 2013).

127. Petti, S., De Giusti, M., Moroni, C. & Polimeni, A. Long-term survival curve of methicillin-resistant *Staphylococcus aureus* on clinical contact surfaces in natural-like conditions. *Am. J. Infect. Control* **40,** 1010–1012 (2012).

128. Williams, C. & Davis, D. L. Methicillin-resistant *Staphylococcus aureus* fomite survival. *Clin. Lab. Sci. J. Am. Soc. Med. Technol.* **22,** 34–38 (2009).

129. Teixeira, L., Carvalho, M. & Facklam, R. in *Manual of clinical microbiology* (eds. Murray, P., Baron, E., Jorgensen, J., Landry, M. & Pfaller, M.) **1,** 430–442 (ASM Press, 2007).

130. de Kraker, M. E. A. *et al.* The changing epidemiology of bacteraemias in Europe: trends from the European Antimicrobial Resistance Surveillance System. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* **19,** 860–868 (2013).

131. Agudelo Higuita, N. I. & Huycke, M. M. in *Enterococci: From Commensals to Leading Causes of Drug Resistant Infection* (eds. Gilmore, M. S., Clewell, D. B., Ike, Y. & Shankar, N.) (Massachusetts Eye and Ear Infirmary, 2014).

132. Guzman Prieto, A. M. *et al.* Global emergence and dissemination of enterococci as nosocomial pathogens: attack of the clones? *Front. Microbiol.* **7,** (2016).

133. Tenorio, A. R. *et al.* Effectiveness of gloves in the prevention of hand carriage of vancomycin-resistant *Enterococcus* species by health care workers after patient care. *Clin. Infect. Dis.* **32,** 826–829 (2001).

134. Roberts, R. R. *et al.* Hospital and societal costs of antimicrobial-resistant infections in a Chicago teaching hospital: implications for antibiotic stewardship. *Clin. Infect. Dis.* **49,** 1175–1184 (2009).

135. Sievert, D. M. *et al.* Vancomycin-resistant *Staphylococcus aureus* in the United States, 2002–2006. *Clin. Infect. Dis.* **46,** 668–674 (2008).

136. Fox, B. & Bland, S. E. *UWHC guidelines for the use of Daptomycin (Cubicin®)*. (University of Wisconsin-Madison Health Center, Antimicrobial Use Subcommittee, 2011).

137. Silveira, E. *et al.* Co-transfer of resistance to high concentrations of copper and first-line antibiotics among *Enterococcus* from different origins (humans, animals, the environment and foods) and clonal lineages. *J. Antimicrob. Chemother.* **69,** 899–906 (2014).

138. Prematunge, C. *et al.* VRE and VSE bacteremia outcomes in the era of effective VRE therapy: a systematic review and meta-analysis. *Infect. Control Hosp. Epidemiol.* **37,** 26–35 (2016).

139. Daikos, G. L. *et al.* First cluster of vancomycin-resistant *Enterococcus faecalis* isolates in Cyprus. *Clin. Microbiol. Infect.* **11,** 232–234 (2005).

140. Leclercq, R., Derlot, E., Duval, J. & Courvalin, P. Plasmid-mediated resistance to vancomycin and teicoplanin in *Enterococcus faecium*. *N. Engl. J. Med.* **319,** 157–161 (1988).

141. CLSI. *Performance standards for antimicrobial susceptibility testing; twenty-third informational supplement*. (Clinical and Laboratory Standards Institute (CLSI), 2013).

142. Sujatha, S., Praharaj, I., Sujatha, S. & Praharaj, I. Glycopeptide resistance in Gram-positive cocci: a review. *Interdiscip. Perspect. Infect. Dis. Interdiscip. Perspect. Infect. Dis.* **2012,** e781679 (2012).

143. O'Driscoll, T. & Crank, C. W. Vancomycin-resistant enterococcal infections: epidemiology, clinical manifestations, and optimal management. *Infect. Drug Resist.* **8,** 217–230 (2015).

144. Roberts, M. C. *et al.* Vancomycin-resistant *Enterococcus* spp. in marine environments from the West Coast of the USA. *J. Appl. Microbiol.* **107,** 300–307 (2009).

145. Jakovac, S. *et al.* Characteristics of vancomycin-resistant *Enterococcus* strains in the West Balkans: a first report. *Microb. Drug Resist. Larchmt. N* (2016). doi:10.1089/mdr.2015.0355

146. López, M., Cercenado, E., Tenorio, C., Ruiz-Larrea, F. & Torres, C. Diversity of clones and genotypes among vancomycin-resistant clinical *Enterococcus* isolates recovered in a Spanish hospital. *Microb. Drug Resist. Larchmt. N* **18,** 484–491 (2012).

147. Thierfelder, C. *et al.* Vancomycin-resistant *Enterococcus*. *Swiss Med. Wkly.* (2012). doi:10.4414/smw.2012.13540

148. Yoon, Y. K. *et al.* Risk factors for prolonged carriage of vancomycin-resistant *Enterococcus faecium* among patients in intensive care units: a case–control study. *J. Antimicrob. Chemother.* **66,** 1831–1838 (2011).

149. Roberts, M. C., Marzluff, J., Delap, J. & Turner, R. Vancomycin resistant *Enterococcus* spp. from crows and their environment in metropolitan Washington State, USA: Is there a correlation between VRE positive crows and the environment? *Vet. Microbiol.* (2016). doi:10.1016/j.vetmic.2016.01.022

150. Hollenbeck, B. L. & Rice, L. B. Intrinsic and acquired resistance mechanisms in *Enterococcus*. *Virulence* **3,** 421–569 (2012).

151. Bhavnani, S. M. *et al.* A nationwide, multicenter, case-control study comparing risk factors, treatment, and outcome for vancomycin-resistant and -susceptible enterococcal bacteremia. *Diagn. Microbiol. Infect. Dis.* **36,** 145–158 (2000).

152. Song, X., MD, MS, Srinivasan, A., MD, Plaut, D., MS & Perl, T. M., MD, MSc. Effect of nosocomial vancomycin-resistant enterococcal bacteremia on mortality, length of stay, and costs. *Infect. Control Hosp. Epidemiol.* **24,** 251–256 (2003).

153. Noble, M. A. *et al.* The toilet as a transmission vector of vancomycin-resistant enterococci. *J. Hosp. Infect.* **40,** 237–241 (1998).

154. Ramadhan, A. A. & Hegedus, E. Survivability of vancomycin resistant enterococci and fitness cost of vancomycin resistance acquisition. *J. Clin. Pathol.* **58,** 744–746 (2005).

155. McAuley, C. M., Gobius, K. S., Britz, M. L. & Craven, H. M. Heat resistance of thermoduric enterococci isolated from milk. *Int. J. Food Microbiol.* **154,** 162–168 (2012).

156. Cui, H., Yang, K., Pagaling, E. & Yan, T. Spatial and temporal variation in enterococcal abundance and its relationship to the microbial community in Hawaii beach sand and water. *Appl. Environ. Microbiol.* **79,** 3601–3609 (2013).

157. Cloutier, D. D., Alm, E. W. & McLellan, S. L. Influence of land use, nutrients, and geography on microbial communities and fecal indicator abundance at Lake Michigan beaches. *Appl. Environ. Microbiol.* **81,** 4904–4913 (2015).

158. Gould, C. & McDonald, L. Bench-to-bedside review: *Clostridium difficile* colitis. *Crit. Care* **12,** 203 (2008).

159. Richardson, C., Kim, P., Lee, C., Bersenas, A. & Weese, J. Comparison of Clostridium difficile isolates from individuals with recurrent and single episode of infection. *Anaerobe* **33,** 105–108 (2015).

160. Carter, G. P., Rood, J. I. & Lyras, D. The role of toxin A and toxin B in *Clostridium difficile*-associated disease. *Gut Microbes* **1,** 58–64 (2010).

161. Tenover, F. C., Tickler, I. A. & Persing, D. H. Antimicrobial-resistant strains of *Clostridium difficile* from North America. *Antimicrob. Agents Chemother.* **56,** 2929–2932 (2012).

162. Dingle, K. *et al.* Clinical *Clostridium difficile* : clonality and pathogenicity locus diversity. *PLOS ONE* **6,** e19993 (2011).

163. Hensgens, M. P. M. *et al.* Diarrhoea in general practice: when should a *Clostridium difficile* infection be considered? Results of a nested case-control study. *Clin. Microbiol. Infect.* **20,** O1067–O1074 (2014).

164. Sethi, A. K., Al-Nassir, W. N., Nerandzic, M. M., Bobulsky, G. S. & Donskey, C. J. Persistence of Skin Contamination and Environmental Shedding of Clostridium difficile during and after Treatment of C. difficile Infection. *Infect. Control Hosp. Epidemiol.* **31,** 21–27 (2010).

165. Tyrrell, K. L., Citron, D. M., Leoncio, E. S., Merriam, C. V. & Goldstein, E. J. C. Evaluation of Cycloserine-Cefoxitin Fructose Agar (CCFA), CCFA with Horse Blood and Taurocholate, and Cycloserine-Cefoxitin Mannitol Broth with Taurocholate and Lysozyme for Recovery of Clostridium difficile Isolates from Fecal Samples. *J. Clin. Microbiol.* **51,** 3094–3096 (2013).

166. Griffiths, D. *et al.* Multilocus sequence typing of *Clostridium difficile*. *J. Clin. Microbiol.* **48,** 770–778 (2010).

167. Gilca, R., Fortin, É., Frenette, C., Longtin, Y. & Gourdeau, M. Seasonal variations in *Clostridium difficile* infections are associated with influenza and respiratory syncytial virus activity independently of antibiotic prescriptions: a time series analysis in Québec, Canada. *Antimicrob. Agents Chemother.* **56,** 639–646 (2012).

168. Lessa, F. C. *et al.* Burden of *Clostridium difficile* infection in the United States. *N. Engl. J. Med.* **372,** 825–834 (2015).

169. Zaiß, N. H. *et al.* Typing Clostridium difficile strains based on tandem repeat sequences. *BMC Microbiol.* **9,** 6 (2009).

170. Feil, E. J., Li, B. C., Aanensen, D. M., Hanage, W. P. & Spratt, B. G. eBURST: inferring patterns of evolutionary descent among clusters of related bacterial genotypes from multilocus sequence typing Data. *J. Bacteriol.* **186,** 1518–1530 (2004).

171. Rotimi, V. O. *et al.* Prevalent PCR ribotypes of clinical and environmental strains of Clostridium difficile isolated from intensive-therapy unit patients in Kuwait. *J. Med. Microbiol.* **52,** 705–709 (2003).

172. Lemee, L., Dhalluin, A., Pestel-Caron, M., Lemeland, J.-F. & Pons, J.-L. Multilocus sequence typing analysis of human and animal *Clostridium difficile* isolates of various toxigenic types. *J. Clin. Microbiol.* **42,** 2609–2617 (2004).

173. Lemee, L. *et al.* Multilocus sequence analysis and comparative evolution of virulence-associated genes and housekeeping genes of *Clostridium difficile*. *Microbiology* **151,** 3171–3180 (2005).

174. Stabler, R. *et al.* Macro and micro diversity of *Clostridium difficile* isolates from diverse sources and geographical locations. *PLOS ONE* **7,** e31559 (2012).

175. Walker, A. *et al.* Characterisation of *Clostridium difficile* hospital ward–based transmission using extensive epidemiological data and molecular typing. *PLOS Med* **9,** e1001172 (2012).

176. Cheng, A. *et al.* Laboratory-based surveillance of *Clostridium difficile* circulating in Australia, September – November 2010. *Pathology (Phila.)* **48,** 257–260 (2016).

177. Profile information: ST-284 (MLST) - *Clostridium difficile* locus/sequence definitions. (2015). Available at: http://pubmlst.org/perl/bigsdb/bigsdb.pl?page=profileInfo&db=pubmlst_cdifficile_seqdef&scheme_id=1&profile_id=284. (Accessed: 21st April 2016)

178. Downey, A. S., Silva, S. M. D., Olson, N. D., Filliben, J. J. & Morrow, J. B. Impact of Processing Method on Recovery of Bacteria from Wipes Used in Biological Surface Sampling. *Appl. Environ. Microbiol.* **78,** 5872–5881 (2012).

179. Claro, T., Daniels, S. & Humphreys, H. Detecting *Clostridium difficile* spores from inanimate surfaces of the hospital environment: which method is best? *J. Clin. Microbiol.* **52,** 3426–3428 (2014).

180. Fijan, S. & Sostar Turk, S. Hospital textiles, are they a possible vehicle for healthcare-associated infections? *Int. J. Environ. Res. Public. Health* **9,** 3330–3343 (2012).

181. Miller, L. & Diep, B. Colonization, fomites, and virulence: rethinking the pathogenesis of community-associated methicillin-resistant *Staphylococcus aureus* infection. *Clin. Infect. Dis.* **46,** 752–760 (2008).

182. Sexton, T., Clarke, P., O'Neill, E., Dillane, T. & Humphreys, H. Environmental reservoirs of methicillin-resistant *Staphylococcus aureus* in isolation rooms: correlation with patient isolates and implications for hospital hygiene. *J. Hosp. Infect.* **62,** 187–194 (2006).

183. Smith, T., Moritz, E., Leedom Larson, K. & Ferguson, D. The environment as a factor in methicillin-resistant *Staphylococcus aureus* transmission. *Rev. Environ. Health* **25,** 121–134 (2010).

184. Uhlemann, A. *et al.* The environment as an unrecognized reservoir for community-associated methicillin resistant *Staphylococcus aureus* USA300: a case-control study. *PLoS ONE* **6,** e22407 (2011).

185. Enright, M., Day, N., Davies, C., Peacock, S. & Spratt, B. Multilocus sequence typing for characterization of methicillin-resistant and methicillin-susceptible clones of *Staphylococcus aureus*. *J. Clin. Microbiol.* **38,** 1008–1015 (2000).

186. Harbottle, H., Thakur, S., Zhao, S. & White, D. G. Genetics of antimicrobial resistance. *Anim. Biotechnol.* **17,** 111–124 (2006).

187. Crawford, S. *et al.* Clinical importance of purulence in methicillin-resistant *Staphylococcus aureus* skin and soft tissue infections. *J. Am. Board Fam. Med.* **22,** 647–654 (2009).

188. Murphy, E. *et al.* Challenges for the evaluation of *Staphylococcus aureus* protein based vaccines: monitoring antigenic diversity. *Hum. Vaccin.* **7 Suppl,** 51–59 (2011).

189. Tenover, F. *et al.* Characterization of nasal and blood culture isolates of methicillin-resistant *Staphylococcus aureus* from patients in United States hospitals. *Antimicrob. Agents Chemother.* **56,** 1324–1330 (2012).

190. Sousa, M. *et al.* Frequent recovery of a single clonal type of multidrug-resistant *Staphylococcus aureus* from patients in two hospitals in Taiwan and China. *J. Clin. Microbiol.* **41,** 159–163 (2003).

191. Ghaznavi-Rad, E. *et al.* Predominance and emergence of clones of hospital-acquired methicillin-resistant *Staphylococcus aureus* in Malaysia. *J. Clin. Microbiol.* **48,** 867–872 (2010).

192. Yu, F. *et al.* Virulence gene profiling and molecular characterization of hospital-acquired *Staphylococcus aureus* isolates associated with bloodstream infection. *Diagn. Microbiol. Infect. Dis.* **74,** 363–368 (2012).

193. University Of Washington. 2014 Antibiogram for U of Washington Harborview Medical Centers. (2015). Available at: http://hsl.uw.edu/files/antibiograms/uwmc-hmc-2014-antibiogram.

194. Cetinkaya, Y., Falk, P. & Mayhall, C. G. Vancomycin-resistant enterococci. *Clin. Microbiol. Rev.* **13,** 686–707 (2000).

195. Novais, C., Coque, T. M., Ferreira, H., Sousa, J. C. & Peixe, L. Environmental contamination with vancomycin-resistant enterococci from hospital sewage in Portugal. *Appl. Environ. Microbiol.* **71,** 3364–3368 (2005).

196. UW Medicine. About Harborview medical center. (2016). Available at: http://www.uwmedicine.org/harborview/about. (Accessed: 27th June 2016)

197. UW Medicine. University of Washington medical center: overview. (2013). Available at: http://www.uwmedicine.org/about/Documents/UWMC-fact-sheet.pdf. (Accessed: 27th June 2016)

198. Tomita, H., Pierson, C., Lim, S. K., Clewell, D. B. & Ike, Y. Possible connection between a widely disseminated conjugative gentamicin resistance (pMG1-like) plasmid and the emergence of vancomycin resistance in Enterococcus faecium. *J. Clin. Microbiol.* **40,** 3326–3333 (2002).

199. Wang, G. *et al.* Identification of a novel clone, ST736, among *Enterococcus faecium* clinical isolates and its association with daptomycin nonsusceptibility. *Antimicrob. Agents Chemother.* **58,** 4848–4854 (2014).

200. CDC. *National and State Healthcare-Associated Infections Progress Report*. (2016).

201. Carling, P. Methods for assessing the adequacy of practice and improving room disinfection. *Am. J. Infect. Control* **41,** S20–S25 (2013).

202. Salgado, C., Farr, B. & Calfee, D. Community-acquired methicillin-resistant *Staphylococcus aureus*: a meta-analysis of prevalence and risk factors. *Clin. Infect. Dis.* **36,** 131–9 (2003).

203. Dulon, M., Peters, C., Schablon, A. & Nienhaus, A. MRSA carriage among healthcare workers in non-outbreak settings in Europe and the United States: a systematic review. *BMC Infect. Dis.* **14,** 363 (2014).

204. Sehulster LM *et al. Guidelines for environmental infection control in health-care facilities recommendations of CDC and the Healthcare Infection Control Practices Advisory Committee (HICPAC)*. (CDC and HICPAC, 2004).

205. European Chemicals Bureau. *Technical guidance document on risk assessment*. (Institute for Health and Consumer Protection, 2003).

206. Kehoe, B. Understanding linens and disease transmission. *Health Facil. Manage.* **28,** 12–13 (2015).

207. Cheng, V. C. C. *et al.* Hospital outbreak of pulmonary and cutaneous zygomycosis due to contaminated linen items from substandard laundry. *Clin. Infect. Dis.* **62,** 714–721 (2016).

208. Fujitani, S., George, W. L., Morgan, M. A., Nichols, S. & Murthy, A. R. Implications for vancomycin-resistant Enterococcus colonization associated with *Clostridium difficile* infections. *Am. J. Infect. Control* **39,** 188–193 (2011).

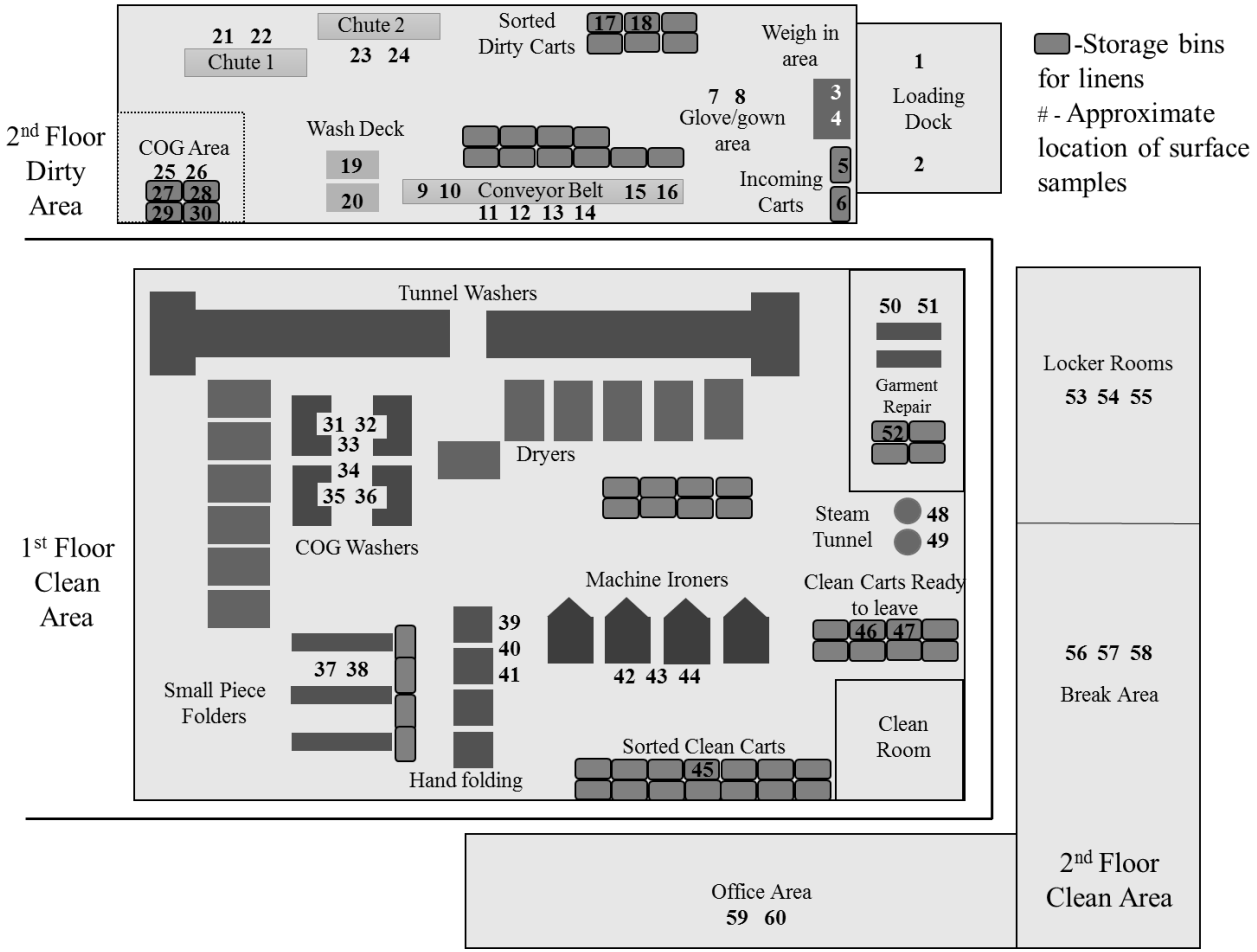
209. Wang, X., Towers, S., Panchanathan, S. & Chowell, G. A population based study of seasonality of skin and soft tissue infections: implications for the spread of CA-MRSA. *PLoS ONE* **8,** (2013).

210. Piepel, G., Amidan, B. & Hu, R. Laboratory studies on surface sampling of *Bacillus anthracis* contamination: summary, gaps and recommendations. *J. Appl. Microbiol.* **113,** 1287–1304 (2012).

211. Noordzij, M. *et al.* Sample size calculations: basic principles and common pitfalls. *Nephrol. Dial. Transplant.* **25,** 1388–1393 (2010).

Chapter 8. APPENDICES

8.1 APPENDIX 1 MAP OF CONSOLIDATED LAUNDRY



Map of Consolidated Laundry. Layout of the two floors of the laundry facility with numbers that correspond to where environmental samples were collected at each time period.

 : Storage bins for linens, #: Approximate location of surface samples

8.2 APPENDIX 2 SURFACE SAMPLE SIZE CALCULATION

x: %difference	n: Sample size in each area with 80% power	n: Sample size in each area with 90% power
10	119	158
20	30	40
30	14	18

8.2.1 *Equation 1*

$$n = \frac{[p_D(1-p_D) + p_C(1-p_C)](z_{1-0.95} + z_{0.8})^2}{x^2}$$

- n= sample size
- p_D=17%
- p_C=1%
- $z_{1-0.95}$=1.96
- $z_{.8}$= 0.842
- $z_{.9}$= 1.28

During the pilot phase of this study, surface samples were collected from both the clean and dirty areas of the laundry to determine differences in prevalence of contamination. The prevalence of positive surface samples from the dirty area (p_D) was 17% and 1% for the clean areas (p_C). A sample size of 30 was chosen to allow 80% power to detect a 20% difference between sampling times using Equation 1 adapted from Noordzij *et al*, 2010 [211].

VITA

Karen Michael completed her BS degree in Biology with a minor in Chemistry at Georgia State University in Atlanta, GA. She then went on to complete her MPH in Environmental and Occupational Health at Drexel University in Philadelphia, PA.