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3. Hess AS, Shardell M, Johnson JK, Thom KA, Strassle P, Netzer G, Harris AD. Methods and recommendations for evaluating and reporting a new diagnostic test. *Eur J Clin Microbiol Infect Dis.* 2012 Sep;31(9):2111-6.
4. Stewart VA, McGrath SM, Walsh DS, Davis S, Hess AS, Ware LA, Kester KE, Cummings JF, Burge JR, Voss G, Delchambre M, Garçon N, Tang DB, Cohen JD, Heppner DG Jr. Pre-clinical evaluation of new adjuvant formulations to improve the immunogenicity of the malaria vaccine RTS,S/AS02A. *Vaccine.* 2006 Oct 30;24(42-43):6483-92.

## Abstracts

1. Stansbury LG, Hess AS, Thompson K, Kramer E, Scalea TM, Hess JR. The clinical significance of platelet counts in the first 24 hours after severe injury. Accepted for Oral Presentation, American Association of Blood Banks, Boston, October 2012
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3. Hess AS, Gill S, Patel PR, Bulens SN, Limbago B, Ray SM, Kreisel K, Roghmann M for the EIP/ABCs Invasive MRSA Surveillance Investigators. Bacterial Genetic Determinants of Virulence Associated with In-Hospital Mortality among Outpatient Dialysis Patients with Bloodstream Methicillin-Resistant Staphylococcus aureus (MRSA) Infections. Fifth Decennial International Conference on Healthcare-Associated Infections, Atlanta, GA, March 2010 (Poster Presentation).

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## Abstract

Title of Dissertation: Anticipation and Prevention of Antimicrobial-Resistant Bacterial Infection in Severely Ill Hospitalized Patients

Aaron R. G. S. Hess, Doctor of Philosophy, 2012

Dissertation Directed by: Anthony Harris MD MPH  
Professor  
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Background: Antibiotic-resistant infections are a common complication of hospital care in the United States and associated with increases in cost, length of stay, and mortality.

Methods proposed to anticipate and prevent these infections include improved hospital cleaning and the use of prior culture data.

Objectives: To test enhanced hospital cleaning as a potential method to reduce patient-to-patient transmission of antibiotic-resistant infections among intensive-care patients, and to evaluate the relationship between prior colonization with antibiotic-resistant bacteria and the subsequent risk of antibiotic-resistant infections among cancer patients.

Methods: We tested the effect of enhanced room cleaning on the contamination of healthcare workers' gowns and gloves with antibiotic-resistant bacteria by conducting a cluster-randomized, controlled trial in four intensive care units. The relationship between prior colonization with antibiotic-resistant bacteria and the subsequent risk of antibiotic-resistant infections among cancer patients was assessed with a matched case-control study of cancer patients with suspected infection who subsequently developed an antibiotic-resistant Gram-negative bacteremia.

Results: The trial enrolled 142 hospital rooms and sampled 4,444 patient-healthcare worker interactions. Improved room cleaning was associated with a reduction in gown

and glove contamination with both methicillin-resistant *S. aureus* (relative risk: 0.89, 95% confidence interval: 0.50 – 1.53) and multidrug-resistant *A. baumannii* (relative risk: 0.77, 95% confidence interval: 0.28 – 2.11). In the case-control study of cancer patients with suspected infection, antibiotic-resistant Gram-negative colonization was associated with subsequent antibiotic-resistant Gram-negative bacteremia after exposure to broad-spectrum antibiotics in the previous month (odds ratio: 8.1, 95% confidence interval: 3.6 – 18.0).

Conclusion: Improved room cleaning may reduce the risk of patient-to-patient transmission of antibiotic-resistant bacteria in the intensive care setting. Prior colonization with antibiotic-resistant Gram-negative bacteria is a risk factor for subsequent bacteremia among cancer patients, and might be used to guide antibiotic therapy.

Anticipation and Prevention of Antimicrobial-Resistant Bacterial Infection in Severely Ill  
Hospitalized Patients

by  
Aaron R. G. S. Hess

Dissertation submitted to the Faculty of the Graduate School of the  
University of Maryland, Baltimore in partial fulfillment  
of the requirements for the degree of  
Doctor of Philosophy  
2012

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## **Dedication**

This dissertation is dedicated to my wife and parents  
for their love, support, and good advice.

Allison L. Lindell  
John R. Hess  
Lynn G. Stansbury

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## **Abbreviations**

ARGN	antibiotic-resistant Gram-negative
CDC	Centers for Disease Control and Prevention
CLSI	Clinical Laboratory Standards Institute
HAI	Hospital Acquired Infections
HCW	Health Care Worker
ICD-9	International Classification of Disease, 9 <sup>th</sup> Revision
ICU	Intensive Care Unit
IDSA	Infectious Disease Society of America
MICU	Medical Intensive Care Unit
MDRAB	Antibiotic-resistant <i>Acinetobacter baumannii</i>
MRSA	Methicillin-resistant <i>Staphylococcus aureus</i>
UMMC	University of Maryland Medical Center

## I. INTRODUCTION AND OBJECTIVES

Hospital-acquired infections (HAIs), a complication of care, are often preventable and associated with substantial morbidity and mortality. In spite of increasing focus on the prevention and treatment of these infections nationwide, there are estimated 250,000 hospital-acquired bloodstream infections, and a similar number of hospital-acquired pneumonias, annually.<sup>(1, 2)</sup> HAIs are associated with significant increases in cost of care, length of hospital stay, and mortality.<sup>(3-7)</sup> Management of these infections is made more difficult by antibiotic-resistant organisms such as methicillin-resistant *Staphylococcus aureus* (MRSA) and multidrug-resistant *Acinetobacter baumannii* (MDRAB). Hospital infection with Antibiotic-resistant bacteria is associated with further increases in costs and mortality compared to drug-susceptible HAIs.<sup>(8-10)</sup> Although resistance to the most powerful antimicrobials is increasing, the pace of new antimicrobial development is not keeping up with need.<sup>(11-14)</sup> Improvements in non-pharmacologic infection control and prevention methods such as environmental cleaning, novel bio-medical interventions, and antimicrobial stewardship are absolutely necessary to address HAIs caused by antibiotic-resistant bacteria.<sup>(11)</sup>

Preventing antibiotic-resistant bacterial transmission in the ICU is a major focus of infection control.<sup>(5)</sup> Regular hand-washing, contact precautions (the use of disposable gloves and gowns, and in some circumstances face masks, respirators, and whole-body suits) and patient isolation are standard techniques.<sup>(10, 15)</sup> Environmental surfaces in patient rooms in the ICU are frequently contaminated with antibiotic-resistant bacteria such as MRSA and MDRAB.<sup>(16)</sup> Patient rooms are also not sanitized thoroughly or frequently enough to keep commonly touched surfaces free of bacteria.<sup>(17-19)</sup> Healthcare

workers frequently touch surfaces in hospital rooms, and their hands, clothing, or personal protective equipment are often contaminated with any antibiotic resistant bacteria present on those surfaces.<sup>(20-22, 64-66)</sup> Gown and glove contamination is therefore an early step in a hypothesized chain of events between environmental contamination and acquisition of antibiotic-resistant bacteria

Several studies have concluded that “enhanced cleaning” (additional cleaning of frequently-touched, frequently-contaminated surfaces while the patient is still in the room) leads to significant reductions in environmental contamination.<sup>(20-24)</sup> To date, two trials have examined the effect of enhanced cleaning on patient-to-patient transmission, but neither had adequate statistical power to detect this outcome.<sup>(23, 24)</sup> The primary outcome of both trials was environmental contamination, and, although the trial conducted by Wilson and colleagues sampled HCW hands as part of this measure, it did not have the statistical power to detect a change. If enhanced cleaning of frequently contaminated surfaces in occupied ICU rooms leads to less HCW contamination, it would justify larger multi-center studies to assess the effect of enhanced cleaning on rarer clinical outcomes such as colonization and infection. Confirmed clinical trial results would in turn justify a change in infection control and cleaning practices that may prevent costly infections and save lives.

Monitoring trends in antimicrobial resistance rates within hospitals is a standard component of infection control programs.<sup>(10, 25-29)</sup> Antibiotic resistance rates for individual bacterial species or genera are often used to assist antimicrobial choices. The latter is particularly important when treating infections in neutropenic cancer patients.

Bloodstream infections are the most common complication of neutropenia in patients receiving chemotherapy, and they are associated with significant mortality.<sup>(2, 8, 30-32)</sup>

Because of the risk posed by infections, fever in the neutropenic patient is often treated “empirically,” that is, before any causative organism and its susceptibilities are known. Empiric treatment is made more difficult by antibiotic-resistant Gram-negative (ARGN) organisms, because by definition these organisms are resistant to one or more commonly used antimicrobials.<sup>(2, 30, 33)</sup> Empiric therapy would be more successful on the first attempt if the physician could anticipate the resistance profile of the infectious organism. Although information about what organisms have colonized a particular patient are frequently available through records of past surveillance and clinical cultures, this information is not routinely used to guide empiric therapy.<sup>(30)</sup> If prior colonization with ARGN organisms were associated with subsequent risk of ARGN bacteremia, then surveillance cultures might be used to anticipate the resistance profile of infectious organisms and improve the chances of correct empiric therapy on the first attempt. In addition, prior culture results might be used to identify patients at increased risk of ARGN bacteremia, and guide empiric therapy choices for cancer patients with suspected infection. In summary, the aims of this dissertation focus on methods for identifying patients at risk for acquiring or transmitting antibiotic-resistant bacterial infections. Anticipation and prevention of antibiotic-resistant bacterial infection is an important component of reducing morbidity and mortality in hospital care.

**A. Research Questions**

1. Can enhanced environmental cleaning in the intensive care unit reduce the risk of contamination of healthcare worker's disposable personal protective equipment with antibiotic-resistant bacteria?
2. Is a history of colonization or infection with antibiotic-resistant organisms, specifically Gram-negative organisms, associated with subsequent antibiotic-resistant Gram-negative bacteremia in cancer patients?

**B. Specific Aim 1**

To determine the effect of enhanced cleaning of frequently contaminated objects on the contamination rate of health-care workers' gloves and gowns with MRSA or MDRAB after providing care. We performed a cluster-randomized, single-blind, controlled trial of enhanced cleaning in intensive care unit rooms occupied by patients colonized with MRSA or MDRAB.

**Hypothesis 1**

Enhanced cleaning of frequently-touched and frequently-contaminated environmental surfaces in intensive care unit rooms is subsequently associated with lower detection rate of MRSA or MDRAB on health-care workers' gloves and gowns, as compared to standard cleaning.

**C. Specific Aim 2**

To examine the relationship between prior colonization with ARGN bacteria and subsequent ARGN bacteremia in cancer patients, using a matched case-control study of patients with suspected bloodstream infection admitted to the UMMC Greenebaum Cancer Center from 2001 through 2010.

**Hypothesis 2**

Among cancer patients with suspected infection, antibiotic-resistant Gram-negative (ARGN) bacteremia is associated with prior colonization with ARGN bacteria

## II. BACKGROUND AND SIGNIFICANCE

### A. Overview

#### Antibiotic-Resistant Healthcare-Associated Infections

Hospital-acquired infections (HAIs) are common complications of care in the United States. In 2007, Klevens and colleagues reported the results of data collected by the National Nosocomial Infection Service in 2002, and found 1.7 million HAIs in the United States during that year, more than any reportable disease.<sup>(5)</sup> These infections are associated with increased costs, length of stay, and mortality. A 2009 Centers for Disease Control and Prevention (CDC) report found that the cost of a HAI varied from \$1,000 to \$25,000, depending on the site of infection, with an attributable direct cost of \$6 to \$7 billion, annually.<sup>(7)</sup> Estimates of the attributable mortality of HAIs vary in the literature. A 2010 study by Eber of 69 million hospital discharges in the Nationwide Inpatient Sample found an attributable mortality of 16% for nosocomial bloodstream infection and 10% for nosocomial pneumonia.<sup>(34)</sup> Recent studies of ventilator-associated pneumonia by Bekaert and of nosocomial bloodstream infection by Prowle estimated the attributable mortality rate of HAIs at approximately 1%.<sup>(3, 4)</sup>

Severely ill patient groups, such as those with cancers or in intensive care, are at greater risk of HAI than the general hospital population. A disproportionate number of HAIs occur in the ICU. The 2007 Klevens report found that 22.7% of HAIs in 2002 occurred in intensive care units (ICU), the occupancy of which accounted for only 18% of all inpatient-days. The highest mortality rates for HAIs (11% – 25%) were among infections in the ICU.<sup>(5)</sup> Cancer patients are also at particular risk for infections since many common cancer chemotherapies cause neutropenia (a severe reduction in the

number of circulating white blood cells).<sup>(30, 32, 35)</sup> Bloodstream infections are of particular concern since 25-30% of cancer patients who have neutropenia will experience a bloodstream infection.<sup>(36)</sup> A number of studies have estimated that the crude mortality from bloodstream infections among cancer patients is around 15%.<sup>(32, 36-38)</sup> In summary, HAIs caused by antibiotic-resistant bacteria are associated with increased healthcare costs and mortality.<sup>(3, 4, 39-43)</sup>

Infection control programs in hospitals are central to preventing HAIs and the spread of antibiotic-resistant bacteria. Recommended components of such policies include isolation of patients colonized or infected with antibiotic-resistant bacteria; use of disposable protective clothing (“contact precautions”) when interacting with patients colonized or infected with antibiotic-resistant bacteria; universal emphasis on good hand hygiene; use of active surveillance cultures to track antibiotic-resistant bacteria of particular interest; and thorough environmental cleaning.<sup>(26, 44, 45)</sup> As of October 1, 2008, the Center for Medicare and Medicaid Services no longer reimburses hospitals for “preventable” complications, among which they include many categories of HAI. Therefore, infection control is not only an issue of patient safety but also a financial incentive.<sup>(46)</sup> This proposal focuses on two of the previously mentioned aspects of infection control: environmental cleaning and active surveillance cultures.

### Environmental Cleaning and Antibiotic-Resistant HAIs

Environmental cleaning is a poorly understood and frequently ignored component of infection control. Although it is a recommended component of infection control programs in the U.S. and abroad, numerous studies have demonstrated that many hospital

cleaning programs do a poor job of removing antibiotic-resistant bacteria.<sup>(19, 47-58)</sup> The task is not impossible, however. Good hospital cleaning has been associated with reductions in the environmental burden of antibiotic-resistant bacteria.<sup>(22, 24, 56, 59)</sup> “Terminal cleaning,” cleaning after one patient has been discharged from a room and before the next is admitted, is particularly important. Patients admitted to rooms previously occupied by a patient colonized or infected with antibiotic-resistant bacteria are at increased risk of infection with the same MDRO, antibiotic-resistant bacteria.<sup>(60-62)</sup> However, good terminal cleaning has been shown to reduce the risk of antibiotic-resistant bacteria infection for other patients in the same unit.<sup>(49, 63)</sup> Contamination of healthcare workers’ hands or disposable protective clothing with antibiotic-resistant bacteria while caring for patients have been documented, but the effect of enhanced cleaning on contamination has not been well described.<sup>(22, 24, 64-66)</sup> Limited studies suggest that enhanced cleaning strongly reduces antibiotic-resistant bacterial contamination in the environment and possibly patient infection rates.<sup>(23, 24)</sup>

#### Surveillance Cultures and Antibiotic-Resistant Gram-Negative Bloodstream Infections in Cancer Patients

Surveillance cultures are used in a number of infection control programs to identify patients colonized with antibiotic-resistant bacteria, isolate them, and place them on contact precautions. The combination of surveillance cultures and isolation with contact precautions has been shown in numerous studies to significantly decrease the incidence of antibiotic-resistant, bacterial HAIs in the intensive care setting.<sup>(26, 67, 68)</sup> Among 16 observational studies of HAIs in the ICU, 13 detected a significant subsequent

drop in HAI rates after implementation of surveillance cultures.<sup>(67)</sup> Using the results of surveillance cultures to identify patients (including neutropenic cancer patients) at risk for antibiotic-resistant bacteria infection and guide empiric therapy has been suggested in the past.<sup>(29, 69, 70)</sup> However, the Infectious Disease Society of America (IDSA) has not endorsed this practice.<sup>(30)</sup> In the 2011 IDSA recommendations it is stated that the choice of empiric antibiotics may be altered in light of the patient's infection or colonization history, but no epidemiological evidence is cited in support of the practice.<sup>(30, 71)</sup>

Infection control and prevention methods appear to be critical components in reducing the burden of disease, but their respective roles are still being evaluated. This research aims at evaluating two aspects of infection control: enhanced cleaning to prevent antibiotic-resistant bacteria transmission in the ICU, and surveillance cultures to identify cancer patients at risk of antibiotic-resistant bacterial infections, specifically bloodstream infections with ARGN bacteria. Evidence that enhanced cleaning of frequently contaminated surfaces in occupied ICU rooms leads to less HCW contamination could change hospital practices. Specifically, it would point out the importance of thorough cleaning while patients are still in their rooms. These changes may in turn reduce patient-to-patient transmission of antibiotic-resistant bacteria. Likewise, evidence that cancer patients who are colonized with ARGN bacteria are more likely to develop ARGN bloodstream infections would reinforce changes in clinical practice, supporting active surveillance of in-patients to guide selection of empiric antimicrobial therapy.

## **B. Aim 1**

### Patients in ICUs Are Frequently Colonized or Infected With Antibiotic-Resistant Bacteria

The intensive care unit is the front line of antimicrobial resistance. Since the 1980s, researchers have recognized that the prevalence of HAIs with antibiotic-resistant bacteria is higher in the ICU than in the general hospital population and that resistance develops rapidly among ICU patients because they are repeatedly treated with antibiotics.<sup>(12, 72-74, 75)</sup> The high prevalence of these infections is attributed to the close confinement of severely ill patients and the large number of invasive devices, such as endotracheal tubes and indwelling catheters, used in their care.<sup>(12, 75)</sup>

### Antibiotic-Resistant Bacteria from Patients Are Found On Environmental Surfaces in the ICU

Antibiotic-resistant bacteria are frequently found on surfaces in the ICU rooms of patients colonized or infected with these organisms. Sexton and colleagues studied the ICU rooms of 25 MRSA-infected patients and found that 53.2% (269/502) of environmental surfaces were contaminated with MRSA; and the infecting strains were identical or closely-related to the isolates from the patients in 70% (14/20) of the cases.<sup>(76)</sup> Hardy and colleagues sampled ICU rooms before and after admission of a MRSA-colonized patient, and found that within 24-hours the previously MRSA-free rooms were contaminated with a strain identical to that carried by the patient.<sup>(77)</sup> Barbolla and colleagues reported the results of a prospective cohort study in a 26-bed medical/surgical ICU in which 82/378 (22%) of environmental surfaces in the unit were contaminated

with *A. baumannii*.<sup>(78)</sup> Thom and colleagues examined 50 ICU rooms occupied by patients colonized with MDRAB and found that 24 (48%) were contaminated with MDRAB on at least one surface; supply carts, infusion pumps, and ventilator controls were the most commonly contaminated surfaces. Patients with a distant history of MDRAB colonization were likely to contaminate their environment.<sup>(79)</sup>

#### Antibiotic-Resistant Bacteria from Environment Contaminates the Hands and Protective Clothing of Healthcare Workers

Healthcare workers personal protective equipment and hands are frequently contaminated with antibiotic-resistant bacteria from caring for patients colonized or infected with these organisms. Snyder and colleagues sampled the hands and disposable gloves and gowns of 137 HCW caring for patient colonized with MRSA or vancomycin-resistant *Enterococcus* (VRE) and found that 23 (17%) had contaminated their gloves or gown, and of those, 2 (9%) had contaminated their hands.<sup>(66)</sup> A study by Morgan and colleagues of 199 interactions with patients colonized by MDRAB and 139 HCW interactions with patients colonized by MDR *Pseudomonas aeruginosa* found that 77 (39%) contaminated their gloves or gown with MDRAB and 11 (8%) contaminated their gloves or gown with MDR *Pseudomonas aeruginosa*, respectively.<sup>(65)</sup> A subsequent analysis of both data sets showed that environmental contamination was an independent risk factor for gown and glove contamination. (OR 4.2, 95% CI 2.7-6.5) Twenty of 22 (91%) gown and glove isolates were compared to environmental isolates from the same room by pulsed-field gel electrophoresis and found to be related.<sup>(80)</sup>

## Antibiotic-Resistant Bacteria in the Environment Can Be Reduced By Cleaning

Although antibiotic-resistant bacteria are frequently found in the environment of patients colonized or infected with these organisms, the burden of contamination can be significantly reduced by cleaning. Carling and colleagues have published a number of studies in which an invisible fluorescent mark is used to identify surfaces that are not adequately cleaned; the marks are abrasion-resistant but are readily removed by wiping with standard cleaning products. In one study that involved 36 acute-care hospitals, the marks were used to provide feedback to cleaning staff about which objects were not being cleaned. Forty eight percent (9,910/20,646) of the surfaces were adequately cleaned prior to implementation of the feedback program, and the number increased to 77% (7,287/9,464) after implementation.<sup>(56)</sup> A similar study in 27 hospitals found comparable pre- and post-intervention levels of cleanliness (50% vs. 82%).<sup>(51)</sup> Wilson and colleagues randomized two ICUs to alternating periods of enhanced and normal cleaning, and found a statistically significant reduction in contamination of environmental surfaces with MRSA (OR 0.6, 95% CI 0.4 – 0.9).<sup>(24)</sup> Otter and colleagues published a study of cleaning of an entire ICU using hydrogen peroxide vapor (HPV); 48% (10/21) of the surfaces were contaminated with ARGN organisms before the intervention, while none were contaminated after HPV cleaning.<sup>(81)</sup> Dancer and colleagues performed a prospective crossover study of room cleaning in which periods of enhanced cleaning were associated with a 33% reduction in the number of MRSA colonies recovered from near-patient sites (95% CI: 20% – 43%).<sup>(23)</sup> These studies demonstrate the feasibility of significant reductions in environmental contamination through improved cleaning and that these gains can be achieved through reinforcement of existing cleaning procedures.

### Terminal Cleaning of Contaminated Rooms Reduces ICU-wide Infection Rates

A few studies have demonstrated that terminal cleaning of a room previously occupied by a patient colonized or infected with antibiotic-resistant bacteria is associated with a reduced risk of infection for other patients. Hacek and colleagues performed a before-after study of terminal cleaning in all the rooms occupied by patients with *Clostridium difficile* infection in three hospitals, substituting dilute bleach for the standard quaternary ammonium cleaner. The authors compared the six months prior to the intervention to the two years after the intervention and found that the hospital-wide *C. difficile* infection rate per 1,000 patient-days fell from 0.85 to 0.45 (48% reduction, 95% CI: 36% – 58%).<sup>(61)</sup> Datta and colleagues reported another before-after study of an improved terminal cleaning protocol in 10 intensive care units in a single hospital. The intervention consisted of cleaning feedback using an invisible fluorescent marker, switching from poured disinfectant to pre-soaked cloths, and emphasizing repeated immersion of the cleaning cloths in the disinfectant while cleaning was performed. The authors reported that the rates of MRSA infection and VRE infection were lower during the intervention period as compared to baseline (1.5.0% vs. 3%,  $p < 0.001$  and 2.2% vs. 3%,  $p < 0.001$ , respectively). Patients in rooms that were previously occupied by MRSA-positive patients were at an increased risk of infection as compared to those in rooms with no previously infected patients (3.9% vs. 2.9%,  $p = 0.03$ ) but not during the intervention period (1.5% vs. 1.5%,  $p = 0.79$ ). Patients in room that were previously occupied by VRE-positive patients were not at any increased risk of VRE-infection.<sup>(49)</sup> Although these data are limited, the existing studies suggest that even the relatively

infrequent act of terminal cleaning, if done well, can have a significant impact on of antibiotic-resistant bacteria infection rates.

### Enhanced Cleaning May Reduce Infections

Dancer and colleagues reported a prospective crossover trial of enhanced cleaning in two different wards of a UK hospital. Both wards selected for the study were surgical wards with a high prevalence of MRSA and identical infection control policies. Each ward had 21 beds, of which two were in single-occupancy isolation rooms and the rest in clusters of three to five in open cubicles. The enhanced cleaning intervention consisted of an additional daily cleaning of frequently touched sites including the patient beds area, nursing stations, and shared clinical equipment using standard UK National Health Service supplies, i.e., detergent-based wipes without other disinfectants. The experimental intervention was implemented in addition to the routine cleaning that was regularly performed by the hospital's cleaning staff. Each ward received enhanced cleaning for six months and regular cleaning for six months in a one-year crossover design. The effect of cleaning on the environment was assessed by weekly microbiological screening of 10 sites in the ward (patient lockers, movable tray tables, bed frames, patient hoists, infusion pump poles, blood pressure monitor stands, nurses' computer keyboards, nurses' desk, nurses' patient notes, and the door handles of the isolation rooms). The effect of cleaning on patients was examined indirectly by noting new infections and colonization as determined by the patient care team.<sup>(23)</sup>

Dancer and colleagues found that enhanced cleaning was associated with a significant reduction in the mean aerobic colony count per sampled site (relative

reduction: 33%, 95% CI: 20% – 43%), whereas the effects in the individual wards were an 8% relative reduction and a 44% relative reduction. There was also a significant reduction in the number of sites with aerobic colony counts greater than 2.5 colony forming units/cm<sup>2</sup> (odds ratio: 0.5, 95% CI: 0.4, 0.7), but not in the absolute number of sites contaminated. The number of expected new MRSA infections was extrapolated from the number of new infections per MRSA-inpatient days during the regular cleaning period. According to the extrapolation, 13.1 MRSA infections were expected and only 4 were observed during the enhanced cleaning period (relative risk: 0.3, 95% CI: 0.1, 0.9). The authors concluded that enhanced cleaning significantly reduces environmental contamination and may reduce infections.<sup>(23)</sup>

The study by Dancer and colleagues examined the effect of enhanced cleaning on environmental contamination in the setting of an open-floor ICU. A major strength of the study is the crossover design, which avoids the potential for cleaning in the intervention arm to artificially reduce the burden of contamination in the control arm. The generalizability of the study is improved by the use of only commercially-available cleaning products that are standard within the UK National Health Service. A limitation of the study is the lack of HCW samples, because in an open ICU ward, contamination of a HCW's hands or disposable protective clothing would be expected to significantly contribute to patient-to-patient transmission of MRDOs. The use of two primarily open-floor ICUs limits the generalizability of the study beyond non-isolation room ICUs.

Wilson and colleagues reported a prospective crossover trial of enhanced cleaning in two ICUs in separate hospitals in the UK. Both ICUs selected for the study were general medical-surgical ICUs: a 35-bed unit with 11 isolation rooms and a 24-bed unit

with 4 isolation rooms. Both wards had similar infection control policies. The intervention consisted of a twice-daily cleaning using microfiber cloths soaked in a copper-based disinfectant (or bleach if cleaning the bed area of a patient with diarrhea). The experimental intervention was performed by study researchers. In addition to the experimental cleaning, routine cleaning using detergents and bleach was performed twice each day by the hospitals' cleaning staff. Each ward received enhanced cleaning for six months and regular cleaning for six months in a one-year crossover design. The effect of cleaning on the environment was assessed by microbiological screening for MRSA at six sites of four randomly selected beds, three times per day, and three times per week. Beds occupied by patients with MRSA were oversampled. The sampled sites were a drawer, the bed rail, the infusion pump, the vital signs monitor, the patient's nurse's hand, and the computer keyboard (at one hospital) and the patient's paper chart (at the other hospital). Patient colonization and infection were monitored prospectively; infections were identified using HELICS or CDC definitions where applicable; and colonization was defined as the presence of pathogens at superficial sites in the absence of infection.<sup>(24)</sup>

Wilson and colleagues found that enhanced cleaning was associated with a significant reduction in the number of times MRSA was recovered per bed per day (81/561 vs. 51/559, OR: 0.6, 95% CI: 0.4 – 0.9). Enhanced cleaning was also associated with a significant reduction in the number of sites contaminated with MRSA (70/10,068 vs. 165/10,141, OR: 0.5, 95% CI: 0.3 – 0.6). The authors found no effect of enhanced cleaning on the rate of MRSA infection (30 vs. 34, OR: 0.98, 95% CI: 0.58 – 1.7). In subgroup analyses, additional cleaning was associated with a significant reduction in how frequently MRSA was isolated from physicians' hands (11/423 vs. 3/425,  $p = 0.025$ ) but

not nurses (28/1,694 vs. 16/1,647,  $p = 0.077$ ). The authors concluded that enhanced cleaning significantly reduces the environmental burden of MRSA compared to standard cleaning. Although they acknowledged that the study was under-powered to detect anything but a large effect of cleaning on colonization and infection rates, they believed that the study results did not justify the additional expense and effort of enhanced cleaning.<sup>(24)</sup>

The study by Wilson and colleagues examined the effect of twice-daily enhanced cleaning on environmental contamination in the setting of an open-floor ICU. Like the study by Dancer and colleagues, a major strength of the Wilson study is the crossover design. Although the generalizability of the study is improved by measuring the primary outcome both before and after the intervention so that the measured levels of contamination represent an average of the effect of cleaning over an entire 24-hour period, the oversampling of bed areas around MRSA patients in combination with statistical analysis at the level of the bed makes interpretation of the effects difficult. In addition, the use of two primarily open-floor ICUs limits the generalizability of the study beyond non-isolation room ICUs.

### Summary of Hospital Cleaning

This aim examined whether enhanced cleaning of intensive care unit rooms occupied by patients colonized with drug-resistant organisms was associated with a reduction in the contamination of health-care workers' gloves and gowns with these pathogens, using a cluster-randomized, single-blind, controlled trial. Two previous trials have used enhanced cleaning as an intervention, but our trial is distinct from these studies

and will advance the state of knowledge in several important respects. The prior studies both used environmental contamination of the near-patient environment as the primary outcome. We examined contamination of HCW's disposable protective clothing, which is the next step in the putative causal chain from one patient to another. The current study took place in the United States, whereas both previous studies were done in the UK. The study population and setting of the current trial is also distinct from that seen in the prior trials, being a mix of dedicated medical and surgical ICUs with only single-occupancy rooms. In this sense the current trial was specific and novel in dealing with isolated patients on contact precautions, while also being more generalizable due to the heterogeneity of patient illnesses. We used the same materials used by the general hospital housekeeping staff, rather than the special cleaning products used by Wilson and colleagues, but cleaning was performed by dedicated study staff, unlike the study by Dancer and colleagues. Finally, the current trial was separately powered to examine the primary outcome for two exemplar pathogens: MRSA and, unlike either previous study, MDRAB (a Gram-negative pathogen of increasing concern in the US). The results of the current trial may justify future trials powered to examine clinical outcomes, and these may in turn alter cleaning practices that will reduce patient-to-patient transmission of antibiotic-resistant bacteria.

### C. Aim 2

#### Cancer Patients Are At Increased Risk Of Infections

Bacteremia is the most common complication of neutropenia in the chemotherapy patient, and represents a life-threatening medical emergency.<sup>(30)</sup> The incidence of

neutropenic bacteremia is not currently known. A retrospective cohort study of 22,631 patients with bloodstream infections at 49 hospitals recorded 251 deaths among the 696 neutropenic patients included, with crude mortality rate ranging from 14% – 48%, depending on the bacterium associated with the infection.<sup>(8)</sup> Since the 1980s, Gram-positive organisms have been the most common cause of bacteremia in this patient population, and currently they cause between 45% and 70% of all infections.<sup>(31)</sup> Mortality remains high among patients with Gram-negative bacteremia, and resistance rates to broad-spectrum antimicrobials among neutropenic cancer patients with Gram-negative infections are rising.<sup>(2, 31, 32)</sup> Not surprisingly, these infections are associated with significant increases in healthcare costs, length of stay, and mortality.<sup>(39, 40, 43)</sup>

### Antibiotic-Resistant Gram-Negative Infections Pose A Serious Risk to Cancer Patients

Among the organisms that cause neutropenic fever, Gram-negative bacteria are the major obstacle to successful empiric therapy. In spite of rising rates of tolerance and resistance, use of tapered empiric vancomycin controls the vast majority of Gram-positive bacteremia in neutropenic patients.<sup>(33)</sup> Antifungal drugs are also used empirically but usually only after 3-5 days duration of fever and neutropenia, and not during the initial course.<sup>(30, 82)</sup> By default, current guidelines for empiric therapy focus on coverage of potential Gram-negative infections. Many attempts have been made to develop a single therapy to cover all potential pathogens, but differences in hospital epidemiology and resistance profiles among organisms prevent any one antimicrobial from being effective

against all Gram-negatives.<sup>(2, 30, 33)</sup> The emergence of ARGN bacteria has complicated decisions about empiric therapy even further.

### Cancer Patients Are At Risk of Infections from Colonizing Bacteria

Gram-negative bacteremia in neutropenic patients is generally believed to arise from the gut. Studies in mouse models suggest that poisoning of the rapidly-proliferating gut mucosa by chemotherapeutic treatments removes most of the physical barriers to infection, allowing bacteria in the intestinal lumen to infiltrate the bloodstream.<sup>(83, 84)</sup> Limited clinical evidence indirectly supports this hypothesis by showing concordance between resistance profiles in stool isolates and isolates subsequently cultured from blood.<sup>(29, 69)</sup> Routine surveillance cultures of the throat, anterior nares, and perirectal area are routine in many cancer centers in the United States, and the results are used for infection control.<sup>(26)</sup> In theory, the results of these cultures could be used to guide empiric therapy decisions. As noted previously, the Infectious Disease Society of America suggests that prior culture results may be used to guide empiric therapy for patients with a history of antibiotic-resistant organisms, but cites no epidemiological evidence for this practice.<sup>(71)</sup>

### Risk Factors for ARGN Infections among Cancer Patients

A few recent studies have examined possible risk factors for Gram-negative and ARGN bacteremia. Some studies detected no associations with bacteremia; others found a link between bacteremia and recent treatment with antibiotics. The results of a meta-analysis by Spanik and colleagues of eight small studies of ARGN bacteremia versus

non-resistant Gram-negative bacteremia were equivocal, and the authors noted that their study was likely confounded by the inclusion of some patients who did not have malignancies or neutropenia.<sup>(85)</sup> A case-control study of 55 Brazilian hematopoietic stem cell transplant patients detected no risk factors between (10 cases with ARGN bacteremia and 44 controls with drug-susceptible Gram-negative infections) in their multivariate model, although ARGN bacteremia was associated with prophylactic antimicrobials in the univariate model.<sup>(86)</sup> In the meta-analysis paper, Spanik reported the results from a case-control study of 153 cancer patients in Bratislava, Slovakia. Fifty-one cancer patients with ARGN were matched to 102 controls with drug-susceptible Gram-negative bacteremia by sex, age, underlying malignancy, and neutropenia. ARGN bacteremia was significantly associated with prophylactic ofloxacin, and prior therapy with first- or second-generation cephalosporins, third-generation cephalosporins, aminoglycosides, ofloxacin, and imipenem ( $p < 0.05$  for all).<sup>(85)</sup> Similarly, a prospective study of 411 hematopoietic stem cell transplant patients in 13 hospitals in Brazil in 2004 found that ARGN bacteremia was associated with prophylactic or empiric use of third-generation cephalosporins (OR: 10.7, 95% CI: 3.8-30.3)<sup>(110)</sup>. A case-control study of 58 cancer patients with MDR *E. coli* infections matched by malignancy and time of admission to 115 controls also found an association with previous antimicrobial therapy in bivariate analysis; whereas, in a multivariate model only prior chemotherapy within 30 days or prior hospital admission within 30 days were significant ( $p < 0.01$  for both).<sup>(87)</sup>

### Surveillance Cultures and ARGN Infection among Cancer Patients

In 1983, Cohen and colleagues published the results of a cohort study of 175 neutropenic bone marrow transplant patients, where they examined the association between nasal, oral, or stool/rectal cultures and the development of bacteremia. Colonization with bacteria was associated with a 17- to 174-fold increased risk of bacteremia, depending on species. Among 15 cases of bacteremia, 12 isolates matched the resistance profiles of the prior surveillance culture isolate. The authors performed no statistical hypothesis tests on their results but noted that the positive predictive value of a positive surveillance culture was less than 25%.<sup>(88)</sup> In 1987, Wells and colleagues published a study exploring the potential association between Gram-negative stool culture isolates resistant to trimethoprim-sulfamethoxazole (TMP-SMX), tobramycin, or ticarcillin and Gram-negative clinical blood culture isolates resistant to the same antimicrobials. Among 20 of 45 bone marrow transplant patients with TMP-SMX-resistant Gram-negative stool isolates, 5 developed bacteremia with an identical organism. Among 25 patients without drug-resistant stool isolates, only 1 developed a bacteremia with a TMP-SMX-resistant Gram-negative organism. The authors concluded that knowledge of the drug susceptibilities of fecal Gram-negative bacteria might be of use in therapy decisions.<sup>(29)</sup> A study published in 1985 by Tancrede and colleagues did not examine surveillance cultures but compared bacterial loads of *P. aeruginosa* and Enterobacteriaceae from fresh, specially-collected stool samples with blood culture isolates from neutropenic patients with hematologic malignancies.<sup>(69)</sup> Of the 55 episodes of bacteremia with complete prior stool cultures, 45 were in patients with stools positive for either *P. aeruginosa*, Enterobacteriaceae, or both. Bacterial loads in fecal matter were

not associated with subsequent bacteremia. The authors concluded that intestinal colonization is the primary risk factor for Gram-negative bacteremia in neutropenic patients with hematologic malignancies.

Summary of Antibiotic-Resistant Gram-Negative Bloodstream Infections among Cancer Patients

Neutropenic patients with ARGN bacteremia represent a limited number of people in the US each year, but the morbidity and mortality associated with these infections are high. The availability of new antimicrobial therapies for these infections is not keeping pace with rising rates of resistance. Finding modifiable risk factors for these infections is an important goal in improving outcomes among these patients. The current study was only the second to examine colonization with drug-resistant Gram-negative bacteria as a risk factor for drug-resistant Gram-negative bacteremia. Like several of the previous studies of risk factors for ARGN bacteremia, use of antibiotics in empiric therapy was a covariate, and this study may support the prior observation that empiric or prophylactic treatment with some antibiotics is a risk factor for subsequent bacteremia with an ARGN organism. The results of this study may justify future experiments utilizing molecular techniques to investigate whether the same strain can be shown in rectal surveillance cultures and subsequent blood cultures. In the long term, risk factors potentially identified in this study may contribute to alterations in the guidelines for empiric treatment of neutropenic fever.

### **III. RESEARCH DESIGN AND METHODS**

The objectives of this research were (1) to evaluate the effect of enhanced cleaning in ICU rooms occupied by patients colonized with methicillin-resistant *S. aureus* (MRSA) and multidrug-resistant *A. baumannii* (MDRAB) on the frequency of healthcare workers (HCW) gowns and gloves contamination with MRSA and MDRAB following routine care in the room, and (2) to examine the association between antibiotic-resistant Gram-negative (ARGN) bacteremia and prior ARGN colonization among cancer patients with suspected infection.

#### **A. Aim 1**

A cluster-randomized, single-blind controlled trial evaluating enhanced cleaning (targeted cleaning of frequently-touched surfaces) in ICU rooms of patients colonized with MRSA and MDRAB to reduce the frequency of HCW's gowns and gloves becoming contaminated with MRSA and MDRAB while in the room.

##### **1. Study Design**

The trial was conducted at the University of Maryland, a 757-bed urban tertiary-care hospital. The trial population was drawn from four ICUs at UMMC: the Medical Intensive Care Unit, the Neurotrauma Critical Care Unit, the Multitrauma Critical Care Unit, and the Select Trauma Critical Care Unit. These units have, respectively, 29, 12, 12, and 12 single-occupancy rooms. The Medical ICU places all patients on contact precautions, and the remaining units place patients colonized with antibiotic-resistant bacteria on contact precautions.

The target population was ICU rooms occupied by patients colonized with MRSA or MDRAB and on contact precautions.

The trial population consisted of 65 rooms in four ICUs at UMMC. Rooms were randomized to the experimental or control group prior to the beginning of the trial, and maintained that allocation until half-way through the trial when all allocations was reversed, i.e., experimental rooms became control rooms and vice-versa. Rooms were treated independently for each half of the trial, so the effective trial population was 130 rooms. On October 11, 2011, the Neurotrauma Critical Care unit was moved in order to accommodate construction of a new hospital wing. The new location was a mirror image of the previous unit. On the day of the move, the old location was removed from screening and the new location was added, effectively adding 12 rooms to the trial population and making a final total of 142 trial rooms.

Rooms were included in the intervention and follow-up when they were occupied by a patient colonized or infected with MRSA, MDRAB, or both. Rooms were permitted to be enrolled and followed multiple times over the course of the trial if occupied by different patients who had not previously occupied a trial room during enrollment.

## **2. Randomization**

All rooms in the trial ICUs were allocated to the intervention or control prior to the start of the trial. Assignment was made using schedule with blocks of 2 to 6, allowing no more than two adjoining rooms to have the same assignment. Rooms maintained their allocation until approximately 50% of the sample was gathered. At the halfway point the intervention and sample collection procedures were suspended for a one-month washout period. After the washout period the room allocations were exactly reversed and the trial resumed.

The unusual allocation scheme was designed to prevent crossover. Previous studies in our hospital and laboratory have found that many multidrug-resistant bacteria can persist in the environment for weeks.<sup>(17, 89)</sup> The effect of a single enhanced cleaning might have a similar duration, and a control room could therefore effectively cross over into the experimental group because of a recent cleaning. By holding all rooms in one group or the other and then exchanging their assignments halfway through the trial, we aimed to prevent crossover while allowing every room equal opportunity to be in either the experimental or the control group. The one-month washout period was included to allow the rooms to return to equilibrium between halves of the trial.

### **3. Screening and Eligibility**

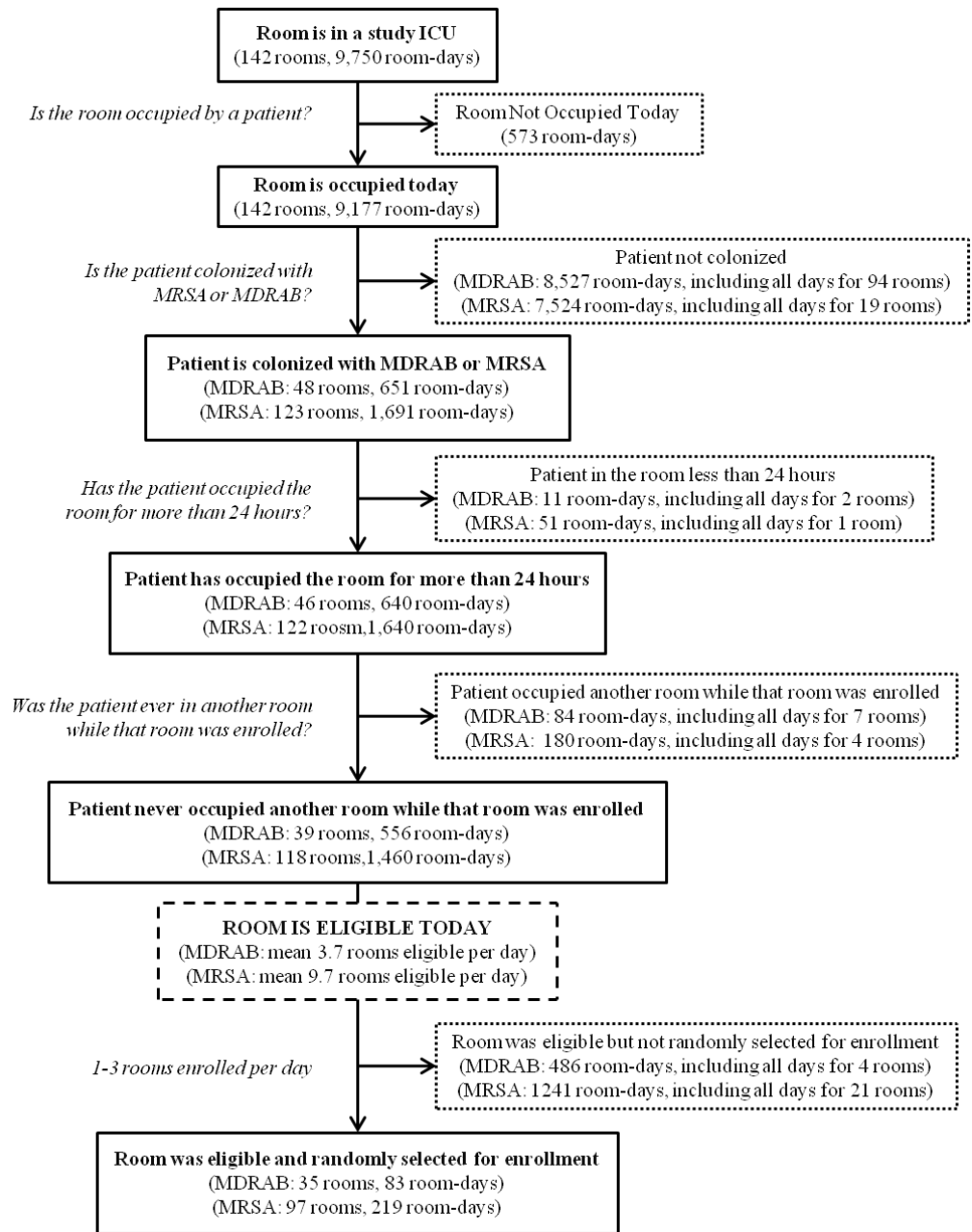
Screening was performed by computer. On the morning of each trial day, an automated screening request was sent to the UMMC Central Data Repository via a specially-created, web-based portal. The request returned a census of all trial rooms, including the room number, the time and date of admission of the current occupant, and a Special Indicator code noting any multidrug resistant organisms associated with the occupant. The census was uploaded into a Microsoft Access database, and the eligibility criteria were applied to all rooms using an automated query. Eligible rooms had to meet two criteria: (1) be currently occupied by a patient whose chart includes a Special Indicator code for MRSA or MDRAB (M, A, AM, AV, AX, X, MG, or XG) and (2) have been occupied by that patient for at least 24 hours. A room was excluded if it was occupied by a patient who had occupied a previously enrolled room during follow-up.

Based on previous admission and infection control records, we expected 5 to 15 rooms would be eligible for follow-up on any given trial day and that a single trial researcher would be able enroll and follow one to two rooms per day.(65, 66)In order to allow a single researcher to enroll and follow rooms in an unbiased fashion, rooms were sub-selected for enrollment and follow-up from the daily list of all eligible rooms using a pseudo-random number generator. Not all eligible rooms were enrolled on all days that they were eligible (Figure 1).

#### **4. Intervention**

The experimental intervention was an enhanced cleaning by a trial researcher. The following items in the ICU were cleaned: (1) the bed rail top bar, (2) the bed electronic control surfaces, (3) bedside table top and control surfaces (4) desktop and sides, (5) IV poles, (6) infusion pump control surfaces, (7) nurse call button/remote control, (8) patient telephone, (9) sink console top, (10) light switches and plates, (11) supply cart top and drawer handles, (12) ventilator control surfaces and desk, (13) vital signs monitor control surfaces. These targets were derived from a CDC list of frequently-touched and frequently-contaminated surfaces.(90) Items were cleaned using paper towels pre-soaked in the same quaternary ammonium solution used by the general housekeeping staff for routine cleaning (Diversey Virex™, Sturtevant, Wisconsin). The control intervention was a sham enhanced cleaning consisting of a mimed cleaning of all the target items by a trial researcher. Regardless of eligibility, enrollment, allocation or follow-up, all rooms in all trial ICUs received standard daily cleaning by UMMC housekeeping staff.

**Figure 1. Algorithm to Identify Rooms Eligible for the Enhanced Cleaning Trial**



Application of the intervention was verified using an invisible fluorescent gel (DAZO™, Ecolab, St. Paul, Minnesota). This gel has been used in several previous studies as a marker of cleaning.<sup>(51, 56, 91)</sup> In experimental rooms, a trial researcher marked all target surfaces with a gel marker, and then used a UV light to check for removal after a second researcher had cleaned the room. In control rooms, target surfaces were marked during the sham cleaning and removal was verified 24 hours later in order to allow the hospital housekeeping staff enough time to clean the room. Intervention verification was planned for 10% of experimental rooms and 100% of control rooms. Results of the intervention verification are presented in Appendix 1.

## **5. Primary Outcome**

The primary outcome measure for the trial was contamination of the disposable gown or gloves of a HCW immediately prior to exiting an enrolled room following any non-emergent activity. Gown and glove cultures were performed using the same method employed in observational studies with the same outcome.<sup>(66)</sup> Sterile, cotton double-tipped applicators (Remel BactiSwab®; Thermo Fisher Scientific, Lenexa, KS) were moistened with liquid Amies transport media. Glove and gown samples were obtained with a standardized process, using a single applicator for both gloves and the gown. For gloves, all inter-digital spaces of each glove were swabbed twice, and the palmar and dorsal surfaces of each glove were swabbed once in a large spiral. For gowns, the length of each sleeve was swabbed twice and then the front of the gown was swabbed once in a large “W” pattern. All samples were collected immediately prior to the HCW removing

their gown and gloves and exiting the room. Based on the previously published finding of low rates of gown and glove contamination prior to entering the room, we did not consider it necessary to pre-screen HCWs and eliminate already-contaminated gloves and gowns from the analysis.<sup>(65, 66)</sup> A detailed intervention and sampling protocol is presented in Appendix 4.

Outcomes were analyzed separately for rooms occupied by patients colonized with MRSA or MDRAB. For patients colonized with either MRSA or MDRAB, one applicator tip was discarded. For rooms occupied by patients colonized by both MRSA and MDRAB, one applicator tip was used for each laboratory analysis. The primary outcome was considered positive if the relevant multidrug resistant organism (i.e. the organism colonizing the room occupant) was isolated. Isolation and speciation of MRSA and *Acinetobacter baumannii* was performed using chromogenic agars (Remel Spectra™ MRSA, Thermo Fisher Scientific, Waltham, MA; CHROMagar™ Acinetobacter, CHROMagar, Paris, France). Antibiotic susceptibilities of *A. baumannii* isolates were established by Kirby-Bauer testing against a panel of ten drugs from seven antibiotic classes. *A. baumannii* isolates were considered multidrug-resistant (and therefore outcome-positive) if susceptible to two or fewer classes of antibiotics. This is the same definition that was used by the UMMC Clinical Microbiology Laboratory during the trial period. A detailed laboratory protocol is presented in Appendix 4.

## **6. Statistical Analysis**

The null hypothesis of this trial is that the proportion of HCWs with contaminated gloves and gowns exiting rooms receiving enhanced cleaning ( $p_1$ ) will not differ from the

proportion of HCWs with contaminated gloves and gowns exiting rooms receiving sham enhanced cleaning ( $p_0$ ). The alternative hypothesis was that these proportions were different:

$$H_0: p_1 = p_0$$

$$H_A: p_1 \neq p_0$$

Data from MRSA and MDRAB rooms were analyzed separately. Rooms occupied by a co-colonized patient contributed independently to both analyses. The primary trial outcome was analyzed using the Wilcoxon-Mann-Whitney test comparing the mean proportions of positive swabs from each room between the two groups. The results of the test were reported as a p-value, and the proportions of contaminated swabs in each group were reported with 95% confidence intervals.

A limitation of the proposed analysis method is the inherently matched nature of the data. The many enrolled rooms appear in both the intervention and control arms, and therefore form a matched pair. Responses coming from each pair are likely to be correlated. This correlation is usually positive. The Wilcoxon-Mann-Whitney test assumes however that the sample is independent, i.e., that there is no correlation. If the correlation between matched pairs is positive, then the Wilcoxon test was conservative. If, however, the correlation between matched pairs is negative then the Wilcoxon test will tend to overstate the statistical significance of the detected effect. In theory, a matched-pair analysis could be conducted using data from each room during the intervention and control periods as a pair. In practice, because of random chance and the limited number of MRSA- and MDRAB-colonized patients present on a given day, many rooms in each

arm will only appear in the control or experimental group. A matched-pair analysis would exclude those rooms, wasting those data and reducing the statistical power of the trial.

In order to evaluate the degree of correlation between matched pairs of rooms, and to determine whether the Wilcoxon-Mann-Whitney test is conservative, a supplemental sensitivity analysis was performed. The proportion of positive swabs from each room in each arm was estimated using the Generalized Estimating Equation method.<sup>(92)</sup> The proportion was modeled as a function of the assigned intervention, with the room specified as the subject and an independent working correlation for observations from the same room. The direction and magnitude of correlation estimated by the model was used to determine whether the result of the Wilcoxon-Mann-Whitney test was conservative or liberal. The results of this sensitivity analysis are presented in Appendix 2.

A secondary analysis was performed using a mixed-effects model.<sup>(93)</sup> This method was used to model the proportion of positive swabs from a particular room occupied by a particular patient on a particular day. The use of random effects accounts for the correlation between observations from the same patient and between patients occupying the same room. The model will specify that the proportion of positive swabs is a linear function of the fixed effect of the experimental intervention and random effects for the room and the patient nested within the room. An advantage of modeling the proportion of positive swabs as a continuous outcome is that the model estimates can be interpreted either at the subject or at the population level.

Two interim analyses were planned for this trial. The first was planned for approximately six weeks into the trial period, the second during the midpoint washout

period. Type I error rates used in the statistical analyses at each point was adjusted using the O'Brien-Fleming method and a nominal two-sided type I error rate of 5%.<sup>(94)</sup> The critical p-value for the final analysis was 0.044. All analyses were conducted on an intention-to-treat basis.

All data management was done using relational database software (MS Access 2010®, Microsoft, Inc., Redmond, Washington). All statistical analyses were performed using commercially available statistical software (SAS 9.2®, SAS Institute, Cary, NC).

## 7. Sample Size

The required sample size was calculated using the methods of Donner. The total number of rooms needed per group is:

$$n = \frac{[Z_{1-\alpha/2} + Z_{1-\beta}]^2 [p_1(1 - p_1) + p_0(1 - p_0)][1 + (m - 1)\rho]}{(p_1 - p_0)^2}$$

Where  $n$  is the number of rooms in each arm,  $Z_{1-\alpha/2}$  is the  $(1 - \alpha/2)^{\text{th}}$  quantile of the Standard Normal distribution,  $Z_{1-\beta}$  is the  $(1 - \beta)^{\text{th}}$  quantile of Standard Normal distribution,  $m$  is the expected cluster size (i.e., the expected number of samples per room),  $\rho$  is the assumed correlation coefficient between observations from the same room,  $p_0$  is the expected proportion of positive swabs from control rooms, and  $p_1$  is the expected proportion of swabs from experimental rooms under  $H_A$ . In order to adjust for the inefficiency of the Wilcoxon-Mann-Whitney test compared to a two-sample test of proportions, the sample size is divided by 0.955, which is the Asymptotic Relative Efficiency of the Wilcoxon-Mann-Whitney test compared to the test of proportions.<sup>(95)</sup>

A 30% relative reduction in contamination in the experimental arm under the alternative hypothesis was assumed for both the MRSA and MDRAB arms, along with an adjusted  $\alpha$  of 0.044 for a two-sided test and a  $\beta$  of 10%. A  $\rho$  of 0.01 was also assumed for both arms. The cluster size  $m$  for both groups was 60. The  $p_0$  for each arm was derived from the results of observational studies of gown and glove contamination with these organisms by Morgan and Snyder.<sup>(65, 66)</sup>

For the MDRAB arm, the total number of swabs is:

$$n = \frac{[Z_{1-\alpha/2} + Z_{1-\beta}]^2 [p_1(1 - p_1) + p_0(1 - p_0)][1 + (m - 1)\rho]}{(p_1 - p_0)^2}$$

$$n = \frac{[2.010 + 1.282]^2 [0.271(1 - 0.271) + 0.387(1 - 0.387)][1 + (60 - 1)0.01]}{(0.271 - 0.387)^2} = 542.7$$

$$n_{WMW} = \frac{n}{A.R.E.} = \frac{542.7}{0.955} = 568.3 \cong \mathbf{569}$$

Or approximately 1138 swabs total for both the experimental and control arms. This figure was rounded to 1200 to allow 60 swabs per room and an even 10 rooms per arm.

For the MRSA arm, the total number of swabs needed is:

$$n = \frac{[2.010 + 1.282]^2 [0.130(1 - 0.130) + 0.186(1 - 0.186)][1 + (60 - 1)0.01]}{(0.130 - 0.186)^2} = 1412.1$$

$$n_{WMW} = \frac{n}{A.R.E.} = \frac{1412.1}{0.955} = 1478.7 \cong \mathbf{1479}$$

Or approximately 2958 swabs total in the experimental and control arms. This figure was rounded to 3240 to allow 60 swabs per room and an even 27 rooms per arm.

As mentioned previously, a major assumption of these calculations is the  $\rho$  of 0.01. The minimum detectable effects given 90% power for a range of  $\rho$  are shown in Table 1.

**Table 1. Detectable difference for a range of intra-cluster correlation coefficients ( $\rho$ ).**

Assumed $\rho$	MDRAB n = 1200	MRSA n = 3240
0	23.2	23.3
0.01	28.8	29.1
0.05	43.6	44.3
0.1	55.5	56.4

## 8. Ethics and Protection of Human Subjects

This trial was approved by the University of Maryland Medical Center Institutional Review Board. Waivers of written, informed consent were granted for both the patients occupying enrolled rooms and the HCW whose gowns and gloves were sampled for the following reasons:

1. This study would answer an important question with implications for patient safety and healthcare practice
2. It would be prohibitively difficult to obtain written informed consent from patients occupying eligible trial rooms due to the acuity of their illness, and contacting legally authorized representatives and attempting to collect proxy consent would not be possible within the time constraints of the trial.
3. Requiring informed consent could potentially lead to a selection bias in the trial population as patients who are more ill are less likely to be consented but are more likely to be colonized with antimicrobial-resistant bacteria.

4. The proposed intervention poses minimal risk to the patient, as only additional cleaning of the patient's room was performed and no samples were taken from the patient.
5. This trial offers no direct or indirect benefit and poses no risk to the HCW; no identifiable information about gown and glove contamination would be collected or revealed to the HCW or their supervisors. Researchers would ask healthcare workers for permission to collect swabs and the HCW would be given the opportunity to decline participation. HCWs would be informed of the trial in advance.

This trial was registered at [ClinicalTrials.gov](https://clinicaltrials.gov). The trial registration number is NCT01481935

## **B. Aim 2**

To compare the frequency of prior colonization with ARGN bacteria between cancer patients with suspected infection and subsequent ARGN bacteremia (cases) and cancer patients with suspected infection but no subsequent ARGN bacteremia (controls).

### **1. Study Design**

We performed two studies and used a matched case-control design for both, because the outcome (ARGN bacteremia) is rare and matched controls was used to improve the comparability of subjects and the statistical efficiency of the study.<sup>(95)</sup>

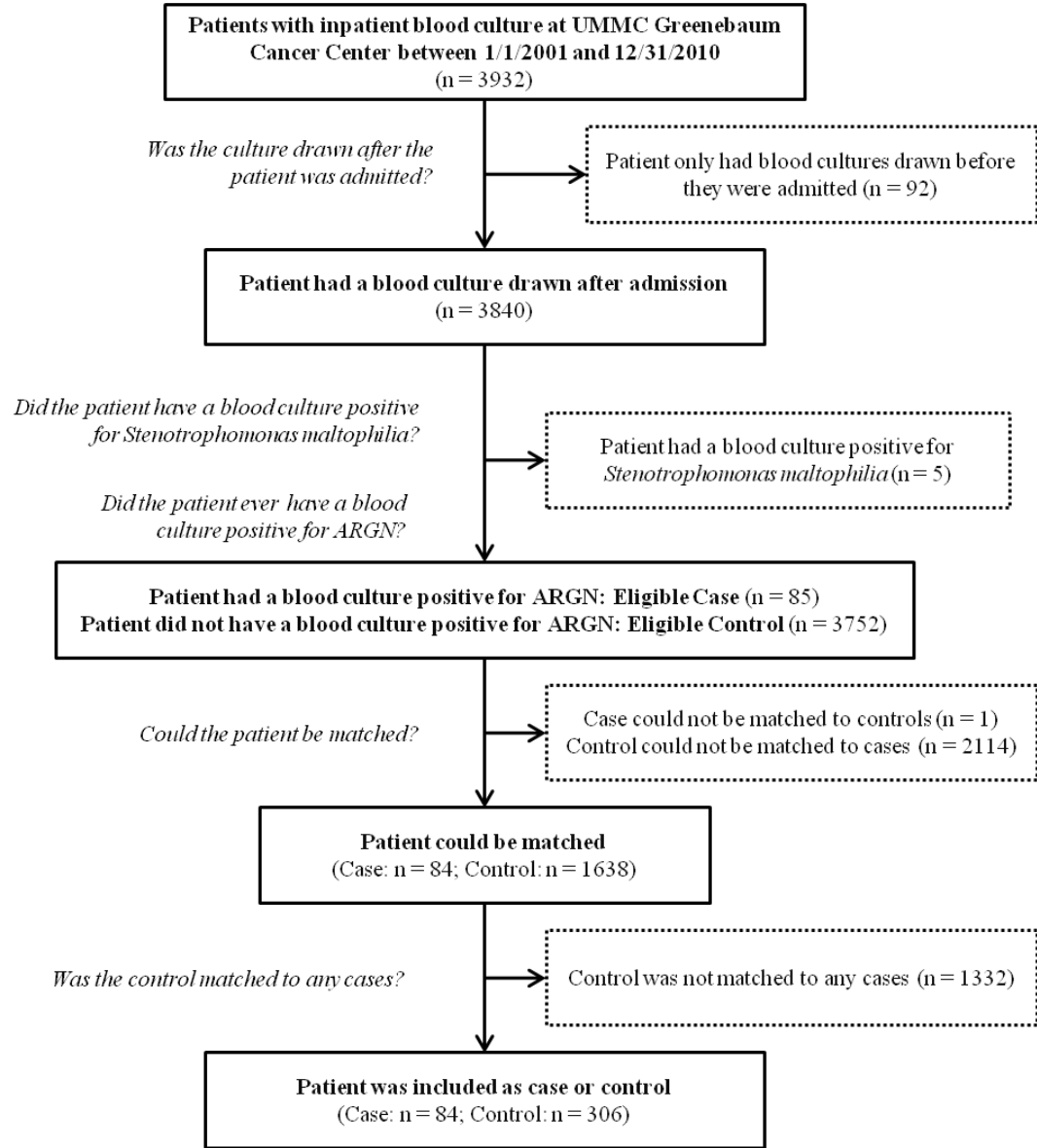
The population for both studies was selected from the UMMC Greenebaum Cancer Center, a National Cancer Institute-designated center in Baltimore, Maryland. The UMMC Central Data Repository (CDR) was searched for medical and laboratory information relating to patients and cultures. Subjects were eligible to be cases or controls if (1) they were patients at the UMMC Greenebaum Cancer Center between January 1<sup>st</sup> 2000 and December 31<sup>st</sup>, 2010, (2) their general type of malignancy was recorded<sup>1</sup>, and (3) they had a blood culture drawn during their eligible admission period.

For the second study, in addition to the above criteria, all potential cases and controls had to have at least one surveillance or non-sterile-site clinical culture 2 to 365 before their blood culture.<sup>(96)</sup> The selection of cases and controls for both studies is presented in Figures 2 and 3.

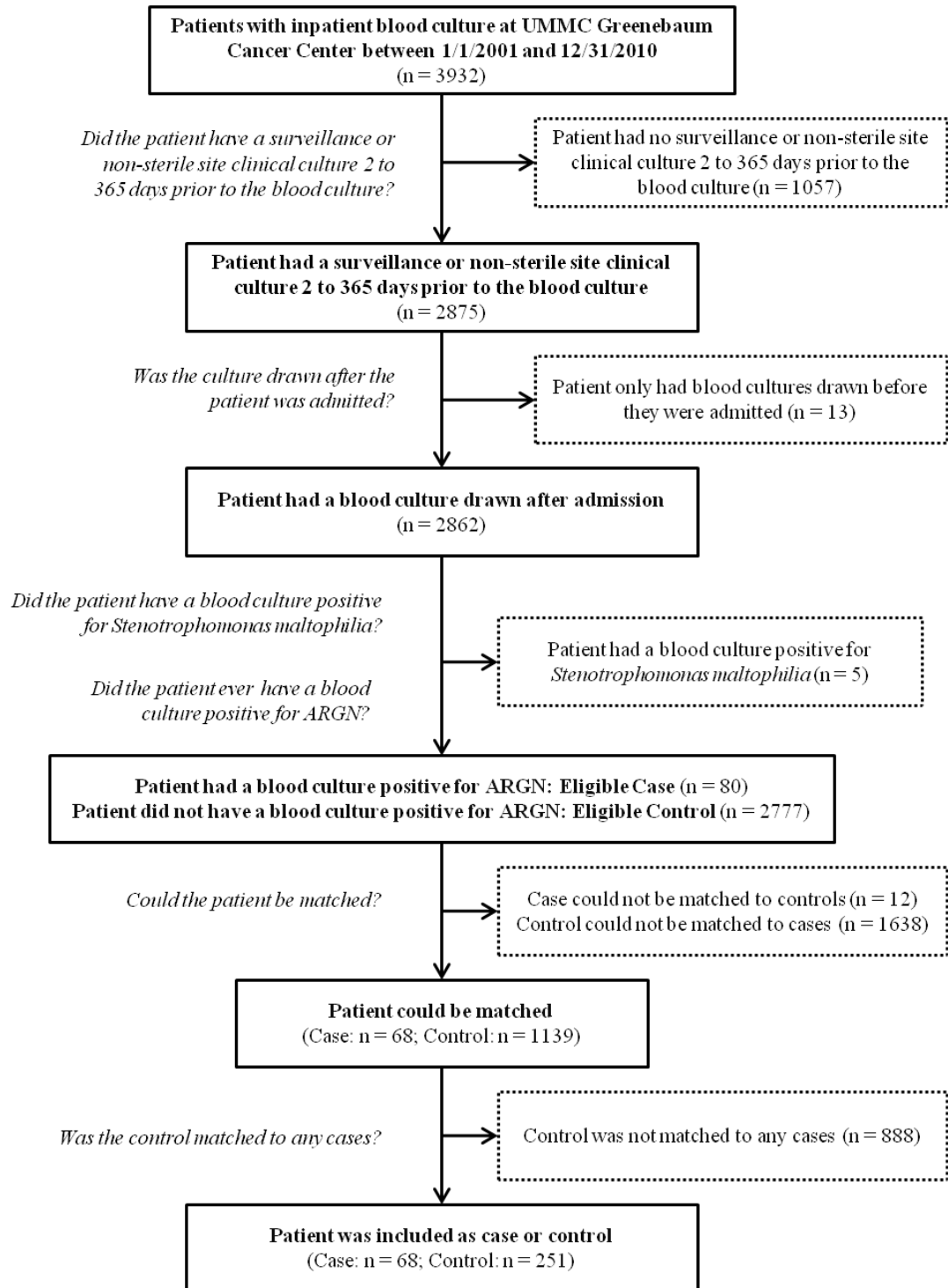
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<sup>1</sup> i.e., ALL (acute lymphocytic leukemia), CLL (chronic lymphocytic leukemia), AML (acute myeloid leukemia), CML (chronic myeloid leukemia), 'other' leukemia, HL (Hodgkin's lymphoma), NHL (non-Hodgkin's lymphoma), myeloma, and solid tumor/other malignancy.

**Figure 2. Algorithm for selection of cases and control for Aim 2.1.**



**Figure 3. Algorithm for selection of cases and control for Aim 2.2.**



Cases were eligible patients with their first blood culture positive for ARGN bacteria. Controls were eligible patients whose blood cultures were negative or grew a non-ARGN organism. Up to four controls were matched to each case. Matching variables included age  $\pm 10$  years, general type of malignancy, year of admission, and days from admission to blood culture. The given matching criteria ensured that controls were comparable to cases with regard to their cancers, (malignancy type) their chemotherapy regimens, (malignancy type, age, year of admission) their surveillance and microbiological analysis, (year of admission) and their time at risk of infection (days from admission to blood culture). Case patients could also be controls if they were eligible at any time more than 48 hours prior to the case-defining blood culture. We excluded all patients with blood cultures positive for *Stenotrophomonas maltophilia* because antimicrobial resistance testing was not routinely performed for this organism at UMMC during the study period. Matching variables are presented in Table 2.

A summary of Central Data Repository codes used to identify and match patients, including International Classification of Diseases, 9<sup>th</sup>-Revision (ICD-9) codes used to identify patient malignancies, is presented in Appendix 5.

## **2. Measures**

ARGN bacteremia was defined as any blood culture isolate positive for a Gram-negative bacterium resistant or with intermediate resistance to cefepime, imipenem, or piperacillin-tazobactam. ARGN colonization was defined as any surveillance culture (a culture of a non-sterile site such as the anterior nares, throat, or peri-rectal skin) or non-sterile site clinical culture (e.g., surgical wound, stool, sputum, urine) positive for Gram-

**Table 2. Matching criteria for case-control studies in Aim 2.1 and Aim 2.2**

Case Variable	Control Criteria
Age	within $\pm$ 10 years
Malignancy type	exact match
Acute lymphocytic leukemia (ALL)	
Chronic lymphocytic leukemia (CLL)	
Acute myeloid leukemia (AML)	
Chronic myeloid leukemia (CML)	
Other leukemia	
Hodgkin's Lymphoma (HL)	
Non-Hodgkin's Lymphoma (NHL)	
Solid tumor/other	
Date of admission	within $\pm$ 180 days
Days from admission to blood culture	
$\leq$ 7 days	within $\pm$ 3 days
$>$ 7 days and $\leq$ 14 days	within $\pm$ 5 days
$>$ 14 days and $\leq$ 21 days	within $\pm$ 7 days
$>$ 21 days and $\leq$ 28 days	within $\pm$ 9 days
$>$ 28 days and $\leq$ 35 days	within $\pm$ 11 days
$>$ 35 days and $\leq$ 42 days	within $\pm$ 13 days
$>$ 42 days and $\leq$ 49 days	within $\pm$ 15 days
$>$ 49 days and $\leq$ 56 days	within $\pm$ 17 days
$>$ 56 days and $\leq$ 63 days	within $\pm$ 19 days

negative bacteria resistant or with intermediate resistance to cefepime, imipenem, or piperacillin-tazobactam (as determined by standard clinical laboratory procedures). It was possible that a positive surveillance or clinical culture was due to the presence of an infection in the patient. We excluded patients with surveillance and non-sterile-site clinical cultures taken less than two days (48 hours) prior to the defining blood culture, in

order to reduce the chance that the results of any prior cultures influenced the choice of empiric antibiotics.

Neutropenia is an important covariate, but since differential counts of white blood cells (WBC) were frequently not available in the medical record, we used leukopenia (defined as any WBC count less than 1,000 cells per mL) as a surrogate measure of neutropenia. Recent exposure to antimicrobials was included to address prior antimicrobial exposure that may have contributed to resistance among colonizing organisms. In order to avoid confusing prior prescription of antimicrobials with recent one in response to early clinical signs of infection, we excluded antimicrobials ordered in the 24 hours prior to the defining blood culture. Covariates examined in both aims are presented in Table 3.

**Table 3. Covariates Examined in Aims 2.1 and 2.2**

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**Demographics**

Age  
Gender  
Race (white, black, other)

**Hospital Stay**

WBC count  $\leq 1 \times 10^3 / \text{mm}^3$  in  $\pm 2$  days of blood culture  
Admitted to medical ICU in 90 days prior to blood culture  
Treatment with antimicrobials 1-28 days prior to blood culture (aztreonam, cefepime, imipenem, doripenem, moxifloxacin, piperacillin-tazobactam).

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**3. Statistical Analysis**

Data for this aim came from the UMMC Central Data Repository (CDR). The data was gathered from successive queries: an initial identification of eligible cases, and

then further queries to identify candidate controls, then full information on all cases and matched controls. Separate matches were performed for the first and second studies. Once the two data sets were assembled, all statistical analyses were performed using commercially available statistical software (SAS 9.2®, SAS Institute, Cary, NC). All bivariate tests and multivariable analyses were performed using conditional logistic regression. Significance of individual covariates and interaction between covariates in multivariable models was assessed using the Likelihood Ratio Test. All associations were reported as odds ratios with 95% confidence intervals.

#### **4. Sensitivity Analyses**

Cases and controls included in the first study included patients who did not have a surveillance or non-sterile-site clinical culture 2 to 365 days prior to their blood culture. The second study addresses the problem by excluding all patients who did not have a surveillance or non-sterile-site clinical culture. In order to rigorously explore the implications of including cases and controls without prior cultures, we performed two sensitivity analyses that tested the extreme consequences of the missing data.

In the first sensitivity analysis, all cases that did not have prior cultures were declared “not colonized” and all controls that did not have prior cultures were declared “colonized”. In the second sensitivity analysis, all cases that did not have prior cultures were declared “colonized” and all controls that did not have prior cultures were declared “not colonized”. The association between exposure and outcome under each of these conditions was tested using a bivariate conditional logistic regression model. The results of the sensitivity analyses are presented in Appendix 3.

## 5. Preliminary Study and Sample Size

The association between prior ARGN colonization and subsequent ARGN bacteremia at the University of Maryland Greenebaum Cancer center was previously examined in a smaller, unpublished study using data from 2000 through 2009. In that study, 71 cases and 284 controls were enrolled and approximately 9% of control patients were previously colonized with ARGN bacteremia. Although a significant association between prior ARGN colonization and subsequent ARGN bacteremia was detected (odds ratio: 7.4, 95% confidence interval: 3.6 – 15.2), the study did not have a sufficient sample size to include a number of important covariates in the initial multivariable model. Based on ARGN bacteremia trends observed in this study, we estimated that 10-20 additional cases and 40-80 additional controls could be added to the study with the addition of 2010-year data. We estimated that with 85 cases and a Type-I error rate of 5%, both studies would have 80% power to detect an odds ratio of 3.0 for prior ARGN colonization between cases and controls.<sup>(96)</sup>

#### IV. A RANDOMIZED, CONTROLLED TRIAL OF ENHANCED CLEANING TO REDUCE CONTAMINATION OF CONTACT PRECAUTIONS WITH MULTIDRUG-RESISTANT BACTERIA IN INTENSIVE CARE UNITS<sup>2</sup>

##### ABSTRACT

**Background.** Multidrug-resistant bacterial infections are a serious complication of intensive care stays in hospitals. The role of environmental cleaning while the patient is in the room in reducing dissemination of multidrug-resistant bacteria infections is unclear.

**Methods.** We performed a cluster-randomized, controlled trial in four intensive care units (ICUs). We enrolled rooms occupied by patients colonized with methicillin-resistant *Staphylococcus aureus* (MRSA) or multidrug-resistant *Acinetobacter baumannii* (MDRAB). The experimental intervention was ‘enhanced cleaning.’ ICU room surfaces frequently touched by healthcare workers were given an additional cleaning. The study outcome was the contamination of healthcare workers’ (HCW) disposable gowns and gloves with MRSA or MDRAB after routine patient care. We calculated the mean proportion of contaminated gowns and gloves from each room and tested the difference between the experimental and control rooms. Results were analyzed separately for the two target organisms.

**Results.** The mean proportion of contaminated gowns and gloves for MDRAB was 16% among control rooms and 12% among experimental rooms (relative risk [RR] 0.77, 95%

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<sup>2</sup> A.S. Hess, M. Shardell, J.K. Johnson, K.A. Thom, M. Roghmann, G. Netzer, S. Amr, A.D. Harris

As submitted to *Clinical Infectious Diseases*.

confidence interval [CI] 0.28 – 2.11,  $p = 0.230$ ). For MRSA, the mean proportions were 22% among control rooms and 19% among experimental rooms (relative risk [RR] 0.89, 95% confidence interval [CI] 0.5 – 1.53,  $p = 0.158$ ).

**Discussion.** Enhanced cleaning of ICU rooms occupied by patients colonized with MRSA or MDRAB was associated with a moderate but non-significant reduction in contamination of HCW gowns and gloves after routine patient-care activities. Our results suggest future research is needed to determine whether intense environmental cleaning will lead to fewer infections with MDR bacteria.

## INTRODUCTION

Environmental surfaces in intensive care unit (ICU) rooms are not cleaned well.<sup>(17-19)</sup> These surfaces are often contaminated with antibiotic-resistant bacteria such as *Staphylococcus aureus* (MRSA) and multidrug-resistant *Acinetobacter baumannii* (MDRAB).<sup>(16, 64)</sup> Improved ICU room cleaning has been shown to be associated with significant reductions in the environmental burden of antibiotic-resistant bacteria.<sup>(20-24)</sup> Proposed methods for improving room cleaning include monitoring and feedback programs, ‘enhanced cleaning’ (extra cleaning targeted at frequently-touched, frequently-contaminated surfaces), and novel cleaning materials such as microfiber cloths, copper biocides, hydrogen peroxide vapor, and UV lamps.<sup>(61, 63, 81, 90, 101, 102, 102, 103, 103-107)</sup>

Contaminated environmental surfaces are a proposed reservoir for patient-to-patient transmission of antibiotic-resistant bacteria via the hands of healthcare workers (HCW),<sup>(20-22)</sup> because antibiotic-resistant bacteria on environmental surfaces have been associated with the appearance of antibiotic-resistant bacteria on the disposable gowns

and gloves of HCW in the ICU.<sup>(64)</sup> A subgroup analysis in one trial found that environmental cleaning with microfiber cloths and copper biocide significantly reduced the contamination of physicians' hands with MRSA (  $p = 0.025$ ).<sup>(24)</sup> Improved environmental cleaning ought to reduce patient-to-patient transmission of antibiotic-resistant bacteria by reducing the opportunities for HCW's hands and clothing to become contaminated.

Prior studies of enhanced cleaning protocols in ICUs have all taken place in units with the majority of beds in a single open area.<sup>(23, 24)</sup> In this study, we examined the effect of enhanced cleaning in a mixture of medical and surgical ICUs with only single-occupant rooms. Our hypothesis is that enhanced cleaning of frequently-contaminated surfaces in occupied ICU rooms would be associated with less HCW contamination. If so, this finding would justify a change in infection control and cleaning practices.

## **METHODS**

***Design.*** We performed a cluster-randomized, single-blind (healthcare workers were blinded) controlled trial of enhanced cleaning in ICU rooms to reduce disposable gown and glove contamination with multidrug resistant bacteria.

***Ethics.*** This trial was approved by the University of Maryland Institutional Review Board. Waivers of written, informed consent were granted for both the patients occupying enrolled rooms and the HCW whose gowns and gloves were sampled. The trial registration number is NCT01481935.

***Participants.*** This trial was conducted at the University of Maryland Medical Center (UMMC), a 757-bed urban tertiary-care hospital. The target population was ICU

rooms occupied by patients colonized with MRSA or MDRAB. The trial was performed in one medical and four surgical ICUs at UMMC, a total of 71 single-occupancy rooms. The trial was conducted in two phases (see *Randomization* section) and for the purposes of the trial each room was counted separately during each phase, bringing the total number of rooms to 142.

A room was considered eligible for enrollment and follow-up if it was occupied by a patient colonized with MRSA or MDRAB and the patient had been in the room for at least 24 hours. A room was excluded if the current patient had been the occupant during intervention and follow-up in another room. Patient colonization with MRSA or MDRAB was determined from a special indicator field in their electronic medical record.

***Intervention.*** The experimental intervention was an ‘enhanced cleaning’ by a trial researcher. The researcher cleaned (1) the bed rail and bed controls, (2) movable over-bed table, (3) built-in desk, (4) IV pole(s), (5) infusion pump, (6) nurse call button, (7) patient telephone, (8) room sink, (9) light switches, (10) supply cart, (11) ventilator desk and controls, and (12) telemetry controls. These targets were chosen based on a CDC list of frequently-touched and frequently-contaminated surfaces.<sup>(90)</sup> Items were cleaned using wipes pre-soaked in the same quaternary ammonium solution used by the general housekeeping staff for routine cleaning (Diversey Virex™, Sturtevant, Wisconsin). The control intervention was a sham enhanced cleaning. A trial researcher entered the room with all necessary cleaning equipment and mimed the action of cleaning the target surfaces, but no additional cleaning was actually performed. Regardless of eligibility, enrollment, allocation or follow-up, all rooms in all trial ICUs received standard daily cleaning by UMMC housekeeping staff. Standard daily cleaning included

cleaning of the doorframe, floors, walls (as needed), furniture, bathroom fixtures, over-bed table, built in desk, nurse call button, patient telephone, sink, light switches, and supply cart.

Application of the intervention was verified using an invisible fluorescent gel (DAZO™, Ecolab, St. Paul, Minnesota). This gel has been used in several previous studies as a marker of cleaning.<sup>(51, 56, 91)</sup> In experimental rooms, a trial researcher marked all target surfaces with a gel marker, and then used an ultraviolet light to check for removal after a second researcher had cleaned the room. In control rooms, target surfaces were marked during the sham cleaning and removal was verified 24 hours later in order to allow the hospital housekeeping staff sufficient time to clean the room. Intervention validation was planned for 10% of experimental rooms and 100% of control rooms.

***Outcome measures.*** The primary outcome measure for the trial was contamination of the disposable gown or gloves of a HCW immediately prior to exiting an enrolled room following any non-emergent patient care. Gown and glove cultures were performed as described previously.<sup>(66)</sup> Sterile, cotton double-tipped applicators (Remel BactiSwab®; Thermo Fisher Scientific, Lenexa, KS) were moistened with liquid Amies transport media. Gloves and gown were cultured with a single applicator. All inter-digital spaces of each glove were swabbed twice, and the palmar and dorsal surfaces of each glove were swabbed once in a large spiral. The length of each gown sleeve was swabbed twice and then the front of the gown was swabbed once in a large “W” pattern. All cultures were collected immediately prior to the HCW removing the gown and gloves and exiting the room. Based on the previously published finding of low rates of gown and glove contamination prior to entering the room and the additional cost, we did not

consider it necessary to pre-screen HCWs and eliminate already-contaminated gloves and gowns from the analysis.<sup>(65, 66)</sup>

Outcomes were analyzed separately for rooms occupied by patients colonized with MRSA or MDRAB. The primary outcome was considered positive if the relevant multidrug-resistant organism was isolated. Isolation and speciation of MRSA and *Acinetobacter baumannii* was performed using chromogenic agars (Remel Spectra™ MRSA, Thermo Fisher Scientific, Waltham, MA; CHROMagar™ Acinetobacter, CHROMagar, Paris, France). Antibiotic susceptibilities of *A. baumannii* isolates were established by Kirby-Bauer test against a panel of ten antibiotics from seven antibiotic classes. *A. baumannii* isolates were considered multidrug-resistant if they were susceptible to two or fewer classes of antibiotics. This is the same definition that was used by the UMMC Clinical Microbiology Laboratory during the trial period.

After application of the assigned intervention, the room was continuously observed and the disposable gowns and gloves of any HCW to exit the room were swabbed for culture. Sampling continued until 15 cultures were obtained or eight hours had elapsed since the intervention.

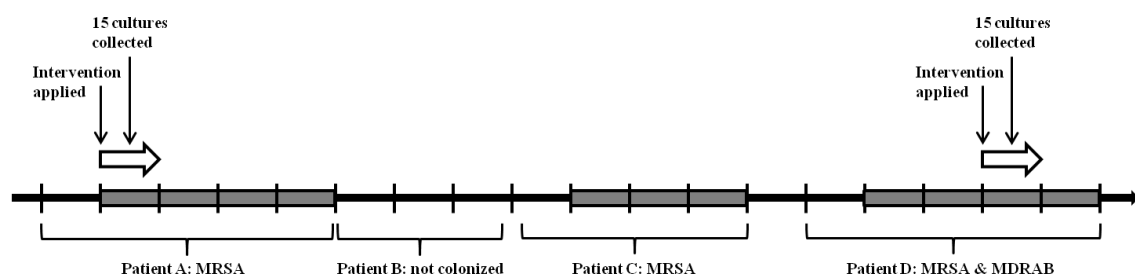
**Sample size.** We estimated that we would need to sample 20 MDRAB rooms in each arm and 54 MRSA rooms in each arm in order to have >90% power to detect a 30% relative reduction in the proportion of gown and glove contamination from each room with a 5% type I error rate.<sup>(95)</sup> These calculations were based on a baseline gown and glove contamination rate of 38% for MDRAB rooms and 18% for MRSA rooms.<sup>(65, 66)</sup> We assumed that we would collect 60 cultures from each room, and that the intra-cluster correlation coefficient ( $\rho$ ) for observations from the same room was 0.01.

**Randomization.** All rooms in the trial ICUs were allocated to the intervention or control arms at a 1:1 ratio prior to the start of the trial. Randomization was in blocks of 2 to 6, allowing no more than two adjoining rooms to have the same assignment. Rooms maintained their allocation until 50% of the sample was gathered. At the half-way point in the study, the intervention and sample collection procedures were suspended for a one-month washout period and the room allocations were exactly reversed.

The allocation scheme was designed to prevent crossover. Many multidrug-resistant bacteria can persist in the environment for weeks.<sup>(17, 89)</sup> Holding all rooms in one group or the other and then exchanging their assignments prevented crossover while allowing every room equal opportunity to be in either the experimental or the control group.

We expected 5 to 15 rooms would be eligible for follow-up on any given trial day, and that a single trial researcher would be able to enroll and follow one to two rooms per day.<sup>(65, 66)</sup> Rooms were sub-selected for enrollment and follow up from the daily list of all eligible rooms using a pseudo-random number generator (Figure 4).

**Figure 4.** Example diagram of eligibility, enrollment, and follow-up for a single study ICU room over an 18-day period.



**Blinding.** Healthcare workers and the occupants of the enrolled ICU rooms were blinded to the intervention by the sham cleaning procedure. Culture results were determined in a separate facility and not reported to the healthcare workers or the occupants of the enrolled ICU rooms.

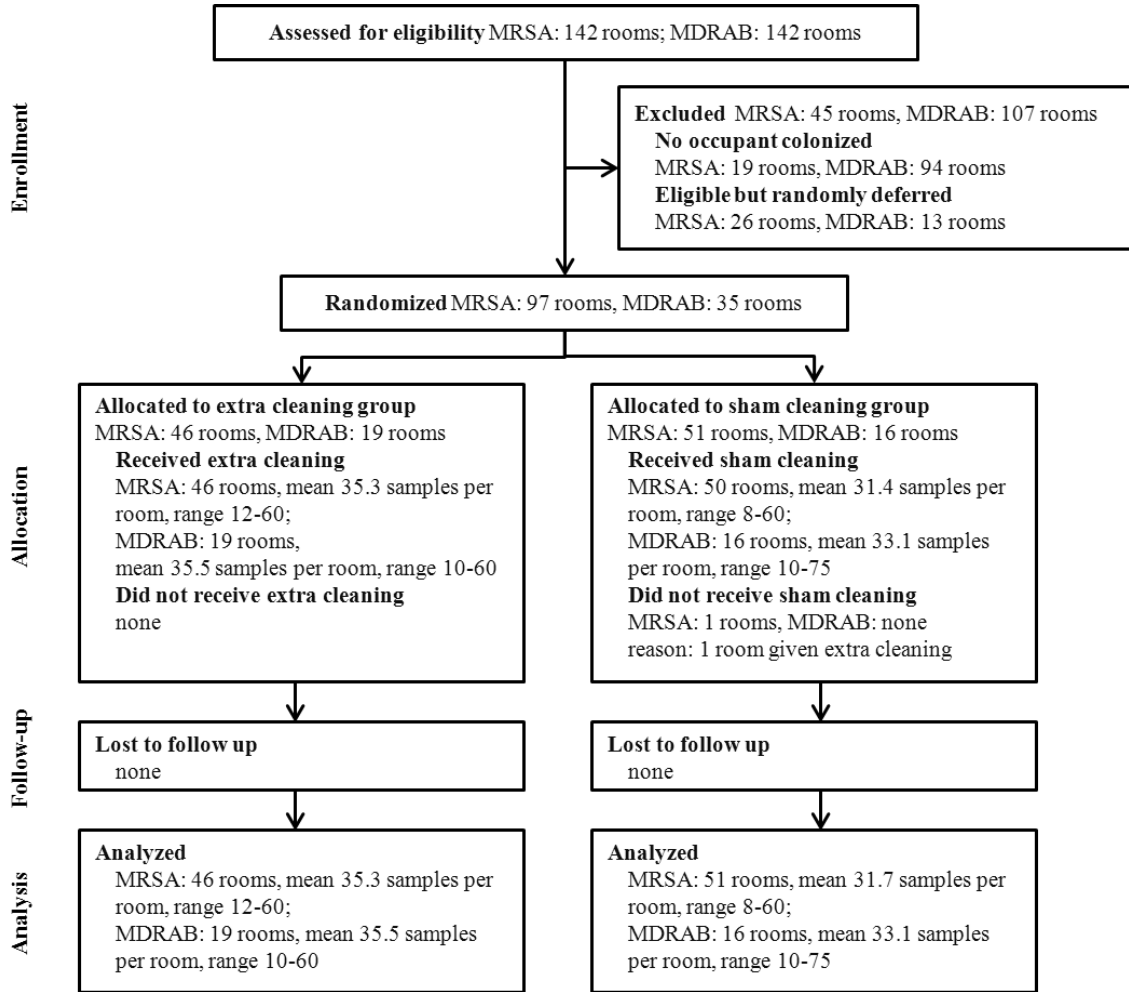
**Statistical analysis.** Results were analyzed on an intention-to-treat basis. Data from MDRAB and MRSA rooms were analyzed separately. For the primary analysis, we calculated the proportion of positive cultures from each room then tested the difference in the mean proportion of positive cultures between control rooms and experimental rooms using the Wilcoxon-Mann-Whitney Test. For the secondary analysis, we calculated the proportion of positive cultures from each room on each day and modeled that proportion in a generalized linear mixed model with the intervention group as a fixed effect and the room and the patient as nested random effects.

We planned two interim analyses at the quarter and halfway points of the trial, adjusted the critical p-values using an O'Brien-Fleming alpha-spending function.<sup>(94)</sup> The final critical p-value was 0.044.

## **RESULTS**

**Participants.** We enrolled 97 MRSA rooms and 35 MDRAB rooms. A total of 4,444 patient-HCW interactions were sampled: 1,206 from MDRAB rooms and 3,240 from MRSA rooms (Figure 5). An average of 3.1 patient-HCW interactions occurred per hour during follow-up. One MRSA control room received the experimental intervention on one day. An average of 1.8 rooms was enrolled on each trial day, and an average of

**Figure 5.** CONSORT diagram for the Enhanced Cleaning Trial.



14.7 cultures were collected per room per trial day. Enrollment began on 21 July 2011 and concluded on 21 May 2012. Rooms randomized to the experimental and control groups were not significantly different with regard to room or patient characteristics (Table 4).

**Intervention verification.** The intervention was verified in 10% of experimental rooms and 100% of control rooms. Among experimental rooms, 168/168 (100%) of fluorescent marks were removed after cleaning. Among control rooms, 521/2030 (26%) of marks were removed after 24 hours.

**Primary analysis.** The mean proportion of contaminated gowns and gloves for MDRAB was 0.16 among control rooms and 0.12 among experimental rooms (attributable risk [AR] -0.04, 95% confidence interval [CI] -0.11 – 0.04;  $p = 0.230$ ). For MRSA, the mean proportions were 22% among control rooms and 19% among experimental rooms (attributable risk [AR] -0.03%, 95% confidence interval [CI] -0.11 – 0.06,  $p = 0.158$ ) (Table 5). The differences in these mean proportions were not statistically significant.

**Secondary analysis.** In a generalized linear mixed model of the proportion of contaminated gowns and gloves contaminated with MDRAB on each study day, including random effects for the room and the patient nested within the room, the effect of enhanced cleaning was an absolute change of -5% in the proportion of contaminated gowns and gloves (95% confidence interval [CI] -13%, 8%,  $p = 0.242$ ). Among MRSA rooms, including random effects for the room and the patient nested within the room, the effect of enhanced cleaning was an absolute change of -2% in the proportion of contaminated gowns and gloves (95% confidence interval [CI] -9%, 6%,  $p = 0.646$ ) (Table 6).

**Table 4.** Baseline characteristics of the study rooms and patient occupants for the Enhanced Cleaning Trial.

<b>Multidrug-resistant <i>A. baumannii</i></b>			
<b>Characteristics</b>	<b>Control</b>	<b>Experimental</b>	<b>p</b>
<i>Rooms</i>			
Rooms sampled ( <i>n</i> )	16	19	-
Cultures collected ( <i>n</i> )	530	674	-
Patients sampled per room ( <i>mean ± SD</i> )	2.3 ± 0.2	2.3 ± 0.1	0.804
Cultures obtained per room ( <i>mean ± SD</i> )	33 ± 5	35 ± 3	0.688
Medical ICU ( <i>n, %</i> )	8 (50)	9 (47)	0.939
<i>Patients</i>			
Patients sampled ( <i>n</i> )	21	24	0.383
Age ( <i>mean ± SD</i> )	55 ± 19	50 ± 19	0.453
Male ( <i>n, %</i> )	11 (52)	18 (75)	0.119
Length of stay in room ( <i>mean ± SD</i> )	8 ± 14	7 ± 9	0.765
Length of stay in hospital ( <i>mean ± SD</i> )	16 ± 19	17 ± 15	0.789
Charlson Comorbidity Index ( <i>mean ± SD</i> )	2.8 ± 1.9	3.4 ± 3.6	0.450
<b>Methicillin-resistant <i>S. aureus</i></b>			
<b>Characteristics</b>	<b>Control</b>	<b>Experimental</b>	<b>p</b>
<i>Rooms</i>			
Rooms sampled ( <i>n</i> )	51	46	-
Cultures collected ( <i>n</i> )	1616	1624	-
Patients sampled per room ( <i>mean ± SD</i> )	2.5 ± 0.1	2.5 ± 0.1	0.947
Cultures obtained per room ( <i>mean ± SD</i> )	32 ± 2	35 ± 2	0.269
Medical ICU ( <i>n, %</i> )	24 (47)	22 (48)	0.939
<i>Patients</i>			
Patients sampled ( <i>n</i> )	76	69	-
Age ( <i>mean ± SD</i> )	56 ± 17	51 ± 19	0.111
Male ( <i>n, %</i> )	45 (59)	45 (65)	0.457
Length of stay in room ( <i>mean ± SD</i> )	4 ± 4	5 ± 5	0.340
Length of stay in hospital ( <i>mean ± SD</i> )	10 ± 9	12 ± 9	0.147
Charlson Comorbidity Index ( <i>mean ± SD</i> )	2.5 ± 2.5	2.6 ± 2.6	0.721

\* Student's T-Test, †Pearson's  $\chi^2$  Test

**Table 5.** Results of the primary analysis for the Enhanced Cleaning Trial.

Arm	Rooms <i>n</i>	Mean Proportion of Contaminated Swabs <i>p</i> (95.6% CI)	Attributable Risk <i>AR</i> (95.6% CI)	Relative Risk <i>RR</i> (95.6% CI)	<i>p</i> * <i>Wilcoxon</i>
<b>Multidrug-resistant <i>Acinetobacter baumannii</i> (MDRAB)</b>					
Control	16	0.16 (0.10, 0.22)			
Experimental	19	0.12 (0.06 – 0.18)	-0.04 (-0.11 – 0.04)	0.77 (0.28 – 2.11)	0.230
<b>Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)</b>					
Control	51	0.22 (0.17 – 0.27)			
Experimental	46	0.19 (0.12 – 0.26)	-0.03 (-0.11 – 0.06)	0.89 (0.50 – 1.53)	0.156

\*A two-tailed  $\alpha$  less than 0.044 is considered significant.

## DISCUSSION

In a randomized, controlled trial we found that enhanced cleaning of ICU rooms occupied by patients colonized with antibiotic-resistant bacteria reduced relative MRSA contamination by 14% and MDRAB by 25%, but these results were not statistically significant. Enhanced cleaning was performed using the same quaternary ammonium solution used by all housekeeping staff in the trial hospital but was done by a researcher in a thorough fashion. One hundred percent of surfaces examined after the experimental intervention were clean, and 26% of surfaces examined after the control intervention were clean. These results suggest that enhanced cleaning in ICU rooms whose occupants are colonized with antibiotic-resistant bacteria may have a modest effect on the contamination of healthcare workers protective clothing with these same pathogens.

**Table 6.** Results of the secondary analysis for the Enhanced Cleaning Trial.

Variable	Estimate <i>p</i> (95.6% CI)	p*
<b>Multidrug-resistant <i>Acinetobacter baumannii</i> (MDRAB)</b>		
<i>Main Effects</i>		
Intercept	0.17 (0.11 – 0.24)	< 0.001
Experimental Intervention	-0.05 (-0.13 – 0.04)	0.242
<i>Random Effects</i>		
Room	0.001 (-0.008 – 0.010)	
Patient (nested in Room)	0.007 (-0.005 – 0.019)	
<b>Methicillin-resistant <i>Staphylococcus aureus</i> (MRSA)</b>		
<i>Main Effects</i>		
Intercept	0.19 (0.14 – 0.24)	< 0.001
Experimental Intervention	-0.02 (-0.09 – 0.06)	0.646
<i>Random Effects</i>		
Room	0	
Patient (nested in Room)	0.023 (0.012 – 0.019)	

\*A two-tailed  $\alpha$  less than 0.044 is considered significant.

In the secondary analysis, the effect of enhanced cleaning was consistent with the direction and magnitude of the effect observed in the primary analysis. The standard error of the random effect of the patient in both models was close to the size of the effect of cleaning. In contrast, the standard error of the random effect of the room was zero or near-zero in both models. These results suggest that the patient has a large effect on how frequently HCW's gowns and gloves are contaminated with antibiotic-resistant bacteria.

Wilson et al. performed a crossover trial in two London hospital ICUs, randomizing the whole unit to two additional cleanings with microfiber cloths soaked in copper biocide or standard cleaning. In subgroup analyses, additional cleaning was associated with a significant reduction in how frequently MRSA was isolated from physicians' hands (11/423 vs. 3/425,  $p = 0.025$ ) but not nurses (28/1,694 vs. 16/1,647,  $p = 0.077$ ).<sup>(24)</sup> Physicians' hand cultures were taken both before and after cleaning and at random locations in the unit, and, given the small number of outcomes for both analyses, drawing conclusions about the relationship between cleaning and contamination is difficult.

Our study has a number of limitations. The mean contamination in MDRAB control rooms, the standard deviation of the mean contamination among MRSA rooms, and the correlation between cultures from the same room were all higher than assumed during sample size calculations. These differences most likely limited the statistical power of the trial.

Strengths of this trial include the cluster randomized design, the use of standard hospital cleaning products, and the mixed population of medical and surgical ICU rooms. Unlike previous trials that examined the effect of enhanced cleaning on surface contamination with multidrug-resistant bacteria, we examined the relationship between enhanced cleaning and contamination of HCW's disposable gowns and gloves. Gown and glove contamination represents an early step in a proposed pathway of patient-to-patient transmission via the hands of HCW and thus potentially quantifies the effect of cleaning on the most interesting causal outcome, namely MRSA and MDRAB colonization acquisition.

Although we were not able to detect a statistically significant reduction in gown and glove contamination with MRSA and MDRAB following enhanced cleaning, we did observe an effect among both organisms. Given the low cost of material and labor for enhanced cleaning, the expense of treating antibiotic-resistant infections in ICU patients, and the frequency of healthcare worker contact, a 10-20% relative reduction in gown and glove contamination may be a cost-effective method for hospital infection control services to reduce the chance of patient-to-patient transmission. Daily enhanced cleaning may produce a cumulative effect that would further reduce the chances of contamination and transmission.

In conclusion, in a cluster-randomized, controlled trial of enhanced cleaning in ICU rooms, gown and glove contamination with MDRAB and MRSA was not significantly reduced. A small effect of cleaning was observed for both organisms. In the control arm, frequently-touched surfaces in ICU rooms were often not clean, and MRSA and MDRAB were often isolated from HCW's disposable gloves and gowns. Healthcare workers in study ICUs had repeated contact with multiple patients each day. Our results suggest future research is needed to determine whether intense environmental cleaning will lead to fewer infections with MDR bacteria.

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## **POTENTIAL CONFLICTS OF INTEREST**

The authors report no potential conflicts of interest.

**V. PRIOR COLONIZATION BY ANTIBIOTIC-RESISTANT GRAM-NEGATIVE BACTERIA IS ASSOCIATED WITH INCREASED RISK OF SUBSEQUENT ANTIBIOTIC-RESISTANT GRAM-NEGATIVE BACTEREMIA IN CANCER PATIENTS<sup>3</sup>**

**ABSTRACT**

**Background:** The incidence of antibiotic-resistant Gram-negative bacterial infection among cancer patients is rising and is associated with increased mortality. We hypothesized that prior colonization with antibiotic-resistant Gram-negative bacteria would be associated with an increased risk of subsequent antibiotic-resistant Gram-negative bacteremia among cancer patients.

**Methods:** We performed two matched case-control studies of patients with suspected bacteremia treated at the University of Maryland Greenebaum Cancer Center from 2001 through 2010. Cases were patients with a blood culture positive for an antibiotic-resistant Gram-negative bacteria. Antibiotic resistance was defined as full or intermediate resistance to cefepime, imipenem, or piperacillin-tazobactam. Controls were patients with a blood culture negative for antibiotic-resistant Gram-negative bacteria. Controls were matched to cases on age, malignancy, admission year, and days from admission to blood culture. Prior colonization was defined as any antibiotic-resistant Gram-negative bacteria in a surveillance or non-sterile-site clinical culture obtained 2 to 365 days prior to the defining blood culture. The first study included all cases and matched controls; the

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<sup>3</sup> A.S. Hess, M. Kleinberg, J.D. Sorokin, G. Netzer, J.K. Johnson, M. Shardell, K.A. Thom, A.D. Harris, M. Roghmann

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As submitted to *Clinical Infectious Diseases*.

second study included cases and matched controls who had cultures to define prior colonization status.

**Results:** We identified 84 cases and matched 306 controls in the first study. 37% of cases and 7% of controls were previously colonized by antibiotic-resistant Gram-negative bacteria. In a conditional logistic model, prior antibiotic-resistant Gram-negative colonization was strongly and positively associated with subsequent antibiotic-resistant Gram-negative bacteremia (odds ratio [OR] 8.1, 95% confidence interval [CI] 3.6 – 18.2) after controlling for use of broad-spectrum antibiotics in the last 28 days. The results were similar in the second study.

**Conclusion:** Prior colonization with antibiotic-resistant Gram-negative bacteria is associated with subsequent antibiotic-resistant Gram-negative bacteremia among cancer patients with suspected infection. Prior cultures may identify cancer patients with suspected infection at increased risk of antibiotic-resistant Gram-negative bacteremia and can be used to select empiric antibiotic therapy.

## INTRODUCTION

Infections with Gram-negative bacteria that are resistant to broad-spectrum antibiotics such as cephalosporins, carbapenems, and piperacillins are increasingly common in the U.S.<sup>(2, 9, 15, 39, 40, 43)</sup> These infections are associated with higher hospital cost, length of stay, and mortality among cancer patients.<sup>(9, 39, 40, 43)</sup> Risk of bacteremia in cancer patients is associated with indwelling devices (e.g. intravenous lines, urinary catheters) and the disruption of mucosal surfaces following chemotherapy.<sup>(29, 69, 71, 83, 84)</sup> Colonization of the skin and mucosal tissue with opportunistic pathogens frequently

precedes infection. Surveillance and clinical cultures are used in many U.S. hospitals to identify patients colonized with antibiotic-resistant organisms for the purposes of infection control, but the results are not routinely used to guide antimicrobial therapy choices and may not be readily available to clinicians.<sup>(26, 30)</sup>

Clinical studies in bone marrow transplant patients have shown that vancomycin-resistant *Enterococcus* colonization is associated with subsequent vancomycin-resistant *Enterococcus* bacteremia and that trimethoprim-sulfamethoxazole-resistant stool isolates are associated with subsequent trimethoprim-sulfamethoxazole-resistant bacteremia.<sup>(29, 108, 109)</sup> Several studies have identified prior antimicrobial exposure as a risk factor for antibiotic-resistant Gram-negative bacteremia among cancer patients.<sup>(85-87, 110)</sup> Identifying risk factors for antibiotic-resistant Gram-negative bacteremia may guide empiric antibiotic therapy and thus improve outcomes among these patients. We hypothesized that prior colonization with antibiotic-resistant Gram-negative bacteria would be associated with increased risk of subsequent antibiotic-resistant Gram-negative bacteremia among cancer patients with suspected infection.

## **METHODS**

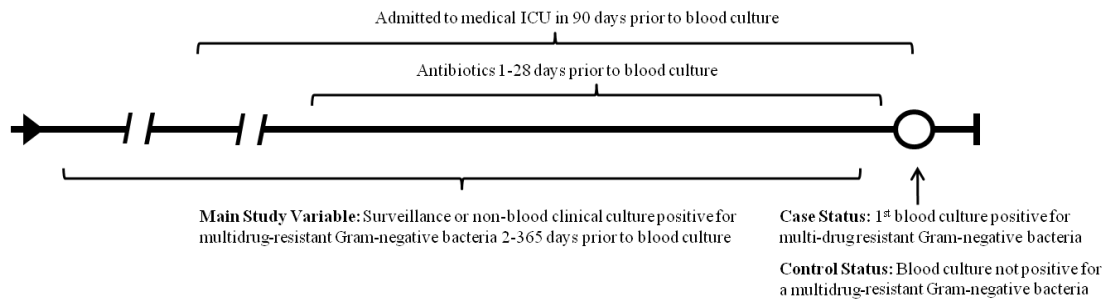
***Patient Population.*** The study population was selected from cancer patients with a suspected infection who received inpatient care at the University of Maryland Medical Center (UMMC) Greenebaum Cancer Center between January 1<sup>st</sup> 2001 and December 31<sup>st</sup> 2010. The UMMC Greenebaum Cancer Center is a National Cancer Institute-designated cancer center in Baltimore, Maryland with 41,000 outpatient and 1,600 inpatient visits annually. To be eligible for our study, we had to be able to determine from

the patient's medical record the type of malignancy the patient had (e.g., leukemia, lymphoma, solid/other) and the patient had to have had at least one blood culture during the course of their hospitalization. Patient data were abstracted from the UMMC Central Data Repository.

**Study Design.** We performed two matched case-control studies. The first study included all cases and matched controls from the population of eligible patients; the second study included cases and matched controls from the population of eligible patients who had cultures to define prior colonization status. Except for the difference in study populations, the studies were performed and analyzed in an identical fashion.

In both studies, cases were patients during the study period with a blood culture that grew antibiotic-resistant Gram-negative bacteria. Eligible controls were patients during the study period with one or more blood cultures that did not grow antibiotic-resistant Gram-negative bacteria. Controls' blood cultures were allowed to have any other result, including no organisms or drug-susceptible Gram-negative bacteria. Antibiotic resistance was defined as full or intermediate resistance to cefepime, imipenem, or piperacillin-tazobactam. These antibiotics were selected because of their role as key antimicrobial agents used in neutropenic fever.<sup>(71)</sup> We excluded all patients with blood cultures positive for *Stenotrophomonas maltophilia* because antimicrobial resistance testing was not routinely performed for this organism at UMMC during the study period. All variables were assessed from the time of the blood culture for both cases and controls (Figure 6).

**Figure 6.** Diagram of the relationship of case and control status to the main study variable and other covariates of interest.



Up to four controls were matched to each case on: age  $\pm 10$  years, type of malignancy, date of admission  $\pm 180$  days, and duration of hospitalization at the time of the blood culture. These criteria were chosen to match cases and controls with respect to changes over time in surveillance and clinical culture practice, changes in microbiology laboratory practice, underlying malignancy, and time at risk for infection in the hospital. Malignancy categories used were acute lymphocytic leukemia (ALL), chronic lymphocytic leukemia (CLL), acute myeloid leukemia (AML), chronic myeloid leukemia (CML), other leukemia, Hodgkin’s lymphoma (HL) non-Hodgkin’s lymphoma (NHL) myeloma, and solid tumor/other. Duration of hospitalization at the time of the blood culture was matched to cases on a sliding scale. For cases whose duration of stay was  $< 7$  days, controls’ duration of hospitalization was required to be within  $\pm 3$  days of the case; for cases whose duration of hospitalization was  $\geq 7$  and  $< 14$  days, controls duration of hospitalization was required to be within  $\pm 5$  days of the case; and so on such that the allowed difference in duration increased by two days for each seven days the case was hospitalized. This approach was chosen instead of categories because it required a closer match when duration of hospitalization was short and small differences may represent

large changes in risk, but did not require as close a match when duration of hospitalization was long and small differences are less likely to represent large changes in risk.

***Study Variables.*** The primary exposure variable was colonization with antibiotic-resistant Gram-negative bacteria from 2 to 365 days prior to the bacteremia-defining blood culture. Patients were considered colonized if antibiotic-resistant Gram-negative bacteria were isolated from any surveillance or non-sterile-site (e.g. wound, urine, sputum) clinical culture. Patients were considered not colonized if no antibiotic-resistant Gram-negative bacteria were isolated from any surveillance or non-sterile-site clinical culture. In the first study, cases and controls without a record of surveillance or non-sterile-site cultures during this period were assumed not colonized. In the second study, cases and controls were selected from a population with at least one surveillance or non-sterile-site clinical culture 2 to 365 days before the blood culture.

Covariates included in the analysis were sex, race, admission to the medical intensive care unit (ICU) in the 90 days prior to the defining blood culture (yes vs. no), white blood cell (WBC) count  $\leq 1.0 \times 10^3$  cells per  $\text{mm}^3$  within  $\pm 2$  days of the defining blood culture (yes vs. no), and treatment with broad-spectrum antimicrobial agents 1-28 days prior to the defining blood culture (yes vs. no). Antimicrobials included in this definition were aztreonam, cefepime, doripenem, imipenem, moxifloxacin, and piperacillin-tazobactam. Antimicrobials used in the 24 hours prior to the defining blood culture were excluded, as they may have been started in response to early clinical signs of infection, i.e. after the outcome. WBC count was used as a surrogate for neutropenia because differential counts were often not available.

**Statistical Analysis.** All statistical analyses were performed using SAS 9.22 (SAS Institute, Cary, NC). All bivariate and multivariable analyses were performed using conditional logistic regression to account for the matched case-control design. The initial multivariable conditional logistic regression was adjusted for variables previously shown to be associated with bacteremia caused by antibiotic-resistant organisms and potential confounders (variables associated with both case-control status and the main study variable at  $p < 0.1$ ). The significance of individual covariates and interaction between covariates was assessed using the Likelihood Ratio Test. All associations are reported as odds ratios with 95% confidence intervals, and a two-tailed  $p$ -value  $< 0.05$  was considered statistically significant.

## RESULTS

**First study.** We identified 84 cases and matched 306 controls. On average, 3.6 controls were matched to each case. Cases and controls were not significantly different with regard to age, days from admission to culture, year of admission, and malignancy type, and sex (Table 7). Controls were more likely to be of white race than cases. Thirty-one (37%) cases and 22 (7%) controls were found to be colonized with antibiotic-resistant Gram-negative bacteria in the prior year.

In bivariate analyses, antibiotic-resistant Gram-negative bacteremia was significantly associated with prior colonization by antibiotic-resistant Gram-negative bacteria (odds ratio [OR] 9.5, 95% confidence interval [CI] 4.2–21.0). Covariates associated with both antibiotic-resistant Gram-negative bacteremia and prior colonization with antibiotic-resistant bacteremia included admission to the medical intensive care unit

in the 90 days prior to blood culture and treatment with antimicrobials in the 1-28 days prior to the blood culture. In a multivariable conditional logistic regression model, antibiotic-resistant Gram-negative bacteremia was independently associated with prior colonization with antibiotic-resistant Gram-negative bacteria and treatment with antibiotics in the 1-28 days prior to the blood culture (Table 8).

**Table 7.** Demographic and hospital-stay characteristics of cancer patients with antibiotic resistant Gram-negative bacteremia (cases) and matched controls from the University of Maryland Greenbaum Cancer Center, 2001 – 2010.

Variable	First Case-Control Study N = 390			Second Case-Control Study N = 319		
	Cases N = 84 n (%)	Controls N = 306 n (%)	p*	Cases N = 68 n (%)	Controls N = 251 n (%)	p*
<b>Main Study Variable</b>						
Colonization with antibiotic-resistant Gram-negative in prior year	31 (37)	22 (7)	< 0.001	24 (35)	17 (7)	< 0.001
Missing	4 (5)	89 (29)		-	-	
<b>Matching Criteria</b>						
Age (years)	51 ± 14†	53 ± 13†	-	50 ± 14†	52 ± 13†	-
Malignancy Type			-			-
ALL	11 (13)	31 (10)		7 (11)	27 (11)	
CLL	2 (2)	2 (1)		2 (3)	2 (1)	
AML	25 (30)	97 (32)		21 (31)	77 (31)	
CML	0	0		0	0	
Other Leukemia	1 (1)	2 (1)		1 (1)	2 (1)	
Hodgkin's Lymphoma	1 (1)	1 (1)		0	0	
Non-Hodgkin's Lymphoma	12 (14)	44 (14)		10 (15)	36 (14)	
Myeloma	7 (8)	28 (9)		6 (9)	24 (10)	
Solid tumor	26 (31)	102 (34)		21 (31)	83 (33)	
Duration of hospitalization at the time of blood culture	5 (0.2, 15) ‡	3 (0.3, 13) ‡	-	4 (0.2, 14) ‡	3 (0.3, 11) ‡	-
<b>Additional Covariates</b>						
Male	54 (64)	188 (61)	0.563	41 (60)	150 (60)	0.971
Race						
White	35 (42)	141 (48)	ref.	27 (40)	125 (50)	ref.
Black	32 (38)	129 (44)	0.046	27 (40)	105 (42)	0.175
Other	17 (20)	23 (8)	0.005	14 (21)	19 (8)	0.003
Missing	0	3 (2)		0	2 (1)	
WBC count ≤1x10 <sup>3</sup> /mm <sup>3</sup> in ±2 days of blood culture	36 (43)	115 (38)	0.544	26 (38)	32 (81)	0.312
Missing	1 (1)	1(1)				
Admitted to medical ICU in 90 days prior to blood culture	14 (17)	22 (7)	0.017	11 (16)	20 (8)	0.062
Treatment with antimicrobials 1-28 days prior to blood culture	51 (61)	114 (37)	< 0.001	41 (60)	90 (36)	< 0.001
Days since last surveillance or non-sterile-site clinical culture	5 (5, 17)‡	7 (31, 28)‡	0.016	7 (3, 25)	8 (3, 50)	0.018
No culture in prior 2 to 365 days	4 (5)	89 (29)		-	-	

\*Conditional logistic regression, †Mean ± SD, ‡Median, 1<sup>st</sup> quartile, 3<sup>rd</sup> quartile  
The distributions of the matching criteria were not tested.  
Due to rounding, some percentages may not add to 100.

Eighty out of eighty-four cases (95%) had at least one surveillance or non-sterile-site clinical culture in the 2 to 365 days prior to their positive blood culture compared to 217 of 306 controls (71%). Cases and controls with prior surveillance or non-sterile-site cultures were more likely than cases and controls without prior surveillance or non-sterile-site cultures to have received antimicrobials in the 1-28 days prior to the blood culture (odds ratio [OR] 5.7, 95% confidence interval [CI] 2.7 – 12.0,  $p < 0.001$ ) and to have been admitted to the medical intensive care unit in the 90 days prior to the blood culture (odds ratio [OR] 3.5, 95% confidence interval [CI] 0.9, 12.4,  $p = 0.058$ ).

Genera represented among antibiotic-resistant Gram-negative blood culture isolates included *Klebsiella* (25%), *Pseudomonas* (23%), *Escherichia* (13%), *Enterobacter* (10%), *Acinetobacter* (8%), *Achromobacter* (5%), *Sphingomonas* (5%), *Chryseobacterium* (3%), *Ochrobactrum* (3%), *Citrobacter* (1%), *Pantoea* (1%), *Roseomonas* (1%), and *Serratia* (1%). Among these isolates, 36 (43%) had full or intermediate resistance to cefepime, 16 (19%) had full or intermediate resistance to imipenem, and 58 (69%) had full or intermediate resistance to piperacillin-tazobactam. In the 31 cases previously colonized with antibiotic-resistant Gram-negative bacteria, 19 (61%) had at least one isolate from a prior surveillance or non-sterile-site clinical culture that was of the same organism and had the same antimicrobial resistances as the blood culture isolate.

**Second study.** There were 68 cases and 251 matched controls with at least one prior surveillance or non-sterile-site clinical culture in the second study. Twelve cases from the first study had prior cultures, but these cases could not be matched to any controls with prior cultures. On average, 3.7 controls were matched to each case. Cases

and controls were not significantly different with regard to age, days from admission to culture, year of admission, and malignancy type, and sex (Table 1). Cases were more likely non-white or non-black than controls. Twenty-four (35%) cases and 17 (7%) controls were colonized with antibiotic-resistant Gram-negative bacteria in the prior year. In a bivariate conditional logistic regression model, antibiotic-resistant Gram-negative bacteremia was strongly associated with antibiotic-resistant Gram-negative colonization in the prior year (odds ratio [OR] 8.5, 95% confidence interval [CI] 3.7–19.2). In a multivariable conditional logistic model including antibiotic-resistant Gram-negative colonization and treatment with antimicrobials in the prior 1-28 days, antibiotic-resistant Gram-negative bacteremia was strongly associated with prior antibiotic-resistant Gram-negative colonization and treatment with antimicrobials in the prior 1-28 days (Table 2).

## **DISCUSSION**

We found that prior colonization with antibiotic-resistant Gram-negative bacteria was strongly and positively associated with subsequent antibiotic-resistant Gram-negative bacteremia among cancer patients. The association was consistent across study designs and independent of recent exposure to antimicrobials. In the 31 cases previously colonized with antibiotic-resistant Gram-negative bacteria, 19 (61%) had at least one isolate from a prior surveillance or non-sterile-site clinical culture that was of the same species and had the same antimicrobial resistances as the blood culture isolate. This suggests that prior detection of antibiotic-resistant Gram-negative bacteria is a significant risk factor for subsequent antibiotic-resistant Gram-negative bacteremia and that review

of prior cultures could be used to select empiric antibiotic therapy in cancer patients with suspected infection.

**Table 8.** Multivariable conditional logistic regression model of antibiotic-resistant Gram-negative bacteremia among cancer patients with suspected infection at the University of Maryland Greenbaum Cancer Center, 2001 – 2010.

Variable	First Case-Control Study <i>N</i> = 390			Second Case-Control Study <i>N</i> = 319		
	OR	(95% CI)	<i>p</i>	OR	(95% CI)	<i>p</i>
Colonization with antibiotic-resistant Gram-negative in prior year	8.1	(3.6 – 18.2)	< 0.001	7.3	(3.2 – 16.8)	< 0.001
Treatment with antimicrobials 1-28 days prior to blood culture	2.3	(1.2 – 4.3)	0.013	2.7	(1.3 – 5.5)	0.007

Cases were matched to controls on age  $\pm$ 10 years, date of admission  $\pm$ 180 days, malignancy type, and hospital days from admission to blood culture.

Our results are similar regardless of study design. Our first study classified cases and controls who did not have surveillance or non-sterile-site clinical cultures in the year before the defining blood culture as not colonized. If all controls missing prior cultures were colonized, the proportion of colonized patients would be nearly equal among cases and controls. However, we consider it unlikely that cases or controls without prior surveillance or non-sterile-site cultures were any more likely to be colonized than patients with prior cultures. Other studies have found that previous hospitalizations, previous intensive care unit stays, and treatment with antibiotics are the strongest predictors of colonization.<sup>(97-100)</sup> In our first study, cases and controls without prior cultures were much less likely to have recently received antibiotics or been in an intensive care unit. In our second study, we restricted the study population to those cancer patients with prior surveillance or non-sterile-site cultures. This design is not open to the same

misclassification bias as the first study, although it is possible that a selection bias is introduced by restricting the population on the presence of cultures. However, the consistency of the results from the two studies along with the implausibility of excess colonization among those without cultures suggests that the observed association is robust to any biases introduced by study design.

Our study is consistent with similar studies in cancer patients. Two previous studies found an association between colonization with Gram-negative bacteria and subsequent Gram-negative bacteremia in cancer patients. Cohen *et al.* assessed hematopoietic stem-cell transplant patients colonized with antimicrobial-sensitive Gram-negative bacteria, and found a 17 to 174-fold increased risk, depending on species.<sup>(88)</sup> Wells *et al.* reported a threefold increase in trimethoprim-sulfamethoxazole-resistant Gram-negative bacteremia among bone marrow transplant patients with prior stool cultures positive for trimethoprim-sulfamethoxazole-resistant Gram-negative bacteria compared to those without positive prior cultures.<sup>(29)</sup> The current study adds to this literature by showing that this association holds true for cefepime, imipenem, and piperacillin-tazobactam.

Our study has limitations. Our population was drawn from a single center, and institutional differences in population and practices may limit the external generalizability of the study, particularly with regard to surveillance culture methods. The use of retrospectively collected clinical data also requires assumptions, particularly that drawing a blood culture is a good surrogate marker of clinical suspicion of infection.

Our results suggest that a review of prior cultures might reduce inappropriate empiric antibiotic therapy in cancer patients with suspected infection. Studies of critically

ill patients including cancer patients have found that inappropriate empiric therapy for Gram-positive or Gram-negative infections is associated with increased mortality and length of stay.<sup>(111-116)</sup> Using prior cultures results might reduce the risk of inappropriate empiric antibiotic therapy and thus reduce mortality or length of stay in settings with high rates of antibiotic resistant Gram-negative bacteremia.

In conclusion, prior antibiotic-resistant Gram-negative colonization is associated with an increased risk of subsequent antibiotic-resistant Gram-negative bacteremia among cancer patients with suspected infection. Future studies should include multiple clinical centers and molecular typing to determine in what proportion of patients the infecting and colonizing isolates are related. If studies indicate that prior antibiotic-resistant Gram-negative colonization predict subsequent antibiotic-resistant Gram-negative bacteremia in cancer patients with suspected infection, surveillance cultures to identify colonization might increase appropriate empiric antibiotic therapy.

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**POTENTIAL CONFLICTS OF INTEREST**

The authors report no conflicts of interest.

## VI. DISCUSSION

The two manuscripts in this dissertation represent respectively the first trial to address the effect of enhanced cleaning on healthcare worker gown and glove contamination rates with antibiotic-resistant bacteria and the first adequately controlled study of the relationship between colonization with antibiotic-resistant Gram-negative bacteria and subsequent antibiotic-resistant Gram-negative bacteremia among cancer patients. We found that enhanced cleaning may modestly reduce gown and glove contamination with antibiotic-resistant bacteria, and that patients colonized with antibiotic-resistant Gram-negative bacteria are at increased risk of subsequent Gram-negative bacteremia.

### A. Current View of Enhanced Environmental Cleaning and Use of Prior Cultures to Guide Empiric Therapy for Cancer Patients

Enhanced-cleaning protocols require that frequently-touched surfaces in occupied hospital rooms are cleaned one or more additional times each day in order to prevent those surfaces becoming reservoirs for patient-to-patient transmission of antibiotic-resistant bacteria. Environmental cleaning in US and UK hospitals has been shown to be inadequate. Monitoring systems, improved training, and novel cleaning technologies have all been shown to significantly reduce antibiotic-resistant bacteria in the hospital environment. The hypothesis that enhanced environmental cleaning will reduce patient-to-patient transmission is widely-supported, but few studies have incorporated clinical outcomes and no conclusive evidence exists.<sup>(117)</sup>

Empiric therapy for a cancer patient with a suspected bloodstream infection requires the physician to guess what combination of antibiotics will successfully treat an unknown organism. Although empiric vancomycin will cover most antibiotic-resistant Gram-positive organisms, no one antibiotic will be effective against all pathogens. The Infectious Disease Society of America suggests that information about prior infections or colonization with antibiotic-resistant bacteria may be used to guide empiric therapy in cancer patients. Current understanding about the pathogenesis of bloodstream infections in cancer patients supports this practice, but epidemiological evidence is limited.

**B. Enhanced Cleaning May Be Associated with Reduced Contamination of Personal Protective Clothing by Antibiotic-Resistant Bacteria**

Enhanced cleaning is associated with significant reductions in environmental contamination with antibiotic-resistant bacteria but its effects on contamination of healthcare workers' protective clothing or hands is not clear. We conducted a single-center randomized trial of enhanced cleaning to reduce contamination of healthcare workers protective clothing with MRSA and MDRAB after caring for patients colonized with these bacteria. We found that enhanced cleaning was associated with a modest reduction in contamination with either organism, but these reductions were not significant.

Subgroup analysis of a study in two UK ICUs found that enhanced cleaning three times per day with copper biocide was associated with significant reductions in MRSA on physicians' hands, but not those of nurses. We did not observe a statistically significant reduction in contamination in our trial, but our outcome was a composite of

all healthcare workers and measured only the effect of a single cleaning with standard ammonium-based cleaner. We also observed a strong correlation between observations from the same patient, which suggests that unobserved patient characteristics are a major determinant of contamination. Large differences between patients in their propensity to contaminate the gowns and gloves of visiting healthcare workers may have overshadowed any effect of cleaning and prevented it from achieving statistical significance.

Our trial measures gown and glove contamination with MRSA and MDRAB. The primary outcome of all previous trials of enhanced cleaning has been environmental contamination. Gown and glove contamination is a more clinically relevant outcome because it represents the first step in a hypothesized chain of events between environmental contamination and acquisition of antibiotic-resistant bacteria. Our research suggests that enhanced cleaning may reduce gown and glove contamination with antibiotic-resistant bacteria but cannot replace other infection control methods.

**C. Prior Colonization with Antibiotic-Resistant Gram-Negative Bacteria is Significantly Associated with Subsequent Antibiotic-Resistant Gram-Negative Bacteremia in Cancer Patients**

Before the current study, epidemiological evidence for a relationship between colonization with antibiotic-resistant Gram-negative bacteria and subsequent antibiotic-resistant Gram-negative bacteremia was limited. We found a significant relationship between the latter and the former after controlling for recent treatment with broad-spectrum antibiotics. Our findings are consistent with previous studies of antibiotic-

resistant Gram-negative bacteremia in cancer patients. Wells and colleagues observed a six-fold increase in the risk of TMP-SMX-resistant Gram-negative infections among patients colonized with TMP-SMX-resistant bacteria compared to those colonized with TMP-SMX-susceptible isolates.<sup>(29)</sup> Cohen and colleagues estimated that prior colonization with Gram-negative pathogens in bone-marrow-transplant patients was associated with a 17-174-fold increase in the risk of Gram-negative bacteremia and that the majority of infecting isolates shared antibiotic resistances with the colonizing isolate.<sup>(88)</sup> Spanik, Oliveira, and Vigil all found a significant association between recent treatment with broad-spectrum antibiotics and subsequent antibiotic-resistant Gram-negative bacteremia.<sup>(85, 87, 110)</sup> The current study is the first to look at the association between antibiotic-resistant Gram-negative colonization and bacteremia while controlling for important confounders, and the first to include several different broad-spectrum antibiotics in the definition of resistance.

The current study found a strong association between colonization with antibiotic-resistant Gram-negative bacteria and subsequent antibiotic-resistant Gram-negative bacteremia. This association provides epidemiological evidence in support of using prior culture results to tailor empiric therapy for cancer patients with suspected infection. More successful initial empiric therapy would be expected to reduce morbidity and mortality among cancer patients.

#### **D. Strengths and Limitations**

This dissertation presents the first trial to examine the relationship between enhanced cleaning and the contamination of healthcare workers' disposable protective

clothing with antibiotic-resistant bacteria and the first adequately controlled study to examine the relationship between colonization with antibiotic-resistant Gram-negative bacteria and subsequent antibiotic-resistant Gram-negative bacteremia.

A limitation of the enhanced-cleaning trial is that it did not anticipate the strong correlation between cultures associated with the same patient. The estimated correlation between cultures taken from the same room was between 0.07 and 0.08. However, the random effects estimated in the secondary analysis of the trial suggest that the propensity for gown and glove contamination varies only slightly between rooms, but there are relatively large variations between patients. This suggests that the correlation among cultures from the same room is driven by the patient, and not the room. By obtaining a large number of cultures per patient from a relatively small pool of patients, the power of the trial to detect the effect of cleaning may have been diminished.

An additional limitation of the enhanced-cleaning trial is the inability to account for statistical non-independence of cultures associated with the same healthcare worker. Accounting for the non-independence of healthcare workers would have required them to give individual, written informed consent. It would have been impossible to obtain individual written consent from the majority of healthcare workers in the study, especially during short care activities involving a large number of persons. Requiring consent would have introduced a significant selection bias into the study.

Strengths of the enhanced-cleaning trial include the randomized design, the outcome measure, and the use of standard hospital cleaning products. Although a number of previous studies of cleaning have used environmental contamination as an outcome, HCW glove and gown contamination more clearly links the results of

enhanced cleaning to patient-to-patient transmission. Previous studies have often combined enhanced cleaning protocols with novel cleaning technologies. It is often unclear which component of a bundled intervention is responsible for any observed effect. Our study used only the hospital-issued quaternary ammonium solution – a common cleaning solution in U.S. hospitals – and applied a single extra cleaning.

A limitation of the study of antibiotic-resistant Gram-negative bacteremia is bias from lack of culture data. The study population includes cases and controls who did not have a prior surveillance or non-sterile-site clinical culture in the year before their defining blood culture. Lack of cultures is a clinical feature of these patients, and a physician for these patients would have to assume that they were not colonized. Including these patients in the study introduces a potential misclassification bias, since they may truly be colonized. Restricting the population to patients with cultures introduces a potential selection bias for the same reason. However, it is unlikely that patients who do not have cultures are colonized, and when both studies are performed they produced the same result.

Strengths of the study of antibiotic-resistant Gram-negative bacteremia include a larger sample size than previous studies and the matched design. Unlike several previous studies of antibiotic-resistant Gram-negative bacteremia, we had sufficient statistical power to control for multiple suspected confounders of the relationship between prior colonization and subsequent bacteremia. The matched design allowed us to compare patients with similar clinical characteristics and risk of infection over a long retrospective study period.

## **E. Summary and Implications**

The studies presented in this dissertation address unresolved issues in the prevention and anticipation of antibiotic-resistant bacterial infection in among severely ill hospital patients. The first aim used a randomized, controlled trial to examine the relationship between enhanced cleaning and patient-to-patient transmission of antibiotic-resistant bacteria. The second aim used two case-control studies to examine whether prior colonization with antibiotic-resistant Gram-negative bacteria is a major risk factor for subsequent antibiotic-resistant Gram-negative bacteremia.

Enhanced cleaning may reduce contamination of healthcare workers' disposable protective clothing. Although the reduction in contamination observed was not statistically significant, given the low cost of enhanced cleaning it may be a cost effective way to modestly reduce patient-to-patient transmission of antibiotic resistant bacteria. The trial results also suggest that patients are a major determinant of glove and gown contamination, and that some patients have a much higher propensity to contaminate than others. These findings in no way reduce the importance of existing infection control methods such as hand hygiene, patient isolation, and contact precautions.

Prior colonization with antibiotic-resistant Gram-negative bacteria may be used to identify cancer patients at increased risk for antibiotic-resistant Gram-negative bacteremia. If these patients become febrile, empiric therapy may be tailored to account for their colonization history, and this may reduce morbidity and mortality.

In conclusion, we found that enhanced cleaning is associated with a modest, non-significant reduction in the contamination of healthcare workers' disposable protective

clothing with antibiotic-resistant bacteria, and that prior colonization with antibiotic-resistant Gram-negative bacteria is a major risk factor for subsequent antibiotic-resistant Gram-negative bacteremia. These results have important implications for infection control professionals and infectious disease physicians.

Future research in enhanced cleaning should incorporate clinical outcomes as well as identifying risk factors for gown and glove contamination. These studies should include multicenter trials of enhanced cleaning adequately powered to detect clinical outcomes and retrospective studies to identify patient characteristics that predict high frequencies of gown and glove contamination. Future research in antibiotic-resistant Gram-negative bacteremia among cancer patients should include molecular studies to verify the match between colonizing and infecting isolates as well as prospective trials to develop and validate prediction rules for antibiotic-resistant Gram-negative bloodstream infection.

## VII. APPENDIX 1 – RESULTS OF INTERVENTION VERIFICATION

In control rooms, target surfaces were marked during the sham cleaning and removal was verified 24 hours later in order to allow the hospital housekeeping staff sufficient time to clean the room. Results of target surface screening are presented in Table 9.

**Table 9. Number of surfaces in control rooms still marked (not clean) or not marked (clean) 24 hours after DAZO placement.**

Surface	Marked (not clean)	Not marked (clean)
	n (%)	n (%)
Bed rail	70 (55)	58 (45)
Bed controls	102 (82)	23 (18)
Over-bed table*	49 (46)	58 (54)
Over-bed table controls*	98 (92)	9 (8)
Desk top*	41 (39)	63 (61)
Desk lip*	78 (74)	27 (26)
IV pole	109 (83)	22 (17)
Infusion pump housing	112 (84)	21 (16)
Nurse call button*	110 (85)	19 (15)
Telephone*	57 (85)	10 (15)
Sink*	105 (78)	30 (22)
Light switches*	107 (78)	30 (22)
Supply cart top*	72 (53)	65 (47)
Supply cart handles*	87 (64)	50 (15)
Ventilator controls	93 (86)	15 (14)
Ventilator desk	88 (84)	17 (16)
Telemetry controls	131 (97)	4 (3)
<b>All</b>	<b>1509 (74)</b>	<b>521 (26)</b>

\*indicates a surface included in routine cleaning by UMMC housekeeping staff

## VIII. APPENDIX 2 – RESULTS OF AIM 1 SENSITIVITY ANALYSES

Two logistic Generalized Estimating Equations (GEE) models were fit to assess whether the use of the unmatched Wilcoxon-Mann-Whitney test was conservative or liberal. One model was fit for the results of MDRAB rooms, and the other was fit for the results of MRSA rooms. For both organisms, the probability that an individual swab was contaminated was modeled as a sole function of the assigned intervention, accounting for repeated measures from the same room. An exchangeable working correlation was assumed for all observations from the same room.

In the GEE model of MDRAB rooms, enhanced cleaning group was not significantly associated with MDRAB contamination (odds ratio 0.67, 95% confidence interval 0.43 – 1.04,  $p = 0.074$ ). The exchangeable working correlation for the model was 0.066.

1. In the GEE model of MRSA rooms, enhanced cleaning group was not significantly associated with MDRAB contamination (odds ratio 0.82, 95% confidence interval 0.42 – 1.60,  $p = 0.560$ ). The exchangeable working correlation for the model was 0.082.

## **IX. APPENDIX 3 – RESULTS OF AIM 2.1 SENSIVITY ANALYSES**

The results of the sensitivity analyses for Aim 2.1 are presented in Table 10.

In the first sensitivity analysis, four cases missing prior surveillance or non-sterile-site clinical cultures were declared “not colonized” and 89 controls missing prior surveillance or non-sterile-site clinical cultures were declared “colonized”. Thirty-one out of 84 cases (37%) and 111 out of 306 controls (36%) were colonized with antibiotic-resistant Gram-negative bacteria. In a bivariate conditional logistic regression model, prior colonization with antibiotic-resistant Gram-negative bacteria was not significantly associated with subsequent risk of antibiotic-resistant Gram-negative bacteremia.

In the second sensitivity analysis, four cases missing prior surveillance or non-sterile-site clinical cultures were declared “colonized” and 89 controls missing prior surveillance or non-sterile-site clinical cultures were declared “not colonized”. Thirty-five out of 84 cases (42%) and 22 out of 306 controls (7%) were colonized with antibiotic-resistant Gram-negative bacteria. In a bivariate conditional logistic regression model, prior colonization with antibiotic-resistant Gram-negative bacteria was significantly associated with subsequent risk of antibiotic-resistant Gram-negative bacteremia.

**Table 10.** Sensitivity analyses for Aim 2.1

<b>Study</b>	<b>OR</b>	<b>(95% CI)</b>	<b><i>p</i></b>
First sensitivity analysis <i>cases without prior cultures = “not colonized”</i> <i>controls without prior cultures = “colonized”</i>	1.0	(0.6 – 1.7)	0.922
Second sensitivity analysis <i>cases without prior cultures = “colonized”</i> <i>controls without prior cultures = “not colonized”</i>	11.3	(5.2 – 24.9)	< 0.001

Cases were matched to controls on age  $\pm 10$  years, date of admission  $\pm 180$  days, malignancy type, and hospital days from admission to blood culture.

## **X. APPENDIX 4 – AIM 1 TRIAL PROCEDURES**

### **Screening and Eligibility Protocol**

1. Log on to the study screening web portal at:  
[http://otisweb.umm.edu:7777/pls/Central Data  
Repositorydoc/aaron\\_adm\\_pkg.web\\_login](http://otisweb.umm.edu:7777/pls/Central%20Data%20Repositorydoc/aaron_adm_pkg.web_login)
2. Download the text file (“aaron\_adm.txt”) containing the daily screen of all study unit rooms
3. Import the text file into the study Access database using the saved import script (“Import Raw Data” under the “Saved Imports” button in the “External Data” tab of the Cleaning Trial Study Database at I:\epid\ALL\infection control\Enhanced Cleaning Trial\)
4. Confirm that the daily list of screened rooms has been appended to the “Screened” table. Close the table.
5. Make sure that the table “Swabs” is up to date, i.e. that it contains a records of all swabs collected to date, including the previous study day’s collection. Complete microbiology data is not necessary, only a record of each swab’s collection with the Accession #, Room ID, Date (of collection) and Time (of collection). If these data are not present in the “Swabs” table, the eligibility algorithm may produce incorrect results.
6. Run the “Apply Eligibility Criteria” macro. Access will prompt the user to allow data to be appended to tables. Click “OK” to confirm all prompts. When the macro is complete, confirm that the day’s eligible rooms have been appended to the “Eligibles” table.
7. The “Today’s Eligibles” form will be automatically generated by the macro. Print a copy of the form for use during sample collection in the study units. When sample collection is complete for the day, this form should be filed with the study records in the designated Enhanced Cleaning Trial filing cabinet.

## Enhanced Cleaning Protocol

NOTE: Rooms are selected for enrollment in the order they appear on the “Today’s Eligibles” sheet. If for any reason a room cannot be enrolled, note the reason in the “Comments” section for that room and proceed to the next room on the list.

Materials:

1. DAZO gel applicator (required for control rooms)
2. Virex WetTask bucket with Virex and wipes (required for all rooms)
3. “Enhanced Cleaning Trial Intervention” logbook

Procedure:

1. Locate the room nurse. Inform the nurse that the room is eligible for the study. Verify that the patient will be in the room during the day, and that the patient is on contact precautions.
2. Wash hands
3. Put on appropriate disposable protective clothing (gloves, gown, and any additional protection required such as a face mask)
4. Collect all necessary materials and clean them with Virex WetTask wipes
5. Bring all study items in to the room

IF ROOM IS IN THE EXPERIMENTAL GROUP:

1. Thoroughly wipe the following surfaces with Virex WetTask wipes. The surfaces should be moistened but not running or dripping with the Virex solution. The order does not matter. Use one wipe for every three or four surfaces. If the wipe is visibly soiled after cleaning a surface, dispose and use a new wipe.
  - bed rail top bar
  - bed electronic control surfaces
  - moveable tray table top
  - moveable tray table adjustment levers
  - desk top
  - desk front lip
  - IV pole
  - IV infusion pump control surfaces
  - nurse call button/remote control

- patient telephone
  - sink console top
  - light switch and plates
  - supply cart top
  - supply cart drawer handles
  - ventilator control surfaces
  - ventilator desk
  - vital signs monitor control surfaces
2. Note which surfaces were cleaned in the “Enhanced Cleaning Trial Intervention” logbook. All surfaces should be cleaned, but some (e.g. the movable tray table, the ventilator) may not be present in the room and should be noted as “not cleaned”.
  3. Clean all study items that were brought into the room with Virex wipes (if patient is not on Enhanced Contact Precautions) or bleach/peroxide wipes (if patient is on Enhanced Contact Precautions).
  4. Exit the room, removing any disposable protective clothing and performing appropriate hand hygiene.
  5. Wait 10 minutes before beginning sample collection.

**ROOM IS IN THE CONTROL GROUP:**

1. Using a Virex WetTask wipe, mime wiping the following surfaces. Do not touch the surfaces with the wipe or allow Virex to drip on to them.
  - bed rail top bar
  - bed electronic control surfaces
  - moveable tray table top
  - moveable tray table adjustment levers
  - desk top
  - desk front lip
  - IV pole
  - IV infusion pump control surfaces
  - nurse call button/remote control
  - patient telephone
  - sink console top
  - light switch and plates
  - supply cart top
  - supply cart drawer handles
  - ventilator control surfaces
  - ventilator desk
  - vital signs monitor control surfaces

6. During the mimed cleaning, mark each surface with a spot of DAZO. Note which surfaces were marked in the “Enhanced Cleaning Trial Intervention” logbook. All surfaces should be marked, but some (e.g. the movable tray table, the ventilator) may not be present in the room and should be noted as “not marked”.
2. Dispose of the DAZO marker in non-biohazard trash.
3. Clean all study items that were brought into the room with Virex wipes (if patient is not on Enhanced Contact Precautions) or bleach/peroxide wipes (if patient is on Enhanced Contact Precautions).
4. Exit the room, removing any disposable protective clothing and performing appropriate hand hygiene.
5. Wait 10 minutes before beginning sample collection.

## Swab Collection Protocol

### Materials

1. 15 Remel Bacti-Swab double-headed applicators with transport tubes.
2. Biohazard sample collection bag
3. “Today’s Eligibles” sheet.

### Procedure

1. Note the time that sampling began on the “Today’s Eligibles” sheet.
2. For each swab:
  - a. Open the top of the swab packet part-way and remove the clear cap on the transport tube
  - b. Remove the applicator from the package without touching the swab tip to any surface outside the packaging and insert the applicator into the transport tube. Press closed.
  - c. Discard the clear cap and packaging.
  - d. Label the transport tube with a pre-printed label marked with the accession number, room ID, and target organism. If the room is occupied by a patient colonized with both MRSA and MDRAB, use two labels with different accession numbers for each organism.
3. Prior to any healthcare worker (HCW) entering the room for routine, non-emergent, approach the HCW and obtain verbal assent for sample collection. Do not approach a HCW during an emergency or any kind of urgent care.
4. As the HCW is preparing to exit the room but before they remove their gloves and gown, sample the gloves and gown with one double-headed swab as follows:
  - a. Run the swab tip once between all the fingers of each hand, the palms of each hand, the dorsum of each hand, and a single sweep across the pads of all the fingers
  - b. Run the swab tip twice along each forearm, and once in a large “W” pattern across the beltline on the front of the gown.
  - c. Place the swab tip back in the transport tube and press closed
  - d. Place the transport tube in a biohazard-labeled sample collection bag
5. When all 15 swabs have been collected, note down the accession numbers on the “Today’s Eligibles” sheet. Make sure that the labels on all swabs have the accession number, room ID number, and the target organism correctly noted

6. After returning from the hospital floor log all collected swabs in the “Cleaning Trial Study Database” using the “Swabs” form. For each swab collected, enter the accession number, the room ID number, the date of collection, and the time of collection. When entry is complete, click the “Update Database” button, and confirm that the swabs were all entered correctly by checking the “Swabs” and “Swab Results” tables.

## Cleaning Trial Microbiology Protocol

### DAY 1

*Samples are collected from the hospital. All sample tubes are labeled with the accession number of the swab, the Room ID (a three-digit code<sup>4</sup>), and the target organism. Swabs from co-colonized rooms count as two separate swabs, and there will be two labels so each organism gets its own accession number.*

#### Materials:

1. 1 “MRSA Reporting Sheet” for every MRSA room swabs and 3 “MDRAB” reporting sheet for every MDRAB room. Rooms where the patient is colonized with both MRSA and MDRAB will need two complete sets of sheets.
2. Three labels for each swab, with the accession number, room ID, and target organism. Swabs from rooms where the patient is colonized with both MRSA and MDRAB will need two complete sets of labels, each with their own accession number and organism.
3. For each MRSA swab, 1 labeled, pre-filled tube with 5mL of tryptic soy broth (TSB). Swabs from rooms where the patient is colonized with both MRSA and MDRAB will need two complete sets of tubes, each with their own accession number and organism.
4. For each MDRAB swab, 1 labeled, pre-filled tube with 5mL of brain-heart infusion (BHI) broth with imipenem at 6µg/mL. Swabs from rooms where the patient is colonized with both MRSA and MDRAB will need two complete sets of tubes, each with their own accession number and organism.
5. For each swab, 1 labeled freezer tube with glycerol. Swabs from rooms where the patient is colonized with both MRSA and MDRAB will need two complete sets of tubes, each with their own accession number and organism.

#### Procedure:

1. Prepare a new spread in the lab book containing:
  - i. (on the left page) summary of the day’s samples, one line per organism per room, noting the number of swabs, the room ID, the range of accession numbers, and the organism
  - ii. (on the right page) all the log sheets – these should be taped together to form a booklet of results

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<sup>4</sup> 3xx = MICU, 4xx = Trauma 4 South/North, 5xx = Trauma 5 South, and 6xx = Trauma 6 South. The last two digits are the actual room number, e.g., 415 = Trauma 4 South, room 15.

2. Remove one swab from the stem and place it in the appropriate broth tube. Vortex for 10 seconds.
3. Break off the swab tip in a pre-labeled freezer tube and cap. Label the cap with the accession number of the swab. Place swab in the corresponding box labeled “Enhanced Cleaning Trial Swabs”. Store freezer tube at -80°C.
4. Incubate loose-capped broth tubes for 18-24 hours at  $35 \pm 2^\circ\text{C}$  in ambient air.  
IF THE SWAB CAME FROM A ROOM WHERE THE PATIENT WAS COLONIZED WITH ONLY ONE ORGANISM:
5. Dispose of the remaining swab tip

IF THE SWAB CAME FROM A ROOM WHERE THE PATIENT WAS COLONIZED WITH BOTH MRSA AND MDRAB:

5. Perform steps 1-4 above for the other swab tip, using the other, appropriate kind of broth and freezing the swab tip in a separate tube.

## DAY 2

### Materials

1. One Remel SpectraMRSA plate for each MRSA broth tube
2. One CHROMAgar Acinetobacter plate for each MDRAB broth tube

### Procedure

#### MRSA

1. For each TSB w/ 6.5% NaCl broth/MRSA tube, label a Remel SpectraMRSA with a pre-printed label.
2. Plate 50 $\mu\text{L}$  of the broth onto its corresponding labeled plate and streak.
3. Incubate broth for 24 hours at  $35 \pm 2^\circ\text{C}$  in ambient air.

#### MDRAB

1. For each BHI w/ 6 $\mu\text{g/ml}$  imipenem/Acinetobacter tube, label a CHROMAgar *Acinetobacter* plate with a pre-printed label..
2. Plate 50 $\mu\text{L}$  of the broth onto its corresponding labeled plate and streak.
3. Incubate plates for 24 hours at  $35 \pm 2^\circ\text{C}$  in ambient air.

## DAY 3

### Materials

1. One TSA agar plate with 5% sheep's blood for every positive chromogenic agar plate.

### Procedure

#### MRSA

1. Read all Remel Spectra MRSA plates 23-25 hours after plating. Denim-blue colonies represent potential MRSA.
2. Document bacterial growth on data sheet: Denim blue, white, no growth (NG) or others.
3. For each plate with a denim blue colony, label a blood agar plate with the accession number of the swab and the date.
4. Using a loop, streak a potential MRSA colony to the blood agar plate for isolation.
4. Incubate plates for 24 hours at  $35 \pm 2^\circ\text{C}$  in ambient air.

#### MDRAB

1. Read all *Acinetobacter* ChromAgar plates. Plump, red colonies represent potential *Acinetobacter* (*Pseudomonas* and *Stenotrophomonas* will appear as red pinpoint).
2. Document bacterial growth on the data sheet: Red colonies, pinpoint red colonies, or no growth (NG)
3. For each plate with potential *A. baumannii*, label a blood agar plate with the accession number.
4. Using a loop, streak a potential *A. baumannii* colony to the blood agar plate for isolation.
5. Incubate plates aerobically for 24 hours at  $35 \pm 2^\circ\text{C}$  in ambient air.
6. If there is no growth, plates must be incubated aerobically for another 24 hours at  $35 \pm 2^\circ\text{C}$  in ambient air. Follow steps 1-4 if plump, red colonies grow.

## DAY 4

### Materials

1. StaphAurex latex agglutination test
2. Oxidase test
3. One freezer tube with glycerol for each confirmed MRSA isolate
4. One Mueller-Hinton plate for each *Acinetobacter* isolate, along with the following antibiotic disks:
  - a.  $\beta$ -lactams:
    - i. Ampicillin-sulbactam\*
    - ii. Piperacillin-tazobactam\*
  - b. Aminoglycosides:
    - i. Amikacin\*
    - ii. Gentamicin\*
  - c. Sulfonamides:
    - i. Sulfamethoxazole-trimethoprim (TMP-SMX)\*
  - d. Fluoroquinolones:
    - i. Ciprofloxacin\*
  - e. Cephalosporins:
    - i. Ceftazidime\*
    - ii. Cefepime\*
  - f. Carbapenems:
    - i. Imipenem\*
    - ii. Meropenem\*
    - iii. Doripenem
  - g. Glycylcyclines:
    - i. Tigecycline
  - h. Polymixins:
    - i. Polymixin B

\*indicates antimicrobials that are considered when judging multidrug-resistance

### MRSA

1. Review the morphology of the colonies on each plate. *Staphylococcus aureus* colonies was small, round, white or gold in color, and will show  $\beta$ -hemolysis (clearing around the colonies).

2. Perform a latex agglutination test using StaphAurex test on denim blue colonies to confirm isolate as *S. aureus*. Record the agglutination results on data sheet.
3. For each plate that has colonies with the correct morphology and positive latex agglutination results, prepare a freeze tube with 1 mL TSB plus glycerol. Label the tube with the organism, assigned freezer number, accession number, and date frozen. Label the cap with the freezer number.
4. Collect multiple colonies using a cotton-tipped applicator. Immerse the tip in the TSB/glycerol and mix vigorously. Remove the applicator and cap the tube.
5. Place all tubes in the corresponding box labeled “Enhanced Cleaning Trial Organisms”. Record the freezer number on the data sheet.

*When all the MRSA samples from a study day are completely assayed, make sure that the data entry in the notebook is complete. Enter the data into the “Cleaning Trial Lab Database” using the “Swab Result Entry Form”. When entry is complete, verify that the data are correctly entered in the “Swab Results” form. NOTE: swab results can only be entered in to the lab database if the swabs have already been logged using the “Swab” form in the “Cleaning Trial Study Database”.*

### MDRAB

1. Review the morphology of the colonies on each plate. *Acinetobacter baumannii* colonies was small, round, white or grey in color, and will have a distinct, sharp smell similar to cat urine.
2. An oxidase test must be performed for each colony. Drop Oxidase on a piece of filter paper.
3. Touch a potential *A. baumannii* colony from the blood plate with a wooden stick. An oxidase-positive isolate will turn purple. *A. baumannii* should be negative (colorless). Record the test results on the data sheet
4. Label a large Mueller-Hinton plate.
5. Suspend a colony or two from the blood plate in 5 ml of normal saline and adjust to a turbidity of 0.06-0.10 on the Microscan turbidity meter. This equates to a 0.5 MacFarland.
6. Dip a sterile cotton swab in the saline and streak a lawn on to the Mueller-Hinton plate.
7. Stamp the Mueller-Hinton plate with the 13 antimicrobials listed above.

8. Incubate plates aerobically for 24 hours at  $35 \pm 2^\circ\text{C}$  in ambient air.

## DAY 5

### Materials

1. Metric ruler
2. One freezer tube with glycerol for each confirmed MDRAB isolate

### Procedure

#### MDRAB

1. Measure clearance zones on each plate in millimeters and record on the data sheet. Assign Susceptible, Intermediate, and Resistant designations. *A. baumannii* isolates are considered antibiotic-resistant if they are susceptible to two or fewer ( $\leq 2$ ) classes of antimicrobials (see list above). Record the test results on the data sheet.
2. Freeze isolate in a tube labeled with organism, assigned freeze number, accession number, and date frozen. Place organism in the corresponding box labeled “Enhanced Cleaning Trial Organisms”. MRSA and MDRAB isolates have separate freezer boxes. Record the freezer number on the data sheet.

*When all the MDRAB samples from a study day are completely assayed, make sure that the data entry in the notebook is complete. Enter the data into the “Cleaning Trial Lab Database” using the “Swab Result Entry Form”. When entry is complete, verify that the data are correctly entered in the “Swab Results” form. NOTE: swab results can only be entered in to the lab database if the swabs have already been logged using the “Swab” form in the “Cleaning Trial Study Database”.*

## Cleaning Trial Experimental Room DAZO Monitoring Protocol

NOTE: DAZO monitoring in experimental rooms should occur at least once per ten experimental rooms enrolled.

### Materials:

1. DAZO gel applicator
2. DAZO or other UV penlight
3. “Enhanced Cleaning Trial Intervention” logbook

### Procedure:

NOTE: DAZO monitoring in experimental rooms requires two study researchers

1. First Researcher: Wash hands, enter the room with the DAZO gel applicator.
2. First Researcher: All study surfaces should be marked once with the DAZO gel applicator. The marks should be placed in an easy-to-view position on the surface. Do not move objects in order to place the gel unless it is necessary to access the surface.
3. First Researcher: Note down which surfaces were marked in the Enhanced Cleaning Trial Quality Control Log.
4. Second Researcher: follow the “Enhanced Cleaning Intervention Protocol (Room is in the experimental group)”.
5. First Researcher: After completion of cleaning (and prior to collection of swabs) re-enter the room and examine the study surfaces. Any surfaces that are not clean should be noted in the log.
6. Second Researcher: If necessary, re-enter the room and re-clean any surfaces that were not clean following the first inspection.
7. First Researcher: Re-inspect any surfaces that did not pass inspection previously.
8. Repeat steps 6 and 7 as necessary until all surfaces are clean.
9. Second Researcher: Continue with sample collection as per protocol.

## **Cleaning Trial Control Room DAZO Monitoring Protocol**

Note: DAZO monitoring is to be done in all enrolled control rooms.

### DAY 1

1. DAZO is applied to the study surfaces during the control intervention process. DAZO application is recorded in the Enhanced Cleaning Intervention Log binder under the room number and the date of enrollment.

### DAY 2

1. 24 hours after application of the DAZO, return to the control room with the Enhanced Cleaning Intervention Log binder and an ECOLAB UV pen light.
2. Scan each of the study the study surfaces with the UV light. Any surface from which DAZO has not been completely removed should be marked under the “24 HR Fail” column in the record for that room. Only complete removal of the DAZO spot should be marked as a successful cleaning: in

## **XI. APPENDIX 5 – DATA REQUEST CODES FOR AIM 2**

Date range for all requests is 1/1/2000-12/31/2010.

### Admitting Services:

1. Cancer Center (CCO)
2. Bone Marrow Transplant unit (CBM)

### Prior MICU Admission Codes:

1. W7A
2. W7B
3. S3U

### Malignancy ICD-9 Codes

1. ALL (Yes/No – any code of 204.0x)
2. CLL (Yes/No – any code of 204.1x)
3. AML (Yes/No – any code of 205.0x)
4. CML (Yes/No – any code of 205.1x)
5. Other Leukemia (Yes/No – any code of 206.xx, 207.xx or 208.xx)
6. HL (Yes/No – any code of 201.xx)
7. NHL (Yes/No – any code of 200.xx or 202.xx)
8. Myeloma (Yes/No – any code of 203.xx)
9. Solid Tumor/Other Malignancy (Yes/No – any patient without at least one of the above codes but with a code of 140.xx-199.xx or 209.xx-239.xx)

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