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Development of Cost Effective Surveillance Program for Carbapenem-Resistant Enterobacteriaceae in a Facility with Low CRE Infection Endemicity

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Development of Cost Effective Surveillance Program for Carbapenem-Resistant *Enterobacteriaceae* in a Facility with Low CRE Infection Endemicity

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ABSTRACT

Background

Carbapenem-resistant *Enterobacteriaceae* (CRE) have emerged as a significant cause of healthcare associated infections (HAI) resulting in significant morbidity and mortality. Identification of CRE colonized and/or infected patients early during care enables implementation of comprehensive infection control measures that limit spread and likely reduces the risk of CRE mediated HAI. Hospitals in areas where CRE is endemic have instituted universal surveillance programs to limit risk, typically employing perianal/perirectal swabs or stool. In our facility, CRE isolation from clinical specimens is so infrequent that a universal surveillance program would not be cost effective. Consequently, in facilities with low CRE prevalence, a targeted approach may be more effective at developing an understanding of the CRE colonization pressure and thus spread represents to hospitalized patients. Prior antibiotic receipt is among the most prevalent predictors of CRE carriage and/or infection. Thus, the use of remnant *Clostridium difficile* diarrhea specimens to assess CRE carriage may be more effective than a universal surveillance program in assessing the risk of CRE to hospitalized patients.

Methods

Remnant diarrhea specimens submitted for *C difficile* toxin PCR (n=600) were compared with remnant perianal swabs collected for vancomycin-resistant *Enterococcus* (VRE) surveillance (n=600) to determine the superiority of one specimen type over the other for CRE carriage. Specimens were analyzed using the two laboratory protocols

recommended by CDC: the direct ertapenem disk method and selective enrichment broth method. Carbapenem resistance was confirmed by disk diffusion testing. Confirmed carbapenem resistant isolates were identified by Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI- TOF).

Results

Using samples collected from patients under care in VRE high-risk units, the CRE colonization rate was 1.8 % (11/600). Samples collected from patients presenting with symptoms associated with antibiotic associated diarrhea, the targeted surveillance arm, were found to have a CRE colonization rate of 6.2% (37/600). The colonization rate difference observed between the two arms was significant (p value of <0.0002, Fisher Exact Test).

Conclusions: The use of targeted specimens was superior in the ability to identify CRE colonized individuals from facilities with low a clinical incidence of these microbes.

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Abbreviations	Meaning	Page
AAD	Antibiotic Associated Diarrhea	7
C. difficile	*Clostridium difficile*	11
CMS	Center for Medicare Services	6
CRE	Carbapenem Resistant *Enterobacteriaceae*	1
DOB	Date of Birth	11
EDTA	Ethylene-diamine-tetraacetic acid	2
ESBL	Extended spectrum beta-lactamase-producing	4
HAC	Hospital Acquired Condition	6
HAI	Healthcare associated infections	1
IMP	metallo-β-lactamases IMP-type carbapenemases	2
KPC	*Klebsiella pneumoniae* carbapenemase	2
LOS	Length of stay	8
MALDI-TOF	Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry	12
MRN	Medical Record Number	11
MRSA	Methicillin-resistant *Staphylococcus aureus*	4
MUH	Medical University Hospital at the Medical University of South Carolina	7
NDM	New Delhi metallo-β-lactamase	2
NPV	Negative Predictive Value	35
OXA	Oxacillinase group of β-lactamases	2
Spp.	Species	1
PPV	Positive Predictive Value	35
TSB	Tryptic Soy Broth	12
UCLA	University of California Los Angeles	5
VIM	Verona integron-encoded metallo-β-lactamase	2
VRE	Vancomycin Resistant *Enterococcus*	4

CHAPTER 1

INTRODUCTION

Carbapenem-Resistant *Enterobacteriaceae* (CRE) have emerged as a significant cause of healthcare associated infections (HAI)[1]. Microbes from this family of facultative anaerobic Gram-negative rods, such as *Klebsiella* species (spp.), *Enterobacter* spp., *Escherichia coli* and others, have acquired resistance to almost all antibiotics thereby limiting our ability to effectively treat patients infected by these microbes. CRE infections are associated with higher morbidity and mortality rates, with an average mortality of 46%, and are one of three of the most life-threatening microbes presently perpetrating HAIs in United States [1-3]. Since its first emergence and recognition in North Carolina in 1996 in *Klebsiella pneumoniae,* the determinants conferring resistance to the carbapenem class of antibiotics have spread rapidly within the *Enterobacteriaceae* as well as across the globe [4]. In order to preserve the clinical utility of this class of antibiotics, it is imperative that we institute measures to curtail the spread of resistant organisms among our patients and within the built environment of our hospitals.

Resistance to the carbapenem class of antibiotics occurs either through the structural modification of the microbe or through an acquisition of enzymes capable of inactivating the drug. Structural modifications to the host conferring resistance often require modifications of a number of genes located within the chromosome. This process occurs principally as a consequence of selective pressure due to exposure of the microbe to the antibiotic resulting in the modification of the host genome through the selection of resistant progeny. This is a slow process requiring an accumulation of mutations and

generally occurs in patients upon exposure to a course of antibiotics. In contrast, enzymes that are capable of inactivating antibiotics are often acquired quickly from other microbes through the acquisition of plasmids or other forms of genetic exchange that readily occur between microbes within the built clinical environment and among the microbes of patients and healthcare workers (reviewed extensively by [5]).

Enterobacteriaceae strains that inactivate carbapenems are classified based on the types of the enzymes they produce. Each enzyme class targets a different structural component of the antibiotic. There are three clinically significant classes of these enzymes, A, B and D, within the *Enterobacteriaceae* [6]. The Class A carbapenemases encompass many different enzymes encoded by genes either on the chromosome or on plasmids, depending on the enzyme. The best known of these in the United States is the plasmid-mediated *Klebsiella pneumoniae* carbapenemase (KPC). KPCs are thought to act by positioning the catalytic residue of the enzyme in order to accommodate the bulky alpha substituents of the carbapenems [7]. The Class B carbapenemases encompass many different enzymes targeting the same structure. They share three distinct functional properties: capability of hydrolyzing the beta-lactam ring, resistance to mechanism-based inhibitors, and susceptibility to chelating agents such as ethylene-diamine-tetra-acetic acid (EDTA) [8]. The clinically relevant enzymes are the Verona integron-encoded metallo-β-lactamase (VIM), the metallo-β-lactamases IMP-type carbapenemases (IMP), and the New Delhi metallo-β-lactamase (NDM) [6]. Class B enzymes are encoded by genes on plasmids in *Enterobacteriaceae* or on the chromosome in non-Enteric organisms such as *Stenotrophomonas maltophilia*. The class D type of

carbapenemases is an oxacillinase group of β-lactamases that have carbapenemase activity (OXA) [6] and were first described in Turkey in 2001[9]. Mobile genetic elements for each carbapenemase class have quickly circled our globe [6, 10]. The rate of carbapenem resistance in *Enterobacteriaceae* species reported is reflected in Figure 1.1 which is an adaptation illustrating the global expansion of carbapenem resistance[11].

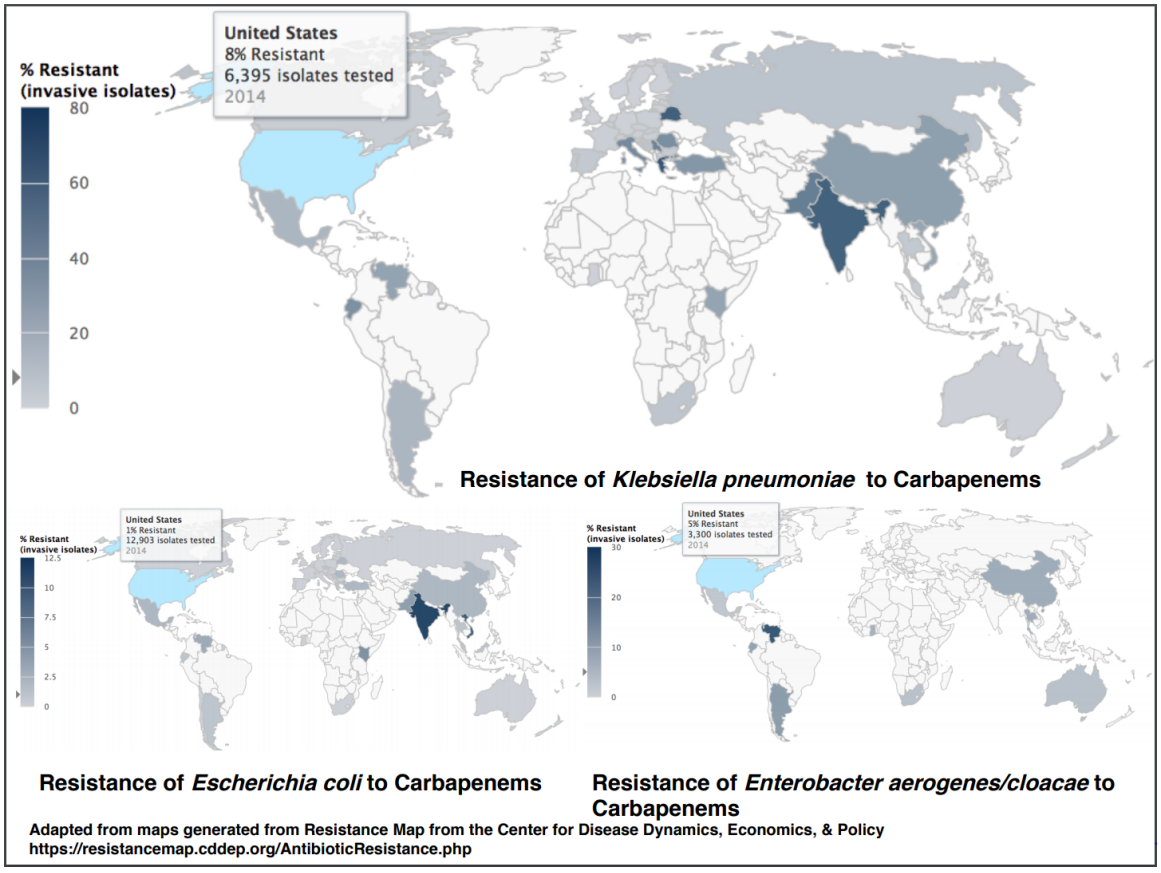


FIGURE 1.1 CARBAPENEM RESISTANCE RATES FOR ISOLATES (*K. PNEUMONIAE*, *E. COLI*, *E. AEROGENES/CLOACAE*) IN UNITED STATES. Adapted from The Center for Disease Dynamics, Economic & Policy. (https://resistancemap.cddep.org/AntibioticResistance.php)

In response to increasing concerns about the presence of antibiotic resistant pathogens within patient care settings, healthcare facilities have implemented

interventions to limit the spread of antibiotic resistant microbes, such as methicillin-resistant *Staphylococcus aureus* (MRSA), vancomycin-resistant enterococci (VRE), extended spectrum beta-lactamase-producing *Enterobacteriaceae* (ESBL), and now CRE among their patients. Such interventions have followed three approaches/strategies. The first intervention often involves a program in antibiotic stewardship. Here the hospital pharmacy in concert with the clinical microbiology laboratory restricts the use of certain antibiotics unless there is a medically demonstrated need for use of the medication. The antibiotic is restricted within the hospital formulary and requires authorization for its use. Antibiotic stewardship programs have had great success in lowering the antibiotic selective pressure within units in hospitals in order to limit the 'spread' of selection pressure and thus the development of resistance to antibiotics through genetic selection as a consequence of avoiding inappropriate use [12, 13]. However, carbapenems are often the last antibiotic available to treat infections caused by multi-drug resistant *Enterobacteriaceae*. Collectively this limits the ability of antimicrobial stewardship programs to control use of this class of drugs thereby limiting the opportunity for this strategy to control the horizontal spread of resistance among the *Enterobacteriaceae*.

The second strategy results from the strict use of advanced infection control measures. Here the intervention limits the transmission of drug resistant pathogens by controlling access to colonized and/or infected patients by requiring the use of protocols prescribing the behavior of clinical staff and visitors entering the colonized/infected patient's room through gowning, gloving, and enhanced disinfection of the care environment subsequent to discharge of the colonized/infected patient. The third

measure requires that hospitals control the horizontal spread of resistance traits through strict adherence to hand hygiene protocols and the routine disinfection of the healthcare environment. Collectively each effort synergistically cooperates and serves to limit both the spread of these microbes and the opportunity to facilitate the horizontal gene transfer among the bacteria resident in the hospital. The overall net effect is to lower the risk of CRE spreading within the facility.

Universal surveillance programs conducted to detect colonization by pathogens such as MRSA, VRE, ESBL, and now CRE at the time of admission, in concert with the routine detection of these pathogens among patients subsequent to admission, have each been shown to limit the spread and thus incidence of drug resistant HAIs within hospitals. Universal surveillance programs are an inherent and unreimbursed cost to the hospital. Recent events argue that in spite of the modest unreimbursed costs associated with surveillance programs the benefits to healthcare well justify the cost. In October of 2014 a women being evaluated for a liver transplant underwent two routine procedures at the University of California Los Angeles (UCLA) hospital employing two separate duodenoscopes [14]. At the time of the procedures she was already infected with CRE and as a result of failure to properly disinfect the two duodenoscopes subsequent to their use, CRE entered the UCLA hospital system exposing others to this highly drug resistant pathogen [14]. The first patient infected with CRE from one of the contaminated duodenoscopes at UCLA required 83 days of hospitalization with subsequent re-hospitalization within 22 days of his first discharge [14].

Lucado and colleagues reported in 2010 that the cost to treat HAIs results in an additional 19.2 days of care and nearly $43,000 in additional charges than stays without HAIs [15]. Further, among patients acquiring an infection during their hospital stay, 29.8% are readmitted within 30 days for an infection or complication, compared to a readmission rate of 6.2% for patients without prior HAI [16]. The Patient Protection and Affordable Care Act of the United States has generated both enhanced scrutiny and added consequence to this alarming rate. For the more than 3,300 U.S. hospitals evaluated by the Center for Medicare Services (CMS), approximately 23 percent of them will lose some funding from Medicare funding as a consequence of the Hospital Acquired Condition (HAC) Reduction Program or 'quality of care' penalty being mandated by section 3008 of the law [17]. While the law can financially incentivize healthcare to do better, the human consequences might have been averted at UCLA if the index patient had only been screened prior to her procedure.

The cost for universal surveillance is not a reimbursable expense to the hospital, thus any effort to limit this cost will increase the likelihood of its adoption. The intent of the study here was to investigate the utility of the secondary screening of specimens for the presence of CRE from two risk populations. Studies evaluating predictors of CRE carriage/infection have found that prior exposure to antibiotics is a significant risk factor for subsequent colonization and/or infection by CRE [2, 18]. One study showed that the risk of acquiring CRE increased 4 % per day while on antibiotic therapy [19]. Consequently, we hypothesize that a CRE surveillance program for patients with a history of antibiotic administration will be a medically effective, more economically

favorable approach to limit the presence and spread of CRE in healthcare settings than from samples collected from every patient entering care at the Medical University Hospital (MUH) at the Medical University of South Carolina.

Three specific aims were pursued in the course of this study. First, an assessment of the CRE colonization rate for patients entering care at MUH was determined using remnant swabs collected from patients admitted to care in high-risk VRE units as part of a modified VRE universal surveillance approach. The results from this aim enabled us to establish the baseline risk of CRE currently impacting care at MUH employing a universal surveillance strategy. The cost of conducting this analysis and common risk factors were also collected and analyzed for significance.

The second aim assessed whether or not the secondary analysis of specimens collected from patients exposed to antibiotics might serve as a superior specimen from which to assess the rate of CRE colonization associated with patients entering care. In contrast to the remnant specimens from patients in a high-risk VRE unit, remnant specimens for this aim were evaluated from patients with an indication of antibiotic associated diarrhea (AAD). The rationale was that exposure to antibiotics that resulted in diarrhea might increase the likelihood of CRE acquisition, as a consequence of antibiotic selection/enrichment, and the spread of microbes bearing this trait within the healthcare setting through the process of 'fugitive' emissions. The primary outcome for this aim was the rate of CRE colonization associated with patients satisfying the criteria of inclusion (AAD). The cost of conducting this analysis and common risk factors were also collected and analyzed for significance.

The third aim conducted a sensitivity analysis and economic evaluation of the methods required to arrive at the primary outcomes for the first and second aims of this study with the intent of determining the cost of surveillance in a circumstance of low CRE infection endemicity. Collectively the data from the three aims of this study should help inform members of the infection control community and hospital leadership whether or not the costs associated with a comprehensive surveillance program for CRE are justified from the perspective of risk resulting in improved outcomes in an era of ever shrinking discretionary budgets.

CHAPTER 2

ASSESSMENT OF THE PREVALENCE COLONIZATION RATE OF CRE ASSOCIATED WITH ADULTS BEING ADMITTED TO CARE WITHIN MUH

RATIONALE

Colonization surveillance programs for pathogens such MRSA and VRE are components of the standard of care in a significant number of hospitals within the United States to limit the spread of these significant nosocomial infections. At MUH, every adult patient admitted for inpatient care with an anticipated length of stay (LOS) of greater than 24 hours is screened for carriage of MRSA and VRE until discharged or a positive culture. Since the inception of the study, MUH moved to a limited form of universal surveillance for VRE, sampling only those patients housed in units where the risk of contracting VRE is considered significant. Because VRE and CRE share the same niche, the gastrointestinal tract, we assessed the prevalence rate of CRE for adults from a secondary analysis of perianal swabs collected for VRE surveillance testing. Repurposing excess clinical material from existing samples will afford us an opportunity to estimate the prevalence rate for CRE in the population served by MUH. Thus, we will be able to assess the relative risk that CRE represents to MUH. This will allow us to assess the value proposition that a surveillance program might offer to limiting the spread and risk that CRE represents to patient safety while limiting the introduction of CRE to our hospital.

The advantage of repurposing existing clinical specimens to both the patient and hospital are two-fold. First, patients need not be subjected to the minimal risk of acquiring an additional sample. Second, the hospital need not incur the cost of collecting

and transporting an additional sample to the laboratory thereby eliminating the cost of collecting a sample solely for CRE assessment.

Based on preliminary studies conducted here at MUH and from published literature [19, 20], a statistical power calculation was conducted from which an estimate was developed for the number of patients required to assess the prevalence rate of CRE for patients entering care at MUH. To that end, 600 unique and randomly selected specimens were evaluated from all patients entering care in the VRE high-risk units. The sample size was selected to detect a CRE colonization rate as low as 1%. Each of the selected patients was followed until discharge in order to assess the frequency with which this patient cohort acquired CRE colonization and/or infection during his/her hospitalization.

METHODS

10. SETTING AND STUDY DESIGN

The study was conducted at MUH, a 700-bed tertiary hospital located in Charleston, South Carolina. Subjects were adult inpatients, ≥18 years of age, from whom a perianal swab was collected to assess for VRE colonization. The patients were not consented for the collection of this specimen, as it is a component of the standard of care for treatment at MUH. Remnant specimens became available to undergo CRE colonization investigation 48 hours after submission to the diagnostic microbiology laboratory. Because CRE screening testing took about five days, all newly available remnant VRE surveillance samples were obtained once every five days until a total of 600 patients were accrued. In addition, follow up swabs associated with each patient that

are routinely collected every 7th day after admission until discharge, or upon transfer out of high-risk VRE units, or until a positive culture for VRE, were also obtained. Primary samples were collected between April 3, 2017 through June 6, 2017; with the last follow up sample collected on July 12, 2017.

II. DATA COLLECTION

Basic demographic information (Name, Medical Record Number (MRN), Date of Birth (DOB), sex, ethnicity (self-reported)) as well as available clinical information (antibiotic use at the time of collection, date of admission, date of discharge, presence/absence of diarrhea, colonization status for VRE, MRSA, ESBL, *Clostridium difficile* (if available) were collected from the current hospitalization period. Collected data were stored electronically and were encrypted at rest (Figure 2.1). The study was registered, reviewed, and approved by the MUH Institutional Review Board as study Pro00057574 on 3/23/2017.

III. MICROBIOLOGICAL TESTING

Perianal swab samples were processed using two distinct methods currently prescribed by the CDC for the microbiological detection of carbapenem resistant or carbapenemase producing *Klebsiella* spp. and *Escherichia coli* [21]. The first method required inoculating the swab onto a selective and differential medium, MacConkey agar, streaking for isolation, depositing a 10-µg ertapenem disk within the first quadrant of the plate, then incubating the plate aerobically at 35°C ± 2°C for 18-24 hours. A flow diagram of specimen processing using this method is described in panel A, Figure 2.2

The second method enriches the carbapenem resistant population resident in the specimen. The swab was inoculated into 5 ml of Tryptic Soy Broth (TSB), a 10-µg ertapenem disk (final concentration of 2 µg/ml of ertapenem) was added to the tube, and incubated at 35°C± 2°C for 18-24 hours. At that time, 100 µl of the bacterial suspension was subsequently subcultured to MacConkey agar, streaked for isolation, and the plate was incubated aerobically at 35°C ± 2°C for 18-24 hours. A flow diagram of specimen processing using this method is described in panel B, Figure 2.2.

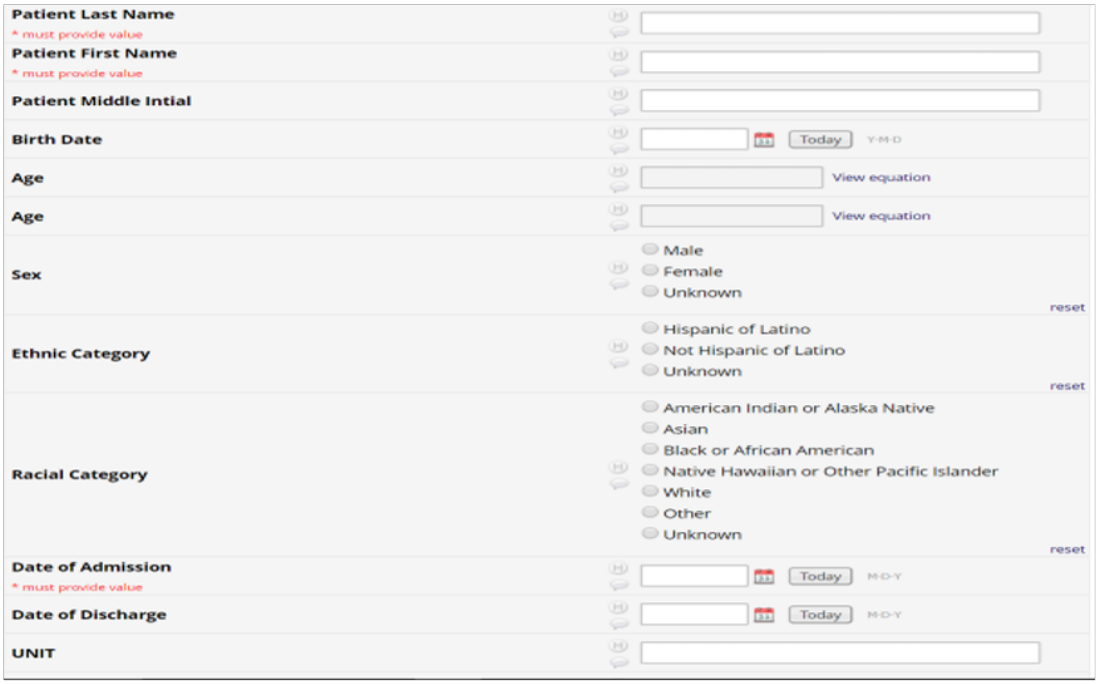
The colonies recovered, from either method were identified by Matrix Assisted Laser Desorption/Ionization-Time of Flight Mass Spectrometry (MALDI-TOF). Resistance to the carbapenem class of antibiotics was confirmed by disk diffusion antibiotic susceptibility testing using 10-µg ertapenem and 10-µg meropenem disks, two carbapenem class antibiotics currently prescribed for patients at MUH. Interpretation of zones of inhibition followed guidelines established by the Clinical and Laboratory Standard Institute (CLSI); zones of inhibition of ≤ 18 mm for ertapenem and ≤19mm for meropenem were qualified as being resistant. Based on the CDC definition, any *Enterobacteriaceae* isolate that is resistant to at least one carbapenem were deemed CRE. The first method, Direct Ertapenem Disk Method, took four days to complete, while the second method, Selective Enrichment Broth Method, required five days to reach a decision.

IV. FINANCIAL ANALYSIS

The cost to perform each culture method was calculated based on the cost of supplies and labor expenses. The cost for each culture method included the price of

media (MacConkey agar, Blood agar plate, Mueller Hinton agar plate, and TSB), and antibiotics (ertapenem disk and meropenem disk) and the cost to identify the confirmed carbapenem resistant isolates by MALDI-TOF. The labor for each procedure included in the analysis and was based on the current market labor rate for a MUH microbiology technologist.

A. Patients' Demographic Data



B. Patients' Risk Factors Data

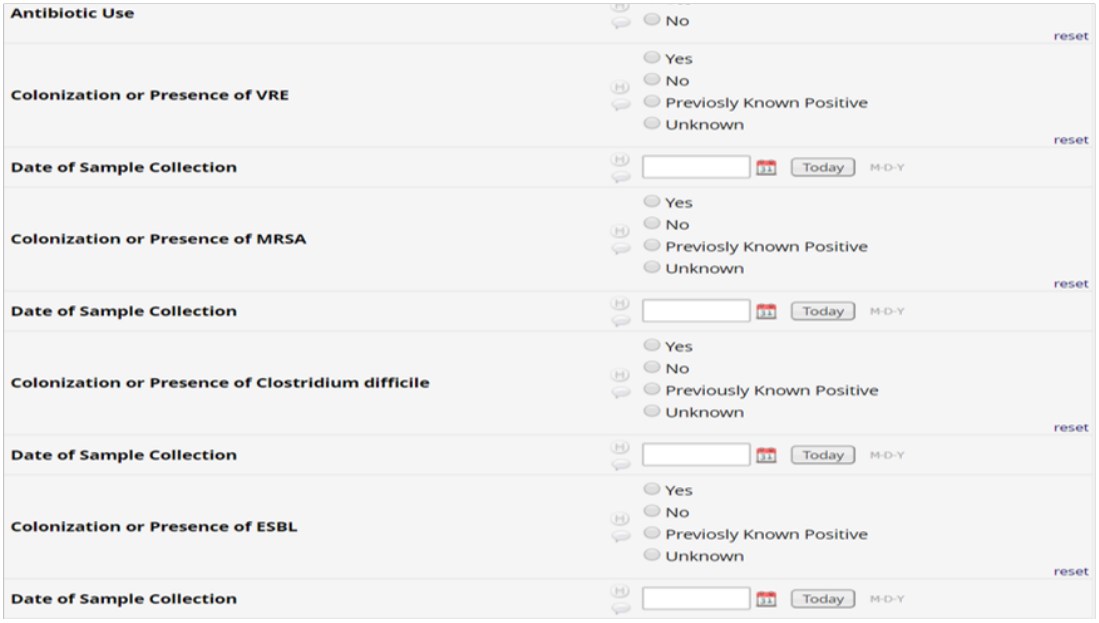
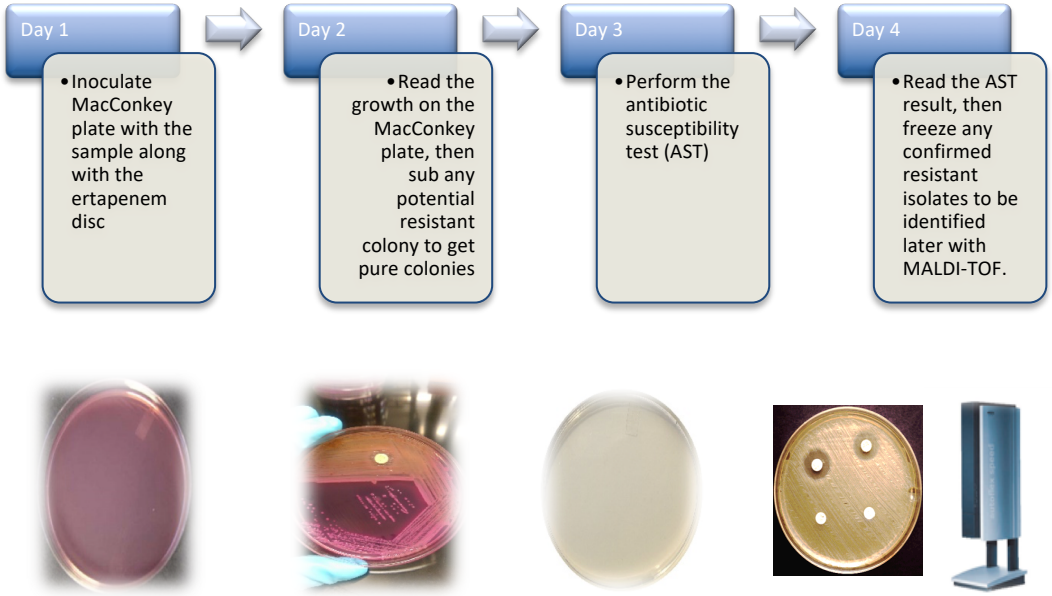


FIGURE 2.1 REDCAP DATA ENTRY SHEET. Basic demographic information and available clinical information were collected for the current hospitalization period. Data were stored electronically and were encrypted at rest.

A. Direct Ertapenem Disk Method



B. Selective Enrichment Broth Method

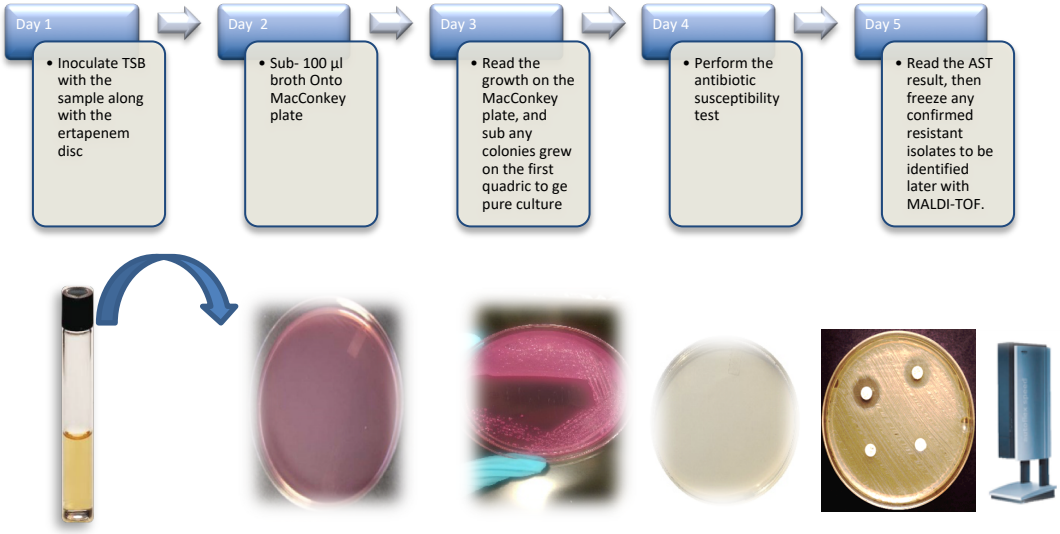


Figure 2.2 Detection Methodologies Used to Assess the Prevalence Rate of CRE Colonization Associated with Adults Being Admitted to Care within MUH. Panel A, the direct ertapenem disk method requires 4 days to reach a decision, while Panel B, the selective enrichment broth method, requires approximately 5 days to reach a decision as to whether or not the samples contain microbes capable of conferring resistance to the carbapenem class of antibiotics.

RESULTS

A total of 600 patients were randomly enrolled through the inclusion of remnant perianal swabs collected from the MUH clinical microbiology laboratory from April 3, 2017 through June 6, 2017. Of the 600 patients, 118 patients who were in residence greater than 7 days were routinely re-screened for VRE colonization subsequent to the 7th day from admission to care. An additional of 181 follow-up remnant specimens were evaluated; 18 follow up samples were not available. The last follow up sample was collected on July 12, 2017.

From the 600 patients evaluated from the universal surveillance arm, 11 patients were found to harbor CRE, yielding an overall base colonization rate of 1.8%. The standard of care requires that the universal surveillance specimens be collected within 48 hours of admission and once weekly for patients who were in residence greater than 7 days. On admission, five of the 11 CRE positive hospitalized patients (600) screened were found to be positive for CRE colonization from their initial VRE-perianal swab. On admission, five (5/11) of the 600 hospitalized patients screened were positive for CRE colonization from their initial VRE perianal swab. The other 5 positive CRE colonizations were detected from patients whose first swab was negative while their subsequent follow up swab was then found to be positive, suggesting that the patients who met this criterion became colonized with CRE as a consequence of hospitalization. One additional patient (1/11) was found to be CRE colonized from the subsequent swab of his/her current admission. However, the patient's admission swab was not obtained,

therefore, we cannot assess whether the patient acquired CRE during hospitalization or whether this individual was previously colonized (Table 2.1).

TABLE 2.1. PERCENTAGE OF REMNANT PERIANAL VRE SPECIMEN HARBORING CRE

Percentage of Remnant Perianal VRE Specimens Harboring CRE		
Overall % Patients CRE Positive	**1.833%**	**11/600**
% Patients CRE Positive at the time of 1° Screening	**0.833%**	**5/600**
% Patients CRE Positive at the time of 2° Screening	**0.833%**	**5/600**
CRE Positive where 1° Colonization Status was Unknown	**0.167%**	**1/600**
45.5% CRE Positive Patients were colonized with CRE at the time of 1° screen/admission		
45.5% CRE Positive Patients were colonized with CRE subsequent to the collection of the 1° specimen suggesting a HAC		

Each of the resistant isolates was identified by MALDI-TOF. The bulk of the isolates, 36%, (4/11) were from the *Enterobacter cloacae* complex, 18% (2/11) were *Enterobacter aerogenes* 18% (2/11) were *Escherichia coli*, 18% (2) were *Klebsiella pneumoniae*, and 9% (1/11) isolate was *Hafnia alvei* (Figure 2.3). All 11 *Enterobacteriaceae* isolates that met the CDC definition, resistant to at least one carbapenem antimicrobial or documented to produce carbapenemase, demonstrated resistance to ertapenem. Meropenem resistance was observed in 6 of 11 isolates while the other 5 isolates were either susceptible or displayed intermediate susceptibility to meropenem. (Table 2.2).

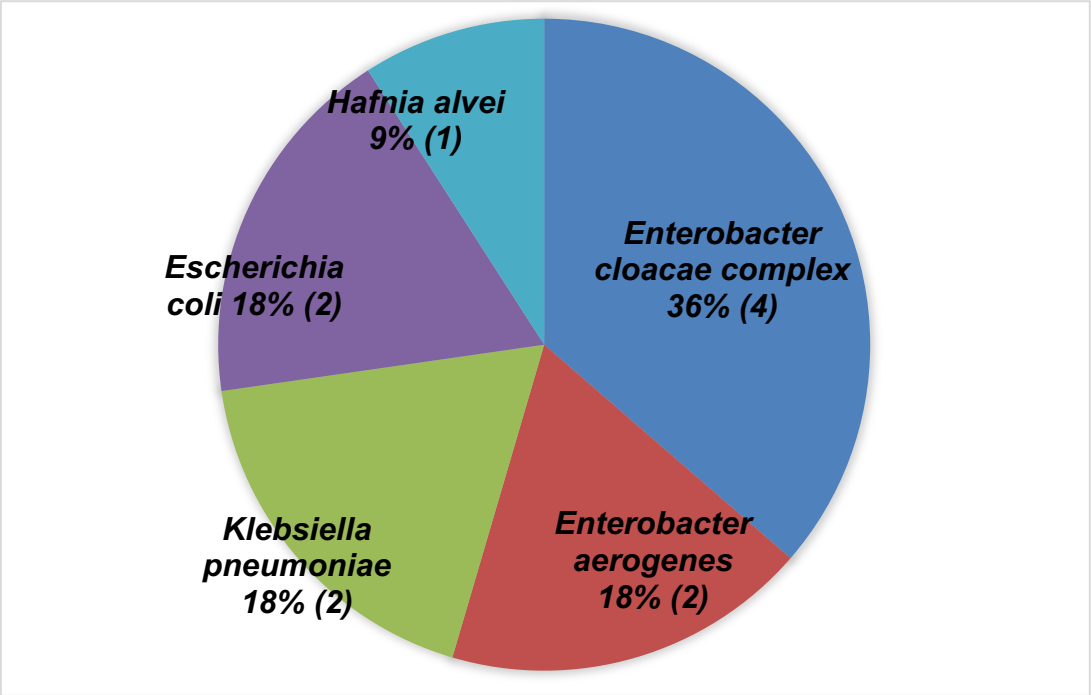


FIGURE 2.3 DISTRIBUTION OF CRE RESISTANCE BY MICROBIAL TYPE, RECOVERED FROM PERIANAL SWABS. Remnant perianal swabs collected for VRE surveillance were screened for the presence of CRE as described in the Materials and Methods section. Positive isolates (11) were identified by MALDI-TOF. The distribution of CRE resistance from the samples evaluated is presented. Members of the *Enterobacter cloacae* complex include, *E. asburiae, E. cloacae, and E. kobei.**

TABLE 2.2. ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *ENTEROBACTERIACEAE* SPECIES ISOLATED FROM PERIANAL SWAB

ISOLATE NUMBER	ORGANISM ID MALDI-TOF	ANTIMICROBIAL SUSCEPTIBILTY	
		ERTAPENEM	MEROPENEM
1	*Enterobacter cloacae*	R	S
2	*Enterobacter aerogenes*	R	S
3	*Enterobacter cloacae*	R	R
4	*Enterobacter cloacae*	R	S
5	*Klebsiella pneumoniae*	R	R
6	*Enterobacter aerogenes*	R	S
7	*Escherichia coli*	R	R
8	*Escherichia coli*	R	S
9	*Enterobacter kobei*	R	I
10	*Hafnia alvei*	R	S
11	*Klebsiella pneumoniae*	R	R

Resistance to the carbapenem class of antibiotics was confirmed by disk diffusion analysis using either an ertapenem (10µg/disk) or meropenem (10µg/disk) impregnated paper disk.

The demographic profiles of the patients (Table 2.3), available clinical information and potential risk factors (Table 2.4) for CRE colonization were evaluated for significance. The rate of CRE colonization was found to be essentially equivalent for men and women at approximately 2% of the population (p= 1.0000). Ethnicity and race of the CRE colonized patients compared to non-colonized patients revealed no bias/significance (ethnicity, p= 0.6568 and race, p= 1, respectively). For the total 600 patients considered in the study, the median age was 59.5 years (range 19.4 - 98.0 years), and the median LOS was 6 days (range 1-138 days). CRE colonized patients were on average two and half years older (61.5 vs. 59 years) and had longer durations of hospitalization (21 vs. 6 days). Wilcoxon sign rank tests were used to compare CRE status for age and LOS. Neither age (p=0.3660) nor LOS (p=0.0647) were significant, although LOS was trending towards significance. (Table 2.3).

Of the CRE colonization risk factors considered, the following were found to be significant: VRE colonization (p<0.0001); ESBL infection (p= 0.0067); and coincident infection with CRE (p=0.031). The VRE colonization rate associated with the 600 patients included in the study was observed to be 5.5% (33/600). Of these VRE colonized patients, 15.2% (5/33) were also colonized with CRE (Table 2.4). Although VRE colonization was found to be significant risk factor for CRE, 55% of CRE colonized were negative for VRE.

A history of prior antibiotic exposure reported for the patients from the time of admission until the collection of samples was also evaluated. Of the 600 patients

analyzed, 70% had an indication of prior antibiotic exposure. Prior antibiotic exposure was not a significant risk factor associated with CRE colonization (p=1.00).

In total, the 600 patients evaluated accounted for 6,294 patient days of care. The CRE colonized patients were responsible for 4.0% of the patient care days (252 days) resulting in a CRE colonization rate for patients admitted to the VRE high risk units of 1.75 CRE colonization per 1,000 patient days. In contrast the VRE colonization rate for this same cohort of patients was 5.24 per 1,000 patient days or a ratio of 1 CRE colonization associated with every 3 VRE colonizations per 1,000 patient days.

TABLE 2.3. DEMOGRAPHIC DETAILS OF ALL PATIENTS IN COMPARISON OF CRE COLONIZED PATIENTS IN THE UNIVERSAL SURVEILLANCE PROGRAM (PERIANAL VRE SWABS)

Demographic Risk Factors Considered Predisposing for Colonization by CRE								
	CRE Colonization Status	**N**	**Mean**	**Median**	**Std Dev**	**Minimum**	**Maximum**	**P Value**
Age	**CRE Negative**	**589**	**57.241**	**59.016**	**16.575**	**19.412**	**98.017**	**0.3660**
	CRE Positive	**11**	**61.880**	**61.562**	**11.824**	**35.141**	**81.532**	
	Total Patients Evaluated	**600**	**57.326**	**59.48**	**16.505**	**19.412**	**98.017**	
Length of Stay (days)	**CRE Negative**	**589**	**10.258**	**6.000**	**12.715**	**1.000**	**138.000**	**0.0647**
	CRE Positive	**11**	**22.909**	**21.000**	**20.002**	**1.000**	**57.000**	
	Total Patients Evaluated	**600**	**10.490**	**6.000**	**12.972**	**1.000**	**138.000**	

		All Patients	**CRE Negative**	**CRE Colonized**	**% Population CRE Negative**	**% Population CRE Colonized**	
Sex	**Female**	**295**	**290**	**5**	**98.31%**	**1.69%**	**1.000**
	Male	**305**	**299**	**6**	**98.03%**	**1.97%**	
	Total Patients	***600***	***589***	***11***	***98.17%***	***1.83%***	
Ethnicity	**Hipanics or Latinos**	**8**	**8**	**0**	**100.00%**	**0.00%**	**0.6568**
	Non-Hispanics or Latinos	**588**	**577**	**11**	**98.13%**	**1.87%**	
	Unknown	**4**	**4**	**0**	**100.00%**	**0.00%**	
	Total Patients	***600***	***589***	***11***	***98.17%***	***1.83%***	
Race	**African American**	**255**	**249**	**6**	**97.65%**	**2.35%**	**1.000**
	American Indian or Alaska Native	**1**	**1**	**0**	**100.00%**	**0.00%**	
	Asian	**2**	**2**	**0**	**100.00%**	**0.00%**	
	Native Hawaiian or another Pacific Islander	**1**	**1**	**0**	**100.00%**	**0.00%**	
	Other	**11**	**11**	**0**	**100.00%**	**0.00%**	
	White	**330**	**325**	**5**	**98.48%**	**1.52%**	
	Total Patients	***600***	***589***	***11***	***98.17%***	***1.83%***	

Wilcoxon sign rank tests were used to compare CRE colonization status with respect to age (years) Neither age nor LOS were significant (age [p=0.3660]; LOS [p=0.0647]). However, LOS was trending towards significance.

TABLE 2.4. RISK FACTORS OF ALL PATIENTS IN COMPARISON OF CRE COLONIZED PATIENTS IN THE UNIVERSAL SURVEILLANCE PROGRAM (PERIANAL VRE SWABS).

Risk Factors Considered Predisposing for Colonization by CRE							
		CRE COLONIZATION					
		NEGATIVE Patients	POSITIVE Patients	TOTAL	% Population CRE negative	% Population CRE Colonized	
VRE COLONIZATION / INFECTION	Negative	561	6	567	98.94%	1.06%	0.0001
	Positive	28	5	33	84.85%	15.15%	
5.50%	Total VRE Assessments	589	11	600	98.17%	1.833%	
MRSA COLONIZATION / INFECTION	Negative	530	11	541	97.97%	2.03%	1.0000
	Previously known MRSA Positive	24	0	24	100.00%	0.00%	
	Unknown	1	0	1	100.00%	0.00%	
	Positive	34	0	34	100.00%	0.00%	
9.67%	Total MRSA Assessments	589	11	600	98.16%	1.836%	
Clostridium difficile Status	Negative	70	3	73	95.89%	4.11%	0.3931
	Previously known *C. difficile* positive	3	0	3	100.00%	0.00%	
	Unknown	501	8	509	98.43%	1.57%	
	Positive	15	0	15	100.00%	0.00%	
3.00%	Total *C. difficile* Assessments	589	11	600	98.16%	1.836%	
ESBL Infection Status	Negative	46	2	48	95.83%	4.17%	0.0067
	Previously known ESBL Infection	1	0	1	100.00%	0.00%	
	Unknown	535	7	542	98.71%	1.29%	
	Positive	7	2	9	77.78%	22.22%	
1.67%	Total ESBL Assessments	589	11	600	98.17%	1.83%	
CRE Infection Status	No	51	4	55	92.73%	7.27%	0.031
	Unknown	537	7	544	98.71%	1.29%	
	Yes	1	0	1	100.00%	0.00%	
0.17%	Total Clinical CRE Infections	589	11	600	98.17%	1.833%	
Antibiotic Utilization at the time of Specimen Collection	No	179	3	182	98.35%	1.65%	1.00
	Yes	410	8	418	98.09%	1.91%	
70%	Total	589	11	600	98.17%	1.83%	

Fisher's Exact Test was conducted on the data set above in order to assess whether or not the respective risk factors are predisposing for CRE colonization. The factors that were found to be significant at predisposing a patient for CRE colonization are highlighted in Light Green.

CHAPTER 3

ASSESSMENT OF THE PREVALENCE RATE FOR CRE IN PATIENTS WITH A HISTORY OF ANTIBIOTIC USE

RATIONALE

Previous studies evaluating predictors of CRE carriage and/ or infection found that prior receipt of a course of antibiotics was among the most prevalent risk factors [2, 18, 19]. Antibiotics increase the likelihood of microbial dysbiosis resulting in loss of susceptible commensal organisms with the subsequent establishment of antibiotic resistant microbes within the intestinal tract of the host (reviewed by [22]). Therefore, samples from a patient population with a history of antibiotic administration are hypothesized to be at an increased risk for colonization by antibiotic resistant organisms including CRE. Because *C. difficile* is well recognized as the leading cause of AAD[23], fecal specimens of patients suspected of displaying symptoms consistent with AAD are routinely tested for the presence of *C. difficile* toxin. A prevalence study of hospitalized patients with AAD demonstrated an ESBL stool carriage rate of 37% [24]. Stools containing *C. difficile* toxin were also significantly more likely to harbor an ESBL microbe than those without *C. difficile* toxin (62% vs 31%,) (p=0.008) [24]. Similarly, VRE colonization has been associated with *C. difficile* infection in up to 50% of patients [25]. Consequently, this "pre-screened" specimen population may be superior in being able to assess the CRE colonization rate risk by significantly reducing the number of surveillance specimens collected from patients unlikely to harbor CRE.

METHODS

I. SETTING AND STUDY DESIGN

The study was conducted at the MUH, a 700-bed tertiary hospital located in Charleston, South Carolina. Subjects were adult inpatients, ≥18 years of age displaying symptoms consistent with AAD. The patients were not consented for the collection of this sample as the remnant sample was collected in concert with the patient's care for which they had previously consented for treatment at MUH. Patients under the age of 18 years of age and outpatients were excluded from the study. All remnant *C. difficile* diarrhea stool samples meeting the above criteria, and submitted to MUH microbiology laboratory between January 8, 2017 and July 1, 2017, were obtained within 24-72 hours of collection and were subjected to CRE isolation as previously described in Chapter 2. Additionally, all diarrhea stool samples collected from patients for *C. difficile* toxin testing during the same hospitalization period were also obtained with the last additional specimen collected on July 21 2017.

II. DATA COLLECTION AND STATISTICAL ANALYSIS

Basic demographic information (Name, MRN, age, sex, ethnicity (self-reported)) as well as available clinical information (antibiotic use at the time of collection, date of admission, date of discharge, presence/absence of diarrhea, colonization status for VRE, MRSA, ESBL, *Clostridium difficile* colonization/infection/toxin status (if available)) were collected for the current hospitalization period. Fisher's exact tests were used because of some of the sample sizes were small. Wilcoxon sign rank tests were used to compare CRE status for patients' age and LOS.

III. MICROBIOLOGICAL TESTING

Diarrhea stool specimens submitted for *C. difficile* toxin testing were screened secondarily for CRE according to the CDC protocol for microbiological recovery as previously described in Chapter 2.

IV. FINANCIAL ANALYSIS

The cost to perform each culture method was calculated based on the cost of supplies and labor expense. The cost for each culture method included the price of media (MacConkey agar, Blood agar plate, Mueller Hinton agar plate, and TSB) and antibiotics (ertapenem disk and meropenem disk) and the cost to identify the confirmed carbapenem resistant isolates by MALDI-TOF. The labor for each procedure was included in the analysis and was based on the current market labor rate, including fringe benefits, for a MUH microbiology technologist.

RESULTS

Six hundred unique patients were enrolled by virtue of the inclusion of their remnant diarrhea stool specimen collected from the MUH clinical microbiology laboratory from January 8, 2017 through July 21, 2017. Seventy-five (75) patients of the 600 recruited in the study had more than one diarrhea stool specimen submitted for testing during the same hospitalization. A total of 717 remnant diarrhea specimens were examined for the presence of CRE.

From the 600 patients included in the study, 37 patients were found to harbor CRE in their GI tracts, yielding a base colonization prevalence rate of 6.2%. Each of the resistant isolates was subjected to MALDI-TOF identification. The bulk of the isolates 51% (19) were from the *Enterobacter* cloacae complex, 16% (6) were *Escherichia coli*, 14% (5) were *Klebsiella pneumoniae*, 8% (3) *Enterobacter aerogenes* 5% (2) *Citrobacter freundii*, 3% (1) *Raoultella ornithinolytica*, and 3% (1) was *Hafnia alvei* (Figure 3.1)

All 37 *Enterobacteriaceae* isolates that met the CDC definition, resistant to at least one carbapenem antimicrobial or documented to produce carbapenemase, demonstrated resistance to ertapenem. Meropenem resistance was observed in 11 of 37 while the other 26 were either susceptible or displayed intermediate susceptibility to meropenem. (Table 3.1)

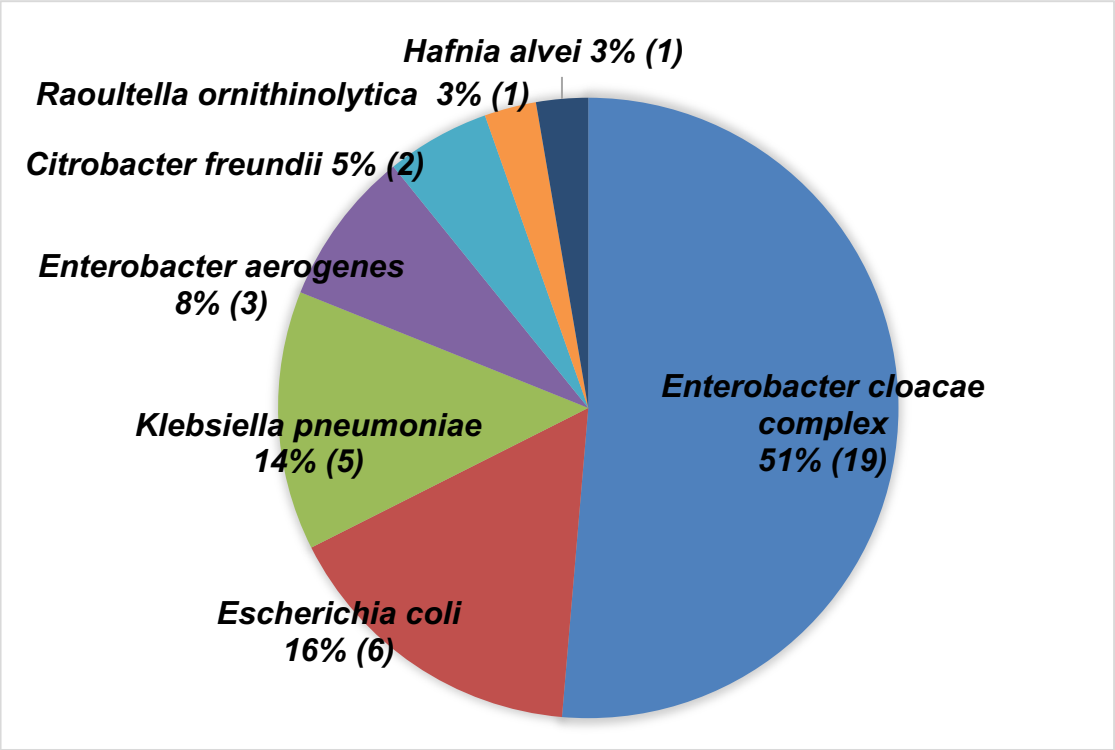


FIGURE 3.1 DISTRIBUTION OF CRE RESISTANCE BY MICROBIAL TYPE, RECOVERED FROM AAD STOOL SPECIMENS FROM 600 PATIENTS ADMITTED TO CARE AT MUH. Remnant AAD Stool Specimens collected for *C. difficile* toxin testing were screened for the presence of CRE as described in the Materials and Methods section of Chapter 2. Positive isolates (37) were subjected to MALDI-TOF identification. The distribution of CRE resistance from the samples evaluated is presented. Members of the *Enterobacter cloacae* complex include: *E. asburiae, E. cloacae and E. kobei.*

TABLE 3.1. ANTIMICROBIAL SUSCEPTIBILITY PROFILE OF *ENTEROBACTERIACEAE* SPECIES ISOLATED FROM DIARRHEAL STOOL SAMPLES

ISOLATE NUMBER	ORGANISM ID MALDI-TOF	ANTIMICROBIAL SUSCEPTIBILTY	
		ERTAPENEM	MEROPENEM
1	*Escherichia coli*	R	R
2	*Escherichia coli*	R	I
3	*Klebsiella pneumoniae*	R	R
4	*Klebsiella pneumoniae*	R	R
5	*Enterobacter cloacae*	R	S
6	*Escherichia coli*	R	R
7	*Raoultella ornithinolytica*	R	R
8	*Enterobacter asburiae*	R	S
9	*Escherichia coli*	R	S
10	*Enterobacter aerogenes*	R	I
11	*Enterobacter asburiae*	R	S
12	*Enterobacter asburiae*	R	S
13	*Enterobacter asburiae*	R	S
14	*Klebsiella pneumoniae*	R	R
15	*Klebsiella pneumoniae*	R	I
16	*Enterobacter cloacae*	R	R
17	*Enterobacter cloacae*	R	R
18	*Enterobacter cloacae*	R	S
19	*Enterobacter cloacae*	R	S
20	*Enterobacter cloacae*	R	I
21	*Enterobacter asburiae*	R	S
22	*Escherichia coli*	R	S
23	*Enterobacter cloacae*	R	S
24	*Enterobacter aerogenes*	R	S
25	*Enterobacter cloacae*	R	S
26	*Enterobacter kobei*	R	R
27	*Enterobacter cloacae*	R	S
28	*Enterobacter cloacae*	R	S
29	*Hafnia alvei*	R	R
30	*Citrobacter freundii*	R	I
31	*Enterobacter cloacae*	R	S
32	*Citrobacter freundii*	R	S
33	*Enterobacter asburiae*	R	I
34	*Escherichia coli*	R	I
35	*Enterobacter cloacae*	R	S
36	*Klebsiella pneumoniae*	R	I
37	*Enterobacter cloacae*	R	S

Resistance to the carbapenem class of antibiotics was confirmed by disk diffusion analysis using either an ertapenem (10µg/disk) or meropenem (10µg/disk) impregnated paper disk.

The demographic profiles of the patients (Table 3.2), available clinical information and potential risk factors (Table 3.3) for CRE colonization were evaluated for significance. The rate of CRE colonization was found to be essentially equivalent for men and women (p=0.866). Ethnicity and race of the CRE colonized patients compared to non-colonized patients revealed no bias/significance (ethnicity, p=0.4735 and race, p=0.078, respectively). The median age for the 600 patients evaluated was 61.7 years (range 19.4 - 96.1 years). The median LOS for the 600 patients was 14 days (range 1-233 days). CRE colonized patients had longer durations of hospitalization, as the median LOS was 18 days compared to 14 days for non-colonized patients. Interestingly, CRE colonized patients were approximately 9 years younger than non-colonized patients (median age 53.0 vs. 61.8 years). Wilcoxon sign rank tests were used to evaluate the significance of difference in age and LOS between colonized and non-colonized patients. Both age (p=0.018) and LOS (p=0.0093) were significant. (Table 3.2).

Of the CRE colonization risk factors considered, the following were found to be significant: VRE colonization (p=0.0312) and coincident infection with CRE (0.0002). Of the diarrheal population whose VRE colonization status was known (N= 410), 17.8% were colonized with VRE. Of these VRE colonized patients, 13.7% were also CRE colonized.

A history of prior antibiotic exposure reported for the patients from the time of admission until the collection of samples was collected. Of the 600 patients analyzed, 88% had an indication of prior antibiotic exposure. Yet, prior antibiotic exposure was not a significant risk factor associated with the patient's CRE colonization status (p=0.11).

TABLE 3.2 DEMOGRAPHIC RISK FACTORS CONSIDERED FOR PREDISPOSING INDIVIDUALS FOR COLONIZATION BY CRE USING REMNANT DIARRHEA SPECIMENS

Demographic Risk Factors Considered Predisposing for Colonization by CRE								
	CRE Colonization Status	**N**	**Mean**	**Median**	**Std Dev**	**Minimum**	**Maximum**	**P Value**
Age	**CRE Negative**	**563**	**59.307**	**61.827**	**16.295**	**19.464**	**96.101**	**0.018**
	CRE Positive	**37**	**53.058**	**53.058**	**16.317**	**23.256**	**87.854**	
	Total Patients Evaluated	**600**	**58.921**	**61.774**	**16.352**	**19.464**	**96.101**	
Length of Stay (days)	**CRE Negative**	**563**	**19.813**	**14.000**	**21.775**	**1.000**	**233.000**	**0.0093**
	CRE Positive	**37**	**27.432**	**18.000**	**24.277**	**2.000**	**121.000**	
	Total Patients Evaluated	**600**	**20.283**	**14.000**	**21.992**	**1.000**	**233.000**	
			All Patients	**CRE Negative**	**CRE Colonized**	**% Population CRE Negative**	**% Population CRE Colonized**	
	Sex	**Female**	**312**	**292**	**20**	**93.59%**	**6.41%**	**0.866**
		Male	**288**	**271**	**17**	**94.10%**	**5.90%**	
		Total Patients	***600***	***563***	***37***	***93.83%***	***6.17%***	
	Ethnicity	**Hipanics or Latinos**	**7**	**6**	**1**	**85.71%**	**14.29%**	**0.4735**
		Non-Hispanics or Latinos	**590**	**554**	**36**	**93.90%**	**6.10%**	
		Unknown	**3**	**3**	**0**	**100.00%**	**0.00%**	
		Total Patients	***600***	***563***	***37***	***93.83%***	***6.17%***	
	Race	**African American**	**217**	**199**	**18**	**91.71%**	**8.29%**	**0.078**
		American Indian or Alaska Native	**0**	**0**	**0**	**0.00%**	**0.00%**	
		Asian	**4**	**4**	**0**	**100.00%**	**0.00%**	
		Native Hawaiian or another Pacific Islander	**1**	**1**	**0**	**100.00%**	**0.00%**	
		Other	**8**	**6**	**2**	**75.00%**	**25.00%**	
		White	**369**	**352**	**17**	**95.39%**	**4.61%**	
		Total Patients	***599***	***562***	***37***	***93.82%***	***6.18%***	

Fisher's exact test was conducted on the data described in the table above in order to assess whether or not the respective risk factors were predisposing for colonization by CRE. Wilcoxon sign rank tests were used to compare CRE colonization status with respect to age (years) and LOS (days). The factors found significant (<0.05) are highlighted in light green.

TABLE 3.3 CLINICAL RISK FACTORS CONSIDERED FOR PREDISPOSING INDIVIDUALS FOR COLONIZATION BY CRE USING REMNANT DIARRHEA SPECIMENS

Risk Factors Considered Predisposing for Colonization by CRE							
		CRE COLONIZATION					
		NEGATIVE Patients	POSITIVE Patients	TOTAL	% Population CRE negative	% Population CRE Colonized	
Clostridium difficile Status	Negative	467	29	496	94.15%	5.85%	
	Positive	96	8	104	92.31%	7.69%	0.5005
17.33%	Total VRE Assessments	563	37	600	93.83%	6.167%	
MRSA COLONIZATION / INFECTION	Negative	482	32	514	93.77%	6.23%	
	Previously known MRSA Positive	27	2	29	93.10%	6.90%	
	Unknown	13	0	13	100.00%	0.00%	0.9068
	Positive	41	3	44	93.18%	6.82%	
12.17%	Total MRSA Assessments	563	37	600	98.16%	1.836%	
VRE COLONIZATION / INFECTION	Negative	318	19	337	94.36%	5.64%	
	Previously known VRE positive	23	3	26	88.46%	11.54%	
	Unknown	182	8	190	95.79%	4.21%	0.0312
	Positive	40	7	47	85.11%	14.89%	
12.17%	Total *C. difficile* Assessments	563	37	600	98.16%	1.836%	
ESBL Infection Status	Negative	70	7	77	90.91%	9.09%	
	Previously known ESBL Infection	8	0	8	100.00%	0.00%	
	Unknown	472	27	499	94.59%	5.41%	0.0994
	Positive	13	3	16	81.25%	18.75%	
4.00%	Total ESBL Assessments	563	37	600	93.83%	6.17%	
CRE Infection Status	No	81	9	90	90.00%	10.00%	
	Unknown	480	25	505	95.05%	4.95%	
	Previously known positive	1	0	1	100.00%	0.00%	0.0002
	Yes	1	3	4	25.00%	75.00%	
0.67%	Total Clinical CRE Infections	563	37	600	93.83%	6.167%	
Antibiotic Utilization at the time of Specimen Collection	No	70	1	71	98.59%	1.41%	
	Yes	493	36	529	93.19%	6.81%	0.11
88%	Total	563	37	600	93.83%	6.17%	

Fisher's exact tests were conducted on the data set above in order to assess whether or not the respective risk factors were predisposing for colonization by CRE. The factors found significant (<0.05) are highlighted in light green.

CHAPTER 4

SENSITIVITY ANALYSIS AND ECONOMIC EVALUATION OF THE SCREENING METHODS USED TO DETERMINE THE NUMBER OF PATIENTS COLONIZED WITH CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE*

RATIONALE

CRE have emerged as significant community acquired infections as well as healthcare associated infections. Accurate and rapid laboratory testing methodologies for CRE are desperately needed to control the spread of this family of multidrug resistant microbes among our patients. Complicating the development of a universal method for detecting if an individual is either colonized or infected by an organism of this family of diverse Gram-negative microbes is the diversity of resistance mechanisms by which resistant members of the *Enterobacteriaceae* facilitate resistance to the carbapenem class of antimicrobials. Presently, the CDC recommends two methods for the microbiological detection of CRE producing *Klebsiella* spp. and *Escherichia coli* [21].

In this chapter we will report on the sensitivity and specificity associated with each CDC method, the time required to confirm resistance of the isolates recovered and the calculated costs associated with each screening methodology. It is anticipated that this analysis will enable us to determine which screening method is most suited to determine the level of CRE colonized patients entering care at MUH. To our knowledge, this analysis will be the first to compare the sensitivity and specificity associated with matched specimens using both CDC methodologies in concert with the time to confirmation of resistance borne by each isolate recovered and the costs associated with the methods.

METHODS

To assess the sensitivity and specificity of the screening methods, data generated from the screening of remnant diarrhea stool specimens and remnant perianal swabs were compared. The first method required inoculation of the specimen onto a selective and differential medium, MacConkey agar, with streaking for isolation, and the deposition of a 10-µg ertapenem disk within the first quadrant of the plate. Incubation followed for 18-24 hours aerobically at 35°C ± 2°C. Putative resistant isolates were those colonies that grew within the zone of inhibition manifested by the antibiotic within that quadrant of the plate. The limitation of using this method was that there was no set zone of inhibition that each isolate was required to grow with respect to the antibiotic impregnated disk in order to be deemed resistant. In an effort to qualify this limitation Blackburn *et al* [26]suggested that growth of a resistant colony should occur within a zone diameter of ≤ 24mm around the disk. Loans *et al* conducting a similar study considered that the resistant zone diameter be ≤27 mm arguing that this would yield greater sensitivity and specificity for detecting KPC[27]. For our study any isolate that grew within a zone of 27mm of an ertapenem disk was considered a candidate resistant isolate. The precise location within the inhibition zone was recorded and contrasted against the findings of Blackburn *et al* [26] and Loans *et al* [27].

The second method employed an enrichment of the CRE population resident in the specimen. Briefly, each specimen was inoculated into 5 ml of TSB containing a 10-µg ertapenem disk yielding a final ertapenem concentration of 2 µg/ml with subsequent incubation at 35°C± 2°C for 18-24 hours. Subsequent to the enrichment phase, 100 µl of

the bacterial suspension was sub-cultured to MacConkey agar and incubated aerobically at 35°C ± 2°C for 18-24 hours. Any colony that grew in the first quadrant of the MacConkey agar was considered to be carbapenem resistant. Colonies growing in the second quadrant were considered sensitive to carbapenem antibiotics. The limitation of this method is the enrichment step adds an additional 18-24 hours to the time required to complete the test.

Candidate resistant isolates were confirmed by disk diffusion antibiotic susceptibility testing using 10-µg ertapenem and 10-µg meropenem disks, two carbapenem class antibiotics currently prescribed for patients at MUH. Interpretation of zones of inhibition followed prescribed guidelines of the Clinical and Laboratory Standard Institute (CLSI); zones of inhibition of ≤ 18 mm for ertapenem and ≤19mm for meropenem were defined as resistant. Based on CDC definition, any *Enterobacteriaceae* isolate that is resistant to at least one carbapenem were deemed CRE. Resistant isolates were then identified by MALDI-TOF. The costs associated with each method, coupled with the time to CRE confirmation, were obtained.

Each isolate recovered from either method was categorized according to the following scheme: (1) no potential resistance (2) oxidase positive, non-lactose fermenter e.g., *Pseudomonas aeruginosa*, (3) confirmed to be susceptible or intermediate to ertapenem, (4) Carbapenem resistant, non-*Enterobacteriaceae*, e.g., *Acinetobacter baumannii* and *Stenotrophomonas maltophilia*, (5) Confirmed CRE.

The costs associated with processing each remnant patient sample confirming carbapenem resistance and identifying the organism recovered is summarized in Table

4.1. Since neither method was selective for *Enterobacteriaceae* species, growth of carbapenem resistant non-*Enterobacteriaceae* isolates were common. This contributed to the total cost associated with surveillance as well as affected the sensitivity, positive predictive value (PPV), and negative predictive value (NPV) of each method. A true positive was operationally defined as a confirmed CRE isolate, while a false positive included carbapenem sensitive isolates and carbapenem resistant non-*Enterobacteriaceae.* True negative isolates were defined as isolates recovered from patient samples that were negative by both methods, while false negatives were the isolates that were negative in one method while CRE was recovered in the other method.

The PPV and NPV for each CDC method were calculated according to the following formulas:

$$\textbf{PPV} = \frac{\textbf{Number of True Positives}}{\textbf{Number of True Positives + Number of False Positives}}$$

$$\textbf{NPV} = \frac{\textbf{Number of True Negative}}{\textbf{Number of True Negatives + Number of False Negatives}}$$

The time required to generate a final result regarding a patient's CRE colonization status was calculated from the time required for preliminary screening, confirmation of potential CRE isolates, and the final identification of the CRE isolates.

TABLE 4.1. COSTS ASSOCIATED WITH PROCESSING REMNANT PATIENT SAMPLES FOR THE DETECTION OF CRE COLONIZATION

Supplies	Direct Ertapenem Disk Method	Enrichment Selective Broth Method
	Cost	Cost
Preliminary Screening		
MacConkey agar plate	$0.30	$0.30
Ertapenem 10µg Disk	$0.20	$0.20
Tryptic Soy Broth (TSB) 5ml	-	$2.07
Labor Expense (2-3 minutes)	$2.82 (1.41 per minute)	$4.23 ($ 1.41 per minute)
Total per patient sample	**$3.32**	**$6.80**
Antimicrobial Resistance Testing		
Ertapenem 10µg Disk	$0.20	$0.20
Meropenem 10µg Disk	$0.20	$0.20
Blood agar plate	$0.36	$0.36
Mueller Hinton plate	$0.70	$0.70
Labor Expense (4 minutes)	$5.64 (1.41 per minute)	$5.64 ($ 1.41 per minute)
Total per putative resistant isolat	$7.10	$7.10
Species Identification Testing		
MALDI-TOF per carbapenem resistant organism	$5.70	$5.70
Total cost PER positve culture	$16.12	$19.60

The costs associated with the materials and labor for processing remnant patient samples for the detection of CRE colonization were market averages for the items listed. In many cases more than one isolate/positive culture could have been recovered from either the direct ertapenem disk or enrichment selective broth methodologies.

RESULTS

In total, 717 diarrhea specimens were evaluated using both methods. There was an 80% agreement between the results observed (Table 4.2). Results for the direct ertapenem disk method were as follows: (1) 571/717 cultures had no potential resistance; (2) 53/717 cultures grew oxidase positive, non-lactose fermenter isolates (*P. aeruginosa*); (3) 48/717 cultures grew isolates that were confirmed to be either intermediate or susceptible to ertapenem by disk diffusion testing; (4) 11/717 cultures grew carbapenem resistant non-*Enterobacteriaceae* isolates such as *A. baumannii* and *S. maltophilia*; (5) 34/717 cultures grew confirmed CRE. (Table 4.2)

Results from the selective, enrichment broth method were as follows: (1) 495/717 patient samples failed to generate an isolate demonstrating resistance to the selective antibiotic (no potential resistance); (2) 114 /717 patient samples resulted in the recovery of oxidase positive, non-lactose fermenting isolates (e.g., *P. aeruginosa*); (3) 67/717 patient samples generated isolates that were confirmed to be either intermediate or susceptible to ertapenem by disk diffusion testing; (4) 13/717 patient samples resulted in the growth of carbapenem resistant non-*Enterobacteriaceae* isolates such as *A. baumannii* and *S. maltophilia* and (5) 28/717 patient samples resulted in the growth of confirmed CRE isolates (Table 4.2).

Out of the 717 diarrhea stool samples evaluated, a total of 41 CRE isolates were recovered. Of the 41 CRE recovered, 21 were coincidentally detected using both methods. Thirteen were detected only using the direct ertapenem disk method, while the remaining 7 were detected only from the selective, enrichment broth method. For stool

specimens, the direct ertapenem disk method had a higher sensitivity (82.93%) and specificity (83.73%). In contrast, the sensitivity and specificity of the enrichment selective broth method were 68% and 72%, respectively. However, the difference was not statistically significant (p= 0.5165). Both methods have low positive predictive value; 23.61% (34/144) for direct ertapenem disk method and 13% (28/215) for selective enrichment method. (Table 4.3)

TABLE 4.2 DISTRIBUTION OF THE RECOVERY PROFILES USING EITHER THE RESULTS OF THE TWO METHODS FOR DIARRHEA SPECIMENS

	Result Category	Direct Ertapenem Disk Method	Selective, Enrichment Broth Method
Carbapenem Sesitive Culture			
	No growth on the plate or no isolates recovered within ≤ 27 mm zone around ertapenem disk	571	495
Carbapenem Resistant Culture			
	Oxidase positive, non-lactose fermenter isolates recovered	53	114
	Carbapenem Susceptible isolates recovered	48	67
	Carbapenem Resistant non-*Enterobacteriaceae* isolates recovered	11	13
	Carbapenem Resistant *Enterobacteriaceae* isolates recovered	34	28
Total Patients Samples Processed		717	717

TABLE 4.3. SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE, AND NEGATIVE PREDICTIVE VALUE OF THE TWO SCREENING METHODS IN DETECTING CRE IN DIARRHEA SPECIMEN

Screening Method	Sensitivity	Specificity	PPV	NPV
Direct Ertapenem Disk Method	82.93%	83.73%	23.61%	98.78%
Enrichment, Selective Broth Method	68%	72%	13%	97%

PPV, Positive Predictive Value; NPV, Negative Predictive Value

In total, 781 perianal swabs were evaluated in both methods. There was 89.6% agreement for the final result between the two methods. Results for the direct ertapenem disk method were as follows: (1) 683/781 patient samples displayed no potential resistance to carbapenems; (2) 35/781 resulted in the growth of oxidase positive, non-lactose fermenting isolates (e.g. *P. aeruginosa*); (3) 45/781 patient samples resulted in the growth of isolates that were confirmed to be either intermediate or susceptible to ertapenem by disk diffusion testing; (4) 8/781 patient samples resulted in the growth carbapenem resistant non-*Enterobacteriaceae* isolates such as *A. baumannii* and *S. maltophilia*; (5) 10/781 patient samples resulted in the growth of confirmed CRE. (Table 4.4)

Results for the selective, enrichment broth method were as follows: (1) 666/781 of the patient samples failed to generate an isolate demonstrating resistance to the selective antibiotic; (2) 54 /781 of the patient samples resulted in the growth of oxidase positive, non-lactose fermenting isolates (e.g., *P. aeruginosa*); (3) 33/781 of the patient samples resulted in the growth of isolates that were confirmed to be either intermediate or

susceptible to ertapenem by disk diffusion testing; (4) 15/781 of the patient samples resulted in the growth of grew carbapenem resistant non-*Enterobacteriaceae* isolates such as *A. baumannii* and *S. maltophilia*; (5) 13/781 of the patient samples resulted in the growth of confirmed CRE. (Table 4.4)

Out of the 781 perianal swabs included in the analysis, a total of 14 CRE isolates were recovered using either method. Both methods equivalently recovered 9 CRE isolates out of the total 14 isolates recovered. One (1/14) of the isolates was recovered only using the direct ertapenem disk method, while 4/14 of the isolates were only detected when using the selective, enrichment broth method. For samples evaluated from perianal swabs, the selective, enrichment broth method resulted in a higher sensitivity (93%) while the sensitivity of direct ertapenem disk method was only found to be 71.43%. Both methods had almost equivalent specificity; 87% for the selective, enrichment broth method and 88.53% for direct ertapenem disk. However, the difference was not statistically significant (p= 0.6753). Both methods displayed low positive predictive values; 10.20% (10/98) for direct ertapenem disk method and 12% (13/113) for selective enrichment method. (Table 4.5)

TABLE 4.4 BREAKDOWN OF THE CULTURE RESULTS OF THE TWO METHODS FOR PERIANAL SWABS

Result Category		Direct Ertapenem Disk Method	Selective, Enrichment Broth Method
Carbapenem Sesitive Culture			
	No growth on the plate or no isolates recovered within ≤ 27 mm zone around ertapenem disk	683	666
Carbapenem Resistant Culture			
	Oxidase positive, non-lactose fermenter isolates recovered	35	54
	Carbapenem Susceptible isolates recovered	45	33
	Carbapenem Resistant non-*Enterobacteriaceae* isolates recovered	8	15
	Carbapenem Resistant *Enterobacteriaceae* isolates recovered	10	13
Total Patients Samples Processed		781	781

TABLE 4.5. SENSITIVITY, SPECIFICITY, POSITIVE PREDICTIVE VALUE, AND NEGATIVE PREDICTIVE VALUE OF THE TWO SCREENING METHODS IN DETECTING CRE IN PERIANAL SWABS.

Screening Method	Sensitivity	Specificity	PPV	NPV
Direct Ertapenem Disk Method	71.43%	88.53%	10.20%	99.41%
Enrichment, Selective Broth Method	93%	87%	12%	100%

PPV, Positive Predictive Value; NPV, Negative Predictive Value

The estimated cost of screening for CRE colonization using the direct ertapenem disk method was calculated as follows: (1) $ 3.62 for no potential resistance; (2) $6.8 for oxidase positive, non-lactose fermenter e.g., *P. aeruginosa*; (3) $10.42 for confirmed susceptible or intermediate isolate to ertapenem; (4) $16.12 for both Carbapenem resistant, non-*Enterobacteriaceae* and Confirmed CRE. (Table 4.6) Results of true negative cultures are being reported within 1 day, while a true positive culture take a total of 3 days.

The estimated cost of surveillance testing using the enrichment selective broth method was calculated as follow; (1) $6.80 for no potential resistance; (2) $9.98 for the identification of an oxidase positive, non-lactose fermenter e.g., *P. aeruginosa*; (3) $13.90 for confirmed susceptible or intermediate isolate to ertapenem; (4) $19.60 for an isolate that was both carbapenem resistant, non-*Enterobacteriaceae* and Confirmed CRE (Table 4.6). Results of true negative cultures were reported within two days, while a true positive culture required a total of four days to reach a definitive identification.

TABLE 4.6 THE ESTIMATED COST AND TIME REQUIRED TO SCREENING ONE SAMPLE FOR CRE COLONIZATION USING EITHER METHOD

	Direct Ertapenem Disk Method		Selective Enrichment Broth Method	
	Cost	**Time**	**Cost**	**Time**
No potential resistance	$3.62	18-24 hrs. (1 day)	$6.80	36-48 hrs. (2 days)
Oxidase positive, non-lactose fermenter	$6.80	36-48 hrs. (2 days)	$9.98	54-72 hrs.(3 days)
Confirmed susceptible or intermediate isolate to ertapenem	$10.42	54-72 hrs.(3 days)	$13.90	72-96 hrs. (4 days)
Both Carbapenem resistant, non-*Enterobacteriaceae* and Confirmed CRE	$16.12	54-72 hrs. (3 days)	$19.60	72-96 hrs. (4 days)

DISCUSSION

Effectiveness, time to decision and cost are major challenges when considering optimal laboratory testing methods for universal surveillance testing. Each of these variables is important especially when the result can immediately alter both the care provided to the patient, namely initiation of isolation and enhanced infection control and the associated costs to provide the care. Screening specimens using a selective enrichment approach, employing TSB containing an ertapenem disk, causes the exposure of the entire patient specimen to ertapenem, thereby limiting the growth of susceptible organisms that might obscure the detection of CRE subpopulation by a non-selective approach. Additionally, as the broth enriches for the growth of resistant isolates such that they should be recovered on a non-selective medium, thus enhancing for sensitivity of the method. However, the additional step of sub-culturing the broth onto a solid medium delayed the recognition that a patient was colonized with CRE by at least a day.

The screening of surveillance specimens employing a MacConkey plate with an ertapenem disk might lead to less sensitivity of the assay for CRE isolates because the plate is only evaluated in an area in proximity to the ertapenem disk. Potential CRE isolates growing in this zone of inhibition around the antibiotic disk should be evident. However, a CRE subpopulation growing distant from the disk may be missed.

Despite the selective advantage of using a broth enrichment to enhance CRE recovery from patient samples, 13 additional isolates were recovered using the direct ertapenem disk method using stool specimens. There are two possible explanations for the failure of the selective enrichment broth to detect CRE. First, a carbapenemase

producing organism, either a CRE or a *non-Enterobacteriaceae,* may have hydrolyzed the ertapenem resulting in the survival of carbapenem susceptible organisms. Second, the microbial concentration in the stool inoculum might be too high and overwhelmed the ability of the antibiotic to limit the growth of the sensitive microbes resident in the broth. For the perianal swab specimens, one CRE isolate was missed when using the selective enrichment broth. That single isolate grew within 25 mm of the ertapenem disk while in the selective enrichment broth there was no detectable growth from that patient specimen subsequent to its subculture to the MacConkey plate.

The CDC recommended that the methods we employed be used for the detection of only *Klebsiella spp*. and *Escherichia coli.* However, since a significant number of CRE isolates recovered from clinical samples at MUH were non-lactose fermenting *Enterobacteriaceae,* such as *Serratia marcescens*, we expanded our surveillance screen to include non-lactose fermenting *Enterobacteriaceae*. This decision lowered the PPV of the CDC methods due to the higher recovery of non-lactose fermenting-non-*Enterobacteriaceae* species such as *A. baumannii* and *P. aeruginosa* (Table 4.3 & Table 4.5)

Given the cost of the additional confirmatory testing for the false positive isolates, the cost to identify one CRE isolate was re-calculated. For screening patient samples recovered from AAD specimens the cost to find one CRE isolate was $115.92 by the direct ertapenem disk method and $246.12 when using the method employing a selective enrichment broth.

For the perianal swabs, the cost to find one CRE isolate was $ 485.69 by the direct ertapenem disk method and $598.21 when using the method employing an enrichment selective broth. Based on this analysis we conclude that the direct ertapenem disk was the more cost-effective method for assessing whether or not a patient entering care was colonized with CRE.

Through screening stool samples and perianal swabs by the two methods recommended by CDC, we found that there was no statistical difference between the two methods. A previous study conducted by Lolans *et al* argued that the direct ertapenem disk method demonstrated higher sensitivity (97% vs. 65.6%) and specificity (90.5% vs. 49.6%) than the method employing a selective enrichment broth [27]. However, they placed only 25 µl of the enriched broth onto each MacConkey plate, which represented 25% of the volume prescribed by CDC protocol. Such a deviation from the CDC protocol may offer an explanation as to why we observed a lower sensitivity and specificity than what was observed when using the direct antibiotic disk methodology.

In retrospect a limitation to our study was that it was not sufficiently powered or designed to consider patients coincidentally screened for CRE using both remnant perianal swabs and remnant AAD samples. Of the 1200 patients evaluated, only 74 of the patients evaluated met this criterion during their first stay. Twenty additional patients fulfilled this criterion subsequent to discharge and readmission. The rate of CRE colonization for the 74 patients in this sub-arm was different from the rates observed in the AAD and perianal sampling arms. Two of the 74 patients meeting the criterion for this sub-arm were positive from both sample types. An additional patient was positive

only from his/her primary perianal swab. The difference was attributed to the fact that this one patient who was colonized with ertapenem resistant and meropenem susceptible *Enterobacteriaceae* species received meropenem in order to treat an ESBL respiratory infection that likely cleared his/her colonization by the time of the collection of the AAD specimen. The clearing of the colonization was evident from the absence of a CRE isolate in this patient's follow up perianal swab collected on the same day as the AAD sample.

CHAPTER 5

ASSESSING THE ECONOMIC AND MEDICAL IMPACT OF A SURVEILLANCE PROGRAM FOR CARBAPENEM-RESISTANT *ENTEROBACTERIACEAE* IN HOSPITALS WITH LOW CRE ENDEMICITY

DISCUSSION

Since the first CRE isolate was reported in 1996 in North Carolina, KPC producing variants of Carbapenem resistant *Enterobacteriaceae* (CRE) have been reported in every state except Idaho[28]. Several studies have reported a constant increase of CRE prevalence over the last two decades. For example, the CRE detection rate increased fivefold (0.26 cases per 100,000 patient days to 1.4 cases per 100,000 patient days) from 2008 to 2012 in community hospitals in the southeastern United States [29]. Higher prevalence rates have been reported in healthcare settings where CRE is endemic (~5%)[19, 30] or during outbreaks (10%)[20]. In contrast, MUH has a CRE infection acquisition rate of 3 per 100,000 patient days. This infection acquisition rate is considered to be a low prevalence rate and at this point in time a rate this low does not warrant universal surveillance.

In spite of the very low incidence rate for CRE infections at the MUH, approximately 0.03 CRE infections per 1,000 patient days, this study nevertheless set out to first determine the rate with which patients entering MUH were already colonized with CRE as well as those patients that would become colonized by these pathogens during their stay subsequent to their 7th day of admission. Second, we elected to evaluate whether or not a secondary analysis of specimens collected from patients considered to be at higher risk of being colonized with CRE might offer a medically superior and more

cost-effective approach to acquire the CRE colonization rate in order to limit the spread of CRE within the setting of healthcare, specifically among hospitalized patients through established infection control methods such as isolation and enhanced environmental cleaning.

Two approaches were conducted. The first required the collection of remnant perianal swabs from a modified universal surveillance program for VRE presently being conducted as a standard of care at MUH. These data served to provide a baseline rate of CRE colonization associated with patients entering care within our hospital. The second approach involved the collection of diarrhea stool samples from all patients presenting with symptoms associated with AAD. Samples collected from the AAD group enabled us to evaluate whether a targeted and more limited surveillance approach might offer hospitals a cost-effective alternative to detect changes to their CRE colonization pressure while the incidence of CRE infections is low.

We learned that in spite of an overall low incidence of CRE infections differences were observed between the rates of CRE colonization between the two patient populations considered. As the collection of samples was conducted under pragmatic conditions we have no definitive explanation to account for the differences observed between the two surveillance programs. In considering the rate of CRE colonization, through a secondary analysis of diarrhea stool specimens, we observed 3.0 colonizations per 1,000 patient days. These data revealed that the rate of colonization associated with hospitalized patients displaying AAD was 100-fold higher than the overall rate observed for patients contracting a CRE infection during their care. In contrast, the CRE

colonization rate observed from the samples collected from patients in the modified universal surveillance arm was found to occur at a rate of 1.8 CRE colonizations per 1,000 patient days, which was 60-fold higher than the overall rate of CRE infections observed.

It was not surprising that a greater percentage of patients screened in the AAD arm of the study were found colonized with CRE (6.2%). It was hypothesized this group of patients would be enriched for CRE carriage enabling us to screen fewer samples to determine the baseline CRE colonization rate. A previous study has reported that a course of antibiotics, within a 2-year window, would increase the likelihood of microbial dysbiosis resulting in loss of susceptible commensal organisms as well as potentially enabling antibiotic resistant microbes to flourish and similarly increasing the likelihood for horizontal gene transfer [31]. Reports in the literature support that the concurrent or prior exposure to a course of antibiotics is a known risk factor for the selection of CRE colonization [22]. However, when considering our CRE colonized patients the prior exposure to a course of antibiotics was not found to be a significant risk factor for CRE colonization ($p = 0.11$). In point of fact 97% of the CRE colonized individuals from this arm had received a course of antibiotics. While this may confirming the observation of an association of antibiotic use and CRE colonization it failed to achieve significance given that the specimens were biased. Further, our data showed that patients displaying symptoms of AAD also had a significantly higher likelihood of being colonized with VRE and/or having an ESBL or a CRE HAI.

Colonization with VRE was found to be a significant risk factor for coincident colonization by CRE (p=0.03). Of the diarrheal population whose VRE colonization status was known, 410 patients, 17.8% of them were VRE colonized and of those 13.7 % of the VRE positive patients were also CRE colonized; 5.6% of VRE negative patients were CRE colonized and 4.2% of VRE status unknown patients were also CRE colonized. Consequently, while coincident colonization was significantly associated between these two antibiotic resistance markers using only VRE colonization as surrogate marker for CRE colonization would have missed a substantial fraction of the CRE colonized individuals.

Of the 600 patients evaluated 4 were infected with CRE at the time of sampling and thus it was not surprising that a CRE infection was a significant predictor for colonization by CRE. The presence of *C. difficile* toxin was not a significant predicator of CRE colonization risk. However, LOS, 18 days vs. 14 days (p=0.01), and the age of the patient, 53.1 years vs. 61.8 years, were found to predispose patient to an increased colonization risk. It has been noted in recent studies that CRE colonized patients were younger than non-colonized patients and the differences were also considered significant but an explanation accounting for this observation is unknown [32, 33].

The advantage of repurposing existing clinical specimens to both the patient and hospital are two-fold. First, patients need not to be subjected to the minimal risk of acquiring an additional sample. Second, the hospital need not incur the cost of collecting and transporting additional samples to the laboratory thereby eliminating the cost of collecting a sample solely for CRE assessment. Of the 600 patients evaluated from

modified universal surveillance arm of the study, 1.83% were found to be CRE colonized. Fifty percent of the CRE colonized patients entered care already colonized. The remaining patients became colonized during care. Like the patients in the AAD arm of the study, here too VRE colonization was found to be significant risk factor for CRE colonization ($p<0.0001$). Of the 600 patients evaluated 5.5% of them were colonized by VRE. Only 15.2% of the VRE positive patients were colonized by CRE while 1.1% of the VRE negative patients were colonized by CRE. An evaluation of the VRE positive samples alone would have missed approximately 55% of CRE colonized patients from this arm of the study. Thus, VRE colonization again could not be used a surrogate for CRE colonization status from this cohort of patients. In contrast to the AAD arm of our study LOS and patient age were not found to be significant risk factors in predicting risk of CRE colonization.

Use of remnant AAD specimens submitted for *C. difficile* toxin testing significantly improved the sensitivity of the surveillance program by increasing the detection rate for CRE colonized patients by approximately 3-fold. An average of 39 VRE perianal swabs were collected each day during the 3-month study period providing us with an initial estimate for CRE colonized patients of approximately 1.8% of the patients seeking care or 1.75 CRE colonizations per 1,000 patient days. In contrast, the average number of AAD samples collected each day was 4.5; the rate of CRE colonization detected using this type of patient sample was higher, yielding a rate of approximately 6.2% or 3.0 CRE colonization per 1,000 patient days. Thus, a greater number of perianal samples would be required to be processed from the modified

universal surveillance arm in order to detect whether or not there was a change to the CRE colonization rate for patients entering care at MUH. Considering the two rates, 67.6 perianal swabs would have required screening to identify one CRE colonized individual from the universal surveillance program. However, just 19.2 AAD specimens would need to be screened in order to detect one CRE colonized individual in the targeted surveillance program. Considering the surveillance program type, the targeted surveillance program was significantly better than the universal surveillance program ($p=0.0002$) at detecting CRE colonization from patients entering care at MUH.

In an effort to calculate the cost associated with CRE infections, Bartsch *et al* found that depending on the infection type, the median cost of a single CRE infection can range from $22,484 to $66,031 for hospitals, $10,440 to $31,621 for third-party payers, and $37,778 to $83,512 for society[34]. This raises a critical question regarding the economic benefits that infection control measures coupled to a universal surveillance program might offer hospitals at first controlling spread and secondly reducing the costs associated with CRE infections. L. Lapointe-Shaw *et al* created a model to assess the cost effectiveness of screening all hospital inpatients for carbapenemase-producing *Enterobacteriaceae* (CPE) at the time of hospital admission[35]. Their model determined that universal screening would be a cost effective or even cost saving approach if the CPE prevalence rate was higher than 0.3%.

Because the cost for surveillance programs is not reimbursable, any effort to limit this cost will increase the likelihood of adoption. The estimated cost for primary screening for a negative culture was found to be between $3.62 and $6.80 based on the

screening method used inclusive of the supplies and labor expenses. The confirmatory testing for each putative resistant isolate added $12.50 to the cost resulting in a total cost of between $16.12 and $19.60 per isolate from each patient sample. Facilities having a higher CRE infection incidence will generate fewer negative cultures that will in turn reduce the overall cost to detect one CRE-positive patient in contrast to the cost required to detect one CRE-positive patient in a facility with a lower prevalence rate for CRE infections. At prevalence rates of between 1.8% and 6.2%, 67.6 perianal swabs specimens and 19.2 diarrhea specimens were required to be tested in order to locate one CRE colonized patient. The cost to identify one CRE carrier thus was between $485.69 to $598.21, when considering samples from an approach requiring universal surveillance, with the cost for the more limited but targeted surveillance approach between $115.92 and $246.12. An approach requiring fewer samples would be more likely to be considered for adoption as a consequence of its lower overall cost as long as the approach was at least as sensitive.

Rapid and accurate recognition of CRE colonized patients would enable infection control an opportunity to limit the risk of intra-hospital transmission of this class of microbes owing to the increased awareness of the presence of these microbes offering the hospital an opportunity to implement the CDC prescribed intervention for enhanced infection control and patient isolation prior to development of infections or an outbreak. In support of this strategy consider that 50% percent of the CRE colonized patients detected from the universal surveillance arm of our study entered care already colonized. The remaining patients became colonized during their care.

Unlike universal surveillance, diarrhea stool specimens were only collected when patients displayed symptoms consistent with AAD, typically 3 diarrhea stools within 24 hours, without other causes for diarrhea, such as laxative administration or underlying clinical disease. These symptoms could be displayed at time of admission, as a consequence of a previous antibiotic exposure, or days later as a result of antibiotic exposure during the patient's current hospitalization. The delay in recognizing patients colonized with CRE while awaiting diarrhea to develop, and then testing the diarrhea stool specimen for CRE colonization, may result in significant environmental contamination thereby increasing the likelihood of spread within the unit currently caring for the patient.

Because MUH currently does not have a CRE surveillance program, Infection Preventionists monitor CRE acquisition rates based on isolates recovered from clinical cultures obtained after 48 hours of admission from patients with no prior history of CRE and no symptoms of infection. Annual HAI and acquisition rates suggest that there is one HAI for every 2 CRE acquisition case. When this ratio was compared to MRSA (1:3.76) and VRE (1: 32.53) HAI: acquisition ratio, it suggests that CRE surveillance is warranted. However, MUH's current CRE acquisition rate may be masking the prevalence of CRE within the hospital. Analyzing the data generated from our study, the mean CRE HAI rate to colonization rate obtained from the modified universal surveillance samples was 1:58.33, while the ratio obtained from the AAD samples was 1:101.33. Since our observed rate for CRE colonization was one-hundred-fold higher than the current CRE infection rate for MUH, we conclude that a universal surveillance

program is not cost effective to implement using either method recommended by the CDC.

Our study was not powered to determine the tipping point at which time the rate of CRE infections would increase in our hospital that would make any type of surveillance program cost effective. Given the effectiveness of screening AAD patients for CRE colonization, a limited, periodic determination of the CRE colonization ratio in comparison to the current CRE infection rate might be sufficiently informative to enable the MUH quality team to address whether or not there are changes to the endemicity of CRE, and thus the risk to our patients of acquiring a CRE infection to their course of stay in our facility.

This study has two limitations. First, between the time that the study was designed and completed, MUH moved from universally patients screening for VRE to a limited form of universal surveillance for VRE, where only those patients housed in units where the risk of VRE colonization was considered to be high were tested. The samples in this arm of the study would no longer be a representative sample of all patients entering care at MUH. However, we nevertheless elected to continue with the study under pragmatic conditions justifying that the patient samples collected from high risk VRE units would yield a similar, if not equivalent, representative sample of patients entering care at MUH.

Second, antibiotic exposure data was collected only during the current admission; antibiotics received during recent prior hospitalization(s) or outpatient visits were excluded. Since 70% of patients in the universal arm had antibiotics during the current

admission, to add data from these excluded sources would increase the numbers of patients on antibiotics making antibiotic exposure even less of a predictor. Thus, conclusions regarding the impact of antibiotics in developing CRE colonization in our patients cannot be made. Future studies regarding the exact impact of antibiotics in developing CRE colonization is needed.

CHAPTER 6

PERSPECTIVE

CRE have emerged as a significant cause of HAI resulting in significant morbidity and mortality. Here we learned that in a facility with low CRE infection endemicity, the use of a targeted surveillance approach for determining the frequency of CRE colonization among patients entering care was found to be more sensitive for determining the CRE colonization risk than an approach using remnant samples collected using a universal surveillance strategy. The rate of CRE colonization was one-hundred fold higher than the current CRE infection rate for MUH. Given this one hundred-fold difference by this family of pathogens we can conclude that presently a universal surveillance program would likely not be cost effective to implement using either method recommended by the CDC. While this study was not powered or designed to consider patients coincidentally screened for CRE the data suggested that a universal surveillance program using a perianal swab would likely be a highly effective approach given a more rapid, less expensive and readily commercially available detection method for isolating members from this family of organisms.

CHAPTER 7

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