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**Predictors of Carbapenem Resistant Gram-negative Bacteria in a Consortium of
Academic Medical Center Hospitals**

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Dedication

This dissertation is dedicated to the wonderful blessing in my life, my son, Kareem. Watching you growing amazingly every day gave me the strength to challenge myself and assured me that I made the right decision.

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This dissertation would not have been possible without the contribution of great people, whom I was lucky to have.

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List of Abbreviations

CR-PA.....	Carbapenem-Resistant <i>Pseudomonas aeruginosa</i>
CR-KP.....	Carbapenem-Resistant <i>Klebsiella pneumoniae</i>
KPC.....	Carbapenamase-producing <i>Klebsiella pneumoniae</i>
ESBLs.....	Extended Spectrum beta-Lactamases
CRE.....	Carbapenem-Resistant <i>Enterobacteriaceae</i>
UHC.....	University HealthSystem Consortium
CMI.....	Case Mix Index
CDC.....	Centers for Disease Control and Prevention
MHT.....	Modified Hodge Test
GEE.....	Generalized Estimating Equations
GLMM.....	Generalized Linear Mixed Models
AIC.....	Akaike's information criterion
QIC.....	Quasi-likelihood under the independence model criterion

Abstract

Background: Gram-negative resistance is a growing problem worldwide. It is generally believed that rates of resistant bacteria within a hospital are a function of antibiotic use, resistant organisms brought into the hospital, infection control efforts, and underlying severity of patient illness. The relative contribution of each to a particular resistance phenotype is unclear. *P. aeruginosa* is responsible for many hospital acquired infections and it may become resistant to carbapenems. In addition, newer threats to the future utility of the carbapenems are carbapenemase-producing *K. pneumoniae*

Purpose: To determine if there is an association between the volume and composition of antibiotic use, geography, severity of illness and rates of carbapenem-resistant *P. aeruginosa* and *K. pneumoniae*.

Methods: This is a retrospective ecological longitudinal investigation within the University HealthSystem Consortium affiliated academic medical centers. Antibiotic use data between January 1, 2006 and December 31, 2009 were obtained from billing records and reported as days of therapy per 1000 patient days (DOT/1000 PD), in addition to hospital characteristics (e.g. geographical location, bed size, case mix index). “Whole house” antibiograms were obtained to determine rates and proportions of carbapenem-resistant *P. aeruginosa* (CR-PA) and carbapenem resistant *K. pneumoniae* (CR-KP). Also, CR-KP isolation was generated as a binary outcome. Generalized estimating equations (GEE) were used to model CR-KP and CR-PA.

Results: CR-KP rates (1000PDs) increased from 0.07 in 2006 to 0.15 in 2009 ($P=0.0118$) and CR-KP proportions increased from 1.3% in 2006 to 3.1% in 2009 (0.0003)

within 40 hospitals over 2006-2009. However, CR-PA rates and proportions were stable over the same period. Geographical location, carbapenems use, and antipseudomonal penicillins use were significantly associated with CR-KP isolation. Thus, for every ten DOT/1000 PDs increase in carbapenem use, the odds of CR-KP isolation increased by 42% (P=0.0149). In contrast, for every ten DOT/1000 PDs increase in antipseudomonal penicillin use, the odds of CR-KP isolation decreased by 14%. However, there was no significant model to explain CR-PA rates and proportions.

Conclusion: Carbapenems, antipseudomonal penicillins, and geographical location were identified as risk factors associated with CR-KP isolation. These findings emphasize the challenges associated with the treatment of multidrug- gram-negative bacteria.

CHAPTER 1

Introduction

1.1 Overview of the document

This dissertation describes a study designed to explain carbapenem resistant gram-negative bacteria in a consortium of academic medical health centers located across the U.S. Antimicrobial drug use, severity of illness, and hospital demographics were investigated as potential predictors of carbapenem-resistant *P. aeruginosa* (CR-PA) and carbapenem resistant *K. pneumoniae* (CR-KP). This chapter provides background information necessary to understand the significance of this project. The second chapter reviews the available literature and provides more extensive background from previous investigations; including epidemiology CR-PA and CR-KP, previously described risk factors, and confounding factors. Chapter 3 describes the methodology used for the dissertation project. The results are provided in Chapter 4, followed by a discussion and concluding remarks in Chapter 5.

1.2 Background

Multidrug-resistant gram negative bacteria

The antimicrobial drug resistance problem is increasing in the U.S. and worldwide. This issue is getting more complicated because of two factors: emergence of multidrug-resistance (MDR) gram-negative organisms; and the shortage of new, effective treatment options against these resistant bacteria.[1-8] The current situation is demanding new methods to improve antibiotic drug use in the clinical setting. Antimicrobial Stewardship Programs (ASPs) are evolving all over the United States hospitals; their mission is to improve the quality of antibiotic use, treatment outcomes, and cost.[9- 12] This is valuable as antibiotic use at both the patient- and aggregate-level has been shown to play a major role in bacterial resistance.[13-16] In addition, the relationship between antibiotic use and bacterial resistance is further complicated by patient risk factors and infection control measures which vary among health care settings. [15, 17] The composition of patients within each hospital varies from one hospital to another. Severely ill patients will presumably be at greater risk to develop infections caused by resistant organisms; because they have impaired host defenses and will receive more antibiotics compared to low risk patients.

Carbapenem antibiotics

Despite the increase in resistance rates against many beta-lactams, carbapenem antibiotics still retain activity against most organisms and are considered the drug of choice against extended-spectrum beta-lactams (ESBLs) producing

Enterobacteriaceae, such as *E. coli* and *K. pneumoniae*. [18-20] Thus, developing resistance against this class of antibiotics is considered a serious problem since the remaining options, colistin or tigecycline, are more toxic and possibly less effective.[21] Moreover, infections with carbapenem resistant gram-negative organisms are associated with high rates of mortality and cost.[22-24]

Carbapenem-resistant gram negative bacteria

Carbapenem-resistant *Klebsiella pneumoniae* (CR-KP) is of special concern because of its rapid spread, microbiological detection challenges, poor health outcomes, and it is resistant to most antimicrobials.[25-29] Outbreaks of *Klebsiella pneumoniae* carbapenamase (KPC)-producing *Enterobacteriaceae* have been reported in the U.S. (with geographical variation) and all over the world. [30-35] According to the Centers for Disease Control and Prevention (CDC), KPC has been reported in 35 states in the U.S. in 2010 compared to one state in 2001.[36] In addition, carbapenem-resistant *P. aeruginosa* (CR-PA) is often a multi-drug resistance bacteria that is associated with significant health outcomes and difficult to be treated with complete eradication.[37,38]

Cofounding in Pharmacoepidemiology

Any epidemiologic investigation attempting to describe the relationship between an exposure and an outcome must consider potential confounders. Confounding occurs when a certain variable(s) influence the relationship between an exposure and outcome.[39] The presence of confounding can result in overestimation of the association of the true association, underestimation of the true association, or change in the direction of the association.[40] Therefore, it is imperative to identify confounders

and control for them. In most situations, confounders are identified in advance based on previous investigations or expert knowledge.[39,40] Severity of illness, hospital bed size, and geographical location were identified as confounders.[15, 16, 41] Multivariable modeling is one way to control for confounders at the level of statistical analysis. [42]

Previous investigations

Prior antibiotic use (carbapenems, fluoroquinolones, and extended-spectrum cephalosporins) and certain patient risk factors (e.g. ICU admission, mechanical ventilation, length of hospitalization) were found to be independent risk factors associated with CR-KP in several single centered case-control studies.[22,29,42-44] These studies were of small sample size, not geographically distributed across the nation which limit their generalizability. In addition, prior carbapenem use and other patient factors (e.g. Foley catheters transfer from outside facility, mechanical ventilation) were identified as independent risk factors associated with CR-PA in case-control studies.[23,24,45,46] One multicenter study did not find an association between carbapenem use and CR-PA in a model that is not adjusting for hospital demographics.[47]

Preliminary results

The preliminary results reported trends in hospital antibacterial drug use in adult patients from 22 US academic medical centers, all of which participated in UHC programs. A significant finding was that broad-spectrum antibiotic use is increasing, in part due to an overall increase in carbapenem use.[48] Also, using the same set of hospitals they found that hospitals which restrict carbapenem antibiotic use (e.g.

imipenem, meropenem) actually use less carbapenem, and rates of CR-Pa are significantly lower.[49]

1.3 Purpose

This study investigated carbapenem-resistance gram-negative bacteria: *Pseudomonas aeruginosa* and *Klebsiella pneumoniae*. The purpose of this investigation is to assess how different “patterns” of antimicrobial drug use, severity of illness, and geography to different patterns of carbapenem resistance. The hypothesis is that a significant portion of the between-hospital variability in rates of CR-PA and CR-KP can be explained by the combination of antimicrobial drug use, severity of illness, and hospital demographics

1.4 Objectives

- 1- Determine trends and patterns of overall gram-negative antibacterial drug use over the study period (2006-2009).
- 2- Determine proportions, rates, and trends of resistance among CR-PA at each of the participating hospitals during the study period.
- 3- Determine proportions, rates, and trends of resistance among CR-KP at each of the participating hospitals during the study period. Also, identify clinical microbiology practices related to the isolation of these organisms.
- 4- Develop a model to determine the magnitude and the significance of association between the use of specific antibiotics, severity of illness, geography, and carbapenem-resistant gram-negative bacteria (CR-PA and CR-KP).

1.5 Significance and practical implications

The preliminary data, as well as data from others, suggest that carbapenem antibiotic use and other antibiotic use might be risk factors for CR-PA and CR-KP, but this relationship is complex. Also, there are no investigations at a national level that have examined at the role of antibacterial drug use in CR-KP in geographically distributed hospitals. It is important to understand the relationships between antibiotic use, patient composition, infection control, and carbapenem resistance to overcome the burden of these resistant pathogens, especially, with the paucity of new antimicrobial drugs. This study is the first multicenter study that attempted to assess the impact of aggregate antibiotic use and other predictors on CR-KP in a relatively large sample of U.S. academic medical centers. This investigation extend previous investigations of CR-PA by adjusting for important hospital characteristics (e.g. geographical location, bed size) The contribution with this study is the assessment of CR-PA and CR-KP current status and associated risk factors in U.S. academic health centers. If modifiable risk factors such as, antibiotic use was identified, this could serve as a target of intervention for antimicrobial stewardship programs to improve resistance patterns in hospitals. In addition, this investigation could provide hypotheses to conduct further studies, such as quasi- experiments to assess the effect of the potential modifiable factors on resistance and to establish a causal relationship, if any.

CHAPTER 2

Literature Review

2.1 Multidrug-resistant gram-negative bacteria and antimicrobial drug use

The selection of antibiotic-resistant bacteria has major consequences at both the patient-and hospital-level. It would increase the risk of colonized patients to develop a true clinical infection and increase the risk of cross-transmission among hospitalized patients and further spread of resistance in the community.[4,50] While antibiotic resistance may develop in the absence of antibiotic pressure, antimicrobial usage has been identified as one of the most important risk factors driving the development of resistance in many studies.[16] Both overuse of a restricted number of antibiotic agents and homogeneous use raise the effect of selection pressure.[4, 16] However, data to support the preceding 'beliefs' are few and the existing data are primarily restricted to single institutions.[13]

Multidrug-resistant (MDR) gram-negative bacteria are often defined as ones that are resistant to three or more classes of antibiotics through different mechanisms.[1, 2, 4]

The molecular mechanism of multidrug resistance is complicated. The selection pressure imposed by the use of one antibiotic can result in the selection of bacteria resistant not only to that particular antibiotic but to other antibiotics, by co-resistance and cross-resistance mechanisms.[50,51] Mechanisms of acquired resistance include: gene mutations resulting in altered porin channels limiting penetration of the antibiotic

into the bacterial cell; production of enzymes that inactivate the antibiotic; alteration of the target site for the antibiotic; or formation of efflux pumps extruding the antibiotic from the cell interior before it can act on its target.[52] One example is the overuse of fluoroquinolone agents, which had led to development of aggressive resistance mechanisms such as “upregulated efflux pumps” in *P. aeruginosa* which was resistant not only to fluoroquinolones but also carbapenems (so-called collateral damage).[51,53] Actually, all classes of antibiotics except polymyxins are susceptible to extrusion by any of these efflux systems.[54]

For gram-negative organisms, the impact of infection control measures seems to be less effective than antibiotic-control measures when compared to gram-positive organisms such as methicillin-resistant *S.aureus* (MRSA); thus, the relative contribution of infection control in nosocomial gram-negative bacteria remains more controversial.[55] The clinical epidemiology of resistant bacteria may alter over time. For example, one study found prior fluoroquinolone use to be associated with fluoroquinolone-resistant *E. coli* colonization in a relationship that varied significantly by the study year.[56] Interventions to improve hospital antibiotic use were associated with a decline in the incidence of antimicrobial resistant bacteria.[57] However, this may differ substantially across institutions due to the variability of the composition of patients.[58]

2.2 Measures of bacterial resistance

Schwaber et al. has argued that the use of proportions of resistant organisms to evaluate the influence of antibacterial use on resistance can be misleading and biased.[59] Proportions are dependent on the susceptible and resistant bacteria populations, whereas the incidence rate of resistance depends only upon the resistant population. He further explained that antibiotic use likely leads to a decrease in the absolute number of susceptible organisms, which results in an increase in the proportion of isolates that are resistant in a certain population. However, this does not necessarily reflect an increase in the absolute number of resistant isolates or the burden of resistance.[59] One investigation looking at the impact of ciprofloxacin restriction on both proportion and incidence rates of resistant isolates of *P. aeruginosa* found that ciprofloxacin use declined by 57% and both incidence rates and the proportion of ciprofloxacin-resistant *P. aeruginosa* isolates declined significantly. [60] In contrast, another investigation reported that the incidence rates of MRSA bacteremia in patients with central lines from 1,684 U.S. intensive care units decreased by nearly 50% over the 10-year period 1997 to 2007, while the proportion (percent) increased by 25%.[61]

2.3 Carbapenem-resistant *P. aeruginosa*: status and implications

Overview

P. aeruginosa is a gram-negative rod that is considered to be one of the leading causes of nosocomial (hospital acquired) infections including healthcare associated pneumonia and ventilator associated pneumonia. [62,63] Carbapenem antibiotics (e.g. imipenem, meropenem, and doripenem) continue to be the most consistent effective agents in the treatment of *P. aeruginosa* infections. However, it is often difficult to treat *P. aeruginosa* with complete eradication due to its ability to develop resistance to multiple classes of antibacterial drugs, including carbapenems, in the process of treating an existing infection.[64,65]

Multidrug-resistant *P. aeruginosa*

Multidrug-resistant *P. aeruginosa* is often defined as resistance to three or more classes of antibiotics through different mechanisms including production of beta-lactamases, upregulation of multidrug efflux pumps, and cell wall mutations. Up-regulated efflux can concurrently compromise fluoroquinolones and most beta-lactams leaving only aminoglycosides and carbapenems susceptible, although the latter have high mutational frequency. A combination of up-regulated efflux, loss of OprD, and impermeability to aminoglycosides compromise all class of antibiotics except the polymyxins.[66] Infections with MDR *P. aeruginosa* are associated with an increase in rates of morbidity, mortality, the need for surgical intervention, length of hospital stay, and overall cost of treatment.[67-69]

Current status

The Surveillance Network Database USA showed an increase in the proportion of *P. aeruginosa* isolates that are resistant to multiple antibiotics over the period 1997-2000. Sixteen percent of these isolates are resistant to ≥ 3 antipseudomonal agents (imipenem, amikacin, ceftazidime, ciprofloxacin, gentamicin, and piperacillin-tazobactam). [66] According to the National Nosocomial Infection Surveillance System (NNIS), the rates of imipenem-resistant *P. aeruginosa* associated with nosocomial infections increased by 15% between 1998 and 2002.[70] In contrast, a 2009 report of 10 year trends in antimicrobial susceptibility among 10-15 U.S. hospitals contributing to the Meropenem Yearly Susceptibility Test Information Collection (MYSTIC) surveillance project found that susceptibility to carbapenems for *P. aeruginosa* has not changed.[71]

2.4 Carbapenem-resistant *K. pneumoniae*: status and implications

K. pneumoniae is a gram-negative, facultative anaerobic bacillus that is responsible for many infections including urinary tract infections, pneumonia, bloodstream infections, and intra-abdominal infections. Resistance to penicillin, cephalosporin, and fluoroquinolone antibiotics has become more common among these organisms over the years. Resistance to extended-spectrum cephalosporins, which occurs through the production of ESBLs, has been described worldwide for the past twenty years.[62,63] Carbapenems have been the most important antimicrobial class for the treatment of these organisms especially with the increasing incidence of fluoroquinolone resistance among *Enterobacteriaceae* including *K. pneumoniae*, and they are the drug of choice to treat serious infections caused by ESBLs. [5, 7]

The two most important phenotypes of carbapenem-resistant *Enterobacteriaceae* in particular for this investigation in *K. pneumoniae* are the production of the serine-carbapenemase (KPC) and the metallo-beta-lactamase VIM.[3] Until recently, resistance to carbapenems has been uncommon among *K. pneumoniae* in the United States. However, the emergence of KPC and an Ambler molecular class A enzyme that utilizes serine at the active site and thereby is able to hydrolyze carbapenems and all other beta-lactam antibiotics have led to the increased prevalence of carbapenem-resistant *K. pneumoniae* in the U.S.[21] *Klebsiella pneumoniae* carbapenemase are encoded by the gene blaKPC, whose potential for inter-species and geographic dissemination is principally explained by its location within a Tn3-type transposon, Tn4401. This transposon is a genetic element which is able of inserting into various

plasmids of gram-negative bacteria. Plasmids carrying blaKPC are frequently associated with resistance determinants for other antibiotics. [26, 27] Although KPCs are identified in many gram-negative species, *K. pneumoniae* is the predominant species carrying this enzyme.[26] The second common mechanism after KPC is the production of metallo-beta-lactamases of amblor class. Acquired metallo-beta-lactamases (MBLs) most commonly IMP- and VIM-type, are zinc-dependent enzymes that have been of growing concern over the last decade. This is due to their capacity to readily hydrolyze most of the beta-lactam antibiotics, including carbapenems, and their increasing dissemination among gram-negative pathogens.[72]

Another mechanism associated with carbapenem resistance in *K. pneumoniae* is the loss of one or both of the porins OmpK35 and OmpK36. Loss of OmpK35 or OmpK36 may increase the MICs of carbapenems, and if the strain also produces beta-lactamases and carbapenemases, it may result in carbapenem resistance.[73,74]

The first KPC-producing *K. pneumoniae* isolate was reported in North Carolina in 2001. Afterwards, outbreaks and transmission of KPC-producing organisms were reported in the U.S. with geographical variations, as the northeastern part of the nation had several outbreaks.[30-32] The initial outbreaks were located in New York City. In one surveillance study conducted in hospitals in Brooklyn in 2003, out of 602 *K. pneumoniae* isolates identified a total of 3.3% were found to carry blaKPC. In the next year, two hospitals in the same city reported outbreaks with an increased number of carbapenem-resistant *K. pneumoniae*. [30] Later on, KPC-producing bacteria have spread throughout the U.S. and worldwide [33-35]. According to CDC, KPC-producing bacteria have been reported in 35 states in the U.S. in 2010 compared to one state in 2001.[36]

Moreover, 8% of all *Klebsiella* isolates reported to CDC at 2008 were resistant to carbapenems compared to less than 1% in 2000. [28] Further, the National Healthcare Safety Network (NHSN) data from 2006 through 2007 reported the percentage of *K. pneumoniae* isolates that were resistant to carbapenems by infection type: central venous catheter associated bloodstream infection (10.8%); catheter associated urinary tract infection (10.1%); surgical site infection (5.2%); and ventilator associated pneumonia (3.6%).[75] In the same MYSTIC surveillance project report mentioned in section 2.3, the incidence of CR-KP was shown to decline in 2008 compared to the steep increase in resistance rates observed from 2004 to 2007. [71]

2.5 Clinical outcomes and treatment options of carbapenem-resistant *K. pneumoniae*

The spread of KPCs has become a significant problem due to poor outcomes such as, increased cost, length of hospitalization, frequent treatment failure, and high mortality rates (30%-57%).[22, 28,29] Poor outcomes resulting from clinical infections with KPC-producing bacteria have been reported since the first outbreaks in New York City hospitals.[30] In one retrospective cohort study, 44% of CR-KP patients died, whereas 12.5% of carbapenem-susceptible *K. pneumoniae* patients died. Also, CR-KP isolation was identified as an independent predictor of in-hospital mortality.[42] Another cohort study found that KPC-producing *K. pneumoniae* cases were associated with a greater than two fold increased risk of death when compared to susceptible *K. pneumoniae* cases.[22]

In addition to poor outcomes associated with KPC-producing *K. pneumoniae*, treatment options are very limited. Clinicians are becoming more dependent on polymyxins, and tigecycline for the treatment of these infections.[76] One literature review, found that treatment with aminoglycosides, polymyxins combinations, and tigecycline had higher rates of success compared to carbapenem and polymyxin monotherapy which had lower rates of success.[77] Polymyxins are in the class of cyclic polypeptides antibiotics; polymyxin B and E (colistin) are available in the market. In vitro susceptibility of KPC-producing isolates to polymyxins is 90-100 %. Polymyxin use is infrequent; mainly because of their associated neurotoxicity and nephrotoxicity.[27, 77] Combining polymyxins with other antibiotics in treating KPC-producing isolates has been associated with a better success rate. In one study, 25% of patients treated with polymyxin monotherapy developed resistance during treatment, whereas none of the patients treated with a polymyxin-tigecycline combination developed resistance.[78]

Tigecycline is a novel semisynthetic glycylicycline, which is used in the treatment of severe infections caused by multidrug-resistant gram-negative bacteria including KPC-producing bacteria. [21, 27] Although in vitro susceptibilities of KPC-producing bacteria are 100% against tigecycline, treatment failure has been reported. According to one literature review, five out of seven patients were treated successfully with tigecycline. One case of recurrent infection was associated with a tigecycline MIC increase from 0.5 to 2 mg/L. However, tigecycline has low serum concentrations and urine concentrations. [26]

Aminoglycosides are considered valuable therapeutic options for treating KPC-producing bacteria, but resistance is increasing towards these agents; susceptibility

should be always evaluated, and preferably these agents should be used in combination. [26, 27]

2.6 Detection of carbapenemase-producing *K. pneumoniae*

Detection of KPCs by the microbiology laboratory represents a challenge as they may not be detected by routine antibiotic susceptibility testing methods. [21, 25, 27] A number of KPC-producing bacteria have been reported as susceptible to carbapenems as they have carbapenem MICs that remain in the susceptible range.[21, 25] The presence of KPC does not always result in high-level resistance to carbapenems, but may represent MIC elevations that stay within the susceptible or intermediate range.[77] Experts consider detection of KPC-producing bacteria susceptibility to be difficult with any microbiological technique.[21] Detection of KPC using automatic antibiotic susceptibility testing methods was shown to provide inconsistent results and considered problematic. Hence, more complicated, confirmatory, and phenotypic methods are needed to confirm KPC. Confirmatory methods include: modified Hodge test; polymerase chain reaction (PCR); inhibitor based test; EDTA-based synergy tests; and Etests.[25, 77, 79-83] The gold standard method to confirm the presence of a KPC is spectrophotometry followed by PCR of the blaKPC gene.[21, 77] However, this method is time consuming, costly, and requires reference laboratories to verify results.[21,77] Susceptibility testing of KPC-producing bacteria using Etest method is considered difficult to interpret as the scattered inner colonies can make the inhibition zone difficult to read.[21, 77, 79] Modified Hodge test has high sensitivity (95%-100%) to detect diffusible carbapenemase and it's the only method recommended by the Clinical

Laboratory Standards Institute (CLSI) in 2009 to confirm KPC-producing bacteria. [84] That is, for an *Enterobacteriaceae* with elevated MICs to carbapenems (2 -4 µg/mL) or a reduced disk diffusion zone, it should be tested with MHT to confirm carbapenemases.[84] The test is performed by culturing susceptible isolates on a Mueller-Hinton plate. Then the carbapenem disk is placed in the center, and isolates suspected of producing carbapenemases are streaked from the disk to the outer margin of the plate. Growth of bacteria like *K. pneumoniae* near the disk or along the isolate streak indicates the presence of carbapenemases (figure 2.1, A).[21, 26] However, the interpretation of this test can be difficult for some isolates resulting in false positive detection. [21, 25, 26] More sophisticated techniques like PCR-based techniques are more specific and sensitive with a major advantage of shorter time to results but require technique knowledge, equipment, and are more costly. [21, 84] Recently, in June 2010 the CSLI lowered carbapenems breakpoints, to better detect KPCs without the need to perform the modified Hodge test. [85] Table 1.1 describes the old and the new breakpoints.

Table 2.1 Clinical and Laboratory Standards Institutes breakpoints for carbapenems and *Enterobacteriaceae* [80]

Agent	Older CLSI breakpoints (M100-S19) MIC($\mu\text{g/mL}$)			Revised CLSI breakpoints (M 100-S20) MIC ($\mu\text{g/mL}$)		
	S	I	R	S	I	R
Imipenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Meropenem	≤ 4	8	≥ 16	≤ 1	2	≥ 4
Ertapenem	≤ 2	4	≥ 8	≤ 0.25	0.5	≥ 1
Doripenem	≤ 1	2	≥ 4

Susceptible = S, Intermediate = I, Resistant=R, Minimum inhibitory concentration= MIC

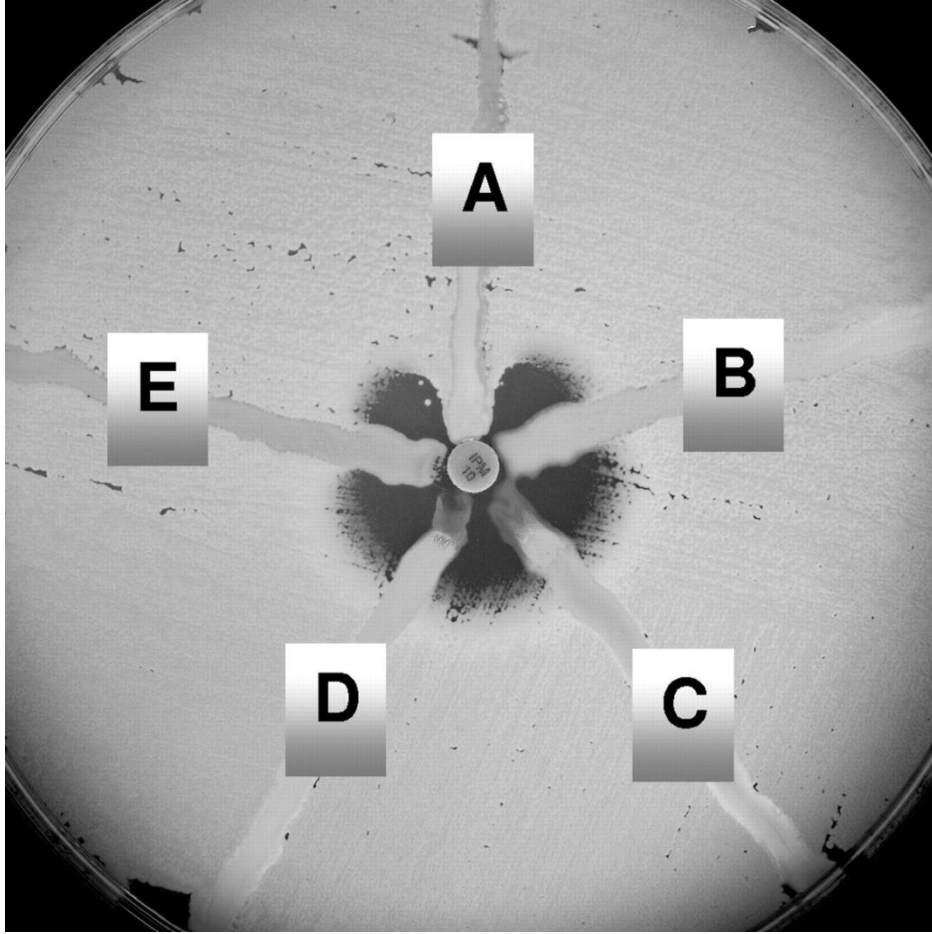


Figure 2.1 The modified Hodge test Mueller-Hinton agar plate. The MHT identified Isolate A as a positive KPC-producing isolate, while isolates B, C, and D were not KPC-producers. Adapted from Anderson et al. (2007). [25]

2.7 The role of hospital infection control

In general, prior antibiotic use has been strongly implicated in the development of resistance. However, exposure to resistant bacteria from other sources, like other patients, healthcare personnel, or inanimate objects has also been associated with the emergence of resistant bacteria.[86]

For gram-negative organisms, the impact of infection control measures seems to be less effective than antibiotic control measures when compared to gram positive organisms such as MRSA, thus the relative contribution of infection control in nosocomial gram-negative bacteria remains more controversial.[61] However, enforced infection control measures were found to decrease the spread and the incidence of gram-negative bacteria like carbapenem-resistant *Enterobacteriaceae* (CRE) in many outbreaks. For example, an implementation of a comprehensive infection control intervention, composed of intensified infection control measures with routine rectal surveillance, was successful in reducing the incidence of CR-KP in an intensive care unit where strains producing the carbapenemase KPC were endemic.[87] A quasi experiment found the use of active surveillance and contact precautions, as part of a multifactorial intervention, to be an effective strategy to decrease rates of nosocomial transmission of carbapenem-resistant *K. pneumoniae* colonization or infection.[88] Another quasi-experiment in a long-term acute care hospital showed the implementation of bundled interventions: daily 2% chlorhexidine gluconate baths for patients; enhanced environmental cleaning, surveillance cultures at admission; serial point prevalence surveillance (PPS); isolation precautions; and training of personnel resulted in preventing horizontal spread of KPC-producing gram-negative rods, despite ongoing

admission of patients colonized with KPC producers.[89] However, there is a lack of information about antimicrobial restriction efforts and their influence on the incidence of CRE, but some researchers believe that antibiotic stewardship should focus on reduction of overall antibiotic use, not on limiting specific agents.[90]

2.8 Confounding factors

Confounding is an important concept in epidemiological studies; it occurs when a third variable(s) influence the relationship between an exposure or treatment and an outcome.[39] This is considered problematic because the estimate of association between exposure and disease include the contribution of both the exposure and the confounder. A confounding variable must be associated with the outcome variable; it is associated with the exposure but not caused by exposure. The presence of confounding can result in overestimation of the true association, underestimation of the true association, or change in the direction of the association between exposure and outcome.[39, 40] Confounding by indication in particular, is the most common type of confounding in observational pharmacoepidemiological studies. It's defined as a "type of selection bias in which the indication of a drug may influence the allocation of a patient into one or another of the comparison groups".[91] Controlling for a confounder variable in study design and/or data analysis is crucial to avoid confounding bias and to rule out its effect on the causal association between exposure (treatment) and the outcome of a pharmacoepidemiological study.

Although antibiotic use is considered a major driver for development of antibiotic resistance, the nature of this relationship is complex.[16] This is due to the presence of other confounding factors that may influence bacterial resistance in a hospital setting. Failure to deal with these confounders will make it difficult to evaluate and interpret the impact of antibiotic use on resistance. Some of these confounding factors are measured by an administrative database, and some are not measured. A brief description of these confounding factors follows.

One potential confounder is the use of antibiotics other than those studied. It is possible that more than an individual class of antibiotics use will aid in promoting resistance. The microbiological outcomes are carbapenem-resistant gram-negative bacteria which are often multidrug resistant. [50] The selection pressure imposed by the use of one antibiotic can result in the selection of bacteria resistant not only to that particular antibiotic but to other antibiotics, by co-resistance and cross resistance mechanisms.[50,51] All broad spectrum antibiotic classes used to treat the bacteria of interest were included in the model selection and adjusting processes.

Geographic location plays an important role in the distribution of many MDR gram-negative bacteria, in particular CR-KPs, which are abundant in the Northeast areas, so elevated rates of resistant bacteria in certain hospitals could be due to their presence in certain geographical locations.[92] Infection control efforts play a major role in bacterial resistance, poor infection control measures can result in the spread of antibiotic resistance.[15] Also, for hospitals with a strict infection control policy, it is expected it will have lower rates of resistance transmission. However, there is no quantitative measure of the quality and quantity of infection control in the hospital. Additionally,

resistant organisms brought into the hospital and cross transmission may confound the relation between antibiotic use and resistance. Patients infected with MDR bacteria transferred from another hospital may impact the resistance rate in that hospital.[24] However, this confounder cannot be adjusted for.

Antimicrobial stewardship programs perform restriction policies for some formulary drugs, cover appropriate choice of empirical therapy, and assign proper dosage and duration of therapy. Proper implementation of stewardship would aid in lowering antibiotic resistance.[11, 12] Thus, it is expect for hospitals with a restriction policy to have lower rates of resistance. Another potential confounder is the underlying severity of patient illness, as severely ill patients will presumably be at greater risk to develop resistance, because they have impaired host defenses and will receive more antibiotics compared to low risk patients.[17] Finally, there are as in any research unknown confounder(s).

2.9 Previous Investigations

2.9. 1 Carbapenem-resistant *P. aeruginosa*

Several studies have been conducted to identify risk factors associated with CR-PA ; most of these investigations were single center case control studies as summarized in table 2.2. One single center case control study found no association between prior antibiotic use (including carbapenems) and acquisition of CR-PA in a multivariable model.[93] The authors considered antibiotic use at the time of study thereby ignoring earlier use of hospital antibiotics. Only admission frequency and presence of Foley catheters were identified as significant risk factors. The authors believed that the observed high proportion of CR-PA in their institution was driven by a consequence of transmission of an organism already resident in their hospital rather than by selective antibiotic pressure. Another investigation in two centers collected 253 isolates of CR-PA between 2001 and 2006, found prior carbapenem, fluoroquinolone, extended-spectrum cephalosporin, and anti-anaerobic agent use in the preceding 30 days of culture sampling to be significantly associated with CR-PA in univariate analysis.[23] However, prior carbapenem use, transfer from outside facility, and duration of hospitalization prior to culture sampling were the only significant independent factors associated with CR-PA in the multivariable model. Also, ICU stay was used as a surrogate for severity of illness. Another single center case control study found that exposure to carbapenems 15 days before isolation and receiving mechanical ventilation for more than 48 hours to be associated with MDR *P. aeruginosa* (defined as resistant to ceftazidime, ciprofloxacin, piperacillin, and imipenem) in a multiple logistic regression

analysis.[45] Exposure to carbapenems within 14 days prior to isolation of positive cultures of CR-PA and renal failure were independently associated with CR-PA in multivariable analysis, in a single center case control study.[46]

The limitations of these case control studies investigating risk factors of CR-PA are the small sample size, single center institutions in which the stewardship and infection control practices may vary, impacting the generalizability of the results. The association between antibiotic use and resistance appears in many of the univariate analysis but when conducting a multivariable model many of these associations seem to fade. Some of these multivariable models adjusted for several confounders, but most relied on ICU stay as a measure of SOI rather than using other standard comorbidity-SOI indices.

One ecological single center study investigated the correlation between antimicrobial use density (AUD), which they used as a surrogate for defined daily dose /1000 patient-days-of carbapenem and carbapenem-susceptible *P. aeruginosa* in patients when followed every six months for the period between 2006 and 2008. They found a significant negative correlation between meropenem susceptibility in *P. aeruginosa* and total AUD of meropenem and doripenem. It wasn't clear how they adjusted for the dependency between their observations and how the correlation analysis was conducted.[94]

One retrospective, longitudinal study conducted was a time series analysis with months as a unit of analysis over the period 2001-2005 that evaluated the association between carbapenem use (defined daily dose) and CR-PA incidence rates and proportions in a single hospital. In their multivariate analysis, the use of group 2 carbapenems

(imipenem, meropenem, and doripenem) was highly associated with CR-PA proportions. However, ertapenem use was not associated with CR-PA.[95]

Finally, a multicenter study (25 hospitals) investigated the role of ertapenem use on CR-PA at an aggregate level and found no significant association between change in ertapenem use, carbapenem use, fluoroquinolone use and CR-PA. [47] They only adjusted for other antipseudomonal antibiotics in their model.

Table 2.2 Carbapenem-resistant *P. aeruginosa* previous studies

Author	Study design	Setting(s)	Risk factors
Eagye et al., 2009	Case-control	Single center	Admission frequency, Foley catheters
Lautenbach et al., 2010	Case-control	Two centers	Carbapenem use, transfer from outside facility, duration of hospitalization
Cao et al., 2004	Case-control	Single center	Carbapenem use, mechanical ventilation
Zavascki et al., 2005	Case-control	Single center	Carbapenem use, renal failure
Carmeli et al., 2011	Ecological, longitudinal study	Single center	Carbapenem use
Eagye et al., 2010	Ecological, longitudinal study	Multicenter	None

2.9.2 Carbapenem-resistant *K. pneumoniae*

CRE was rare until 2006; however, the incidence has been increasing since then, with outbreaks reported in the northeastern United States and spread of CRE described in other countries over the world.[90] Summary of the studies that investigated CR-KP are displayed in table 2.3.

One case control study where cases were patients with KPC-producing *K. pneumoniae* compared with control subjects with carbapenem-susceptible *K. pneumoniae* was conducted in two medical centers. In the multivariable analysis, only APR-DRG extreme illness category, prior fluoroquinolone use, and prior extended spectrum cephalosporin use were significantly associated with isolation of KPC-producing *K. pneumoniae*. [42] Prior antibiotic exposure was defined as at least two days of therapy administered during the 30 days prior to the culture. The authors mentioned that a very few patients had a history of carbapenem use (1.1%). As such, prior use of carbapenems as a risk factor for KPC production in *K. pneumoniae* isolates cannot be excluded.

In a single center case control study, patient conditions like malignancy, neurologic disease, poor functional status, high Charlson comorbidity index score, presence of central venous line, Foley catheters, mechanical ventilation, and ICU stay were associated with carbapenem-resistant *K. pneumoniae* in univariate analysis.[29] In addition, cases were shown to have received more antibiotics than controls. However, the multivariable model identified prior use of fluoroquinolones to be the only antibiotic independent predictor of carbapenem-resistant *K. pneumoniae* isolation along with poor

functional status and ICU stay. Carbapenem use could not be included in the model as none of the controls received carbapenems.

Another matched case control study in two health centers compared carbapenem – resistant *K. pneumoniae* to carbapenem-susceptible *K. pneumoniae*. The following were identified as predictors of CR-KP in univariate analysis: history of Chronic Obstructive Pulmonary Disease (COPD); admission to the ICU; mechanical ventilation; prior use of antipseudomonas penicillin; fluoroquinolones; glycopeptides; carbapenems; presence of tracheotomy; and having surgery with the use of a foreign body.[43] Prior antibiotic use was identified as an exposure that occurred only during the hospitalization in which the infection developed and the antibiotic had been administered for at least 3 consecutive days prior to the development of the infection. The multivariable analysis for matched data showed that prior use of fluoroquinolones and antipseudomonal penicillins were independent risk factors for CR-KP infections. Another matched case control study identified 99 cases of CR-KP and 99 control with carbapenem-susceptible *K. pneumoniae*. [22] In the univariate analysis, transplant recipient, mechanical ventilation, ICU stay, length of stay before infection, prior use of cephalosporin, beta-lactam and/or beta-lactamase inhibitor, carbapenem, and aminoglycosides were associated with CR-KP. The multivariable model had shown prior exposure to cephalosporins, carbapenems, and longer length of stay before infection to be significantly associated with CR-KP acquisition. Antibiotic exposure was identified during patient hospitalization or within 3 months before the diagnosis of *K. pneumoniae* infection. Finally, a case control study design found that ICU admission within two weeks, tracheal intubation, mechanical ventilation, exposure to carbapenems, fourth generation

cephalosporins, piperacillin-tazobactam, and glycopeptides to be associated with the isolation of CR-KP in univariate analysis.[44] The multivariable model found only ICU admission, prior exposure to carbapenems, and glycopeptides to be independently associated with CR-KP isolation. However, the sample size was relatively small, and resistance mechanisms were not identified.

To summarize, prior use of carbapenems, fluoroquinolones, and extended-spectrum cephalosporins are associated with isolation of carbapenem resistant CR-KP as identified in several case control studies. Most of the studies had a small sample size and were single centered which limit their generalizability, especially as infection control measures vary among these institutions.

Table 2.3 Carbapenem-resistant *K. pneumoniae* summary studies

Author	Study design	Setting(s)	Risk factors
Gasink et al., 2009	Case-control	Two centers	APR-DRG extreme category, fluoroquinolones, extended-spectrum cephalosporins
Schwaber et al., 2008	Case-control	Single center	Fluoroquinolones, poor functional status, ICU stay
Falagas et al., 2007	Case-control	Two centers	Fluoroquinolones, antipseudomonal penicillins
Patel et al., 2008	Case-control	Single center	Carbapenems, cephalosporins, length of hospital stay
Wu et al., 2011	Case-control	Single center	ICU admission, carbapenems, glycopeptides

CHAPTER 3

METHODS

3.1 Study design

This was an ecological longitudinal observational study of hospitals within the University Health System Consortium (UHC) database. The outcomes of interest were carbapenem-resistant *Pseudomonas aeruginosa* (CR-PA) and carbapenem-resistant *Klebsiella pneumoniae* (CR-KP). The exposures of interests included hospitals' antibacterial drug use, case mix index (CMI), aggregated 3M All Patient Refined Diagnosis Related Group (APR-DRG) Classification System, and hospital demographics (bed size and geographical location).

3.2 Data source

The UHC is a national alliance of 103 academic medical centers and 206 of their affiliated hospitals representing approximately 90% of the nation's non-profit academic medical centers. The UHC provides a mechanism for collaboration for research across academic medical centers, which are geographically distributed throughout the U.S. A subset of UHC hospitals participate in the Clinical Resource Manager (CRM) Database and will serve as the data source. It contains Uniform Billing (UB-92) data, inpatient medication use from charge transaction masters, and billing files from participating UHC members. Patient records contain detailed information on inpatient care, and this includes primary and secondary diagnoses (in *International Classification of Diseases*,

9th Revision, Clinical Modification [ICD-9-CM] format), inpatient procedure codes (in ICD-9-CM format), patient demographic information (age, race, and gender), and hospital demographic information (bed size and geographical location). [96] The database also contains admission and discharge dates as well as information on comorbidities, severity of illness, physician specialty, length of stay (LOS), costs, and clinical outcomes such as inpatient mortality and complications rates. Antibacterial drug use was used from this database; the validation of UHC database and its assessment of hospital antibacterial drug use have been described. [48]

3.3 Antibacterial drug Use

The UHC database provides antibiotic use data that are based on charges for patients not the overall amounts the institution purchases. Antibacterial agents whose use has been reported as risk factors for the development of CR-Pa and CR-KP as identified in the literature review are listed in table 3.1. The systemic use of these antibiotics in adult (≥ 18) inpatients discharged between January 1, 2006 and December 31, 2009 was obtained from billing records and reported as days of therapy per 1000 patient days (DOT/1000 PDs). Any single dose of antibiotics that was received by a patient was reported as one DOT regardless of multiple dose administration. For example, administration of gentamicin every 8 hours for 3 doses, or administration of the entire daily dose every 24 hours, would be counted as one DOT. The defined daily dose (DDD) method of reporting drug use is adopted by World Health Organization (WHO), but important deficiencies were presented when compared to DOT method.[97] In

addition, CMI, geographic location, proportion of patients in each of the four APR-DRG categories, and bed size for each hospital were obtained for the study period.

Table 3.1 Systemic use of antibacterial drugs categorized into groups

Antibacterial Group	Antibacterial agents
Carbapenems	Imipenem, meropenem, ertapenem, doripenem
Fluoroquinolones	Ciprofloxacin, levofloxacin, moxifloxacin, gatifloxacin
Third-and fourth-generation cephalosporins, monobactams	Ceftriaxone, cefotaxime, ceftazidime, cefepime, aztreonam
Aminoglycosides	Gentamicin sulfate, tobramycin, amikacin
Antipseudomonal Penicillins	Piperacillin-tazobactam, ticarcillin-clavulanate

3.4 Severity of illness

Measuring severity of illness using aggregate level data is not a well established concept. Overall hospital CMI [the average of DRG weights for all of an individual hospital's DRG-paid Medicaid claims] had been considered an indicator of the intensity of hospital resource utilization and can be used to compare hospital performance, but it is less sensitive to the severity of illness from a clinical perspective. [98, 99] In addition to CMI, another severity of illness measure based on the APR-DRG classification system was used in this present investigation; this measure reflects the clinical complexity of a patient population. The underlying clinical principle of the APR-DRGs is that the severity of illness and risk of mortality of a patient depends to a great extent on the patient's underlying characteristics. This system assesses the relative severity of a patient's illness based on the severity level of the secondary diagnoses and interactions between secondary diagnoses, age, principal diagnosis, and certain procedures. There are four subclasses of SOI (minor, moderate, major, extreme) based on the presenting DRG.[100, 101] The UHC database includes the number of patients within each of the four categories for each participating hospital for each year. Hence, the proportions of patients within each category for each year were calculated. The APR-DRG proportions and CMI were highly correlated, especially the extreme category of the APR-DRG.

3.5 Antimicrobial susceptibility

“Whole house” antibiograms from all hospitals for which antibacterial drug use data were obtained were requested from 2006 through 2009. An email request was sent to 50 hospitals accompanied by an online survey for which antibiotic use data from 2006 to 2009 were obtained. Only antibiograms with a full calendar year of susceptibility data from all clinical sources, at least 30 isolates for each organism and those including total number of isolates and proportion of susceptible isolates were included in the final dataset. The desired outcomes, carbapenem (imipenem or meropenem)-resistant *P. aeruginosa* and *K. pneumoniae* were calculated from these antibiograms. Both proportions and rates of resistance for CR-Pa and CR-KP were recorded. The resistant proportion was the number of resistant isolates divided by the total number of isolates, and the resistant incidence rate was the number of resistant isolates per 1000 adult patient discharges, and per 1000 adult patient days (PDs). The number of adult discharges and adult patient days were obtained from the UHC database.

3.6 Survey

Hospitals that participated in the UHC-CRM for which the antibacterial use data were obtained from 2006 to 2009 were invited to complete a survey in January 2011 (with follow-up requests to non-responders in April 2011 and August 2011). The online survey was sent to each of the 50 hospitals along with the request for new antibiograms. Each hospital contact, usually clinical pharmacy infectious disease specialists but may also include infectious disease physicians, clinical microbiologists, or infection control

practitioners, received an online survey by REDCap (Research electronic data capture) to obtain data that are not available to us through UHC. REDCap is a secure, web-based application designed to support data capture for research studies, providing: 1) an intuitive interface for validated data entry; 2) audit trails for tracking data manipulation and export procedures; 3) automated export procedures for seamless data downloads to common statistical packages; and 4) procedures for importing data from external sources.[102] The online survey was divided into three parts. The first part requested additional information regarding susceptibility testing methods and antibiograms construction, including the inclusion/exclusion of duplicate isolates, method(s) of routine susceptibility testing, policy regarding surveillance cultures and intermediate susceptible isolates. The second part requested information about carbapenem-resistant *Enterobacteriaceae* and carbapenem-resistant *K. pneumoniae* including hospital encounter of such organisms in the past five years, rate of isolation in the past year, source (e.g. transfer from another hospital and/or nursing home), and microbiological technique used to identify and/or confirm CRE. In order to compare the number of isolates of carbapenem-resistant *K. pneumoniae* obtained from hospital antibiograms to the number of resistant isolates obtained from the clinical microbiology laboratory in each hospital a question about the reflection of the rate of isolation of these bacteria in hospitals antibiograms was included in the survey. The third component was about antimicrobial stewardship programs (ASP), specifically about the role of ASP in managing CR-KP cases, isolation procedures and measures with such cases, and restriction policies for carbapenems. It was also asked if there had been any

changes in the ASP policies over the current study period. The survey is provided in appendix A.

3.7 Data collection

Each hospital was offered \$100 per year of antibiograms and for the completion of the online survey. Antibiotic use data, CMI, APR-DRG and other demographics were available for 50 hospitals for the period of 2006-2009. The antibiograms/survey requests were sent to all contacts of the 50 hospitals in the period of January 2011, a second round of requests was sent to non-responders by April 2011, and a final round of reminders was sent by August 2011. Of the total 50 hospitals approached, 40 hospitals provided antibiograms, thirty of which completed the online survey. The outcome data, proportions and rates of CR-PA and CR-KP were calculated directly from the antibiograms. The UHC obtained variables are listed in table 3.2.

Table 3.2 Description of the analysis variables obtained by UHC

UHC data variable	Unit	Symbol used SAS code
Fluoroquinolones	DOT/1000 PDs	FQ
Fluoroquinolones (except moxifloxacin)		FQMo
Carbapenems	DOT/1000 PDs	CB
Carbapenems (except ertapenem)		CBE
Aminoglycosides	DOT/1000 PDs	AG
3 rd & 4 th generation cephalosporins	DOT/1000 PDs	CEPHt
Antipseudomonal cephalosporins		CEPHn
Piperacillin-tazobactam, ticarcillin-clavulanate	DOT/1000 PDs	ExPen
Hospital bed size	Number of beds	Bedsizes
Hospital geographic location	Name	Location
	Mid-Western	1
	Mid-Continent	2
	Southeastern	3

	Mid-Atlantic	4
	Western	5
	New-England	6
Hospital CMI	No unite	CMI
Proportion of patients with APR-DRG score minor	Proportion	minor
Proportion of patients with APR-DRG score moderate	Proportion	moderate
Proportion of patients with APR-DRG score major	Proportion	major
Proportion of patients with APR-DRG score extreme	Proportion	extreme
Carbapenem restriction	Yes/ No	restriction
Removal of duplicate isolates	Yes/No	dup
Including surveillance cultures	Yes/No	surv

3.8 Modeling longitudinal (repeated measures) data

Longitudinal data

This analysis involves longitudinal measures of bacterial resistance and antibiotic use over a four-year period. In this set of longitudinal data (repeated measures), the assumptions of independence between any two observations from different hospitals are valid, as this sample represents a random sample of UHC hospitals. In contrast, any two observations from the same hospital are correlated as they will have the same basic characteristics and accordingly they will not be independent.[103] Modeling correlated data as if they were independent can result in incorrect inferences of the regression parameters due to underestimation of standard errors and insufficient estimators.[103,104] Therefore, there are specific statistical methods to model longitudinal data.

Mixed models, generalized mixed models, and generalized estimating equations are all methods used to analyze longitudinal data and will be explained in this section. All of these methods share one common feature, which is the predetermination of the covariance structure to account for correlation between observations. [105,106] Table 3.3 compares these methods and their use in SAS.

Table 3.3 Comparison of most common statistical methods to analyze longitudinal data

Model	Output variables	Types of inputs, effects	Assumptions	Method to estimate parameters	Goodness of fit criteria	Missing data
LMM	Interval	Categorical, interval, random effects	Normality	Need to be specified in the model	AIC, AICu, BIC	MAR
GEE	Categorical, interval	Categorical, interval, combine fix and random effects	Exponential family	Need to be specified in the model	QIC , QICu	MCAR
GLMM	Categorical, interval	Categorical, interval, random effects	Exponential family	Need to be specified in the model	Pseudo-AIC Pseudo-BIC	MAR

Note: Linear mixed models (MM), Generalized estimating equations (GEE), Generalized Linear mixed models (GLMM), Akaike's information criterion (AIC), Schwarz's Bayesian information criterion (BIC), pseudo-Akaike's information criterion (pseudo-AIC), pseudo-Schwarz's Bayesian information criterion (pseudo-BIC), quasi-likelihood under the independence model criterion (QIC), missing at random (MAR), missing completely at random (MCAR).

The following section will provide an overview of each of these statistical methods.

Linear Mixed models (LMM)

These models contain both fixed and random effects and perform analysis by way of structured covariance models. The LMM procedure estimates parameters by restricted maximum likelihood (REML) technique which was introduced by Patterson and Thompson (1971). The restricted likelihood is maximized under the assumption of normal distribution of the data. All available data can be used with LMM as long as any missing data are missing at random (MAR).[107,108] Covariance parameters are estimated by the method of moments though solving expressions for expected mean squares.[107,108] Model fit criteria usually involves Akaike's information criterion (AIC) and Schwarz's Bayesian information criterion (BIC). Both can be used in the model selection process. [107-109]

The notation for linear mixed models is:

$$y = X\alpha + Z\beta + e$$

Where y , X , α and e are as defined in the fixed effects model, and

$\beta = (\beta_1, \beta_2, \dots, \beta_q)$ = random effect/coefficient parameters. The Z is a second design matrix with dimension $n \times q$ giving the values of random effects corresponding to each observation.[108]

A covariance structure must be explicitly specified to account for within-subject correlation by specifying how observations within a subject or cluster are correlated with

each other. Misspecification of the covariance structure can yield to invalid results.[107-110] Examples of most commonly used correlation matrices are described in table 3.4

Generalized linear mixed models (GLMM)

The GLMM is an extension of the family of generalized linear models (GLM). These models have the ability to account for within-subject correlations by explicitly specifying a working correlation matrix and are considered subject-specific models.[108,111] The GLMM inflates GLMs by including some variables as random effects. It allows the within subjects dependence to differ from one subject to another by means of the random variation of the linear combination of covariates and the residual variation. The random variation can be expressed in the random intercept and random slope. [108,111,112] The change of an individual's response can be evaluated by including these random effects in the model; consequently they are considered to be subject-specific models. Similar to linear mixed models, GLMM handles missing data if they were missing at random. Thus, for missing data, the working correlation from data containing missing values can be estimated by using all the available pairs of data. Moreover, generalized mixed models by include variables of non-normal distribution, in particular when the response variable has a distribution from one of the exponential family of distributions. For example, binary, binomial, Poisson, negative binomial, normal, beta, gamma and inverse Gaussian distributions are all members of the exponential family.[108,111, 112]

The notation for GLMM is:

$$g(\mu) = X\alpha + Z\beta + e$$

Where μ is the vector of expected means of the observations and is linked to the model parameters by a link function, g , X , and Z are the fixed and random effects design matrices. Moreover, α is the vector of fixed parameters and β is the vector of random effects parameters.[108]

The GLMM implements the estimation technique using residual pseudo-likelihood and was proposed by Wolfinger and O'Connell (1993) with a subject specific expansion.[112-114] This method is referred to as 'pseudo-likelihood' because the likelihood function maximization is based on the pseudo variable (pseudo data) and not that of the original data. Consequently, model fitting criteria such as pseudo-AIC and pseudo-BIC are not comparable to the log-likelihood criteria used in mixed models and should be interpreted cautiously.[108,112-114] In order to fit GLMM models, an appropriate distribution, link function, and working correlation structure should be specified.

The Generalized Estimating Equations (GEE)

The GEE method was introduced by Liang and Zeger 1986 for analyzing correlated response variables in longitudinal data. The GEE model extended generalized linear models by assuming a known function of the marginal expectation of the outcome variables by using the quasi-likelihood approach.[115] The GEE model accounts for within-subject correlations by explicitly specifying a working correlation matrix for different types of response data, in particular the exponential family of distributions.[115,116] Also, the GEE method handles missing data as if they were missing completely at random (MCAR). [115,116]

The notation for GEE is:

$$S(\beta) = \sum_{i=1}^K D_i' V_i^{-1} (Y_i - \mu_i(\beta)) = 0$$

Where

$$D_i = \frac{\partial \mu_i}{\partial \beta}$$

Since

$$g(\mu_{ij}) = x_{ij}' \beta$$

where g is the link function, the $p \times n$ matrix of partial derivatives of the mean with respect to the regression parameters β for the i th subject.[115,117]

The GEE model is also known as a “marginal” model as it models a known function of the marginal expectation of the response variable as a linear function of the explanatory variables.[116,118] The effect of the between-subjects factor is modeled separately from the within-subjects correlation. Thus, the interpretation of the parameters does not depend on a particular subject, but rather it is valid for the whole population of possible subjects in the study. Accordingly, these parameters can be referred to as population-averaged parameters.[115,116,118] Regression coefficients of such model describe the average population response curve. One unique advantage with GEE models is the robust covariance matrix concept proposed by Liang and Zeger (1986) which entails that as long as the mean model is correct, the parameter estimates are consistent as the number of subjects (hospitals) becomes large.[115,116,119,120] This is of considerable importance in cases of correlation structure misspecification where one can still obtain consistent parameters estimates. The quasi-likelihood under the independence model criterion (QIC) proposed by Pan (2001) is an extension of the AIC and can be used in model fit assessment.[121,122]

Table 3.4 Commonly used correlation matrices with correlated observations [105,110]

Correlation structure	Definition
Independence	Each observation in a subject is completely uncorrelated with every other observation in that subject
Exchangeable (Compound Symmetry)	Each observation within a subject is equally correlated with every other observation in that subject
Autoregressive	For two observations taken close in time within a subject, they will be more closely correlated than two observations taken far apart for the same individual.
Unstructured	No assumption is made about the magnitude of correlation between any two pairs of observation.

3.9 Statistical analysis

3.9.1 Software

Statistical analyses were performed using SAS (version 9.2; SAS Institute, Cary, NC) and JMP (version 8.0; SAS Institute, Cary, NC). JMP was mainly used for descriptive statistics while SAS was used for the GEE analysis. All tests were two-tailed; a p-value < 0.05 was considered significant.

3.9.2 Summary statistics

Descriptive statistics including the mean, standard deviation, and full range were calculated for antibiotic use groups. The median, interquartile range, and full range were calculated for CR-KP rates and proportions and the mean, standard deviation, and full range were calculated for CR-PA rates and proportions.

Mixed model ANOVA method was used to report trends in antibiotic use and resistance rates and proportions of CR-PA and CR-KP over the study period. The mean use of carbapenem use and rates of CR-PA were compared among hospitals that did vs. did not restrict carbapenem agents using the Wilcoxon Rank-Sum Test.

3.9.3 Modeling carbapenem-resistance gram-negative bacteria

The analytical approach used in this investigation was generalized estimating equations. The QIC value was used to determine the best distribution and link functions,

as well as working correlation structure. [121,122] For the CR-PA rates (1000 PDs), a normal distribution, identity link, and first-order autoregressive working correlation structure were selected after comparing QIC values (the smaller the better). CR-PA proportions are considered limited range variables; they are limited between 0 and 1. Falsely assuming normality in such cases can lead to incorrect results.[119] Hence, a binomial distribution, logit identity, and first-order autoregressive working correlation structure were selected for CR-PA proportions.

Given that almost half of the CR-KP data were of zero value, using proportion or incidence rate was not feasible. Therefore, CR-KP data were treated as a binary outcome (present vs. absent). First, a cut point of a proportion $\leq 1\%$ was considered an “absence” response and $>1\%$ was considered a “present” response, however, only 24% of the observations were “present” outcome and this resulted in some convergence/ iteration issues in running the model through SAS. Thus, the raw number of isolates was used to generate a binary categorical variable as follows: if a hospital (according to its antibiogram) encountered less than 5 isolates per year then it was considered as an “absent” response, if the hospital encountered 5 or more isolates per year then it was considered as a “present”. Consequently, 41% of observations had the outcome “present”. Binary distribution, logit link, and 1st order autoregressive working correlation structure were chosen for CR-KP.

Each of the three outcomes was analyzed separately. Variables were added to the model based on their importance according to previous findings in literature and preliminary analysis of the data. For CR-PA rates and proportions the following explanatory variables were included in the model selection process: fluoroquinolones

(ciprofloxacin, levofloxacin, gatifloxacin); carbapenems (imipenem, meropenem, doripenem); antipseudomonal penicillins (piperacillin-tazobactam, ticarcillin-clavulanate); antipseudomonal cephalosporins (ceftazidime, cefepime, aztreonam); aminoglycosides (gentamicin, tobramycin, amikacin); and hospital CMI, patient-proportion within the APR-DRG categories. The patient-proportions within the APR-DRG categories were not combined with CMI. Variables were entered and models were compared using QIC_u . QIC_u is a simplified version of QIC that can be used to select the most parsimonious model, that is, the best model is the one with smallest QIC_u . [122] A Huber-White sandwich estimator (robust estimator) was used as a way to ensure that the variances were robust. Specifically, robust variances are important as they provide accurate assessments of the sample-to-sample variability of the parameter estimates even if the model is misspecified. [115,116,118] After selection of the “best model”, adjusting for potential confounders was performed. Each of the confounding variables (hospital bed size, geographical location, and variables that were not included in the “best” model, e.g. CMI, other antibiotic classes) were added to the model to assess their impact on parameter estimates. A confounding variable that changed parameter estimates by $\geq 20\%$ was added to the selected model. [123] For the test of model effects, Type III, was selected for all analysis as it does not depend on the entry order of the variables like Type I does. Test Type III is typically preferred unless the order of the variables is necessary. [117]

For CR-KP dependent variable, the following explanatory variables were included in the model selection process: fluoroquinolones (ciprofloxacin, levofloxacin, gatifloxacin, moxifloxacin); carbapenems (imipenem, meropenem, doripenem, ertapenem);

antipseudomonal penicillins (piperacillin-tazobactam, ticarcillin-clavulanate); third-and fourth-generation cephalosporins (ceftazidime, cefepime, aztreonam, ceftriaxone, cefotaxime); aminoglycosides (gentamicin, tobramycin, amikacin); hospital CMI; patient-proportion within the APR-DRG categories; and geographical location.

After selecting the model; the adjustment for potential confounders was performed in a similar fashion to the CR-PA modeling process.

3.10 Human subjects' protection and data privacy

VCU IRB exemption was obtained for this study. A dataset was constructed from the University Health System Consortium (UHC) data warehouse. Access to the dataset was restricted to those individuals listed on the study protocol, and the dataset was centrally maintained in a password-protected environment. Disclosure of any kind of information did not take place without the expressed written permission of UHC or as required by law. Results will be published in such a way that no hospital will be individually identifiable. Data within UHC's Clinical Resource Manager is compliant with the Health Insurance Portability and Accountability Act of 1996 (HIPAA). This study qualified for exemption according to 45 CFR 46.101(b) Category 4 at Virginia 56 Commonwealth University internal review board VCU IRB#: 12377. A copy of the IRB Approval form can be found in the Appendix B.

CHAPTER 4

Results

4.1 Descriptive statistics of hospitals

Forty hospitals were included in the analysis of this project out of the 50 hospitals approached. Six of the total 50 hospitals did not provide any antibiograms nor did they respond to the survey. One hospital responded to the survey but did not provide any antibiograms. Three hospitals provided antibiograms that cannot be used; two of them did not include carbapenem susceptibility results and one antibiogram did not include the total number of isolates for organisms of interest.

The 40 participating hospitals were located in different geographical locations across the continental U.S. Eight of the participating hospitals were located in the Mid-Western region, eight hospitals were located in the Mid-Continent region, ten hospitals were located in the Mid-Atlantic region, both the South-Western and Western regions had six hospitals in each, and two hospitals were located in the New-England region. Table 4.1 lists patient and hospital demographics for 2009 at 40 UHC hospitals.

The observations in the study period represent a total of 22,224,512 total patient days and 3,960,380 discharges. During this period, 2,022,956 patients received antibiotics.

Table 4.1 Characteristics of 40 member hospitals of the UHC in 2009

Characteristics	Mean (SD)	Range
Adult patient age	52 ± 4	44 - 59
Bed size	558 ±190	216 - 1156
Case mix index	1.67 ± 0.16	1.21 – 1.99
Mean length of stay, days	5.59 ± 0.45	4.74 – 6.79
Total Patient Discharges	25283 ± 8615	11594 - 54192
Total Patient Days	141509 ± 48733	59763 - 282327
Surgical procedures per 1000 discharges ^a	356 ± 56	194 - 502
Diagnosis per 1000 discharges ^a		
Urinary Tract infections	86 ± 15	49 - 119
Pneumonias	62 ± 12	40 - 94
Blood stream infections	54 ± 11	31- 88
Bone marrow transplants	3.5 ± 3.2	0 - 15
Solid organ transplants	10.4 ± 6.3	0 - 29

^aone hospital was excluded for these characteristics

4.2 Summary statistics of antibiotic use

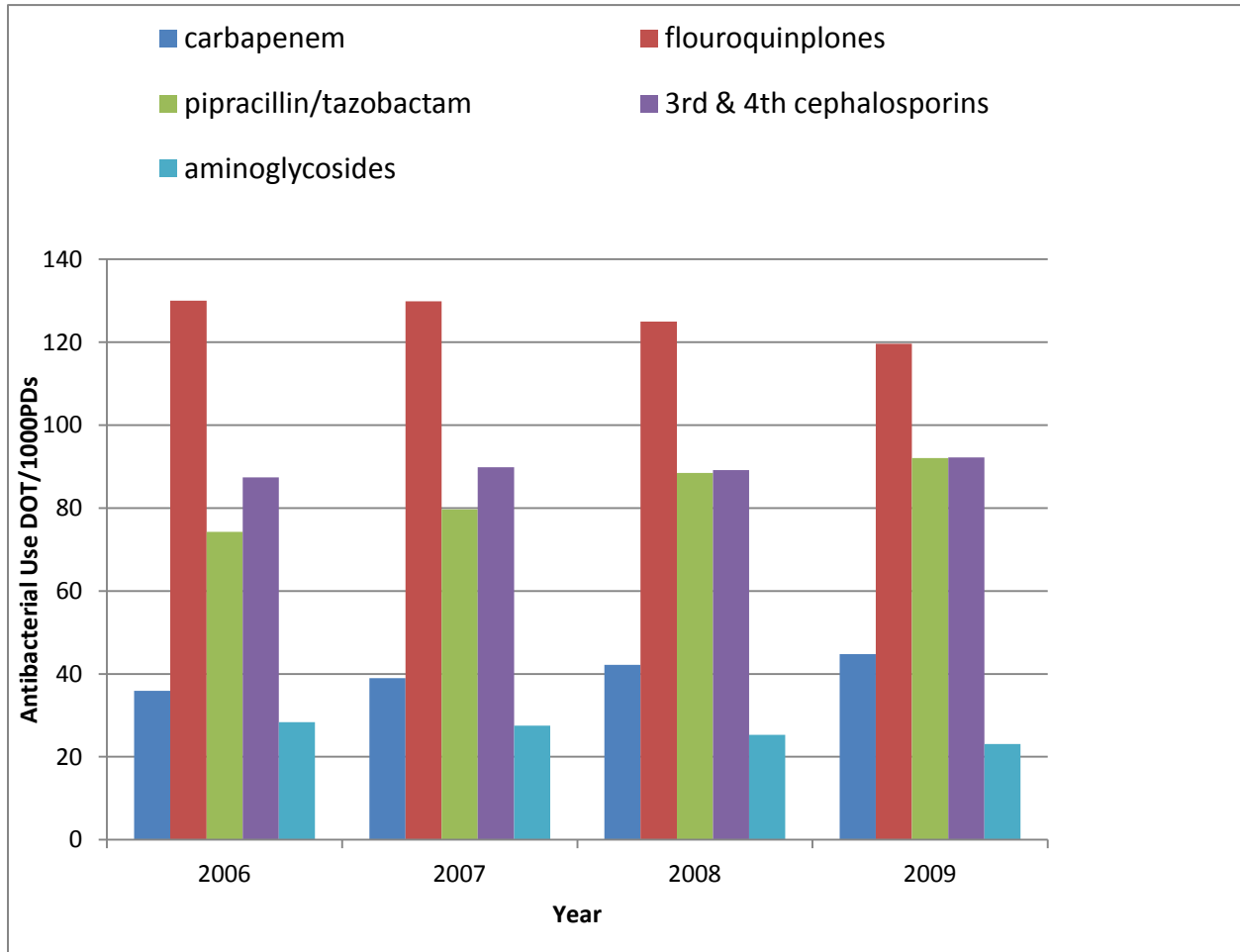
Antibiotics use data were available for all 40 hospitals over the study period. Each antimicrobial group was assessed for trends over the study period. The mean (\pm SD) of antibiotics under this investigation are presented in table 4.2 and figure 4.1. One hospital's antibiotic use data for 2009 was excluded because the data for that year were not correct. For antipseudomonal penicillins, piperacillin-tazobactam was mainly used in most hospitals. Total use of five broad spectrum antibiotic classes increased significantly over the study period, from 354.0 (\pm 72.2) DOT/1000 PDs in 2006, to 369.7 (\pm 80.3) DOT/1000 PDs in 2009 ($P=0.0206$). This increase is driven primarily by an increase in carbapenem and piperacillin-tazobactam use. Total carbapenem use increased by 24% over the study period, from 36.1 (\pm 21.7) DOT/1000 PDs in 2006, to 44.8 (\pm 24.4) DOT/1000 PDs in 2009, ($P <.0001$). A similar increase of 25% was observed with piperacillin-tazobactam use 73.8 (\pm 39.6) DOT/1000 PDs in 2006 to 92.3 (\pm 37.2) DOT/ 1000 PDs in 2009 ($P <.0001$). On the other hand, total fluoroquinolone use decreased by 8% over the study period; from 130.0 (\pm 40.6) DOT/1000 PDs in 2006, to 119.6 (\pm 36.9) DOT/ 1000 PDs in 2009 ($P= 0.0013$). Total aminoglycoside use decreased by 18% over the period of this investigation, from 28.3 (\pm 9.5) DOT/1000 PDs in 2006, to 23.1 (\pm 8.7) DOT/1000 PDs in 2009 ($P<0.0001$). Total third-and fourth-generation cephalosporin use did not change significantly over the study period. It is worth mentioning that fluoroquinolone use represented the highest proportion of broad spectrum antibiotics. For example, in 2009 fluoroquinolone use was 32%, followed by piperacillin-tazobactam 25%, total third-and fourth-generation cephalosporins 25%, carbapenem use was 12%, and finally aminoglycosides use was 6% of total use.

Table 4.2 Changes in antibacterial drug use among 5 classes of broad spectrum antibiotics from 2006 -2009

Antibiotic	Year	Mean use (DOT/1000PDs)	SD	Range
Carbapenems	2006	35.92	21.71	7.00- 98.8
	2007	38.96	21.78	11.32 -98.54
	2008	42.19	24.8	10.80 – 121.72
	2009	44.80	24.38	8.51 – 160.56
Fluoroquinolones	2006	130.01	40.55	52.93 – 212.53
	2007	129.85	37.89	51.19 – 217.70
	2008	124.96	39.71	44.01 – 219.31
	2009	119.62	36.87	41.73 – 200.63
Third-and fourth-generation cephalosporins	2006	87.44	35.50	24.97 – 226.37
	2007	89.84	34.87	27.80 – 228.55
	2008	89.77	33.14	26.09 – 217.43
	2009	92.24	37.47	23.37 – 253.17
Aminoglycosides	2006	28.33	9.53	16.25- 61.85
	2007	27.49	9.04	14.85 – 58.11

	2008	25.34	8.72	12.10 – 53.54
	2009	23.05	8.65	10.11 – 46.17
Antipseudomonal penicillins	2006	76.69	37.30	1.66 – 175.75
	2007	81.83	37.53	1.91 – 155.88
	2008	89.13	36.54	3.05 – 149.15
	2009	93.53	38.69	3.67 – 161.31

Figure 4.1 Changes in antibacterial drug use and carbapenem-resistant *P. aeruginosa* over 4 years



4.3 Summary statistics of resistance

Antibiograms were available for 40 hospitals. Of the total 160 possible hospital years, antibiograms that met the criteria (identified in methods chapter, section 3.5) were available for 146 (91.25%). Four years of antibiograms from 30 hospitals, three years of data from eight hospitals, and two years of data from 2 hospitals were obtained. CR-PA and CR-KP rates and proportions are summarized in tables 4.3 and 4.4 respectively.

4.3.1 Carbapenem-resistant *P. aeruginosa*

The mean, standard deviation, and range calculated values are presented in table 4.3. The mean proportion and incidence rate for carbapenem-resistant *Pseudomonas aeruginosa* (per 1000 patient days and discharges) did not change significantly over the study period ($P = 0.30, 0.17, 0.12$; respectively, figure 4.3).

Table 4.3 Changes in carbapenem susceptibility among *P. aeruginosa*

Bacteria-resistance measure	Year	Mean	SD	Range
CR-PA proportion (%)	2006	20	6.9	7 - 37
	2007	20	7.7	6 - 36
	2008	19	7.7	5 - 36
	2009	19	5.7	6 - 30
CR-PA rate (1000 PDs)	2006	1.02	0.65	0.10 – 2.65
	2007	1.00	0.72	0.20- 2.80
	2008	0.91	0.72	0.15 – 2.66
	2009	0.88	0.57	0.19 – 2.12
CR-PA rate (1000 discharges)	2006	5.73	3.87	0.42– 15.02
	2007	5.67	4.30	1.18 – 16.30
	2008	5.31	4.61	0.83 – 18.78
	2009	4.93	3.43	1.09 – 12.89

4.3.2 Carbapenem-resistant *K. pneumoniae*

The median, interquartile range and total range were calculated for each of the four years and are presented in table 4.4. Microbiology results obtained from antibiograms for CR-KP varied among hospitals and the years of this study. Almost one half of the participating hospitals (22 hospitals) reported 0.5 to 2 percent carbapenem-resistant *K. pneumoniae* in at least one year of the study period. Nine hospitals reported a zero percent of CR-KP for all years, while 4 hospital hospitals reported a CR-KP percent that is more than 2 and less than 10 in at least one year of the study period. Twenty seven hospitals had zero percent/rate of CR-KP as reported in their antibiograms; this number decreased over the study period as follows: 22 hospitals in 2006, 20 hospitals in 2007; 17 hospitals in 2008; and 10 hospitals in 2009. Further, five hospitals reported a CR- KP proportion of more than 10% in at least one year of the study period. The five hospitals with the highest proportions of CR-KP were all located in the Mid-Atlantic region of the U.S. The total number of CR-KP isolates was calculated for all the participating hospitals over the study period to show the increase in these isolates over time, as shown in figure 4.2.

The mean proportion of carbapenem-resistant *K. pneumoniae* increased by 130%, from a mean of 1.3% in year 2006 to a mean of 3.1% in year 2009 ($P=0.003$, Table 4.5, figure 4.3) and the mean incidence rate for carbapenem-resistant *K. pneumoniae* (per 1000 patient days) increased by 120%, from 0.07 in year 2006 to 1.52 in 2009 ($P=0.0118$, table 4.6, figure 4.3).

Table 4.4 Changes in carbapenem-resistant *K. pneumoniae* over the study period

Bacteria-resistance measure	year	Median	IQR	range
CR-KP proportion (%)	2006	0.0	0 - 1	0 - 14
	2007	0.0	0 - 1	0 - 20
	2008	1.0	0 - 2	0 - 20
	2009	1.0	0 - 2	0 - 23
CR-KP rate (1000 PDs)	2006	0.0	0 - 0.05	0 - 1.054
	2007	0.0	0 - 0.05	0 - 0.548
	2008	0.02	0 - 0.06	0 - 1.002
	2009	0.04	0 - 0.07	0 - 1.049
CR-KP rate (1000 discharges)	2006	0	0- 0.26	0 - 5.98
	2007	0	0 - 0.30	0 - 3.48
	2008	0.12	0 - 0.34	0 - 6.27
	2009	0.21	0 - 0.40	0 - 5.78

Figure 4.2 Total number of carbapenem-resistant *K. pneumoniae* isolates over the study period

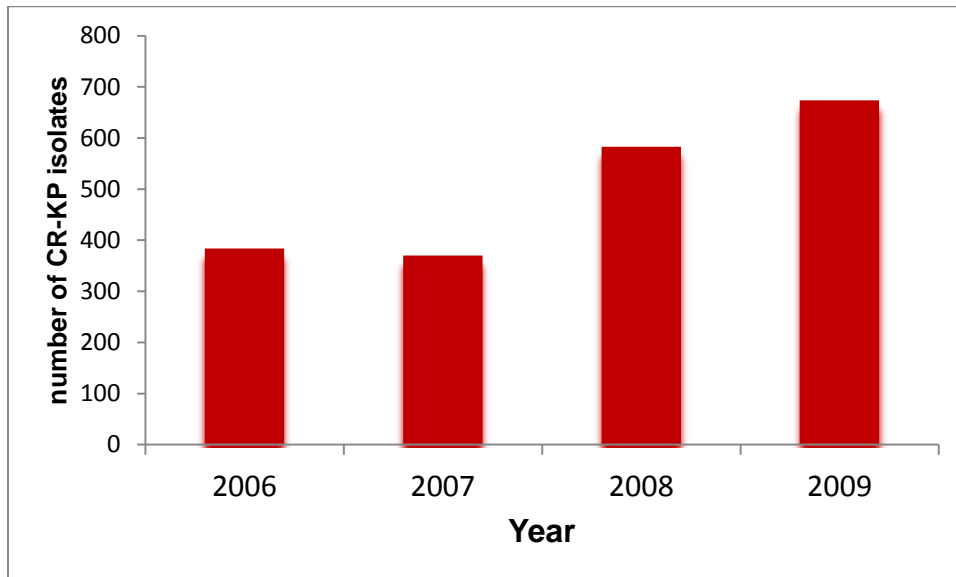


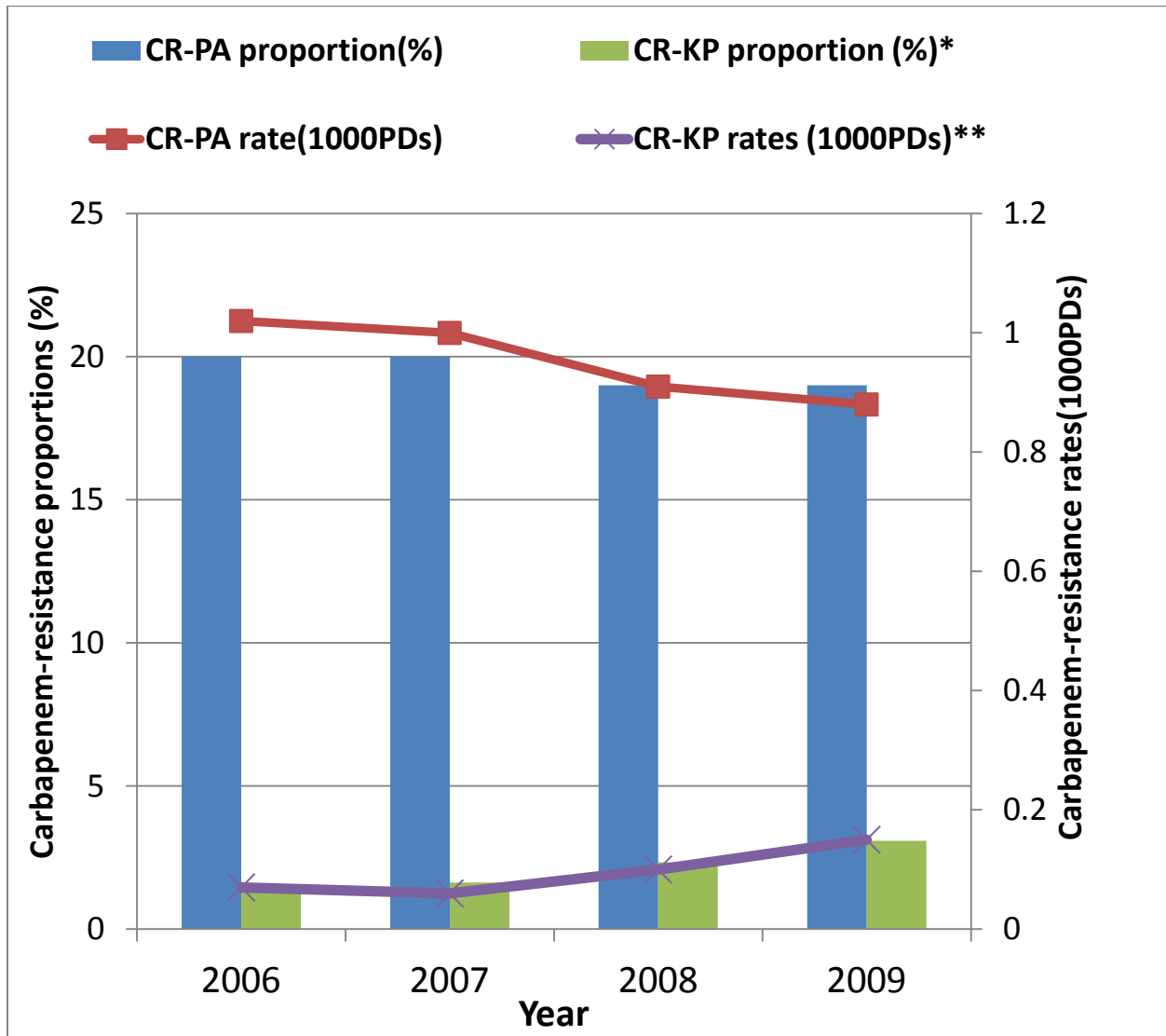
Table 4.5 Changes in carbapenem-resistant *K. pneumoniae* proportions (%)

Year	LS means	SE	Lower CI 95%	Upper CI 95%
2006	1.33	0.69	-0.057	2.721
2007	1.63	0.69	0.245	3.013
2008	2.33	0.69	0.943	3.713
2009	3.08	0.72	1.644	4.519

Table 4.6 Changes in carbapenem-resistant *K. pneumoniae* rates/1000 PDs

Year	LS means	SE	Lower 95% CI	Upper 95% CI
2006	0.07	0.03	0.002	0.138
2007	0.06	0.03	-0.006	0.130
2008	0.10	0.03	0.031	0.166
2009	0.15	0.04	0.081	0.224

Figure 4.3 Changes in carbapenem-resistant *P. aeruginosa* and *K. pneumoniae* over the four years



4.4 Survey

The response rate for the online survey was 64%. Among the thirty-two hospitals responded two hospitals were excluded because one hospital did not provide any antibiograms and the second hospital antibiograms were not including number of isolates. For the first part of the survey (antibiogram construction), data from previous projects were used.

4.4.1 Antibiogram construction

Antibiograms construction information was available for 38 hospitals out of the total 40 hospitals included in the study. Data about antibiogram construction from another project were used for this study. Multiple methods were employed in year 2009 for routine susceptibility testing, including MicroScan (n = 9), Vitek 2 (n = 11), disk diffusion (n = 5), Phoenix (n = 9), Vitek (n=3) and Sensititre (n=1). Thirty-one hospitals reported removing duplicate isolates from the same patient from their annual antibiograms; while seven hospitals included duplicates (two of them included all duplicates while the other five included some). Thirty-two hospitals reported that surveillance cultures were excluded, and six hospitals included surveillance cultures in their annual antibiograms.

The next two sections described responses of thirty hospitals to the remaining components of the survey.

4.4.2 Carbapenem-resistant *K. pneumoniae*

The survey results of this section are summarized in table 4.7. Twenty-seven hospitals reported that they have encountered clinical isolates of carbapenem-resistant *Enterobacteriaceae* including carbapenem-resistant *K. pneumoniae* within the past 5 years, while three hospitals reported they had not. Of the twenty seven hospitals, eighteen hospitals reported their CRE isolates represented both colonized and clinical (caused clinical infection) organisms. Five hospitals reported that they were clinical isolates only, two reported they were colonizing isolates only, and two hospitals were not sure. The approximate rate of isolation of carbapenem-resistant *Enterobacteriaceae* (all unique isolates from individual patients) varied in 2010 among hospitals. Around 50% of hospitals (14 hospitals) reported an isolation rate of < 5 patients in the past year, seven hospitals encountered CRE in 5 - 20 patients and six hospitals reported more the 20 patients. These isolates were from patients transferred from another hospital and /or long-term care facility in 12 hospitals. Thirteen hospitals were not sure of the source, while just two hospitals reported that these isolates were not from transferred patients. Microbiological tests used to identify and confirm CRE including carbapenem-resistant *K. pneumoniae* varied among hospitals. Most hospitals used imipenem, meropenem, or ertapenem (mainly imipenem) resistance on routine susceptibility test to identify the CRE isolates and then confirmed by the following microbiological methods: Modified Hodge test (n=14); PCR (n=3); E-test (n=1); Modified Hodge test and PCR (n=2); Modified Hodge test, Etest, and PCR (n=1); Modified Hodge test, indirect phenotypic test, and PCR (n=1); other (n=1). However, four hospitals did not use any confirmatory

testing and relied on imipenem or meropenem resistance on routine susceptibility testing.

Part of this survey was to compare if these hospital antibiograms reflected the approximate rate of isolation of CR-KP. According to the survey, twenty hospitals reported that their antibiograms for 2009 and/or 2010 accurately reflected the approximate rate of isolation of CRE organisms, and they reflected the rate of isolation even with a small percentage. They were asked in particular, “For example, if you isolate CREs ‘only rarely’, does your antibiogram show that a small percentage of isolates (e.g., 1 or 2%) of *K. pneumoniae* are resistant to imipenem or meropenem?” Five hospitals reported that they did not include such a percentage in their antibiograms. However, four of these hospitals reported an isolation rate of less than five isolates in the last year which would not affect this study. Additionally, when the rates obtained from antibiograms in 2009 and the rates obtained via the survey were compared they were highly correlated ($r = 0.85$, $P < 0.0001$).

Table 4.7 Survey summary results: Carbapenem-resistant *K. pneumoniae*

Criteria	results			
CR-KP encounter	27/30			
Type of isolates	Colonized & clinical (18/27)	Clinical only (5/27)	Colonized only (2/27)	Unknown (2/27)
Rate of isolation in the past year	< 5 (14/27)	5-20 (7/27)	>20 (6/27)	
Source	Transferred from another hospital / long-term care facility (12/27)	Not sure of the source (13/27)	Not from transferred patients (2/27)	
Microbiology test used to confirm KPC	MHT (14)	PCR (3)	E-test (1)	MHT,PCR (2)
	MHT, PCR, E-test (1)	MHT, PCR, indirect phenotypic test (1)	Other (1)	Routine susceptibility only (4)
ASP	Yes (24/30)	No (6/30)		
ASP role in KPC	Monitor & intervene (21/24)	Don not monitor of intervene (3/24)		
Isolation procedures for infected/colonized patients	(24/24)			

4.4.3 Antimicrobial stewardship efforts

Twenty-four hospitals reported having a formal ASP, while six hospitals reported not having an antimicrobial stewardship program in their institution. Of the twenty-four hospitals, twenty-one hospitals had their antimicrobial stewardship personnel monitor and intervene with CRE including CR-KP infections and/or colonization cases in accordance with infection control personnel, whereas the other three hospitals antimicrobial stewardship personnel do not intervene in these cases. All of the hospitals reported conducting isolation procedures for patients infected or colonized with CRE including CR-KP and implementing strict infection control measures. Most of the CRE cases were handled on a case-by-case basis. Finally, more than half of the responders reported no significant changes in their antimicrobial stewardship activities/program during the years 2006-2009. The other hospitals reported significant improvements in the enforcement of their ASP activities.

One of the survey questions was about the restriction policy of carbapenems. A total of 19 hospitals responded that they restricted carbapenems. Of these, eleven hospitals had restriction policies that entailed a preauthorization requirement. A preauthorization restriction policy was one in which prior approval was required by either pharmacy or medical personnel before the carbapenem agent was dispensed for at least some patient populations. The other eight hospitals stated that they restricted carbapenems, but did not require preauthorization. For example, a restriction policy may entail that a carbapenem could be dispensed upon a physician order provided appropriate criteria

for use were met, such as febrile neutropenia, but the restriction policy did not require preauthorization or confirmation that the carbapenem order met the usage criteria before the drug was released from the pharmacy.

It was not known how long the restriction policy had been implemented; thus, 2009 mean carbapenem use and CR-PA rates and proportions were compared for hospitals that did (n = 19) vs. those that did not restrict carbapenems (n =11), as displayed in table 4.8. Although carbapenem use was lower in hospitals that restricted carbapenems, there were not any statistical differences in either CR-PA and CR-KP rates or proportions for 2009.

Table 4.8 Carbapenem use (DOT/1000PDs) and resistance based on restriction status of carbapenems in 29 hospitals in 2009^a

	Restriction	No restriction	Wilcoxon rank test p-value
Carbapenems	Mean \pm SD	Mean \pm SD	
	34.96 \pm 19.53	62.05 \pm 26.58	0.0111
CR-PA rate (1000 PDs)	Mean \pm SD	Mean \pm SD	
	0.75 \pm 0.42	0.96 \pm 0.75	0.4568
CR-PA proportion (%)	Mean \pm SD	Mean \pm SD	
	17 \pm 5	19 \pm 7	0.6523

^aone hospital data was excluded.

4.5 Carbapenem-resistant *P. aeruginosa* rates/1000 PDs explanatory model

A GEE model utilizing normal distribution and identity link was fitted to explain CR-PA rates/1000 PDs. The model (with lowest QIC_u) to explain CR-PA rates included the variables of fluoroquinolone use (ciprofloxacin, levofloxacin, gatifloxacin), carbapenem use (imipenem, meropenem, doripenem), antipseudomonal penicillin use, and antipseudomonal cephalosporin use. Most of the other proposed models had a very similar QIC_u score to the best model, $\Delta\text{QIC}_u = 1.0$. The full model had a $\Delta\text{QIC}_u = 3.0$ from the best model. Hence, the simplest model identified above was chosen model to explain CR-PA rates as shown in table 4.9. According to the GEE analysis, none of the antipseudomonal antibiotics included in the model were associated significantly with CR-PA rates/1000 PDs.

After selecting the model, adjusting for each of the potential confounders (hospital bed size, CMI, aminoglycoside use, and hospital geographical location) was conducted. The change in the parameter estimates were less than 1% after adding bed size, less than 10% after adding aminoglycosides, and $\geq 20\%$ after adding, CMI, and geographical location. Consequently, the last two confounding variables were added to the model and the adjusted parameter estimates are displayed in table 4.10. According to the GEE analysis, none of the antipseudomonal antibiotics included in the model were associated significantly with CR-PA rates/1000 PDs.

Table 4.9 GEE analysis with carbapenem-resistant *P. aeruginosa* rates/1000 PDs as the dependent variable (crude estimates)

Parameter	β	SE	Z	P value
Intercept	1.015	0.257	3.94	<0.0001
Flourquinolines	0.002	0.001	1.51	0.1305
Carbapenems	-0.002	0.004	-0.52	0.6007
Antipseudomonal cephalosporins	-0.0004	0.003	-0.16	0.8750
Antipseudomonal penicillins	-0.002	0.00	-1.55	0.1220

Table 4.10 GEE analysis with carbapenem-resistant *P. aeruginosa* rates/1000 PDs as the dependent variable (adjusted estimates).

Parameter	β	SE	Z	P value
Intercept	-0.693	0.850	-0.81	0.4151
Carbapenems	-0.004	0.0034	-1.25	0.2093
Fluoroquinolones	0.002	0.0014	1.53	0.1250
Antipseudomonal cephalosporins	-0.001	0.0022	-0.28	0.7827
Antipseudomonal penicillins	-0.003	0.0018	-1.57	0.1171
CMI	1.099	0.551	1.99	0.0461
Geographical region (Mid-Western vs. Mid-Atlantic)	0.1004	0.356	0.28	0.7780
Geographical region (Mid-Continent vs. Mid-Atlantic)	0.1429	0.316	0.45	0.6506
Geographical region (Southeastern vs. Mid-Atlantic)	-0.1672	0.279	0.60	0.5488
Geographical region (Western vs. Mid-Atlantic)	-0.0503	.387	-0.13	0.8967
Geographical region (New-England vs. Mid-Atlantic)	-0.209	0.555	-0.38	0.7063

The steps that were performed after selecting the persimmons model were repeated, but with the proportion of patients in each of the four APR-DRG grouper scores. The model (with lowest QIC_u) to explain CR-PA rates included the variables of fluoroquinolone use (ciprofloxacin, levofloxacin, gatifloxacin), carbapenem use (imipenem, meropenem, doripenem), antipseudomonal penicillin use, and antipseudomonal cephalosporin use. This was followed by adjusting for potential confounders (hospital bed size, aminoglycoside use, hospital geographical location, and APR-DRG scores proportions). Consequently, the APR-DRG groups and geographical location were added to the model, and the adjusted parameter estimates are displayed in table 4.11. According to GEE analysis, there was no significant association between CR-PA rates/ PDs and any of the variables proposed by the model.

Table 4.11 GEE analysis with carbapenem-resistant *P. aeruginosa* rate/1000 PDs as the dependent factor and APR-DRG independent variables (adjusted estimates)

Parameter	β	SE	Z	P value
Intercept	1.103	1.067	1.03	0.3015
Antipseudomonal cephalosporins	-0.003	0.002	-1.39	0.1647
Carbapenems	-0.004	0.004	0.90	0.3674
Fluoroquinolones	0.002	0.002	1.23	0.2169
Antipseudomonal cephalosporins	-0.001	0.003	-0.31	0.7549
Geographical region (Mid-Western vs. Mid-Atlantic)	0.057	0.362	0.16	0.8755
Geographical region (Mid-Continent vs. Mid-Atlantic)	0.080	0.338	0.24	0.8139
Geographical region (Southeastern vs. Mid-Atlantic)	-0.116	0.275	-0.42	0.6729
Geographical region (Western vs. Mid-Atlantic)	0.007	0.350	0.02	0.9837
Geographical region (New-England vs. Mid-Atlantic)	-0.183	0.524	-0.35	0.7268
APR-DRG proportions (base-minor)				
Extreme	2.640	5.779	0.46	0.6478
Major	-0.669	2.324	-0.29	0.7736
Moderate	-0.113	2.542	-0.04	0.9645

4.6 Carbapenem-resistant *P. aeruginosa* proportion explanatory model

A GEE model utilizing binomial distribution and logit link was fitted to explain CR-PA proportions. The model (with lowest QIC_u) to explain variability in CR-PA proportions included the variables of fluoroquinolone use, carbapenem use, and antipseudomonal cephalosporin use. The second best model had a QIC_u score close to the best model, ΔQIC_u 2.15, and included antipseudomonal penicillins in addition to the variables identified in the previous model. Using the principle of parsimony, the model with the lowest QIC_u and fewest explanatory variables was selected. According to the GEE analysis, none of the antipseudomonal antibiotics included in the model were associated significantly with CR-PA proportions over the study period as displayed in table 4.12.

Each of the potential confounding variables (case mix index, bed size, geographical location, aminoglycoside use, and antipseudomonal penicillins) was adjusted for in a similar fashion to the method used in section 4.5. Table 4.13 represents the parameter estimates for the adjusted explanatory model. The parameter estimates were exponentiated for interpretation purposes. According to the GEE analysis, none of the antipseudomonal antibiotics included in the model were associated significantly with CR-PA proportions over the study period.

Table 4.12 GEE analysis with carbapenem-resistant *P. aeruginosa* proportions as the dependent variable (crude estimates)

Parameter	β	Exp(β)	SE	Z	P value
Intercept	-1.508	0.221	0.172	-8.76	<0.0001
Carbapenems	0.003	1.003	0.003	0.97	0.3317
Fluoroquinolones	0.002	1.002	0.001	1.14	0.2524
Antipseudomonal cephalosporins	-0.002	0.998	0.003	-0.42	0.6737

Table 4.13 GEE analysis with carbapenem-resistant *P. aeruginosa* proportion as the dependent variable (adjusted estimates)

Parameter	β	Exp(β)	SE	Z	P value
Intercept	-2.451	0.086	0.668	-3.67	0.0002
Carbapenems	0.004	1.004	0.003	1.26	0.2076
Fluoroquinolones	0.002	1.002	0.001	1.48	0.1388
Antipseudomonal cephalosporins	-0.003	0.997	0.002	-1.29	0.1974
Antipseudomonal penicillins	-0.003	0.997	0.002	-2.02	0.0437
CMI	0.703	2.020	0.463	1.52	0.1291
Geographical region (Mid-Continent vs. Mid-Western)	0.342	1.408	0.211	1.62	0.1051
Geographical region (Southeastern vs. Mid-Western)	-0.337	0.714	0.257	-1.31	0.1900
Geographical region (Mid-Atlantic vs. Mid-Western)	0.295	0.745	0.223	1.16	0.2455
Geographical region (Western vs. Mid-Western)	-0.343	0.710	0.182	-1.88	0.0596
Geographical region (New-England vs. Mid-Western)	-0.209	0.811	0.191	-1.10	0.2733

4.7 Carbapenem-resistant *K. pneumoniae* explanatory model

A GEE model utilizing a binomial distribution and logit link was used to model CR-KP. The best model for explaining CR-KP isolates presence in the 40 hospitals included the variables of carbapenem use, fluoroquinolone use, aminoglycoside use, antipseudomonal penicillins, and hospital geographical location. The second best model had a QIC_u score very similar to the best model, ΔQIC_u 1.80, and included carbapenem use, fluoroquinolone use, third-and forth-cephalosporin use, aminoglycoside use, antipseudomonal penicillins, and geographical location. Using the principle of parsimony, the model with lowest QIC_u and fewest explanatory variables was preferred. The parameter estimates were exponentiated for interpretation purposes

After selecting the "best" model, it was adjusted for the potential confounders (bed size, CMI, third and fourth-generation cephalosporins) similar to the method used in section 4.5. None of the confounding variables changed the parameter estimate by more than 20%; hence, the crude estimates were used. Table 4.14 represents the parameter estimates for the explanatory model.

According to GEE analysis, carbapenem antibiotic use was significantly ($P=0.0149$) associated with the presence of CR-KP isolates. Thus, the estimated change in the odds of carbapenem-resistant *K. pneumoniae* for a one-unit increase in carbapenem antibiotic use is 1.04 with a 95% confidence interval of (1.02, 1.06). This means that for ten DOT/1000 PDs increase in carbapenem use, the odds of carbapenem-resistant *K. pneumoniae* isolation increase by 42%. Hospitals located in the Midwestern, Midcontinent, Western, and New -England regions of the nation were less likely to

encounter CR-KP isolates when compared to hospitals located in the Mid-Atlantic region of the nation ($P = 0.0002, 0.0140, 0.0001, 0.0304$, respectively).

Finally, antipseudomonal penicillin use was significantly associated with CR-KP isolation ($P=0.0477$). Thus, the estimated change in the odds of carbapenem-resistant *K. pneumoniae* for a one unit increase in antipseudomonal penicillin antibiotic use is 0.985 with 95% confidence interval of (-0.0298,-0.0002). This means that for a ten DOT/1000 PDs increase in antipseudomonal penicillin use, the odds of CR-KP isolation decreased by 14%.

Table 4.14 GEE analysis with carbapenem-resistant *K. pneumoniae* as the dependent variable

Parameters	β	Exp(β)	SE	Z score	P value
Intercept	4.002	54.407	1.477	2.71	0.0067
Carbapenems	0.035	1.036	0.014	2.43	0.0149
Fluoroquinolones	-0.009	0.991	0.006	-1.63	0.1033
Antipseudomonal Penicillins	-0.015	0.985	0.008	-1.98	0.0477
Aminoglycosides	-0.053	0.948	0.027	-1.93	0.0537
Geographical region (Mid-Western vs. Mid-Atlantic)	-2.922	0.054	0.779	-3.75	0.0002
Geographical region (Mid-Continent vs. Mid-Atlantic)	-2.688	0.068	1.049	-2.56	0.0140
Geographical region (Southeastern vs. Mid-Atlantic)	-0.781	0.458	0.968	-0.81	0.4199
Geographical region (Western vs. Mid-Atlantic)	-4.399	0.012	1.138	-3.86	0.0001
Geographical region (New-England vs. Mid-Atlantic)	-3.057	0.047	1.413	-2.16	0.0304

*bolded = P value < 0.05

CHAPTER 5

Discussion

5.1 Summary of findings

This chapter summarizes the study, providing a discussion of the study results, conclusions, limitations, and suggestions for future research.

In this current investigation, it was attempted to explain carbapenem-resistant *Pseudomonas aeruginosa* and *Klebsiella pneumoniae* in a consortium of academic medical centers over the period from 2006-2009.

Objective 1 described trends of antimicrobial use in 40 hospitals over the study period. Total broad spectrum antibiotics for five classes of gram-negative antibiotics increased over time. Carbapenem and piperacillin-tazobactam use increased by 25%, while fluoroquinolone and aminoglycoside use decreased over time.

Objective 2 described rates and proportions of CR-PA and showed rates and proportions of CR-PA to be stable over four years.

Objective 3 described rates, proportions, and number of CR-KP and showed an increase of CR-KP over the four-year period. Moreover, the CR-KP isolation rate, isolation procedure, source, microbiological identification techniques, and isolation rate were described in thirty hospitals via a survey. CR-KP rates obtained from antibiograms were strongly correlated with rates obtained by the survey. Hospitals conducted strict measures with regard to isolation of such organisms.

Objective 4 identified important predictors of CR-KP isolation within the 40 participating hospitals over four-year period among broad spectrum gram-negative antibiotic classes, CMI, and hospital demographics (bed size, geographical location). The GEE analysis utilizing binary distribution and logit link showed a significant association between hospital geographical location and carbapenem use, and antipseudomonal penicillin use. However, no significant predictors were associated with CR-PA rates/1000 PDs or proportions over the study period.

5.2 Discussion of results by objective

Objective 1

Descriptive statistics were used to assess current broad spectrum gram-negative antibiotic use in U.S. academic medical centers. The observations of this investigation are updated and consistent with prior investigation of antibiotic use in UHC consortium of hospitals from 2002 to 2006 conducted by Pakyz et al.[48] The previous investigation showed a 59% increase in carbapenem use and an 84% increase in the use of piperacillin-tazobactam between 2002 and 2006. However, this investigation showed a lower rate of increase in carbapenem use and piperacillin-tazobactam over the period of 2006 to 2009, 24% and 25% respectively. Further exploration assessed 19 hospitals that restricted carbapenem use and found they used less carbapenems than hospitals that did not restrict carbapenems, but had a significant ($P=.0001$) increase in carbapenem use, from 29.31 DOT/1000 PDs in 2006 to 39.80 DOT/1000 PDs in 2009. Carbapenem use increased presumably because of increasing resistance among gram-

negative organisms, including ESBLs producing *Enterobacteriaceae*, to other more commonly used antimicrobial agents.[124, 125] The proportion of piperacillin-tazobactam use was similar to the proportion of total third-and fourth-generation cephalosporin use. Fluoroquinolone use decreased slightly over the study period; however, they remained the most commonly prescribed class of antibiotics among broad spectrum antimicrobials throughout the study period. The previous investigation showed stability in fluoroquinolone use over 2002 -2006; however, this investigation includes twice as many hospitals. Similar to Pakyz et al., third- and fourth-generation cephalosporin use did not change during the study period. Finally, aminoglycoside use was decreasing in the current study sample.

Objective 2

In the current investigation, rates and proportions of CR-PA remained stable or even declined over four years, while the mean use of carbapenems increased. This observation is supported by similar recent investigation of CR-PA proportion over six years in 25 hospitals.[47] CR-PA resistance is stable over time, although carbapenem use is increasing over the same period. However, most of these organisms are multidrug resistant, and the contributions of other antibacterial compounds to their prevalence remains to be fully explained.[50] In the same context, when hospitals which restricted the availability of carbapenem antibiotics were compared to those which did not restrict, it was found that hospitals restricted carbapenem used significantly less than hospitals that did not restrict use, but restriction was not associated with lower rates of CR-PA.

Objective 3

The rates and proportions of CR-KP varied among the participating hospitals in this investigation. Proportions and rates of CR-KP increased by 120% and 130%, respectively, between 2006 and 2009. However, the magnitude of this increase varied considerably among hospitals, as some hospitals reported outbreaks (more than 100 isolate per year), while some hospitals reported the isolation of less than 5 isolates. However, this observation is not consistent with the MYSTIC report that found the incidence of KPC to be declined in 2008 compared to the steep increase in resistance rates observed from 2004 to 2007 [71]. This is likely due to the larger number of participating hospitals of this investigation compared to the MYSTIC report (40 vs. 15). Furthermore, the CDC reported, as of December 2010, that KPC-producing isolates have been received or identified from 36 states, which indicates the spread of these organisms. [28,36] Carbapenem-resistant *K. pneumoniae* is the most common carbapenem-resistant *Enterobacteriaceae* in the United States. Based on the survey results for 30 hospitals, antibiograms were found to reflect the approximate rate of isolation of CR-KP, specifically, both rates from antibiograms and the survey were highly correlated for 2009. Almost one half of the responders confirmed the isolation of CRE to be of transferred patients from another hospital and/or long-term care facility which is the true case in most outbreaks reported as patients come to the hospital from another healthcare facility carrying these organisms. However, the other half of the responders were not sure about the source of CRE isolates; these could be from hospital patients. The presence of CR-KP carriage has been described in a number of

studies involving patients from long-term acute care hospitals.[126-128] One investigation found that more than half of the patients with carbapenem-resistant gram-negative bacteria were admitted from post-acute care facilities implying that these health-care settings may be significant reservoirs for transmission and spread of these bacteria. [127] According to the survey results, most of the hospital's CRE isolates were both colonizing and clinical isolates which can occur with the presence of a small number of CR-KP clinical cases. For example, one investigation of three patients with CR-KP infection transferred from long-term acute care hospital showed that 49% of the residents were having colonized with CRE isolates.[27] The majority of the hospitals used phenotypic method along with the imipenem (in some hospitals meropenem or ertapenem) resistance routine susceptibility test to confirm CR-KP. Modified Hodge test was the most common confirmatory technique used either alone, or in combination with other techniques, mainly PCR method. Although the CLSI recommendation to change (lower) carbapenem breakpoints were corrected in 2010, it appears that hospitals' microbiology laboratories continue to depend on the older breakpoints in addition to phenotypic confirmatory tests (e.g. MHT). This is likely due to the delay in the Food and Drug Administration (FDA) approval process, consequently the automated system manufacturers have not been able to provide microbiology laboratories with validated equipments with the new CLSI breakpoints.[129] However, with the lowering of the carbapenem breakpoints and exclusion of the need to perform MHT testing, it remains questionable whether some patients colonized/infected with carbapenemase-producing organisms might be missed. [129]

One item that all hospitals were consistent about is the requirement for universal precautions and infection control measures including patient isolation being performed as early as colonized or infected patients with CRE were identified. Strict infection control measures have been shown to decrease and contain the spread and the incidence of CRE in many outbreaks. [88-90]

Objective 4

Carbapenem-resistant *K. pneumoniae*

The determinants of bacterial resistance are complex and multifactorial; no single comprehensive model explaining resistance has yet been developed. This investigation used generalized estimating equations (GEE) with binary distribution and logit link to examine the relationship between some potential predictor variables, in particular aggregated antimicrobial drug use, SOI (CMI), hospital demographics, and aggregated carbapenem-resistance in clinical isolates of *K. pneumoniae* in a consortium of UHC university teaching hospitals. CR-KP is resistant to most available antibiotic drugs, leaving few options such as colistin or tigecycline, which are more toxic and possibly less effective. [21, 26, 76] Infections with CR-KP are associated with poor outcomes and a high mortality rate.[22,28,29] The results of this investigation showed that carbapenem antibiotic use, geographical location, and antipseudomonal penicillins were significantly associated with CR-KP isolation, with carbapenem use being positively associated with CR-KP isolation. While there was no ecological study at the hospital level to compare the findings with, this observation was in general agreement with other single center case control studies in which prior carbapenem use was identified as an independent risk factor for the isolation of CR-KP.[22, 29, 42, 43, 44] This investigation

observed a significant increase in CR-KP rates and proportions over the period of 2006 to 2009 in a relatively large sample of US hospitals. Carbapenem use continued to increase in the same set of hospitals over the study period. This increase is likely due to the wide use of carbapenems for the treatment of severe infections caused by ESBL producing *Enterobacteriaceae* including *K. pneumoniae* as carbapenems considered the drug of choice in these infections. Carbapenems have been broadly used to treat *Enterobacteriaceae* species which may increase antibiotic selection pressure, consequently promoting carbapenem-resistance bacteria development.[51-53] The specific mechanisms of CR-KP include production of KPC, metallo-beta-lactamases and loss of porins as described in detail in chapter 2, section 2.5. This investigation included all CR-KP isolates, regardless of the mechanism of resistance as this information could not be obtained. Therefore, the variation in resistance mechanisms, which may be associated with different risk factors among the CR-KP isolates, could not be incorporated in the model to better clarify the role of carbapenem use in the isolation of CR-KP. Additionally, hospital geographical location was identified as an independent predictor of the CR-KP isolation. Hospitals located in the Mid-Atlantic region were more likely to encounter CR-KP isolation when compared to hospitals located in the Midwestern, Mid-Continent, Western, and New-England regions of the continental U.S. The first KPC producing isolate was identified in North Carolina in 2001. Reports soon followed from other regions of the U.S., mostly on the East Coast. Subsequently, the escalating prevalence of CR-KP infection in the Mid-Atlantic coastal region of the United States was documented and some of these sporadic outbreaks have turned into an endemic spread.[30-32] The CDC reported as of December 2010, that KPC-producing

isolates have been received or identified from 36 states.[36] Hospitals located in the Southeastern region were not different from the Mid-Atlantic region indicating the endemic spread of these isolates in this region.

The antipseudomonal penicillins and CR-KP association was in a negative direction, implying a favorable effect of the increase in the use of antipseudomonal penicillins (mainly piperacillin-tazobactam) on CR-KP. The favorable impact of agents like piperacillin-tazobactam on CR-KP could imply that hospitals using more piperacillin-tazobactam could be using less of other classes of antibiotics with known effect on CR-KP. CMI and APR-DRG categories were not part of the final model to explain CR-KP isolation, but adjusting for each of them was considered. However, when they were forced into the model, there was no significant association with CR-KP isolation.

Carbapenem-resistant *P.aeruginosa*

This investigation used generalized estimating equations (GEE) to examine the relationship between some potential predictor variables, in particular aggregated broad spectrum antipseudomonal antimicrobial drug use, SOI (CMI, APR-DRG), hospital demographics and aggregated carbapenem-resistance *P. aeruginosa* in a consortium of UHC university teaching hospitals. Binomial distribution and logit link were used to model carbapenem-resistant *P. aeruginosa* proportions; normal distribution and identity link were used to model carbapenem-resistant *P. aeruginosa* rates/1000 PDs. The results of this investigation showed no significant association between CR-PA rates/1000 PDs, proportions, and any of the predictors identified in each model. Previous single center case control studies identified prior carbapenem use,

fluoroquinolone use, and extended-spectrum cephalosporins as risk factors for CR-PA; however, none of the other antipseudomonal antibiotic classes identified in the model selection process were significantly associated with CR-PA proportions and rates. Similarly, Eagye et al. found no significant association between carbapenem use, fluoroquinolone use, and CR-PA incidence rates in a multivariable model in 25 hospitals. [47]

Finally, when CMI was forced into the model as a predictor, there was a positive association with CR-PA rates/1000 PDs. Hospitals with an elevated CMI have sicker patients and perform more complicated procedure. Also, none of the APR-DRG categories were associated with CR-PA rates or proportions when forced into the model.

5.2 Limitations

First, the current study design is a longitudinal ecological study. Using an ecological approach has some limitations. By definition, ecologic data contain only marginal observations on the common distribution of individually defined confounders and outcomes.[130] “Ecological fallacy” occurs when the results of an ecological study are interpreted as being applied to individuals.[131] In this current investigation, the role of aggregated antibiotic use on carbapenem-resistant gram-negative bacteria in U.S. academic hospitals was assessed; the resulting findings do not necessarily reflect to the patient level. Ecological (hospital-level) variables weaken the linkage between patient-level variables including outcome, exposure, and covariates, and may result in a complex misrepresentation at the patient-level. For example, if this same project was conducted with patient-level data, stronger linkages would be found compared to

ecological study results.[91] According to Harbarth et al., ecological studies in general can identify trends, but lack sufficient details to make the case for causality between antibiotic exposure and resistance in gram-negative bacteria. He suggested including patient-level data analysis in these studies to provide more valid results.[131] In contrast, Turnidge et al. stated that “given the complexity of resistance ecology.....when correlations are shown they are almost certainly of major importance and suggest that reduction in consumption of the correlated antibiotic class will reduce resistance”. [132] Despite the limitations of ecological studies there was adjustments for some confounders.

Second, the response rate for the survey or antibiogram requests was not 100%. It is possible that those that did not respond to the survey would have different patterns of antibacterial drug use and/or carbapenem-resistance. However, the non-responding hospitals had similar demographic characteristics to responding hospitals; the mean bed size, CMI, and patient age were similar among responders and non-responders. The non-respondent rate can be explained, in part, due to changes in the infectious diseases clinical pharmacists or physicians positions over the study period. A contact from previous studies is more likely to respond compared to a new clinical pharmacist, who may not be aware of the UHC CRM database.

Third, this investigation used aggregated data at the hospital-level including aggregated susceptibility data obtained from whole-house annual antibiograms. The literature is conflicting as to whether antibiograms are truly reflective of nosocomial resistance rates.[133,134] However, a network of hospitals such as described in this investigation

may be able to link changes in antimicrobial drug use to changes in resistance using whole-house antibiograms because of greater statistical power and the potential to adjust for confounders and some methodological differences in antibiogram reporting methods. This interpretation is supported by the findings from a MYSTIC surveillance project. [135] In 10 -15 medical centers observed over a 3 year period, significant positive relationships in aggregated mean drug use and antimicrobial resistance rates were observed for a number of “drug-bug” pairs. However, at the institution level no significant relationships were observed. Although additional research is necessary to determine if this approach is valid, this investigation and the limited available data suggest that results from annual antibiograms correlate well to more established surveillance programs. [134,136]

Additionally, there was variability between hospitals with respect to the method of measuring susceptibility to carbapenem antibiotics. While most hospitals used CLSI breakpoints to determine susceptibility, the source of clinical isolates between hospitals was likely to be variable depending upon, for example, the number of specimens obtained from outpatients and the proportion of cultures obtained from pediatric patients. Additionally, while most hospitals did not include surveillance cultures in the antibiogram and most deleted duplicate cultures from the same patients, this was not always the case. Within a hospital, these sources of variability may be acceptable since the practice is likely to be consistent, but these differences limit the ability to compare across hospitals. However, the incorporation of some antibiogram construction data (e.g. removal of duplicates, surveillance cultures) as covariates did not change the results of this investigation.

Fourth, hospital infection control measures and the impact of patient-to-patient transmission were not measured. Patients infected with MDR bacteria from another hospital may impact the resistance rate in that hospital.[126-128]

Finally, this study used hospital data from the UHC. Secondary databases can be convenient since the researcher does not have to wait for the data to be prospectively collected. However, problems with inadequate or inaccurate codes in databases may introduce bias in the results.[137,138] While potential predictors were considered, there may be other factors that influence the prediction of carbapenem-resistant bacteria that were not available. Factors such as non-formulary antibiotic drug use, antimicrobial combination therapy, and infection control measures cannot be assessed, as the UHC database does not capture such information. Hence, these factors which contribute to drug use and bacterial resistance were not included in the study. Further, the UHC consists of all-payer hospital discharge data from most of the United States academic medical centers. However, community hospitals are not represented.

5.4 Future research

This study provides the basis for some potential future research. While, many of these findings were in agreement with other studies identified, some of the findings were not. The modifiable risk factors identified in this investigation could be a potential target of intervention by antimicrobial stewardship programs. Further, future studies that adopt a multilevel approach would provide a better understanding between antibiotic use and bacterial resistance. A multilevel approach takes into consideration the nested

hierarchies of data and allows integration of observations on all available levels: physiological (which examines exposures and responses of systems within individuals); individual (which examines exposures and responses of individuals); and aggregate or contextual (which examines exposures and responses of aggregates or clusters of individuals, such as hospitals).[130,139] Incorporation of patient-level data, including microbiology data, would potentially provide a better explanation of resistance.[131]

5.5 Conclusions

The results of this investigation did not show significant relationships between antipseudomonal antibacterial drug use and rates or proportions of carbapenem-resistant clinical isolates of *P. aeruginosa* in 40 UHC academic medical centers over a four-year period. However, continued examination of these relationships will remain important since both antibacterial drug use and resistance among many clinical isolates will continue to evolve over time, although the direction is unclear and the interrelationships are currently uncertain. The increasing spread of carbapenem-resistant *K. pneumoniae* over time and across different regions of the U.S., and the significant relationship between carbapenem antibiotics use and carbapenem-resistant *K. pneumoniae*, emphasize the challenges associated with the treatment of multidrug-gram-negative bacteria.

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Appendix A

UHC-VCU Antimicrobial Stewardship Research Network

Survey questionnaire: Antimicrobial stewardship and carbapenem-resistant Enterobacteriaceae.

Hospital name _____

Name of Person Completing the Survey _____

1. What is your current (2009/2010) method for susceptibility testing of routine clinical isolates?

- MicroScan
- Vitek
- Vitek 2
- Phoenix
- Sensititre
- Etest
- Disk diffusion
- Other

2. Are all duplicate isolates from the same patient removed from the annual antibiogram?

- Yes
- No

2.a. Are all duplicates included, or is there an algorithm for including some duplicate isolates, e.g. a second isolate > 30 days following the first is included?

- Yes, all duplicates are included.
- No, only some are included

Please summarize the algorithm:

3. Are surveillance cultures included in the antibiogram?

- Yes
- No

4. How are "intermediately susceptible" isolates categorized for the antibiogram?

- Susceptible
- Resistant

5. Has your hospital encountered clinical isolates of carbapenem-resistant enterobacteriaceae (CRE) [including carbapenem -resistant K.pneumonia (KPC)] within the past 5 years? If your answer is 'Yes' you will be taken to a few additional questions, If you answer is 'No', you will skip to question 6.

- Yes
- No

5.a. Have these CREs been colonizing organisms or have they caused clinical infection, or both?

5.b. What is an approximate rate of isolation of CREs (all unique isolates from individual patients) in the past year (2010)?

- Only rarely(from < 5 patients in the past year)
- Occasionally (from 5-20 patients in the past year)
- Commonly (from > 20 patients in the past year)

5.c. Were any of these CREs brought in by patients transferred from another hospital and/or nursing home?

- Yes
- No
- Don't Know

5.d. How were these CREs identified / confirmed?

- Imipenem-resistance on routine susceptibility tests
- modified Hodge test
- E-test
- Inhibitor based test
- PCR based test
- EDTA-based synergy test
- Others

UHC-VCU Antimicrobial Stewardship Research Network

Survey questionnaire: Antimicrobial stewardship and carbapenem-resistant Enterobacteriaceae.

Hospital name _____

Name of Person Completing the Survey _____

1. What is your current (2009/2010) method for susceptibility testing of routine clinical isolates?

- MicroScan
 Vitek
 Vitek 2
 Phoenix
 Sensititre
 Etest
 Disk diffusion
 Other

2. Are all duplicate isolates from the same patient removed from the annual antibiogram?

- Yes
 No

2.a. Are all duplicates included, or is there an algorithm for including some duplicate isolates, e.g. a second isolate > 30 days following the first is included?

- Yes, all duplicates are included.
 No, only some are included

Please summarize the algorithm:

3. Are surveillance cultures included in the antibiogram?

- Yes
 No

4. How are "intermediately susceptible" isolates categorized for the antibiogram?

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 Resistant

5. Has your hospital encountered clinical isolates of carbapenem-resistant enterobacteriaceae (CRE) [including carbapenem -resistant K.pneumonia (KPC)] within the past 5 years? If your answer is 'Yes' you will be taken to a few additional questions. If you answer is 'No', you will skip to question 6.

- Yes
 No

5.a. Have these CREs been colonizing organisms or have they caused clinical infection, or both?

5.b. What is an approximate rate of isolation of CREs (all unique isolates from individual patients) in the past year (2010)?

- Only rarely(from < 5 patients in the past year)
 Occasionally (from 5-20 patients in the past year)
 Commonly (from > 20 patients in the past year)

5.c. Were any of these CREs brought in by patients transferred from another hospital and/or nursing home?

- Yes
 No
 Don't Know

5.d. How were these CREs identified / confirmed?

- Imipenem-resistance on routine susceptibility tests
 modified Hodge test
 E-test
 Inhibitor based test
 PCR based test
 EDTA-based synergy test
 Others

5.e. Do your antibiograms for 2009 and/or 2010 accurately reflect the approximate rate of isolation of CRE organisms? For example, if you isolate CREs "only rarely", does your antibiogram show that a small percentage of isolates (eg., 1 or 2%) of K. pneumoniae are resistant to imipenem or meropenem?

- Yes
 No

6. Do you have a "formal" antimicrobial stewardship program (ASP), and do ASP personnel monitor or intervene in cases of CRE infection and/or colonization?

- Yes - Yes
 Yes - No
 No - No

Please explain.

6.a. Are there isolation procedures for patients infected or colonized with CRE ?

- Yes
 No

6.b. Did the ASP team have a pre-prepared intervention plan or its case by case bases? please explain.

- Yes
 No

7. Does your hospital restrict the availability of carbapenem antimicrobials?

- Yes
 No

7.a. Please describe your restriction policy?

8. Have any of your antimicrobial stewardship activities/program elements changed significantly during the years 2006 - 2009?

- Yes
 No

Please explain

9. Please provide any additional relevant information or comments.

Appendix B

VCU

MCV CAMPUS

V i r g i n i a C o m m o n w e a l t h U n i v e r s i t y

Office of Research
Office of Research Subjects' Protection

DATE: February 23, 2012

TO: Ronald E. Polk, PharmD
Department of Pharmacy
Box 980533

FROM: John D. Roberts, MD
Chairperson, VCU IRB Panel A
Box 980568

Biotech Research Park, Bldg. 1
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*BVT R JDR
2/23/12*

RE: VCU IRB #: HM12377
Title: Predictors of Rates of Resistant Gram-Negative Bacteria in a Consortium of Academic Medical Center Hospitals

On February 20, 2012, this research study was approved for continuation by expedited review according to 45 CFR 46.108(b) and 45 CFR 46.109(e) and 45 CFR 46.110 Category 5.

PROTOCOL: Predictors of Rates of Resistant Gram-Negative Bacteria in a Consortium of Academic Medical Center Hospitals (VCU Research Plan; Version 2, dated 06/24/10; received 1/23/12; VCU IRB Study Personnel Roster, dated 2/19/12)

CONSENT/ASSENT:

- All four conditions for waiver of consent have been met. See §45 CFR 46.116(d). The IRB Panel has waived all elements of consent.

This approval expires on January 31, 2013. Federal Regulations/VCU Policy and Procedures require continuing review prior to continuation of approval past that date. Continuing Review report forms will be mailed to you prior to the scheduled review.

The Primary Reviewer assigned to your research study is Benjamin Van Tassell, PharmD. If you have any questions, please contact Dr. Van Tassell at bvantassell@vcu.edu or 828-4583; or you may contact Stephan Hicks, IRB Coordinator, VCU Office of Research Subjects Protection, at hickssa2@vcu.edu or 828-9876.

Attachment – Conditions of Approval

Vita

Name: Mera Abdel-Karim Ababneh

Birth date/ Place: December, 29, 1982 / Irbid-Jordan

Citizenship: Jordanian

Education:

2006: Pharm.D degree/ Jordan University of Science and Technology

Teaching experience:

September 2006 - July 2008:

Clinical Preceptor/ Cardiology rotations/ King Abdallah University Hospital

Teaching and Research Assistant/ Clinical pharmacy department/ School of Pharmacy/

Jordan University of Science and Technology

Honors:

2006 : Full scholarship for a graduate degree by Jordan University of Science for 4 years.

2009 : Best poster presentation award/13th Annual Graduate Student Symposium and Exhibit/VCU

