Risk of hospital-acquired infections and drug resistance caused by gram-negative bacteria in patients with multiple hospitalizations

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Submitted in partial fulfillment of the
requirements for the degree of
Doctor of Philosophy
under the Executive Committee
of the Graduate School of Arts and Sciences

COLUMBIA UNIVERSITY

2017

ABSTRACT

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Patients who experience multiple hospitalizations over short periods of time may be at greater risk of hospital-acquired infections (HAIs). While it is known that prior hospitalizations are associated with HAIs, there is a gap in knowledge regarding which factors of prior hospitalizations have an impact on the risk of HAIs in subsequent hospitalizations. HAIs caused by gram-negative bacteria (GNB) are of particular concern due to their propensity to develop drug resistance and the limited antibiotics available to treat them. The aims of this dissertation are to: 1) examine clinical and patient risk factors associated with acquiring at least one gram-negative hospital-acquired infection in adult patients with multiple hospitalizations; 2) systematically review the literature assessing the association between repeat gram-negative bacterial infections and changes in antibiotic susceptibility patterns; and 3) assess the association between repeat infections with three common gram-negative pathogens and risk of subsequent drug resistant infections with the same species among patients with multiple hospitalizations.

A retrospective cohort study was conducted to identify risk factors from prior hospitalizations associated with incident HAIs caused by three common GNB. Of the 129,372 patients with multiple hospitalizations, 1,672 (1.3%) acquired *K. pneumoniae*, 1,127 (0.9%) acquired *P. aeruginosa*, and 262 (0.2%) acquired *A. baumannii* infections. In survival analyses, older age, mechanical ventilation, history of chronic diseases, and increasing days of use of

antibiotics decreased the time to infection for all 3 pathogens. This study highlights potential modifiable risk factors for infection control.

Patients with multiple hospitalizations are also inherently at greater risk for repeat HAIs which may result in decreased antibiotic susceptibility, making them more difficult to treat. A systematic review was conducted to evaluate if there is an association between repeat GNB HAIs and drug resistance. From 2000 to 2015, only seven studies explicitly examined repeat GNB HAIs and change in antibiotic susceptibility, five of which reported decreased susceptibility in later infections.

The association between repeat GNB HAIs and risk of drug resistance among patients with multiple hospitalizations was then investigated with available electronic medical record data. The risk of a drug-resistant *K. pneumoniae* HAI increased by 1.14 times (95%CI: 1.04-1.24) with each prior *K. pneumoniae* HAI, after adjusting for potential confounders and antibiotic use. Similarly, patients with repeat *P. aeruginosa* infections had a 1.23 times increased risk of a subsequent drug-resistant infection (95%CI: 1.12-1.36) with each prior *P. aeruginosa* HAI as compared to patients with only one infection. Repeat *A. baumannii* infections were not analyzed due to limited sample size.

The studies in this dissertation demonstrate that patients with multiple hospitalizations are a high-risk population for GNB HAIs. Prevention of GNB HAIs in this group is critical in order to reduce complications to medical care and limit transmission of infections to others in healthcare facilities and the community. Patient medical history can be used for infection risk assessment and to guide future medical care to reduce risk of infection in patients with multiple hospitalizations.

TABLE OF CONTENTS

LIST OF FIGURES

LIST OF TABLES

Acknowledgements

First and foremost, I would like to thank my advisor and dissertation sponsor, Dr. Elaine Larson. It has been an honor to be her PhD student these last few years. I am particularly appreciative of her time, dedication to her students, and quality feedback and guidance. I have learned much about research, infections and infection control from Dr. Larson.

I am also grateful for my internal committee, Dr. Stephen Morse and Dr. Barun Mathema for their time, interest, and helpful comments. I'd also like to thank the other members of the committee, Dr. Neil Schluger and Dr. Christine Kubin as well as the proposal readers, Dr. Dana March and Dr. Tiffany Harris for their insightful questions and interest in this research.

I would like to express my gratitude for the funding sources that made my Ph.D. work possible. I was funded by the Hearst Foundation through their Doctoral Fellowship in Aging and Public Health for my first three years in the program and was honored to be TIRI fellow for my last year.

Through working with Dr. Larson, I met and worked with other researchers as part of the HIT-HAI research team. These researchers, including Dr. Amanda Hessels and Dr. Jianfang Liu, were instrumental in helping me understand the field of infection control and in honing in on my research questions.

In addition, countless family and friends supported me through the past five years – without them, none of this would have been possible. While too many to list here, I heartily thank each and every one.

Dedication

This work is dedicated to my family that have supported me, without hesitation, throughout the years. My parents Mukesh & Manjula, husband Sumeet, brother Mayank, and of course the pups Suki and Henna that are so dear to my heart, I could not have made it this far without all of you.

1. Introduction

Hospital-acquired infections (HAIs) are often preventable and yet continue to account for a significant number of complications in hospitalized patients. In 2014, a landmark study reported that 1 in every 25 patients hospitalized in the United States acquired a HAI [1]. In addition to the $5.7 to $6.5 billion in attributable annual healthcare costs for the US, HAIs are associated with substantial complications and deaths in hospitalized patients, particularly those in intensive care units (ICUs) [2, 3]. In older adults, such infections cause a two-fold increase in 90-day mortality rate and an average of 10 additional days in the hospital [4].

1.1 Gram-negative bacteria

Gram-negative bacteria (GNB) are implicated as the causative organisms in 20% of HAIs in hospital settings and 60% of HAIs in intensive care units [1, 5]. In hospital settings, three of the primary GNB of interest are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* [6], all three of which cause opportunistic infections and have unique features that make them particularly pathogenic in hospital settings. GNB differ from gram-positive bacteria in that their cell structure has only a thin peptidoglycan cell wall and an outer membrane, with the latter acting as an extra protective layer against antibiotics. This additional protection means that a limited number of antibiotic classes are effective in destroying GNB [6]. Once drug resistance develops in GNB, there are few options available for treatment.

K. pneumoniae are encapsulated gram-negative rods commonly found in the environment such as soil and water and more importantly, in patients, staff and on surfaces in medical settings. As a common nosocomial pathogen, *K. pneumoniae* are frequently implicated in urinary tract and respiratory tract infections [7]. The organism has several virulence factors that make it difficult to

treat and destroy. One of the most studied is the capsule; *Klebsiella* form polysaccharide capsules that prevent phagocytosis, which make them harder to destroy in the hospital environment [7, 8]. In addition, the outer membrane of the bacteria secretes endotoxins called lipopolysaccharide that assist in evading phagocytosis. Other resistance mechanisms include pili that help adhere the bacteria to surfaces and the use of siderophores that transport iron to the bacteria for increased bacterial growth and dissemination [9]. β-lactam antibiotics such as penicillins, cephalosporins and carbapenems are typically the first line of treatment for *K. pneumoniae* and other gram-negative bacteria. However, the production of extended-spectrum β-lactamases (ESBLs) and *K. pneumoniae* carbapenemase (KPC) by *K. pneumoniae* have made the bacteria resistant to most available drugs [10].

Infections caused by *Pseudomonas aeruginosa* have been frequently found in patients suffering from chronic diseases, particularly in the respiratory tract such as chronic obstructive pulmonary disease (COPD) and cystic fibrosis (CF) [11]. *P. aeruginosa* causes ventilator-associated pneumonia (VAP), a major source of morbidity and mortality in hospitalized patients, particularly those in ICUs [12]. With the widespread use of antibiotics, selective pressure has led to an increase in ESBL- and carbapenemase-producing *P. aeruginosa* [13]. Few antibiotic therapy options remain effective and clinicians have returned to the use of colistin, a potentially toxic antibiotic, to treat patients with multi-drug resistant (MDR) and extensively drug-resistant (XDR) pseudomonal infections [14].

Acinetobacter baumannii are gram-negative coccobacilli that have been commonly isolated in hospitals in the last two decades. Like other hospital-acquired infections, *A. baumannii* can be transmitted through hand contact or medical devices and equipment. Similar to other bacteria, *A. baumannii* are able to form biofilms on a variety of surfaces and environments but can

also survive in dry environments [15, 16]. It can also spread through airborne droplets making it very difficult to control [17].

1.2 Drug resistance in GNB

Gram-negative bacteria are intrinsically resistant to some antibiotics and may develop resistance to others. For example, most GNB, particularly *P. aeruginosa*, are intrinsically resistant to many classes of antibiotics because of the low permeability of the outer membrane and selective intake due to porins and efflux pumps [18]. Another method in which GNB acquire drug resistance is through the exchange of resistance genes. Genes that encode resistance factors are frequently found on plasmids, small strands of self-replicating DNA within the cytoplasm of the bacteria. Through horizontal gene transfer, resistance genes are spread among bacterial cells. For example, genes that produce ESBLs, resulted from the increased prevalence of plasmid-encoded β-lactamases among GNB and the subsequent use of other β-lactam antibiotics such as quinolones and third generation cephalosporins [19].

GNB also use biofilms, aggregate cell colonies that adhere tightly to surfaces, to harbor resistance genes [20, 21]. Biofilms are groups of tightly connected cells in which horizontal gene transfer can occur quickly and unhindered. Biofilms prevent the entry of antibiotics through their thick cover and produce toxins that reduce the hosts immune system response [22]. These bacterial biofilms are able to grow on inanimate surfaces such as ventilators, catheters, implants, and other medical devices, making drug resistant cells more pervasive in hospital environments.

Another method by which GNB evade antibiotics is through persister cells. Persister bacterial cells are non-dividing cells that are able to evade the immune system response and antimicrobials because of their slow metabolic processes [22]. These cells usually retain the same

resistance pattern as the original population. Bacteria considered as high-persistence (*hip*) mutants have specific *hip* genes that cause the formation of persisters in a bacterial population [23]. *Hip* mutants, usually a tiny proportion of the bacterial population, remain in the host when other bacteria are killed by antibiotics. They then reactivate to become regular growing cells and can cause re-infection in the host. When bacteria have both resistance genes and *hip* genes, they can recolonize the host with drug-resistant organisms or continue to reside on surfaces.

Starting from the late 1980s and early 1990s, GNB, particularly *Klebsiella* spp., with acquired resistance to a range of cephalosporins through the production of ESBLs, spread throughout US hospitals [18, 24, 25]. Carbapenems used to treat ESBL- producing GNB were also rendered ineffective through the emergence of bacteria producing carbapenemases [25]. Bacteria with MDR and XDR have also emerged in recent times. From 1993 to 2004, the prevalence of MDR *K. pneumoniae, P. aeruginosa*, and *A. baumannii* in ICUs in US hospitals increased from 5.1% to 13.3%, 1.7% to 9.3%, and 6.7% to 29.9%, respectively [19]. Recently, the National Healthcare Safety Network released a report stating that in 2014, 17.8% of all *Enterobacteriaceae* infections in the US were of the EBSL phenotype and 3.6% were carbapenem-resistant [26]. Furthermore, by 2014, 12.6% and 32.7% of all *P. aeruginosa* infections in short-term acute care hospitals and long-term acute care hospitals,2 respectively, were MDR [26]. That same year, almost 45% and 80% of all *A. baumannii* infections in short-term acute hospitals and long-term acute hospitals in the United States, respectively, were found to be MDR [26]. Due to the fast development of resistance in GNB, it is urgent to slow the spread of drug resistance, particularly in health care settings where outbreaks can have tragic outcomes.

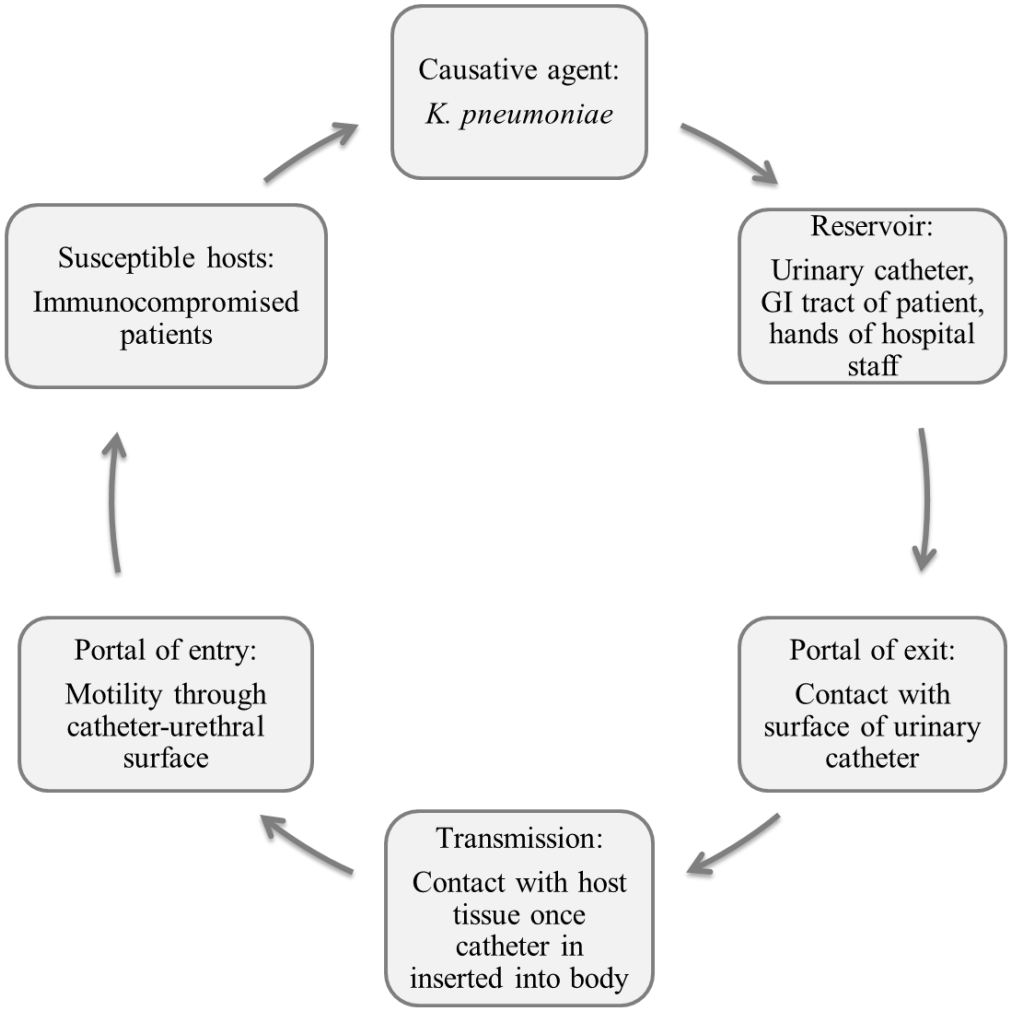
1.3 Conceptual Framework

1.3.1 Hospital transmission of GNB infections

Patients who have a higher risk of HAIs need to be identified and protected early in their hospitalizations. Methods of preventing HAIs such as infection control precautions including hand hygiene and contact isolation, health care worker education, active screening, and antimicrobial stewardship are well known [25, 27, 28]. These methods target the portal of exit and entry, mode of transmission, and the reservoir based on the chain of transmission [29]. Identification of characteristics of susceptible hosts is also important in order to better allocate resources to patients at higher risk of HAIs.

The causative agents highlighted in this dissertation will be *K. pneumoniae, P. aeruginosa,* and *A. baumannii* for reasons previously mentioned. As an example, the chain of transmission for a catheter-associated urinary tract infection (CAUTI) with *K. pneumoniae* is depicted in Figure 1. The reservoirs for *K. pneumoniae* in hospital settings include surfaces of medical equipment, hands of hospital staff, and the gastrointestinal tract of patients and hospital staff [7]. Through inadequate hygiene and sanitary practices of hospital staff or the patient's own peri-urethral area, *K. pneumoniae* can attach to the urinary catheter and be transmitted to the patient. The GNB then enters the patient's urinary tract by travelling up the catheter-urethral surface. Colonization and/or infection by *K. pneumoniae* occurs if the host immune response cannot destroy the bacteria. *K. pneumoniae* will form capsules around itself, release enzymes that break down host cells, and create biofilms that are harder to remove by antimicrobials. Susceptible hosts such as patients with impaired immune function may be at higher risk for CAUTIs and characterizing these susceptible patients can help provide one more opportunity to prevent infections through focused use of infection prevention methods [30].

Figure 1: Chain of transmission for K. pneumoniae CAUTI.



1.3.2 Risk factors for GNB HAIs

Many studies have examined risk factors for acquiring GNB and MDR GNB infections in hospital settings [20-22, 31-33]. General risk factors for HAIs include use of invasive devices, surgery, immunosuppressive agents, previous antibiotic use, older age, length of hospital stay, and chronic diseases such as diabetes and cancer [34]. Risk factors for drug resistant GNB are similar: previous antibiotic use, residence in long-term acute care facilities, stay in an intensive care unit, use of indwelling medical devices, poor functional status, older age, and organ or stem cell

transplants [25]. Prior hospitalization is also a significant risk factor for GNB and drug resistant GNB HAIs [35-37]. The higher risk of infection due to prior hospitalization may be related to the increased contact with healthcare workers and equipment. Patients who are hospitalized multiple times may have an even greater risk of HAIs with repeated exposure to hospital settings and may have different additional risk factors for GNB infections.

1.3.3 Patients with multiple hospitalizations

Multiple hospital admissions pose a considerable burden on healthcare costs and patient morbidity and mortality. In the United States, 12-19% of all discharged patients are re-hospitalized for all causes within 30 days of discharge [38-40]. Despite an overall decline in readmissions in the last decade, hospitalization and readmission rates for patients with multiple chronic conditions have increased [41]. Chronically ill patients such as those with multiple chronic diseases are also at high risk of developing HAIs and may fall into a recurring hospitalization cycle since HAIs are known to increase risk of subsequent hospitalizations [42, 43]. Healthcare costs for chronically ill patients account for over half of the total healthcare expenditures in the US [44]. Thus, it is of particular importance to study the risk factors that influence risk of HAIs in this special population who have multiple hospitalizations. Prediction models have been created to identify these patients at high risk of infection and mortality due to HAIs based on known risk factors and comorbidities [45-47]. However, these models do not take into account the longitudinal and changing trajectory of adult patients who are repeatedly hospitalized. This dissertation will assess risk factors for GNB HAIs that change across hospitalizations and may better identify patients at risk of infection.

1.3.4 Repeat HAIs

Repeat HAIs likely result from a combination of general host risk factors such as compromised immune function and/or extremes of age, inappropriate or prolonged antibiotic therapy and/or failed treatment [20, 48, 49]. However, repeat infections may also be the result of organism persistence factors such as biofilms and persister cells [23, 50, 51]. Patients may carry persister cells that become activated when exposed to the pressure of hospital environments, leading to re-colonization and relapse of the original infection. When exposed to the hospital environment multiple times, it is possible that these persistence factors put patients at greater risk of acquiring a drug-resistant infection. Previous research on recurrent HAIs have been focused on *Clostridium difficile*, gram-positive bacteria found naturally in the human gut and spread through fecal-oral transmission [52]. This research has shown that about half of recurrent *C. difficile* cases are due to reinfection of the original or a new strain and the other half may be through reactivation of persistent cells within the host [52, 53]. Both *K. pneumoniae* and *P. aeruginosa* are naturally part of the human microbiome and may result in the same distribution of reinfections and relapses. In contrast, *A. baumannii* is naturally found in the soil and water surfaces and rarely on human skin and may not cause repeat infections through the same mechanisms as *K. pneumoniae* and *P. aeruginosa* [54].

Extensive antibiotic exposure in hospitals could select for drug resistant infections in future hospitalizations. While summaries of antimicrobial susceptibility patterns prevalent in a hospital are often used to guide empirical antibiotic therapy, these reports are based on aggregate counts and may not provide relevant data for an individual patient [55]. Using infection history from prior hospitalizations may help to minimize antimicrobial resistant GNB HAIs. One of the goals of this

dissertation will be to assess if a larger number of repeat HAIs is associated with an increased prevalence of resistant infections within individual patients.

The specific aims of this dissertation are:

1) Examine clinical and patient risk factors associated with acquiring at least one gram-negative hospital acquired infection in adult patients with multiple hospitalizations.

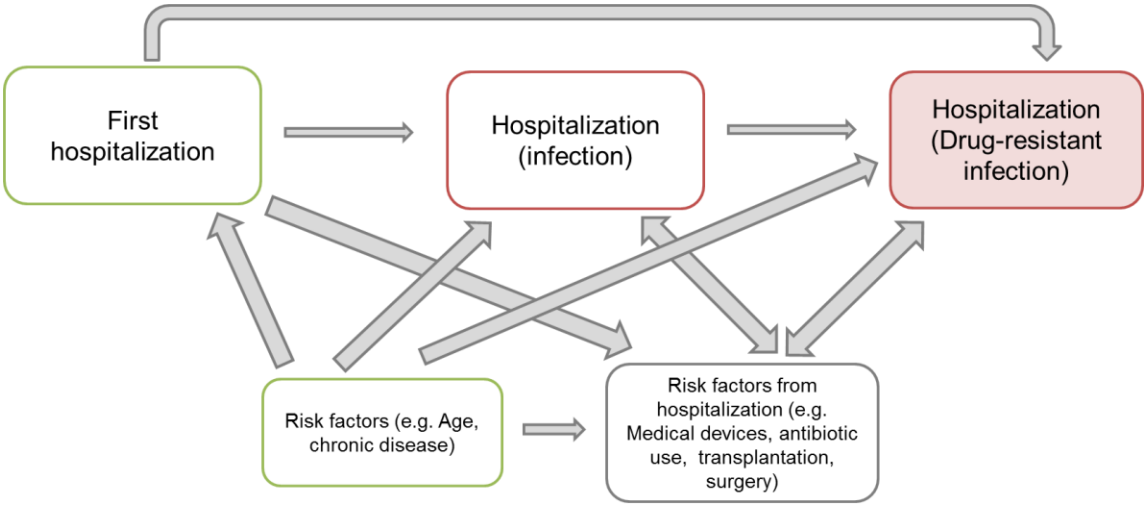
2) Systematically review the literature assessing the association between repeat gram-negative bacterial infections and changes in antibiotic susceptibility patterns.

3) Assess the association between repeat gram-negative infections and risk of drug-resistant gram-negative infection among patients with multiple hospital admissions.

The conceptual framework of the project is shown in

Figure 2. Individuals may enter the hospital system due to a variety of different factors including age, chronic diseases, injury, etc. The patients then may develop risk factors for GNBs within the hospital such as use of invasive medical devices, antibiotic use, staying in an ICU and surgeries. When these patients experience a second or multiple hospitalizations, their prior hospitalizations in addition to risk factors from their prior hospitalizations and current hospitalization become risk factors for GNB HAIs. Aim 1 will investigate which of these factors across hospitalizations increase the risk of GNB HAIs. Once a GNB HAI has occurred, patients are at risk of developing repeat infections. When patients are re-hospitalized after acquiring a HAI in previous hospitalizations, they may be at greater risk of drug-resistant GNB HAIs. Aims 2 and 3 will evaluate if there is an association between repeat GNB HAIs and risk of a subsequent drug-resistant GNB HAI.

Figure 2: Theoretical framework describing hypothesized risk factors for GNB and hypothesized risk factors for drug-resistant GNB HAIs in patients with multiple hospitalizations.



1.3.5 Summary

The overall goal of this dissertation is to identify risk factors for GNB and drug resistant GNB infections among patients with multiple hospital admissions. Patients who have multiple admissions may be at an increased risk of HAIs due to frequent exposure to the hospital environment as well as potential chronic conditions that bring them back to the hospital. However, there are few studies that examine the risk of HAIs among patients with multiple hospitalizations. Chapter 2 of this dissertation summarizes a retrospective cohort study in which I examined risk factors associated with acquiring at least one GNB HAI in adult patients with multiple hospitalizations using a longitudinal approach by incorporating prior medical history.

Chapter 0 summarizes a systematic review of the literature that evaluated the association between repeat GNB infections and changes in antibiotic susceptibility patterns. It informs the hypothesis that repeat GNB HAIs in patients with multiple hospitalizations result in greater risk of a subsequent drug resistance HAI. The review specifically examined studies in which change in antibiotic susceptibility in HAIs was evaluated at the individual patient level.

I then investigated the relationship between repeat GNB HAIs and antibiotic susceptibility patterns. Chapter 4 presents the study conducted to assess the association between repeat GNB HAIs and risk of drug resistance among patients with multiple hospital admissions. Finally, Chapter 5 summarizes the findings and discusses the public health implications of this research.

2. Risk factors for gram-negative hospital-acquired infections in patients with multiple hospitalizations

2.1 Abstract

Risk factors for acquiring *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii* infections have been widely examined but few studies have incorporated multiple hospitalizations into risk models. This study aimed to longitudinally evaluate risk factors for hospital-acquired infections (HAI) caused by the three pathogens over multiple hospitalizations. Identifying these risk factors will assist in focusing infection control resources on the highest risk patients and in decreasing the alarming growth of drug resistance in these three pathogens.

Electronic medical records of adult patients hospitalized ≥2 times in three New York City hospitals between 2006-2014 were used to assess the impact of potential risk factors on the time to the first GNB HAI. A time-dependent Cox model was developed for each organism. Risk factors examined included demographic and clinical factors such as age, sex, insurance, admission source, Charlson Comorbidity Index (CCI), chronic diseases, major surgeries, invasive medical device use, and antibiotic use.

Of the 129,372 unique patients, 1,672 (1.3%) acquired *K. pneumoniae*, 1,127 (0.9%) acquired *P. aeruginosa*, and 262 (0.2%) acquired *A. baumannii* infections. Older age, mechanical ventilation, and increasing days of use of antibiotics decreased the time to infection for all 3 pathogens. In the *K. pneumoniae* model, a history of cancer, congestive heart failure, and higher CCI also decreased time to infection. In addition, *P. aeruginosa* infections were associated with

admission from a non- healthcare facility and *A. baumannii* infections with a cancer diagnosis and dementia. This study identifies several longitudinal risk factors that can be used to guide infection control resources for patients with multiple hospitalizations. Incorporating clinical risk factors that change across patients' medical histories into risk models can enhance targeted infection control measures and prevent transmission.

2.2 Introduction

Hospital-acquired infections (HAIs) are often preventable and yet continue to account for a significant number of complications in hospitalized patients. In 2014, a landmark study reported that 1 in every 25 patients hospitalized in the United States acquired a HAI [1]. HAIs are associated with substantial complications and deaths, particularly among those in intensive care units (ICUs) [2, 3]. In older adults, such infections are associated with a two-fold increase in 90-day mortality rate and an average of 10 additional days in the hospital [4]. Gram-negative bacteria (GNB) are implicated in 20% of HAIs in all hospital settings and 60% of HAIs in ICUs [1, 5]. In hospital settings, three of the primary GNB of interest are *Klebsiella pneumoniae*, *Pseudomonas aeruginosa*, and *Acinetobacter baumannii*, all of which cause opportunistic infections and are named by the World Health Organization as critical pathogens for which new antibiotics are urgently needed [6, 27].

Patients with multiple hospitalizations, the so-called 'high-cost high-risk' patients, pose a great burden on the hospital system in terms of the associated care and costs [56]. These patients tend to be older, sicker and more likely to get HAIs [57-59]. While risk factors for HAIs due to *K. pneumoniae*, *P. aeruginosa*, or *A. baumannii* have been widely studied, these factors have been identified mostly through cross-sectional studies, which disregard the frequent healthcare

exposure, the dynamic health status, and antibiotic use of these high-risk patients [35, 60, 61]. General risk factors for GNB HAIs include use of invasive devices, surgery, use of immunosuppressive agents, antibiotic use, older age, longer hospital stays, and chronic diseases such as diabetes and cancer [1, 62-64]. Many of these factors can change across hospitalizations, making cross-sectional risk models problematic for patients with multiple hospitalizations. Furthermore, patients with multiple hospitalizations may have different risk profiles than the general hospitalized population because of the greater exposure to the hospital environment and greater opportunity for infection transmission [65, 66]. By incorporating longitudinal methods in infection risk assessment, the complex medical histories of patients can be utilized to accurately assess risk over the long-term.

With availability of electronic medical records, more data are available for longitudinal infection risk assessment in hospitals. This study aims to evaluate risk factors for the first infection caused by *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* in patients with multiple hospitalizations and demonstrate the use of long-term electronic medical records in identifying high-risk patients.

2.3 Methods

2.3.1 Study population

The population consisted of adult patients aged 18 years or older hospitalized for any cause two or more times between 2006 and 2014 at three New York City (NYC) hospitals that are part of a large academic healthcare system, including a 221-bed community hospital and two tertiary care hospitals with 657 and 914 beds. These hospitals had over 713,700 discharges of adult patients within the 9-year period during which 3.6% of all discharges acquired a HAI. Electronic medical

records for these patients were collected from a large clinical research database that included all adult patients (≥18 years) discharged between January 1, 2006 and December 31, 2014. The database, the development of which has been described in Apte, et. al. [67], included information from patient administrative records, clinical and medication records, lab- oratory and antibiotic susceptibility reports from blood, urine, respiratory, and wound cultures, and International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnosis and procedure codes from admission and discharge. The database was developed as part of a federally funded project, "Health Information Technology to Reduce Healthcare-Associated Infections" (National Institute of Nursing Research, R01NR010822). The study was approved by the study sites institutional review boards.

2.3.2 Data measures

HAIs were defined as infections that were culture positive three or more calendar days after hospital admission and not known to be incubating at the time of hospital admission [68]. The outcome was defined as the first HAI caused by *K. pneumoniae*, *P. aeruginosa*, or *A. baumannii* which first occurred in the second or subsequent hospitalizations. Patients who had a HAI with any of the three GNB in their first hospitalization were excluded from the analysis pertaining to the specific GNB.

Potential risk factors/predictors assessed included age, sex, year of first admission, any stay in the ICU, days spent in ICU during each hospitalization, Charlson Comorbidity Index (CCI) [69], and chronic diseases present on admission to hospital including diabetes, renal failure, malignancies, myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular diseases, dementia, chronic pulmonary disease, rheumatic disease, peptic ulcer disease, paraplegia, liver disease, HIV/AIDS, hypertension, and hypothyroidism. Also included in

the dataset were major surgical procedures (operating room procedure, biopsy, dialysis, transplant), insurance (Medicare, Medicaid, commercial, other), admission source (clinic referral, non-health care facility, other health care facility, unknown), presence of other infections (other GNB, other gram positive infection, other infection), use of invasive medical devices (mechanical ventilator, feeding tube, cardiac catheter, vascular stent, CV/IV line), and antibiotic use during each hospitalization prior to the occurrence of the GNB infection. Admission from a non-healthcare facility included walk-ins and patients coming from home.

Antibiotics were grouped into the following classes: penicillins, cephalosporins, carbapenems, monobactams, polymyxins, anti-MRSA agents (vancomycin, daptomycin, ceftaroline, linezolid), aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and other (fosfomycin, nitrofurantoin, metronidazole, clindamycin). Penicillins were categorized into four groups: natural penicillins, B-lactamase resistant penicillins, aminopenicillins/extended spectrum penicillins, and penicillin/inhibitor combinations. Cephalosporins were also categorized by generation. Drug resistance was defined by organism as resistant to imipenem and meropenem for *K. pneumoniae,* levofloxacin for *P. aeruginosa*, and ampicillin/sulbactam for *A. baumannii*.

2.3.3 Statistical analysis

Separate analyses were conducted for *K. pneumoniae*, *P. aeruginosa*, and *A. baumannii* to delineate the different risk profiles for each GNB [70]. Bivariate descriptive statistics were conducted to assess the association between potential risk factors and specific GNB infection through the use of Students t tests, X^2 tests, and Fishers exact tests as appropriate. Based on the results of these analyses, significant covariates were added in a stepwise fashion in multivariate models. The final models for each organism retained covariates significant at the $p<0.05$, plus age, sex, and CCI as *a priori* covariates.

To accommodate the longitudinal nature of multiple hospitalizations and the risk factors that vary between each hospitalization, time-varying Cox proportional hazards models were used to calculate hazard ratios. Time intervals were set up so that each hospitalization represented one interval for each patient. All covariates except sex were allowed to vary over time. The proportional hazards assumption was tested and met for each covariate using cox.zph function in R [71]. Patients were followed until the hospital day during the second or subsequent hospitalization in which the first GNB HAI occurred. Patients who died in the hospital were censored to the day of death. Crude and adjusted hazards ratios were calculated for each of the potential risk factors. Robust sandwich estimators for the standard errors were used to account for any model fitting errors and correlations between the variance due to repeated measures from the same patient. Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC) and R software, version 3.3.2.

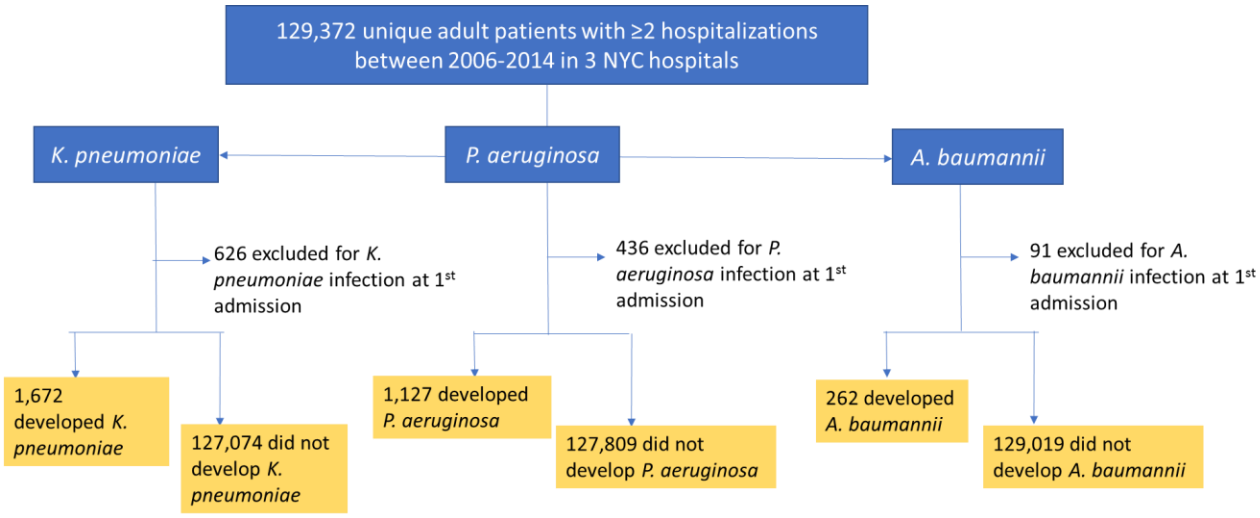
2.4 Results

2.4.1 Incidence of infection

Between 2006-2014, 129,372 patients were hospitalized two or more times (Figure 3). After excluding patients who acquired the primary GNB in their first hospitalization, the final study sample included 128,746 patients (n=1672 with infection) with 100,285,918 patient-days for the *K. pneumoniae* analysis, 128,936 patients (n=1127 with infection) with 100,576,147 patient-days for the *P. aeruginosa* analysis, and 129,281 patients (n=262 with infection) with 101,047,094 patient-days for the *A. baumannii* analysis. The incidence rate of *K. pneumoniae* infections over the nine- year period was 1.67 infections per 100,000 patient-days. For *P.*

aeruginosa and *A. baumannii*, the incidence rates were 1.12 and 0.26 infections per 100,000 patient-days, respectively.

Figure 3: Study sample of patients with multiple hospitalizations by infection group.



Patients had on average 4.6 hospitalizations before acquiring one of the three GNB (Table 1). The majority of *K. pneumoniae* and *P. aeruginosa* infections were urinary tract infections (UTI) while *A. baumannii* infections were primarily pneumonia (PNU) (40%) and UTIs (35%).

Table 1: Characteristics of study populations infected with one of the three study GNB.

	K. pneumoniae	***P. aeruginosa***	***A. baumannii***
Number of patients	1672	1127	262
Number of hospital days	118367	79975	23806
Mean number of hospitalizations to infection (range)	4.6 (2-44)	4.4 (2-49)	4.6 (2-41)
Site of infection, n (%)			
BSI	296 (18%)	95 (8%)	58 (22%)
UTI	1056 (63%)	593 (53%)	92 (35%)
PNU	284 (17%)	373 (33%)	104 (40%)
SSI	36 (2%)	66 (6%)	8 (3%)
Number of drug resistant infections (%)	613 (37%)	330 (29%)	116 (44%)

Of the *K. pneumoniae* infections, 613 (37%) were resistant to imipenem and meropenem. Twenty-nine percent (n=330) of *P. aeruginosa* infections were resistant to levofloxacin and almost half of *A. baumannii* infections (n=116, 44%) were resistant to ampicillin/sulbactam.

2.4.2 Antibiotic use

Table 2 shows antibiotic use among the full study population. Among the antibiotics given to the study population, anti-MRSA agents were most frequently given, followed by first generation cephalosporins, macrolides, other antibiotics, and third generation cephalosporins. First generation cephalosporins were given to the most number of patients, followed by anti-MRSA agents, other antibiotics, third generation cephalosporins, and macrolides. Polymyxins and carbapenems, typically used to drug-resistant GNB pathogens, were given for the longest average duration within hospitalizations.

Table 2: Distribution of antibiotics.

	Number of hospitalizations during which drug given, n (%)	**Number of unique patients who received drug, n (%)**	**Number of days drug given over all hospitalizations, mean (SD)**
Natural penicillins	360 (0.2)	248 (0.3)	5.1 (5.4)
B-lactamase resistant penicillins	2705 (1.5)	2053 (2.5)	8.4 (8.8)
Amino/extended spectrum penicillins	21377 (11.5)	17292 (21.3)	3.7 (4.7)
Penicillin/inhibitor combo	1311 (0.7)	1248 (1.5)	1.6 (3.2)
1st gen Cephalosporins	49746 (26.8)	36088 (44.5)	2.8 (3.6)
2nd gen Cephalosporins	3119 (1.7)	2874 (3.5)	2.8 (2.7)
3rd gen Cephalosporins	29049 (15.7)	20711 (25.5)	3.8 (5.0)
4th gen Cephalosporins	9194 (5.0)	6657 (8.2)	8.2 (12.5)
Carbapenems	9004 (4.9)	6121 (7.5)	11.2 (16.4)
Monobactams	5420 (2.9)	4015 (4.9)	6.8 (14.9)
Polymyxins	1478 (0.8)	1059 (1.3)	14.4 (22.9)
Anti-MRSA agents	59728 (32.2)	35147 (43.3)	7.4 (13.3)
Aminoglycosides	19756 (10.7)	14608 (18.0)	5.2 (11.0)

Tetracyclines	4378 (2.4)	3377 (4.2)	7.2 (12.3)
Macrolides	32670 (17.6)	20431 (25.2)	4.2 (5.6)
Fluoroquinolones	25778 (13.9)	18060 (22.2)	4.9 (8.5)
Other*	31566 (17.0)	21873 (26.9)	6.6 (11.4)

*Other category contained antimicrobials used to treat fungal infections and topical drugs.

2.4.3 Baseline characteristics

Table 3 shows the distribution of clinical characteristics of patients at their first hospitalization, grouped by GNB and whether the patient developed an infection. Patients who had a HAI with any of the three GNB by the end of the study period were generally older, had higher CCIs, were more likely to have diabetes, renal failure, malignancies, congestive heart failure, peripheral vascular disease, cerebrovascular disease, chronic pulmonary disease, rheumatic disease, hemiplegia/paraplegia, liver disease, HIV/AIDS, and hypertension. For all three pathogens, patients were more likely to have stayed in an ICU, taken antibiotics, used mechanical ventilation, a feeding tube or a CV line, had a major procedure, have Medicare insurance, and be admitted through a clinic referral for their first hospitalization.

Table 3: Characteristics of patients at their first hospitalization and associations with GNB infection, by organism.

	K. pneumoniae			*P. aeruginosa*			*A. baumannii*		
	Infection	**No infection**	**p value**	**Infection**	**No infection**	**p value**	**Infection**	**No infection**	**p value**
Age, mean (range)	62.9 (18-99)	57.8 (18-112)	<0.0001	66.2 (18-101)	57.8 (18-112)	<0.0001	63.0 (18-98)	57.9 (18-112)	0.003
Female, n (%)	892 (53.4)	71152 (56.0)	0.03	552 (49.0)	71618 (56.0)	<0.0001	109 (41.6)	72247 (56.0)	<0.0001
CCI, mean (range)	2.6 (0-13)	1.6 (18.0)	<0.0001	2.3 (0-14)	1.6 (0-18)	0.54	2.7 (0-11)	1.6 (0-18)	0.003
Conditions POA, n (%)									
Diabetes	463 (27.7)	25289 (19.9)	<0.0001	290 (25.7)	25519 (20.0)	<0.0001	70 (26.7)	25830 (20.0)	0.007
Renal failure	396 (23.7)	16125 (12.7)	<0.0001	253 (22.5)	16333 (12.8)	<0.0001	73 (27.9)	16593 (12.9)	<0.0001
Malignancies	424 (25.4)	17531 (13.8)	<0.0001	243 (21.6)	17764 (13.9)	<0.0001	60 (22.9)	18037 (14.0)	<0.0001
Myocardial infarction	117 (7.0)	6523 (5.2)	0.0008	86 (0.07)	6576 (5.2)	0.0001	16 (6.1)	6684 (5.2)	0.52
Congestive heart failure	338 (20.3)	14069 (11.2)	<0.0001	233 (20.8)	14231 (11.2)	<0.0001	58 (22.1)	14498 (11.3)	<0.0001
Peripheral vascular disease	111 (6.7)	6676 (5.3)	0.014	81 (7.2)	6704 (5.3)	0.004	18 (6.9)	6816 (5.3)	0.27
Cerebrovascular disease	125 (7.5)	7230 (5.7)	0.002	104 (9.3)	7278 (5.8)	<0.0001	25 (9.5)	7396 (5.8)	0.009
Dementia	22 (1.3)	2328 (1.9)	0.11	30 (2.7)	2323 (1.8)	0.04	9 (3.4)	2349 (1.8)	0.06
Chronic Pulmonary Disease	316 (19.0)	19210 (15.3)	<0.0001	274 (24.4)	19267 (15.2)	<0.0001	64 (24.2)	19573 (15.3)	<0.0001
Any liver disease	146 (8.8)	5543 (4.4)	<0.0001	58 (5.2)	5664 (4.5)	0.26	15 (5.7)	5718 (4.5)	0.33
AIDS/HIV	27 (1.6)	1595 (1.3)	0.2	18 (1.6)	1609 (1.3)	0.32	6 (2.3)	1625 (1.3)	0.15
Hypertension	875 (52.6)	58050 (46.1)	<0.0001	622 (55.4)	58439 (46.2)	<0.0001	131 (50.0)	59078 (46.2)	0.22
Hypothyroidism	139 (8.4)	9335 (7.4)	0.15	108 (9.6)	9382 (7.4)	0.005	19 (7.3)	9503 (7.4)	0.91
Days in ICU, mean (range)	2.62 (0-146)	0.60 (0-443)	<0.0001	3.45 (0-443)	0.61 (0-391)	<0.0001	4.87 (0-192)	0.64 (0-443)	<0.0001
Antibiotic use, n (%)	718 (42.9)	44009 (34.6)	<0.0001	548 (48.6)	44281 (34.7)	<0.0001	118 (45.0)	44996 (34.9)	0.0006
Medical device use, n (%)									
Mechanical vent	107 (6.4)	2870 (2.3)	<0.0001	101 (9.0)	2896 (2.3)	<0.0001	38 (14.5)	3056 (2.8)	<0.0001
Feeding Tube	49 (2.9)	1135 (0.9)	<0.0001	38 (3.4)	1152 (0.9)	<0.0001	15 (5.7)	1219 (0.9)	<0.0001
Card Catheter	165 (9.9)	12874 (10.1)	0.72	117 (10.4)	12974 (10.2)	0.8	20 (7.6)	13110 (10.2)	0.18
Vascular stent	86 (5.1)	9390 (7.4)	0.0005	37 (3.3)	9458 (7.4)	<0.0001	9 (3.4)	9495 (7.4)	0.02
CV Line	323 (19.3)	12171 (9.6)	<0.0001	210 (18.6)	12347 (9.7)	<0.0001	62 (23.7)	12696 (9.8)	<0.0001
Major procedures, n (%)									
OR procedure	447 (26.7)	31481 (22.8)	<0.0001	309 (27.4)	31656 (24.8)	<0.0001	71 (27.1)	32095 (24.9)	0.01
Biopsy	87 (5.2)	2970 (2.3)	<0.0001	45 (4.0)	3029 (2.4)	0.0004	13 (5.0)	3079 (2.4)	0.006
Dialysis	84 (5.0)	2628 (2.1)	<0.0001	32 (2.8)	2698 (2.1)	0.09	14 (5.3)	2746 (2.1)	0.0003
Transplant	52 (3.1)	2106 (1.7)	<0.0001	36 (3.2)	2137 (1.7)	<0.0001	7 (2.7)	2187 (1.7)	0.22
Insurance									
Medicare	927 (55.4)	56311 (44.3)	<0.0001	719 (63.8)	56631 (44.3)	<0.0001	156 (59.5)	57405 (44.5)	<0.0001
Medicaid	306 (18.3)	29101 (22.9)		186 (16.5)	29252 (22.9)		46 (17.6)	29443 (22.8)	

Commercial/Blue Cross	410 (24.5)	39008 (30.7)		210 (18.6)	39250 (30.7)		57 (21.8)	39483 (30.6)	
Other	29 (1.7)	2654 (2.1)		12 (1.1)	2676 (2.1)		3 (1.2)	2688 (2.1)	
Admission Source									
Clinic	659 (39.4)	45303 (35.7)	<0.0001	483 (42.9)	45543 (35.6)	<0.0001	123 (46.9)	46024 (35.7)	<0.0001
Non-health care facility	618 (37.0)	50235 (39.5)		366 (32.5)	50551 (39.6)		86 (32.8)	50932 (39.5)	
Other health care facility	222 (13.8)	12222 (9.6)		166 (14.7)	12316 (9.6)		36 (13.7)	12506 (9.7)	
Unknown	173 (10.4)	19314 (15.2)		112 (9.9)	19399 (15.2)		17 (6.5)	19557 (15.2)	

2.4.4 Time-varying Cox models for first GNB infection

The multivariate cox models developed for the first HAI due to *K. pneumoniae*, *P. aeruginosa*, or *A. baumannii* and statistically significant risk factors are summarized in Table 4: Time-varying Cox models for acquiring a GNB HAI across multiple hospitalizations, by infecting organism.

	K. pneumoniae		***P. aeruginosa***		***A. baumannii***	
	HR	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Age	1.009	1.006 - 1.012	1.024	1.020 - 1.028	1.008	1.000 - 1.016
Female	1.315	1.191 - 1.451	1.035	0.919 - 1.166	0.783	0.605 - 1.012
Charlson Comorbidity Index	1.051	1.030 - 1.073	1.007	0.984 - 1.031	1.021	0.966 - 1.080
Malignancies	1.368	1.198 - 1.561	--		1.596	1.163 - 2.189
Congestive heart failure	1.161	1.034 - 1.303	--		--	
Dementia	--		--		2.495	1.557 - 3.999
Stay in an ICU	2.019	1.797 - 2.270	1.949	1.673 - 2.270	--	
Mechanical ventilation	1.495	1.298 - 1.720	2.404	2.039 - 2.833	1.019	1.003 - 1.035
Admission from non-healthcare facility	--		0.821	0.722 - 0.934	--	
B-lactamase resistant penicillins	1.035	1.017 - 1.054	--		--	
Amino/extended spectrum penicillins	1.016	1.006 - 1.026	0.986	0.973 - 0.998	1.019	1.003 - 1.035
Penicillin/inhibitor combo	1.046	1.015 - 1.079	--		1.055	1.022 - 1.089
1st gen Cephalosporins	--		1.022	1.007 - 1.038	--	
2nd gen Cephalosporins	--		--		--	
3rd gen Cephalosporins	--		1.012	1.005 - 1.020	1.016	1.006 - 1.027
4th gen Cephalosporins	--		--		0.982	0.968 - 0.996
Anti-MRSA agents	--		1.006	1.004 - 1.009	1.009	1.004 - 1.013
Aminoglycosides	--		1.007	1.005 - 1.009	1.015	1.007 - 1.022
Carbapenems	1.011	1.006 - 1.016	0.988	0.983 - 0.994	--	
Fluoroquinolones			1.010	1.005 - 1.015	--	
Monobactams	0.995	0.991 - 0.999	--		--	
Tetracyclines	1.019	1.011 - 1.027	1.009	1.001 - 1.016	--	
Macrolides	1.015	1.009 - 1.022	--		0.977	0.960 - 0.994
Other	1.006	1.003 - 1.010	0.997	0.994 - 0.999	--	

In the *K. pneumoniae* model, stay in an ICU (Hazard Ratio: 2.019, 95%CI: 1.797-2.27), mechanical ventilation (HR: 1.495, 95%CI: 1.298-1.72), malignancies (HR: 1.368, 95%CI: 1.198-1.561), and female sex (HR: 1.315, 95%CI: 1.191-1.451) were strongly associated with incident infection after adjustment of all other significant factors. Older age was also a predictor for *K. pneumoniae* infection. For every one day increase in the use of penicillin/inhibitor combination drugs, there was an associated 4.6% increase (95%CI: 1.5-7.9%) in risk of incident *K. pneumoniae* infection, in addition to a 3.5% increase (95%CI: 1.7-5.4%) with β-lactamase resistant penicillins, 1.19% increase (95%CI: 1.1-2.7%) with tetracyclines and 1.6% increase (95%CI: 0.6-2.6%) with aminopenicillins/extended spectrum penicillins. There was a slight protective effect associated with monobactam use (HR: 0.995, 95%CI: 0.991- 0.999).

Table 4: Time-varying Cox models for acquiring a GNB HAI across multiple hospitalizations, by infecting organism.

	K. pneumoniae		*P. aeruginosa*		*A. baumannii*	
	HR	**95% CI**	**HR**	**95% CI**	**HR**	**95% CI**
Age	1.009	1.006 - 1.012	1.024	1.020 - 1.028	1.008	1.000 - 1.016
Female	1.315	1.191 - 1.451	1.035	0.919 - 1.166	0.783	0.605 - 1.012
Charlson Comorbidity Index	1.051	1.030 - 1.073	1.007	0.984 - 1.031	1.021	0.966 - 1.080
Malignancies	1.368	1.198 - 1.561	--		1.596	1.163 - 2.189
Congestive heart failure	1.161	1.034 - 1.303	--		--	
Dementia	--		--		2.495	1.557 - 3.999
Stay in an ICU	2.019	1.797 - 2.270	1.949	1.673 - 2.270	--	
Mechanical ventilation	1.495	1.298 - 1.720	2.404	2.039 - 2.833	1.019	1.003 - 1.035
Admission from non-healthcare facility	--		0.821	0.722 - 0.934	--	
B-lactamase resistant penicillins	1.035	1.017 - 1.054	--		--	
Amino/extended spectrum penicillins	1.016	1.006 - 1.026	0.986	0.973 - 0.998	1.019	1.003 - 1.035
Penicillin/inhibitor combo	1.046	1.015 - 1.079	--		1.055	1.022 - 1.089
1st gen Cephalosporins	--		1.022	1.007 - 1.038	--	
2nd gen Cephalosporins	--		--		--	
3rd gen Cephalosporins	--		1.012	1.005 - 1.020	1.016	1.006 - 1.027
4th gen Cephalosporins	--		--		0.982	0.968 - 0.996
Anti-MRSA agents	--		1.006	1.004 - 1.009	1.009	1.004 - 1.013
Aminoglycosides	--		1.007	1.005 - 1.009	1.015	1.007 - 1.022
Carbapenems	1.011	1.006 - 1.016	0.988	0.983 - 0.994	--	
Fluoroquinolones			1.010	1.005 - 1.015	--	
Monobactams	0.995	0.991 - 0.999	--		--	
Tetracyclines	1.019	1.011 - 1.027	1.009	1.001 - 1.016	--	
Macrolides	1.015	1.009 - 1.022	--		0.977	0.960 - 0.994
Other	1.006	1.003 - 1.010	0.997	0.994 - 0.999	--	

Patients who were older (HR: 1.024, 95%CI: 1.02-1.028), experienced an ICU stay (HR: 1.949, 95%CI: 1.673-2.27), or received mechanical ventilation (HR: 2.404, 95% CI: 2.039-2.833) had increased risk of *P. aeruginosa* infection. Admission from any location other than a healthcare facility (e.g., home) and use of aminopenicillins/extended spectrum penicillins, carbapenems, and other antibiotics were associated with a decreased risk of *P. aeruginosa* infection. Patients who were given first generation cephalosporins had a 2.2% increased risk for incident infection with

each day of use, higher than all other antibiotics. Female sex, the CCI, and other comorbidities were not associated with *P. aeruginosa* infection after controlling for other significant factors.

Age, sex, and the CCI were not significantly associated with acquiring an *A. baumannii* infection. Instead, patients who had malignancies (HR: 1.596, 95%CI: 1.163-2.189), dementia (HR: 2.495, 95%CI: 1.557-3.999), and mechanical ventilation (HR: 1.019,95%CI: 1.003-1.035) were significantly associated with *A. baumannii* infection. Prior to infection, use of aminopenicillins/extended spectrum penicillins, penicillin/inhibitor combinations, third generation cephalosporins, anti-MRSA agents, and aminoglycosides increased the hazard of *A. baumannii* infection. Use of fourth generation cephalosporins and macrolides was associated with a decreased risk of infection.

2.5 Discussion

Patients with multiple hospitalizations represent a unique high-risk group for their frequent exposure to a high transmission environment and antibiotic pressure. This study leveraged longitudinal electronic medical data to describe a population of patients who experienced multiple hospitalizations across a nine-year period and evaluated possible time-varying risk factors for acquiring a *K. pneumoniae*, *P. aeruginosa*, or *A. baumannii* infection in the hospital. To our knowledge, this study is the first to examine variable risk factors for GNB HAIs across multiple years and hospitalizations in these high-risk patients.

As with prior studies, we found that older age, having an ICU stay, mechanical ventilation, and antibiotic use prior to infection were associated with incident GNB HAI after adjusting for other variables [61, 72-76]. Increased transmission of GNB infections in the ICU and through the use of medical devices such as mechanical ventilators is well-documented [62, 77]. Most recent literature examines prior antibiotic use only in determining risk of drug resistance and mortality

among hospitalized patients. Among studies that have investigated antibiotic use as a risk factor for any GNB HAI, very few have incorporated the duration of treatment into statistical models [61, 75]. This study provides evidence that even a single day of a particular antibiotic in the hospital can result in an increased risk of a HAI.

Although there was only a small increased risk of infection with every day of antibiotic use, this risk compounds when patients are repeatedly exposed to empirical therapy or treatment for other infections. These results may apply to patients with little or no hospitalization history due to the widespread outpatient use of antibiotics [78]. Patients who have had extensive exposure to antibiotics prior to entering the hospital system may have high risk of developing a drug-resistant GNB HAI. Antibiotic stewardship is crucial in both hospital and outpatient settings to reduce patients' risk of GNB HAIs.

There was no consistent pattern of association between any one antibiotic group and any of the three pathogens. For example, in contrast to previous studies, this study found no association between the use of cephalosporins with incident *K. pneumoniae* infection although there was increased risk of *P. aeruginosa* and *A. baumannii* infections [64, 79, 80]. This may reflect the reduction of cephalosporin use due to increased drug resistance in HAIs as well as the prescribing patterns for empirical therapy at these particular hospitals [79].

Overall, the incidence of *K. pneumoniae, P. aeruginosa,* or *A. baumannii* was relatively low in our population as compared to other studies [76, 81, 82]. However, among patients with multiple hospitalizations, the proportion of drug resistant infections was higher than the national average [83]. Not surprisingly, greater use of antibiotics, particularly cephalosporins and fluoroquinolones, and long durations of carbapenem and polymyxin use prior to the incident infection were associated with increased drug resistance within this population.

This study was limited by the use of available electronic medical records from a single urban three-hospital system and the fact that data on patients' medical history outside of these hospitals or beyond 2006-2014 were unavailable. We did conduct post- hoc analyses comparing patients who had their first hospitalization between 2006-2009 and those between 2010-2014 and found little or no difference in the Cox models, indicating that our results may be robust to recent time periods (data not shown). In addition, there were slight variations in GNB infection rates by year but did not make a statistically significant change in our results.

Another limitation was that only HAIs detected during hospitalization were included; data on HAIs that may have been diagnosed after discharge were not identified unless patients were re-hospitalized within 30 days. In this study, it was not possible to identify the indications for the prior use of antibiotics which may help explain the extended use of carbapenems and polymyxins in the sample.

Despite these limitations, the types of data collected electronically in this study are similar to those collected by hospital infection prevention and control systems; therefore, the use of these data will help assess the extent to which routinely-collected and electronically-available clinical and administrative data can be used for infection risk assessment. Long term electronic medical records are being used within automated systems for antibiotic stewardship [84, 85]. However, there seems to be a gap in the literature in using long-term patient data for identification of infection risk. Future studies to investigate how electronic medical records can be used with automated prediction models to improve infection control are indicated. This study provides the first step in doing so by demonstrating how infection risk can be predicted in patients with multiple hospitalizations. Using such data, it is possible to account for patient changes over time and utilize their full hospitalization history to identify high-risk patients.

In conclusion, this study quantifies the risk associated with daily use of antibiotics on GNB HAI amongst a vulnerable population of hospitalized patients. There is a strong case for improving antibiotic stewardship efforts in hospitals to prevent infections as well as to control drug resistance.

3. Repeat gram-negative hospital-acquired infections and antibiotic susceptibility: a systematic review

3.1 Abstract

Repeat hospital-acquired infections (HAIs) among frequently hospitalized patients contribute to the high rates of antibiotic resistance among gram-negative bacteria (GNB) in hospital settings. This systematic review examines the state of the literature assessing the association between repeat GNB HAIs and changes in antibiotic susceptibility patterns. A systematic search of English language published literature was conducted to identify studies in peer-reviewed journals from 2000-2015. Studies must have assessed drug resistance in repeat GNB infections longitudinally at the patient level. Two researchers independently reviewed search results for papers meeting inclusion criteria and extracted data. Risk of bias was assessed using a modified quality assessment tool based on the *Checklist for Measuring Study Quality* and the *Quality Assessment Checklist for Cases Series*.

From 3,385 articles identified in the search, seven met inclusion criteria. Five reported lower antibiotic susceptibility in repeat infections, one found a change but did not specify in which direction, and one reported no change. All studies were of low to average quality. Despite the dearth of studies, evidence suggests that repeat infections are associated with lower antibiotic susceptibility among hospitalized patients. Larger scale studies with strong methodology are warranted.

3.2 Introduction

Over 648,000 patients in US hospitals develop hospital-acquired infections (HAIs), with approximately 75,000 of those patients dying due to related complications each year [1]. Almost one-third of all HAIs and 60% of HAIs in intensive care units are caused by gram-negative bacteria (GNB) [1, 5, 19, 86]. GNB are becoming increasingly resistant to available antibiotics as widespread antibiotic use has surged globally [87, 88]. Patients who are repeatedly hospitalized are at greater risk for GNB infections and may contract multiple infections throughout their hospitalization history [35, 89, 90]. Repeat HAIs among frequently hospitalized patients may be contributing to the high rates of antibiotic resistance seen in GNB in hospital settings. Multiple infections likely result from a combination of general host risk factors, invasive therapeutic interventions, inappropriate or prolonged antibiotic treatment, and organism persistence factors such as biofilms and persister cells [20, 23, 48-51]. When exposed to the hospital environment multiple times, it is possible that these factors put patients at greater risk of acquiring or developing a drug-resistant infection.

The current literature has extensively described the effect of prior antibiotic use on antibiotic resistance and the use of antibiotic stewardship in reducing resistance [91, 92]. However, less is known about the role of previous GNB HAIs on antibiotic susceptibility in subsequent HAIs with the same organism. This systematic review examines the state of the literature assessing the association between repeat GNB HAIs and changes in antibiotic susceptibility patterns.

3.3 Methods

To be included in the systematic review, articles must have met the following criteria: 1) published in a peer-reviewed journal between January 1, 2000 and December 31, 2015, 2) primary

research written in English, 3) have an abstract and full text available, 4) assess drug resistance in repeat GNB infections longitudinally as a primary or secondary outcome. The search start date of January 2000 was chosen to account for the increasing incidence of drug resistant GNB infections beginning in the early 2000s in the United States [18, 87]. Articles assessing the effect of treatment on an infection were excluded as well as studies that were done solely in CF or COPD patients, who have increased risk of recurrent colonization and infection. Other exclusions included single patient case studies, articles only studying community-acquired infections, articles focused on salmonella and other food-borne diseases, and articles without data at the individual patient level. Conference presentations and dissertations were also excluded.

The search was conducted using the PubMed and Embase databases with a combination of Medical Subject Headings (MeSH) and keywords. The search terms are shown in Table 5. A professional medical librarian was consulted to review and refine search terms and strategy.

Table 5: Literature search terms.

Database	Date of search	Search terms	Number of results
Pubmed	08/05/2016	((("Gram-Negative Bacteria"[MeSH] and "Drug Resistance"[MeSH] AND "Bacterial Infections"[MeSH] AND ("Recurrence"[MeSH] or repeat or previous or chronic or persistent or persistence or longitudinal))) AND ("2000/01/01"[PDat] : "2015/12/31"[PDat]) AND Humans[MeSH] AND English[lang])	1367
Embase	08/05/2016	'drug resistance'/exp OR 'drug resistance' AND ('gram negative bacteria'/exp OR 'gram negative bacteria') AND ('recurrence'/exp OR 'recurrence' OR 'repeat' OR 'persistent' OR 'longitudinal' OR 'chronic') AND [humans]/lim AND [english]/lim AND [2000-2015]/py	2316

Relevant articles were extracted and stored using reference management software (EndNote X7, Thomas Reuters) and duplicates were deleted. Two reviewers, both doctoral candidates in epidemiology, independently screened titles and abstracts of articles to determine

whether the inclusion criteria were met. Full-text articles were then reviewed by the same two reviewers and reference lists of those articles were searched for potentially relevant publications. Inconsistencies regarding eligibility assessments between the two reviewers were discussed and resolved by consensus.

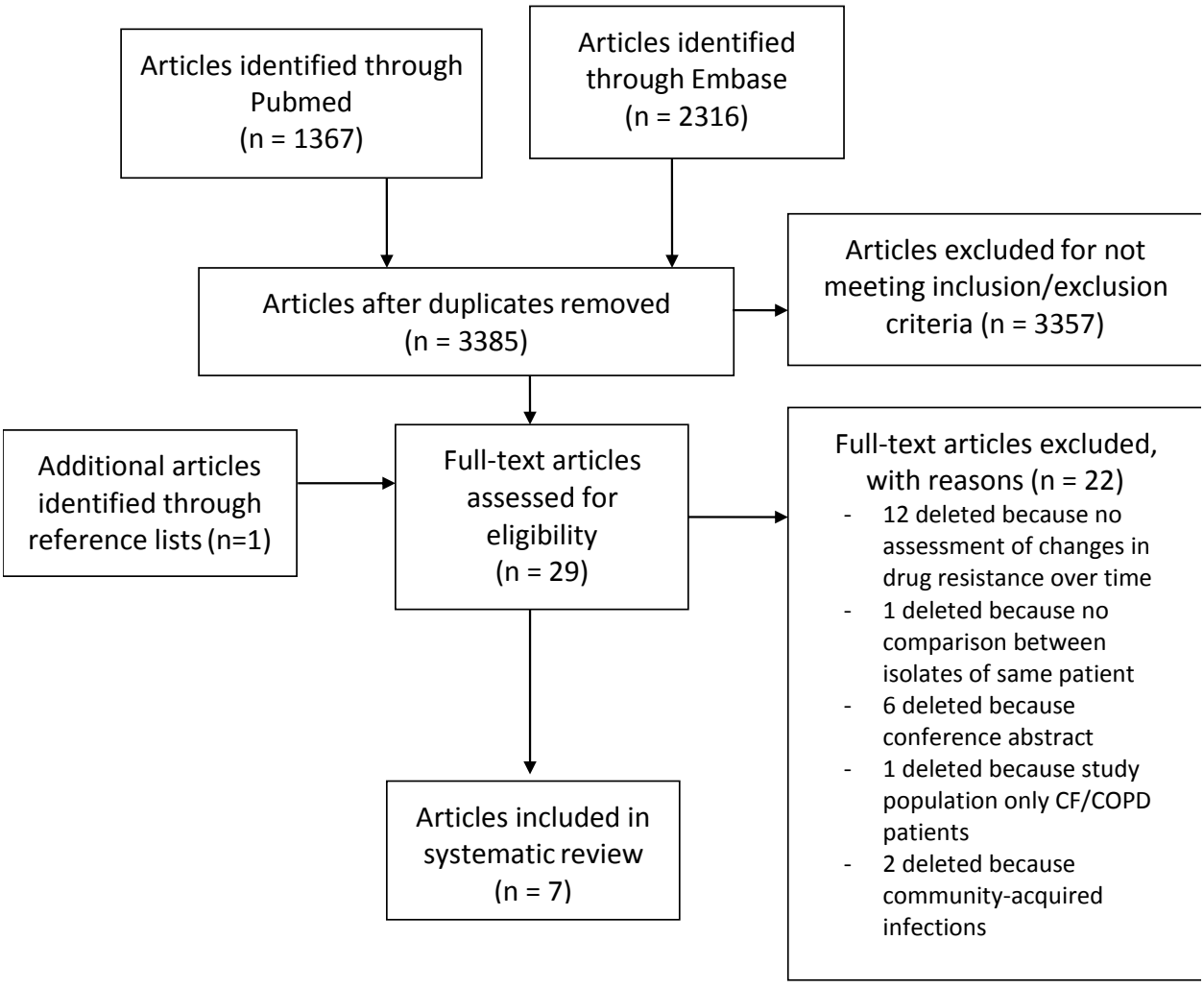
The following data were extracted from the final articles: publication journal, publication year, country in which the study was conducted, research questions, study time frame, sample, study design, analytic approach, organism, determination and definition of repeat infection, and findings related to antibiotic susceptibility changes. The quality of the selected studies was assessed by both reviewers using a modified quality assessment tool based on the *Checklist for Measuring Study Quality* and the *Quality Assessment Checklist for Case Series* [93, 94] which were developed for observational case series and case-control study designs, respectively. Questions relating to study randomization, blinding and interventional aspects were removed as they did not apply to the studies in this review. Items were scored as Yes (Y), No (N), Partial (P), Unclear/unable to determine (U) and Not applicable (NA) and weighted equally. The overall quality of the studies was based on the following scores: Good (G): at least 80% of criteria met; average (A): between 50% and 80% of criteria met; and poor (P): 50% of criteria met.

3.4 Results

The search yielded 3,385 potential articles (Figure 4). After applying the inclusion and exclusion criteria, 29 articles were selected for full text review. Seven final articles were identified after exclusion for the following reasons: 1) no assessment of antibiotic susceptibility changes over time (n=12), 2) only conducted among patients with CF or COPD who have differential and higher risk of recurrent infection (n=1) 3) conference abstracts (n=6), 4) no comparison of antibiotic

susceptibility changes within the same individual (n=1), and 5) primarily focused on community-acquired infections (n=2).

Figure 4: Flow diagram of article search.



3.4.1 Characteristics of included studies

The included studies were conducted in Australia, Canada, Israel, South Korea, Switzerland, Taiwan, and the United States (Table 6) [95-101].

Table 6: Summary of key characteristics of publications included in the systematic review.

Authors	Year	Setting	Inclusion/exclusion criteria	Sample size	Study design	Analytic approach	Examined organisms	Definition of repeat infection	Findings on change in antibiotic susceptibility
Patel, O'Toole, Larson	2012	New York City	Patients 18 years of age or younger who were hospitalized from January 1, 2006, to December 31, 2008	56,235	Retrospective cohort	Descriptive, $\chi 2$, Fisher Exact tests, t-tests	All GNB	isolation of more than 1 GNB collected over more than 1 date	39 /147 patients had additive DR, including 10/39 with additive DR from cultures across 2 or more admissions, and 6/39 patients who developed later infection/colonization with MDR GNB
Qi, Scheetz, & Malczynski	2009	Chicago, IL	Patients with multiple positive clinical cultures of *A. baumannii*	41	Retrospective cohort	Descriptive, logistic regression	*A. baumannii*	$\geq$2 clinical isolations of *A. baumannii* separated by at least 30 days	Patients with initial carbapenem-resistant isolates had more closely related isolates obtained for subsequent cultures than patients with non–carbapenem-resistant isolates, whereas patients with initial susceptible isolates frequently lost the initial strain and developed colonization/infection with a resistant and genetically distinct *A. baumannii*.
Ram R, Farbman L, Leibovici L, et al.	2012	Petah-Tikva, Israel	All consecutive hospitalized patients with fever of unknown origin, clinically documented infection or microbiologically documented infection after intensive chemotherapy or hematopoietic cell transplantation	271	Prospective cohort	$\chi 2$ or Fisher Exact test, multivariate logistic regression for mortality	All bacteria	infections developing during or after antibiotic treatment	Higher antibiotic susceptibilities were observed with initial infections compared with subsequent infections in patients with GNB infection
Reinhardt, Kohler, Wood, et al.	2007	Geneva, Switzerland	Intubated patients in surgical and medical intensive care units with respiratory tract colonization by *P. aeruginosa*	2	Prospective cohort	Ratios, correlations	*Pseudomonas aeruginosa*	Colonization of *P. aeruginosa* despite multiple antibiotic treatments	Resistant isolates appeared 6-10 days after treatment, persistent colonization was due to mutations in original strain and not cross-colonization with new *P. aeruginosa*
St. Denis, Ramotar, Vandemheen, et al.	2007	Ten Canadian and two Australian sites	Confirmed diagnosis of cystic fibrosis, >=12 years old, able to spontaneously produce sputum, and chronically infected with MDR Bcc., *P. aeruginosa*, *Sten. maltophilia*, or *Ach. xylosoxidans* bacteria	36	Prospective cohort	Paired t tests, Fisher Exact test	*Burkholderia cepacia* complex	$\geq$2 sputum cultures within the past 12 months	Bcc. isolates retrieved during exacerbations were less sensitive to meropenem, ciprofloxacin, chloramphenicol, piperacillin, and tobramycin compared to isolates retrieved during clinically stable periods.

Yang Y, Siu LK, Yeh K, et al.	2009	Taiwan	Patients with recurrent KLA at least 1 year after the onset of the first KLA	6	Retrospective cohort	Descriptive	*Klebsiella pneumoniae*	KLA occurring at least 1 year after the onset of the first KLA	No change in antibiotic resistance in recurrent infection
Yum H, Park I, Shin B, et al.	2014	Seoul, Korea	Patients with 2 or more different periods of recurrences	18	Retrospective cohort	T-tests, one-way analysis of variance	*Pseudomonas aeruginosa*	Occurrence after ≥2 months of complete treatment of previous pneumonia without evidence of extrapulmonary source of infection	7/24 recurrent cases had different antibiotic phenotype

*Abbreviations: KLA: *Klebsiella*-infected liver abscesses; MDR: multi-drug resistant organisms.

All of them were cohort studies, three prospective and four retrospective. The inclusion and exclusion criteria varied across studies; all but one study included only patients with confirmed recurrent infection or colonization [96-101]. The Patel et al. study included only patients 18 years while the Ram et al., St. Denis et al., Yang et al., and Yum et al. studies included only adults ≥18 years [95, 96, 98, 100, 101]. The Qi et al. and Reinhardt et al. studies did not provide details on the study patients' age [97, 99]. Two studies recruited patients from multiple hospitals [95, 100]. Six of the seven studies included ≤41 patients with recurrent infections; Ram *et al.* included data from 271 patients [95-101] and Patel *et al.* included 39 patients with drug-resistant isolates collected on different dates [95]. One study included only two patients [99]. Organisms examined included exclusively *Burkholderia cepacia complex* (n=1), exclusively *Pseudomonas aeruginosa* (n=2), exclusively *Acinetobacter baumannii* (n=1), exclusively *Klebsiella pneumoniae* (n=1), all GNB (n=1), and all bacteria (n=1).

The definition of the timing of repeat infections differed among the studies. One study defined recurrent infection as positive cultures at least 30 days apart, another defined recurrence as occurring at least one year apart, and another as at least two months after the completion of antibiotic therapy [96, 97, 101]. The St. Denis et al. required two or more positive cultures within

12 months [100]. Three studies did not describe a specific time difference between positive isolates [95, 98, 99].

One study did not report any type of statistical analyses [101]. Of the other six studies, one used correlations and three used bivariate analyses including $\chi 2$ tests, Fisher Exact tests and Student's t-tests [95, 96, 99, 100]. Two studies used logistic regression to calculate odds ratios [97, 98].

3.4.2 Antibiotic susceptibility changes in recurrent infections

Six of the seven studies found that the GNB causing recurrent infections had reduced antibiotic susceptibility patterns as compared to initial or early infections [95, 97-100]. The Yang et al. study found no change in susceptibility for *K. pneumoniae* liver abscesses and the Yum et al. study did not specify the direction of antibiotic susceptibility changes among the 30% of recurrent cases that had a change [96].

3.4.3 Quality assessment

Based on the quality assessment tool, five studies were of average quality and two were poor quality (Table 7). None of the studies had adequate power to detect statistically significant differences, reported loss-to-follow up rates, or adjusted for different follow up times in their analyses or discussions. Only one publication examined potential confounders [98].

Table 7: Risk of bias assessment for included studies.

First Author		Patel	Qi	Ram	Reinhardt	St. Denis	Yang	Yum
Study question								
1.	Is the hypothesis/aim/objective of the study stated in the abstract, introduction, or methods section?	Y	Y	Y	Y	Y	P	Y
Study population								
2.	Are the characteristics of the patients included in the study clearly described? (number, gender, age, etiology).	Y	Y	Y	Y	Y	Y	Y
3.	Was the case series collected in more than one center? (If the study is multicenter, the question should be answered 'yes'.)	Y	N	N	N	Y	N	N
Comparability of subjects/samples								
4.	Are the eligibility criteria explicit and appropriate? (Inclusion and exclusion criteria should be stated.)	Y	P	Y	Y	Y	Y	N
5.	Were data collected prospectively?	N	N	Y	Y	Y	N	N
6.	Were patients recruited consecutively?	Y	U	Y	U	U	U	U
7.	Did patients enter the study at a similar point in the disease?	Y	U	Y	Y	N	N	U
8.	Were the subjects recruited during the same period of time?	Y	Y	Y	Y	Y	Y	Y
9.	Was there loss to follow-up reported?	N	N	N	NA	N	N	N
Outcome measurement (change in antibiotic susceptibility)								
10.	Are outcomes (primary and secondary) clearly defined in the introduction or methodology section?	Y	Y	Y	Y	Y	N	Y
11.	Did the authors use accurate (standard, valid, reliable) objective methods to measure the outcomes? (Systematic, repeatable methods of case finding and appropriate lab definitions used?)	Y	Y	Y	Y	Y	P	Y
12.	Was there assessment of outcome before and after the study?	Y	Y	Y	Y	Y	Y	Y
13.	Was the length of follow-up clearly described/reported?	Y	Y	N	Y	Y	P	N
Statistical analysis								
14.	Were the statistical tests used to assess the primary outcomes appropriate? (No if no statistical tests)	Y	Y	Y	N	Y	N	Y
15.	Have actual probability values been reported (e.g. 0.035 rather than <0.05) for the primary outcome measurements except where the probability value is less than 0.001? (NA if Q14 is N)	Y	Y	Y	NA	Y	NA	N
16.	Does the study provide estimates of the random variability in the data for the primary outcomes? (e.g. standard error, standard deviation, confidence intervals)	Y	Y	Y	NA	Y	N	N
17.	Was there a discussion/assessment of possible confounders?	N	N	Y	N	N	N	N
Results								
18.	Are the main findings of the study clearly described?	Y	Y	Y	Y	Y	Y	Y
19.	Do the analyses adjust for different lengths of follow-up of patients? If follow-up is differential between groups, was this controlled for in the design or analysis?	N	N	N	N	N	N	N
20.	Do the study's findings respond to research objectives/question(s)?	Y	Y	Y	Y	Y	Y	Y
21.	If any of the results of the study were based on “data dredging”, was this made clear?	NA	N	Y	Y	Y	N	N
Discussion/Conclusion								
22.	Are the conclusions supported by results?	Y	Y	Y	Y	Y	Y	Y
23.	Are the limitations of the study taken into consideration?	Y	Y	Y	Y	P	N	N

24.	Did the study have sufficient power to detect a clinically important effect where the probability value for a difference being due to chance is less than 5%? (No if no mention of power)	N	N	N	N	N	N	N
Total								
	Yes	18	14	19	15	17	7	10
	No	5	7	5	5	5	12	12
	Partial	0	1	0	0	1	3	0
	Unclear/unable to determine	0	2	0	2	1	1	2
	Quality rating*	A	A	A	A	A	P	P

*The studies rated with respect to quality criteria as follows: Good (G): at least 80% of criteria met; average (A): between 50% and 80% of criteria met; poor (P): ≤ 50% of criteria met.

3.5 Discussion

Hospitalized patients who experience repeat HAIs are understudied yet represent an increasingly important group in the effort to slow the spread of antibiotic resistance. Repeat infections are due either to an infection with a new strain of an organism or from a relapse of the prior infection-causing organism. Both may result in patients developing future drug resistant infections. Several studies have examined whether repeat infections represent re-infections with a different strain or relapse of prior infections [102-104]. Yet surprisingly, only a few studies have examined repeat infections of GNB, despite the devastating outcomes associated with these organisms in hospital settings [105-107]. One study published in 1999 examined recurrent gram-negative bacteremia among patients to identify relapsed infections versus reinfections with a different strain of the same organism [108]. However, in this study, 60% of the infections were community-acquired. There is a need to assess repeat GNB infections within hospital settings since there is a greater chance of acquiring a drug-resistant GNB infection in hospitals [109-111].

In this review, we found only seven published studies that examined changes in antibiotic susceptibility in GNB among patients with repeat infections since 2000. Increased drug resistance was generally found in patients with more repeat infections, suggesting that if initial infections were contained, subsequent resistant infections could be prevented. Studies focused on

Staphylococcus aureus have identified patients with initial antibiotic-susceptible infections who are at higher risk of subsequent resistant infections [4, 112].

The quality of the literature included in this review limits our ability to determine with strong evidence that there is an increase in antibiotic resistance in repeat hospital-acquired GNB infections. The studies, which were of average to low quality, did not have adequate sample sizes or statistical power to evaluate if there was a change in antibiotic susceptibility in repeat GNB infections. They also differed in the definition of a repeat infection, for example whether the later infection occurred 30 days or a year after the initial infection. Additionally, all but one of the studies provided only descriptions of occurrence of repeat infections as opposed to risk of occurrence of repeat infections with and without drug resistance. Hence, the published literature, while suggestive, does not strongly substantiate the association between repeat infections and antibiotic susceptibility.

Future research should not only assess whether there is increased drug resistance in repeat GNB infection, but also what patient and hospital risk factors are associated with repeat infections and drug resistance. Patients who are older and/or have chronic illnesses are likely at disproportionately higher risk of repeat HAI. For example, the Patel et al. paper examined risk factors for acquiring a multi-drug resistant infection or multiple infections with resistance to multiple drugs and found that patients with more admissions, stay in a long-term care facility, higher number of days in an intensive care unit, and higher number of days with a catheter had a greater risk of having repeat infections with drug resistance [95]. Identifying risk factors that put patients at greater risk for a repeat infection as well greater risk of a drug-resistant infection will help in focusing infection prevention and care resources. Future studies should identify larger samples of hospitalized patients with repeat infections by using electronic medical records or large

national data sets that would contain long-term infection history in order to ensure sufficient statistical power to identify associations. In addition, future research can assess whether an association between repeat GNB infections and lower antibiotic susceptibility is due primarily to prior antibiotic exposure in order to guide antibiotic stewardship and other infection control initiatives.

This systematic review has limitations. The inclusion criteria for the review were relatively narrow; nevertheless, studies that met our inclusion criteria were heterogeneous in patient samples and in their definitions of repeat infections so it was not possible to do a meta-analysis. We may have missed articles in the literature search due to other variations in terminology. Finally, there is the possibility of publication bias, as we did not include conference abstracts, dissertations, other grey literature, articles not published in English, and articles not available through library services.

3.6 Conclusion

Despite the limitations, this review suggests that repeat GNB infections contribute to drug resistance in hospitals and highlights the need for further research. Repeat GNB HAIs most likely affect patients with multiple hospitalizations or those who have extensively long hospital stays. Repeat GNB infections in patients with multiple hospitalizations, particularly older patients, can result in increased complications, higher mortality, increased hospital and patient costs, and greater risk of future infections [4]. At a time when drug resistance among GNB is increasingly prevalent, reducing repeat infections may lead to fewer drug resistant infections in hospitals and improve outcomes in patients.

4. Risk of drug resistance in repeat gram-negative infections among patients with multiple hospitalizations

4.1 Abstract

Drug resistance in gram-negative bacterial hospital-acquired infections (GNB HAIs) has become ubiquitous in recent years. Patients who experience multiple hospitalizations are at high risk of developing repeat GNB HAIs. This study aims to evaluate the relationship between repeat HAIs with two GNB, *Klebsiella pneumoniae* and *Pseudomonas aeruginosa*, and antibiotic susceptibility patterns in this high-risk group.

Using electronic medical records from 3 NYC hospitals, 4,053 patients were identified with at least one HAI caused by *K. pneumoniae* or *P. aeruginosa* over multiple hospitalizations in a 9-year period. Modified Poisson regression was used to evaluate the risk of drug resistance with increasing number of prior infections. Drug resistance was defined as resistant to carbapenems for *K. pneumoniae* and resistant to levofloxacin for *P. aeruginosa*.

In patients with repeat infections by the same organism, almost 15% of consecutive infections changed from susceptible to drug resistant. Patients with *K. pneumoniae* infections had a 1.14 times increased risk of acquiring a drug-resistant HAI (95%CI:1.04-1.24) with each prior HAI, after adjusting for potential confounders and antibiotic use prior to infection. Patients with *P. aeruginosa* infections had a 1.23 times increased risk of a future drug-resistant infection (95%CI: 1.12-1.36) with each prior *P. aeruginosa* HAI. Prevention of repeat infections in chronically ill patients may be important in reducing drug resistance in this population.

4.2 Introduction

Rising rates of drug resistance in gram-negative bacteria (GNB) have threatened the gains made in reducing hospital-acquired infections (HAIs) around the world [113, 114]. Patients who develop drug-resistant HAIs are at greater risk for longer hospital stays, complications, and mortality [115-117]. Prevention of infections caused by GNB including *K. pneumoniae* and *P. aeruginosa*, are particularly critical due to the limited treatment options. In early 2017, the World Health Organization declared that *P. aeruginosa* and *Enterobacteriaceae*, in particular extended-spectrum *β*-lactamase (ESBL) producing *K. pneumoniae,* were priority pathogens that urgently required new antibiotic development due to widespread multi-drug resistance [118].

Patients with multiple hospital admissions are a vulnerable group with high risk of HAIs [119-121]. These patients are exposed repeatedly to the hospital environment, specifically to intensive care units (ICUs), where the combination of compromised immune systems, empiric antibiotic therapy and medical devices creates the ideal haven for drug-resistant infections. These high-risk patients may experience repeat infections by the same organism, potentially resulting in an increased likelihood of developing a drug-resistant infection.

Repeat infections can lead to drug resistance through several mechanisms such as inappropriate antibiotic therapy, biofilms, and persister cells [23, 122, 123]. A number of studies have focused on repeat infections due to pathogens such as *Escherichia coli* and *Staphylococcus aureus* [124-126]. The few studies that have examined repeat GNB infections have primarily aimed at identifying risk factors for recurrence and less on antibiotic resistance patterns in the repeat infections [127-129]; changes in antibiotic susceptibility with repeat GNB HAIs has been largely overlooked. We recently reviewed peer-reviewed literature published between 2000 and 2015 and found only seven studies that examined changes in drug resistance in GNB HAIs [95-

101]. Although one recently published study described an association between multidrug resistance and history of GNB bloodstream infections [130], the primary objective of the study was to measure costs associated with multidrug resistance rather than to focus on recurrent infections.

The aim of our study was to evaluate the association between repeat GNB HAIs and antibiotic susceptibility patterns in chronically ill patients who have repeated hospitalizations. It is hypothesized that as the number of infections by the same organism increases, there is a greater likelihood of developing a drug-resistant infection with that same organism.

4.3 Methods

4.3.1 Sample and Setting

Electronic medical record (EMR) data were obtained from a data warehouse, the development of which has been described in Apte, et. al. [67], which includes information from patient administrative records, clinical and medication records, laboratory and antibiotic susceptibility reports from blood, urine, respiratory, and wound cultures, and International Classification of Diseases, Ninth Revision, Clinical Modification (ICD-9-CM) diagnosis and procedure codes from admission and discharge. The data were from a large healthcare system in New York City which consisted of a community hospital and two tertiary care hospitals. The three hospitals had over 713,700 discharges of adult patients between 2006 and 2014 and 3.6% of patient discharges experienced at least one HAI. The database was developed as part of a federally funded project, "Health Information Technology to Reduce Healthcare- Associated Infections" (R01NR010822), and was approved by the institutional review boards of the study sites.

A subset of data containing records of all adult inpatients (≥ 18 years) discharged between January 1, 2006 to December 31, 2014 was used for this study. Patients who were hospitalized two or more times during the study period and had at least one HAI caused by *K. pneumoniae* or *P. aeruginosa* were included in our analyses. These two pathogens were chosen due to their pervasiveness in hospital settings. *Acinetobacter baumannii* was also considered but there were not enough patients with repeat infections with the pathogen to warrant analysis.

4.3.2 Data Measures

An infection was considered to be a HAI if there was a positive culture with the organism on the third or later calendar day following hospital admission, the infection was not known to be incubating at the time of admission, and it met the criteria defined by the National Healthcare Safety Network of the Centers for Disease Control and Prevention [68]. In this study, repeat HAIs were defined as HAIs that occurred at least 30 days after a previous hospital discharge. Exposure to repeat infections was defined as the count of HAI with the same causative organism across ≥2 hospitalizations during the study period. Patients were considered unexposed to repeat infections if they had ≥2 hospital visits but only one GNB HAI across all hospitalizations. In the case of multiple GNB infections with the same organism within the same hospitalization, only the first infection was included in the analyses to limit bias due to an unresolved initial infection.

The outcome measure was a binary variable denoting any antimicrobial-resistant infection versus an antimicrobial-susceptible infection for the last HAI caused by each organism. *K. pneumoniae* was considered to be drug resistant if it was not susceptible to imipenem or meropenem. *P. aeruginosa* was considered to be drug resistant if it was not susceptible to levofloxacin. Among exposed patients (those with ≥ 2 GNB HAIs), only the last HAI with the

same organism was assessed for drug resistance. For unexposed patients (those with only one GNB HAI across all their hospitalizations), the one GNB HAI was assessed for drug resistance.

Variables assessed for potential confounding included age, sex, cumulative length of hospital stay prior to development of the last GNB HAI, number of total hospitalizations prior to the outcome infection, any stay in the intensive care unit (ICU), cumulative number of days in the ICU prior to the outcome infection, admission source from hospitalization with the outcome infection (clinic referral, non- health care point of origin (e.g., home), other health care facility, unknown), major procedures (operating room (OR) procedure, biopsy, dialysis, transplants), use of invasive medical devices (mechanical ventilator, feeding tube, cardiac catheter, vascular stent, CV/IV line), and co-infections (other GNB, other gram positive infection, other infection). Also, included in the dataset were change in the Charlson Comorbidity Index (CCI) [131] from first visit to the visit with the last infection as a proxy to measure change in health status across hospitalizations, comorbidities (chronic obstructive pulmonary disease (COPD), cystic fibrosis (CF), diabetes, malignancies, renal failure, and liver disease) at the last infection, insurance (Medicare, Medicaid, commercial, other), and antibiotic use across hospitalizations prior to the occurrence of the outcome infection.

Antibiotics were grouped as follows: penicillins, cephalosporins, carbapenems, monobactams, polymyxins, anti-MRSA agents (vancomycin, daptomycin, ceftaroline, linezolid), aminoglycosides, tetracyclines, macrolides, fluoroquinolones, and other (fosfomycin, nitrofurantoin, metronidazole, clindamycin).

4.3.3 Statistical Analyses

Separate analyses were carried out for each organism, *K. pneumoniae* and *P. aeruginosa*. Bivariate analyses were conducted to identify any differences between exposed and unexposed

patients using Student's t tests, X^2 tests, and Fishers exact tests, as appropriate. A modified Poisson regression model was used to analyze the relationship between repeat GNB HAIs and drug resistance. In our study population, 33.3% of patients with multiple hospitalizations and at least one GNB HAI of interest had a drug-resistant infection so the rare disease assumption was not valid. The Poisson model, modified through use of a generalized estimating equations (GEE) approach, was used to obtain unbiased robust standard errors for the relative risks [132, 133].

Confounding was assessed though calculating the effect size of the β coefficient with and without inclusion of the potential confounders, those with a p value ≤ 0.05 in the bivariate analyses. Covariates that resulted in a 10% or greater change in the effect estimate were retained in the final model in addition to all antibiotic use. Statistical analyses were performed using SAS, version 9.4 (SAS Institute, Cary, NC).

4.4 Results

There were 2,416 patients who had at least one *K. pneumoniae* HAI and 1,637 who had at least one *P. aeruginosa* HAI across multiple hospitalizations between 2006 and 2014. Of the patients who acquired *K. pneumoniae*, 97 (4%) had repeat *K. pneumoniae* HAIs. Of the patients who acquired *P. aeruginosa*, 79 (4.8%) had repeat infections.

Table 8 summarizes the patient demographic, clinical, laboratory, and medication data based on whether they had repeat infections by the end of the study period. In the *K. pneumoniae* analysis, patients with two or more infections were younger, had more hospitalizations prior to last infection, spent a greater number of days in the hospital and in an ICU, more likely diagnosed with COPD, and more likely to have had an OR procedure, biopsy, or transplantation as compared to patients who only had one HAI caused by *K. pneumoniae*. Patients with repeat infections were

also more likely to have experienced a feeding tube, mechanical ventilation, intubation, urinary catheterization and IV line as well as a greater number of days on all types of antibiotics as compared to unexposed patients.

Table 8: Descriptive statistics of study sample, by exposure status.

	K. pneumoniae			***P. aeruginosa***		
Covariate	**One infection N=2319**	**Repeat infections N=97**	***P-value***	**One infection N=1558**	**Repeat infections N=79**	***P-value***
Age, mean (SD)	64.82 (17.04)	61.22 (17.47)	0.042	67.01 (17.12)	63.63 (18.66)	0.088
Female sex	1283 (55.33)	46 (47.42)	0.125	789 (50.64)	31 (39.24)	0.048
Prior hospitalizations, mean (SD)	2.78 (3.93)	6.79 (6.81)	<.001	2.62 (3.8)	6.73 (5.76)	<.001
Total prior days in hospital, mean (SD)	56.58 (60.37)	145.05 (129.87)	<.001	56.64 (62.32)	135.47 (120.59)	<.001
Total prior days in ICU, mean (SD)	10.72 (21.63)	28.54 (37.41)	<.001	14.23 (28.65)	28.42 (46.24	<.001
Drug resistance in last infection	822 (35.45)	55 (56.7)	<.001	427 (27.41)	46 (58.23)	<.001
Admission source						
Clinic	658 (28.37)	24 (24.74)	0.018	448 (28.75)	17 (21.52)	0.03
Non-healthcare point of origin	773 (33.33)	23 (23.71)		482 (30.94)	29 (36.71)	
Other facility	303 (13.07)	12 (12.37)		232 (14.89)	5 (6.33)	
Other	585 (25.23)	38 (39.18)		396 (25.42)	28 (35.44)	
Insurance						
Medicare	1429 (61.62)	62 (63.92)	0.548	1040 (66.75)	59 (74.68)	0.416
Medicaid	400 (17.25)	20 (20.62)		244 (15.66)	11 (13.92)	
Commercial	459 (19.79)	14 (14.43)		260 (16.69)	9 (11.39)	
Other	31 (1.34)	1 (1.03)		14 (0.9)	0 (0)	
Prior clinical history*						
Prior OR procedure	1123 (48.43)	62 (63.92)	0.003	839 (53.85)	52 (65.82)	0.037
Prior feeding tube	182 (7.85)	14 (14.43)	0.02	172 (11.04)	24 (30.38)	<.001
Prior mechanical ventilation	571 (24.62)	39 (40.21)	<.001	505 (32.41)	38 (48.1)	0.004
Prior biopsy	268 (11.56)	20 (20.62)	0.007	139 (8.92)	13 (16.46)	0.024
Prior intubation	473 (20.4)	36 (37.11)	<.001	410 (26.32)	33 (41.77)	0.003
Prior cardiac catheter	399 (17.21)	20 (20.62)	0.384	227 (14.57)	15 (18.99)	0.281
Prior vascular stent	181 (7.81)	8 (8.25)	0.874	92 (5.91)	3 (3.8)	0.621
Prior transplant	168 (7.24)	17 (17.53)	<.001	103 (6.61)	9 (11.39)	0.101
Prior urinary catheter	1837 (79.22)	90 (92.78)	0.001	1295 (83.12)	73 (92.41)	0.03
Prior CV or IV line	1220 (52.61)	76 (78.35)	<.001	861 (55.26)	69 (87.34)	<.001
Chronic conditions						
COPD	950 (40.97)	54 (55.67)	0.004	703 (45.12)	42 (53.16)	0.161

Cystic Fibrosis	77 (3.32)	2 (2.06)	0.769	75 (4.81)	11 (13.92)	0.002
Diabetes	955 (41.18)	46 (47.42)	0.222	582 (37.36)	33 (41.77)	0.429
Renal disease	971 (41.87)	49 (50.52)	0.091	612 (39.28)	43 (54.43)	0.007
Any malignancy	830 (35.79)	33 (34.02)	0.721	494 (31.71)	27 (34.18)	0.646
HIV/AIDS	56 (2.41)	4 (4.12)	0.302	36 (2.31)	3 (3.8)	0.432
Hypertension	1798 (77.53)	76 (78.35)	0.85	1197 (76.83)	59 (74.68)	0.66
Hypothyroidism	395 (17.03)	21 (21.65)	0.238	269 (17.27)	16 (20.25)	0.495
Change in CCI from 1st hospitalization to last hospitalization, mean (SD)	0.71 (2.27)	0.4 (2.23)	0.185	0.58 (2.07)	0.34 (2.12)	0.316
Prior antibiotic use (days)						
Penicillins, mean (SD)	1.94 (7.6)	4.37 (10.35)	0.002	1.89 (8.82)	2.7 (6.47)	0.421
Cephalosporins, mean (SD)	5.86 (15.16)	16.01 (24.08)	<.001	9.94 (27.91)	21.77 (33.05)	<.001
Carbapenems, mean (SD)	3.83 (16.99)	12 (24.68)	<.001	5.36 (27.51)	15.48 (29.28)	0.001
Monobactams, mean (SD)	1.02 (14.16)	3.87 (23.46)	0.061	1.17 (12.93)	8.85 (38.52)	<.001
Polymyxins, mean (SD)	0.81 (7.91)	5.32 (15.87)	<.001	1.49 (11.34)	16.77 (54.78)	<.001
Anti-MRSA agents, mean (SD)	15.42 (38.56)	45.61 (53.75)	<.001	20.05 (59.25)	56.05 (91.14)	<.001
Aminoglycosides, mean (SD)	2.82 (13.26)	8.84 (24.77)	<.001	4.37 (15.44)	25.11 (49.52)	<.001
Tetracyclines, mean (SD)	1.16 (9.72)	5.16 (12.73)	<.001	1.5 (15.55)	7.9 (33.63)	0.001
Macrolides, mean (SD)	2.27 (9.12)	10.55 (34.98)	<.001	3.28 (11.91)	10.77 (40.47)	<.001
Fluoroquinolones, mean (SD)	2.85 (12.52)	6.35 (14.23)	0.007	3.15 (16.58)	13.44 (29.72)	<.001
Other, mean (SD)	6.07 (21.12)	16.61 (26.43)	<.001	6.92 (20.99)	24.47 (53.31)	<.001
Levofloxacin, mean (SD)	2.83 (12.52)	6.35 (14.23)	0.007	3.12 (16.61)	13 (29.51)	<.001
Meropenem/Imipenem, mean (SD)	3.83 (16.99)	12 (24.68)	<.001	5.36 (27.51)	15.48 (29.28)	0.001

*Prior history refers to any exposure across all hospitalizations in study time frame prior to the last GNB HAI.

Patients who had two or more *P. aeruginosa* infections compared to patients with only one infection were younger, had more hospitalizations, spent a greater number of days in the hospital and in an ICU, had a prior OR procedure, had a biopsy, underwent transplantation, were exposed to invasive medical devices, and had a diagnosis of cystic fibrosis, and renal disease. These patients were also more likely to have taken all antibiotics, except penicillins, for a greater number of days compared to patients with one *P. aeruginosa* infection.

Patients who had one or more *K. pneumoniae* HAIs had a total of 2,570 infections (Table 9). The majority of these were bloodstream infections (63%), followed by pneumonia (19%),

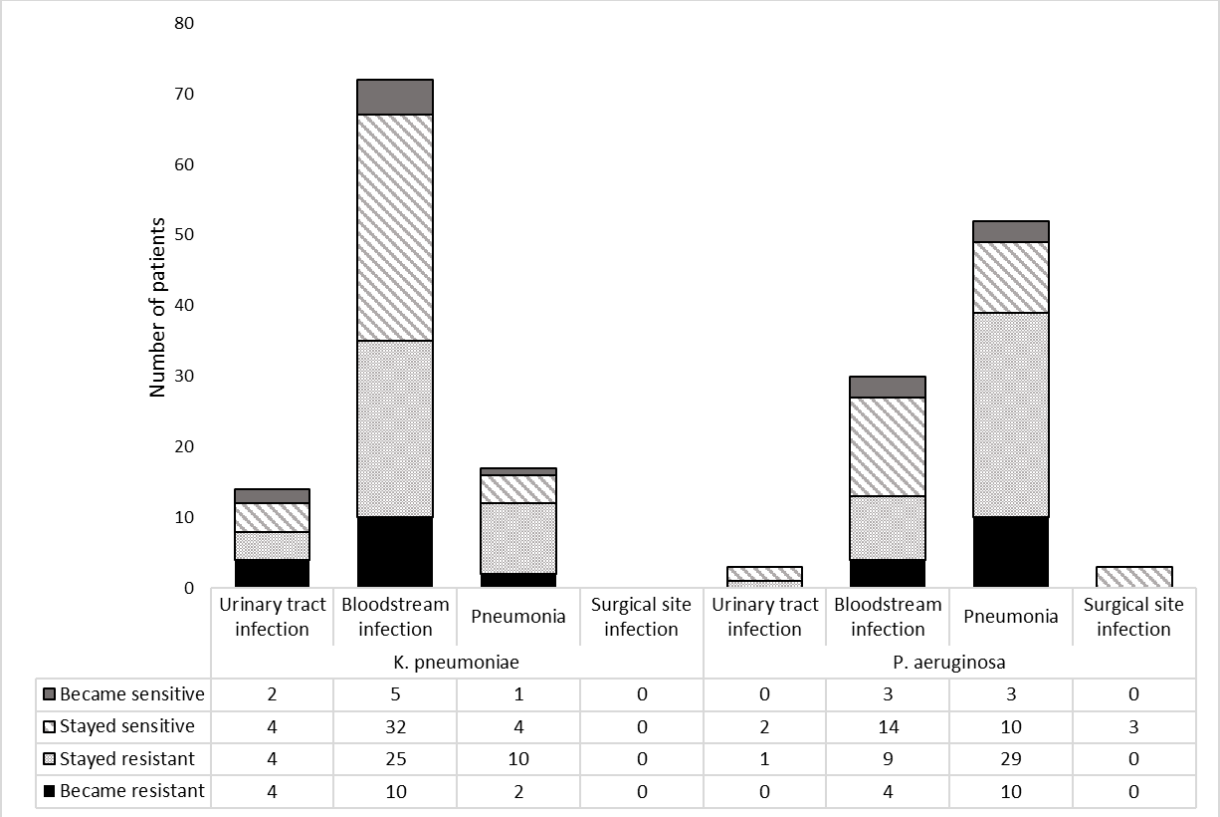
urinary tract infections (15%), and surgical site infections (2.8%). However, 65% of surgical site infections were drug-resistant while only 35% of bloodstream pathogens were drug-resistant. For *P. aeruginosa*, there were a total of 1,757 HAIs amongst all patients who had at least one infection. *P. aeruginosa* infections tended to be bloodstream (50%) or pneumonia (37%). Pneumonia caused by *P. aeruginosa* had the highest proportion of drug resistance (41%) as compared to other types of infection.

Table 9: Number of total infections in study population, by body site.

	K. pneumoniae		***P. aeruginosa***	
	N = 2570		***N = 1757***	
	n	**% resistant**	**n**	**% resistant**
Urinary tract infection	373	36.73	122	23.77
Bloodstream infection	1627	34.54	878	23.80
Pneumonia	496	41.33	644	40.68
Surgical site infection	74	64.86	113	23.01

When changes in antibiotic susceptibility were evaluated (Figure 5), there was minimal change in consecutive *K. pneumoniae* HAIs; 80% (n=82) remained susceptible or resistant from the prior infection. In 15% (n=16) of paired *K. pneumoniae* infections, the organism was resistant in the second infection. In consecutive *P. aeruginosa* infections, a large proportion of infections remained resistant (44%) and 16% (n=14) of second infections were drug-resistant.

Figure 5: Consecutive infections and antibiotic susceptibility in patients who had 2 or more HAIs, by organism and site.



	K. pneumoniae				P. aeruginosa			
	Urinary tract infection	Bloodstream infection	Pneumonia	Surgical site infection	Urinary tract infection	Bloodstream infection	Pneumonia	Surgical site infection
Became sensitive	2	5	1	0	0	3	3	0
Stayed sensitive	4	32	4	0	2	14	10	3
Stayed resistant	4	25	10	0	1	9	29	0
Became resistant	4	10	2	0	0	4	10	0

Table 10 and Table 11 summarize the crude and adjusted relative risks for drug- resistant infection for *K. pneumoniae* and *P. aeruginosa,* respectively. Poisson models with robust standard errors showed that a greater number of prior HAIs resulted in a higher likelihood of drug-resistant *K. pneumoniae* infection (Relative Risk: 1.14, 95%CI: 1.04-1.24) and a greater likelihood of a drug-resistant *P. aeruginosa* infection (RR: 1.23, 95%CI: 1.12-1.36) after adjusting for other confounders and antibiotic use. Mechanical ventilation (RR: 1.39, 95%CI: 1.24-1.57) and admission from another healthcare facility (RR:1.61, 95%CI: 1.02-1.32) were also associated with increased risk of *K. pneumoniae* infection after adjustment. In the *P. aeruginosa* model, cystic fibrosis (RR: 2.7, 95%CI: 1.88-2.73) was associated with drug-resistant infection.

Table 10: Crude and adjusted risk ratios of having a drug resistant *K. pneumoniae.*

	Crude		Adjusted*	
	RR	***95% CI***	***RR***	***95% CI***
Number of infections	1.242	1.141 - 1.352	1.136	1.038 - 1.244
Prior biopsy	1.205	1.042 - 1.394	1.115	0.956 - 1.300
Prior transplant	1.311	1.112 - 1.546	1.116	0.939 - 1.326
Prior days in ICU	1.004	1.003 - 1.006	0.998	0.995 - 1.000
Prior mechanical ventilation	1.463	1.315 - 1.627	1.391	1.237 - 1.565
Admission source				
Non-healthcare point of origin	*Ref*		*Ref*	
Clinic	0.743	0.618 - 0.894	0.766	0.660 - 0.890
Other healthcare facility	1.138	0.963 - 1.344	1.161	1.021 - 1.320
Other	0.970	0.820 - 1.146	0.981	0.831 - 1.157
Prior antibiotic use (days)				
Penicillins	1.009	1.006 - 1.013	1.004	1.001 - 1.008
Cephalosporins	1.006	1.004 - 1.009	1.001	0.998 - 1.004
Carbapenems	1.006	1.004 - 1.008	1.003	1.000 - 1.005
Monobactams	1.002	1.001 - 1.003	0.999	0.997 - 1.002
Polymyxins	1.010	1.007 - 1.013	1.001	0.998 - 1.004
Anti-MRSA agents	1.003	1.002 - 1.004	0.999	0.997 - 1.001
Aminoglycosides	1.006	1.004 - 1.008	1.001	0.999 - 1.004
Tetracyclines	1.008	1.005 - 1.010	1.004	1.002 - 1.006
Macrolides	1.005	1.003 - 1.007	1.001	0.998 - 1.004
Fluoroquinolones	1.007	1.004 - 1.009	1.002	0.998 - 1.005
Other	1.004	1.002 - 1.006	1.004	1.001 - 1.006

*Adjusted for all other potential confounders shown in table.

Table 11: Crude and adjusted risk ratios of having a drug resistant *P. aeruginosa.*

	Crude		Adjusted*	
	RR	***95% CI***	***RR***	***95% CI***
Number of infections	1.395	1.284 - 1.516	1.232	1.121 - 1.355
Cystic fibrosis	2.471	2.081 2.934	2.272	1.884 2.739
Prior antibiotic use (days)				
Penicillins	1.004	0.997 - 1.010	1.006	0.999 - 1.014
Cephalosporins	1.002	1.000 - 1.004	0.997	0.995 - 1.000
Carbapenems	1.0037	1.0016 - 1.0059	1.003	1.000 - 1.006
Monobactams	1.003	1.000 - 1.006	0.998	0.995 - 1.002
Polymyxins	1.006	1.004 - 1.008	0.997	0.992 - 1.001
Anti-MRSA agents	1.002	1.001 - 1.003	1.000	0.998 - 1.001
Aminoglycosides	1.008	1.006 - 1.010	1.003	1.000 - 1.006
Tetracyclines	1.002	0.999 - 1.005	0.997	0.992 - 1.002
Macrolides	1.005	1.003 - 1.007	1.004	1.002 - 1.007
Fluoroquinolones	1.007	1.005 - 1.009	1.004	1.001 - 1.007
Other	1.006	1.005 - 1.007	1.004	1.001 - 1.007

*Adjusted for all other potential confounders shown in table.

4.5 Discussion

In a population of patients with multiple hospitalizations, more repeat GNB HAIs were associated with higher risk of drug-resistant infection. To the best of our knowledge, this study is the first to describe the association between repeat GNB HAIs and drug resistance, taking into account prior antibiotic use. For each prior hospitalization with a *K. pneumoniae* HAI, there was a 14% increased risk of having a drug-resistant *K. pneumoniae* HAI in a subsequent hospitalization. For patients with repeat *P. aeruginosa* infections, the risk of drug resistance increased by 23% with each prior HAI with *P. aeruginosa*.

Repeat GNB HAIs increased risk of drug-resistant infections after adjusting for antibiotic use and other risk factors, indicating that there is some other mechanism through which prior infections influence the development of drug resistance. Persister cells are likely a cause for this phenomenon as they have been shown to be resistant to antibiotics and difficult to detect and

eradicate [134]. Most studies examining persistence factors in bacterial infections have been lab-based studies focused on *S. aureus, E. coli* and *P. aeruginosa* [135-137]; however, there are very few patient-based studies. This study demonstrates at the patient level that persistence factors may play a role in repeat infections and drug resistance in repeat GNB infections.

In this study, about 15% of consecutive infections changed from antibiotic susceptible to resistant and 38% of consecutive *K. pneumoniae infections* and 44% of consecutive *P. aeruginosa* infections remained drug resistant. Because our definitions of drug resistance were carbapenems for *K. pneumoniae* and fluoroquinolones for *P. aeruginosa*, typically considered one of the last lines of treatment for infections caused by these organisms, prevention of the first drug resistant infection is particularly important. This may be achieved through prioritizing prevention of all GNB HAIs to reduce patients' risk of subsequent infection with drug resistance.

There are several limitations to this study. The use of electronic medical records from a single hospital system does not capture patient medical history from other facilities nor provide an in-depth background on the patient. Our data covered a relatively small time frame for many patients who may have had hospitalizations outside the time period studied. In addition, there was no genotyping data available so it was not possible to assess strain relatedness or confirm whether a repeat infection was indeed a new infection. However, in this study, we defined a repeat infection as a HAI occurring in a hospitalization at least 30 days after a prior hospital discharge, it is unlikely that the infection was an unresolved prior infection. Although the number of patients who had repeat HAIs with the same GNB was relatively small, power calculations confirmed that there was >80% power to detect a significant association between repeat infections and drug resistance in this sample.

Using patient history over a nine-year period at three New York City hospitals, this study demonstrated the high threat of drug-resistant infections in patient with multiple admissions and repeat infections. These high-risk patients may serve as a reservoir for drug-resistant infections in hospitals; hence, patients with history of GNB HAIs should be prioritized to prevent repeat infections and slow the spread of drug resistance in GNB HAIs.

5. Conclusion

5.1 Summary of findings

Patients with multiple hospitalizations are at considerable risk for poor hospital outcomes, particularly hospital-acquired infections (HAIs), given their high volume of days in the hospital environment, exposure to broad spectrum antibiotics, and increased exposure to invasive medical devices and procedures. With increasing rates of multidrug resistant gram-negative bacteria (GNB) in hospitals, patients with multiple hospitalizations are at particular risk for loss of effective treatment options. The overarching goal of this dissertation was to evaluate the risk of acquiring GNB and drug resistant GNB HAIs amongst this vulnerable population. Few studies to date have investigated the risk of GNB infections in hospitalized patients with a history of prior hospitalizations. For three GNBs that are frequently implicated in HAIs–
K. pneumoniae, *P. aeruginosa*, and *A. baumannii* –with 9 years of electronic health records, I used a longitudinal approach to test the hypothesis that infections and antibiotic use in prior hospitalizations increase the risk of infections and drug resistance in future hospitalizations.

In Chapter 2 of this dissertation, I presented an investigation of risk factors associated with an incident GNB HAI after a prior hospitalization. Findings corroborated other studies in that older age, exposure to an intensive care unit (ICU), antibiotic use, and mechanical ventilation are significant risk factors for a GNB HAI. More importantly, analyses demonstrated that risk for infection increased with each prior day of antibiotic use. Prior use of carbapenems, cephalosporins, fluoroquinolones and polymyxins were significantly associated with a drug-resistant incident GNB infection.

As summarized in Chapter 3, I conducted a systematic literature review to assess studies that attempted to find an association between repeat GNB HAIs and increasing drug resistance. Between 2000 and 2015, only seven studies were found to have studied changes in antibiotic susceptibility in GNB HAIs at the patient level. Despite the high risk of bias in these studies, the majority reported increased drug resistance in subsequent infections, suggesting that repeated infections by GNB could result in lower susceptibility to available drugs.

To further investigate the association between repeat GNB HAIs and decreased antibiotic susceptibility, I analyzed clinical records of patients who had multiple hospitalizations and at least one GNB HAI. The analysis in Chapter 4 illustrated the strong association between repeat GNB infections caused by *K. pneumoniae* or *P. aeruginosa* and higher drug resistance in subsequent infections.

5.2 Limitations

As with all observational studies, this dissertation is not without its limitations. The data warehouse used in the empirical analyses was developed through the combination of electronic medical records, pharmacy records, and laboratory records and spanned nine years across three hospitals [67]. The data went through a rigorous cleaning process and has been used by researchers to answer critical questions pertaining to HAIs [138-140]. Nevertheless, as the data were not collected for research purposes, there may have been missing data and data entry errors.

The possibility of unmeasured confounding in the analyses is high due to the complex relationship between hospital environments, immunocompromised patients, and antibiotic pressure. Although many factors associated with acquiring a GNB HAI have been identified in the literature, factors such as previous bed occupants with infection, contaminated water, and level of

patient care were not included in these current analyses because these data elements were not available [141-143]. Lack of genotyping data also prohibited a comprehensive analysis of repeat GNB infections in patients. GNB strain relatedness could not be established to assess if repeat infections were new infections or relapses of prior infections.

The data also contained minimal patient demographic information which prevented any in-depth analysis of socio-economic status and risk of infection. This lack of data may have led to unmeasured confounding in the results. However, insurance status was evaluated and was not found to be associated with acquiring a GNB HAI or a repeat infection.

With very few prior studies examining patients with multiple hospitalizations and repeat GNB HAIs, it is difficult to evaluate the external validity of our results. However, the risk factors found in Chapter 2 are consistent with what other studies have found to be associated with incident GNB HAI including specific antibiotic use [61, 64, 72, 73]. Furthermore, based on the results of the systematic review and the basic science literature on bacterial persistence, it is likely that there is an association between repeat GNB HAIs and increasing drug resistance [96, 99, 144-147].

5.3 Public health relevance

Despite improvements in antibiotic stewardship and standard safety precautions in the last decade, drug resistance among GNB in hospitals has been increasing [1, 148]. For patients who experience multiple hospitalizations, this presents a potentially serious concern. These patients are at high risk for GNB HAIs but have been largely under-studied. This dissertation is one of the first and largest studies examining HAIs in patients with multiple hospitalizations. Using a large electronic database with a sizable and diverse patient population, results demonstrated that

longitudinal analysis of patient risk factors is feasible and can provide a thorough understanding of how infections can be prevented.

It is important to examine risk of GNB HAIs using a longitudinal or life-course perspective to 1) identify high risk patients and 2) identify potentially modifiable risk factors to prevent incident and repeat GNB infections. As electronic medical records become more widely utilized, it will be easier to evaluate patient risk throughout their medical history. Despite governmental support including the 2009 Health Information Technology for Economic and Clinical Health (HITECH) Act, only about 55% of healthcare providers had implemented fully integrative electronic records by 2014 [149]. In addition, although most healthcare facilities now have some form of electronic record, the usability and interoperability of these systems remains suboptimal [150].

Identification of high-risk patients can help focus infection prevention and control resources on these patients [17]. Given that prior antibiotic pressure and persistence factors are associated with the development of antibiotic resistance among GNBs, discerning patients' prior medical history would provide guidance for effective care and treatment. Having complete pharmacy records will be important to evaluate patients' life-time exposure to antibiotics and which remain effective. Future studies can evaluate if such data can be used to predict antibiotic susceptibility among patients who are given empiric therapy. Patients' detailed infection and antibiotic use history can also help to guide transitional care for patients at the time of hospital discharge to prevent reinfection as well as limit infection transmission within the community, nursing homes, and other health care facilities [151, 152].

As our population grows and lives longer with chronic disease, multiple hospitalizations are becoming the norm. Further research on high risk patients and their risk for drug-resistant GNB infections is critical if there is any hope to slow the rates of drug resistance in hospitals.

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