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Approval Sheet

Predictors for Developing a Carbapenem-Resistant Enterobacteriaceae Invasive Infection from Bacteriuria

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Abstract Cover Page

Predictors for Developing a Carbapenem-Resistant Enterobacteriaceae Invasive Infection from Bacteriuria

By

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ScB, Brown University, 2008

MD, David Geffen School of Medicine at University of California Los Angeles, 2013

Advisor: Jesse T. Jacob, MD, MSc

An abstract of

A thesis submitted to the Faculty of the

James T. Laney School of Graduate Studies of Emory University

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ABSTRACT

Predictors for Developing a Carbapenem-Resistant Enterobacteriaceae

Invasive Infection from Bacteriuria

By Jessica Howard-Anderson

Background
Infections with carbapenem-resistant Enterobacteriaceae (CRE) have limited treatment options and high mortality. Patients with CRE isolated only from urine (bacteriuria) have better outcomes than patients with CRE from a sterile site (invasive infection). This study describes the clinical epidemiology of CRE bacteriuria in metropolitan Atlanta and evaluates if urinary catheters increase the risk of "progression" from CRE bacteriuria to an invasive CRE infection.

Methods
We used active, laboratory- and population-based surveillance data from the Georgia Emerging Infections Program to identify patients with CRE bacteriuria in metropolitan Atlanta between 2012 - 2017. We calculated the annual incidence of CRE bacteriuria using census data and described the clinical characteristics of this cohort through chart review. We used univariable analyses to identify risk factors associated with progression to an invasive CRE infection within one year of CRE bacteriuria and multivariable logistic regression modeling to estimate the association between urinary catheters and progression. In an exploratory aim, we assessed the relatedness between urine and sterile site isolates from the same patient with pulsed-field gel electrophoresis and whole genome sequencing. Analyses were performed in SAS 9.4.

Results
We identified 464 patients with CRE bacteriuria, with a yearly incidence of 1.96 cases/100,000 population. Most patients had a urinary catheter (56%), and many resided in long term care facilities (49%), had a Charlson comorbidity index (CCI) >3 (37%), or had a decubitus ulcer (36%). 25 (6%) patients had progression. Risk factors for progression included black race, high CCI, presence of a urinary catheter, central venous catheter or another indwelling device, decubitus ulcer and having a culture obtained in an inpatient facility. In multivariable models, having a urinary catheter was associated with an increased the risk of progression (OR 4.1 95% CI 1.1 – 14.5). Most (6, 75%) patients with available isolates had highly related urine and sterile site CRE strains.

Conclusions
Patients with CRE bacteriuria are chronically ill and frequently have indwelling devices. Because urinary catheters may increase the risk of progression from CRE bacteriuria to an invasive infection, future interventions should target reducing inappropriate insertion and improving early appropriate removal of urinary catheters.

Cover Page

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Disclaimer: The primary dataset used in this project was collected by the Georgia Emerging Infections Program (EIP). The Georgia EIP was not involved in the analyses presented in this thesis.

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INTRODUCTION

Enterobacteriaceae are a large family of bacteria, including *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp., that frequently cause community-acquired and healthcare-associated infections (HAI) ranging from simple urinary tract infections to life-threatening bloodstream infections (1,2). Carbapenems are an antibiotic class often reserved for severely ill patients with multidrug-resistant infections. Enterobacteriaceae can become resistant to carbapenems through several different mechanisms, but the most concerning is mediated through carbapenemases that can hydrolyze carbapenems and are easily transmissible between bacteria (3,4). In 2019, the U.S. Centers for Disease Control and Prevention (CDC) declared carbapenem-resistant Enterobacteriaceae (CRE) an urgent threat, especially in healthcare facilities (5). Medical devices have been implicated as an important risk factor for CRE infections, and CRE remains an important cause of both central line–associated bloodstream infections and catheter-associated urinary tract infections (CAUTI) in the U.S. (2,6).

Partly due to limited and toxic therapeutic options for CRE infections, the mortality in patients with an invasive (culture positive from a sterile site) CRE infection is estimated to be greater than 50% (7). Less is known about the outcomes for patients with CRE bacteriuria (culture positive from urine), although the mortality is estimated to be much lower (6 – 19%) (7–9). Patients who develop an invasive CRE infection have worse outcomes, and patients with CRE bacteremia are over twice as likely to die in the hospital than those with CRE urinary colonization (10), making it imperative to better understand which patients with CRE bacteriuria are likely to develop an invasive infection. Urinary catheters represent potentially modifiable prevention targets in patients with CRE bacteriuria.

The Georgia Emerging Infections Program (EIP) conducts active laboratory- and population-based surveillance for CRE in metropolitan Atlanta. Since 2011, they have collected

CRE isolates from urine and sterile sites and performed chart reviews to determine patient demographics, comorbidities, healthcare exposures and other risk factors for CRE. In this study, we have utilized Georgia EIP data to 1) describe the epidemiology of patients with CRE bacteriuria in metropolitan Atlanta, 2) identify risk factors associated with developing an invasive CRE infection in patients with prior CRE bacteriuria ("progression"), and 3) determine the proportion of patient with CRE progression that have highly related CRE strains in both the urine and sterile site cultures. We hypothesized that in patients with CRE bacteriuria, urinary catheters increase the risk of progression to an invasive CRE infection.

BACKGROUND

Significance and Epidemiology of Carbapenem-Resistant Enterobacteriaceae

Enterobacteriaceae are a family of gram-negative bacteria including *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. These bacteria commonly colonize (presence of bacteria without associated disease) the human gastrointestinal tract. However they can also cause a wide spectrum of infections (presence of bacteria causing disease) in the gastrointestinal, urinary, and respiratory tracts, bloodstream, and central nervous system (1). Carbapenem resistance among Enterobacteriaceae is concerning since carbapenems are often the last line of defense against multidrug-resistant infections. In addition to carbapenems, CRE often develop resistance to additional antimicrobial agents including most cephalosporins, fluoroquinolones and aminoglycosides. Treatment options for patients with CRE are very limited and often involve using antibiotics that can cause significant toxicities (11).

In the last twenty years, the incidence of CRE has increased worldwide (12–14). Both the World Health Organization and CDC consider CRE among the most concerning multidrug-resistant pathogens because the resistance genes are highly transmissible and infections are associated with a high mortality (5,15). Largely due to improvements in infection control, the prevalence of CRE in the U.S. has stabilized in the last five years; however it remains an important cause of HAIs (2,5,16). According to a recent systematic review, the incidence of CRE in the U.S. ranges from 0.3 – 2.93 infections per 100,000 patient years, with the highest incidence in long-term acute-care hospitals (LTACHs) (17). An estimated 7% of device-associated HAIs caused by *Klebsiella* species are resistant to carbapenems, and in LTACHs 23% of CAUTIs caused by *Klebsiella* species are carbapenem resistant (16).

Mechanisms of Carbapenem-Resistance

The production of carbapenemases, enzymes that hydrolyze carbapenem antibiotics, is the most common and concerning mechanism for carbapenem resistance. Less commonly, mutations in genes encoding porins (channels in the membrane of bacteria) or efflux pumps can also result in carbapenem resistance (4,18). Carbapenemase-producing organisms are particularly worrisome because carbapenemases are often encoded on mobile genetic elements that can be transmitted between bacteria and cause nosocomial outbreaks (3,12,19–21).

Carbapenemases can be classified into 3 major classes of β-lactamases (Ambler class A, B, and D) that tend to be geographically distinct. *Klebsiella pneumoniae* carbapenemase (KPC), is a class A β-lactamase. It is the most common carbapenemase in the U.S., but is also endemic in Greece, Italy and South America. Class B β-lactamases, or metallo-β-lactamases (MBLs), including Verona integron-encoded MBL (VIM), imipenemase MBL (IMP) and New Delhi MBL (NDM), are endemic in the Indian subcontinent. Oxacillinase (OXA)-48-like β-lactamase, a class D β-lactamase, is endemic in Turkey and northern Africa (3,19). CRE dissemination in the U.S. has predominantly been attributed to a particular strain (sequence type 258) which produces a KPC that helped facilitate its spread (22,23). Laboratory techniques such as pulsed-field gel electrophoresis (PFGE) and whole genome sequencing (WGS), which can identify relatedness of CRE strains, have also become essential in in tracing and containing CRE outbreaks (21,24,25).

CRE Bacteriuria

In the U.S., CRE is most commonly identified in the urine, however dedicated research on the epidemiology of CRE bacteriuria is sparse (26,27). Differentiating urinary tract infections from urinary colonization is challenging, although one study showed that the mortality of patients with CRE urinary colonization is likely similar to that of patients with a urinary tract infection (10). In one of the largest cohorts of 105 patients with CRE bacteriuria, the 30-day mortality was low (6%) and deaths were unrelated to CRE (9). Yet in other similarly large cohorts of patients with CRE bacteriuria or urinary tract infections the 1-month all-cause

mortality ranged from 16-19%, with a relapse rate as high as 62% (8,27). Additionally, in solid organ transplant patients, CRE bacteriuria is associated with increased microbiologic treatment failure, ICU admission, and mortality (28,29).

Outcomes of Patients with CRE Differ by Site of Infection

The mortality associated with an invasive CRE infection (CRE cultured from a normally sterile site such as blood) is higher than that associated with CRE bacteriuria and can range from 18 – 82% (7). In one recent meta-analysis of over 20 studies, the pooled mortality for patients with carbapenem-resistant *K. pneumoniae* bacteremia was 54%, but only 14% for patients with urinary tract infections (7). Compared with carbapenem-susceptible organisms, bacteremic patients with carbapenem-resistant organisms have worse outcomes, but this may not be true for non-bacteremic infections (30). It is important to understand which patients with CRE bacteriuria will progress to an invasive CRE infection and therefore have a higher risk for a poor outcome.

CRE Colonization and Progression

The risk of progression to an invasive infection in patients with CRE bacteriuria is unknown. In a cohort of 105 patients with bacteriuria, no patients developed an invasive CRE infection within 90 days, although this study was limited to a single hospital (9). Two other studies showed a small but clinically significant rate of subsequent bacteremia, ranging from 6 - 15% in patients followed for up to 30 days (27,28). By utilizing Georgia EIP data in this study, we were able to determine how frequently patients in metropolitan Atlanta progress to an invasive infection within one year of the diagnosis of CRE bacteriuria.

While we are not aware of prior studies assessing risk factors for progression from CRE bacteriuria to an invasive CRE infection, we can extrapolate from research done in patients with CRE rectal colonization, which is also a risk factor for developing an invasive CRE infection (31).

One recent meta-analysis estimated that patients with CRE gastrointestinal colonization have a 16.5% chance of developing a CRE infection, although only 13% of the infections were invasive (32). Risk factors for developing CRE bacteremia in patients with rectal colonization include: admission to the ICU; presence of a tracheostomy, urinary catheter or central venous catheter; abdominal procedures; receipt of chemotherapy or radiation; comorbidities including diabetes and solid tumor malignancies; prior antibiotic administration; and CRE colonization at additional sites (33–35). Colonization with carbapenemase-producing CRE is also more likely to lead to a clinical CRE infection than non-carbapenemase-producing CRE (36). While potentially useful, screening for rectal CRE carriage can be resource intensive and is not routinely done in the U.S.(19,37). Urine cultures, however, are frequently obtained by clinicians allowing their use in this study to improve understanding of the outcomes of patients with CRE urinary colonization.

CRE and Urinary Catheters

CRE infections predominantly occur in patients with significant prior healthcare exposures (13,14,26). A 2018 meta-analysis of 69 studies found that patients with medical devices are at the highest risk of CRE acquisition (6). As noted above, patients with CRE rectal colonization and devices including urinary catheters and central venous catheters are at increased risk for developing an invasive infection (34,35). CDC recommends minimizing inappropriate medical device use to decrease the incidence and transmission of CRE (19,37). Urinary catheters in particular are medical devices that are often placed and retained without a clear indication (38) and are therefore more likely to be modifiable than other devices such as tracheostomy and central venous catheters. This study investigates if urinary catheters increase the risk of progression from CRE bacteriuria to an invasive CRE infection, as removing catheters could be a potential target for future infection prevention efforts.

METHODS

Study Aims:

This study aims to: 1) describe the epidemiology of patients with CRE bacteriuria in Atlanta, and 2) identify risk factors associated with developing an invasive CRE infection within one year of having CRE bacteriuria ("progression"). We hypothesized that in patients with CRE bacteriuria, urinary catheters would be associated with an increased risk of progression to an invasive CRE infection. In a third exploratory aim, we sought to determine the proportion of patients with progression that have highly related strains of CRE isolated from both the urine and sterile site cultures and evaluate if this was associated with the time between cultures. We hypothesized that patients with the similar CRE strains would have a shorter time to progression than patients who had different CRE strains in urine and sterile site cultures.

Study Population, Design, and CRE Definition:

We used data from the CDC-funded EIP's Multi-state Gram Negative Surveillance Initiative (MuGSI). Since 2011, Georgia EIP has been performing active, population- and laboratory-based surveillance of CRE in 8 counties of metropolitan Atlanta. CRE cases are identified by running routine queries of automated testing instruments used by laboratories in the Atlanta catchment area. The surveillance database includes all carbapenem-resistant *E. coli*, *K. pneumoniae*, *Klebsiella oxytoca*, *Klebsiella* (formerly *Enterobacter*) *aerogenes, and Enterobacter cloacae* isolated from a sterile site or urine culture. From 2011 – 2015, an isolate was defined as carbapenem-resistant if it was non-susceptible to imipenem, meropenem or doripenem (defined by the Clinical and Laboratory Standards Institute (CLSI) as a minimum inhibitory concentration (MIC) ≥ 2 μg/mL) and resistant to all tested 3rd generation cephalosporins. In 2016, the surveillance definition of CRE was changed to include any isolate that was resistant to doripenem, imipenem, or meropenem (defined by CLSI as MIC ≥ 4

μg/mL), or ertapenem (defined by CLSI as MIC ≥ 2 μg/mL) to improve the sensitivity of detection (Figure 1).

For every CRE case identified, an EIP surveillance epidemiologist reviews the patient's electronic medical record and completes a case report form which includes physical location at time of collection and place of residence four days prior, demographic information, comorbidities, risk factors for CRE including presence of invasive devices, patient outcome, specimen source, and results of antibiotic susceptibility testing including cephalosporins and carbapenems. When feasible, the CRE isolates from participating laboratories are also collected and stored by EIP for further laboratory testing.

We used the EIP surveillance database to identify a retrospective study cohort which included any patient with CRE first identified in a urine culture from 1/1/2012 – 12/31/2017. We excluded patients that had CRE identified in any other culture site prior to the identification of CRE in urine, including cultures identified in the pilot data collection period (8/1/2011 – 12/31/2011). Because the CDC surveillance definition of CRE changed in 2016, we used a CRE definition that could be unified over all available surveillance periods for the study cohort: resistant to at least one non-ertapenem carbapenem (doripenem, imipenem, or meropenem MIC ≥ 4 μg/mL), and resistant to all tested 3rd generation cephalosporins (Figure 1).

Variable Definitions:

For aims one and two, all variables pertaining to demographics, comorbid conditions, and CRE risk factors were obtained through manual review of the patient's medical record by EIP surveillance epidemiologists. Demographic variables included the patient's age at the time of culture identification and race. Comorbidity burden was quantified by the Charlson comorbidity index (CCI), which is a validated scoring system for comorbid conditions and can be used to predict mortality (39). Conditions included in the CCI include history of myocardial infarction, congestive heart failure, peripheral vascular disease, cerebrovascular disease,

dementia, chronic pulmonary disease, connective tissue disease, ulcer disease, liver disease, diabetes, hemiplegia, moderate to severe renal disease, malignancy, and acquired immunodeficiency syndrome. We dichotomized the CCI variable and categorized patients with CCI of ≤ 3 as "low" and CCI >3 as "high." This breakpoint was selected because it was the median for our study and was associated with an estimated 10-year survival of < 55% in the original validation study (39). We assessed for the presence of an indwelling urethral or suprapubic urinary catheter, central venous catheter or other indwelling device in place at the time the culture was obtained or in the two prior calendar days. Other indwelling devices were defined as devices implanted into an inner body space for the purpose of promoting drainage, providing a route for administration of food or medications, or administering oxygen, including endotracheal or nasotracheal tubes, gastrostomy tubes, nasogastric tubes, nephrostomy tubes, or tracheostomies. Additional covariates included the patient's residence (inpatient facility, LTACH, long-term care facility (LTCF), or private residence) four days prior to the culture, location where the CRE culture was obtained (inpatient facility, LTACH, LTCF, or outpatient clinic), and if the patient was in the intensive care unit (ICU) in the 7 calendar days prior to the culture. The presence of decubitus ulcers, dementia, hemi- or paraplegia or underlying urinary tract abnormalities (structural or functional abnormalities that lead to obstruction or retention of urine) at the time of CRE culture were captured as part of the standard EIP chart review because these characteristics may be more common in patients with urinary catheters, causing confounding.

For aim two, we defined progression as any patient in the study cohort that had CRE isolated from a sterile site (blood, cerebrospinal fluid, pericardial fluid, pleural fluid, peritoneal fluid, synovial fluid, bone or internal body site) between one day and one year after the original CRE urine culture. The time between the first CRE urine culture and the first CRE sterile site culture (time to progression) was defined in days. The main exposure variable was the presence of an indwelling urethral or suprapubic urinary catheter at the time the culture was obtained or

in the two prior calendar days. We captured 90-day mortality through Georgia Vital Statistics records.

Laboratory Analysis:

For patients with progression in which the GA EIP laboratory had both the urine and sterile site CRE isolates available, we performed PFGE to help determine strain relatedness. For one patient, the original CRE urine isolate was not available for analysis, but there was a similar CRE isolated from urine four days later which was used. We adapted the CDC PulseNet PFGE protocol for *E. coli*, *Salmonella* and *Shigella* spp. (https://www.cdc.gov/pulsenet/pdf/ecoli-shigella-salmonella-pfge-protocol-508c.pdf) and used Xbal as the restriction enzyme. The gels were run in 0.5X TBE (Tris-borate-EDTA) on a CHEF Mapper (Bio-Rad, Hercules, CA) with an initial switch time of 2.2 seconds and a final switch time of 54.2 seconds at 6 volts and an angle of 120 degrees. We used BioNumerics software v 7.6 (Applied Maths, Kortrijk, Belgium) to assess relatedness between paired isolates using an unweighted-pair group method of arithmetic averages and Dice coefficients with band position tolerance and optimization set at 1.5%.

Whole Genome Sequencing:

For the paired urine and sterile site isolates we also performed and analyzed WGS. For four of the isolates we used sequencing data from the CDC that was publicly available through National Center for Biotechnology Information (NCBI) Sequence Read Archive (BioProject PRJNA288601). For the remaining 12 isolates, genomic sequencing was performed using Illumina HiSeq by Omega Bioservices (Norcross, GA). We processed each sample through the Bactopia (https://github.com/bactopia/bactopia) analysis pipeline. The genomes were assembled by SKESA (40) and annotated with Prokka (41). To assess relatedness between the paired urine and sterile site isolates, we created pairwise pan-genomes using Roary (42). From the core genome alignment, we calculated single nucleotide polymorphism (SNP) distances

through snp-dists (43). We considered urine and sterile site CRE isolates to be highly related if they had < 21 SNPs between core genomes (44).

Sample Size and Power Calculations:

The sample size calculation was determined for our primary hypothesis that urinary catheters are associated with an increased risk of progression to an invasive CRE infection. By extrapolating from prior Georgia EIP data, we anticipated that approximately 50% of the patients with CRE bacteriuria would have a urinary catheter, and 5% would have progression (45). Medical devices can increase the risk of CRE acquisition with an odds ratio (OR) of 3.4 – 7.7, and patients with CRE rectal colonization and a urinary catheter have almost 5 times the odds of developing a CRE infection than those without a catheter (6,34). In this study, we estimated that 2.5% of patients without a urinary catheter and 9% of patients with a urinary catheter would progress to an invasive infection (OR of ~4 for urinary catheter), requiring a sample size of 404 patients with CRE bacteriuria to achieve 80% power with an alpha of 0.05 (openepi.com).

Analytic Plan by Aim:

Aim 1:

We calculated the yearly incidence of CRE bacteriuria in metropolitan Atlanta by dividing the number of new CRE bacteriuria cases by census population estimates of the 8-county metropolitan Atlanta catchment area. We used mean and standard deviation (SD) for continuous variables and proportions for categorical variables to characterize demographics, comorbid conditions, and risk factors for CRE infections and urinary catheters.

Aim 2:

We compared patients with and without the presence of a urinary catheter using chi-square and Fisher's exact tests as appropriate for categorical variables, and Student's t-tests for continuous variables. We performed univariable logistic regression to identify risk factors associated with progression to an invasive CRE infection and reported ORs with 95% confidence intervals (CIs). We also reported absolute risk differences (labeled as absolute risk increase) between the risk of progression with a selected characteristic of interest and the risk of progression without that characteristic (e.g. presence of a urinary catheter).

We created multivariable logistic regression models to estimate the association between urinary catheter (exposure) and progression (primary outcome). The first multivariable model included age and sex as covariates. The second model included potential confounding covariates that were significantly associated ($p < 0.1$) with the presence of a urinary catheter and progression. We assessed for interaction between urinary catheter and all covariates in this full model and used the Wald test to remove non-significant interaction terms through backward elimination. This model was then adjusted based on clinical plausibility and all combinations of covariates were tested in order to create the most parsimonious model that preserved accuracy and precision of the estimate of association between urinary catheter and progression. The final multivariable model is stated below, where P equals the probability of progression to an invasive CRE infection within 1 year. The exposure variable was the presence of a urinary catheter, and the covariates included the presence of a central venous catheter, the presence of another indwelling device, the location where the culture was obtained (inpatient facility, long term care location including LTACH or LTCF, or outpatient clinic), and whether the patient had a decubitus ulcer.

$$Logit\ (P) = \beta_\theta + \beta_1(\boldsymbol{Urinary\ catheter}) + \beta_2(\boldsymbol{Central\ venous\ catheter}) +$$
$$\beta_3(\boldsymbol{Other\ indwelling\ device}) + \beta_4(\boldsymbol{Location\_inpatient}) +$$
$$\beta_5\ (\boldsymbol{Location\ \_longterm\ care}) + \beta_6(\boldsymbol{Decubitus\ ulcer})$$

Missing data were infrequent in this study and a complete case analysis was used. In the multivariable model, the largest proportion of missing data from one variable was only 2% and believed to be missing at random.

Two sensitivity analyses were performed. The first was a propensity score analysis in which we performed a multivariable logistic regression to create a propensity score, or probability, that a patient in our cohort would have a urinary catheter. All covariates included in this model were significantly associated with urinary catheter in univariable analyses ($p < 0.1$). We then inversely weighted the propensity score to make the distribution of covariates independent of urinary catheter and performed a new logistic regression model with urinary catheter as the exposure and progression as the outcome. The second sensitivity analysis considered that patients may die before they progress to an invasive infection, a competing event. Since we only had mortality data available for up to 90 days after the CRE culture, we only included progression events up to 90 days in this analysis. We performed a cause-specific proportional hazards model for progression and patients who either died or did not progress by 90 days were censored. The proportional hazards assumption was met for urinary catheter and we used this model to estimate a crude hazards ratio for urinary catheter and progression at 90 days. Due to limitations in sample size we did not perform a multivariable hazard model.

All statistical analyses were performed in SAS version 9.4 (SAS Institute, Cary, NC). P-values < 0.05 were considered statistically significant except as noted above in model formation.

Aim 3:

We used descriptive statistics to calculate the proportion of patients with progression that had highly related CRE strains identified in both the urine and sterile site cultures. We compared the median time to progression in days between patients with highly related CRE strains and those with different CRE strains. No formal statistical testing was performed given the small number of isolates available for testing.

Institutional Review Board Approval

The Georgia EIP surveillance, data collection and analysis are approved by the Emory University Institutional Review Board.

RESULTS

Incidence of CRE Bacteriuria in Metropolitan Atlanta

Between 2012 – 2017 we identified 464 patients with their first episode of CRE bacteriuria in metropolitan Atlanta. The number of new CRE bacteriuria cases per year was relatively stable, although the annual incidence appeared to modestly decline over the study period (Table 1, Figure 2). The mean (SD) annual incidence rate over the 6 years was 1.96 (0.19) and ranged from 1.68 – 2.20 cases per 100,000 people in metropolitan Atlanta.

Characteristics of the Study Cohort

The majority of patients with CRE bacteriuria in metropolitan Atlanta had *K. pneumoniae* (69%), followed by *E. coli* (17%), *E. cloacae* (10%), *K. aerogenes* (3%), and *K. oxytoca* (2%). 428 patients (92%) had chart review data available. The mean (SD) age was 64.6 (17.0) years and 234 (55%) were female. Many patients were chronically ill; 159 (37%) had a high CCI and more than one-third of the patients had a decubitus ulcer. Medical devices were common: 238 (56%) patients had an indwelling urinary catheter, 124 (29%) had a central venous catheter and 163 (38%) had another indwelling device. Almost half (49%) of the patients resided in a LTACH or LTCF prior to identification of CRE bacteriuria (Table 1).

Differences in Patients with a Urinary Catheter

There were many significant differences between patients with and without the presence of a urinary catheter (Table 3). Notably, patients with urinary catheters were more likely to be male (49% vs 41%, $p = 0.07$), have central venous catheters (39% vs 17%, $p < 0.001$) or other indwelling devices (49% vs 25%, $p < 0.001$), have a decubitus ulcer (45% vs 25%, $p < 0.001$), and underlying urinary tract abnormalities (19% vs 11%, $p = 0.01$). Patients with urinary catheters more commonly had a CRE culture obtained in an inpatient facility (41% vs 28%, $p = 0.01$) and had been admitted to the ICU in the week prior (20% vs 4%, $p < 0.001$).

Frequency and Risk Factors for Progression

Twenty-five (6%) patients in our study cohort had progression from CRE bacteriuria to an invasive CRE infection within one year. The median (interquartile range [IQR]) time to progression was 34 (15 – 110) days. All but one patient with progression had *K. pneumoniae,* and the remaining patient had *E. coli*. Compared to all other CRE, *K. pneumoniae* was significantly associated with progression (unadjusted OR 10.7, 95% CI 1.4 – 79.7) .

Black race (OR 3.2, 95% CI 1.1 – 9.5), high CCI (OR 2.7, 95% CI 1.2 – 6.1), presence of a urinary catheter (OR 6.3, 95% CI 1.9 – 21.5), presence of a central venous catheter (OR 4.0, 95% CI 1.8 – 9.3), presence of another indwelling device (OR 2.3, 95% CI 1.3 – 7.2), decubitus ulcer (OR 2.3, 95% CI 1.0 – 5.3), and CRE culture obtained in an inpatient location (OR 4.2, 95% CI 1.2 – 15.1) were all significantly associated with progression in univariable logistic regression (Table 4). Having a urinary catheter, the primary exposure variable of interest, increased the absolute risk of progression by 7.6%.

Association Between Urinary Catheter and Progression

To estimate the association between urinary catheters and the risk of progression to an invasive CRE infection we created multiple models through multivariable logistic regression. In the first model, we adjusted only for sex and age and the OR for urinary catheter was 6.3 (95% CI 1.9 – 21.6). In the second model we controlled for all covariates that were suspected to be confounders from univariable analyses and the OR for urinary catheter decreased to 4.1 (95% CI 1.2 – 14.7). Our third model revealed a similar OR (4.2, 95% CI 1.2 – 14.9) for urinary catheter after removing *K. pneumoniae* as a covariate because of the wide CI in univariable analysis due to only one non-*K. pneumoniae* case of progression. In our final model (model 4), we removed the ICU covariate which allowed for a more parsimonious model and improved precision (urinary catheter OR 4.1 95% CI 1.1 – 14.5) (Table 5).

In both sensitivity analyses, the presence of a urinary catheter was also associated with progression. Using a propensity score analysis with inverse probability weighting, urinary catheters increased the odds of progression by 4.8 (95% CI 1.5 – 14.9). When accounting for death as a competing risk, the presence of a urinary catheter increased the risk of progression at 90 days with an unadjusted OR of 6.1 (95% CI 1.4 – 26.6) in a cause-specific proportional hazards model.

Relatedness between Urine and Sterile Site Cultures

For 8 patients with progression, we had both the CRE urine and sterile site isolates ("paired isolates"). Seven (88%) patients had paired isolates that were > 90% similar on PFGE. Using WGS allowed for further discrimination between isolates and we determined that 6 (75%) patients with CRE bacteriuria had an invasive infection with a CRE strain that was highly related to the original urine strain. The median (IQR) time to progression was 29 (25 – 35) days in patients with highly related CRE strains and 200.5 (IQR 101 – 300) days in patients with different CRE strains.

DISCUSSION

We estimated that the average annual incidence of CRE bacteriuria in metropolitan Atlanta per 100,000 population was 1.96 cases, well within the range of 0.5 – 2.93 infections reported in a recent systematic review of CRE epidemiology in the U.S. (17). Over the six year study period, this incidence remained stable, also consistent with recent national data showing a flat trend when assessing both invasive and non-invasive CRE (2,5,16). Carbapenem-resistant *K. pneumoniae* is the most common CRE in the U.S. (14,26) and globally (4,46) and was identified in more than two-thirds of our cohort.

Similar to findings from national CRE surveillance (26), our study, to our knowledge the largest CRE cohort to focus only on patients with bacteriuria, showed that these patients have high frequencies of chronic illnesses and indwelling devices, though patients with invasive infections tend to have less favorable clinical outcomes (7). CRE infections most commonly occur in patients with prior healthcare exposure, especially among those residing in LTACHs (17,18,47). In our study, almost half of the patients resided in an LTACH or LTCF four days prior to identification of CRE bacteriuria. However, only 33% of the patients had their culture obtained at an LTACH or LTCF, suggesting patients may have been admitted to an inpatient facility prior to the culture being obtained. This finding highlights the interconnectedness of healthcare systems and demonstrates how multidrug-resistant organisms can easily be transferred from one setting to another.

Six percent of patients with CRE bacteriuria developed an invasive CRE infection within one year. This relatively rare but clinically significant event is important since CRE bacteremia is associated with up to a 40% increase in mortality (7). While we were only able to sequence eight paired urine and sterile site isolates, most patients with progression had very similar strains of CRE in both their sterile site and original urine culture, especially if the invasive infection occurred within 35 day of the urine culture. This suggests that invasive CRE infections developing in patients with CRE bacteriuria are related to the prior bacteriuria episode,

plausibly more likely from incomplete treatment or persistence of colonization than acquisition of a new CRE infection. Alternatively, patients may be re-exposed to the same CRE strain multiple times, particularly if they return to the same living environment in which they were originally exposed to CRE. In one patient, the urine and sterile site isolates were similar on PFGE (91%) but not on WGS (776 SNP differences). This discrepancy is not surprising as PFGE relies on identifying patterns in large DNA fragments and is subject to variability both in the restriction enzyme being used and in visual estimates of relatedness. WGS provides a more exact method of comparing DNA sequences and can better differentiate between two isolates that may appear similar on PFGE (48).

Through multivariable logistic regression modeling we found that in patients with CRE bacteriuria, urinary catheters increase the odds of developing an invasive CRE infection by at least four times; an absolute risk increase of almost 8%. We confirmed this finding in a propensity score analysis which can help to minimize confounding by indication as patients who had urinary catheters were different from those that did not. Additionally, when accounting for the time to progression and death as a competing event, urinary catheters increased the risk of progression at 90 days.

Both the Society for Hospital Medicine and the Society for Healthcare Epidemiology of America recommend minimizing the use of urinary catheters, daily assessments of necessity, and removing catheters when no longer needed as part of the Choosing Wisely campaign (49,50). Our findings support this approach as a critical aspect in caring for patients with CRE bacteriuria. Most patients in our cohort had a urinary catheter (56%), but fewer than observed in the national EIP surveillance study of patients with CRE in both urine and sterile sites from 2012 – 2013 (74%) (26). This difference may be due to geographic variability and local infection control practices in Atlanta as well as increased awareness of the risk of urinary catheters. Patients with bacteremia may also be more acutely ill and require a urinary catheter while in the

ICU or for hemodynamic monitoring, which could contribute to the higher proportion of urinary catheters in the invasive infection group.

Unfortunately, our surveillance dataset does not capture how long urinary catheters remain in place after identification of CRE bacteriuria. This area deserves future attention since urinary catheters may be removed in less than a third of patients with CRE bacteriuria (27). We suspect that the risk of progression is related to how long a catheter remains in place, but how much risk, if any, each additional day confers is unknown. In a South African study, each additional urinary catheter day was associated with a 7% increase in the odds of CRE acquisition; however this was during a CRE outbreak and may not be directly applicable to our patient population or setting (51). The risk of progression may also differ depending on the indication for the urinary catheter, and if the catheter was chronic or placed due to an acute illness.

Additional risk factors for CRE progression included black race, the presence of a central venous catheter or other indwelling medical devices, high CCI, decubitus ulcers, and being in the ICU within one week prior to CRE culture identification. Most of these characteristics, especially the presence of medical devices, have been identified in prior studies analyzing risk factors for CRE infection in patients with prior CRE rectal colonization (33–35). We are not aware of prior literature reporting race as a risk factor for developing an invasive CRE infection, and this finding warrants further investigation surrounding access to care or health disparities as potential explanations. Decubitus ulcers are infrequently studied in patients with CRE, although this subgroup of patients likely does have a high risk of developing CRE colonization and invasive infection given frequent healthcare exposures and often limited mobility. Strategies already employed at many hospitals to minimize medical devices and prevent decubitus ulcers should be particularly emphasized in patients that are already colonized with CRE as a way to decrease the risk of an invasive infection.

Patients with carbapenem-resistant *K. pneumoniae* bacteriuria also had a higher risk of progression compared to patients with other species of CRE. However, all except one of the patients with progression had *K. pneumoniae*, limiting our ability to draw conclusions. The 95% CI associated with the odds of progression for *K. pneumoniae* species was wide (1.4 – 79.7), indicating a large degree of uncertainty. To our knowledge *K. pneumoniae* has not been previously reported as a risk factor for progression, but *in vitro* data suggests that multidrug-resistant *K. pneumoniae* may produce more biofilm than other drug-resistant Enterobacteriaceae (52). Biofilm production on urinary catheters could be one reason patients with *K. pneumoniae* may be at increased risk for progression and this could be better elucidated in a larger sample of patients with CRE bacteriuria.

Study Strengths

A major strength of this study is that we used active, population-based surveillance data over six years, creating one of the largest cohorts of patients with CRE. With over 400 patients, we had the power to evaluate relatively rare outcomes such as progression within one year. Unlike hospital-based surveillance studies, we could identify patients with subsequent CRE infections after discharge if the culture was obtained anywhere within the 8-county metropolitan Atlanta area, regardless of setting. We were able to assess for progression up to one year after the original identification of CRE bacteriuria, a longer follow-up time than used in prior studies (9,27,28). This study is the first to examine factors that may increase a patient's risk for developing an invasive CRE infection in an easily identifiable, high-risk group of patients—those with CRE bacteriuria—with a potentially modifiable characteristic (urinary catheters).

Study Limitations

This study has some limitations. First, as previously noted, we were not able to assess how long a urinary catheter remained in place after diagnosis of CRE bacteriuria and duration of catheterization is known to be associated with bacteriuria. Second, patients in this study cohort did not have routine surveillance cultures obtained nor were they prospectively followed for any specific time period. Instead, EIP relies on automated laboratory queries to identify all patients with a CRE culture, and therefore case ascertainment depends to some degree on clinical practice patterns and diagnostic intensity that may vary across the spectrum of healthcare. While it is possible that we may have underestimated CRE bacteriuria, the generally low threshold for urinary cultures makes this less of a limitation than with cultures of other anatomic sites. Third, population-based surveillance may miss cases in non-residents or in those who had care delivered outside of the metropolitan Atlanta area. Fourth, in this fragile population, patients may die before surveillance can capture the outcome of progression. We attempted to account for this in a sensitivity analysis, although due to limitations in sample size a multivariable hazards model was not performed. Finally, while the study was powered to assess the effect of urinary catheters on progression, we were unable to clearly assess some additional potential risk factors (such as organism) because a relatively small proportion of patients had this outcome and few paired isolates were available for WGS.

Future Directions

This study adds to existing knowledge while raising several new questions about patients with CRE bacteriuria, and design interventions aimed at decreasing invasive CRE infections in this population. To increase our sample size, we may be able to combine our data with the other nine EIP sites throughout the U.S. This would allow us to validate our findings with regards to urinary catheters and to help clarify if *K. pneumoniae* is truly a risk factor for CRE progression.

Future studies are needed to evaluate how often urinary catheters are removed or exchanged in patients with CRE bacteriuria, and reasons why they may not be removed.

Interventions could then be designed to educate healthcare providers and caregivers about minimizing urinary catheter use whenever possible. LTACHs may be an ideal place to consider instituting such an intervention, as many patients in our study resided in an LTACH immediately prior to being diagnosed with CRE bacteriuria and intervention efforts focused entirely in LTACHs can reduce the majority of CRE transmissions (53). Lastly, future research could elucidate the mechanism for why urinary catheters increase the risk of CRE progression, including investigating CRE biofilms on urinary catheters to ascertain if this contributes to the pathogenesis of CRE infection in patients with underlying CRE bacteruria.

REFERENCES

1. Donnenberg MS. Enterobacteriaceae [Chapter 220]. In: Mandell, Douglas, and Bennett's *Principles and Practice of Infectious Diseases*. 8th ed. Philadelphia, PA: Saunders, an imprint of Elsevier; 2015:2503-2517.

2. Weiner LM, Webb AK, Limbago B, et al. Antimicrobial-Resistant Pathogens Associated With Healthcare-Associated Infections: Summary of Data Reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2011–2014. *Infect. Control Hosp. Epidemiol.* 2016;37(11):1288–1301.

3. Bonomo RA, Burd EM, Conly J, et al. Carbapenemase-Producing Organisms: A Global Scourge. *Clin. Infect. Dis.* 2018;66(8):1290-1297.

4. Nordmann P, Poirel L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria. *Clin. Infect. Dis.* 2019;69(Supplement_7):S521–S528.

5. CDC. Antibiotic Resistance Threats in the United States, 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2019.

6. Loon K van, Holt AFV in 't, Vos MC. A Systematic Review and Meta-analyses of the Clinical Epidemiology of Carbapenem-Resistant Enterobacteriaceae. *Antimicrob. Agents Chemother.* 2018;62(1):e01730-17.

7. Xu L, Sun X, Ma X. Systematic review and meta-analysis of mortality of patients infected with carbapenem-resistant Klebsiella pneumoniae. *Ann. Clin. Microbiol. Antimicrob.* 2017;16(18):2–12.

8. Önal U, Sipahi OR, Pullukçu H, et al. Retrospective evaluation of the patients with urinary tract infections due to carbapenemase producing Enterobacteriaceae. *J. Chemother.* 2020;32(1):15–20.

9. Qureshi ZA, Syed A, Clarke LG, et al. Epidemiology and clinical outcomes of patients with carbapenem-resistant Klebsiella pneumoniae bacteriuria. *Antimicrob Agents Chemother*. 2014;58(6):3100–4.

10. Hauck C, Cober E, Richter SS, et al. Spectrum of Excess Mortality due to Carbapenem-Resistant Klebsiella pneumoniae Infections. *Clin. Microbiol. Infect. Off. Publ. Eur. Soc. Clin. Microbiol. Infect. Dis.* 2016;22(6):513–519.

11. Nordmann P, Cuzon G, Naas T. The real threat of Klebsiella pneumoniae carbapenemase-producing bacteria. *Lancet Infect. Dis.* 2009;9(4):228–236.

12. Nordmann P, Naas T, Poirel L. Global Spread of Carbapenemase-producing Enterobacteriaceae. *Emerg. Infect. Dis. J.* 2011;17(10):1791.

13. Jacob JT, Klein E, Laxminarayan R, et al. Vital Signs: Carbapenem-Resistant Enterobacteriaceae. *MMWR Morb. Mortal. Wkly. Rep.* 2013;62(9):165–170.

14. Thaden JT, Lewis SS, Hazen KC, et al. Rising Rates of Carbapenem-Resistant Enterobacteriaceae in Community Hospitals: A Mixed-Methods Review of Epidemiology and Microbiology Practices in a Network of Community Hospitals in the Southeastern United States. *Infect. Control Hosp. Epidemiol.* 2014;35(8):978–983.

15. World Health Organization. Global priority list of antibiotic-resistant bacteria to guide research, discovery and development of new antibiotics. Geneva: World Health Organization;2017

16. Weiner-Lastinger LM, Abner S, Edwards JR, et al. Antimicrobial-resistant pathogens associated with adult healthcare-associated infections: Summary of data reported to the National Healthcare Safety Network, 2015–2017. *Infect. Control Hosp. Epidemiol.* 2020;41(1):1–18.

17. Livorsi DJ, Chorazy ML, Schweizer ML, et al. A systematic review of the epidemiology of carbapenem-resistant Enterobacteriaceae in the United States. *Antimicrob. Resist. Infect. Control*. 2018;7(1):55.

18. Logan LK, Weinstein RA. The Epidemiology of Carbapenem-Resistant Enterobacteriaceae: The Impact and Evolution of a Global Menace. *J. Infect. Dis.* 2017;215(suppl_1):S28–S36.

19. Friedman ND, Carmeli Y, Walton AL, et al. Carbapenem-Resistant Enterobacteriaceae: A Strategic Roadmap for Infection Control. *Infect. Control Hosp. Epidemiol.* 2017;38(5):580–594.

20. Bratu S, Landman D, Haag R, et al. Rapid spread of carbapenem-resistant Klebsiella pneumoniae in New York City: a new threat to our antibiotic armamentarium. *Arch. Intern. Med.* 2005;165(12):1430–1435.

21. Epstein L, Hunter JC, Arwady MA, et al. New Delhi metallo-β-lactamase-producing carbapenem-resistant Escherichia coli associated with exposure to duodenoscopes. *JAMA*. 2014;312(14):1447–1455.

22. Kitchel B, Rasheed JK, Patel JB, et al. Molecular Epidemiology of KPC-Producing Klebsiella pneumoniae Isolates in the United States: Clonal Expansion of Multilocus Sequence Type 258. *Antimicrob. Agents Chemother.* 2009;53(8):3365–3370.

23. Munoz-Price LS, Poirel L, Bonomo RA, et al. Clinical epidemiology of the global expansion of Klebsiella pneumoniae carbapenemases. *Lancet Infect. Dis.* 2013;13(9):785–796.

24. Yang S, Hemarajata P, Hindler J, et al. Evolution and Transmission of Carbapenem-Resistant Klebsiella pneumoniae Expressing the blaOXA-232 Gene During an Institutional Outbreak Associated With Endoscopic Retrograde Cholangiopancreatography. *Clin. Infect. Dis.* 2017;64(7):894–901.

25. Goering RV. Pulsed field gel electrophoresis: A review of application and interpretation in the molecular epidemiology of infectious disease. *Infect. Genet. Evol.* 2010;10(7):866–875.

26. Guh AY, Bulens SN, Mu Y, et al. Epidemiology of Carbapenem-Resistant Enterobacteriaceae in 7 US Communities, 2012-2013. *JAMA*. 2015;314(14):1479–1487.

27. Satlin MJ, Kubin CJ, Blumenthal JS, et al. Comparative Effectiveness of Aminoglycosides, Polymyxin B, and Tigecycline for Clearance of Carbapenem-Resistant Klebsiella pneumoniae from Urine. *Antimicrob. Agents Chemother.* 2011;55(12):5893–5899.

28. Pouch S m., Kubin C j., Satlin M j., et al. Epidemiology and outcomes of carbapenem-resistant Klebsiella pneumoniae bacteriuria in kidney transplant recipients. *Transpl. Infect. Dis.* 2015;17(6):800–809.

29. Brizendine KD, Richter SS, Cober ED, et al. Carbapenem-Resistant Klebsiella pneumoniae Urinary Tract Infection following Solid Organ Transplantation. *Antimicrob. Agents Chemother.* 2015;59(1):553–557.

30. Falagas ME, Tansarli GS, Karageorgopoulos DE, et al. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. *Emerg Infect Dis*. 2014;20:1170–5.

31. McConville TH, Sullivan SB, Gomez-Simmonds A, et al. Carbapenem-resistant Enterobacteriaceae colonization (CRE) and subsequent risk of infection and 90-day mortality in critically ill patients, an observational study. *PloS One*. 2017;12(10):e0186195.

32. Tischendorf J, de Avila RA, Safdar N. Risk of infection following colonization with carbapenem-resistant Enterobactericeae: A systematic review. *Am J Infect Control*. 2016;44:539–43.

33. Giannella M, Trecarichi EM, Rosa FGD, et al. Risk factors for carbapenem-resistant Klebsiella pneumoniae bloodstream infection among rectal carriers: a prospective observational multicentre study. *Clin. Microbiol. Infect.* 2014;20(12):1357–1362.

34. Borer A, Saidel-Odes L, Eskira S, et al. Risk factors for developing clinical infection with carbapenem-resistant Klebsiella pneumoniae in hospital patients initially only colonized with carbapenem-resistant K pneumoniae. *Am. J. Infect. Control*. 2012;40(5):421–425.

35. Schechner V, Kotlovsky T, Kazma M, et al. Asymptomatic rectal carriage of blaKPC producing carbapenem-resistant Enterobacteriaceae: who is prone to become clinically infected? *Clin. Microbiol. Infect.* 2013;19(5):451–456.

36. Tamma PD, Kazmi A, Bergman Y, et al. The Likelihood of Developing a Carbapenem-Resistant Enterobacteriaceae Infection during a Hospital Stay. *Antimicrob. Agents Chemother.* 2019;63(8): e00757-19.

37. CDC. Facility Guidance for Control of Carbapenem-resistant Enterobacteriaceae (CRE). Atlanta, GA: U.S. Department of Health and Human Services, CDC; 2015.

38. Saint S, Wiese J, Amory JK, et al. Are physicians aware of which of their patients have indwelling urinary catheters? *Am. J. Med.* 2000;109(6):476–480.

39. Charlson ME, Pompei P, Ales KL, et al. A new method of classifying prognostic comorbidity in longitudinal studies: Development and validation. *J. Chronic Dis.* 1987;40(5):373–383.

40. Souvorov A, Agarwala R, Lipman DJ. SKESA: strategic k-mer extension for scrupulous assemblies. *Genome Biol.* 2018;19(1):153.

41. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinforma. Oxf. Engl.* 2014;30(14):2068–2069.

42. Page AJ, Cummins CA, Hunt M, et al. Roary: rapid large-scale prokaryote pan genome analysis. *Bioinforma. Oxf. Engl.* 2015;31(22):3691–3693.

43. Seemann, T. snp-dists - Pairwise SNP distance matrix from a FASTA sequence alignment. (Github).

44. David S, Reuter S, Harris SR, et al. Epidemic of carbapenem-resistant Klebsiella pneumoniae in Europe is driven by nosocomial spread. *Nat. Microbiol.* 2019;1–11.

45. Sexton ME. Evaluation of Risk Factors for Invasive Carbapenem Resistant Enterobacteriaceae Infections and Resultant Mortality in Atlanta, 2011-2015 [master's thesis]. Atlanta,GA: Emory University;2017.

46. Grundmann H, Glasner C, Albiger B, et al. Occurrence of carbapenemase-producing Klebsiella pneumoniae and Escherichia coli in the European survey of carbapenemase-producing Enterobacteriaceae (EuSCAPE): a prospective, multinational study. *Lancet Infect. Dis.* 2017;17(2):153–163.

47. Lin MY, Lyles-Banks RD, Lolans K, et al. The Importance of Long-term Acute Care Hospitals in the Regional Epidemiology of Klebsiella pneumoniae Carbapenemase–Producing Enterobacteriaceae. *Clin. Infect. Dis.* 2013;57(9):1246–1252.

48. Pulsed-field Gel Electrophoresis (PFGE). Centers for Disease Control and Prevention. 2016; (https://www.cdc.gov/pulsenet/pathogens/pfge.html) (Accessed 2020 Feb 26).

49. Bulger J, Nickel W, Messler J, et al. Choosing wisely in adult hospital medicine: five opportunities for improved healthcare value. *J. Hosp. Med.* 2013;8(9):486–492.

50. Morgan DJ, Croft LD, Deloney V, et al. Choosing Wisely in Healthcare Epidemiology and Antimicrobial Stewardship. *Infect. Control Hosp. Epidemiol.* 2016;37(7):755–760.

51. de Jager P, Chirwa T, Naidoo S, et al. Nosocomial Outbreak of New Delhi Metallo-β-Lactamase-1-Producing Gram-Negative Bacteria in South Africa: A Case-Control Study. *PLoS ONE*. 2015;10(4).

52. Ramos-Vivas J, Chapartegui-González I, Fernández-Martínez M, et al. Biofilm formation by multidrug resistant Enterobacteriaceae strains isolated from solid organ transplant recipients. *Sci. Rep.* 2019;9(1):8928.

53. Toth DJA, Khader K, Slayton RB, et al. The Potential for Interventions in a Long-term Acute Care Hospital to Reduce Transmission of Carbapenem-Resistant Enterobacteriaceae in Affiliated Healthcare Facilities. *Clin. Infect. Dis.* 2017;65(4):581–587.

TABLES AND FIGURES

Table 1: Annual Number of CRE Bacteriuria Cases and Incidence Rates in Metropolitan Atlanta

Year	Number of New Cases	Census Population	Annual Incidence Rate/100,000 people
2012	84	3,821,534	2.20
2013	79	3,864,091	2.04
2014	77	3,925,130	1.96
2015	71	3,991,607	1.78
2016	84	4,036,982	2.08
2017	69	4,098,115	1.68

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae

Table 2: Demographics and Clinical Characteristics of Patients with CRE Bacteriuria in Metropolitan Atlanta

Variable	Study Cohort (n = 428)
Age (mean years [SD])	64.5 (17.0)
Female	234 (55)
Race (n = 407)	
Black	258 (63)
White	137 (34)
Other	12 (3)
Charlson comorbidity index >3 (n = 425)	159 (37)
Urinary catheter[2]	238 (56)
Central venous catheter[1]	124 (29)
Other indwelling device[1,2]	163 (38)
Decubitus ulcer	156 (36)
Dementia	108 (25)
Hemi- or paraplegia	64 (15)
Underlying urinary tract abnormalities	66 (15)
Patient residence 4 days prior to culture (n = 422)	
Inpatient	82 (19)
LTCF or LTACH	206 (49)
Private residence	134 (32)
Location where culture was obtained (n = 427)	
Inpatient	149 (35)
LTCF or LTACH	143 (33)
Outpatient	135 (32)
ICU prior to the culture[3] (n = 418)	54 (13)

All values are presented as number (%) unless otherwise stated

1. At the time culture was obtained or in the prior 2 calendar days
2. Endotracheal tube, gastrostomy tube, nasogastric tube, tracheostomy, or nephrostomy tube
3. Any time in the 7 calendar days prior to the culture

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae; SD, standard deviation; LTCF, long-term care facility; LTACH, long-term acute care hospital; ICU, intensive care unit

Table 3: Demographics and Clinical Characteristics of Patients with CRE Bacteriuria Stratified by the Presence of a Urinary Catheter

Variable	No Urinary Catheter (n = 190)	Urinary Catheter[1] (n = 238)	P-value[2]
Age (mean years [SD])	66.8 (16.3)	62.7 (17.3)	0.02
Female	113 (59)	121 (51)	0.07
Race (n = 407)			0.2
Black	106 (59)	152 (67)	
White	67 (37)	70 (31)	
Other	7 (4)	5 (2)	
Charlson comorbidity index >3 (n = 425)	74 (40)	85 (36)	0.41
Central venous catheter[1]	32 (17)	92 (39)	<0.001
Other indwelling device[1,3]	47 (25)	116 (49)	<0.001
Decubitus ulcer	48 (25)	108 (45)	<0.001
Dementia	61 (32)	47 (20)	0.004
Hemi- or paraplegia	20 (11)	44 (18)	0.02
Underlying urinary tract abnormalities	20 (11)	46 (19)	0.01
Patient residence 4 days prior to culture (n = 422)			<0.001
Inpatient	24 (13)	58 (24)	
LTCF or LTACH	85 (46)	121 (51)	
Private residence	76 (41)	58 (24)	
Location where culture was obtained (n = 427)			0.01
Inpatient	53 (28)	96 (41)	
LTCF or LTACH	65 (34)	78 (33)	
Outpatient	72 (38)	63 (27)	
ICU prior to the culture[4] (n = 418)	7 (4)	47 (20)	<0.001

All values are presented as number (%) unless otherwise stated

1. At the time culture was obtained or in the prior 2 calendar days
2. Comparison of patients with and without a urinary catheter. Categorical variables were analyzed by Chi-square tests and continuous variables were analyzed by Student's t-tests
3. Endotracheal tube, gastrostomy tube, nasogastric tube, tracheostomy, or nephrostomy tube
4. Any time in the 7 calendar days prior to the culture

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae; SD, standard deviation; LTCF, long-term care facility; LTACH, long-term acute care hospital; ICU, intensive care unit

Table 4: Univariable Logistic Regression Analysis Assessing Risk Factors for Progression from CRE Bacteriuria to an Invasive CRE Infection

Variable	No Progression (n = 403)	Progression (n = 25)	Absolute Risk Increase[1]	Univariable OR (95% CI)	P-value
Age (mean [SD])	64.6 (16.8)	62.9 (19.6)	N/A	0.99 (0.97 – 1.0)	0.62
Female	221 (55)	13 (52)	-0.6%	0.9 (0.4 – 2)	0.78
Race (n = 407)					
Black	237 (62)	21 (84)	5.5%	3.2 (1.1 – 9.5)[2]	0.04
White	133 (35)	4 (16)	Ref	Ref	
Other	12 (3)	0 (0)	Ref	Ref	
CCI >3 (n = 425)	144 (36)	15 (60)	5.7%	2.7 (1.2- 6.1)	0.02
Urinary Catheter[3]	216 (54)	22 (88)	7.6%	6.3 (1.9 – 21.5)	0.003
Central venous catheter[3]	109 (27)	15 (60)	8.8%	4.0 (1.8 – 9.3)	0.001
Other indwelling device[3,4]	147 (36)	16 (64)	6.4%	3.1 (1.3 – 7.2)	0.01
Decubitus ulcer	142 (35)	14 (56)	4.9%	2.3 (1.0 - 5.3)	0.04
Dementia	104 (26)	4 (16)	-2.9%	0.5 (0.2 – 1.6)	0.3
Hemi- or paraplegia	60 (15)	4 (16)	0.5%	1.1 (0.4 – 3.3)	0.88
Underlying urinary tract abnormalities	64 (16)	2 (8)	-3.3%	0.5 (0.1 – 2.0)	0.3
Patient residence 4 days prior to culture (n = 422)					0.43
Inpatient	76 (19)	6 (24)	3.6%	2.0 (0.6 – 6.9)	0.25
LTCF or LTACH	192 (48)	14 (56)	3.1%	1.9 (0.7 – 5.4)	0.24
Private residence	129 (32)	5 (20)	Ref	Ref	
Location where culture was obtained (n = 427)					0.09
Inpatient	136 (34)	13 (52)	6.5%	4.2 (1.2 -15.1)	0.03
LTCF or LTACH	134 (33)	9 (36)	4.1%	3.0 (0.8 -11.2)	0.11
Outpatient	132 (33)	3 (12)	Ref	Ref	
ICU prior to the culture[5] (n = 418)	48 (12)	6 (24)	5.9%	2.3 (0.9 – 6.0)	0.10

All values are presented as number (%) unless otherwise stated

1. Difference in risk of progression between those with the variable of interest and those without the variable of interest

2. Odds ratio was calculated for black race versus any other race
3. At the time culture was obtained or in the prior 2 calendar days
4. Endotracheal tube, gastrostomy tube, nasogastric tube, tracheostomy, or nephrostomy tube
5. Any time in the 7 calendar days prior to the culture

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae; OR, odds ratio; CI, confidence interval; SD, standard deviation; Ref, reference; CCI, Charlson comorbidity index; LTCF, long term care facility; LTACH, long term acute care hospital; ICU, intensive care unit

Table 5: Multivariable Models Estimating the Association Between Urinary Catheter and Progression to an Invasive Infection in Patients with CRE Bacteriuria

Variable	Model 1 aOR (95% CI)	Model 2 aOR (95% CI)	Model 3 aOR (95% CI)	Model 4 aOR (95% CI)
Urinary catheter[1]	**6.3 (1.9–21.6)**	**4.1 (1.2–14.7)**	**4.2 (1.2–14.9)**	**4.1 (1.1–14.5)**
Sex	1.0 (0.4–2.3)	--	--	--
Age	1.0 (0.98–1.0)	--	--	--
Central venous catheter[1]	--	2.1 (0.8–5.6)	2.2 (0.8–5.9)	2.1 (0.8–5.6)
Other indwelling device[1,2]	--	1.3 (0.5–3.5)	1.5 (0.5–4.1)	1.4 (0.5–3.7)
Location where culture was obtained				
Inpatient	--	2.7 (0.7–10.6)	2.7 (0.7–10.5)	2.4 (0.6–9.0)
LTCF or LTACH	--	2.3 (0.6–9.2)	1.9 (0.5–7.4)	1.9 (0.5–7.6)
Outpatient	--	Ref	Ref	Ref
Decubitus ulcer	--	1.5 (0.7–3.7)	1.6 (0.7–3.8)	1.6 (0.7–3.8)
ICU prior to the culture[3]	--	0.7 (0.2–2.4)	0.7 (0.2–2.3)	--
Klebsiella pneumoniae in culture[4]	--	7.9 (1.0–60.5)	--	--

1. At the time culture was obtained or in the prior 2 calendar days
2. Endotracheal tube, gastrostomy tube, nasogastric tube, tracheostomy, or nephrostomy tube
3. Any time in the 7 calendar days prior to the culture
4. Compared to patients with other CRE organisms (*E. coli*, *Klebsiella oxytoca*, *Klebsiella aerogenes, and Enterobacter cloacae*)

Abbreviations: aOR, adjusted odds ratio; CI, confidence interval; Ref, reference; LTCF, long-term care facility; LTACH, long-term acute care hospital; ICU, intensive care unit

Table 6: Pulsed-Field Gel Electrophoresis and Whole Genome Sequencing Results on Paired Urine and Sterile Site CRE isolates in Patients with Progression to an Invasive Infection in Patients with CRE Bacteriuria

Patient	Organism	Time to Progression (days)	Percent Similarity on PFGE	Core-Genome SNPs	Highly Related Strain
1	*Klebsiella pneumoniae*	22	100%	3	Yes
2	*Klebsiella pneumoniae*	25	91%	7	Yes
3	*Klebsiella pneumoniae*	27	100%	12	Yes
4	*Klebsiella pneumoniae*	31	100%	18	Yes
5	*Klebsiella pneumoniae*	35	100%	9	Yes
6	*Klebsiella pneumoniae*	101	75%	27,369	No
7	*Escherichia coli*	118	100%	9	Yes
8	*Klebsiella pneumoniae*	300	91%	776	No

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae; PFGE, pulsed-field gel electrophoresis; SNP, single-nucleotide polymorphism

Figure 1: Definitions of CRE

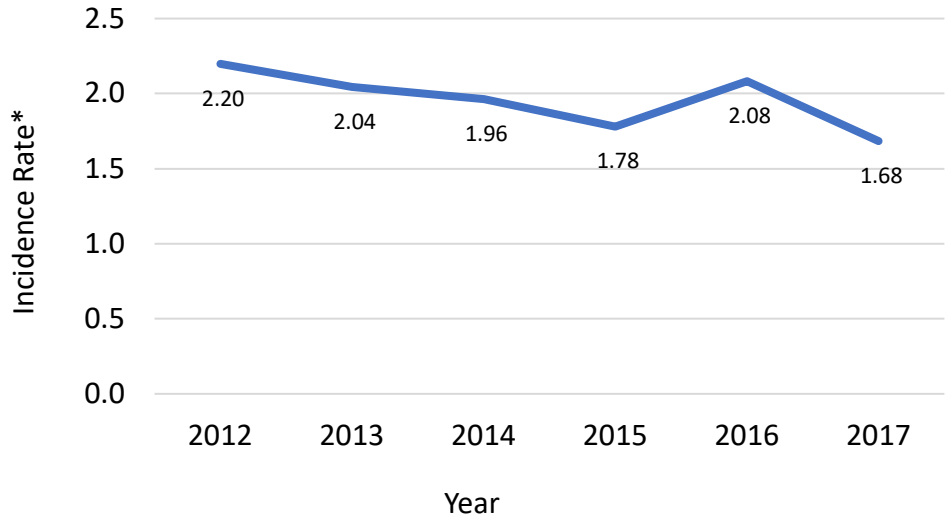
Definition	Period	Species	Carbapenem Susceptibility Phenotype
Initial CDC surveillance definition	2011 - 2015	*Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Enterobacter cloacae* *Klebsiella aerogenes*[1]	Intermediate or resistant to: - Imipenem (MIC ≥2 μg/mL), or - Meropenem (MIC ≥2 μg/mL), or - Doripenem (MIC ≥2 μg/mL) AND resistant to: - Ceftazidime (MIC ≥16 μg/mL), and - Ceftriaxone (MIC ≥4 μg/mL), and - Cefotaxime (MIC ≥4 μg/mL)
Revised CDC surveillance definition	2016 - present	*Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Enterobacter cloacae* *Klebsiella aerogenes*[1]	Resistant to: - Imipenem (MIC ≥4 μg/mL), or - Meropenem (MIC ≥4 μg/mL), or - Doripenem(MIC ≥4 μg/mL), or - Ertapenem (MIC ≥2 μg/mL)
Present Study	2011 - 2017	*Escherichia coli* *Klebsiella pneumoniae* *Klebsiella oxytoca* *Enterobacter cloacae* *Klebsiella aerogenes*[1]	Resistant to: - Imipenem (MIC ≥4 μg/mL), or - Meropenem (MIC ≥4 μg/mL), or - Doripenem(MIC ≥4 μg/mL), or AND resistant to: - Ceftazidime (MIC ≥16 μg/mL), and - Ceftriaxone (MIC ≥4 μg/mL), and - Cefotaxime (MIC ≥4 μg/mL)

Figure 1 presents the different phenotypic definitions of CRE that were used for surveillance from 2011 – 2015 and then from 2016 onward. We created a study definition for CRE that unified the study cohort under one definition and allowed us to make comparisons across the entire study period.

1. Formerly *Enterobacter aerogenes*

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae; CDC, Centers for Disease Control and Prevention; MIC, minimum inhibitory concentration

Figure 2: Annual Incidence Rates of CRE Bacteriuria in Metropolitan Atlanta, 2012-2107



*Cases per 100,000 population

Figure 2 displays the trend in annual incidence rates of CRE bacteriuria from 2012 to 2017 in Metropolitan Atlanta using surveillance data from the Georgia Emerging Infections Program.

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae

Figure 3: Differences in Time to Progression from Bacteriuria to an Invasive Infection between Patients with Different and Highly Related CRE Strains

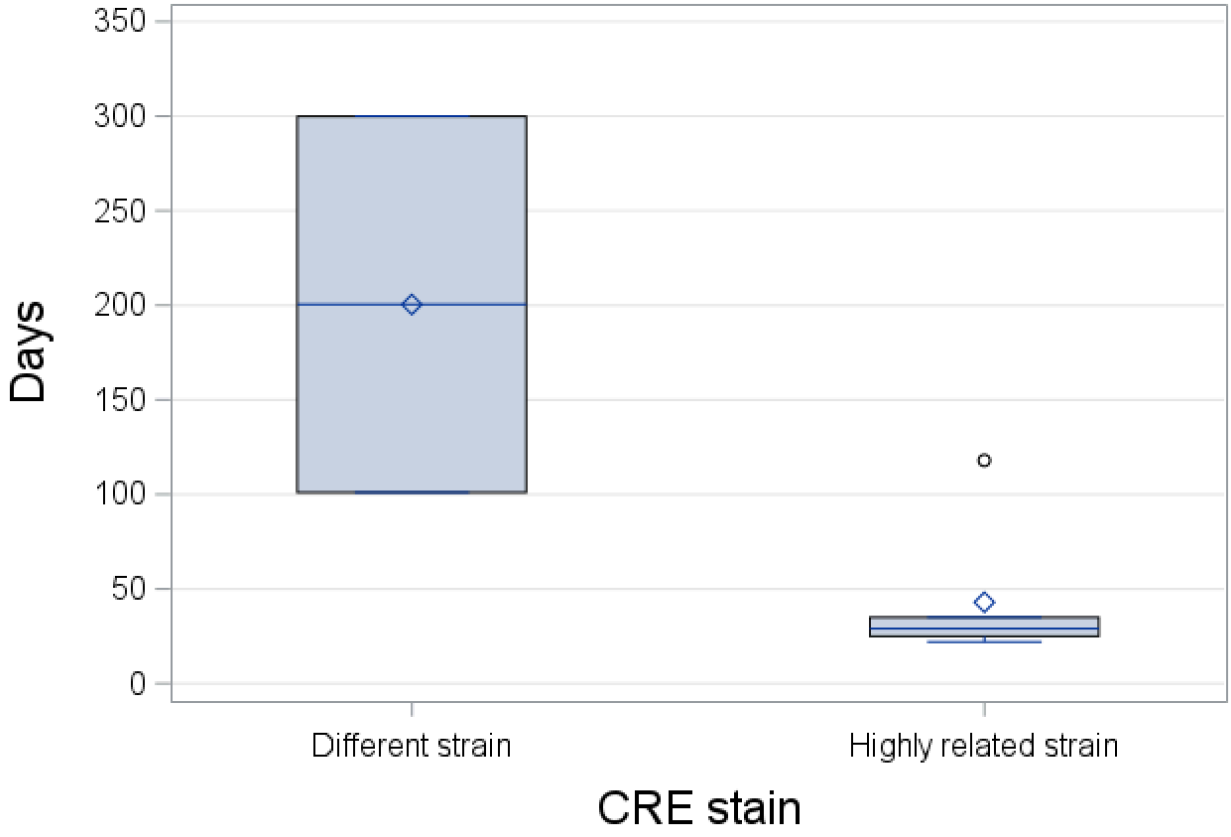


Figure 3 is a box and whisker plot that displays the difference in progression time between patients that had different (n =2) and highly related (n = 6) CRE strains in the paired urine and sterile site isolates.

Abbreviations: CRE, carbapenem-resistant Enterobacteriaceae