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Surveillance of Carbapenem-Resistant *Klebsiella pneumoniae*: Tracking Molecular Epidemiology and Outcomes through a Regional Network

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Carbapenem resistance in Gram-negative bacteria is on the rise in the United States. A regional network was established to study microbiological and genetic determinants of clinical outcomes in hospitalized patients with carbapenem-resistant (CR) *Klebsiella pneumoniae* in a prospective, multicenter, observational study. To this end, predefined clinical characteristics and outcomes were recorded and *K. pneumoniae* isolates were analyzed for strain typing and resistance mechanism determination. In a 14-month period, 251 patients were included. While most of the patients were admitted from long-term care settings, 28% of them were admitted from home. Hospitalizations were prolonged and complicated. Nonsusceptibility to colistin and tigecycline occurred in isolates from 7 and 45% of the patients, respectively. Most of the CR *K. pneumoniae* isolates belonged to repetitive extragenic palindromic PCR (rep-PCR) types A and B (both sequence type 258) and carried either bla_{KPC-2} (48%) or bla_{KPC-3} (51%). One isolate tested positive for bla_{NDM-1}, a sentinel discovery in this region. Important differences between strain types were noted; rep-PCR type B strains were associated with bla_{KPC-3} (odds ratio [OR], 294; 95% confidence interval [CI], 58 to 2,552; $P < 0.001$), gentamicin nonsusceptibility (OR, 24; 95% CI, 8.39 to 79.38; $P < 0.001$), amikacin susceptibility (OR, 11.0; 95% CI, 3.21 to 42.42; $P < 0.001$), tigecycline nonsusceptibility (OR, 5.34; 95% CI, 1.30 to 36.41; $P = 0.018$), a shorter length of stay (OR, 0.98; 95% CI, 0.95 to 1.00; $P = 0.043$), and admission from a skilled-nursing facility (OR, 3.09; 95% CI, 1.26 to 8.08; $P = 0.013$). Our analysis shows that (i) CR *K. pneumoniae* is seen primarily in the elderly long-term care population and that (ii) regional monitoring of CR *K. pneumoniae* reveals insights into molecular characteristics. This work highlights the crucial role of ongoing surveillance of carbapenem resistance determinants.

Antimicrobial resistance is "one of the greatest threats to human health worldwide" (1). The additional annual cost associated with treating antimicrobial-resistant pathogens in the United States is estimated to be between $21 billion and $34 billion (1). Carbapenem-resistant (CR) *Klebsiella pneumoniae* infections are especially worrisome, as very few treatment options remain for patients and outcomes are generally poor.

Central to the control of the problem of CR *K. pneumoniae* is the institution of effective regional surveillance networks. To address this, we formed a multicenter consortium to prospectively study the epidemiology, molecular characteristics, and clinical outcomes of infections due to CR *K. pneumoniae* in hospitalized patients in northeastern Ohio. Here, we report the initial findings of this consortium and describe the genomic analysis of a bla_{NDM-1}-carrying strain that was discovered as a result of organized surveillance.

MATERIALS AND METHODS

Establishment of the Great Lakes CR *K. pneumoniae* consortium. A multicenter consortium was established that consists of three health care systems that include 18 hospitals serving more than 2 million people in northeastern Ohio. These included both community-based hospitals and tertiary-care referral centers ranging in size from 25 to >1,400 beds. Each health care system participating in this study is served by one or more clinical microbiology laboratories that process an average of 200,000 bacterial cultures from clinical samples yearly. None of the hospitals performed screening for asymptomatic carriage of CR *K. pneumoniae* during the study period.

Microbiology. We defined CR *K. pneumoniae* as isolates with decreased susceptibility to meropenem or imipenem (MIC, >1 μg/ml). Bacterial identification to the species level and routine antimicrobial susceptibility testing were performed with the MicroScan (Siemens Healthcare Diagnostics) or Vitek2 (bioMérieux) automated system supplemented by additional testing if required. In this study, MIC results were interpreted according to breakpoints defined by the Clinical and Laboratory Standards Institute (CLSI), with the exception of tigecycline and colistin, where criteria set forth by the European Committee on Antimicrobial Susceptibility Testing (EUCAST) were used because of a lack of

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validated CLSI breakpoints. At each site, study personnel were notified by the local microbiology laboratory upon the identification of a CR *K. pneumoniae* isolate from a hospitalized patient. Clinical data were obtained from the medical record and entered into a centralized clinical database. The Institutional Review Boards of all of the health care systems involved approved this study.

Patients. All of the hospitalized patients from whom CR *K. pneumoniae* was isolated were included if their hospitalization began and ended within the study period, which lasted from 12/24/2011 until 3/1/2013. All of the analyses are based on the first culture positive for CR *K. pneumoniae* during the index hospitalization of each individual patient.

Definitions of infection. A patient with a culture positive for CR *K. pneumoniae* was deemed to have an infection if CR *K. pneumoniae* was isolated from blood or any other sterile source. For patients with positive respiratory cultures, the criteria outlined by the American Thoracic Society and the Infectious Diseases Society of America were used (2, 3). For patients with positive cultures from urine or surgical wounds, the CDC National Healthcare Safety Network criteria were applied (4). Patients with cultures from nonsurgical wounds were considered infected only if the treating physician documented infection in the medical record and evidence of systemic inflammation on the day the positive culture was documented; this was defined as an abnormal systemic white cell count (either $>10 \times 10^3$ or $<4 \times 10^3$ cells/μl) and/or an abnormal body temperature (either >99.5°F or <96°F). All of the other culture episodes were designated colonizations.

Clinical data collection and definitions. Clinical data collection included the following: demographics, prehospital origin and posthospital disposition, comorbidities, including the Charlson comorbidity index (5), CR *K. pneumoniae* treatment variables, previous antibiotic use, and culture site-specific variables. Previous antibiotic use was recorded as the class of antibiotic with documented exposure in the 14 days leading up to the day of the first positive culture. Infections were defined as nosocomial if the first CR *K. pneumoniae*-positive culture was obtained >48 h after hospital admission. Health care-associated infections were defined as infections in patients admitted from long-term care facilities or other hospitals who had a first CR *K. pneumoniae*-positive culture within the first 48 h. Patients with a first CR *K. pneumoniae*-positive culture within 48 h who were admitted from home were deemed to have community-acquired infections. Renal insufficiency was defined as any serum creatinine level of ≥2 mg/dl. The Pitt bacteremia score (PBS) was calculated as described previously (6). If patients were intubated and sedated and no further information on mental status was available, the mental status was coded as "stuporous" for the purpose of calculating this score only. This score has been validated as a predictor of the outcome of CR *K. pneumoniae* bloodstream infection (7).

Molecular detection of carbapenemases in CR *K. pneumoniae*. All of the available CR *K. pneumoniae* isolates were sent to a central research laboratory in this consortium for further testing. PCR amplification of the bla_{KPC}, bla_{NDM} bla_{VIM}, bla_{IMP}, and bla_{OXA-48} genes was performed with established primers and methods (8, 9). Amplicons were sequenced at a commercial sequencing facility (MCLAB, San Francisco, CA) and analyzed as previously described (8, 9).

Molecular typing of CR *K. pneumoniae*. Genetic similarity among isolates was investigated by repetitive extragenic palindromic PCR (rep-PCR) by using the DiversiLab strain typing system (Bacterial BarCodes, bioMérieux, Athens, GA). Isolates with ≥95% similarity were considered to be of the same rep-PCR type. Rep-PCR has previously been shown to have adequate discriminatory power for CR *K. pneumoniae* strain typing (10). Multilocus sequence typing (MLST) of the CR *K. pneumoniae* isolates of the predominant rep-PCR types was performed as described previously (11). The sequences of seven housekeeping genes (*rpoB*, *gapA*, *mdh*, *pgi*, *phoE*, *infB* and *tonB*) were compared to the MLST database (http://www.pasteur.fr/recherche/genopole/PF8/mlst).

Genome sequencing. To generate a finished accurate CR *K. pneumoniae* bla_{NDM-1} genome, independent *de novo* assemblies were generated from sequence data from Pacific Biosciences and Illumina instruments. CR *K. pneumoniae* bla_{NDM-1} genomic DNA was sheared, SMRTbell libraries were generated, XL polymerase-bound complexes were loaded onto SMRT cells, and sequencing was performed with the RSII system as suggested by the manufacturer (Pacific Biosciences, Menlo Park, CA). Assemblies were generated with the Celera Assembler and then postprocessed with Quiver for realignment and polishing as described previously (12). In brief, we utilized the suggested hierarchical genome assembly process, which consisted of (i) selecting long sequencing reads as seed data, (ii) using seed sequences as references to recruit consensus shorter reads, (iii) assembling long seed data and short reads with the Celera Assembler adapted for SMRT sequencing data, (iv) polishing the assembly with Quiver to generate consensus sequences, and (v) manually trimming overlapping identical end regions to create circular finished chromosomes and plasmids.

For independent sequence comparison, a Nextera library was generated from the same genomic DNA and sequenced to an ~100× depth on an Illumina MiSeq instrument; this was followed by assembly with MIRA as described previously (13). Draft annotation was performed with the RAST pipeline and finalized with PGAP.

Analysis. Univariate logistic regression was used to estimate associations between CR *K. pneumoniae* strain types and microbiological and clinical variables. Cox proportional hazard modeling was used to estimate associations between potential predictors and time to death in the hospital. The time in days from the first positive culture to death in the hospital was displayed in a Kaplan-Meier plot. A log rank test was used to compare groups. Patients were censored at the time of discharge or on postculture day 30 if still in the hospital and alive. All of the analyses were performed with JMP software (SAS Inc., Cary, NC).

Nucleotide sequence accession numbers. Sequences have been deposited in GenBank, and the accession numbers can be found via NCBI BioSample record number 2777842.

RESULTS

Patients. During the study period, 251 individual patients were included (Table 1 and Fig. 1). Patients were generally elderly (median age, 70 years; interquartile range [IQR], 58 to 81 years) and had several comorbid conditions (median Charlson score, 4; IQR, 2 to 6). Of note, 71 patients (28%) were admitted from home, whereas the remainder came from skilled-nursing facilities (SNFs) (49%) or long-term acute-care facilities (10%) or were transferred from other hospitals (13%).

CR *K. pneumoniae* infection and colonization. Urine (63%) was the predominant anatomic source of CR *K. pneumoniae* (Fig. 2), followed by respiratory (12%), wound (11%), blood (10%), and other (4%) samples. Patients culture sources grouped as "other" included abscess ($n = 2$), vascular catheter tip ($n = 3$), ascites ($n = 2$), bile ($n = 2$), and stool ($n = 1$). Antibiotic use prior to the first positive culture was common; only 29% had no documented antibiotic exposure in the preceding 14 days. CR *K. pneumoniae* infection was present in 45% of the patients. Of patients with CR *K. pneumoniae* infections, 43/114 (38%) had nosocomial infections, 48/114 (42%) had health care-associated infections, and 23 (20%) had community-acquired infections.

Most (74%) of the patients with CR *K. pneumoniae* colonizations had urinary tract colonizations, and half of the patients with infections had urinary tract infections. The use of a Foley catheter was documented in 53 and 39% of the patients with CR *K. pneumoniae* urinary tract colonizations and infections, respectively.

The antibiotic susceptibilities determined are summarized in Fig. 2. Colistin susceptibilities were determined for the first isolates of 114 patients; of these, 8/114 (7%) were nonsusceptible. In contrast, tigecycline nonsusceptibility was more common; 83/182 (45%) isolates tested were nonsusceptible to tigecycline. However,

TABLE 1 Demographic characteristics of the patients in this study

Patient characteristic	All	Infected	Colonized
Total no.	251	114	137
Median age, yr (IQR)	70 (58–81)	67 (54–81)	73 (64.5–83)
No. (%) female	150 (60)	67 (59)	83 (61)
No. (%) who were:			
White, non-Hispanic	145 (58)	63 (55)	82 (60)
Black, non-Hispanic	95 (38)	49 (43)	46 (34)
Hispanic	7 (3)	2 (2)	5 (4)
Other	4 (2)	0	4 (3)
No. (%) with:			
Diabetes mellitus	140 (56)	66 (58)	74 (54)
Heart disease[a]	142 (57)	62 (55)	80 (58)
Renal insufficiency	65 (26)	31 (27)	34 (25)
COPD[b]	73 (29)	30 (27)	43 (31)
Malignancy	36 (14)	18 (16)	18 (13)
Dementia	54 (22)	22 (19)	32 (23)
Immunocompromise	25 (10)	14 (12)	11 (8)
Prednisone therapy[c]	12 (5)	6 (5)	6 (4)
Solid-organ transplant	10 (4)	5 (4)	5 (4)
HIV infection	2 (1)	2 (2)	0
Hematopoietic stem cell transplant	1 (0)	1 (1)	0
Median Charlson comorbidity index (IQR)	4 (2–6)	4 (2–6)	4 (2–6)
No. (%) from:			
SNF	123 (49)	50 (44)	73 (53)
Home	71 (28)	34 (30)	37 (27)
Another hospital	32 (13)	20 (18)	12 (8)
Long-term acute-care facility	25 (10)	10 (9)	15 (11)

[a] Coronary artery disease and/or congestive heart failure.
[b] COPD, chronic obstructive pulmonary disease.
[c] Prednisone or equivalent corticosteroid at 10 mg/day.

for most of these isolates (56/182, 31%) the MIC was 2 μg/ml (intermediate susceptibility). Three isolates that were colistin nonsusceptible were also nonsusceptible to tigecycline (intermediate, $n = 1$; resistant, $n = 2$).

Outcomes. The outcomes of the index hospitalizations are summarized in Table 2. Hospital stays were prolonged (median length of stay [LOS], 9 days; IQR, 5 to 17 days) and complicated. About half of the patients (51%) were admitted to the intensive care unit (ICU), and the median ICU LOS in those admissions was 6 days (IQR, 3 to 15 days). Most of the patients were discharged to SNFs (43%), and only 17% of the patients were discharged home. Of the 71 patients who were admitted from home, only 48% could be discharged home. Thirty-nine patients died, and an additional six were discharged to hospice care. In patients with CR *K. pneumoniae* infections versus colonizations, the hospital (including hospice care) mortality rate was 23% versus 14%.

Of the 26 patients with bacteremia, 21 (81%) received either monotherapy (tigecycline [$n = 4$], colistin [$n = 4$], aminoglycosides [$n = 3$], or trimethoprim-sulfamethoxazole [$n = 1$]) or combination therapy (tigecycline-gentamicin [$n = 5$], amikacin-colistin [$n = 3$], or tigecycline-colistin [$n = 1$]). Five patients with bloodstream infections died before antibiograms were available and did not receive antibacterial therapy with activity against CR *K. pneumoniae*.

Antibacterial therapy active against CR *K. pneumoniae* was used in 65% of the patients with urinary tract infections and 60% of the patients with other sources of infection. Of note, 46% of the patients with urinary colonization were also treated with agents active against CR *K. pneumoniae*.

In patients with CR *K. pneumoniae* infection ($n = 114$), the source of infection was associated with the time to death in the hospital within 30 days of admission (Fig. 3). Variables associated with the time to death in the hospital in an age-corrected Cox proportional hazard model included severe acute disease, as defined by a PBS of ≥4 (hazard ratio [HR], 3.82; 95% CI, 1.49 to 11.11; $P = 0.005$) and a nonurinary source of infection (HR, 2.7; 95% CI, 1.04 to 8.49; $P = 0.040$). Receipt of antibiotics with *in vitro* susceptibility within 48 h of a positive culture was not associated with improved survival in this analysis (HR, 1.46; 95% CI, 0.41 to 4.13; $P = 0.52$).

Molecular strain typing and mechanisms of resistance. Molecular strain typing by rep-PCR was performed for all of the 113 patients whose CR *K. pneumoniae* isolates were available, i.e., 52

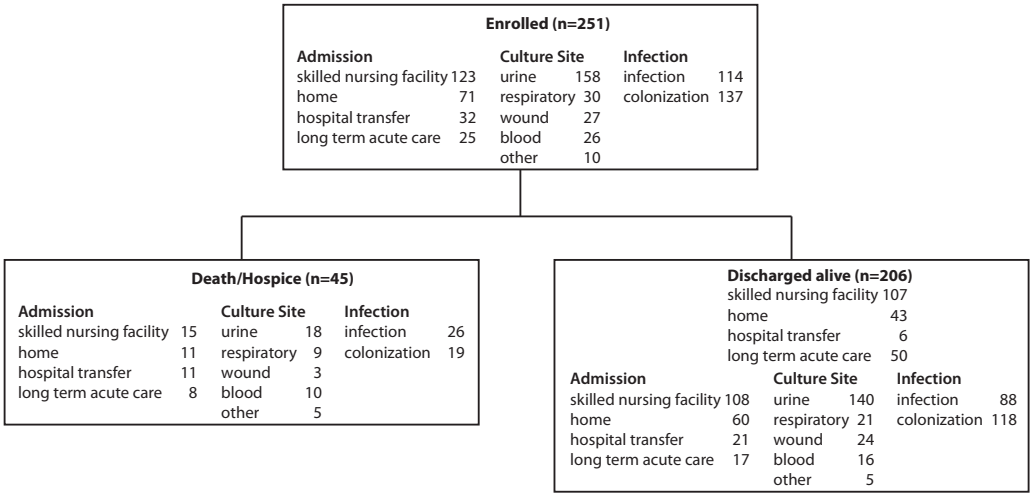


FIG 1 Flow diagram outlining the characteristics of the patients in this study. In each box, the origin of admission for the index hospitalization, the first culture site, and whether the first culture represented infection or colonization are shown.

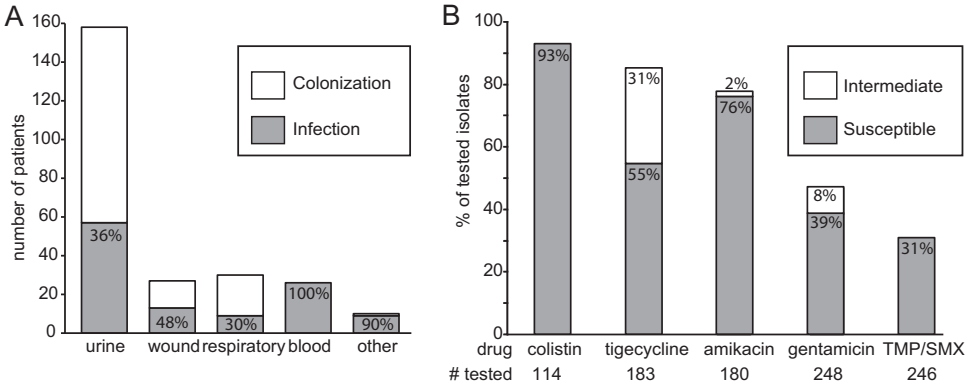


FIG 2 (A) Numbers of colonizations and infections per anatomical site of CR *K. pneumoniae*-positive cultures. Percentages are the proportions of patient with infections. (B) Selected antimicrobial susceptibility rates. Note that breakpoints defined by the CLSI were used for aminoglycosides and criteria set forth by the EUCAST were used for colistin and tigecycline. TMP/SMX, trimethoprim-sulfamethoxazole.

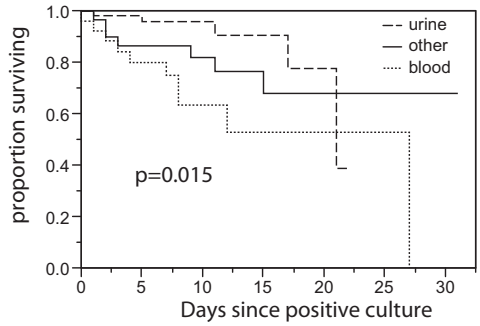
patients with CR *K. pneumoniae* colonizations and 61 patients with CR *K. pneumoniae* infections. The two most common types were A (36/113, 29%) and B (52/113, 46%), which were both identified as belonging to MLST sequence type 258 (ST258). The remaining 22 isolates (19%) represented 16 different rep-PCR types, including strains belonging to ST15, ST17, ST299, and ST395.

Carbapenemase genes were detected in isolates from 109/113 patients, while none of the carbapenemase genes tested (bla_{KPC}, bla_{NDM} bla_{VIM}, bla_{IMP}, and bla_{OXA-48}) were detected in the four isolates tested. Furthermore, these four isolates did not phenotypically express a carbapenemase, according to a modified Hodge test (data not shown); the carbapenem resistance of these isolates is likely due to a combination of extended-spectrum beta-lactamases (ESBLs) and changes in outer membrane proteins (14). All but one of the carbapenemase-harboring isolates carried either bla_{KPC-2} (51/109, 48%) or bla_{KPC-3} (57/109, 51%).

TABLE 2 Outcome of first admission per patient[a]

Patient characteristic	All	Infected	Colonized	*P* value[b]
Total no.	251	114	137	
No. (%) at following location at time of culture:				0.48
Emergency department	66 (26)	26 (23)	40 (29)	
Ward	108 (43)	50 (44)	58 (42)	
ICU	77 (31)	38 (33)	39 (28)	
Median PBS (IQR)	2 (1–4)	2 (1–4)	2 (0–4)	0.10
Median no. of days to first positive culture (IQR)	1 (0–4)	1 (0–6)	0 (0–4)	0.08
No. (%) with CR *K. pneumoniae* isolated:				
Within first 24 h	124 (49)	54 (47)	70 (51)	
On days 2–5	69 (27)	29 (25)	40 (29)	
After day 5	58 (23)	31 (27)	27 (20)	
Median LOS, days (IQR)	9 (5–17)	10 (6–20)	8 (5–15)	0.11
No. (%) with ICU admission	129 (51)	65 (57)	64 (47)	0.13
Median no. of days in ICU (IQR)[a]	6 (3–15)	7 (3.5–15.5)	6 (3–15)	0.31
No. (%) with:				
Surgery	60 (24)	38 (33)	22 (16)	<0.01
Renal insufficiency	91 (36)	43 (38)	48 (35)	0.69
Renal replacement therapy	46 (18)	24 (21)	22 (16)	0.33
Mechanical ventilation	85 (34)	47 (41)	38 (28)	0.03
Tracheostomy	39 (16)	17 (15)	22 (16)	0.86
No. (%) with following disposition:				0.05
Death/hospice	45 (18)	26 (23)	19 (14)	
Home	43 (17)	19 (17)	24 (18)	
SNF	107 (43)	39 (35)	68 (50)	
Other	56 (22)	30 (26)	26 (19)	

[a] For patients with any ICU admission.
[b] Fisher's exact test for binomial variables, *t* test for normally distributed continuous variables, and likelihood ratio test for distributions.



Number at risk

urine	57	43	20	13	3	0	0
other	31	25	16	9	8	5	3
blood	26	17	9	6	4	2	0

FIG 3 Kaplan-Meier survival curves showing times from the first positive culture to death in the hospital for patients with CR *K. pneumoniae* infections by source of infection, censoring at 30 days, or time of discharge, whichever occurred first ($P = 0.015$ by log rank test).

The remaining carbapenemase-harboring isolate tested positive for bla_{NDM-1}, a sentinel finding in this geographic region. The patient in whom this bla_{NDM-1}-positive CR *K. pneumoniae* strain was found was a male from Egypt who was admitted for cardiac surgery. Nine days postoperatively, he developed abdominal pain, turbid urine, a fever to 38.7°C, and leukocytosis. Urine analysis showed more than 25 white cells per high-power field, and his urine culture grew CR *K. pneumoniae*, as well as an ESBL-producing *Escherichia coli* strain that was susceptible to carbapenems. He was treated with a single dose of gentamicin and 7 days of meropenem. His symptoms resolved, and he was discharged 8 days after his first positive CR *K. pneumoniae* culture. He did not have a readmission during the study period. No other CR *K. pneumoniae* isolates obtained during the study period tested positive for bla_{NDM-1}, suggesting that no transmission to other patients occurred (see below).

As ST258 is the most common CR *K. pneumoniae* sequence type worldwide, we further explored clinical and microbiological differences between the A and B types identified by rep-PCR (Table 3). Antibiotic susceptibility patterns were significantly associated with rep-PCR strain types; type A CR *K. pneumoniae* was more likely than type B to be tigecycline (odds ratio [OR], 5.34; 95% CI, 1.30 to 36.41; $P = 0.018$) and gentamicin (OR, 24.11; 95%

TABLE 3 Comparison of patients with rep-PCR type A and B strains

Patient characteristic	A	B	OR[a] (95% CI)	*P* value[b]
Total no.	39	52		
No. (%) with:				
Any carbapenemase	39 (100)	51 (98)		
KPC-2	36 (92)	2 (4)	294.00 (58.16–2552.68)	<0.001
KPC-3	3 (7)	49 (96)	0.00 (0.00–0.02)	<0.001
No. (%) with isolate from:				0.135
Urine	19 (49)	36 (69)		
Blood	11 (28)	8 (15)	2.61 (0.90–7.81)	
Other	9 (23)	8 (15)	2.13 (0.70–6.57)	
No. (%) infected	22 (56)	27 (51)	1.20 (0.52–2.78)	0.67
No. with susceptible isolates/no. with isolates tested (%)				
Colistin	32/35 (91)	37/39 (95)	0.57 (0.07–3.69)	0.66
Tigecycline	23/25 (92)	28/41 (68)	5.34 (1.30–36.41)	0.018
Amikacin	7/18 (39)	42/48 (88)	0.09 (0.02–0.31)	<0.001
Gentamicin	30/38 (79)	7/52 (13)	24.11 (8.39–79.38)	<0.001
Trimethoprim-sulfamethoxazole	9/36 (25)	22/52 (42)	0.45 (0.17–1.13)	0.091
No. (%) from:				
SNFs	9 (23)	25 (48)		0.043
Home	15 (38)	15 (29)	2.78 (0.99–8.16)	
Other	15 (38)	12 (23)	3.47 (1.21–10.55)	
No. (%) with strain present at admission	14 (36)	28 (54)	0.48 (0.20–1.11)	0.088
Median LOS (IQR)	12 (6–32)	8 (5–19.75)	1.02[c] (1.00–1.04)	0.043
No. (%) with following disposition:				
Death/hospice	11 (28)	10 (19)		0.72
Home	7 (18)	13 (25)	0.49 (0.13–1.69)	
SNF	15 (38)	20 (38)	0.68 (0.22–2.02)	
Other	6 (15)	9 (17)	0.61 (0.15–2.29)	

[a] OR for A versus B.
[b] Univariate logistic regression.
[c] OR per day increase in LOS.

CI, 8.39 to 79.38; $P < 0.001$) susceptible and less likely to be susceptible to amikacin (OR, 0.09; 95% CI, 0.02 to 0.31; $P < 0.001$). bla_{KPC} was present in all of the type A and in all but one of the type B CR *K. pneumoniae* isolates. KPC-2 and KPC-3 were the two carbapenemases found. A significant association between the rep-PCR type and the KPC enzyme found was also discovered; type A CR *K. pneumoniae* isolates were associated with KPC-2, whereas type B isolates were associated with KPC-3 (OR, 294.00; 95% CI, 58.16 to 2552.68; $P < 0.001$).

On clinical grounds, patients with type B CR *K. pneumoniae* were more likely to be admitted from an SNF (OR, 3.09; 95% CI, 1.26 to 8.08; $P = 0.013$) and to have a shorter LOS (OR, 0.98/day increase; 95% CI, 0.95 to 1.00; $P = 0.043$) than patients with type A CR *K. pneumoniae*.

Genome sequencing of bla_{NDM-1}-producing strain. As the isolation of a bla_{NDM-1}-producing CR *K. pneumoniae* strain represented a "sentinel event" in our community, the bacterial genome of this strain was sequenced in order to provide a reference sequence for the development of clinical diagnostics for these newly emerging organisms. In brief, the chromosome of this *K. pneumoniae* strain is 5,284,834 nucleotides (nt) in length, has a 57% GC content, and was determined to belong to ST395 (11), which is unique in our region.

Two plasmids were also found in this strain. Plasmid 1 was 94,656 nt long and present at 1.6× relative to the chromosome. Plasmid 2 carried the bla_{NDM-1} gene, was 118,033 nt long, and was present at 2.2× relative to the chromosome. Plasmid 2 is an IncF replicon with similarity to other IncF plasmids, like pKPN4 (accession no. CP000649.1). Notably, the bla_{NDM-1} gene environment is adjacent to a bleomycin resistance protein-encoding gene, a finding that is common in bla_{NDM-1}-producing strains and is thought to impact the overall survival value of this strain (15).

DISCUSSION

Through this comprehensive effort, a clear picture of the origin, acute and chronic morbidity, hospitalization characteristics, and outcomes of hospitalized patients with CR *K. pneumoniae* in our region has emerged. Hospitalized patients with CR *K. pneumoniae* originate primarily from the long-term care population (16–19). Skilled nursing and long-term acute-care facilities clearly play an important role in the epidemiology of CR *K. pneumoniae*. Whether this is simply a reflection of the overlap of risk factors for multidrug-resistant (MDR) organisms with risk factors for admission to long-term care facilities, such as comorbidities and invasive device use, or whether this is an indication of transmission within long-term care facilities remains to be seen (19).

Importantly, a substantial minority of the patients we studied were admitted from home. These patients may have had prior health care or long-term care exposure but were admitted from home for their index hospitalization during the study period. This has an impact for hospitals that screen patients for MDR organisms upon admission. It may not be sufficient to screen only those who are admitted from other hospitals.

Patients with CR *K. pneumoniae* are both chronically and acutely ill and have prolonged and complicated hospitalizations. Moreover, patients are unlikely to be discharged home, and the overall hospital mortality rate was 16%, with an additional 2% of the patients discharged to hospice care. In patients with CR *K. pneumoniae* infections, clinical factors associated with time to death in the hospital included expected variables such as the source of infection and severe acute illness, as previously described (7).

A troublesome trend was observed in the susceptibilities of CR *K. pneumoniae* to the "last-resort" antimicrobials colistin, tigecycline, and aminoglycosides. Colistin nonsusceptibility occurred in a small subset of the patients, and tigecycline and aminoglycoside nonsusceptibility was common. Most concerning was the fact that two patients presented with colistin- and tigecycline-resistant isolates. This is clear evidence that new antimicrobials directed against CR *K. pneumoniae* are urgently needed, as well as ongoing efforts to stem the rise in MDR pathogens.

The great majority of isolates belonged to ST258, which is the predominant strain of CR *K. pneumoniae* in the United States and worldwide (20). Within this sequence type, however, we were able to further identify two predominant separate strains by rep-PCR. Importantly, these two strains differed in their susceptibilities to aminoglycosides and tigecycline. In addition, differences in clinical outcomes were observed in patients possessing the two strains.

A very important finding in our cohort was the presence of a single patient during the study period who was infected with bla_{NDM-1}-harboring CR *K. pneumoniae*. NDM-1 remains an uncommon mechanism of carbapenem resistance in the United States but is widespread in India, Pakistan, Bangladesh, and other parts of the world (21, 22). Fortunately, continued vigilance through this network has not detected further evidence of dissemination of bla_{NDM-1}-harboring CR *K. pneumoniae* in our region.

Our study has several limitations. While we have used standardized definitions of infection, the distinction between infection and colonization remains a challenge. We suspect that at least some patients with infections were misclassified as having colonizations. However, when standardized definitions are used, the likelihood of the patients with colonizations being misclassified as having infections is low. In addition, our definition of CR *K. pneumoniae* may have missed some non-KPC-producing *K. pneumoniae* isolates with lower carbapenem MICs. The lack of active surveillance means that we are likely to underestimate the true number of CR *K. pneumoniae*-colonized patients. We categorized 20% of the infections as community acquired. However, data on prior health care exposures that may have preceded the current admission from home are not available.

The heterogeneity of antibiotic treatment in our cohort precluded comparative effectiveness analysis. The observed lack of association between the risk of death and CR *K. pneumoniae*-directed treatment in the first 48 h is probably secondary to both confounding by indication and other unmeasured covariables and the limited efficacy of currently available agents. Several studies have provided preliminary data suggestive of a benefit of combination therapy (23, 24).

Our findings illustrate the ongoing and increasing threat that CR *K. pneumoniae* poses to our vulnerable population and the clear benefits of real-time surveillance and molecular epidemiology in tracking the dissemination and clinical impact of resistance genes in specific pathogens.

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