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Molecular Characterization of Carbapenem-Resistant Enterobacterales Collected in the United States

Maria Karlsson,¹ Joseph D. Lutgring,¹ Uzma Ansari,¹ Adrian Lawsin,¹ Valerie Albrecht,¹ Gillian McAllister,¹ Jonathan Daniels,¹ David Lonsway,¹ Susannah McKay,¹ Zintars Beldavs,² Chris Bower,³ Ghinwa Dumyati,⁴ Anastasia Gross,⁵ Jesse Jacob,^{3,6} Sarah Janelle,⁷ Marion A. Kainer,⁸ Ruth Lynfield,⁵ Erin C. Phipps,⁹ Kyle Schutz,⁷ Lucy Wilson,¹⁰ Medora L. Witwer,⁵ Sandra N. Bulens,¹ Maroya Spalding Walters,¹ Nadezhda Duffy,¹ Alexander J. Kallen,¹ Christopher A. Elkins,¹ and J. Kamile Rasheed¹

Carbapenem-resistant Enterobacterales (CRE) are a growing public health concern due to resistance to multiple antibiotics and potential to cause health care-associated infections with high mortality. Carbapenemase-producing CRE are of particular concern given that carbapenemase-encoding genes often are located on mobile genetic elements that may spread between different organisms and species. In this study, we performed phenotypic and genotypic characterization of CRE collected at eight U.S. sites participating in active population- and laboratory-based surveillance of carbapenem-resistant organisms. Among 421 CRE tested, the majority were isolated from urine ($n=349$, 83%).

Klebsiella pneumoniae was the most common organism ($n=265$, 63%), followed by *Enterobacter cloacae* complex ($n=77$, 18%) and *Escherichia coli* ($n=50$, 12%). Of 419 isolates analyzed by whole genome sequencing, 307 (73%) harbored a carbapenemase gene; variants of *bla*_{KPC} predominated ($n=299$, 97%). The occurrence of carbapenemase-producing *K. pneumoniae*, *E. cloacae* complex, and *E. coli* varied by region; the predominant sequence type within each genus was ST258, ST171, and ST131, respectively. None of the carbapenemase-producing CRE isolates displayed resistance to all antimicrobials tested; susceptibility to amikacin and tigecycline was generally retained.

Keywords: carbapenem-resistant Enterobacterales, carbapenemase, KPC

Introduction

CARBAPENEM-RESISTANT ENTEROBACTEREALES (CRE) represent a major threat to public health and modern health care.¹ According to the Centers for Disease Control and Prevention (CDC), CRE are associated with ~13,100 health care-associated infections (HAIs) annually in the United States, of which ~1,100 result in death.¹ In addition to β -lactams, CRE are often resistant to multiple other anti-

biotic classes resulting in few therapeutic options.² These organisms can cause a number of different types of disease, including urinary tract infections, bloodstream infections, intra-abdominal infections, wound infections, and pneumonia.

Infections with these organisms are associated with a higher mortality than infections with carbapenem-susceptible organisms.^{3,4} Patients with medical devices, undergoing invasive procedures, or admitted to the intensive care unit are particularly at risk for CRE.⁵

¹Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia, USA.

²Oregon Health Authority, Portland, Oregon, USA.

³Georgia Emerging Infections Program, Atlanta, Georgia, USA.

⁴New York Emerging Infections Program at the University of Rochester Medical Center, Rochester, New York, USA.

⁵Minnesota Department of Health, St. Paul, Minnesota, USA.

⁶Emory University School of Medicine, Atlanta, Georgia, USA.

⁷Colorado Department of Public Health and Environment, Denver, Colorado, USA.

⁸Tennessee Department of Public Health, Nashville, Tennessee, USA.

⁹New Mexico Emerging Infections Program, Santa Fe, New Mexico, USA.

¹⁰Maryland Department of Health, Baltimore, Maryland, USA.

Phenotypic resistance to carbapenems can be mediated by a number of different mechanisms: through the production of a carbapenemase β -lactamase, production of an extended-spectrum β -lactamase or AmpC enzyme in combination with changes in membrane permeability, or efflux pumps. Carbapenemase-producing CRE are of particular concern given that carbapenemase-encoding genes often are located on mobile genetic elements that can transfer between organisms and species, increasing potential for rapid transmission.²

In the United States, the epidemic of CRE began in the early 2000s with the emergence of *Klebsiella pneumoniae* isolates producing the *K. pneumoniae* carbapenemase (KPC). These isolates, first identified in North Carolina in 1996, have since spread and caused multiple outbreaks across the United States.^{6–9} Over the past decade, an increasing number of CRE-producing carbapenemases other than KPC, including New Delhi metallo- β -lactamase (NDM) and OXA-48-like enzymes, have been observed in the United States.^{10,11}

Since 2011, CDC administers the Multisite Gram-negative Surveillance Initiative (MuGSI) as a part of the Emerging Infections Program (EIP) Healthcare-Associated Infections Community Interface (HAIC) activity. Through MuGSI, CDC conducts active population- and laboratory-based surveillance of carbapenem-resistant organisms with the goal to provide resistance data and describe the molecular epidemiology for Gram-negative bacteria that cause infections at the intersection of health care facilities and the general community.¹² In this study, we describe the molecular epidemiology of CRE isolates collected at eight MuGSI-participating U.S. communities.

Materials and Methods

During 2011–2015, eight U.S. sites (Colorado, Georgia, Maryland, Minnesota, New York, New Mexico, Tennessee, and Oregon) participated in MuGSI. Test results from local clinical laboratories were used to identify *Escherichia coli*, *Enterobacter cloacae* complex, *Klebsiella aerogenes* (formerly *Enterobacter aerogenes*), *K. pneumoniae*, and *Klebsiella oxytoca* isolates that were not susceptible to meropenem, imipenem, or doripenem and resistant to all third-generation cephalosporins. To meet the inclusion criteria, isolates further had to be collected from a normally sterile site or urine.

Antimicrobial susceptibility test methods varied among the clinical laboratories although the majority reported the use of an automated test system (MicroScan, Beckman Coulter Diagnostics, Brea, CA; Vitek, bioMérieux, Marcy-l'Étoile, FR, or BD Phoenix, Becton Dickinson, Franklin Lakes, NJ).

A convenience sample of up to 30 isolates per site per quarter meeting the inclusion criteria were forwarded to CDC for species identification and antimicrobial susceptibility testing. At CDC, isolates were cultured on nonselective media and incubated in ambient air at 35°C overnight. Species-level identification was obtained by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-ToF MS) using a Biotyper 3.1 MALDI-ToF System (Bruker Daltonics, Billerica, MA).

Antimicrobial susceptibility testing was performed using reference broth microdilution for the following drugs: ami-

kacin, ampicillin, aztreonam, cefazolin, cefepime, cefotaxime, ceftazidime, ceftazidime, ceftriaxone, ciprofloxacin, colistin, doripenem, ertapenem, gentamicin, imipenem, levofloxacin, meropenem, piperacillin/tazobactam, tetracycline, tigecycline, tobramycin, and trimethoprim/sulfamethoxazole.

Testing was performed according to guidelines from the Clinical and Laboratory Standards Institute (CLSI) using *E. coli* ATCC 25922, *E. coli* ATCC 35218, *K. pneumoniae* ATCC 700603, *K. pneumoniae* BAA-2146, and *Pseudomonas aeruginosa* ATCC 27853 as quality control strains.¹³ The panels were read after 16–20 hours of incubation at 35°C and results interpreted according to CLSI-established criteria, where available.¹⁴ Breakpoints from the U.S. Food and Drug Administration (FDA) were used for tigecycline. Only isolates meeting CDC's current surveillance definition (Enterobacterales resistant to ertapenem, imipenem, doripenem, or meropenem) based on susceptibility testing performed at CDC were included in this analysis.¹⁵

Whole genome sequence analysis

For each isolate, genomic DNA was extracted using the Promega Maxwell[®] 16 Cell Low Elution Volume DNA Purification Kit and the Maxwell 16 MDx Instrument (Madison, WI). Genomic libraries were prepared using the NuGEN Ovation[®] Ultralow System V1 or V2 Assay Kit (San Carlos, CA) and the PerkinElmer Zephyr[®] G3 NGS Workstation (Waltham, MA).

Whole genome sequencing was performed using an Illumina MiSeq (San Diego, CA); raw reads were filtered for phiX using bbdduk and trimmed using Trimmomatic.¹⁶ *De novo* assembly of the sequence raw reads was performed with the SPAdes assembler using default parameters.¹⁷ Short contiguous sequences, consisting of <500 bases, were removed from the assembly. For each isolate, the sequence type (ST) was determined using the program mlst on the assembly; for any unknown ST-types, srst2 was used on the trimmed reads.¹⁸

To determine the presence of acquired antimicrobial resistance genes, c-SSTAR and srst2 were used along with a combined database of ARG-ANNOT¹⁹ and ResFinder²⁰; genes with 100% ID and 100% coverage were reported. Loss of expression of porin-encoding genes was assessed using c-SSTAR; all identified porins (based on $\geq 80\%$ identity and $\geq 80\%$ gene coverage) with an internal stop codon were considered truncated. Porin genes assessed included *ompC* and *ompF* (*E. coli*); *ompK35* and *ompK36* (*K. pneumoniae*); *omp35* and *omp36* (*K. aerogenes*); and *ompF2* and *ompC2* (*E. cloacae* complex).

This activity was reviewed by the human subjects advisors in the CDC's National Center for Emerging and Zoonotic Infectious Diseases and determined to constitute a non-research public health surveillance activity. Therefore, CDC institutional review board (IRB) review was not required. The project also underwent review in each of the participating EIP sites and was either approved by IRB with waiver of informed consent or was considered a non-research public health activity.

Results

In 2011–2015, 643 Enterobacterales were collected by the eight EIP-participating sites and forwarded to CDC. Upon

confirmatory testing at CDC, 421 (65%) met CDC's current surveillance definition for CRE (resistant to ertapenem, imipenem, doripenem, or meropenem). *K. pneumoniae* was the most common organism ($n=265$, 63%), followed by *E. cloacae* complex ($n=77$, 18%), *E. coli* ($n=50$, 12%), *K. aerogenes* ($n=26$, 6%), and *K. oxytoca* ($n=3$, 1%). The majority of CRE were isolated from urine ($n=349$, 83%); the remaining isolates were obtained from blood ($n=58$, 14%) or other normally sterile sites ($n=14$, 3%). None of the isolates was recovered from a respiratory tract source. Of the 421 CRE, 419 were available for whole genome sequencing (Supplementary Table S1). Among these, a carbapenemase gene was identified in 307 (73%).

Carbapenemase-producing CRE

The proportion of CRE harboring a carbapenemase-encoding gene differed by site, with the highest frequency observed in Maryland (91%) followed by Georgia (90%), New York (72%), Minnesota (57%), New Mexico (40%), Colorado (37%), Tennessee (25%), and Oregon (16%) (Table 1). The predominant carbapenemase-producing (CP) species was *K. pneumoniae* ($n=242$, 79%) followed by *E. cloacae* complex ($n=37$, 12%), *E. coli* ($n=23$, 7%), *K. aerogenes* ($n=3$, 1%), and *K. oxytoca* ($n=2$, 1%). The proportion of CP-CRE was 92% (242/264) among carbapenem-resistant *K. pneumoniae*, 48% (37/77) among carbapenem-resistant *E. cloacae* complex and 47% (23/49) among carbapenem-resistant *E. coli*. *K. pneumoniae* was the most common carbapenemase producer at every site, except Minnesota where *E. cloacae* complex predominated.

A variant of *bla*_{KPC} was the most frequently detected carbapenemase gene ($n=299$, 97%). Other carbapenemase genes detected included *bla*_{NDM} variants ($n=5$), *bla*_{NMC-A} ($n=1$), *bla*_{VIM-1} ($n=1$), and *bla*_{OXA-48} ($n=1$) (Table 1).

Among the CP-CRE, the predominant *K. pneumoniae* ST was ST258 ($n=153$; 63%) followed by ST15 ($n=12$; 5%), ST11 ($n=9$; 4%), ST16 ($n=9$; 4%), and ST340 ($n=5$; 2%). The predominant *E. coli* ST was ST131 ($n=7$; 30%). All of these were collected 2012–2014; none of the CP *E. coli* ($n=9$) detected in 2015 belonged to ST131. Of the nine CP *E. coli* detected in 2015, three belonged to STs previously encountered in 2013 and 2014 (ST38, ST617, and ST393); the remaining CP *E. coli* included six unique STs (ST345, ST354, ST641, ST685, ST3057, and ST5498) (Fig. 1). The predominant *E. cloacae* complex ST was ST171 ($n=14$; 38%) (Fig. 1 and Table 1).

Antimicrobial susceptibility testing results for the 307 CP-CRE are presented in Table 2. Among the carbapenemase-producing isolates, 99% displayed resistance to ertapenem while resistance to imipenem, meropenem, and doripenem was observed in 93%, 86%, and 79% of the isolates, respectively. All isolates were resistant to ampicillin, cefazolin, cefotaxime, and ceftiraxone. The majority of isolates also displayed resistance to piperacillin/tazobactam (98%), aztreonam (99%), ceftazidime (99%), cefepime (91%), and ceftiofloxacin (81%).

While most CP-CRE isolates were resistant to ciprofloxacin (88%), levofloxacin (83%), tobramycin (69%) and trimethoprim/sulfamethoxazole (80%), resistance to amikacin (7%), gentamicin (24%), tetracycline (29%), and tigecycline (2%) was less common (Table 2). Among all

CP-CRE tested, 35 (11%) displayed resistance to colistin. However, none of the isolates harbored the colistin resistance gene *mcr*.

Noncarbapenemase-producing CRE

Whole genome sequencing identified 112 isolates without a known carbapenemase gene, constituting 27% of all CRE (Table 3). The top species was *E. cloacae* complex ($n=40$, 36%) followed by *E. coli* ($n=26$, 23%), *K. pneumoniae* ($n=22$, 20%), *K. aerogenes* ($n=23$, 21%), and *K. oxytoca* ($n=1$, 1%).

There were no predominant *E. cloacae* complex STs; ST50 ($n=4$, 10%), ST108 ($n=3$, 8%), and ST41 ($n=3$, 8%) were most common. The predominant *E. coli* ST was ST131 ($n=8$, 31%). There were no predominant *K. pneumoniae* STs, but ST15 ($n=3$, 14%) was most common. Among the non-CP *E. coli*, *K. pneumoniae* and *K. oxytoca*, 59% harbored an acquired extended-spectrum β -lactamase and 31% an acquired AmpC-type gene. Among the non-CP *E. cloacae* complex and *K. aerogenes* isolates, 8% harbored an acquired extended-spectrum β -lactamase and 11% an acquired AmpC-type gene. Among all non-CP Enterobacteriales, 43 (38%) carried at least one truncated porin gene.

Antimicrobial susceptibility testing results for 112 non-CP CRE are presented in Table 2. A high proportion of these isolates were resistant to ertapenem (99%), while resistance to imipenem, meropenem, and doripenem was observed in 37%, 42%, and 38% of isolates, respectively. All isolates were resistant to ampicillin, cefazolin, cefotaxime, and ceftiraxone. Most isolates were resistant to aztreonam (98%), ceftazidime (97%), and ceftiofloxacin (96%). Piperacillin/tazobactam resistance was observed in 79% of isolates and cefepime resistance in 49% of isolates. Low or moderate levels of resistance were observed for amikacin (4%), tobramycin (26%), gentamicin (19%), and tigecycline (3%). Resistance to colistin was observed in 16% of the isolates (Table 2); no isolates harbored an *mcr* gene.

Discussion

CRE are an important public health problem and a priority for action to control their spread. A better understanding of the molecular epidemiology of CRE, and especially CP-CRE, might help target interventions and guide priorities for public health practice and research.

By performing molecular characterization of 419 CRE collected from 8 EIP sites performing active population- and laboratory-based surveillance, we found that *K. pneumoniae* is the most common CRE and that most CRE, when confirmed by CDC, harbor a carbapenemase gene (73%). In our study *K. pneumoniae* harboring a *bla*_{KPC} gene accounted for most CRE, with other carbapenemase genes, such as *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{VIM-1}, and *bla*_{NMC-A} occurring less frequently.

Although the overall frequency of CP-CRE was high among the CRE isolates collected, regional variation was observed with the highest frequencies detected in Maryland, Georgia, and New York. The sites with the highest proportion of CP-CRE also had the highest incidence of CRE [12]. The lowest frequencies of CP-CRE were observed in Colorado (37%), Tennessee (25%), and Oregon (16%) (Table 1), but the total number of isolates submitted from

TABLE 1. CHARACTERISTICS OF 307 CARBAPENEMASE-PRODUCING ENTEROBACTERIALES COLLECTED THROUGH ACTIVE POPULATION- AND LABORATORY-BASED SURVEILLANCE THROUGH THE EMERGING INFECTIONS PROGRAM, 2011–2015

<i>EIP site</i>	<i>No. of CRE</i>	<i>No. of CP-CRE (%)</i>	<i>Species of CP-CRE (n)</i>	<i>Carbapenemase genes (n)</i>	<i>ST (n)^a</i>
Colorado	41	15 (37)	<i>Klebsiella pneumoniae</i> (13)	KPC-3 (10), KPC-2 (1), KPC-4 (1), NDM-1 (1)	ST258 (9), ST307 (1), other (3)
Georgia	132	119 (90)	<i>Escherichia coli</i> (2)	NDM-1 (1), NDM-5 (1)	other (2)
			<i>K. pneumoniae</i> (103)	KPC-3 (94), KPC-2 (8), NDM-4 (1)	ST258 (89), ST15 (4), ST11 (1), ST13 (1), ST307 (2), other (6)
			<i>E. coli</i> (9)	KPC-3 (9)	ST131 (2), ST617 (3), other (4)
Maryland	119	108 (91)	<i>Enterobacter cloacae</i> complex (5)	KPC-3 (4), KPC-4 (1)	ST175 (1), other (4)
			<i>Klebsiella aerogenes</i> (1)	KPC-3 (1)	
			<i>Klebsiella oxytoca</i> (1)	KPC-3 (1)	
			<i>K. pneumoniae</i> (89)	KPC-3 (59), KPC-2 (30)	ST258 (30), ST15 (7), ST11 (8), ST16 (9), ST340 (5), ST13 (3), ST48 (4), ST307 (1), other (22)
Minnesota	61	35 (57)	<i>E. coli</i> (6)	KPC-3 (4), KPC-2 (2)	ST131 (2), other (4)
			<i>E. cloacae</i> complex (12)	KPC-3 (8), KPC-2 (3), KPC-4 (1)	ST175 (1), other (11)
			<i>K. aerogenes</i> (1)	KPC-3 (1)	
			<i>K. pneumoniae</i> (16)	KPC-3 (11), KPC-2 (4), KPC-4 (1)	ST258 (14), other (2)
			<i>E. coli</i> (2)	KPC-3 (1), KPC-4 (1)	ST131 (1), other (1)
New Mexico	10	4 (40)	<i>E. cloacae</i> complex (17)	KPC-3 (14), KPC-6 (1), NMC-A (1), VIM-1 (1)	ST171 (14), other (3)
			<i>K. pneumoniae</i> (2)	KPC-2 (2)	ST258 (1), other (1)
			<i>E. cloacae</i> complex (1)	KPC-2 (1)	other (1)
			<i>K. aerogenes</i> (1)	KPC-2 (1)	
New York	29	21 (72)	<i>K. pneumoniae</i> (14)	KPC-3 (9), KPC-2 (5)	ST258 (8), ST418 (4), other (2)
			<i>E. coli</i> (4)	KPC-2 (2), KPC-3 (1), NDM-4 (1)	ST131 (2), other (2)
			<i>E. cloacae</i> complex (2)	KPC-3 (1), KPC-4 (1)	ST175 (1), other (1)
Oregon	19	3 (16)	<i>K. oxytoca</i> (1)	KPC-3 (1)	ST258 (2), ST15 (1)
			<i>K. pneumoniae</i> (3)	KPC-3 (2), OXA-48 (1)	other (2)
			<i>K. pneumoniae</i> (2)	KPC-3 (1), KPC-2 (1)	ST258 (153), ST15 (12), ST11 (9), ST16 (9), ST340 (5), ST13 (4), ST48 (4), ST307 (4), ST418 (4), other (38)
			<i>K. pneumoniae</i> (242)	NDM-1 (1), NDM-4 (1), OXA-48 (1)	ST131 (7), ST617 (3), other (13)
Tennessee	8	2 (25)	<i>E. coli</i> (23)	KPC-3 (15), KPC-2 (4), KPC-4 (1), NDM-1 (1), NDM-4 (1), NDM-5 (1)	ST171 (14), ST175 (3), other (20)
			<i>E. cloacae</i> complex (37)	KPC-3 (27), KPC-2 (4), KPC-4 (3), KPC-6 (1), NMC-A (1), VIM-1 (1)	
			<i>K. aerogenes</i> (3)	KPC-3 (2), KPC-2 (1)	
Total	419	307 (73)	<i>K. oxytoca</i> (2)	KPC-3 (2)	

^aThe category “other” includes STs with an $n \leq 2$.CRE, carbapenem-resistant Enterobacterales; CP-CRE, carbapenemase-producing carbapenem-resistant Enterobacterales; EIP, Emerging Infections Program; KPC, *K. pneumoniae* carbapenemase; NDM, New Delhi metallo- β -lactamase; NMC-A, nonmetallocarbapenemase class A; ST, sequence type; VIM, Verona integron-encoded metallo- β -lactamase.

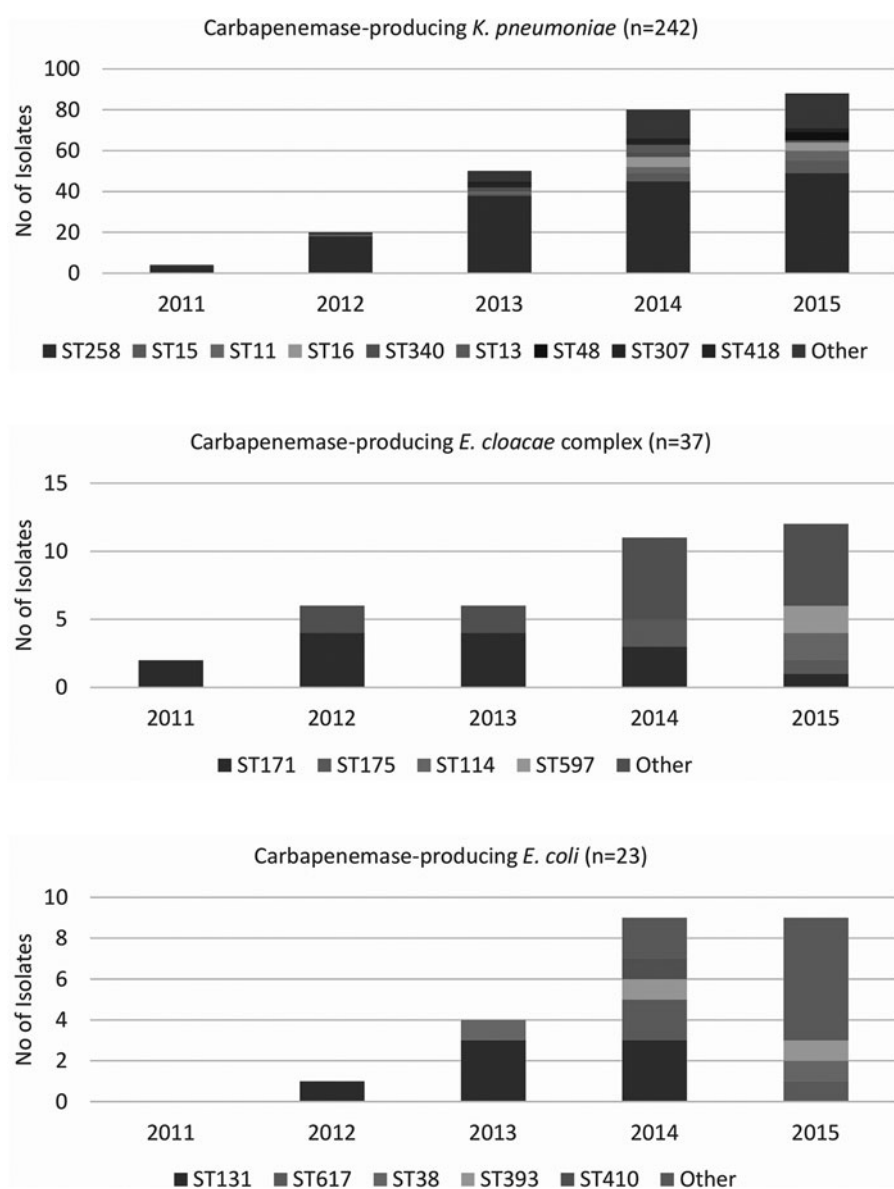


FIG. 1. ST distribution among carbapenemase-producing isolates of *Klebsiella pneumoniae*, *Enterobacter cloacae* complex, and *Escherichia coli* collected through active population- and laboratory-based surveillance through the Emerging Infections Program, 2011–2015. ST, sequence type.

these sites was low (41, 8, and 19, respectively). The variability in CP-CRE frequency among the different sites is likely a reflection of when CP-CRE first emerged in the area and the epidemiological stage of CRE outbreaks.²¹

Information on national and regional variations in the prevalence of carbapenem nonsusceptibility among certain genera of Enterobacterales isolated from several HAI types is available through CDC's Antibiotic Resistance & Patient Safety Portal (<https://www.cdc.gov/hai/data/portal/AR-Patient-Safety-Portal.html>). This system provides open and interactive data about antimicrobial-resistant organisms reported to CDC through the National Healthcare Safety Network and other sources.

One of the most important factors influencing the regional distribution of CP-CRE is the occurrence of successful clones that may facilitate and drive the expansion within a region. In the present set of surveillance isolates, the most prevalent CP sequence types among *K. pneumoniae*, *E. cloacae* complex, and *E. coli* were ST258, ST171, and

ST131, respectively. *K. pneumoniae* ST258 is a well-established clone in the United States, commonly associated with *bla*_{KPC} carriage and multidrug resistance.²² This sequence type, which has been associated with worldwide expansion of KPC-producing *K. pneumoniae*, was detected at seven of the eight EIP-participating sites. The occurrence of ST258 was particularly high in Georgia and Minnesota where 86% and 88%, respectively, of all carbapenemase-producing *K. pneumoniae* were ST258.

In contrast to *K. pneumoniae* ST258, the most common sequence type among *E. cloacae* complex, ST171, was only detected at a single site, Minnesota. This clone, initially reported from a western Pennsylvania Hospital in 2014, has since been reported from multiple sites, including a New York City Hospital and a Boston Hospital.^{23–25} In 2015, Hargreaves *et al.* reported on the emergence of KPC-3-producing ST171 in upper Midwestern United States.²⁶ It is likely that several of the isolates in the Hargreaves *et al.* study were included in the present surveillance set.

TABLE 2. ANTIMICROBIAL SUSCEPTIBILITY TESTING RESULTS FOR CARBAPENEM-RESISTANT ENTEROBACTERIALES COLLECTED THROUGH ACTIVE POPULATION- AND LABORATORY-BASED SURVEILLANCE THROUGH THE EMERGING INFECTIONS PROGRAM, 2011–2015

Antimicrobial agent	Total no. of resistant CRE (n=419) (%)	No. of resistant CP-CRE (n=307) (%)	No. of resistant non-CP-CRE (n=112) (%)
Ertapenem	416 (99)	305 (99)	111 (99)
Imipenem	327 (78)	287 (93)	41 (37)
Meropenem	309 (74)	263 (86)	47 (42)
Doripenem	283 (68)	241 (79)	43 (38)
Ampicillin	419 (100)	307 (100)	112 (100)
Piperacillin/tazobactam	390 (93)	302 (98)	89 (79)
Aztreonam	415 (99)	305 (99)	110 (98)
Cefepime	332 (79)	278 (91)	55 (49)
Ceftazidime	411 (98)	303 (99)	109 (97)
Ceftriaxone	419 (100)	307 (100)	112 (100)
Cefotaxime	418 (100)	307 (100)	112 (100)
Cefoxitin	356 (85)	248 (81)	108 (96)
Cefazolin	419 (100)	307 (100)	112 (100)
Colistin	53 (13)	35 (11)	18 (16)
Ciprofloxacin	321 (77)	269 (88)	52 (46)
Levofloxacin	301 (72)	254 (83)	47 (42)
Amikacin	26 (6)	21 (7)	4 (4)
Tobramycin	240 (57)	211 (69)	29 (26)
Gentamicin	94 (22)	73 (24)	21 (19)
Tetracycline	132 (32)	89 (29)	43 (38)
Tigecycline	9 (2)	6 (2)	3 (3)
Trimethoprim/sulfamethoxazole	290 (69)	246 (80)	46 (41)

Data presented for all CRE undergoing reference antimicrobial susceptibility testing and whole genome sequencing at Centers for Disease Control and Prevention, $n=419$. Antimicrobial susceptibility test results were interpreted according to CLSI-established criteria, where available. Breakpoints from the U.S. Food and Drug Administration were used for tigecycline.

CLSI, Clinical and Laboratory Standards Institute; Non-CP-CRE, noncarbapenemase-producing carbapenem-resistant Enterobacterales.

Continued surveillance will be needed to evaluate dissemination of ST171; spread of carbapenemase-producing ST171 could increase the proportion of *E. cloacae* complex resistant to carbapenems, which would limit treatment options.

ST131 was the predominant clone among carbapenemase-producing *E. coli* isolates. First described in 2008, ST131 has become widely disseminated and established as a high-risk clone with increased epidemic potential.^{27,28} ST131 is reported globally, and in contrast to *K. pneumoniae* ST258, which mainly is associated with HAIs, ST131 is reported both in health care and community settings.²⁹ ST131 has mainly been associated with resistance to extended-spectrum β -lactam drugs and fluoroquinolones.^{27–29} Carbapenem-resistant isolates remain less common but have previously been reported from the United States.³⁰

Among the fifteen carbapenem-resistant ST131 collected in this study, 47% were carbapenemase producers harboring a *bla*_{KPC} gene. Continued monitoring of CP-CRE clones will be important for the detection of new, emerging high-risk clones and for assessing antimicrobial resistance development among the epidemic clones already circulating in the United States.

Treatment options are limited for most CP-CRE infections since these isolates commonly display a multidrug-resistant phenotype. In this study, we confirmed CP-CRE to be more resistant than non-CP-CRE isolates (Table 2). However, no CP-CRE isolate displayed resistance to all antimicrobials tested and susceptibility was generally preserved to amikacin, tigecycline, and colistin. Although these drugs represent valid treatment options, all three have been associated with substantial toxicities or reduced efficacy.^{31–34}

The high frequency of carbapenemase producers detected among CRE in the present evaluation highlights the continued need for containment strategies that will help prevent transmission between patients in health care settings and slow the spread of this important multidrug-resistant organism. A strategy to respond to and contain even single isolates of CP-CRE when detected has been outlined by CDC and serves as an important complement to other foundational CDC strategies, including antibiotic stewardship, in the national fight against antimicrobial resistance (<https://www.cdc.gov/hai/containment/guidelines.html>; <https://www.cdc.gov/vital/signs/containing-unusual-resistance>).

As part of this strategy, CDC has taken measures to facilitate rapid and early detection of emerging resistant organisms. In 2016, CDC established the Antibiotic Resistance Laboratory Network (AR Lab Network) with expanded capabilities to respond to emerging resistance threats, including CP-CRE, through state and local public health laboratories trained and equipped to detect resistant organisms from human samples throughout the United States.³⁵

Building capacity to enhance laboratory detection of carbapenemase production among CRE is important. Infection control programs can use the results of carbapenemase production testing to target interventions in their facilities toward the most concerning strains.^{36,37} However, laboratory detection of CP-CRE is challenging due to the many resistance mechanisms and bacterial genera involved and the fact that the epidemiology of CP-CRE may vary by region.^{38,39}

Recently developed laboratory tests such as the modified carbapenem inactivation method (mCIM) and Carba NP test

TABLE 3. CHARACTERISTICS OF 112 NONCARBAPENEMASE-PRODUCING CARBAPENEM-RESISTANT ENTEROBACTERIALES COLLECTED THROUGH ACTIVE POPULATION- AND LABORATORY-BASED SURVEILLANCE THROUGH THE EMERGING INFECTIONS PROGRAM, 2011–2015

Site	No. of non-CP-CRE	Species of CRE (n)	Multilocus STs ^a (n)
Colorado	26	<i>Klebsiella pneumoniae</i> (4) <i>Escherichia coli</i> (2) <i>Enterobacter cloacae</i> complex (12) <i>Klebsiella aerogenes</i> (7) <i>Klebsiella oxytoca</i> (1)	Other (4) ST410 (1), other (1) ST50 (2), ST108 (2), other (8)
Georgia	13	<i>K. pneumoniae</i> (4) <i>E. coli</i> (4) <i>E. cloacae</i> complex (5)	ST15 (1), other (3) ST410 (1), other (3) Other (5)
Maryland	11	<i>K. pneumoniae</i> (3) <i>E. coli</i> (4) <i>E. cloacae</i> complex (2) <i>K. aerogenes</i> (2)	ST15 (2), other (1) ST131 (2), ST410 (1), other (1) Other (2)
Minnesota	26	<i>K. pneumoniae</i> (2) <i>E. coli</i> (8) <i>E. cloacae</i> complex (7) <i>K. aerogenes</i> (9)	Other (2) ST131 (2), other (6) ST41 (1), other (6)
New Mexico	6	<i>K. pneumoniae</i> (2) <i>E. coli</i> (1) <i>E. cloacae</i> complex (3)	Other (2) ST131 (1) ST50 (2), other (1)
New York	8	<i>K. pneumoniae</i> (3) <i>E. coli</i> (2) <i>E. cloacae</i> complex (2) <i>K. aerogenes</i> (1)	other (3) ST131 (1), other (1) other (2)
Oregon	16	<i>K. pneumoniae</i> (4) <i>E. coli</i> (2) <i>E. cloacae</i> complex (7) <i>K. aerogenes</i> (3)	Other (4) ST131 (1), other (1) ST108 (1), ST41 (1), other (5)
Tennessee	6	<i>E. coli</i> (3) <i>E. cloacae</i> complex (2) <i>K. aerogenes</i> (1)	ST131 (1), other (2) ST41 (1), other (1)
Total	112	<i>K. pneumoniae</i> (22) <i>E. coli</i> (26) <i>E. cloacae</i> complex (40) <i>K. aerogenes</i> (23) <i>K. oxytoca</i> (1)	ST15 (3), other (19) ST 131 (8), ST410 (3), other (15) ST50 (4), ST108 (3), ST41 (3), other (30)

^aOther: the ST classified as other all have an $n \leq 2$.

have, however, greatly improved clinical microbiology laboratories' ability to phenotypically identify CP organisms.^{40,41} There are also several rapid molecular tests, which laboratories can use to obtain information regarding the presence of various carbapenemase genes.^{42–44}

Information regarding the underlying mechanism of carbapenem resistance in an isolate has become increasingly relevant since it can help predict the *in vitro* susceptibility to the newly developed β -lactam– β -lactamase inhibitor combinations ceftazidime–avibactam, meropenem–vaborbactam, and imipenem–relebactam. Avibactam, vaborbactam, and relebactam inhibit the KPC enzyme but not the metallo- β -lactamases (MBLs), such as NDM, active on imipenem metallo- β -lactamase (IMP), or verona integron-encoded metallo- β -lactamase (VIM).⁴⁵ However, the combination of aztreonam, a monobactam stable to MBLs, and avibactam is one of few treatments in clinical development with promising activity against MBL Enterobacterales coproducing extended-spectrum β -lactamase or AmpC-type enzymes.

Caution is needed, however, on using molecular mechanism data to guide treatment decisions since acquired re-

sistance mechanisms affecting novel β -lactam– β -lactamase inhibitor combinations have been described.^{46–48} Through the AR Lab Network, CDC is building capacity for state and public health laboratories to screen CRE isolates for phenotypic carbapenemase activity and the most common carbapenemase genes circulating in the United States (*bla*_{KPC}, *bla*_{NDM}, *bla*_{OXA-48-like}, *bla*_{IMP}, and *bla*_{VIM}).³⁵

This investigation has some limitations. A significant number of isolates that were sent to CDC as CRE were not confirmed as CRE ($n = 222$, 35%). Possible reasons include misidentification by the local clinical laboratory, loss of plasmid in transport to CDC, or decreased genetic expression of resistance mechanisms in transport to CDC. In addition, not all CRE isolates at each participating site were sent to CDC. Finally, all FDA-approved drugs with activity against CRE were not evaluated (*e.g.*, ceftazidime–avibactam, imipenem–relebactam, meropenem–vaborbactam, cefiderocol, and eravacycline were not tested).

In summary, carbapenemase-producing organisms made up a significant proportion of confirmed CRE collected at eight geographically diverse sites in the United States. *K.*

pneumoniae is found across all the EIP-participating sites and remains the most commonly encountered carbapenem-resistant species. The occurrence of carbapenemase production among CR *K. pneumoniae*, *Enterobacter*, and *E. coli* varies by region, but is driven, in part, by the presence of three specific clones: ST258, ST171, and ST131. Continued monitoring of CRE will be critical to follow the expansion of these three clones as well as the emergence of new, potential high-risk clones. Through the recently established AR Lab Network, CDC is increasing the capacity for CRE characterization and early detection of CP-CRE nationwide.

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Disclaimer

The findings and conclusions in this article are those of the authors and do not necessarily represent the official position of the Centers for Disease Control and Prevention.

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Supplementary Material

Supplementary Table S1

References

- Centers for Disease Control and Prevention. 2019. Antibiotic Resistance Threats in the United States 2019. Atlanta, GA: U.S. Department of Health and Human Services, CDC. Available at <https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf> (accessed October 30, 2021).
- Bonomo, R.A., E.M. Burd, J. Conly, *et al.* 2018. Carbapenemase-producing organisms: a global scourge. *Clin. Infect. Dis.* 66:1290–1297.
- Falagas, M.E., G.S. Tansarli, D.E. Karageorgopoulos, and K.Z. Vardakas. 2014. Deaths attributable to carbapenem-resistant Enterobacteriaceae infections. *Emerg. Infect. Dis.* 20:1170–1175.
- Kohler, P.P., C. Volling, K. Green, E.M. Uleryk, P.S. Shah, A. McGeer. 2017. Carbapenem resistance, initial antibiotic therapy, and mortality in *Klebsiella pneumoniae* bacteremia: a systematic review and meta-analysis. *Infect. Control Hosp. Epidemiol.* 38:1319–1328.
- van Loon, K., A.F. Voor In 't Holt, and M.C. Vos. 2018. A systematic review and meta-analyses of the clinical epidemiology of carbapenem-resistant Enterobacteriaceae. *Antimicrob. Agents Chemother.* 62:e01730-17.
- Bradford, P.A., S. Bratu, C. Urban, *et al.* 2004. Emergence of carbapenem-resistant *Klebsiella* species possessing the class A carbapenem-hydrolyzing KPC-2 and inhibitor-resistant TEM-30 beta-lactamases in New York City. *Clin. Infect. Dis.* 39:55–60.
- Kanamori, H., C.M. Parobek, J.J. Juliano, *et al.* 2017. A prolonged outbreak of KPC-3-producing *Enterobacter cloacae* and *Klebsiella pneumoniae* driven by multiple mechanisms of resistance transmission at a Large Academic Burn Center. *Antimicrob. Agents Chemother.* 61:e01516-16.
- Snitkin, E.S., A.M. Zelazny, P.J. Thomas, *et al.* 2012. Tracking a hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* with whole-genome sequencing. *Sci. Transl. Med.* 4:148ra116.
- Yigit, H., A.M. Queenan, G.J. Anderson, *et al.* 2001. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 45:1151–1161.
- Epstein, L., J.C. Hunter, M.A. Arwady, *et al.* 2014. New Delhi metallo-beta-lactamase-producing carbapenem-resistant *Escherichia coli* associated with exposure to duodenoscopes. *JAMA* 312:1447–1455.
- Lutgring, J.D., W. Zhu, T.J.B. de Man, *et al.* 2018. Phenotypic and genotypic characterization of Enterobacteriaceae producing oxacillinase-48-like carbapenemases, United States. *Emerg. Infect. Dis.* 24:700–709.
- Guh, A.Y., S.N. Bulens, Y. Mu, *et al.* 2015. Epidemiology of carbapenem-resistant Enterobacteriaceae in 7 US communities, 2012–2013. *JAMA* 314:1479–1487.
- Clinical and Laboratory Standards Institute. 2018. Methods for Dilution Antimicrobial Susceptibility Tests for Bacteria That Grow Aerobically; 11th ed. CLSI Standard M07. Wayne, PA: Clinical and Laboratory Standards Institute.
- Clinical and Laboratory Standards Institute. 2021. Performance Standards for Antimicrobial Susceptibility Testing. 31st ed. CLSI Supplement M100. Clinical and Laboratory Standards Institute.
- Chea, N., S.N. Bulens, T. Kongphet-Tran, *et al.* 2015. Improved phenotype-based definition for identifying carbapenem producers among carbapenem-resistant Enterobacteriaceae. *Emerg. Infect. Dis.* 21:1611–1616.
- Bolger, A.M., M. Lohse, and B. Usadel. 2014. Trimmomatic: a flexible trimmer for Illumina sequence data. *Bioinformatics* 30:2114–2120.
- Bankeovich, A., S. Nurk, D. Antipov, *et al.* 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. *J. Comput. Biol.* 19:455–477.
- Inouye, M., H. Dashnow, L.A. Raven, *et al.* 2014. SRST2: rapid genomic surveillance for public health and hospital microbiology labs. *Genome Med.* 6:90.
- Gupta, S.K., B.R. Padmanabhan, S.M. Diene, *et al.* 2014. ARG-ANNOT, a new bioinformatic tool to discover antibiotic resistance genes in bacterial genomes. *Antimicrob. Agents Chemother.* 58:212–220.
- Zankari, E., H. Hasman, S. Cosentino, *et al.* 2012. Identification of acquired antimicrobial resistance genes. *J. Antimicrob. Chemother.* 67:2640–2644.
- Grundmann, H., C. Glasner, B. Albiger, *et al.* 2017. 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.* 17: 153–163.S
22. Kitchel, B., J.K. Rasheed, J.B. Patel, *et al.* 2009. Molecular epidemiology of KPC-producing *Klebsiella pneumoniae* isolates in the United States: clonal expansion of multilocus sequence type 258. *Antimicrob. Agents Chemother.* 53: 3365–3370.
 23. Ahn, C., A. Syed, F. Hu, J.A. O'Hara, J.I. Rivera, and Y. Doi. 2014. Microbiological features of KPC-producing Enterobacter isolates identified in a U.S. hospital system. *Diagn. Microbiol. Infect. Dis.* 80:154–158.
 24. Gomez-Simmonds, A., M.K. Annavajhala, Z. Wang, *et al.* 2018. Genomic and geographic context for the evolution of high-risk carbapenem-resistant *Enterobacter cloacae* complex clones ST171 and ST78. *MBio* 9:e00542-18.
 25. Pecora, N.D., N. Li, M. Allard, *et al.* 2015. Genomically informed surveillance for carbapenem-resistant Enterobacteriaceae in a health care system. *MBio* 6:e01030.
 26. Hargreaves, M.L., K.M. Shaw, G. Dobbins, *et al.* 2015. Clonal dissemination of *Enterobacter cloacae* harboring blaKPC-3 in the upper Midwestern United States. *Antimicrob. Agents Chemother.* 59:7723–7734.
 27. Nicolas-Chanoine, M.H., J. Blanco, V. Leflon-Guibout, *et al.* 2008. Intercontinental emergence of *Escherichia coli* clone O25:H4-ST131 producing CTX-M-15. *J. Antimicrob. Chemother.* 61:273–281.
 28. Petty, N.K., Ben N.L. Zakour, M. Stanton-Cook, *et al.* 2014. Global dissemination of a multidrug resistant *Escherichia coli* clone. *Proc. Natl. Acad. Sci. U S A.* 111: 5694–5699.
 29. Nicolas-Chanoine, M.H., X. Bertrand, and J.Y. Madec. 2014. *Escherichia coli* ST131, an intriguing clonal group. *Clin. Microbiol. Rev.* 27:543–574.
 30. Peirano, G., P.A. Bradford, K.M. Kazmierczak, *et al.* 2014. Global incidence of carbapenemase-producing *Escherichia coli* ST131. *Emerg. Infect. Dis.* 20:1928–1931.
 31. Beaubien, A.R., E. Ormsby, A. Bayne, *et al.* 1991. Evidence that amikacin ototoxicity is related to total perilymph area under the concentration-time curve regardless of concentration. *Antimicrob. Agents Chemother.* 35:1070–1074.
 32. Lim, L.M., N. Ly, D. Anderson, *et al.* 2010. Resurgence of colistin: a review of resistance, toxicity, pharmacodynamics, and dosing. *Pharmacotherapy* 30:1279–1291.
 33. Anthony, K.B., N.O. Fishman, D.R. Linkin, L.B. Gasink, P.H. Edelstein, and E. Lautenbach. 2008. Clinical and microbiological outcomes of serious infections with multidrug-resistant gram-negative organisms treated with tigecycline. *Clin. Infect. Dis.* 46:567–570.
 34. Prasad, P., J. Sun, R.L. Danner, and C. Natanson. 2012. Excess deaths associated with tigecycline after approval based on noninferiority trials. *Clin. Infect. Dis.* 54:1699–1709.
 35. Centers for Disease Control and Prevention. Lab Capacity: Antibiotic Resistance Laboratory Network (AR Lab Network). Available at <https://www.cdc.gov/drugresistance/solutions-initiative/ar-lab-network.html> Accessed October 30, 2018.
 36. Hayden, M.K., M.Y. Lin, K. Lolans, *et al.* 2015. Prevention of colonization and infection by *Klebsiella pneumoniae* carbapenemase-producing enterobacteriaceae in long-term acute-care hospitals. *Clin. Infect. Dis.* 60:1153–1161.
 37. Munoz-Price, L.S., M.K. Hayden, K. Lolans, *et al.* 2010. Successful control of an outbreak of *Klebsiella pneumoniae* carbapenemase-producing *K. pneumoniae* at a long-term acute care hospital. *Infect. Control. Hosp. Epidemiol.* 31: 341–347.
 38. Humphries, R.M., J.A. Hindler, E. Epton, *et al.* 2018. Carbapenem-resistant Enterobacteriaceae detection practices in California: what are we missing? *Clin. Infect. Dis.* 66:1061–1067.
 39. Lutgring, J.D., and B.M. Limbago. 2016. The problem of carbapenemase-producing-carbapenem-resistant-Enterobacteriaceae detection. *J. Clin. Microbiol.* 54:529–534.
 40. Pierce, V.M., P.J. Simner, D.R. Lonsway, *et al.* 2017. Modified carbapenem inactivation method for phenotypic detection of carbapenemase production among Enterobacteriaceae. *J. Clin. Microbiol.* 55:2321–2333.
 41. Vasoo, S., S.A. Cunningham, P.C. Kohner, *et al.* 2013. Comparison of a novel, rapid chromogenic biochemical assay, the Carba NP test, with the modified Hodge test for detection of carbapenemase-producing Gram-negative bacilli. *J. Clin. Microbiol.* 51:3097–3101.
 42. Pogue, J.M., E.L. Heil, P. Lephart, *et al.* 2018. An antibiotic stewardship program blueprint for optimizing verigene BC-GN within an institution: a tale of two cities. *Antimicrob. Agents Chemother.* 62:e02538-17.
 43. Southern, T.R., T.C. VanSchooneveld, D.L. Bannister, *et al.* 2015. Implementation and performance of the BioFire FilmArray(R) Blood Culture Identification panel with antimicrobial treatment recommendations for bloodstream infections at a midwestern academic tertiary hospital. *Diagn. Microbiol. Infect. Dis.* 81:96–101.
 44. Tato, M., P. Ruiz-Garbajosa, M. Traczewski, *et al.* 2016. Multisite evaluation of cepheid Xpert Carba-R assay for detection of carbapenemase-producing organisms in rectal swabs. *J. Clin. Microbiol.* 54:1814–1819.
 45. Wright, H., R.A. Bonomo, and D.L. Paterson. 2017. New agents for the treatment of infections with Gram-negative bacteria: restoring the miracle or false dawn? *Clin. Microbiol. Infect.* 23:704–712.
 46. Nelson, K., P. Hemarajata, D. Sun, *et al.* 2017. Resistance to ceftazidime-avibactam is due to transposition of KPC in a porin-deficient strain of *Klebsiella pneumoniae* with increased efflux activity. *Antimicrob. Agents Chemother.* 61: e00989-17.
 47. Wang, Y., J. Wang, R. Wang, and Y. Cai. 2020. Resistance to ceftazidime-avibactam and underlying mechanisms. *J. Glob. Antimicrob. Resist.* 22:18–27.
 48. Gaibani, P., D. Lombardo, L. Bussini, *et al.* 2021. Epidemiology of meropenem/vaborbactam resistance in KPC-producing *Klebsiella pneumoniae* causing bloodstream infections in Northern Italy, 2018. *Antibiotics (Basel)*. 10: 536.

Address correspondence to:

Maria Karlsson, PhD

Division of Healthcare Quality Promotion

Centers for Disease Control and Prevention

1600 Clifton Road

Atlanta, GA 30329

USA

E-mail: fwt4@cdc.gov