JAC Antimicrob Resist
doi:10.1093/jacamr/dlab137

JAC-Antimicrobial Resistance

Carbapenemase production among less-common Enterobacterales genera: 10 US sites, 2018

Alicia Shugart [1]*, Garrett Mahon[1], Jennifer Y. Huang[1], Maria Karlsson[1], Ann Valley[2], Megan Lasure[2], Annastasia Gross[3], Brittany Pattee[3], Elisabeth Vaeth[4], Richard Brooks[1,4], Tyler Maruca[4], Catherine E. Dominguez[4], David Torpey[4], Drew Francis[5], Rachana Bhattarai[5], Marion A. Kainer[6]†, Allison Chan[6], Heather Dubendris[7]‡, Shermalyn R. Greene[7], Sara J. Blosser[8], D. J. Shannon[8]||, Kelly Jones[9], Brenda Brennan[9], Sopheay Hun[10], Marisa D'Angeli[10], Caitlin N. Murphy[11]§, Maureen Tierney[12]¶, Natashia Reese[1], Amelia Bhatnagar[1,13], Alex Kallen[1], Allison C. Brown[1] and Maroya Spalding Walters[1]

[1]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, GA, USA; [2]Wisconsin State Laboratory of Hygiene, Madison, WI, USA; [3]Minnesota Department of Health, St Paul, MN, USA; [4]Maryland Department of Health, Baltimore, MD, USA; [5]Arizona Department of Health Services, Phoenix, AZ, USA; [6]Tennessee Department of Health, Nashville, TN, USA; [7]North Carolina Department of Health and Human Services, Raleigh, NC, USA; [8]Indiana State Department of Health, Indianapolis, IN, USA; [9]Michigan Department of Health and Human Services, Lansing, MI, USA; [10]Washington State Department of Health, Tumwater, WA, USA; [11]University of Nebraska Medical Center, Department of Pathology and Microbiology, Omaha, NE, USA; [12]Nebraska Department of Health and Human Services, Lincoln, NE, USA; [13]Goldbelt C6 Inc, Juneau, AK, USA

*Corresponding author. E-mail: ashugart@cdc.gov
†Present address: Melbourne, Australia.
‡Present address: Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, U.S. Department of Health and Human Services, Atlanta, GA, USA.
||Present address: Eskenazi Health, Indianapolis, IN, USA.
§Present address: Health Network Laboratories, Allentown, PA, USA.
¶Present address: School of Medicine, Creighton University, Omaha, NE, USA.

Received 11 May 2021; accepted 30 July 2021

Background: Historically, United States' carbapenem-resistant Enterobacterales (CRE) surveillance and mechanism testing focused on three genera: *Escherichia*, *Klebsiella*, and *Enterobacter* (EsKE); however, other genera can harbour mobile carbapenemases associated with CRE spread.

Objectives: From January through May 2018, we conducted a 10 state evaluation to assess the contribution of less common genera (LCG) to carbapenemase-producing (CP) CRE.

Methods: State public health laboratories (SPHLs) requested participating clinical laboratories submit all Enterobacterales from all specimen sources during the surveillance period that were resistant to any carbapenem (Morganellaceae required resistance to doripenem, ertapenem, or meropenem) or were CP based on phenotypic or genotypic testing at the clinical laboratory. SPHLs performed species identification, phenotypic carbapenemase production testing, and molecular testing for carbapenemases to identify CP-CRE. Isolates were categorized as CP if they demonstrated phenotypic carbapenemase production and $\geq$1 carbapenemase gene (bla_{KPC}, bla_{NDM}, bla_{VIM}, bla_{IMP}, or $bla_{OXA\text{-}48\text{-}like}$) was detected.

Results: SPHLs tested 868 CRE isolates, 127 (14.6%) were from eight LCG. Overall, 195 (26.3%) EsKE isolates were CP-CRE, compared with 24 (18.9%) LCG isolates. LCG accounted for 24 (11.0%) of 219 CP-CRE identified. *Citrobacter* spp. was the most common CP-LCG; the proportion of *Citrobacter* that were CP (11/42, 26.2%) was similar to the proportion of EsKE that were CP (195/741, 26.3%). Five of 24 (20.8%) CP-LCG had a carbapenemase gene other than bla_{KPC}.

Conclusions: Participating sites would have missed approximately 1 in 10 CP-CRE if isolate submission had been limited to EsKE genera. Expanding mechanism testing to additional genera could improve detection and prevention efforts.

Introduction

Enterobacterales (which has now replaced the former Family Enterobacteriaceae) is a large taxonomic Order encompassing seven families and more than 80 genera of Gram-negative bacteria.[1–3] It includes pathogens from three genera associated with 30% of healthcare-associated infections (HAIs) in adults in the United States: *Escherichia coli*, *Klebsiella* spp., and *Enterobacter* spp.,[4] and many less-common pathogens that can cause complicated infections, such as *Proteus* spp., *Citrobacter* spp., and *Serratia* spp.[5–7] Carbapenems are broad-spectrum antibiotics and a mainstay of treatment for serious Enterobacterales infections; however, their efficacy can be compromised by multiple distinct resistance mechanisms.[8–11] Carbapenemase enzymes, the most

concerning of these mechanisms, are β-lactamases that inactivate most or all β-lactam antibiotics. Most carbapenemases are encoded by genes located on mobile genetic elements (MGEs), which can be efficiently transferred between bacterial taxa.[8–13] These MGEs also frequently carry additional genes that confer resistance to non-β-lactam antibiotics, further limiting treatment options for carbapenemase-producing carbapenem-resistant Enterobacterales (CP-CRE) infections.[8,10,14] Owing to the potential for rapid spread of multidrug resistance, CP-CRE surveillance and prevention has been a US public health priority since cases were first identified domestically.[11,12,15] Overall, 34.7% of CRE from US patients have a carbapenemase gene detected.[15] Among CP-CRE tested through the Antibiotic Resistance (AR) Laboratory Network, *Klebsiella pneumoniae* carbapenemase (bla_{KPC}) is the most-common gene identified by far, found in 85.7% of CP-CRE isolates. Other carbapenemase genes are more rare: 9.8% of CP-CRE isolates harbour New Delhi metallo-β-lactamase (bla_{NDM}), 3.9% carry oxacillinase ($bla_{OXA\text{-}48\text{-}like}$), 1.3% carry active-on-imipenem (bla_{IMP}), and 0.8% carry Verona integron-encoded metallo-β-lactamase (bla_{VIM}).[15]

In the United States, increased reports of carbapenem-resistant *Klebsiella pneumoniae* and *E. coli* HAIs in the early 2000s, followed by more recent decreases, have been attributed in part to the initial spread of, and subsequent public health efforts to control, CP-CRE.[11,12,16] These control efforts prioritize early detection of clinical cases and contact screening to identify asymptomatic carriage, even for single cases of emerging carbapenemases.[11,12,16] Public health surveillance programmes have been especially critical to detection and control of CP-CRE because of limited clinical laboratory testing. In a national survey of US hospitals, only half reported being served by a laboratory that tests CRE for carbapenemases in 2016;[17] this proportion remained similar through the 2020 survey year (CDC, unpublished data). Although many Enterobacterales genera are now known to harbour MGE-encoded carbapenemases, historically, United States CRE surveillance and carbapenemase detection focused on *E. coli*, *Klebsiella* spp., and *Enterobacter* spp. (EsKE).[18,19] Other CRE genera were not routinely targeted because of limited carbapenemase testing capacity and the presence of additional carbapenem resistance mechanisms that can complicate identification of mobile carbapenemases. For example, organisms in the Morganellaceae family (*Proteus*, *Providencia*, and *Morganella* spp.) have intrinsic low-level imipenem resistance.[3,6] Other less-common genera (LCG) can harbour carbapenemase genes that are not targeted by public health due to their limited host organism range, such as the chromosomally encoded carbapenemase, *Serratia marcescens* enzyme (bla_{SME}), found in some *Serratia marcescens*.[9,13,20] However, these LCG contribute to the burden of HAIs, cause difficult-to-treat infections,[4,6,21,22] and have potential to serve as reservoirs for transfer of the high-concern carbapenemase genes to carbapenem-susceptible Gram-negative bacilli.[11,19]

In 2016, CDC launched the AR Lab Network to improve detection of and response to emerging antibiotic resistant threats, including CP-CRE.[11,12] Although carbapenem-resistant *Klebsiella* spp., *Enterobacter* spp., and *E. coli* were targeted for mechanism testing (i.e., a combination of phenotypic testing for carbapenemase enzymatic activity and molecular testing for carbapenemase genes), less-common Enterobacterales species were accepted. *Ad hoc* submissions over the first 9 months of isolate collection in 2017 identified carbapenemases in 21% of CRE from LCG, suggesting carbapenemases of public health concern might be more common in these organisms than previously recognized.[11] We conducted a 5 month, 10 state surveillance project to determine what proportion of CRE from LCG were carbapenemase-producing, and the overall contribution of LCG to the burden of CP-CRE in the areas under surveillance.

Materials and methods

From 1 January through 31 May, 2018, 10 state public health laboratories (SPHLs) volunteered to conduct systematic surveillance, aligned with US public health laboratory capabilities and mission (https://www.cdc.gov/drugresistance/laboratories.html), to assess carbapenemase production in all species of carbapenem-resistant Enterobacterales identified at clinical laboratories. Arizona, Minnesota, Nebraska and Wisconsin included isolates from all clinical laboratories statewide. The remaining states identified a total of 25 sentinel clinical laboratories to participate, including four laboratories in Indiana, six in Maryland, two in Michigan, four in North Carolina, six in Tennessee, and four in Washington.

All states except for North Carolina had a public health CRE reporting mandate during the study period and six states required isolate submission to the SPHL; however, CRE definitions and target organism (e.g., CRE versus CP-CRE) varied by jurisdiction.

We defined CRE as any Enterobacterales resistant to any carbapenem antibiotic (MIC $\geq$4 mg/L for doripenem, imipenem, and meropenem, and $\geq$2 mg/L for ertapenem)[3] or demonstrating the presence of a carbapenemase by a phenotypic or genotypic test at the clinical laboratory. For organisms from the Morganellaceae family with intrinsic low-level imipenem resistance, resistance to doripenem, ertapenem, or meropenem was required for submission. SPHLs requested their participating clinical laboratories to submit all CRE isolated from any specimen source; in addition to clinical cultures, CRE isolated from active surveillance cultures at facilities served by the participating laboratories may have been forwarded to SPHLs.

SPHLs performed species identification using MALDI-ToF MS [nine SPHLs used Bruker (Billerica, MA); the MD SPHL used bioMérieux (Marcy-l'Étoile, France)]. Confirmed Enterobacterales species underwent phenotypic carbapenemase production testing using the modified carbapenem inactivation method (mCIM)[23] and broth microdilution AST using Sensititre™ GNX2F or GN4F plates (Thermo Fisher Scientific, Waltham, MA). Isolates with carbapenemase activity were tested by PCR for genes encoding KPC, NDM, OXA-48-like, VIM, and IMP carbapenemases.[24,25] PCR-based methods varied by state and included in-house laboratory developed assays, CDC-developed assays, and GeneXpert Carba-R® (Cepheid, Sunnyvale, CA). Isolates that demonstrated carbapenemase activity via the mCIM test, but did not have a carbapenemase gene detected, were re-tested at CDC by PCR for the five common carbapenemases; *S. marcescens* and *Enterobacter*

spp. were additionally tested by conventional PCR for the presence of bla_{SME} and imipenem-hydrolysing-β-lactamase (bla_{IMI})/non-metallo-carbapenemase (bla_{NMC}) genes, respectively (CDC, unpublished data).

SPHLs submitted testing data to CDC for collation and analysis. We excluded isolates not tested according to the specified algorithm and included only the first isolate per organism–mechanism combination per patient. Organisms reported as *Enterobacter aerogenes* were re-categorized as *Klebsiella aerogenes*.[2] Two VIM-producing isolates without a definitive species identification, reported to CDC as either *Klebsiella oxytoca* or *Raoultella ornithinolytica*, were categorized as *K. oxytoca*. Isolates were defined as carbapenemase-producing if they showed carbapenemase activity by mCIM test and had a KPC, NDM, OXA-48-like, VIM, or IMP-encoding gene identified. For the primary analysis, *S. marcescens* with bla_{SME} and *Enterobacter* spp. with bla_{IMI}/bla_{NMC} were grouped with non-CP-CRE because these genes are not associated with the same risk for spread of carbapenem resistance; these genes have generally not been associated with spread outside of their host organisms and therefore the recommended public health and infection control response is more similar to non-CP-CRE than to CP-CRE with one of the five targeted carbapenemase genes.[9,13,20,26] We then performed a secondary sensitivity analysis grouping these isolates with CP-CRE to reflect their genotypic classification.

For patients with CP-CRE from LCG, state health departments reported to CDC age, inpatient healthcare history, and international travel history for the 12 months prior to specimen collection. These data are routinely collected during public health investigations of CP-CRE.[16]

State health departments reported to CDC known or suspected CRE outbreaks from submitting healthcare facilities during the surveillance period. We performed a sensitivity analysis excluding outbreak-associated isolates to assess their impact on our findings.

Differences in frequency were assessed using the Chi-square test, or Fisher's exact test for cell sizes ≤5, with significance assessed at $P < 0.05$ using a two-tailed test. Analyses were performed with SAS version 9.4 (SAS Institute, Cary, NC).

This activity was reviewed by the human subjects' advisors in the National Center for Emerging and Zoonotic Infectious Diseases at the CDC and was determined to constitute public health surveillance.

Results

Overall, 877 CRE isolates were submitted and 868 (99.0%) were tested according to the project algorithm. Among the 868 isolates, 127 (14.6%) were LCG (Table 1). In total, 219 (25.2%) CRE isolates met the definition of CP. The proportion of CP-CRE did not differ significantly between EsKE and LCG (195/741, 26.3%, versus 24/127, 18.9%). Among the 219 CP-CRE identified, 24 (11.0%) were LCG.

SPHLs each contributed a median of 62 CRE isolates, ranging from 21 isolates from Nebraska to 187 isolates from Wisconsin (Table 1). SPHLs that conducted statewide surveillance (AZ, MN, NE, WI) accounted for 59.8% (519/868) of CRE isolates and 67.7% (86/127) of LCG identified, but only 37.0% (81/219) of all CP-CRE. Although laboratories that did statewide surveillance had a greater proportion of CRE from LCG (86/519, 16.6%) than SPHLs that did sentinel surveillance (41/349, 11.7%, $P = 0.049$), their proportion of LCG that were CP-CRE was lower (statewide 11/86, 12.8%, versus sentinel, 13/41, 31.7%, $P = 0.011$). SPHLs in the Midwest census division (IN, MI, MN, NE, and WI) identified more LCG among submitted CRE (84/454, 18.5%) than the SPHLs outside the Midwest (43/414, 10.4%, $P = 0.003$). Midwestern SPHLs also found that LCG accounted for a greater proportion of CP-CRE (14/78, 17.9%) compared with the other sites (10/141, 7.1%,

Table 1. Total carbapenem-resistant Enterobacterales (CRE) submitted and carbapenemase-producing (CP)[a] CRE identified, with isolates grouped by the three most-common genera (EsKE; *Escherichia*, *Klebsiella*, and *Enterobacter*) and less-common genera (LCG), by submitting state, $N = 868$

	CRE Isolates					CP-CRE Isolates[a]				
	Total	EsKE CRE		LCG CRE		Total	EsKE CP-CRE		LCG CP-CRE	
State	*N*	*N*	%	*N*	%	*N*	*N*	%	*N*	%
AZ	150	131	87.3%	19	12.7%	37	33	89.2%	4	10.8%
IN	37	29	78.4%	8	21.6%	22	16	72.7%	6[b]	27.3%
MD	108	99	91.7%	9	8.3%	51	48	94.1%	3	5.9%
MI	48	39	81.3%	9	18.8%	12	11	91.7%	1	8.3%
MN	161	130	80.7%	31	19.3%	16	14	87.5%	2	12.5%
NC	59	57[c]	96.6%	2	3.4%	19	18[d]	94.7%	1	5.3%
NE	21	17	81.0%	4	19.0%	1	1	100.0%	0	0.0%
TN	64	54	84.4%	10	15.6%	28	26	92.9%	2	7.1%
WA	33	30	90.9%	3	9.1%	6	6	100.0%	0	0.0%
WI	187	155	82.9%	32	17.1%	27	22	81.5%	5	18.5%
Total	868[e]	741	85.4%	127	14.6%	219	195	89.0%	24	11.0%

[a]Isolates were defined as carbapenemase-producing if they had both carbapenemase activity by mCIM test and had a bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{VIM}, or bla_{IMP} gene identified.
[b]Four *Serratia* spp. with bla_{KPC} outbreak isolates were identified at one facility.
[c]Two non-CP-*Klebsiella* spp. outbreak isolates were identified at one facility.
[d]Three *Klebsiella* spp. with $bla_{OXA-48-like}$ outbreak isolates identified at one facility.
[e]Three states, IN, NE, WI, additionally reported 80 isolates with intermediate susceptibility to carbapenems. Of these, 11/80 (13.8%) isolates were LCG and 2 (2.5%) isolates, both EsKE, were CP-CRE. A sensitivity analysis in which these isolates were included found no significant difference in the frequency of carbapenemase-production between EsKE (197/810, 24.3%) and LCG (24/138, 17.4%).

Table 2. Frequency of carbapenemase-production and carbapenemase genes with known epidemiological significance to public health detected among carbapenem-resistant Enterobacterales (CRE) isolates by genus, $N = 868$

Organisms	No. CP-CRE[b]/Total No. CRE *n*/*N* (%)	Carbapenemase genes detected by isolate[a]						
		bla_{KPC}	$bla_{OXA-48-like}$	bla_{NDM}	bla_{VIM}	bla_{IMP}	$bla_{NDM}/bla_{OXA-48-like}$	bla_{KPC}/bla_{VIM}
More-common genera[c]	195/741 (26.3)	154	16	15	6	1	2	1
Enterobacter	29[d]/308 (9.4)	25		1	2	1		
Escherichia	36/136 (26.5)	18	9	9				
Klebsiella	130[e]/297[f] (43.8)	111	7	5	4		2[c]	1
Less-common genera[g]	24/127 (18.9)	19	1			3	1	
Citrobacter	11/42 (26.2)	10	1					
Hafnia	0/4 (0.0)							
Morganella	0/13 (0.0)							
Proteus	2/19 (10.5)	1				1		
Providencia	3/8 (37.5)					2	1	
Raoultella	0/5 (0.0)							
Serratia	8[h]/36[d] (22.2)	8[h]						
Total	219/868 (25.2)	173	17	15	6	4	3	1

[a]Some laboratories employed hierarchical molecular testing for isolates showing carbapenemase activity by mCIM test. 147 isolates (67.1%) were tested for all five carbapenemase genes. 61 isolates (27.9%) were tested for 4 genes (bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, and bla_{VIM}); 8 isolates (3.7%) were tested for bla_{KPC} and bla_{NDM} only; 2 isolates (1.0%) were tested for bla_{KPC} only; and 1 isolate (0.5%) was tested for bla_{KPC}, *bla*NDM, and $bla_{OXA-48-like}$ genes only.

[b]Isolates were defined as carbapenemase-producing if they had both carbapenemase activity by mCIM test and had a bla_{KPC}, bla_{NDM}, $bla_{OXA-48-like}$, bla_{VIM}, or bla_{IMP} gene identified.

[c]More common genera species included *E. cloacae* complex (304), *E. coli* (137), *K. aerogenes* (63), *K. oxytoca* (16), *K. pneumoniae* (214), and *K. variicola* (2). Six *Enterobacter* spp. isolates and 6 *Klebsiella* spp. isolates could not be definitively speciated.

[d]One *Enterobacter* spp. with bla_{IMI}/bla_{NMC} carbapenemases and 7 *S. marcescens* with bla_{SME} carbapenemase are excluded from CP-CRE calculations. If *E. cloacae* with bla_{IMI}/bla_{NMC} and *S. marcescens* with bla_{SME} were categorized as CP, then 26.5% (196/741) of EsKE and 24.4% (31/127) LCG would have been CP-CRE.

[e]Three *Klebsiella* spp. with $bla_{OXA-48-like}$ outbreak isolates identified at one facility.

[f]Less common genera species included *Citrobacter amalonaticus* (2), *C. freundii* complex (36), *C. koseri* (2), *Hafnia alvei* (4), *Morganella morganii* (13), *Proteus mirabilis* (18), *P. vulgaris* (2), *Providencia rettgeri* (5), *P. stuartii* (3), *Raoultella ornithinolytica* (3), *R. planticola* (1), *Serratia marcescens* (35), and *S. ureilytica* (1). Two *Citrobacter* spp., one *Raoultella* spp., and one *Serratia* spp. could not be definitively speciated.

[g]Two non-CP-Klebsiella spp. outbreak isolates were identified at one facility.

[h]Four *Serratia* spp. with bla_{KPC} were identified at one facility.

$P = 0.014$); Indiana had the highest proportion of CP-CRE that were LCG (6/22, 27.3%) (Table 1).

Table 2 describes, by genus, the proportion of isolates that were CP-CRE and the carbapenemase genes identified. The proportion of isolates that were CP-CRE among three less-common genera, *Providencia* (3/8, 37.5%), *Citrobacter* (11/42, 26.2%), and *Serratia* (8/36, 22.2%), was similar to that of the EsKE genera overall (195/741, 26.3%) and greater than the proportion among *Enterobacter* (29/308, 9.4%; *Enterobacter* versus *Providencia* $P = 0.037$; versus *Citrobacter* $P = 0.001$; versus *Serratia* $P = 0.019$) (Table 2). Among CP-CRE from the LCG, 11 (46%) isolates were *Citrobacter* spp., of which 10 harboured bla_{KPC}. bla_{IMP} was more commonly identified in the LCG (3/24, 12.5%) compared with the EsKE genera (1/194, 0.5%, $P = 0.004$) (Table 2); the distribution of other carbapenemases did not differ. Of the 219 CP-CRE, 72 (32.9%) underwent hierarchical PCR testing for carbapenemase genes, which may have decreased detection of isolates carrying >1 carbapenemase gene. Overall, 61 (27.9%) CP-CRE were tested for four of five genes, but not bla_{IMP}, and 11 (5.0%) CP-CRE were tested for fewer than four genes. Ten isolates showed carbapenemase activity by mCIM test, but had none of the five common carbapenemase genes detected; of these, seven *Serratia* isolates harboured bla_{SME}, one *Enterobacter* isolate harboured bla_{IMI}/bla_{NMC}, and two *Enterobacter* isolates had unknown mechanisms of carbapenemase production, although one AST phenotype was consistent with hyper-AmpC production (i.e., carbapenem-resistant, cefepime-susceptible).

Two states reported three suspected or confirmed CRE outbreaks at participating sites during the surveillance period. Nine outbreak-associated isolates were reported: three OXA-48-like-producing *Klebsiella* spp. and two non-CP-*Klebsiella* spp., from North Carolina, and four KPC-producing *Serratia* spp. from Indiana. Excluding these nine isolates, 16.3% (20/123) of LCG were CP-CRE compared with 26.1% (192/736) of EsKE genera and the difference between the proportions reached statistical significance ($P = 0.019$). Also in the sensitivity analysis, the difference in proportions of LCG that were CP-CRE that varied by surveillance methodology (statewide: 11/86, 12.8%, versus sentinel sites: 9/37, 24.3%) and by geography (Midwest SPHLs: 10/74, 13.5%, versus non-Midwest SPHLs: 10/138, 7.2%) were no longer statistically significant; and Wisconsin supplanted Indiana as the state with the highest proportion of LCG among CP-CRE (5/27, 18.5%).

Among the 24 patients with CP-CRE from LCG, median patient age was 59.5 years (range: 21–88 years). Excluding outbreak isolates, the most common specimen sources were respiratory ($n = 6$, 30%) and urine ($n = 6$, 30%), followed by wounds ($n = 5$, 25%), blood ($n = 1$, 5%), ear ($n = 1$), and rectum ($n = 1$). Compared with CP-CRE from the EsKE genera, CP-CRE from LCG were more likely to be from respiratory specimens (18, 12.2%, $P = 0.032$) and wounds (13, 8.8%, $P = 0.044$) and less likely to be from urine (86, 58.1%, $P = 0.018$). Two (8.3%) patients, one with OXA-48-like-producing *Citrobacter koseri* and one with NDM- and OXA-48-like-producing *Providencia rettgeri*, had a history of inpatient hospitalization outside of the United States in the 12 months prior to specimen collection; both had been hospitalized in India. Among the 22 remaining patients, 20 (90.9%) had been hospitalized in the United States in the 12 months prior to specimen collection. Two patients (8.3%), one with IMP-producing *Proteus mirabilis* and one with KPC-producing *Citrobacter freundii* complex, had no prior inpatient healthcare exposures identified during medical record review.

Discussion

Among participating laboratories, if CRE mechanism testing had been limited to the EsKE genera that were targeted by the US national testing programme in 2018, approximately one in 10 CP-CRE identified during the surveillance period would have been missed. We observed geographic variability in the contribution of LCG to the total burden of CP-CRE, consistent with the heterogeneous epidemiology of CRE in the United States.[15] The highest burden of CP-CRE from LCG was observed among Midwestern states, but there was considerable variability even within this region. Among both carbapenem-resistant and CP-LCG organisms, *Citrobacter* was the most common genus, with a frequency of CP-CRE no different than *E. coli* and substantially higher than *Enterobacter*. Other LCG, such as *Providencia* and *Serratia,* although identified less often, were similarly likely to harbour transmissible carbapenemase genes. To our knowledge, this is the first formal assessment of carbapenemase production across a broad range of Enterobacterales species. Taken together, these findings suggest that strategic CRE testing beyond the three most-common genera, accounting for local epidemiology and targeting specific organisms, could improve CP-CRE detection and control.

Ten SPHLs volunteered to participate in this evaluation: half from the Midwestern census region (IN, MI, MN, NE, WI), three from the South (MD, NC, TN), two from the West (AZ, WA), and none from the Northeast. This geographic subset did not include several major metropolitan areas where KPC-producing CRE are endemic,[27] which might explain why the overall proportion of CP-CRE (25.2%) in our assessment was lower than the 32% identified through the AR Lab Network nationally.[11] Our systematic evaluation found 18.9% of LCG were CP-CRE, similar to the proportion (21%) identified from a convenience sample of 346 LCG isolates submitted to the AR Lab Network,[11] confirming that carbapenemase production in these organisms is not uncommon. We observed variation within and between geographic regions, including in neighbouring states. Wisconsin and Minnesota had similar overall proportions of CRE that were CP (27/187, 14.4%, and 16/161, 9.9%, respectively) and both used statewide surveillance. However, in Wisconsin, nearly one in five CP-CRE were from LCG, almost 50% more than the burden in Minnesota. The variable burden of CP-CRE from the LCG within the Midwest, which was overrepresented in our assessment, and across states from other regions, is consistent with the diversity of CP-CRE nationally.[15,18] It also highlights that the burden of CP-CRE from LCG varies geographically and cannot be generalized even within broad geographic areas.

Citrobacter and *Serratia* commonly carried bla_{KPC}, which is the most widely disseminated carbapenemase gene among CRE overall in the United States.[11,15,27] These two genera are already intrinsically multidrug resistant.[3,5] With the addition of bla_{KPC}, which we observed in approximately 1 in 4 isolates, these organisms have potential to cause infections with few treatment options.[5] Additionally, both KPC-producing *Citrobacter* spp. and KPC-producing *Serratia* spp. have caused outbreaks in healthcare settings.[28–33] Mechanism testing of these organisms could help to prevent further spread of bla_{KPC} in the United States. Half ($n = 4$) of the KPC-producing *Serratia* we identified were from a single facility respiratory outbreak. Notably, even when this outbreak is excluded, the proportion of *Serratia* that were CP (4/32, 12.5%) still exceeded that of *Enterobacter*. Although the outbreak may have elevated the frequency of CP-*Serratia* relative to a random sample, it underscores the propensity for CP-*Serratia* to cause healthcare-associated outbreaks, and the role for expanded carbapenemase testing to facilitate a public health response to prevent spread.[16]

The frequency of bla_{IMP} carriage was notably different between LCG and EsKE genera. Three of the four IMP-producing CRE were among the LCG, all within the Morganellaceae family. Although these organisms with intrinsic low-level imipenem resistance contribute a relatively small number of isolates to the burden of CP-CRE, data from this and other studies indicate Morganellaceae frequently harbour transmissible carbapenemase genes, most commonly metallo-β-lactamases such as bla_{IMP}.[11,19,34] The number of *Providencia* isolates was very small, but more than a third were CP-CRE. Although Morganellaceae are associated with a small proportion of healthcare-associated infections in hospitals, they are epidemiologically important in other healthcare settings such as nursing homes, where they can cause complex, persistent infections[6,21,22] and have been associated with large outbreaks.[11,19,35]

We did not collect extensive medical histories for patients with CP-CRE in LCG and the overall numbers are small, but the assessed risk factors yielded some interesting observations. First, two patients (8.3%) had no known recent healthcare exposures, indicating they might be community-associated cases. Cases of community-associated CP-CRE have been documented,[36] but overall, community-associated CRE are rare.[18] Second, among the five patients with CP-LCG producing carbapenemases other than KPC, only two had been hospitalized outside the United States. Hospitalization outside the United States has historically been a risk factor for non-KPC carbapenemases;[8–10] however, our findings are consistent with recent reports of domestic acquisition and transmission of metallo-β-lactamases.[11,19,29,35–40] As carbapenemase testing among LCG increases, it will better inform the epidemiology of these organisms.

This analysis is subject to multiple limitations. We conducted CRE mechanism testing for a relatively short timeframe in ten states, therefore, these results are not nationally generalizable. Additionally, in states that conducted sentinel surveillance, participating clinical laboratories might have served catchments with different underlying epidemiology from the state overall. Clinical

laboratories' adherence to the isolate submission protocol could have varied by state, clinical laboratory, and organism submitted, and may have caused unrecognized biases. Although most states have a legal requirement for healthcare facilities to report outbreaks to public health authorities, it is possible that outbreaks, especially of non-CP-CRE, might have been underrecognized and underreported. When sensitivity analysis was performed by removing known outbreak isolates, the proportion of LCG that were CP-CRE declined from 18.9% to 16.3%, and the difference in proportions of EsKE and LCG that were CP-CRE became statistically significant. This illustrates that outbreaks can be highly influential in analyses such as this, but also emphasizes the importance of early detection and response to limit CP-CRE spread. Finally, hierarchical molecular testing of some isolates, wherein PCR testing for less commonly identified carbapenemase genes (e.g., bla_{IMP}) may not be conducted if a more common gene is identified first, may have limited our ability to detect CP-CRE carrying multiple carbapenemase genes. Further characterization, including whole genome sequencing, is required to determine the distribution of carbapenemase gene variants and assess the contribution of species' clones to outbreaks and expansion of CP-CRE among the LCG.

Based on these findings, we recommend that clinical and public health laboratories consider strategic expansion of carbapenem resistance mechanism testing to additional genera that frequently harbour carbapenemase genes, such as *Citrobacter* and *Providencia*. As of January 2019, AR Lab Network jurisdictions were encouraged to expand mechanism testing to include all CRE genera overall, and *Providencia*, *Proteus*, *Morganella*, *Citrobacter*, and *Serratia*, in particular. Testing from additional sites over a longer timeframe will expand our knowledge of the relative frequency of carbapenemase genes circulating in these LCG as well as our understanding of regional differences and temporal variations. Most importantly, however, these actions are anticipated to enhance rapid identification of CP-CRE, which when coupled with prompt implementation of appropriate infection control measures, is critical to preventing spread.[11,12]

Acknowledgements

Preliminary results from this study were presented at the Society for Healthcare Epidemiology of America (SHEA), abstract 247: 'Carbapenemase Production among the Less Common Enterobacteriaceae Genera—10 U.S. Sites, 2018'.

Funding

This paper was produced as part of the routine work of the United States Centers for Disease Control and Prevention.

Transparency declarations

None to declare.

Disclaimer

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

References

1 NamesforLife. Abstract for Enterobacterales (Garrity and Holt 2001) Adeolu et al. 2016. doi:10.1601/nm.29303.

2 Adeolu M, Alnajar S, Naushad S *et al.* Genome-based phylogeny and taxonomy of the 'Enterobacteriales': proposal for Enterobacterales ord. nov. divided into the families Enterobacteriaceae, Erwiniaceae fam. nov., Pectobacteriaceae fam. nov., Yersiniaceae fam. nov., Hafniaceae fam. nov., Morganellaceae fam. nov., and Budviciaceae fam. nov. *Int J Syst Evol Microbiol* 2016; **66**: 5575–99.

3 CLSI. *Performance Standards for Antimicrobial Susceptibility Testing—Thirtieth Edition: M100*. 2020.

4 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**: 1–18.

5 Gajdacs M, Urban E. Resistance trends and epidemiology of Citrobacter-Enterobacter-Serratia in urinary tract infections of inpatients and outpatients (RECESUTI): a 10-year survey. *Medicina (Kaunas)* 2019; **55**: 285.

6 O'Hara CM, Brenner FW, Miller JM. Classification, identification, and clinical significance of *Proteus*, *Providencia*, and *Morganella*. *Clin Microbiol Rev* 2000; **13**: 534–46.

7 Sabbuba NA, Mahenthiralingam E, Stickler DJ. Molecular epidemiology of *Proteus mirabilis* infections of the catheterized urinary tract. *J Clin Microbiol* 2003; **41**: 4961–5.

8 Tangden T, Giske CG. Global dissemination of extensively drug-resistant carbapenemase-producing Enterobacteriaceae: clinical perspectives on detection, treatment and infection control. *J Intern Med* 2015; **277**: 501–12.

9 Nordmann P, Poirel L. The difficult-to-control spread of carbapenemase producers among Enterobacteriaceae worldwide. *Clin Microbiol Infect* 2014; **20**: 821–30.

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

11 Woodworth KR, Walters MS, Weiner LM *et al*. Vital Signs: Containment of Novel Multidrug-Resistant Organisms and Resistance Mechanisms — United States, 2006-2017. *MMWR Morb Mortal Wkly Rep* 2018; **67**: 396–401.

12 Centers for Disease Control and Prevention. Antibiotic Resistance Threats in the United States, 2019. 2019. https://www.cdc.gov/drugresistance/pdf/threats-report/2019-ar-threats-report-508.pdf.

13 Queenan AM, Bush K. Carbapenemases: the versatile β-lactamases. *Clin Microbiol Rev* 2007; **20**: 440–58.

14 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**: 55.

15 Centers for Disease Control and Prevention. Antibiotic Resistance & Patient Safety Portal: Antibiotic Resistance–All Carbapenem-resistant Enterobacteriaceae. https://arpsp.cdc.gov/profile/geography/united-states.

16 Centers for Disease Control and Prevention. Interim guidance for a public health response to contain novel or targeted multidrug-resistant organisms. 2019. https://www.cdc.gov/hai/pdfs/containment/Health-Response-Contain-MDRO-H.pdf.

17 Shugart A, Walters MS, Weiner LM *et al*. Hospital microbiology laboratory practices for Enterobacteriaceae: Centers for Disease Control and Prevention National Healthcare Safety Network (NHSN) annual survey, 2015 and 2016. *Infect Control Hosp Epidemiol* 2018; **39**: 1115–7.

18 Guh AY, Bulens SN, Mu Y *et al*. Epidemiology of Carbapenem-Resistant Enterobacteriaceae in 7 US Communities, 2012-2013. *JAMA* 2015; **314**: 1479–87.

19 Walters MS, Witwer M, Lee YK *et al*. Notes from the Field: Carbapenemase-Producing Carbapenem-Resistant Enterobacteriaceae from Less Common Enterobacteriaceae Genera — United States, 2014-2017. *MMWR Morb Mortal Wkly Rep* 2018; **67**: 668–9.

20 Deshpande LM, Jones RN, Fritsche TR *et al*. Occurrence and characterization of carbapenemase-producing Enterobacteriaceae: report from the SENTRY Antimicrobial Surveillance Program (2000-2004). *Microb Drug Resist* 2006; **12**: 223–30.

21 Aliyu S, Smaldone A, Larson E. Prevalence of multidrug-resistant gram-negative bacteria among nursing home residents: A systematic review and meta-analysis. *Am J Infect Control* 2017; **45**: 512–8.

22 Nicolle LE. Resistant pathogens in urinary tract infections. *J Am Geriatr Soc* 2002; **50**: S230–5.

23 Pierce VM, Simner PJ, Lonsway DR *et al*. Modified Carbapenem Inactivation Method for Phenotypic Detection of Carbapenemase Production among Enterobacteriaceae. *J Clin Microbiol* 2017; **55**: 2321–33.

24 Lutgring JD, Zhu W, de Man TJB *et al*. Phenotypic and Genotypic Characterization of Enterobacteriaceae Producing Oxacillinase-48-Like Carbapenemases, United States. *Emerg Infect Dis* 2018; **24**: 700–9.

25 Rasheed JK, Kitchel B, Zhu W *et al*. New Delhi metallo-β-lactamase-producing Enterobacteriaceae, United States. *Emerg Infect Dis* 2013; **19**: 870–8.

26 Boyd DA, Mataseje LF, Davidson R *et al*. *Enterobacter cloacae* Complex Isolates Harboring bla_{NMC-A} or bla_{IMI}-Type Class A Carbapenemase Genes on Novel Chromosomal Integrative Elements and Plasmids. *Antimicrob Agents Chemother* 2017; **61**: e02578-16.

27 Arnold RS, Thom KA, Sharma S *et al*. Emergence of *Klebsiella pneumoniae* carbapenemase-producing bacteria. *South Med J* 2011; **104**: 40–5.

28 Gomez-Gil MR, Pano-Pardo JR, Romero-Gomez MP *et al*. Detection of KPC-2-producing *Citrobacter freundii* isolates in Spain. *J Antimicrob Chemother* 2010; **65**: 2695–7.

29 Jimenez A, Castro JG, Munoz-Price LS *et al*. Outbreak of *Klebsiella pneumoniae* carbapenemase-producing *Citrobacter freundii* at a tertiary acute care facility in Miami, Florida. *Infect Control Hosp Epidemiol* 2017; **38**: 320–6.

30 Rodriguez C, Brengi S, Caceres MA *et al*. Polyclonal dissemination of KPC-2 in *Serratia marcescens*, including a clone with epidemic behaviour in the nosocomial niche. *Int J Antimicrob Agents* 2017; **49**: 657–8.

31 Silva KE, Cayo R, Carvalhaes CG *et al*. Coproduction of KPC-2 and IMP-10 in Carbapenem-Resistant *Serratia marcescens* Isolates from an Outbreak in a Brazilian Teaching Hospital. *J Clin Microbiol* 2015; **53**: 2324–8.

32 Venditti C, Fortini D, Villa L *et al*. Circulation of bla_{KPC-3}-carrying IncX3 plasmids among *Citrobacter freundii* isolates in an Italian hospital. *Antimicrob Agents Chemother* 2017; **61**: e00505-17.

33 Zhang R, Zhou HW, Cai JC *et al*. Plasmid-mediated carbapenem-hydrolysing β-lactamase KPC-2 in carbapenem-resistant *Serratia marcescens* isolates from Hangzhou, China. *J Antimicrob Chemother* 2007; **59**: 574–6.

34 Yang JH, Sheng WH, Hsueh PR *et al*. Antimicrobial susceptibility and distribution of extended-spectrum β-lactamases, AmpC β-lactamases and carbapenemases among *Proteus*, *Providencia* and *Morganella* isolated from global hospitalised patients with intra-abdominal and urinary tract infections: Results of the Study for Monitoring Antimicrobial Resistance Trends (SMART), 2008-2011. *J Glob Antimicrob Resist* 2020; **22**: 398–407.

35 Dubendris H, MacFarquhar J, Kornegay R *et al*. Imipenemase-producing carbapenem-resistant Enterobacteriaceae transmission in a long-term-care facility during a community-wide multidrug resistant organism outbreak-North Carolina, 2017. *Am J Infect Control* 2020; **48**: 320–3.

36 Janelle SJ, Kallen A, de Man T *et al*. Notes from the field: New Delhi metallo-β-lactamase-producing carbapenem-resistant Enterobacteriaceae identified in patients without known health care risk factors — Colorado, 2014-2016. *MMWR Morb Mortal Wkly Rep* 2016; **65**: 1414–5.

37 Potter RF, Wallace MA, McMullen AR *et al*. bla_{IMP-27} on transferable plasmids in *Proteus mirabilis* and *Providencia rettgeri*. *Clin Microbiol Infect* 2018; **24**: 1019.e5–e8.

38 Dixon N, Fowler RC, Yoshizumi A *et al*. IMP-27, a unique metallo-β-lactamase identified in geographically distinct isolates of *Proteus mirabilis*. *Antimicrob Agents Chemother* 2016; **60**: 6418–21.

39 Chae SR, Yaffee AQ, Weng MK *et al*. Notes from the field: investigation of carbapenemase-producing carbapenem-resistant Enterobacteriaceae among patients at a community hospital — Kentucky, 2016. *MMWR Morb Mortal Wkly Rep* 2018; **66**: 1410.

40 Shannon DJ, Blosser S, Walters M *et al*. Notes from the Field: domestically acquired verona integron-mediated metallo-β-lactamase-producing Enterobacteriaceae — Indiana, 2016-2017. *MMWR Morb Mortal Wkly Rep* 2018; **67**: 727–8.