Phenotypic and Molecular Characteristics of Carbapenem-Resistant *Enterobacteriaceae* in a Health Care System in Los Angeles, California, from 2011 to 2013

S. Pollett,^a S. Miller,^b J. Hindler,^b D. Uslan,^c M. Carvalho,^b R. M. Humphries^b

Marie Bashir Institute for Infectious Diseases and Biosecurity, University of Sydney, Sydney, Australia^a; Pathology & Laboratory Medicine, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA^b; Division of Infectious Diseases, David Geffen School of Medicine, University of California, Los Angeles, Los Angeles, California, USA^c

Carbapenem-resistant *Enterobacteriaceae* (CRE) are a concern for health care in the United States but remain relatively uncommon in California. We describe the phenotype, clonality, and carbapenemase-encoding genes present in CRE isolated from patients at a Californian tertiary health care system. CRE for this study were identified by evaluating the antibiograms of *Enterobacteriaceae* isolated in the UCLA Health System from 2011 to 2013 for isolates that were not susceptible to meropenem and/or imipenem. The identification of these isolates was subsequently confirmed by matrix-associated laser desorption/ionization–time of flight, and broth microdilution tests were repeated to confirm the CRE phenotype. Real-time PCR for *bla*_{KPC}, *bla*_{SME}, *bla*_{IMP}, *bla*_{NDM-1}, *bla*_{VIM}, and *bla*_{OXA-48} was performed. Clonality was assessed by repetitive sequence-based PCR (repPCR) and multilocus sequence typing (MLST). Of 15,839 nonduplicate clinical *Enterobacteriaceae* isolates, 115 (0.73%) met the study definition for CRE. This number increased from 0.5% (44/8165) in the first half of the study to 0.9% (71/7674) in the second ($P = 0.004$). The most common CRE species were *Klebsiella pneumoniae*, *Enterobacter aerogenes*, and *Escherichia coli*. A carbapenemase-encoding gene was found in 81.7% (94/115) of CRE and included *bla*_{KPC} (78.3%), *bla*_{NDM-1} (0.9%), and *bla*_{SME} (2.6%). The majority of *bla*_{KPC} genes were in *K. pneumoniae* isolates, which fell into 14 clonal groups on typing. *bla*_{KPC} was identified in more than one species of CRE cultured from the same patient in four cases. Three *bla*_{SME}-carrying *Serratia marcescens* isolates and one *bla*_{NDM-1} carrying *Providencia rettgeri* isolate were detected. CRE are increasing in California, and carbapenemases, particularly KPC, are a common mechanism for carbapenem resistance in this region.

Carbapenems are a critical reserve class of β -lactam antimicrobials for severe Gram-negative infections, particularly as resistance to other β -lactam classes is increasingly common (1, 2). The emergence of carbapenem-resistant *Enterobacteriaceae* (CRE) is a rapidly evolving global public health dilemma with recent intense media exposure and calls for urgent action within the international scientific community (3–6). In industrialized regions like Europe and the United States, CRE infections are typically associated with health care and include urinary tract infections, central line-associated bloodstream infections, medical device infections, wound infections, and pneumonia (3, 5).

Treatment options for CRE are extremely limited, and the mortality of CRE health care-associated infections (HAI) exceeds 50% in some case series (7, 8). An escalating incidence of CRE HAI in U.S. hospitals has been noted over the last decade, with 4.6% of acute-care hospitals in the United States and more than 17.8% of long-term-acute-stay hospitals (LTACH) having at least one CRE HAI in 2012 (3).

Enterobacteriaceae are common colonizers of the human intestinal tract, and CRE have been shown to colonize patients for prolonged periods, facilitating spread (9). The current CRE epidemic is thought to be fuelled by both clonal dissemination (patient-patient transmission) of CRE and horizontal transfer of carbapenemase-encoding genes between isolates (1, 5, 10). In the United States, KPC carbapenemases are the more prevalent of these enzymes (3). Shortly after the report of *bla*_{KPC} in an isolate of *Klebsiella pneumoniae* on the East Coast (North Carolina) in 2001 (11), *bla*_{KPC} was identified in isolates from hospitals across the United States, with rapid spread to the West Coast, including Cal-

ifornia, by 2009 (11, 12). Compounding this spread of *bla*_{KPC} is the recent sporadic but increasingly reported introduction of other carbapenemases, in particular NDM-1, from developing countries via medical tourism, migration, and travel (13).

Creating a standardized definition of CRE for research and surveillance purposes has been challenging. In 2012, the Centers for Disease Control and Prevention (CDC) issued an interim surveillance CRE definition, which includes isolates of *Enterobacteriaceae* that are not susceptible (i.e., MICs $>1 \mu\text{g/ml}$) to imipenem (except for *Providencia*, *Proteus*, or *Morganella species*), meropenem, or doripenem and excludes isolates if they are susceptible or intermediate to the third-generation cephalosporins (14). This CDC definition differs from some other CRE definitions used in the literature, which may include ertapenem resistance and would exclude carbapenemase-encoding isolates with a carbapenem-susceptible phenotype (5, 15). Moreover, the traditional phenotypic tests for carbapenemases, such as the modified Hodge test,

Received 15 May 2014 Returned for modification 18 June 2014

Accepted 5 September 2014

Published ahead of print 10 September 2014

Editor: D. J. Diekema

Address correspondence to R. M. Humphries, rhumphries@mednet.ucla.edu.

Supplemental material for this article may be found at <http://dx.doi.org/10.1128/JCM.01397-14>.

Copyright © 2014, American Society for Microbiology. All Rights Reserved.
doi:10.1128/JCM.01397-14

are now known to be associated with poor sensitivity and specificity for carbapenemases but remain the “reference” phenotypic method for CRE confirmation by the Clinical and Laboratory Standards Institute (CLSI), although new-generation phenotypic tests, such as the CarbaNP test, appear promising (16, 17).

The spread of CRE in the United States is potentially preventable or could be at least mitigated, particularly on the West Coast, where a lower incidence of CRE has been documented than in Eastern States (3). Region-specific detailed microbiological and molecular characterization of CRE isolates would aid such control efforts by creating a useful benchmark for future CRE surveillance and infection control interventions. While some state- and county-level surveillance of CRE occurs in California (18), detailed description of the phenotypic, molecular, and clonal characteristics of CRE in California or the United States is limited. Thus, the objectives of this study were the following: (i) to describe the proportion of clinically significant *Enterobacteriaceae* in a large Californian tertiary health care system that were CRE, (ii) to describe the phenotypic characteristics (including antibiograms) and clonality of CRE in this setting, and (iii) to determine the frequency of selected carbapenemase-encoding genes among these CRE.

(This study was presented in part as a poster at the 114th General Meeting of the American Society for Microbiology, Boston, MA, 2014.)

MATERIALS AND METHODS

Study setting and study population. UCLA Health is composed of two hospitals and associated community clinics located in West Los Angeles, California. Ronald Regan UCLA Medical Centre is a 540 patient bed referral facility with tertiary level surgical, transplant, pediatric and intensive care services. UCLA Medical Center, Santa Monica is an associated secondary-level 266-patient-bed hospital and services a region with a high density of skilled nursing facilities (SNFs) (<http://www.uclahealth.org/>; accessed September 2013). Both hospitals are associated with several long-term-acute-care hospitals (LTACHs).

Identification and inclusion of CRE from archived clinical isolates. Antimicrobial susceptibility test results for clinical isolates of *Enterobacteriaceae* (i.e., from patient cultures, not surveillance specimens) from patients receiving care at UCLA Health between 1 January 2011 and 31 January 2013 were reviewed. During this study period, the UCLA Health System was not performing surveillance cultures for CRE. The isolates were cultured from a variety of specimen types, including urine (midstream and catheter), blood, body fluid, pus, nasotracheal suction aspirate, tracheal aspirate, sputum, wound swabs, central venous line tips, and arterial line tips. Isolates were defined as CRE and included in the study if they were not susceptible to meropenem (i.e., MIC > 1 µg/ml) or imipenem (MIC > 1 µg/ml), both carbapenems which were routinely tested with all clinically significant isolates of *Enterobacteriaceae* in the UCLA clinical laboratory. Susceptibility testing at the time of isolation was performed using either Vitek2 (bioMérieux, Durham NC) for urine isolates or reference CLSI broth microdilution (BMD) for nonurine isolates, as described below. Per laboratory policy, all *Enterobacteriaceae* with a meropenem and/or imipenem MIC of > 1 µg/ml were retested by BMD to confirm the CRE phenotype prior to reporting final patient results.

CRE which demonstrated an MIC in the susceptible or intermediate range for third-generation cephalosporins were not excluded (unlike the recent CDC definitions of CRE), in order to potentially capture *bla*_{SME} and *bla*_{OXA-48} carbapenemases, carriers of which often test susceptible to these agents (14, 19). Isolates belonging to the *Providencia*, *Proteus*, or *Morganella* genus that demonstrated an MIC of > 1 µg/ml for imipenem alone were excluded from the study, since these species are intrinsically less susceptible to imipenem, with MICs of > 1 µg/ml (14). Duplicate CRE isolates (i.e., those of the same species, irrespective of specimen type) from

the same patient were excluded from the study. When duplicate isolates existed, the first CRE isolate cultured was included in the study. In cases of coinfection with two or more distinct CRE species, all nonduplicate species were included. Isolates were identified to the species level at the time of isolation from clinical specimens by routine methods, including a Vitek2 GNI card (bioMérieux, Durham NC), API 20E (bioMérieux), or other phenotypic tests.

Identifications of all isolates enrolled in this study were confirmed by matrix-assisted laser desorption/ionization–time of flight (MALDI-TOF) using the Vitek MS system (bioMérieux). MALDI-TOF analysis was performed as previously described (20).

Antimicrobial susceptibility testing. Isolates stored at −70°C were subcultured twice and tested using the CLSI reference BMD method with panels prepared in-house (Difco, Detroit MI) (21). The following antimicrobials were tested: meropenem, imipenem, ertapenem, ceftriaxone, ceftazidime, ceftipime, ciprofloxacin, levofloxacin, trimethoprim-sulfamethoxazole, gentamicin, tobramycin, and amikacin. MICs were interpreted using CLSI M100-S24 interpretive criteria (22). In addition, the ceftipime MIC for each isolate was interpreted with the current CLSI susceptible breakpoint of ≤ 2 µg/ml and the former CLSI susceptible breakpoint of ≤ 8 µg/ml (23). Isolates with carbapenem MICs repeatedly and significantly discordant from the original MIC results of the banked isolate (i.e., loss of carbapenem-nonsusceptible phenotype) were removed from the study.

Detection of carbapenemases. Genomic DNA was isolated using the NucliSENS easyMAG system (bioMérieux). Three multiplexed TaqMan-based real-time PCRs were performed to investigate the presence of carbapenemase. PCR 1 consisted of amplification of *bla*_{KPC} and *bla*_{NDM-1}, PCR 2 of *bla*_{VIM} and *bla*_{IMP}, and PCR 3 of *bla*_{OXA-48} and *bla*_{SME}. Each reaction also included a third target for the universal bacterial 16S rRNA-encoding gene as an endogenous control for DNA amplification (24). PCRs were carried out using the Roche LightCycler 480 instrument (Roche, Indianapolis, IN). Each PCR included 1 U AmpliTaq DNA polymerase (Invitrogen, Grand Island, NY), 1× PCR buffer mix supplemented with magnesium chloride (3 mM for primer mix 1 or 4 mM for primer mixes 2 and 3), 0.2 mM deoxynucleoside triphosphate (dNTP) mix, a combined primer/probe mix, and 2 µl of template (see Table S1 in the supplemental material). All isolates were screened individually by mix 1 but were pooled ($n = 10$ per pool) for screening by mix 2 and mix 3, after verification that this did not impact the sensitivity of the PCRs (not shown). Positive pools were broken down to determine which isolates harbored the carbapenemase-encoding gene. Positive controls included for each carbapenemase primer/probe mix were as follows: *bla*_{KPC} control, *K. pneumoniae* ATCC BAA-1705; *bla*_{NDM-1} control, *K. pneumoniae* ATCC BAA-2146. For *bla*_{VIM}, *bla*_{IMP}, *bla*_{SME}, and *bla*_{OXA-48}, well-characterized clinical isolates were used as controls. The negative control for each primer/probe mix was the positive control for the opposing carbapenemase in the mix. Internal verification studies ensured that both carbapenemases would be detected if present in a single bacterium (not shown). Cycling conditions were a 3-min enzyme activation step at 95°C, followed by 40 cycles of 10 s at 95°C and 30 s at 60°C and a final cycle of 1 min at 40°C.

All CRE isolates negative by real-time PCR for the carbapenemase genes evaluated were further tested using two phenotypic carbapenemase assays, the CarbaNP and the Rosco Rapid CARB screen (Rosco Diagnostica A/S, Taastrup, Denmark), according to the manufacturer's instructions and/or methods described elsewhere (17, 25).

Determination of CRE clonality among isolates harboring *bla*_{KPC}. DiversiLab typing was performed with *K. pneumoniae* isolates with *bla*_{KPC}. Extracted DNA was quantified using a NanoDrop-2000 spectrophotometer (NanoDrop, Wilmington, DE). The repetitive sequence-based PCR (repPCR) amplification was performed using the *Klebsiella* DL fingerprinting kit according to the manufacturer's instructions (bioMérieux) and as previously described (26). Data were analyzed using Pearson's correlation coefficient to establish distance matrices and unweighted-pair

TABLE 1 Identities and antimicrobial agent susceptibilities of all CRE isolates studied^a

Characteristic or group	No. (%) of isolates
Total nonduplicate CRE	115 (100)
Species	
<i>Klebsiella pneumoniae</i>	87 (75.7)
<i>Klebsiella oxytoca</i>	3 (2.6)
<i>Enterobacter aerogenes</i>	9 (7.8)
<i>Enterobacter cloacae</i>	2 (1.7)
<i>Citrobacter freundii</i>	4 (3.5)
<i>Escherichia coli</i>	5 (4.3)
<i>Serratia marcescens</i>	4 (3.5)
<i>Providencia rettgeri</i>	1 (0.9)
Susceptible to antimicrobial	
Meropenem	5 (4.3)
Imipenem	2 (1.7)
Ertapenem	0 (0.0)
Ceftriaxone	5 (4.3)
Ceftazidime	4 (3.5)
Cefepime ^b	21 (18.3)
Cefepime ^c	9 (7.8)
Ciprofloxacin	21 (18.3)
Piperacillin-tazobactam	3 (2.6)
Levofloxacin	21 (18.3)
Trimethoprim-sulfamethoxazole	33 (28.7)
Gentamicin	72 (62.6)
Tobramycin	18 (15.7)
Amikacin	78 (67.8)

^a CRE, carbapenem-resistant *Enterobacteriaceae*.^b MIC $\leq$ 8 μ g/ml, CLSI 2013 breakpoint.^c MIC $\leq$ 2 μ g/ml, CLSI 2014 breakpoint.

group method using average linkages (UPGMA) to create dendrograms. Criteria used to define isolates as indistinguishable, similar, or unrelated were as follows: indistinguishable is 0 to 1 band difference and $>97\%$ similarity; similar is 2 band differences and $\geq 95\%$ similarity; unrelated is ≥ 3 band differences and $<95\%$ similarity.

Multilocus sequence typing (MLST) was performed with representative isolates from each repPCR clonal type according to the protocol described by the *K. pneumoniae* MLST website and by Diancourt et al. (27, 28). Allele sequence types (STs) were determined by querying MSLT results against this international database.

Statistical analyses. Descriptive analyses were performed using the SPSS software program (version 21; IBM, New York). Ninety-five percent confidence intervals of all proportions were determined. Paired proportion and independent proportion comparisons were determined by binomial testing, McNemar's test, chi-square test, or Fisher's exact test, where appropriate.

Ethical considerations. This study was approved by the UCLA Institutional Review Board (IRB).

RESULTS

Incidence of CRE from January 2011 through January 2013. Of 15,841 nonduplicate *Enterobacteriaceae* isolated from patients attending UCLA Health between 1 January 2011 and 31 January 2013 and tested for antimicrobial susceptibility, 126 met the study criteria for CRE. Eleven were eliminated because the original carbapenem results and/or isolate identification were not confirmed upon repeat testing.

One hundred fifteen of 15,839 *Enterobacteriaceae* (0.73% [0.61% to 0.88%, 95% confidence interval]), which were isolated

TABLE 2 Carbapenemase-encoding genes identified in CRE isolates studied^a

Species	No. of isolates:			
	Total	With carbapenemase gene detected		
		<i>bla</i> _{KPC}	<i>bla</i> _{SME}	<i>bla</i> _{NDM-1}
<i>Klebsiella pneumoniae</i>	87	80		
<i>Klebsiella oxytoca</i>	3	2		
<i>Enterobacter aerogenes</i>	9			
<i>Enterobacter cloacae</i>	2			
<i>Citrobacter freundii</i>	4	3		
<i>Escherichia coli</i>	5	4		
<i>Serratia marcescens</i>	4	1	3	
<i>Providencia rettgeri</i>	1			1
All	115	90	3	1

^a CRE, Carbapenem-resistant *Enterobacteriaceae*.^b Isolates did not harbor any of the following genes: *bla*_{KPC}, *bla*_{NDM-1}, *bla*_{SME}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{OXA-48}.

from 110 patients, thus ultimately met the study definition for CRE. A statistically significant increase in CRE frequency during the study period was noted, with 0.5% (44/8,165) CRE in the first half of the study period (1 January 2011 to 16 January 2012), increasing to 0.9% (71/7,674) in the second half of the study period (17 January 2012 to 31 January 2013) ($P = 0.004$).

Cumulative antibiogram of CRE. Table 1 presents the identities and cumulative antibiograms of the 115 CRE isolates studied. There was no statistically significant difference in the percentages of CRE susceptible to meropenem and those susceptible to imipenem (4.3% versus 1.7%; $P = 0.16$). All CRE were ertapenem nonsusceptible (MIC > 0.5 μ g/ml). Of 5/115 (4.3%) isolates with susceptibility to ceftriaxone, ceftazidime, or both, 3 were *Serratia marcescens* (all susceptible to ceftriaxone with MICs of ≤ 0.5 μ g/ml and to ceftazidime with MICs of ≤ 0.5 μ g/ml) and the remaining 2 were *Klebsiella* species (*K. oxytoca* and *K. pneumoniae*, both susceptible to ceftriaxone [MIC = 1 μ g/ml] and nonsusceptible to ceftazidime [MIC = 8 μ g/ml]). Only 21/115 isolates (18.3%) were susceptible to ciprofloxacin and levofloxacin, and 33/115 isolates (28.7%) were susceptible to trimethoprim-sulfamethoxazole. Applying the 2013 CLSI cefepime breakpoint (≤ 8 μ g/ml), 21/115 (18.3%) CRE were susceptible to cefepime, but with the 2014 breakpoint (≤ 2 μ g/ml), only 9/115 (7.8%) were susceptible. No significant difference was noted in susceptibility to amikacin (67.8%) compared to that to gentamicin (62.6%) ($P = 0.52$), whereas a significantly greater proportion of CRE were susceptible to gentamicin than to tobramycin (15.7%) ($P < 0.001$). Nine of one hundred fifteen (7.8%) isolates were interpreted as intermediate or resistant to all antimicrobials tested. Colistin, tigecycline, aztreonam, and fosfomycin were not tested in this study.

Resistance mechanism and antibiograms by carbapenemase gene. A carbapenemase-encoding gene was detected in 94/115 (81.7%) of CRE isolates (Table 2), with 78.3% (90/115) of all CRE study isolates harboring *bla*_{KPC}, 2.6% (3/115) harboring *bla*_{SME}, and 1 isolate harboring *bla*_{NDM-1}. All CRE negative for the presence carbapenemase-encoding genes by real-time PCR were also negative for carbapenemase production by both the CarbaNP and Rosco Rapid CARB phenotypic assays.

Antimicrobial susceptibilities for the *bla*_{KPC}-carrying CRE are listed in Table 3. The antimicrobial agents to which these isolates

TABLE 3 Antimicrobial susceptibility, by carbapenemase-encoding gene detected

Antimicrobial agent	No. (%) of susceptible isolates, by carbapenemase gene detected ^c			
	<i>bla</i> _{KPC}	<i>bla</i> _{SME}	<i>bla</i> _{NDM-1}	None ^d
Carbapenems				
Meropenem	2 (2)	0 (0)	0 (0)	3 (14)
Imipenem	0 (0)	0 (0)	0 (0)	2 (10)
Ertapenem	0 (0)	0 (0)	0 (0)	0 (0)
Cephems				
Ceftriaxone	0 (0)	3 (100)	0 (0)	2 (10)
Ceftazidime	1 (1)	3 (100)	0 (0)	0 (0)
Cefepime ^a	7 (8)	3 (100)	0 (0)	11 (52)
Cefepime ^b	0 (0)	3 (100)	0 (0)	6 (29)
β-Lactamase inhibitor combinations				
Piperacillin-tazobactam	0 (0)	3 (100)	0 (0)	0 (0)
Fluoroquinolones				
Ciprofloxacin	8 (9)	3 (100)	1 (100)	9 (43)
Levofloxacin	8 (9)	3 (100)	1 (100)	9 (43)
Aminoglycosides				
Gentamicin	51 (57)	3 (100)	1 (100)	17 (81)
Tobramycin	2 (2)	3 (100)	1 (100)	12 (57)
Amikacin	58 (64)	3 (100)	1 (100)	16 (76)
Trimethoprim-sulfamethoxazole	20 (22)	3 (100)	0 (0)	10 (48)

^a MIC ≤ 8 μg/ml, CLSI 2013 breakpoint.^b MIC ≤ 2 μg/ml, CLSI 2014 breakpoint.^c For *bla*_{KPC}, *n* = 90; for *bla*_{SME}, *n* = 3; for *bla*_{NDM-1}, *n* = 1; for "None," *n* = 21.^d Isolates did not harbor any of the following genes: *bla*_{KPC}, *bla*_{NDM-1}, *bla*_{SME}, *bla*_{VIM}, *bla*_{IMP}, and *bla*_{OXA-48}.

tested susceptible most frequently were amikacin (58/90, 64%) and gentamicin (51/90; 57%), followed by trimethoprim-sulfamethoxazole (20/90; 22%). When the 2014 CSLI cefepime breakpoints were applied (MIC ≤ 2 μg/ml), no *bla*_{KPC}-carrying CRE were susceptible to cefepime. Two *bla*_{KPC}-containing isolates (*K. oxytoca* and *Escherichia coli*) originally tested as meropenem non-susceptible (MIC = 2 μg/ml) but tested as meropenem susceptible (MIC = 1 μg/ml) in a repeat test on BMD. Since original and repeat test results were within 1 dilution, they did not warrant further testing. Additionally, these isolates met the study CRE definition by consistently testing as imipenem nonsusceptible (MIC = 4 and 2 μg/ml, respectively).

Compared to non-carbapenemase-carrying CRE, *bla*_{KPC}-carrying CRE were less frequently susceptible to non-β-lactam antimicrobial agents, including tobramycin (*P* < 0.001), ciprofloxacin (*P* < 0.001), levofloxacin (*P* < 0.001), and trimethoprim-sulfamethoxazole (*P* = 0.018). The median MIC for imipenem and meropenem was the same (8 μg/ml) in non-*bla*_{KPC}- and *bla*_{KPC}-carrying *K. pneumoniae* isolates.

Table 3 also presents the antibiograms of CRE harboring carbapenemases other than *bla*_{KPC}. All four isolates were susceptible to aminoglycosides and fluoroquinolones. All three *bla*_{SME} *S. marcescens* isolates were susceptible to ceftriaxone, ceftazidime, and cefepime (MIC ≤ 0.5 μg/ml). Two additional CRE isolates (*K. pneumoniae* with a meropenem MIC of 2 μg/ml, imipenem MIC of 1 μg/ml, and ceftriaxone MIC of 1 μg/ml and *K. oxytoca*

TABLE 4 Multilocus sequence typing results for representative *bla*_{KPC}-containing *K. pneumoniae* isolates^a

repPCR clonal type	No. of isolates	Allele no. ^b							ST
		<i>rpoB</i>	<i>mdh</i>	<i>pgi</i>	<i>phoE</i>	<i>infB</i>	<i>gapA</i>	<i>tonB</i>	
1	35	1	1	1	1	3	3	79	258
2	11	1	1	1	1	3	3	79	258
3	7	1	1	1	1	3	3	79	258
4	6	1	1	1	1	3	3	79	258
5	5	9	1	1	1	3	3	79	1084
6	3	1	1	1	1	3	3	79	258
7	3	1	1	1	1	3	3	79	258
8	3	1	1	1	1	3	3	79	258
9	2	1	1	1	1	3	3	79	258
10	1	1	1	1	1	3	3	79	258
11	1	1	1	2	5	1	12	36	133
12	1	7	6	75	9	3	2	137	643
13	1	22	21	27	47	24	16	NS	NT
14	1	22	21	ND	47	24	16	NS	NT

^a ST, sequence type; NS, not able to sequence; NT, nontypeable due to missing allele sequence(s); ND, not determined since no matching allele type in database.^b Refers to an allele profile which corresponds to a match in the MLST database.

with a meropenem MIC of 8 μg/ml, imipenem MIC of 4 μg/ml, and ceftriaxone MIC of 1 μg/ml) were susceptible to ceftriaxone, but no carbapenemase was detected in either of these isolates. Thus, of the five *Enterobacteriaceae* which were susceptible to 3rd-generation cephalosporins and would have been excluded by CDC definitions for CRE, three carried *bla*_{SME}.

*bla*_{NDM-1} was detected in a single isolate, of *Providencia rettgeri*. Given the rarity of this carbapenemase in this region, clinical information was derived from the medical file, with IRB approval. This isolate was related to a catheter-associated urinary tract infection in a functionally poor patient with a history of hospitalization in South India for an ankle fracture 2 years prior to presentation at UCLA and recovery of the isolate.

Isolation of multiple CRE from individual patients. In four patients, more than one CRE genus was isolated during the study period, and all harbored the *bla*_{KPC} gene. In the first patient, *E. coli* and then *K. pneumoniae* were isolated (15 days apart). In the second patient, *Enterobacter cloacae* and then *K. pneumoniae* were isolated (44 days apart). In the third patient, *K. pneumoniae* and then *S. marcescens* were isolated (36 days apart). In the fourth patient, 3 different genera of CRE were isolated: an *E. coli* isolate, followed by a *Citrobacter freundii* isolate 1 day later, and then *K. pneumoniae* 19 days thereafter.

Clonal analysis and typing of *K. pneumoniae* isolates positive for *bla*_{KPC}. The dendrogram of *bla*_{KPC}-expressing *K. pneumoniae* is presented in Fig. S1 in the supplemental material. Most *bla*_{KPC}-carrying *K. pneumoniae* isolates belonged to clonal groups on typing. Five of the eighty isolates were unrelated to any others recovered over the study period. The remaining 75 isolates fell into 9 clonal groups, containing the following numbers of isolates: 2, 3, 3, 3, 5, 6, 7, 11, and 35 (Table 4). MLST analysis showed that 9 out of 14 (64%) representative isolates from each clonal group had an MLST type that matched ST 258. Two isolates could not be typed based on missing sequence at the *tonB* allele (repPCR clonal types 13 and 14). In one case (repPCR clonal type 14), the *pgi* allele was not defined because it did not match any of the previously characterized STs; however, it is a single-locus variant of *pgi* allele type 66.

DISCUSSION

While CRE remained uncommon in our health care system, the frequency of CRE increased over the 25-month study period, a trend observed across the United States (3). The incidence of CRE would appear to be lower at our institution than in other regions of the United States and the overall national prevalence, which was recently estimated at 1.4% to 4.2% (3).

Caution is needed in the comparison of our results with those of others, due in some cases to different study populations and denominators, different carbapenem breakpoints, and different definitions of CRE (29, 30). Surveillance data recorded by the California State Public Health Department in 2010 showed that overall, 5.1% (out of 24,611 isolates) of *Klebsiella* species were CRE (31), but this is also difficult to compare with our results, since reporting of CRE is not mandatory on a state level. Furthermore, such statewide surveillance data are likely to be derived from labs using different methods of antimicrobial susceptibility testing and interpretive breakpoints. It is well recognized that some automated susceptibility systems may under- or overreport carbapenem resistance, compared to the reference BMD used in the present study (32).

The most common CRE species observed in this study were *K. pneumoniae*, followed by *E. aerogenes* and *E. coli*. This distribution of CRE species is consistent with that of other CRE studies in California, the United States, and Europe (3, 33–35) and is consistent with the CDC's recommendations that *K. pneumoniae*, *E. coli*, and *Enterobacter* spp. are the key health care-associated pathogens to focus on in the control of the U.S. CRE epidemic (14).

Carbapenemases were detected in 81.7% (94/115) of CRE isolates. The remaining 18.3% of isolates were likely carbapenem nonsusceptible due to a combination of a cephalosporinase with weak carbapenemase activity, such as *ampC*, and a porin mutation in the *ompK35* or *ompK36* gene (36). The other possibility includes rarer carbapenemases, such as *bla_{NMC-A}*, and *bla_{GES}*, although these have not been reported to date in the United States. In addition, all isolates that were negative for the carbapenemase genes assayed in our study were negative for carbapenemase by the phenotypic Carba NP assay (2). The fact that 18.3% of CRE were carbapenemase negative suggests that an imipenem or meropenem MIC in the intermediate or resistant range may be relatively nonspecific in predicting carbapenemase carriage.

bla_{KPC} was the most common carbapenemase-encoding gene detected, as is the case in the rest of the United States (2, 3). Those CRE carrying *bla_{KPC}* were more likely to be resistant to multiple classes of antimicrobial agents than those CRE not carrying *bla_{KPC}* or other carbapenemases. This has been noted in other studies, with *bla_{KPC}* often accompanied by other genes that encode resistance to fluoroquinolones or aminoglycosides (37, 38). Most of the *bla_{KPC}* *K. pneumoniae* strains seen in this study clustered into multiple clonal groups. Additionally, the majority of isolates tested by MLST (representing 71 of 80 total isolates) matched to ST 258, a result consistent with reports of other KPC-carrying *K. pneumoniae* isolates spread via clonal expansion in the United States (12). Given the frequency of the ST 258 clone in *bla_{KPC}*-carrying *K. pneumoniae* in the central and eastern U.S. states, this finding may support a model of dissemination of *bla_{KPC}*-bearing CRE from eastern to western states (10, 12). However, since KPC-producing ST 258 *K. pneumoniae* isolates are well described for

other global regions, such clones may also have been introduced by international travel (7, 12, 29).

These data support a model of *bla_{KPC}* transmission in this region at least partly explained by clonal dissemination and patient-to-patient spread, as has been described for other regions of the world (36, 39–41). Such clones may be circulating in our region, within nearby long-term-care facilities or perhaps in the community in those who have previously been hospitalized. The often lengthy continued CRE carriage in patients would facilitate this clonal dissemination (9). That multiple clones are cocirculating suggests that the CRE seen in this health system is not simply a prolonged outbreak from one source.

The presence of the *bla_{KPC}* in 2 to 3 different genera of *Enterobacteriaceae* within some patients is consistent with the transfer of resistance genes between bacteria within a host. Such a mixture of horizontal gene transfer and clonal expansion has been demonstrated elsewhere in the United States with plasmid linkage and clonal typing (42–44). *bla_{SME}* and *bla_{NDM-1}* carbapenemases were detected in this study. Detection of *bla_{SME}* is clinically important because its presence suggests that 3rd- and 4th-generation cephalosporins might be used as therapy when isolates test susceptible to cephalosporins, although the success of such therapy has not been well validated (19). The detection of *bla_{NDM-1}* was of crucial importance for epidemiological and clinical reasons. While *bla_{NDM-1}*-expressing *P. rettgeri* has been found in Latin America, this is the first such isolate reported in the United States (45, 46). *bla_{NDM-1}* is most commonly found in *K. pneumoniae* and *E. coli* isolates but has been found in an increasing number of *Enterobacteriaceae* genera, a testament to its particularly mobile encoding plasmid (13, 47, 48). *bla_{NDM-1}* isolates typically coexpress resistance to aminoglycosides (unlike the isolate seen here), quinolones, and trimethoprim-sulfamethoxazole (47), and resistance to last-line agents such as colistin is well described (49). *bla_{NDM-1}* still remains rare in this region, with only 5 cases reported previously in California (13, 50, 51) and just over 40 isolates in total reported within the whole of the United States (13, 52, 53). Given the rapid spread of the *bla_{NDM-1}* carbapenemase since its discovery in Europe only 5 years ago (5, 54, 55), timely detection and aggressive infection control approaches to both colonized or infected cases in this region are crucial to prevent its establishment as an endemic carbapenemase in California hospitals or long-term-care facilities. The *bla_{NDM-1}* isolate in this study was associated with both a chronic indwelling urinary device and hospitalization in South Asia, two key risk factors described in other cases both in the United States and other Western regions (13, 56–58).

This study had several limitations. First, epidemiological and clinical data were not collected. Such data would have been useful not only for clinicians and public health organizations but for microbiologists in interpreting these results. Second, we did not test for susceptibility to last-line agents, such as colistin, tigecycline, and fosfomycin. While parenteral fosfomycin is not available in the United States, including such agents in susceptibility results for future CRE studies in this health system and region would be useful for clinicians choosing empirical therapy for undifferentiated septic shock in patients with a high risk for CRE. Resistance to such agents, in addition to concerns regarding efficacy and/or toxicity, have been well described for other regions (36). Third, we only tested those isolates that demonstrated imipenem or meropenem MICs in the intermediate or resistant ranges. Carbapenemases have been detected in carbapenem-sus-

ceptible *Enterobacteriaceae*, particularly *bla*_{OXA-48} (5, 59). Intuitively, such carbapenemases associated with a susceptible phenotype may not be clinically significant, although animal data suggest carbapenem treatment failure with such carbapenemase producers regardless of the susceptible-range MICs (60). Finally, we only considered clinically significant isolates, and this likely underestimates the burden of colonized patients, which may effectively spread CRE through the region, LTACH, and SNFs (43). Given that CRE are increasing in this region, increasing escalation of local infection control measures beyond core measures to active surveillance may be useful, as suggested by the CDC (14).

Our findings create a useful benchmark for future CRE and carbapenemase surveillance and future CRE infection control interventions. The above limitations could be addressed in future studies of CRE in this region. Such studies will be useful in the approach to local CRE control before they become endemic within Californian hospitals.

ACKNOWLEDGMENTS

We thank Amy Mathers for the *bla*_{OXA-48} reference strain. We also thank Myra Maldonado for her technical assistance.

REFERENCES

- Queenan AM, Bush K. 2007. Carbapenemases: the versatile beta-lactamases. *Clin. Microbiol. Rev.* 20:440–458. <http://dx.doi.org/10.1128/CMR.00001-07>.
- Castanheira M, Farrell SE, Deshpande LM, Mendes RE, Jones RN. 2013. Prevalence of beta-lactamase-encoding genes among *Enterobacteriaceae* bacteremia isolates collected in 26 U.S. hospitals: report from the SENTRY Antimicrobial Surveillance Program (2010). *Antimicrob. Agents Chemother.* 57:3012–3020. <http://dx.doi.org/10.1128/AAC.02252-12>.
- Centers for Disease Control and Prevention. 2013. Vital signs: carbapenem-resistant *Enterobacteriaceae*. *MMWR Morbid. Mortal. Wkly. Rep.* 62:165–170.
- Bush K. 2013. Proliferation and significance of clinically relevant beta-lactamases. *Ann. N. Y. Acad. Sci.* 1277:84–90. <http://dx.doi.org/10.1111/nyas.12023>.
- Nordmann P, Naas T, Poirel L. 2011. Global spread of Carbapenemase-producing *Enterobacteriaceae*. *Emerg. Infect. Dis.* 17:1791–1798. <http://dx.doi.org/10.3201/eid1710.110655>.
- Nordmann P, Cornaglia G. 2012. Carbapenemase-producing *Enterobacteriaceae*: a call for action! *Clin. Microbiol. Infect.* 18:411–412. <http://dx.doi.org/10.1111/j.1469-0691.2012.03795.x>.
- Chen LF, Anderson DJ, Paterson DL. 2012. Overview of the epidemiology and the threat of *Klebsiella pneumoniae* carbapenemases (KPC) resistance. *Infect. Drug Resist.* 5:133–141. <http://dx.doi.org/10.2147/IDR.S26613>.
- Mammina C, Bonura C, Di Bernardo F, Aleo A, Fasciana T, Sodano C. 2012. Ongoing spread of colistin-resistant *Klebsiella pneumoniae* in different wards of an acute general hospital, Italy, June to December 2011. *Euro Surveill.* 17:pi=20248.
- Zimmerman FS, Assous MV, Bdoah-Abram T, Lachish T, Yinnon AM, Wiener-Well Y. 2013. Duration of carriage of carbapenem-resistant *Enterobacteriaceae* following hospital discharge. *Am. J. Infect. Control* 41:190–194. <http://dx.doi.org/10.1016/j.ajic.2012.09.020>.
- Castanheira M, Farrell SE, Wanger A, Rolston KV, Jones RN, Mendes RE. 2013. Rapid expansion of KPC-2-producing *Klebsiella pneumoniae* isolates in two Texas hospitals due to clonal spread of ST258 and ST307 lineages. *Microb. Drug Resist.* 19:295–297. <http://dx.doi.org/10.1089/mdr.2012.0238>.
- Yigit H, Queenan AM, Anderson GJ, Domenech-Sanchez A, Biddle JW, Steward CD. 2001. Novel carbapenem-hydrolyzing beta-lactamase, KPC-1, from a carbapenem-resistant strain of *Klebsiella pneumoniae*. *Antimicrob. Agents Chemother.* 45:1151–1161. <http://dx.doi.org/10.1128/AAC.45.4.1151-1161.2001>.
- Kitchel B, Rasheed JK, Patel JB, Srinivasan A, Navon-Venezia S, Carmeli Y. 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. <http://dx.doi.org/10.1128/AAC.00126-09>.
- Rasheed JK, Kitchel B, Zhu W, Anderson KF, Clark NC, Ferraro MJ, Savard P, Humphries RM, Kallen AJ, Limbago BM. 2013. New Delhi metallo-beta-lactamase-producing *Enterobacteriaceae*, United States. *Emerg. Infect. Dis.* 19:870–878. <http://dx.doi.org/10.3201/eid1906.121515>.
- Centers for Disease Control and Prevention. 2012. Guidance for control of carbapenem-resistant *Enterobacteriaceae*. Centers for Disease Control and Prevention, Atlanta, GA. <http://www.cdc.gov/hai/pdfs/cre/cre-guidance-508.pdf>. Accessed May 2014.
- Braykov NP, Eber MR, Klein EY, Morgan DJ, Laxminarayan R. 2013. Trends in resistance to carbapenems and third-generation cephalosporins among clinical isolates of *Klebsiella pneumoniae* in the United States, 1999–2010. *Infect. Control Hosp. Epidemiol.* 34:259–268. <http://dx.doi.org/10.1086/669523>.
- Carvalhoes CG, Picao RC, Nicoletti AG, Xavier DE, Gales AC. 2010. Cloverleaf test (modified Hodge test) for detecting carbapenemase production in *Klebsiella pneumoniae*: be aware of false positive results. *J. Antimicrob. Chemother.* 65:249–251. <http://dx.doi.org/10.1093/jac/dkp431>.
- Vasoo S, Cunningham SA, Kohner PC, Simner PJ, Mandrekar JN, Lolans K, Hayden MK, Patel R. 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. <http://dx.doi.org/10.1128/JCM.00965-13>.
- Marquez P, Terashita D, Dassey D, Mascola L. 2013. Population-based incidence of carbapenem-resistant *Klebsiella pneumoniae* along the continuum of care, Los Angeles County. *Infect. Control Hosp. Epidemiol.* 34:144–150. <http://dx.doi.org/10.1086/669087>.
- Bush K, Pannell M, Lock JL, Queenan AM, Jorgensen JH, Lee RM, Lewis JS, Jarrett D. 2013. Detection systems for carbapenemase gene identification should include the SME serine carbapenemase. *Int. J. Antimicrob. Agents* 41:1–4. <http://dx.doi.org/10.1016/j.ijantimicag.2012.08.008>.
- Richter SS, Sercia L, Branda JA, Burnham CA, Bythrow M, Ferraro MJ, Garner OB, Ginocchio CC, Jennemann R, Lewinski MA, Manji R, Mochon AB, Rychert JA, Westblade LF, Procop GW. 2013. Identification of *Enterobacteriaceae* by matrix-assisted laser desorption/ionization time-of-flight mass spectrometry using the VITEK MS system. *Eur. J. Clin. Microbiol. Infect. Dis.* 32:1571–1578. <http://dx.doi.org/10.1007/s10096-013-1912-y>.
- Clinical and Laboratory Standards Institute. 2013. Methods for dilution antimicrobial susceptibility tests for aerobic bacteria. CLSI document M07-A9. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2014. Performance standards for antimicrobial susceptibility testing: 24th informational supplement. CLSI document M100-S24. Clinical and Laboratory Standards Institute, Wayne, PA.
- Clinical and Laboratory Standards Institute. 2013. Performance standards for antimicrobial susceptibility testing: 23rd informational supplement. CLSI document M100-S23. Clinical and Laboratory Standards Institute, Wayne, PA.
- Yang S, Lin S, Kelen GD, Quinn TC, Dick JD, Gaydos CA, Rothman RE. 2002. Quantitative multiprobe PCR assay for simultaneous detection and identification to species level of bacterial pathogens. *J. Clin. Microbiol.* 40:3449–3454. <http://dx.doi.org/10.1128/JCM.40.9.3449-3454.2002>.
- Huang TD, Berhin C, Bogaerts P, Glupczynski Y. 2014. Comparative evaluation of two chromogenic tests for rapid detection of carbapenemase in *Enterobacteriaceae* and in *Pseudomonas aeruginosa* isolates. *J. Clin. Microbiol.* 52:3060–3063. <http://dx.doi.org/10.1128/JCM.00643-14>.
- Deplano A, Denis O, Rodriguez-Villalobos H, De Ryck R, Struelens MJ, Hallin M. 2011. Controlled performance evaluation of the DiversiLab repetitive-sequence-based genotyping system for typing multidrug-resistant health care-associated bacterial pathogens. *J. Clin. Microbiol.* 49:3616–3620. <http://dx.doi.org/10.1128/JCM.00528-11>.
- Pasteur Institute. 2014. *Klebsiella pneumoniae* MLST Database. Pasteur Institute, Paris, France. <http://www.pasteur.fr/recherche/genopole/PF8/mlst/Kpneumoniae.html>. Accessed July 2014.
- Diancourt L, Passet V, Verhoef J, Grimont PA, Brisse S. 2005. Multilocus sequence typing of *Klebsiella pneumoniae* nosocomial isolates. *J. Clin. Microbiol.* 43:4178–4182. <http://dx.doi.org/10.1128/JCM.43.8.4178-4182.2005>.
- Woodford N, Eastaway AT, Ford M, Leanord A, Keane C, Quayle RM, Steer JA, Zhang J, Livermore DM. 2010. Comparison of BD Phoenix, Vitek 2, and MicroScan automated systems for detection and inference of mecha-

- nisms responsible for carbapenem resistance in *Enterobacteriaceae*. J. Clin. Microbiol. 48:2999–3002. <http://dx.doi.org/10.1128/JCM.00341-10>.
30. Vading M, Samuelsen O, Haldorsen B, Sundsfjord AS, Giske CG. 2011. Comparison of disk diffusion, Etest and VITEK2 for detection of carbapenemase-producing *Klebsiella pneumoniae* with the EUCAST and CLSI breakpoint systems. Clin. Microbiol. Infect. 17:668–674. <http://dx.doi.org/10.1111/j.1469-0691.2010.03299.x>.
 31. California Department of Public Health. 2010. California AntibioGram Project: aggregate susceptibility data of selected pathogens, 2010. California Department of Public Health, Sacramento, CA. <http://www.cdph.ca.gov/programs/hai/Documents/CAAntibiogram2010.pdf>. Accessed September 2013.
 32. Kulah C, Aktas E, Comert F, Ozlu N, Akyar I, Ankarali H. 2009. Detecting imipenem resistance in *Acinetobacter baumannii* by automated systems (BD Phoenix, Microscan WalkAway, Vitek 2); high error rates with Microscan WalkAway. BMC Infect. Dis. 9:30. <http://dx.doi.org/10.1186/1471-2334-9-30>.
 33. Hidron AI, Edwards JR, Patel J, Horan TC, Sievert DM, Pollock DA, Fridkin SK. 2008. NHSN annual update: antimicrobial-resistant pathogens associated with healthcare-associated infections: annual summary of data reported to the National Healthcare Safety Network at the Centers for Disease Control and Prevention, 2006–2007. Infect. Control Hosp. Epidemiol. 29:996–1011. <http://dx.doi.org/10.1086/591861>.
 34. Nordmann P, Cuzon G, Naas T. 2009. The real threat of *Klebsiella pneumoniae* carbapenemase-producing bacteria. Lancet Infect. Dis. 9:228–236. [http://dx.doi.org/10.1016/S1473-3099\(09\)70054-4](http://dx.doi.org/10.1016/S1473-3099(09)70054-4).
 35. Akova M, Daikos GL, Tzouveleklis L, Carmeli Y. 2012. Interventional strategies and current clinical experience with carbapenemase-producing Gram-negative bacteria. Clin. Microbiol. Infect. 18:439–448. <http://dx.doi.org/10.1111/j.1469-0691.2012.03823.x>.
 36. Kim SY, Shin J, Shin SY, Ko KS. 2013. Characteristics of carbapenem-resistant *Enterobacteriaceae* isolates from Korea. Diagn. Microbiol. Infect. Dis. 76:486–490. <http://dx.doi.org/10.1016/j.diagmicrobio.2013.04.006>.
 37. Huang S, Dai W, Sun S, Zhang X, Zhang L. 2012. Prevalence of plasmid-mediated quinolone resistance and aminoglycoside resistance determinants among carbapenem non-susceptible *Enterobacter cloacae*. PLoS One 7:e47636. <http://dx.doi.org/10.1371/journal.pone.0047636>.
 38. Canton R, Akova M, Carmeli Y, Giske CG, Glupczynski Y, Gniadkowski M, Livermore DM, Miriagou V, Naas T, Rossolini GM, Samuelsen O, Seifert H, Woodford N, Nordmann P. 2012. Rapid evolution and spread of carbapenemases among *Enterobacteriaceae* in Europe. Clin. Microbiol. Infect. 18:413–431. <http://dx.doi.org/10.1111/j.1469-0691.2012.03821.x>.
 39. Dai W, Sun S, Yang P, Huang S, Zhang X, Zhang L. 2013. Characterization of carbapenemases, extended spectrum beta-lactamases and molecular epidemiology of carbapenem-non-susceptible *Enterobacter cloacae* in a Chinese hospital in Chongqing. Infect. Genet. Evol. 14:1–7. <http://dx.doi.org/10.1016/j.meegid.2012.10.010>.
 40. Huang SR, Liu MF, Lin CF, Shi ZY. 2014. Molecular surveillance and clinical outcomes of carbapenem-resistant *Escherichia coli* and *Klebsiella pneumoniae* infections. J. Microbiol. Immunol. Infect. 47:187–196. <http://dx.doi.org/10.1016/j.jmii.2012.08.029>.
 41. Cuzon G, Naas T, Truong H, Villegas MV, Wisell KT, Carmeli Y, Gales AC, Venezia SN, Quinn JP, Nordmann P. 2010. Worldwide diversity of *Klebsiella pneumoniae* that produce beta-lactamase blaKPC-2 gene. Emerg. Infect. Dis. 16:1349–1356. <http://dx.doi.org/10.3201/eid1609.091389>.
 42. Mathers AJ, Cox HL, Kitchel B, Bonatti H, Brassinga AK, Carroll J, Scheld WM, Hazen KC, Sifri CD. 2011. Molecular dissection of an outbreak of carbapenem-resistant enterobacteriaceae reveals intergenus KPC carbapenemase transmission through a promiscuous plasmid. mBio 2(6):e00204–11. <http://dx.doi.org/10.1128/mBio.00204-11>.
 43. Viau RA, Hujer AM, Marshall SH, Perez F, Hujer KM, Briceno DF, Dul M, Jacobs MR, Grossberg R, Toltzis P, Bonomo RA. 2012. “Silent” dissemination of *Klebsiella pneumoniae* isolates bearing *K. pneumoniae* carbapenemase in a long-term care facility for children and young adults in Northeast Ohio. Clin. Infect. Dis. 54:1314–1321. <http://dx.doi.org/10.1093/cid/cis036>.
 44. Sheng JF, Li JJ, Tu S, Sheng ZK, Bi S, Zhu MH, Shen XM, Li LJ. 2012. blaKPC and rmtB on a single plasmid in *Enterobacter amnigenus* and *Klebsiella pneumoniae* isolates from the same patient. Eur. J. Clin. Microbiol. Infect. Dis. 31:1585–1591. <http://dx.doi.org/10.1007/s10096-011-1481-x>.
 45. Carvalho-Assef AP, Pereira PS, Albano RM, Beriao GC, Chagas TP, Timm LN, Da Silva RC, Falci DR, Asensi MD. 2013. Isolation of NDM-producing *Providencia rettgeri* in Brazil. J. Antimicrob. Chemother. 68:2956–2957. <http://dx.doi.org/10.1093/jac/dkt298>.
 46. Barrios H, Garza-Ramos U, Reyna-Flores F, Sanchez-Perez A, Rojas-Moreno T, Garza-Gonzalez E, Llaca-Diaz JM, Camacho-Ortiz A, Guzman-Lopez S, Silva-Sanchez J. 2013. Isolation of carbapenem-resistant NDM-1-positive *Providencia rettgeri* in Mexico. J. Antimicrob. Chemother. 68:1934–1936. <http://dx.doi.org/10.1093/jac/dkt124>.
 47. Castanheira M, Deshpande LM, Farrell SE, Shetty S, Shah N, Jones RN. 2013. Update on the prevalence and genetic characterization of NDM-1-producing *Enterobacteriaceae* in Indian hospitals during 2010. Diagn. Microbiol. Infect. Dis. 75:210–213. <http://dx.doi.org/10.1016/j.diagmicrobio.2012.10.017>.
 48. Nordmann P, Poirel L, Walsh TR, Livermore DM. 2011. The emerging NDM carbapenemases. Trends Microbiol. 19:588–595. <http://dx.doi.org/10.1016/j.tim.2011.09.005>.
 49. Kumarasamy KK, Toleman MA, Walsh TR, Bagaria J, Butt F, Balakrishnan R, Chaudhary U, Doumith M, Giske CG, Irfan S, Krishnan P, Kumar AV, Maharjan S, Mushtaq S, Noorie T, Paterson DL, Pearson A, Perry C, Pike R, Rao B, Ray U, Sarma JB, Sharma M, Sheridan E, Thirunarayan MA, Turton J, Upadhyay S, Warner M, Welfare W, Livermore DM, Woodford N. 2010. Emergence of a new antibiotic resistance mechanism in India, Pakistan, and the UK: a molecular, biological, and epidemiological study. Lancet Infect. Dis. 10:597–602. [http://dx.doi.org/10.1016/S1473-3099\(10\)70143-2](http://dx.doi.org/10.1016/S1473-3099(10)70143-2).
 50. Mochon AB, Garner OB, Hindler JA, Krogstad P, Ward KW, Lewinski MA, Rasheed JK, Anderson KF, Limbago BM, Humphries RM. 2011. New Delhi metallo-beta-lactamase (NDM-1)-producing *Klebsiella pneumoniae*: case report and laboratory detection strategies. J. Clin. Microbiol. 49:1667–1670. <http://dx.doi.org/10.1128/JCM.00183-11>.
 51. Green DA, Srinivas N, Watz N, Tenover FC, Amieva M, Banaei N. 2013. A pediatric case of New Delhi metallo-beta-lactamase-1-producing *Enterobacteriaceae* in the United States. Pediatr. Infect. Dis. J. 32:1291–1294. <http://dx.doi.org/10.1097/INF.0b013e31829eca34>.
 52. Centers for Disease Control and Prevention. 2013. Notes from the field: hospital outbreak of carbapenem-resistant *Klebsiella pneumoniae* producing New Delhi metallo-beta-lactamase—Denver, Colorado, 2012. MMWR Morb. Mortal. Wkly. Rep. 62:108.
 53. Centers for Disease Control and Prevention. 2010. Detection of *Enterobacteriaceae* isolates carrying metallo-beta-lactamase—United States, 2010. MMWR Morb. Mortal. Wkly. Rep. 59:750.
 54. Bushnell G, Mitrani-Gold F, Mundy LM. 2013. Emergence of New Delhi metallo-beta-lactamase type 1-producing *Enterobacteriaceae* and non-*Enterobacteriaceae*: global case detection and bacterial surveillance. Int. J. Infect. Dis. 17:e325–e333. <http://dx.doi.org/10.1016/j.ijid.2012.11.025>.
 55. Yong D, Toleman MA, Giske CG, Cho HS, Sundman K, Lee K, Walsh TR. 2009. Characterization of a new metallo-beta-lactamase gene, bla(NDM-1), and a novel erythromycin esterase gene carried on a unique genetic structure in *Klebsiella pneumoniae* sequence type 14 from India. Antimicrob. Agents Chemother. 53:5046–5054. <http://dx.doi.org/10.1128/AAC.00774-09>.
 56. Prabaker K, Lin MY, McNally M, Cherabuddi K, Ahmed S, Norris A, Lolans K, Odeh R, Chundi V, Weinstein RA, Hayden MK. 2012. Transfer from high-acuity long-term care facilities is associated with carriage of *Klebsiella pneumoniae* carbapenemase-producing *Enterobacteriaceae*: a multihospital study. Infect. Control Hosp. Epidemiol. 33:1193–1199. <http://dx.doi.org/10.1086/668435>.
 57. Poirel L, Lagrutta E, Taylor P, Pham J, Nordmann P. 2010. Emergence of metallo-beta-lactamase NDM-1-producing multidrug-resistant *Escherichia coli* in Australia. Antimicrob. Agents Chemother. 54:4914–4916. <http://dx.doi.org/10.1128/AAC.00878-10>.
 58. Arpin C, Noury P, Boraud D, Coulange L, Manetti A, Andre C, M’Zali F, Quentin C. 2012. NDM-1-producing *Klebsiella pneumoniae* resistant to colistin in a French community patient without history of foreign travel. Antimicrob. Agents Chemother. 56:3432–3434. <http://dx.doi.org/10.1128/AAC.00230-12>.
 59. Nordmann P, Gniadkowski M, Giske CG, Poirel L, Woodford N, Miriagou V. 2012. Identification and screening of carbapenemase-producing *Enterobacteriaceae*. Clin. Microbiol. Infect. 18:432–438. <http://dx.doi.org/10.1111/j.1469-0691.2012.03815.x>.
 60. Mimoz O, Gregoire N, Poirel L, Marliat M, Couet W, Nordmann P. 2012. Broad-spectrum beta-lactam antibiotics for treating experimental peritonitis in mice due to *Klebsiella pneumoniae* producing the carbapenemase OXA-48. Antimicrob. Agents Chemother. 56:2759–2760. <http://dx.doi.org/10.1128/AAC.06069-11>.