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Identification of a Carbapenemase-Producing Hypervirulent *Klebsiella pneumoniae* Isolate in the United States

Maria Karlsson,[a] Richard A. Stanton,[a] Uzma Ansari,[a] Gillian McAllister,[a] Monica Y. Chan,[a,b] Erisa Sula,[a,b] Julian E. Grass,[a] Nadezhda Duffy,[a] Melissa L. Anacker,[c] Medora L. Witwer,[c] J. Kamile Rasheed,[a] Christopher A. Elkins,[a] Alison Laufer Halpin[a,d]

[a]Division of Healthcare Quality Promotion, Centers for Disease Control and Prevention, Atlanta, Georgia, USA
[b]Oak Ridge Associated Universities, Oak Ridge, Tennessee, USA
[c]Minnesota Department of Public Health, St. Paul, Minnesota, USA
[d]U.S. Public Health Service, Rockville, Maryland, USA

ABSTRACT We report on a carbapenemase-producing hypervirulent *Klebsiella pneumoniae* (CP-hvKP) isolate collected from a U.S. patient at an outpatient clinic. The isolate was identified as *K. pneumoniae* serotype K1 sequence type 23 and included both a hypervirulence (with *rmpA*, *rmpA2 iroBCDN*, *peg*-344, and *iucABCD-iutA* genes) and a carbapenemase-encoding (bla_{KPC-2}) plasmid. The emergence of CP-hvKP underscores the importance of clinical awareness of this pathotype and the need for continued monitoring of CP-hvKP in the United States.

KEYWORDS *Klebsiella*, hypervirulence, plasmid-mediated resistance

Carbapenemase-producing *Klebsiella pneumoniae* (CP-KP) isolates exhibit broad resistance to most β-lactams, including the carbapenems ertapenem, imipenem, meropenem, and doripenem, which often serve as last-line therapeutic options for infections caused by highly resistant *Enterobacteriaceae* (1). Resistance to β-lactams in CP-KP is frequently mediated by *K. pneumoniae* carbapenemase (KPC), a gene that commonly resides on a mobile genetic element, allowing the gene to spread horizontally between different bacteria (2, 3). Carbapenem-resistant *Enterobacteriaceae* (CRE) strains, such as CP-KP, can cause a number of serious infections, including intra-abdominal infections, pneumonia, urinary tract infections, and device-associated infections (1). The U.S. Centers for Disease Control and Prevention (CDC) estimates that >9,000 health care-associated infections are caused by carbapenem-resistant *Escherichia coli* and *Klebsiella* species each year in the United States (4). Importantly, infections caused by CRE are associated with mortality rates as high as 47% (5, 6).

Over the past decade, hypervirulent variants of *K. pneumoniae* (hvKP) have emerged. These isolates, which often display a mucoid or hypermucoviscous phenotype, are concerning because they are associated with severe infections, such as pyogenic liver abscesses and osteomyelitis, in immunocompetent healthy individuals (7). Although CP-KP and hvKP have both been identified across the globe, they typically occupy nonoverlapping clonal groups and strain types (8). However, recent reports of carbapenemase-producing hvKP (CP-hvKP) in China (9, 10) and Argentina (11) have signaled the concerning convergence of CP-KP and hvKP, with the potential for increased pathogenicity and mortality.

Aware of the public health threat presented by CP-hvKP, we searched for five virulence markers (*peg*-344, *iroB*, *iucA*, *rmpA*, and *rmpA2*) associated with hvKP (12), using whole-genome sequence data from 600 *K. pneumoniae* isolates collected through CDC surveillance and reference activities from March 2015 to May 2018.

A *K. pneumoniae* isolate was cultured in 2016 from a urine specimen collected from a patient >65 years of age at an outpatient clinic. The patient had recently traveled to

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Address correspondence to Maria Karlsson, fwt4@cdc.gov.

M.K. and R.A.S. contributed equally to this work.

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TABLE 1 Antimicrobial susceptibility test results for *K. pneumoniae* isolate DHQP1701672

Antimicrobial agent	Minimum inhibitory concentration (μg/ml)	Interpretation[a]
Amikacin	≤1	S
Ampicillin	>32	R
Aztreonam	8	I
Cefotaxime	4	R
Ceftriaxone	>32	R
Ceftazidime	≤1	S
Ceftazidime-avibactam	≤0.5	S
Cefepime	2	S
Ciprofloxacin	≤0.25	S
Colistin	≤0.25	NWT
Doripenem	1	S
Ertapenem	2	R
Gentamicin	≤0.25	S
Imipenem	2	I
Levofloxacin	≤0.125	S
Meropenem	2	I
Piperacillin-tazobactam	128/4	R
Tetracycline	≤2	S
Tigecycline	≤0.5	S
Trimethoprim-sulfamethoxazole	≤0.5	S

[a]Interpretative criteria were applied according to CLSI document M100 (14), except for tigecycline, for which criteria established by the U.S. Food and Drug Administration were used. NWT, non-wild type; R, resistant; S, susceptible.

South America but was not hospitalized while traveling. No signs or symptoms of disease were reported, indicating probable asymptomatic bacteriuria. The isolate was submitted to the CDC as part of the Emerging Infections Program Multi-site Gram-negative Surveillance Initiative (MuGSI), (13), which has conducted active population- and laboratory-based surveillance for carbapenem-resistant pathogens since 2012.

Isolate DHQP1701672, identified as a *K. pneumoniae* isolate by using matrix-assisted laser desorption ionization–time of flight mass spectrometry, underwent antimicrobial susceptibility testing using reference broth microdilution according to Clinical and Laboratory Standards Institute (CLSI) guidelines (14). DHQP1701672 displayed resistance to ampicillin, ceftriaxone, cefotaxime, ertapenem, and piperacillin-tazobactam but remained susceptible or intermediate to all other drugs tested (Table 1). Carbapenemase activity was confirmed using the modified carbapenem inactivation method (15), and a bla_{KPC} gene was detected using multiplex real-time PCR (16). Colonies grown from the isolate were not hypermucoviscous, as the string test was negative (viscous strings were <5 mm) (17). Whole-genome sequencing of DHQP1701672 was performed using both the Illumina MiSeq (San Diego, CA) and Oxford Nanopore Technologies MinION (Oxford, UK) platforms.

DHQP1701672 was identified as *K. pneumoniae* serotype K1 sequence type 23 (ST23, per multilocus sequence typing definitions from http://bigsdb.pasteur.fr/klebsiella/klebsiella.html), which belongs to the globally disseminated hypervirulent clonal complex 23 (18) and is associated with life-threatening liver abscesses (19–21). The chromosome of DHQP1701672 was 5,393,085 bp and included multiple virulence factors associated with ST23 isolates (22), such as *allS* (allatonin metabolism), *kfu* (iron uptake), *magA* (mucoviscosity), *mrkD* (type 3 fimbrial adhesion), *wcaG* (capsule biosynthesis), and *ybtS* (yersiniabactin siderophore). Four antimicrobial resistance genes were detected on the chromosome: the β-lactamase bla_{SHV-36}, the fosfomycin resistance gene *fosA*, and the multidrug efflux pump genes *oqxA* and *oqxB*.

The genome of DHQP1701672 included two plasmids. The larger plasmid (pDHQP1701672_hv) was 227,239 bp and had IncFIB and IncHI1B replicons (Fig. 1). Among the 259 open reading frames were multiple virulence factors, including the mucoid phenotype transcription factors *rmpA* and *rmpA2* (truncated at residue 99), the salmochelin siderophore *iroBCDN*, the metabolite transporter *peg*-344, and the ferric

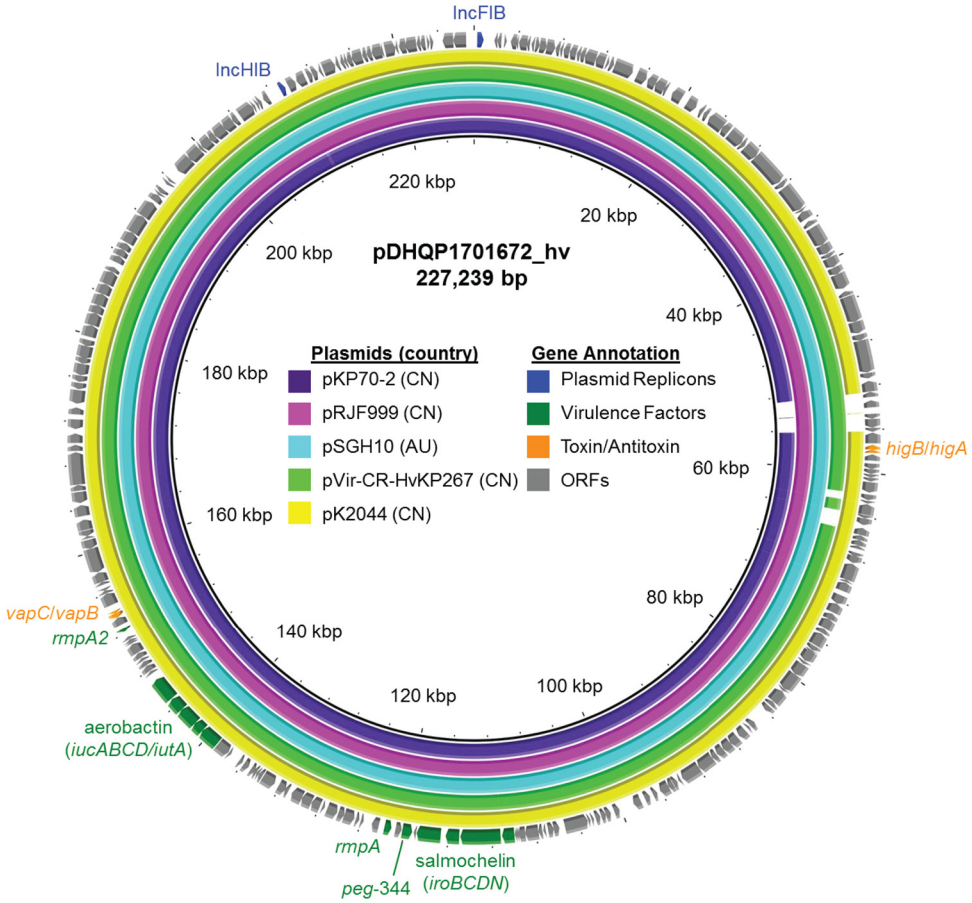

FIG 1 Sequence alignment of the 227-kb pDHQP1701672_hv hypervirulence plasmid to the five most similar plasmids from an NCBI BLAST search. pVir-CR-HvKP267 was found in a *K. pneumoniae* ST11 isolate, and the other plasmids were from *K. pneumoniae* ST23. Similar plasmids were found in isolates from China (CN) and Australia (AU). Open reading frames (ORFs) of pDHQP1701672_hv are shown as the outermost ring, with plasmid replicons, virulence factors, and toxin-antitoxin systems highlighted.

aerobactin and receptor *iucABCD-iutA*. The plasmid carried two type II toxin-antitoxin systems, *higB-higA* and *vapC-vapB* (23). A BLAST search revealed that pDHQP1701672_hv shared >98% sequence identity and coverage with previously reported hypervirulent plasmids from China and Australia (24–26). Like pDHQP1701672_hv, two of these plasmids originated from CP-hvKP isolates; pKP70-2 (GenBank accession no. MF398271.1) was from an ST23 isolate and carried a mobile element that included bla_{KPC-2}, making it both a carbapenemase-producing and a hypervirulent plasmid (24), and pVir-CR-hvKP267 (GenBank accession no. MG053312.1) was from an ST11 isolate with bla_{KPC-2} on a separate plasmid (26).

The smaller plasmid from DHQP1701672 (pDHQP1701672_amr) was 121,239 bp long and included IncFIB and IncFII replicons (Fig. 2). The plasmid had 135 open reading frames and two antimicrobial resistance genes, bla_{KPC-2} and bla_{TEM-1A}, as well as a copy of bla_{OXA-9} truncated at residue 118 by a nonsense mutation. Carbapenemase gene bla_{KPC-2} was found on a Tn*4401* transposon, the mobile genetic element most commonly associated with bla_{KPC} genes (27). Two toxin-antitoxin systems were also found on the plasmid, the type I *hok-sok* and the type II v*apC-vapB* systems. A BLAST search revealed that the plasmid shared >99% sequence identity with 69% to 95% coverage of multiple pKpQIL-like plasmids (28), which have been largely responsible for the spread of bla_{KPC} genes among *Enterobacteriaceae* in the United States and elsewhere (29, 30).

Previous studies identified bla_{KPC-2} in ST23 isolates from Argentina (11), China (10,

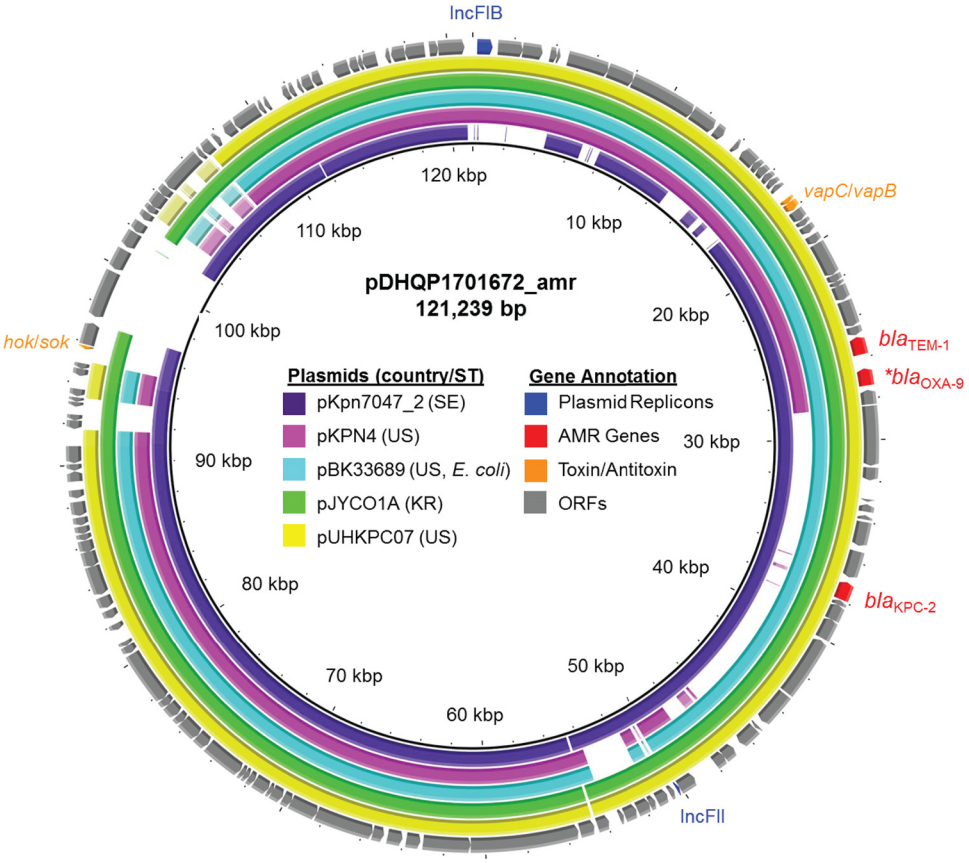

FIG 2 Sequence alignment of the 121-kb pDHQP1701672_amr carbapenemase-encoding plasmid to the five most similar plasmids from an NCBI BLAST search. All of the plasmids were from CRE, four from *K. pneumoniae* and one (pBK33689) from *E. coli* isolates. Similar plasmids were found in isolates from Sweden (SE), the United States (US), and South Korea (KR). Open reading frames (ORFs) of pDHQP1701672_amr are shown as the outermost ring, with plasmid replicons, antimicrobial resistance (AMR) genes (*, including truncated bla_{OXA-9}), and toxin-antitoxin systems highlighted.

31), and Poland (32). In contrast to DHQP1701672, these isolates displayed resistance to all β-lactams tested, including carbapenems. The low-level carbapenem MICs associated with DHQP1701672 may be the result of numerous genetic factors affecting the level of KPC production (e.g., copy number, level of expression), as well as the absence of other mechanisms (e.g., porin mutations) contributing to a carbapenem-resistant phenotype (33). Similar to DHQP1701672 and one of the isolates from China (24), the ST23 isolate from Argentina was positive for the virulence gene *rmpA* (as determined by PCR) (11), suggesting that it may have also harbored a hypervirulence and an antimicrobial resistance plasmid.

Although DHQP1701672 did not have a positive string test, it did carry five biomarkers (*peg*-344, *iroB*, *iucA*, *rmpA*, and *rmpA2*) described as more accurate predictors of hypervirulence in *K. pneumoniae* than the string test (12). All of these markers were located on the same IncFIB/IncHI1B plasmid (pDHQP1701672_hv), suggesting that horizontal acquisition of this virulence gene cluster is a possibility. The presence of toxin-antitoxin systems on both plasmids further increases the likelihood that these will be maintained across generations.

Other reports indicated different distributions of virulence factors in *Klebsiella* isolates associated with invasive infections (34). Thus, further studies are needed to fully understand the elements underlying hypervirulence and how they are related to a hypermucoviscous phenotype (34). To what extent DHQP1701672 is associated with enhanced *in vivo* virulence remains to be determined.

Although infections due to hvKP have been reported in the United States (7), this

report represents the first documented CP-hvKP isolate. Together, the global distribution of ST23 (18) and the fact that plasmids carrying bla_{KPC} genes are ubiquitous in many U.S. health care settings (35) represent potential stages on which additional CP-hvKP strains may emerge in the United States. Such emergence of CP-hvKP strains underscores the importance of clinical awareness of this pathotype and the need for continued monitoring of CP-hvKP in the United States and abroad. Given the patient's history of travel to South America and that CP-hvKP ST23 has been reported from Argentina, it is possible that this isolate was imported. However, with high rates of human mobility, the recognition that these CP-hvKP strains are being imported and potentially circulating domestically is critical for containing their spread, especially given their potential for increased pathogenicity and mortality.

The present CP-hvKP isolate was identified through population-based surveillance conducted by the Emerging Infections Program, a network of 10 state health departments and academic partners working with the CDC to track the incidence and describe the epidemiology of infections caused by resistant or health care-associated pathogens. To enhance the ability to rapidly detect and contain emerging antimicrobial-resistant organisms, the CDC recently established the Antibiotic Resistance Laboratory Network (AR Lab Network), which supports infrastructure in 56 state and local public health laboratories to detect emerging antimicrobial-resistant pathogens, including carbapenemase-producing *Enterobacteriaceae* (36). Thus, the AR Lab Network allows for identification and characterization of CRE nationwide and complements the Emerging Infections Program's intensive epidemiological efforts. At the CDC, whole-genome sequence analysis is routinely applied to surveillance isolates and a subset of CRE collected through the AR Lab Network. This report demonstrates the broad utility of whole-genome sequencing and emphasizes its role as a tool for detecting emerging pathotypes of public health importance, such as hypervirulence.

Accession number(s). All whole-genome sequencing data are deposited in the NCBI database under BioSample accession number SAMN11054834.

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