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Multidrug-Resistant *Klebsiella pneumoniae* ST307 in Traveler Returning from Puerto Rico to Dominican Republic

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We report *bla*$_{KPC-2}$–harboring carbapenem-resistant *Klebsiella pneumoniae* in an emerging sequence type 307 lineage in a traveler returning from Puerto Rico to the Dominican Republic. Phylogenetic analyses indicate regional dissemination of this highly drug-resistant clone across the Americas, underscoring the need for adequate surveillance and infection control efforts to prevent further spread.

Carbapenemase-resistant *Enterobacteriaceae* (CRE), in particular carbapenem-resistant *Klebsiella pneumoniae* (CRKp), represent a serious threat to public health (*1*). CRKp infections have been associated with high mortality rates, up to 50% in some studies (*2*). In resource-limited regions, such as the Dominican Republic, multiple challenges hinder efforts to contain CRE infections, including lack of novel antimicrobial drugs, inability to monitor drug levels of potentially toxic treatment regimens, and absence of molecular tools to investigate outbreaks and potential spread.

In fall 2015, a 66-year-old woman with diabetes mellitus, hepatitis C virus infection, and end-stage renal disease on hemodialysis was admitted to a hospital in the Dominican Republic for fever, anorexia, chills, and myalgia. On day 3, her blood culture tested positive for *K. pneumoniae.* She had been admitted to a hospital in Puerto Rico a few months before and had been treated for a multidrug-resistant bacterial infection.

The *K. pneumoniae* isolate from the patient was nonsusceptible to all tested antimicrobial drugs except polymyxins (Appendix Table 1, http://wwwnc.cdc.gov/EID/article/25/8/17-1730-App1.pdf). We began combination therapy with a loading dose of colistin, then 100 mg postdialysis, plus ertapenem (150 mg postdialysis) and fosfomycin (2 g 3×/d). We implemented infection control measures by placing the patient in a single room and using gloves, gowns, masks, and a dedicated stethoscope. Despite initial improvement, the patient died on day 25 after admission.

Whole-genome sequencing revealed that the patient isolate, NR6025, was of the emerging sequence type 307 (ST307) (*3*) and closely related (≤185 SNPs) to several international ST307 isolates of similar phenotype (Figure). Of note, this isolate was most closely related, within 36 SNPs, to an isolate recovered from a patient in New York, NY, USA, who also had been hospitalized in Puerto Rico in 2016 (*4*). This finding raises the possibility that both patients acquired CRE in Puerto Rico and their infections subsequently developed in their home countries.

In silico resistance gene detection demonstrated that *bla*$_{KPC-2}$, on Tn*4401e*, was likely the mechanism of carbapenem resistance for this isolate. Moreover, the meropenem MIC was >32 μg/mL, consistent with high carbapenem MICs observed in the ST307 Tn*4401e* isolates (*4*) from New York, suggesting association with a strong promoter. In addition, the isolate harbored a large repertoire of acquired-resistance genes, including additional β-lactamase genes CTX-M-15, SHV-100, OXA-1, and TEM-1D (Appendix Table 1). The isolate contained IncFIBK, ColRNA1, and IncA/C2 plasmid replicons; IncA/C plasmid encodes for *bla*$_{KPC-2}$, *bla*$_{TEM}$, *sulI*, *aadB*, *aac6*, and *qacE*, which has been implicated in chlorhexidine resistance.

A case of CRKp was described from Medellin, Colombia, in 2005, and subsequent CRKp infections have been reported in Mexico, in South America in Brazil, Argentina, and Venezuela, and in the Caribbean in Cuba, Puerto Rico, and Trinidad and Tobago (*5*–*7*). In many of these studies, CRKp isolates were mainly accounted for

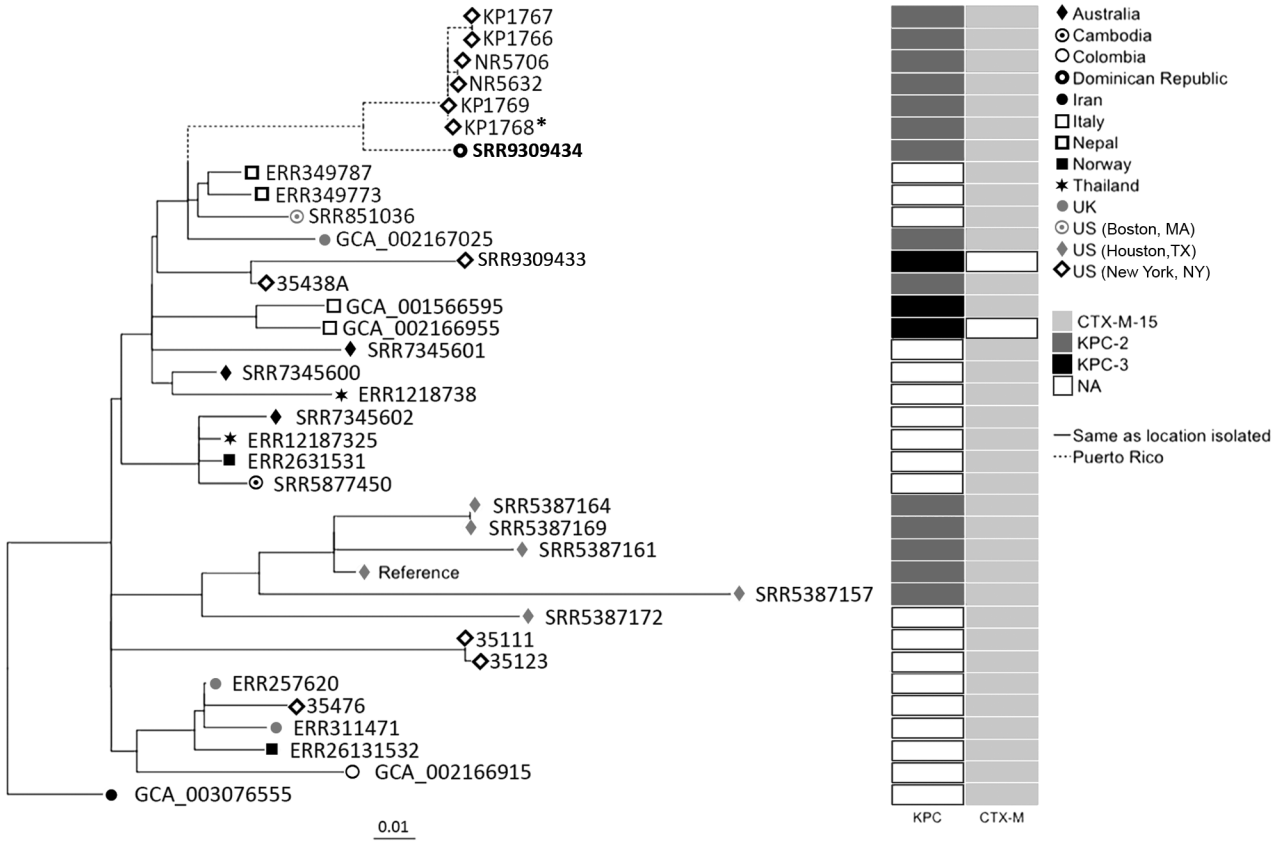

Figure. Maximum-likelihood phylogenetic tree of geographically diverse *Klebsiella pneumoniae* sequence type 307 isolates based on 860 concatenated single-nucleotide polymorphisms, extracted from an alignment length of 5,248,133 bp. Bold indicates isolate from a traveler from Puerto Rico to the Dominican Republic (this study). Asterisk (*) indicates an isolate recovered from a patient admitted to a hospital in Puerto Rico during the same year as the case-patient in this study (*4*). *bla* gene types (KPC, CTX-M) are indicated. Scale bar indicates nucleotide substitutions per site. NA, not applicable.

by ST258 and ST512. The SENTRY Antimicrobial Surveillance Program showed that bla_{KPC-2}-harboring CRE accounted for most CRE infections in Latin America and that the incidence rate has been rising sharply (*8*). These organisms also are prevalent in Puerto Rico, where a 6-month, PCR-based, islandwide hospital surveillance study conducted in 2011 found that 333/2,805 (11.9%) *K. pneumoniae* isolates harbored bla_{KPC} (*9*). However, little is known about CRKp genotypes in Puerto Rico.

Our case highlights the many challenges for controlling CRE infections in resource-limited countries like the Dominican Republic and accentuates the potential for international spread of CRKp through travel, particularly between resource-limited regions. Rapid molecular diagnostic tests for CRKp are not widely available, which can delay treatment. Optimal treatment regimens for CRKp remain controversial, but combination therapy could reduce risk for death compared with monotherapy (*10*). Our facility lacked the resources needed to monitor colistin drug levels, a major concern in particular in patients with underlying renal dysfunction.

Risk factors for acquisition of CRE in resource-limited settings are not well defined, potentially delaying diagnosis and implementation of infection control strategies. In our case, recent travel, healthcare contact, and unspecified exposure to antimicrobial drugs might have played a role in the patient's CRE infection. We did not observe additional CRKp infections at our institution during a 6-month follow-up period after this case. However, we were unable to institute an active molecular surveillance program. We cannot rule out silent transmission and colonization of other hospitalized patients or contacts. Even though 2 cases have now been linked to travel to Puerto Rico, no molecular epidemiologic data are available from that island. Future studies should target active surveillance for CRKp in the Caribbean.

Of note, although ST258 has been the dominant genotype of the CRE epidemic globally, the ST307 clone could be expanding disproportionately in some locations. For example, in Houston, Texas, USA, ST307 now accounts for more *K. pneumoniae* infections than ST258 (*3*). Moreover, ST307 Tn*4401e* bla_{KPC-2} isolates showed high carbapenem

MICs. Taken together, our data suggest that ST307 is highly drug resistant and harbors an extended repertoire of antimicrobial resistance genes, which might have accelerated its recent emergence and wide dissemination.

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Feast of Sacrifice and Orf, Milan, Italy, 2015–2018

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Orf (ecthyma contagiosum) is an infection of the skin caused by a DNA virus belonging to the genus *Parapoxvirus*. We recently observed 7 cases of orf in Muslim men living in the metropolitan area of Milan, Italy, who acquired the infection after the Feast of Sacrifice.

Orf (ecthyma contagiosum) is an infection of the skin caused by a DNA virus of the genus *Parapoxvirus*, family *Poxviridae*. Skin lesions (e.g., vesicles, blisters, pustules, erosions, ulcers, papules, nodules) occur at sites of inoculation of the virus 3–15 days after infection. Hands are usually affected (*1*). The differential diagnosis for orf includes milker's nodule, anthrax, tularemia, fish tank granuloma, cutaneous leishmaniasis, pyogenic granuloma, and keratoacanthoma (*1*). The disease spontaneously heals within 6 weeks, although pain, bacterial superinfections, and regional lymphadenitis are possible (*1*). Treatment is based on topical antiseptics (*1*).

Orf virus usually infects sheep and goats. Humans are infected by handling infected meat from these animals; orf is considered an occupational disease in shepherds, shearers, veterinarians, butchers, and cooks (*1*).

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Multidrug-Resistant *Klebsiella pneumoniae* ST307 in Traveler from Puerto Rico to Dominican Republic

Appendix

Detailed Clinical Case

The patient and family did not recall the source of the infection in Puerto Rico or treatment regimen, but endorsed that she had experienced adverse reactions to her antimicrobial treatments. Further workup, including transthoracic echocardiogram, revealed a vegetation of 0.4 X 0.5 cm on the tip of the hemodialysis catheter on the right side of the heart, which was subsequently removed. The patient remained bacteremic for 6 days after initiation of treatment. She clinically improved and her procalcitonin levels decreased from ≥200 ng/mL to 29 ng/mL. We did not have the ability to measure colistin levels in our laboratory. However, on day 11 of treatment, the patient began to experience neuropathy and diarrhea and fosfomycin was discontinued. On day 25 of admission the patient experienced cardiac arrest and died.

Antimicrobial Susceptibility Testing

We performed antimicrobial susceptibility testing by using Vitek2 Compact (bioMérieux, https://www.biomerieux.com) and interpreted susceptibilities according to Clinical and Laboratory Standards Institute (CLSI) guidelines (*1*). The patient's isolate was nonsusceptible to all tested antimicrobial drugs, except polymyxin (Table 1). Fosfomycin susceptibility testing and broth microdilution testing for polymyxin was not initially available at our institution and was performed after the patient died according to CLSI guidelines (*1*). Modified Hodge's test was positive, indicating presence of a carbapenemase.

Whole Genome Sequencing and Bioinformatics Analyses

We extracted DNA from bacteria cultured overnight by using the UltraClean Microbial DNA Isolation Kit (QIAGEN, https://www.qiagen.com). We prepared libraries by using Nextera XT DNA Library Prep Kit and sequenced on MiSeq (Illumina, https://www.illumina.com).

We performed SRST2 analysis (*2*) for multilocus sequence typing and characterization of resistance determinants. For comparative sequence analyses, we mapped Illumina reads against a *K. pneumoniae* ST307 reference genome (GenBank accession no. GCA_002148835.1) and included additional, previously published sequences for comparative analyses (Appendix Table 2) (*3*–*5*). We performed variant calling by using Snippy 3.1 after exclusion of mobile genetic elements with PHASTER and IslandViewer 3 (*6*–*8*).

For phylogenetic analyses, we generated a core chromosomal single nucleotide variant alignment by using Snippy 3.1 (*6*). We used a maximum likelihood approach with RAXML to construct a phylogenetic tree based on 860 concatenated SNPs (*9*). We assessed support for nodes by using 1,000 rapid bootstrap inferences and then by a thorough maximum likelihood search. We estimated free model parameters by RAxML and evaluated and optimized likelihood of the final tree under GAMMA (*10*). We created the phylogenetic tree in R 3.4.3 by using the ggtree R package (*11*). The tree was rooted on isolate GCA_003076555, the earliest isolate in the collection.

To determine the location of resistance genes, we used SPAdes (http://cab.spbu.ru/software/spades) for assembly and mapped contigs to the NYC ST307, isolate KP1768, core chromosome and plasmids. This indicated that the IncA/C plasmid harbored *bla*$_{\text{KPC}}$, *bla*$_{\text{TEM}}$, *sulI*, *aadB*, *aac6*, as well as *qacE*, implicated in chlorhexidine resistance. *bla*$_{\text{OXA-1}}$, *bla*$_{\text{CTX-M-15}}$ and *bla*$_{\text{SHV100}}$, as well as *catB*, *fosA*, *tet*, *aac6*, and *aadB* mapped to the ColR replicon, putatively integrated into the core chromosome. No major resistance genes mapped to the IncFIB(K) plasmid, which contained many elements for encoding resistance to diverse metals.

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Table 1. Results of antimicrobial susceptibility testing and molecular mechanisms of resistance of *Klebsiella pneumoniae* in patient in Dominican Republic with recent travel to Puerto Rico*

Antimicrobial drug	Mean inhibitory concentration (µg/mL) and EUCAST interpretation	Molecular mechanism
Meropenem	≥32 R	bla_{KPC-2}
Ceftriaxone	≥64 R	$bla_{CTX-M-15}$, $bla_{SHV-100}$, bla_{OXA-1}, bla_{TEM-1D}
Piperacillin/Tazobactam	≥64 R	
Cefepime	≥64 R	
Ciprofloxacin	≥4 R	*gyrA* Y83I, *parC* S80I
Gentamicin	≥16 R	*aac(3)-Iia, aac(6')-Ib, aac(6')-33, aadB*
Tobramicin	≥16 R	
Amikacin	16 I	
Colistin	<0.5 S	
Polymyxin	1 S	
Fosfomycin	NA R	*fosA3*
Trimethoprim/Sulfamethoxazole	≥320 R	*dfrA14, sulI, sulII*
Tetracycline	≥16 R	*tetD*
Minocycline	≥16 R	
Tigecycline	≥8 R	
Chloramphenicol	32 R	*catB4*

*Susceptibility testing for fosfomycin was performed by using disc diffusion testing; the zone diameter was 19 mm. Polymyxin B susceptibility testing was performed by using broth microdilution and meropenem testing was performed by using Etest (bioMérieux, https://www.biomerieux.com). Clinical and Laboratory Standards Institute breakpoints are not available for intravenous fosfomycin, however EUCAST criteria interpret the isolate as resistant (*12*). EUCAST, European Committee on Antimicrobial Susceptibility Testing; I, intermediate; NA, not available; R, resistant; S, susceptible.

Table 2. ST307 reference genomes from GenBank and metadata for previously published sequences used for comparative analyses of *Klebsiella pneumoniae* in patient in Dominican Republic with recent travel to Puerto Rico*

Isolate	Accession number	Location	Year	bla_{KPC}	bla_{CTX-M}
35111	SRR6892777	U.S. (New York, NY)	2014		CTX-M-15
35123	SRR6892773	U.S. (New York, NY)	2014		CTX-M-15
35476	SRR6892718	U.S. (New York, NY)	2015		CTX-M-15
35438A	SRR6892699	U.S. (New York, NY)	2015	KPC-2	CTX-M-15
ERR1218732	ERR1218732	Thailand	2015		CTX-M-15
ERR1218738	ERR1218738	Thailand	2015		CTX-M-15
ERR257620	ERR257620	UK	2010		CTX-M-15
ERR2631531	ERR2631531	Norway	2013		CTX-M-15
ERR2631532	ERR2631532	Norway	2012		CTX-M-15
ERR311471	ERR311471	UK	2012		CTX-M-15
ERR349773	ERR349773	Nepal	2012		CTX-M-15
ERR349787	ERR349787	Nepal	2012		CTX-M-15
GCA_001566595	GCA_001566595	Italy	2014	KPC-3	CTX-M-15
GCA_002166915	GCA_002166915	Colombia	2013		CTX-M-15
GCA_002166955	GCA_002166955	Italy	2014	KPC-3	
GCA_002167025	GCA_002167025	UK	2015	KPC-2	CTX-M-15
GCA_003076555	GCA_003076555	Iran	2009		CTX-M-15
KP1766	SRR6844958	U.S. (New York, NY)	2016	KPC-2	CTX-M-15
KP1767	SRR6844959	U.S. (New York, NY)	2016	KPC-2	CTX-M-15
KP1768	SRR6845005	U.S. (New York, NY)	2016	KPC-2	CTX-M-15
KP1769	SRR6845004	U.S. (New York, NY)	2016	KPC-2	CTX-M-15
NR0970	SRR9309433	U.S. (New York, NY)	2014	KPC-3	
NR5632	SRR6348596	U.S. (New York, NY)	2016	KPC-2	CTX-M-15
NR5706	SRR6348592	U.S. (New York, NY)	2016	KPC-2	CTX-M-15
NR6025	**SRR9309434**	**Dominican Republic**	**2015**	**KPC-2**	**CTX-M-15**
Reference	GCA_002148835.1	U.S. (Houston, TX)	2011	KPC-2	CTX-M-15
SRR5387157	SRR5387157	U.S. (Houston, TX)	2015	KPC-2	CTX-M-15
SRR5387161	SRR5387161	U.S. (Houston, TX)	2015	KPC-2	CTX-M-15
SRR5387164	SRR5387164	U.S. (Houston, TX)	2015	KPC-2	CTX-M-15
SRR5387169	SRR5387169	U.S. (Houston, TX)	2015	KPC-2	CTX-M-15
SRR5387172	SRR5387172	U.S. (Houston, TX)	2015		CTX-M-15
SRR5877450	SRR5877450	Cambodia	2013		CTX-M-15
SRR7345600	SRR7345600	Australia	2013		CTX-M-15
SRR7345601	SRR7345601	Australia	2013		CTX-M-15
SRR7345602	SRR7345602	Australia	2014		CTX-M-15
SRR851036	SRR851036	U.S. (Boston, MA)	2012		CTX-M-15

*Bold text indicates isolate in this case.