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Population Structure of KPC-Producing *Klebsiella pneumoniae* Isolates from Midwestern U.S. Hospitals

Meredith S. Wright,[a] Federico Perez,[b] Lauren Brinkac,[a] Michael R. Jacobs,[c] Keith Kaye,[d] Eric Cober,[e] David van Duin,[f] Steven H. Marshall,[b] Andrea M. Hujer,[b,g] Susan D. Rudin,[b,g] Kristine M. Hujer,[b,g] Robert A. Bonomo,[b,g,h,i] Mark D. Adams[a]

J. Craig Venter Institute, La Jolla, California, USA[a]; Research Service, Louis Stokes Cleveland Department of Veterans Affairs Medical Center, Cleveland, Ohio, USA[b]; Department of Pathology, Case Western Reserve University and University Hospitals Case Medical Center, Cleveland, Ohio, USA[c]; Detroit Medical Center, Detroit, Michigan, USA[d]; Infectious Diseases Department, Cleveland Clinic, Cleveland, Ohio, USA[e]; Division of Infectious Diseases, University of North Carolina, Chapel Hill, North Carolina, USA[f]; Departments of Medicine,[g] Pharmacology,[h] and Molecular Biology and Microbiology,[i] Case Western Reserve University, Cleveland, Ohio, USA

Genome sequencing of carbapenem-resistant *Klebsiella pneumoniae* isolates from regional U.S. hospitals was used to characterize strain diversity and the bla_{KPC} genetic context. A phylogeny based on core single-nucleotide variants (SNVs) supports a division of sequence type 258 (ST258) into two distinct groups. The primary differences between the groups are in the capsular polysaccharide locus (*cps*) and their plasmid contents. A strict association between clade and KPC variant was found. The bla_{KPC} gene was found on variants of two plasmid backbones. This study indicates that highly similar *K. pneumoniae* subpopulations coexist within the same hospitals over time.

Carbapenem-resistant *Enterobacteriaceae* (CRE), including *Klebsiella pneumoniae*, are an increasing clinical challenge for health care facilities worldwide (1–3). Several *K. pneumoniae* sequence types are identified as carrying bla_{KPC}, a gene conferring resistance to carbapenems (4). One prevalent multidrug-resistant (MDR) multilocus sequence type, ST258, is disseminated worldwide (5–7). Recent analysis of genetic diversity within the ST258 lineage suggested the separation of isolates into two distinct groups, in which the primary chromosomal difference centered around the capsular polysaccharide locus (*cps*) region (8). However, the diversity of genetic elements mobilizing bla_{KPC} in *K. pneumoniae* strains within and among hospitals is not known, and knowledge of it would provide insight into dispersal processes for these organisms and genetic elements and their evolutionary history.

We characterized the population structure of bla_{KPC}-positive clinical strains from a consortium that includes four tertiary-care hospitals in the midwestern United States (three in Cleveland, OH, and one in Detroit, MI) (9). Strains were collected from 2004 to 2012, were primarily of the ST258 sequence type (10), and were isolated from patients with a variety of infections. Draft genome sequences of 57 MDR *K. pneumoniae* strains (see Table S1 in the supplemental material) were determined, and five representative genomes were determined at a higher quality by using Pacific Bioscience SMRT sequencing (see Supplemental Methods in the supplemental material). Publicly available strains from GenBank were used as references to place the 57 genomes in a phylogenetic context.

A phylogeny scheme based on *k*-mer alignments of consensus contigs (kSNP software) (11) was used to determine the population structure and strain relatedness. The 33,833 core single-nucleotide variants (SNVs) for all genomes, or 976 SNVs when only ST258 genomes were analyzed, resulted in ST258 strains grouping together in the tree (Fig. 1). Within the ST258 group, there were two well-supported clades (ST258a and ST258b) corresponding to clade 1 and clade 2 in the study by DeLeo et al. (8). Two distinct ST258 groups were also found by repetitive sequence-based PCR (rep-PCR) of the midwestern U.S. consortium strains from Ohio and Michigan (9). Each clade consisted of strains isolated from all four hospitals, collected during many years and from multiple infection sites. Reference ST258 strains reported from Maryland (KPNIH strains) (12), New Jersey (NJST258) (8), and Italian (ST258-K26BO and ST258-K28BO) (13) hospitals formed separate branches within the ST258b lineage. Interior branches within the ST258a and ST258b lineages suggest some clustering of strains by location but were generally poorly resolved with low bootstrap support.

A 50-kb region of elevated SNV density between the two ST258 clades was centered around and extended beyond the capsular polysaccharide (*cps*) locus to the *mdtABC* locus (1.78 to 1.86 Mb in NJST258-1) and was identified using BRATnextgen software (14) as a likely recombination hot spot. The *cps* locus is a major source of variability among *K. pneumoniae* isolates in part because of its role in host-pathogen interactions (15). The *cps* locus in ST258b strains has one nucleotide difference from the *wzi*-81 allele in the typing scheme of Brisse et al. (16) and bears rhamnose-utilizing genes. The *cps* locus in these strains was most similar to that in the bla_{KPC-2}-producing Kp13 strain from Brazil (17, 18) and clade 2 strains in reference 8, cps_{BO-4} in reference 19, and to the *cps* locus in the KPNIH strains, with more than 99.99% nucleotide identity throughout the 50-kb region. The *cps* locus in ST258a strains (*wzi*-29) was nearly identical to the *cps* locus in the ST258 clade 1 (8), in VA360 (ANGI00000000), a bla_{KPC-2} ST258 strain from Ohio, in KpMDU1 (AMWO00000000), a bla_{KPC-2} strain

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Address correspondence to Mark D. Adams, madams@jcvi.org, or Robert A. Bonomo, Robert.Bonomo@va.gov.

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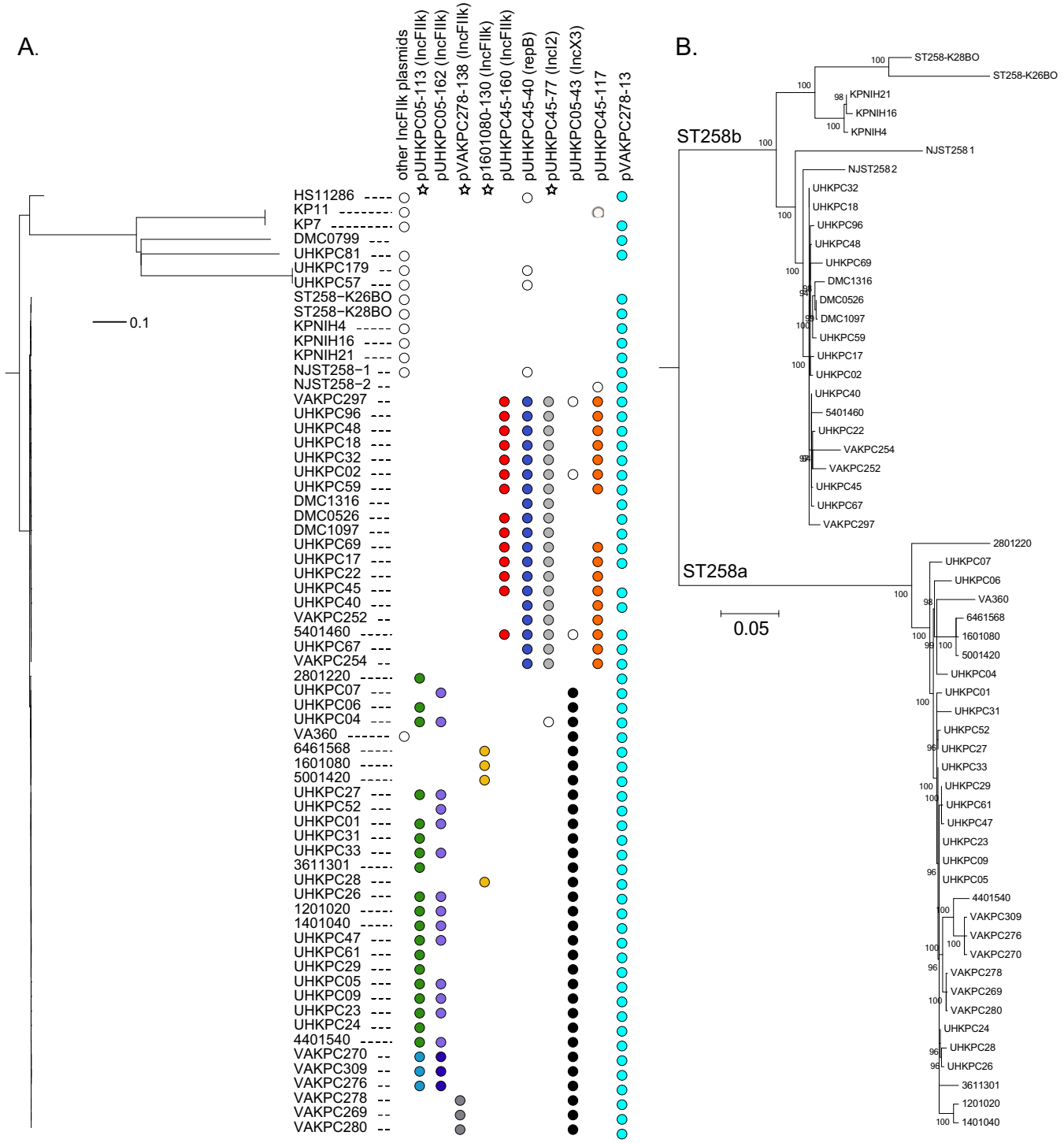

FIG 1 Phylogenetic trees and plasmid content. (A) Maximum likelihood tree generated from core SNVs using kSNP (11) for *K. pneumoniae* strains sequenced as part of this project and reference strains. The presence of plasmids is indicated by colored or open circles. Colored circles indicate substantially identical plasmids as inferred from draft genome sequences. Open circles indicate genomes that contain the same *repA* type but whose plasmid content and organization were not similar. Plasmids carrying the bla_{KPC} gene are noted with a star under the plasmid name. (B) Core SNV tree constructed as in panel A but using only ST258 strains as input. Numbers at nodes represent bootstrap support (>50%).

from Australia, and to $cps_{207\text{-}2}$ (19). Common to these ST258a strains is a shared IS*1* insertion within the *cps* region. An additional cluster of 28 SNVs is present over an 8-kb span (1.98 to 1.99 Mbp in NJST258-1). These two regions together were identified by DeLeo et al. as a 215-kb region of divergence (RD). Based on a sequence comparison, the ST258b genomes are more similar across this region to the non-ST258 Kp13 strain, while the ST258a genomes are most similar to HS11286 (20) (NC_016845), a

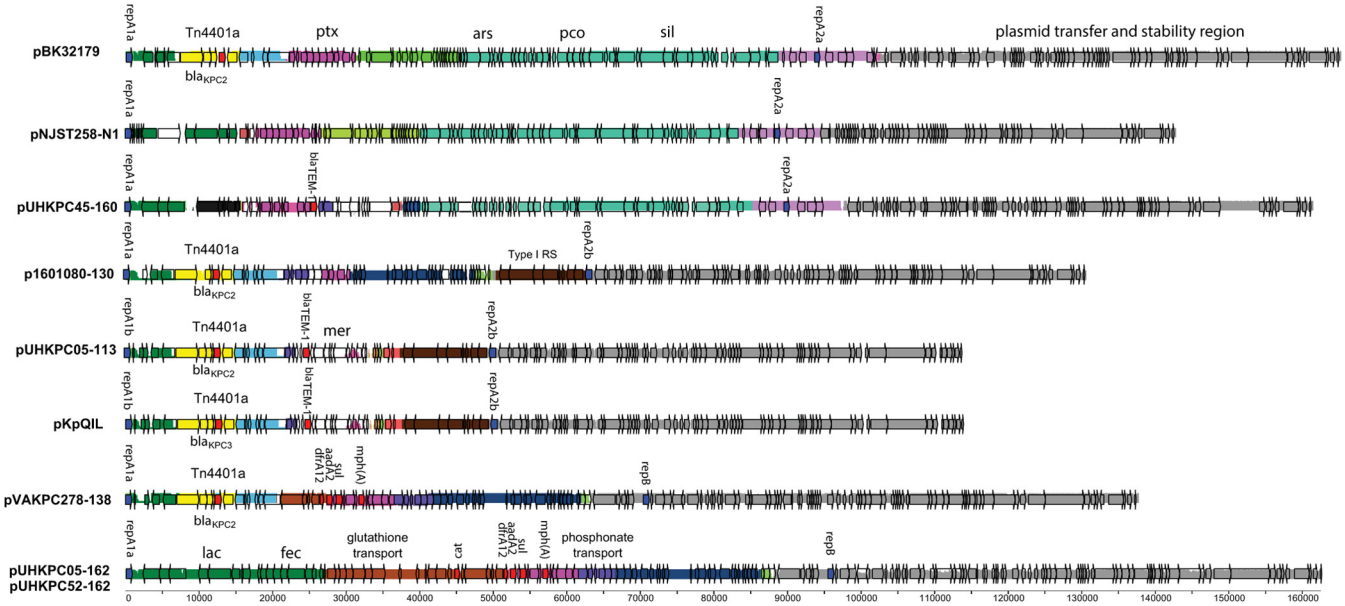

FIG 2 Comparison of IncFII$_k$ plasmid content and organization of *K. pneumoniae* ST258 strains. IncFII$_k$ plasmid sequences from PacBio Hierarchical Genome Assembly Pipeline (HGAP) assemblies and reference plasmids pBK32179, pNJST258-N1, and pKpQIL were aligned using Mauve (31). Colors indicate shared locally colinear blocks. Blue, replication genes (nomenclature as described in reference 26); yellow, Tn*4401*; red, antibiotic resistance genes.

closely related non-ST258 strain. HS11286 differs across the *cps* region (*wzi*-74 type) from both ST258 groups though, suggesting that the RD was created by at least two recombination events.

Chromosomal and mobile gene content comparisons also supported the separation of the two ST258 lineages. In addition to differences in the *cps* region, several chromosomal gene content differences between the ST258 lineages were found. There is a 97-bp deletion in the *entS* gene, which encodes the enterobactin exporter (21), in the ST258b consortium strains but not in other ST258b strains (KPNIH and two NJST258 strains). Phage-associated and IS-mediated gene losses included one region that is present in every ST258a strain but absent from every ST258b strain corresponding to 2.530 to 2.545 Mb in the HS11286 genome and that contains genes predicted to be involved in competence induction and two-component regulatory systems. Both ST258 lineages have an IS*5* insertion at this location, but only strains within ST258b are missing the adjacent sequences. The KPNIH strains in ST258b carry this sequence, so it appears that the region was lost after the split from the KPNIH strains. The ST258a strains are missing open reading frames (ORFs) predicted to be involved in maltose metabolism (HS21186, 2.805 to 2.814 Mb), also adjacent to an IS*5* insertion common to both lineages; this region is also missing from VA360.

The primary source of gene content variability, however, is in plasmid content and *bla*$_{KPC}$ context. The *bla*$_{KPC}$ gene can be carried on plasmids of several different types in *K. pneumoniae*, typically embedded within the Tn*4401* transposon (22). The *bla*$_{KPC}$ gene in the study genomes was Tn*4401* associated but found on different plasmid backbones (Fig. 1A and 2). The *bla*$_{KPC-2}$ variant was always on the Tn*4401*a isoform (23), while the *bla*$_{KPC-3}$ variant was always on Tn*4401*b. Furthermore, there was strict fidelity between lineage and *bla*$_{KPC}$ and Tn*4401* type, where ST258a strains always carried *bla*$_{KPC-2}$ and Tn*4401*a and ST258b strains always carried *bla*$_{KPC-3}$ and Tn*4401*b, except for UHKPC22, which carried the *bla*$_{KPC-7}$ variant. This is quite surprising given the observed dynamic nature of Tn*4401*, as other *K. pneumoniae* strains were shown to carry diverse *bla*$_{KPC}$ variants on Tn*4401* isoform a or b (24). The plasmid context for Tn*4401* also varied by lineage, where in the ST258a clade, Tn*4401*a was found on variants of IncFII$_k$ plasmids, with pUHKPC05_113 being nearly identical to pKpQIL (NC_014016) (25), and others sharing significant segments with pBK32179 (NC_020132 [26]) (e.g., p1601080-130 and pVAKPC278-138). Tn*4401*b was found on an IncI2 plasmid in all ST258b strains (e.g., pUHKPC45-77) that is nearly identical to pBK15692 (NC_022520 [27]).

Deeper investigation into plasmid carriage revealed that there is both overlap and segregation of plasmid types between the ST258 groups (Fig. 1B). For example, strains from both clades carry IncFII$_k$ plasmids. Some ST258a strains carry two IncFII$_k$ plasmids (e.g., UHKPC05), while others have only pUHKPC05-113, only pUHKPC05-162, or a hybrid of the two (e.g., pVAKPC278-138 or p1601080-130). However, there is only one IncFII$_k$ plasmid variant in each ST258b strain (pUHKPC45-160). While the IncFII$_k$ plasmids are highly variable among the ST258a strains, the content and organization of other plasmids are more consistent across genomes. For example, the IncX3 plasmid (pUHKPC05-43) is nearly identical within the ST258a strains, with a backbone similar to that of pKPC-NY79 (28). Strains from both lineages carry this IncX3 *repA*, but the plasmid backbones are different between the clades. The plasmid pUHKPC45-40 has a backbone similar to that of pNJST258C1 and is present only in the ST258b clade. pUHKPC45-117 has a backbone similar to that of the *bla*$_{KPC-3}$-carrying plasmid pNJST258N2 in the ST258 clade 2 strains described in reference 8, but the *bla*$_{KPC-3}$ gene in our ST258b/clade 2 strains is on pUHKPC45-77. pVAKPC278_13 is very similar to pColEST258 (JN247853) and is widely present in *K. pneumoniae* strains. The considerable variability in plasmid structure and content among strains cooccurring in the same hospitals

suggests that additional plasmid types and arrangements remain to be described in *K. pneumoniae*.

The fidelity between sublineage *cps* type and bla_{KPC} type was maintained across geographic and temporal distances even though these strain types were present in all locations during the same time period. Reference strains from other geographic areas had the same association between *cps* type and bla_{KPC} type, which suggests that this population structure (i) is not restricted to strains in the geographic region examined here and (ii) has been maintained during global dispersal. However, SNV patterns, gene content comparisons, and plasmid carriage suggest that there is regional divergence occurring among strains within each clade, likely due to founder effects. For example the KPNIH strains and NJST258 genomes form separate branches with longer branch lengths from the ST258b strains in this study, while ST258b study strains have distinct gene losses and carry Tn*4401* on different plasmids. Clinical data from van Duin et al. (9) indicate that patients infected with ST258a experience longer hospital stays than patients infected with ST258b strains, and ST258b/KPC2 strains have higher MIC values for tested carbapenems (see Table S1 in the supplemental material). However, the full implications for the linkage of bla_{KPC} type and distinct chromosomal backgrounds with physiological differences in infection dynamics between ST258a and ST258b strains remain to be explored. For example, an investigation into iron uptake differences between ST258 strains missing full-length *entS* genes may reveal potential fitness costs during host infection (29). As additional variation within the *cps* region among ST258 strains has now been detected (30), additional genomic analysis of ST258 strains would further our understanding of carbapenem-resistant *K. pneumoniae* evolution.

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