Epidemic *Klebsiella pneumoniae* ST258 Is a Hybrid Strain

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ABSTRACT **Carbapenem-resistant *Enterobacteriaceae* (CRE), especially *Klebsiella pneumoniae* carbapenemase (KPC)-producing *K. pneumoniae*, pose an urgent threat in health facilities in the United States and worldwide. *K. pneumoniae* isolates classified as sequence type 258 (ST258) by multilocus sequence typing are largely responsible for the global spread of KPC. A recent comparative genome study revealed that ST258 *K. pneumoniae* strains are two distinct genetic clades; however, the molecular origin of ST258 largely remains unknown, and our understanding of the evolution of the two genetic clades is incomplete. Here we compared the genetic structures and single-nucleotide polymorphism (SNP) distributions in the core genomes of strains from two ST258 clades and other STs (ST11, ST442, and ST42). We identified an ~1.1-Mbp region on ST258 genomes that is homogeneous to that of ST442, while the rest of the ST258 genome resembles that of ST11. Our results suggest ST258 is a hybrid clone—80% of the genome originated from ST11-like strains and 20% from ST442-like strains. Meanwhile, we sequenced an ST42 strain that carries the same K-antigen-encoding capsule polysaccharide biosynthesis gene (*cps*) region as ST258 clade I strains. Comparison of the *cps*-harboring regions between the ST42 and ST258 strains (clades I and II) suggests the ST258 clade I strains evolved from a clade II strain as a result of *cps* region replacement. Our findings unravel the molecular evolution history of ST258 strains, an important first step toward the development of diagnostic, therapeutic, and vaccine strategies to combat infections caused by multidrug-resistant *K. pneumoniae*.**

IMPORTANCE **Recombination events and replacement of chromosomal regions have been documented in various bacteria, and these events have given rise to successful pathogenic clones. Here we used comparative genomic analyses to discover that the ST258 *K. pneumoniae* genome is a hybrid—80% of the chromosome is homologous to ST11 strains, while the remaining 20% is homologous to that of ST442. Meanwhile, a recent study indicated that ST258 strains can be segregated into two ST258 clades, with distinct capsule polysaccharide gene (*cps*) regions. Our analysis suggests ST258 clade I strains evolved from clade II through homologous recombination of *cps* region. Horizontal transfer of the *cps* region appears to be a key element driving the molecular diversification in *K. pneumoniae* strains. These findings not only extend our understanding of the molecular evolution of ST258 but are an important step toward the development of effective control and treatment strategies for multidrug-resistant *K. pneumoniae*.**

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Klebsiella pneumoniae carbapenemase (KPC) has emerged as a serious clinical challenge in health care facilities in the United States and worldwide (1). The bla_{KPC}-harboring plasmid encoding the carbapenemase has been found in numerous *K. pneumoniae* sequence types (STs)/clones as well as in other Gram-negative species; however, the vast majority of the global KPC-producing *K. pneumoniae* isolates are associated with a single multilocus sequence type—ST258 (2–4). *K. pneumoniae* ST258 emerged as a notable clinical problem in the middle 2000s in the United States and remains the main ST in the United States and elsewhere (3–6).

Recently, two KPC-harboring *K. pneumoniae* ST258 clinical isolates were sequenced to closure (7). These genomes were used as references for a comparative genome analysis of 83 ST258 clinical isolates collected between 2002 and 2012 from geographically diverse sources. Phylogenetic analysis of the core genome of these isolates revealed that ST258 *K. pneumoniae* strains are comprised of two distinct genetic clades (ST258 clades I and II), largely due to an ~215-kb region of divergence (RD) that includes genes involved in capsular polysaccharide (CPS) biosynthesis (7). Further genotyping analysis with 2 *cps*-associated genes, *wzi* and *wzy*, in ST258 and other unrelated *K. pneumoniae* strains identified the ST258 clade I genotype in genetically distinct ST42 strains (7). Interestingly, a GenBank BLAST search using nucleotides encompassing the ST258 clade II *cps* region indicated this region is highly similar to that of a Brazilian ST442 strain, Kp13, which harbors cps_{Kp13} (8, 9).

TABLE 1 Features of completely sequenced ST258, ST11, and ST442 genomes

Parameter[a]	Result for strain:					
	NJST258_1	NJST258_2	HS11286	JM45	ATCC BAA-2146	Kp13
ST no.	258	258	11	11	11	442
Yr	2010	2010	2011	2010	2010	2009
Country	United States	United States	China	China	United States	Brazil
bla genes	bla_{KPC-3}, bla_{OXA-9}, bla_{TEM-1}	bla_{KPC-3}, bla_{SHV-11}	bla_{KPC-2}, $bla_{CTX-M-14}$, bla_{TEM-1}	bla_{KPC-2}, $bla_{CTX-M-24}$, bla_{VEB-3}	bla_{NDM-1}, $bla_{CTX-M-15}$, bla_{CMY-6}, bla_{OXA-1}, bla_{TEM-1}, bla_{SHV-11}	bla_{KPC-2}, $bla_{CTX-M-2}$, bla_{OXA-9}, bla_{TEM-1}, bla_{SHV-12}, $bla_{SHV-110}$
Size (bp)	5,263,329	5,293,301	5,333,942	5,273,813	5,435,369	5,307,003
G+C content (%)	57.4	57.5	57.5	57.5	57.3	57.5
No. of CDS	5,475	5,434	5,316	4,872	5,315	5,189
rRNA (*n*)	25	25	25	25	25	24
16S	8	8	8	8	8	8
23S	8	8	8	8	8	8
5S	9	9	9	9	9	8
tRNA (*n*)	77	86	87	83	85	86
Plasmids (*n*)	5	3	6	2	4	6
Prophages (*n*)	8	7	7	7	8	2
ICEs (*n*)	2	2	2	1	3	1
IS elements (*n*)	22	19	23	14	23	31
IS family (*n*)	IS*1294* (3), IS*1400* (1), IS*5* (7), IS*5075* (1), IS*903* (2), IS*Kpn1* (5), IS*Kpn18* (2), IS*Sm1* (1)	IS*1294* (2), IS*1400* (1), IS*5* (7), IS*903* (2), IS*Kpn1* (5), IS*Kpn18* (2)	IS*1* (1), IS*5* (1), IS*903* (7), IS*Ec22* (1), IS*Ecp1* (6), IS*Kpn1* (6), IS*Kpn18* (1)	IS*1400* (1), IS*5* (1), IS*Ecp1* (1), IS*Kpn1* (6), IS*Kpn18* (6)	IS*1* (1), IS*1222* (1), IS*1400* (1), IS*2* (2), IS*26* (2), IS*6100* (1), IS*66* (3), IS*Ec36* (2), IS*Ecp1* (2), IS*Kpn1* (5), IS*Kpn18* (2), IS*Kpn21* (1)	IS*1* (7), IS*10* (1), IS*1222* (1), IS*4321* (2), IS*3* (1), IS*5* (3), IS*903* (7), IS*Ec11* (2), IS*Ecp1* (1), IS*Kpn1* (5), IS*Kpn21* (1)

[a] ICE, integrated conjugative element; IS, insertion sequence; CDS, coding sequences.

The emergence and global spread of ST258 and recent reports that this clone has diversified as a result of recombination and replacement of the *cps* region raise the question of its evolutionary history. One speculation is that ST11 (allelic profile 3-3-1-1-1-1-4), a highly predominant multidrug-resistant clone in Asia and South America (10–12), and a single-locus variant of ST258 (allelic profile 3-3-1-1-1-1-79) gave rise to the ST258 clone through the acquisition of the *tonB79* allele (13).

To better understand the phylogeny of the ST11 and ST258 lineages, we compared the genomes of three ST11 strains (HS11286, JM45, and ATCC BAA-2146), three ST258 strains (NJST258_1, NJST258_2, and Kp1787 [a representative ST258 clade I strain present in our collection]), and an ST42 strain, Kp1832. The comparative analysis of these genomes indicates that large and repeated chromosomal exchanges in *K. pneumoniae* have occurred between ST11 and ST258, with a significant role for ST442 in the recent molecular evolution of epidemic ST258 strains.

RESULTS

Large ~1.1-Mbp recombination region in ST258. To elucidate the phylogenetic relationship among ST258, ST11, and ST442 strains, we first compared the genome sequences of six closed *Klebsiella pneumoniae* strains (Table 1). The size of the chromosomes was on average ~5.3 Mbp, but the number of mobile genetic elements (MGEs), including plasmids, prophages, integrated conjugative elements (ICEs), and insertion sequences (IS), varied (Table 1; see Fig. S1 in the supplemental material). Consistent with multilocus sequence typing (MLST) (Fig. 1A), which indicates ST11 and ST258 differ by a single locus (the *tonB* allele distinguishes the two sequence types), the 3 ST11 and 2 ST258 genomes have 7 of 8 prophages in common, and all harbor ICEKp258.1 (see Fig. S1). Sequence comparison among the *tonB* alleles shows *tonB79* (in ST258) differs from *tonB4* (in ST11) by four single-nucleotide polymorphisms (SNPs) and differs from *tonB14* (in ST442) by a single SNP. Of note, the three ST11 strains (HS11286, JM45, and ATCC BAA-2146) harbor three different *cps* operons, a finding similar to the distinguishing *cps* genotypes in ST258 clade I and II strains and which supports the observation that *cps* switching provides *K. pneumoniae* the plasticity to change its antigenic nature (Fig. 1B).

Comparative genome and SNP distribution analyses of the core chromosome region, as depicted in Fig. 1B, uncovered a number of surprising findings given the MLST results for ST11 and ST258. Except for differences in the *tonB* allele and the region encoding the capsular polysaccharide biosynthetic machinery, the 6 genomes of ST11 and ST258 have a high degree of identity (regions of the same color in Fig. 1B). However, further analysis of the RD and flanking nucleotides revealed that the differences between ST11 and ST258 were expansive, covering an ~1.1-Mbp contiguous region corresponding to nucleotide positions 1,660,631 to 2,723,681 in strain NJST258_1 (Fig. 1). Significantly, the ~1.1-Mbp region identified in ST258 clade I and II strains has identical chromosomal nucleotide boundaries (Fig. 2).

Analysis of SNPs in the genomes of ST258 strains (NJST258_1, NJST258_2, and Kp1787) and ST11 strains (HS11286, JM45, and ATCC BAA-2146) indicated that these strains differ by an average of 9,647 SNPs, and 98.1% (9,460 SNPs) of these polymorphisms are concentrated in the contiguous ~1.1-Mbp region (identified above), which represents 20% of the genome (Fig. 3). By comparison, the genomes of ST258 strains and ST442 strain Kp13 differed by 21,095 SNPs, consistent with their genetically distinct MLST

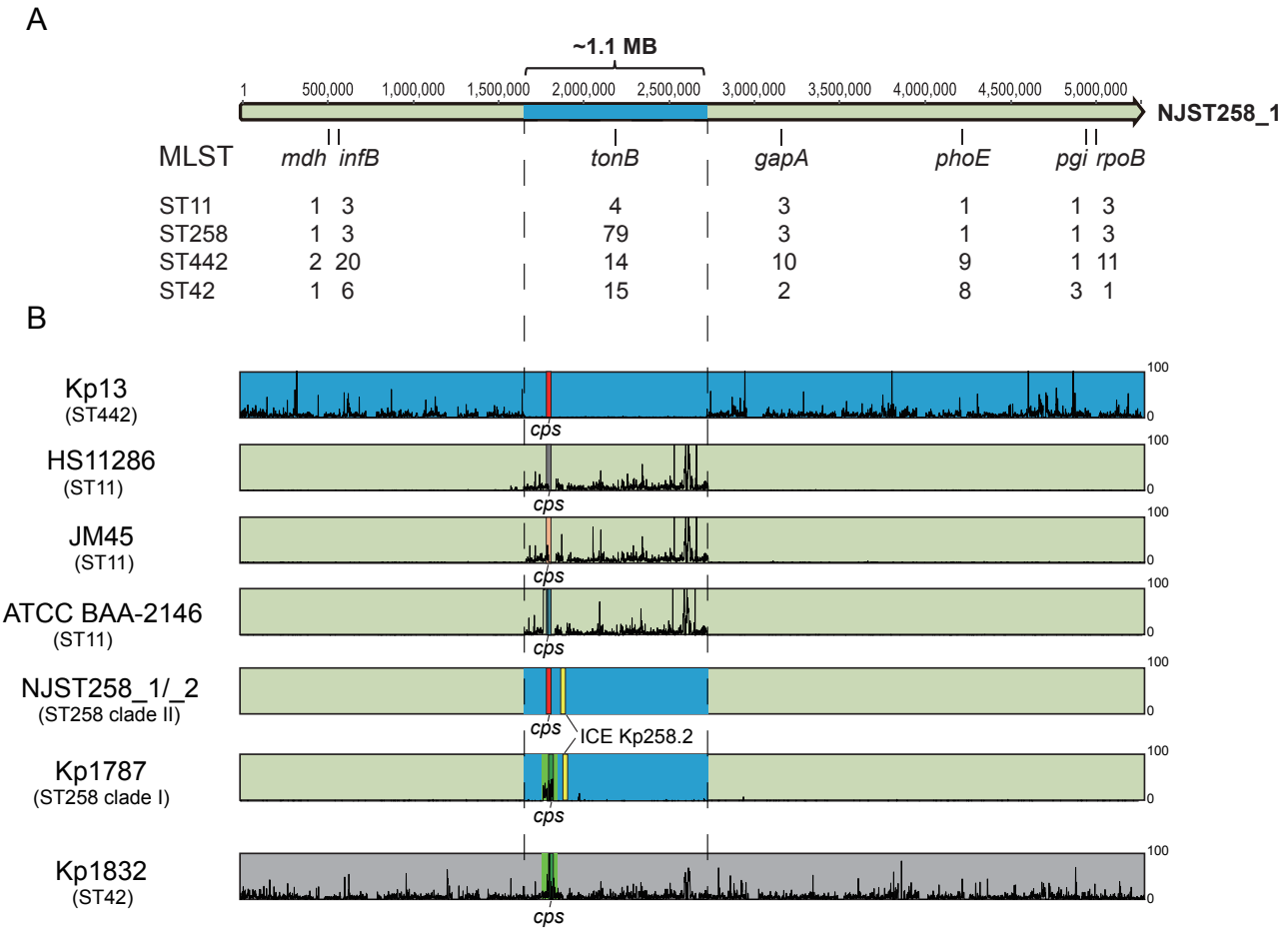

FIG 1 (A) MLST allele locations on NJST258_1 genome. The light green arrow denotes the genome of NJST258_1, and the light blue region shows the ~1.1-Mbp putative recombination region between the ST11 and ST442 genomes. The chromosomal positions of the seven MLST housekeeping genes (*gapA*, *infB*, *mdh*, *pgi*, *phoE*, *rpoB*, and *tonB*) are illustrated beneath the genome arrow of NJST258_1, and the corresponding allele numbers for ST11, ST258, ST442, and ST42 are listed below the gene names. (B) Core genome SNP distributions in the ST11, ST258 (clades I and II), ST442, and ST42 strains. The number of SNPs (*y* axis) per 1,000 nt is plotted according to the position on the NJST258_1 genome (*x* axis). Different homogeneous regions (>98% identity based on SNP comparisons) are color coded. Specifically, the ~52-kb *cps*-containing regions in Kp1787 (ST258 clade I) and Kp1832 (ST42), which are nearly identical in these strains, are shaded in green. ICEKp258.2 and *cps* are illustrated by small vertical bars, and the same *cps* regions are shown in the same color.

profiles (Fig. 1B and SNP matrix in Fig. 3). Most significantly, the SNP mapping revealed contiguous ~1.1-Mbp regions that were nearly identical in ST258 clade II (NJST258_1 and NJST258_2) and ST442 (Kp13) strains, differing by only 206 SNPs (1.0%). As depicted in Fig. 1B, the comparative genomic organization and SNP results provide additional support to the idea that the ST258 clade II strain is a hybrid strain containing 80% (~4.2 Mbp) of the chromosome from ST11 and 20% (~1.1 Mbp) from ST442 (Fig. 1B).

The ~1.1-Mbp chromosomal region in ST258 clade I and II strains contains the ~215-kb RD and the *cps* gene cluster (Fig. 2). ICEKp258.2 is common to the prototype ST258 strains shown in Fig. 1B but is absent from ST442 strain Kp13. To determine the level of conservation of ICEKp258.2 among ST258 clinical isolates, we analyzed the DNA contigs of 83 additional ST258 genomes sequenced in our previous study (7). We found that ICEKp258.2 is conserved in all of the queried ST258 genomes, and the insertion of this element in ST258 clade I and II genomes is at the same tRNA-Asn site (data not shown).

***cps* replacement in ST258 strains.** In our previous study, we identified seven ST42 strains that harbored *cps* genetic markers (*wzy* and *wzi*) that are identical to those in ST258 clade I strains (7), and we hypothesized that this unrelated sequence type (ST42) was the donor for the *cps* region in ST258 clade I strains. As a first step toward testing this hypothesis, we used Illumina Miseq to sequence the DNA in the *cps* region of ST42 and ST258 clade I strains and that in strain Kp1832, a representative ST42 isolate in our strain collection. The gross organization between ST42 and ST258 strains indicates their distal genetic relatedness (Fig. 1B), a finding consistent with MLST data. An SNP analysis confirmed that there was significant genome divergence between ST258 and ST42 strains. A total of 31,157 SNPs distinguished the three ST258 strains from the ST42 strain, Kp1832, and 27% of these SNPs (8,444 SNPs) were located in the ~1.1-Mbp recombination region (Fig. 3).

Since the two sequenced reference strains (NJST258_1 and NJST258_2) were genotyped as ST258 clade II strains, we created a *de novo* genome sequence of the prototypic ST258 clade I strain

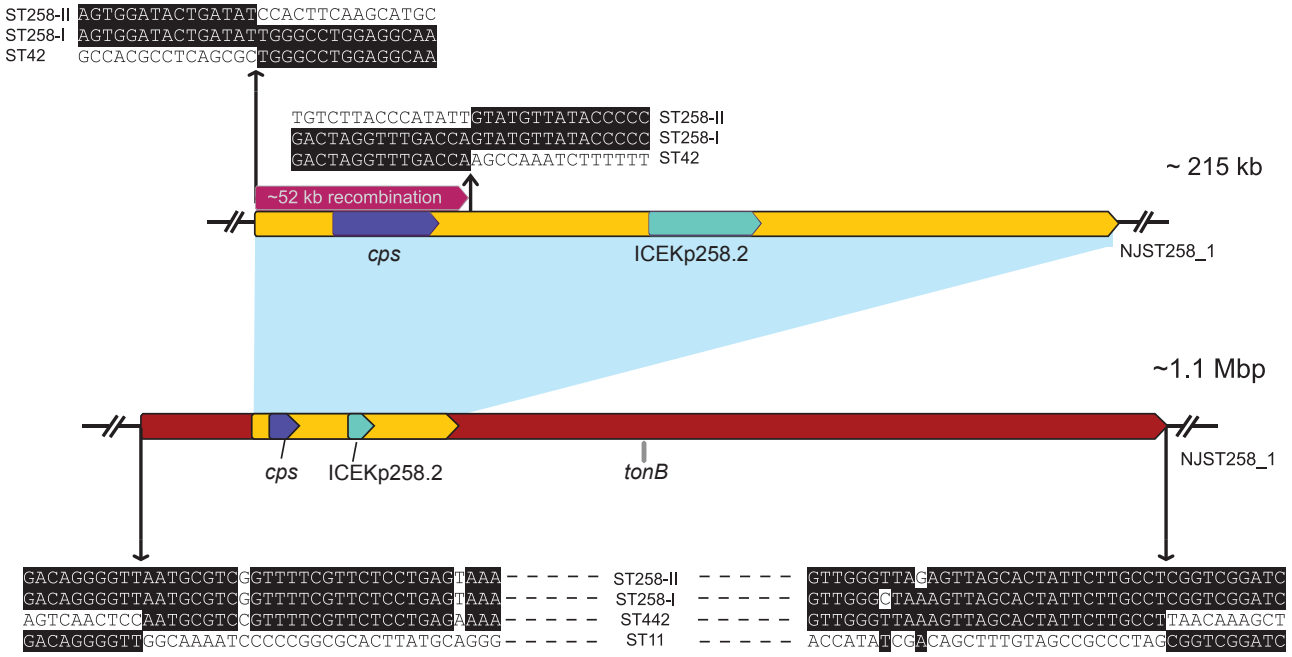

FIG 2 Upstream and downstream junction SNPs for the ~1.1-Mbp recombination fragment and *cps* region in ST258, ST442, and ST42 strains. The start site of the replacement of the ~52-kb *cps*-harboring region is the same as that of the ~215-kb RD in ST258 II clades (7). Sequences were obtained from Kp1878 (an ST258 clade I strain), NJST258_1 (an ST258 clade II strain), JM45 (an ST11 strain), Kp1832 (an ST42 strain), and Kp13 (an ST442 strain).

(Kp1787) to use as a clade I reference genome. An ~450-kb region of the Kp1787 genome, which contains the entire ~215-kb RD, was used as a reference to examine recombination events between Kp1832 (ST42) and Kp1787 (an ST258 clade I strain). The comparison revealed a nearly identical region (2 SNPs) spanning ~52 kb that contains the *cps* region; the alignment of the two regions maps to the start of this DNA replacement at the same location in the RD region (Fig. 2). In addition, the ICEKp258.2 element is absent from strain Kp1832, and there is nucleotide divergence outside the aforementioned ~52-kb region (Fig. 1 and 2).

Comparative sequence analysis further showed that the neighboring sequences upstream and downstream from this ~52-kb region are identical in ST258 clade I and II strains (Fig. 2). In addition, the SNP distribution among the 85 ST258 genomes reported in our previous study revealed that 592 (89%) of the 664 SNPs in the RD are located within the ~52-kb *cps*-harboring region (7). Together, the genomic findings are consistent with the hypothesis that clade I evolved rapidly through the acquisition of the *cps* region from an ST42 strain. The evidence provided above strongly suggests that replacement of the original (presumably ST258 clade II) *cps* region in clade I contributes largely to the noted phylogenetic difference between the two ST258 clades.

***bla*$_{KPC}$-harboring genetic element.** We and others have reported that the *bla*$_{KPC}$ gene in ST258 strains is carried exclusively by a Tn*3*-based transposon, Tn*4401* (5, 7, 14). Tn*4401* is 10 kb in length, delimited by two 39-bp imperfect inverted repeat (IR) sequences, and harbors the *bla*$_{KPC}$ gene, a Tn*3* transposase gene (*tnpA*), a Tn*3* resolvase gene (*tnpR*), and two insertion sequences, IS*Kpn6* and IS*Kpn7* (15) (see Fig. S2 in the supplemental material). In contrast, ST11 and ST442 strains harbor *bla*$_{KPC}$-containing elements that are distinct from those in ST258 strains (see Fig. S2) and share only ~2 kb of sequence with Tn*4401*. Collectively, these findings suggest ST258 strains are hybrid strains that arose from an ancestral ST11 strain that acquired an ~1.1-Mbp contiguous chromosomal segment from an ST442-like strain by DNA recombination/replacement. The identification of distinct *bla*$_{KPC}$-harboring elements in ST258, ST11, and ST442 strains indicates *bla*$_{KPC}$ was acquired by ST258 strains via horizontal gene transfer (rather than by vertical gene transmission from ST11 or ST442 parental strains) after the recombination events.

DISCUSSION

The current rise of KPC-producing *K. pneumoniae* infections in U.S. health care facilities has been overwhelmingly associated with strains typed as ST258. To better understand the evolutionary history of this epidemic clone, we compared the genome sequences of ST258 strains, single-locus variant ST11 strains, and other selected *K. pneumoniae* strain types. Notably, we discovered that ST258 strains are hybrid strains comprised of genomic DNA from ST11 (~80%) and ST442 (~20%)-like strains—presumably the product of a large chromosomal replacement event.

Recombination events and replacement of large chromosomal regions have been documented in various bacteria, and there are reported examples where the hybrid strains are associated with epidemiological success. Robinson and Enright were the first to report a naturally occurring bacterial hybrid—in this case, in a *Staphylococcus aureus* strain known as ST239 (16). This hybrid strain is a pandemic methicillin-resistant *S. aureus* (MRSA) strain responsible for ~90% of the nosocomial infections throughout mainland Asia and much of South America (17). ST239 is comprised of large chromosomal regions from two distantly related lineages, ST8 and ST30. Approximately 20% of the ST8 genome was replaced with an ~550-kb contiguous chromosomal fragment from an ST30 donor strain, thereby creating ST239. This apparently rare molecular event has not been explained or reproduced

A

	NJST258_2	Kp1787	JM45	HS11286	ATCC BAA-2146	Kp13	Kp1832
NJST258_1	175/43	508/360	8180/8027	8056/7742	12829/12661	21035/217	31304/8566
NJST258_2		445/337	8117/8004	7993/7719	12766/12638	20966/194	31240/8543
Kp1787			8087/7968	8056/7771	12743/12609	21284/503	30927/8222
JM45				3621/3370	6784/6681	28841/8086	30395/7718
HS11286					8183/7915	28522/7761	30080/7414
ATCC BAA-2146						33493/12721	35032/12338
Kp13							31848/8590

B

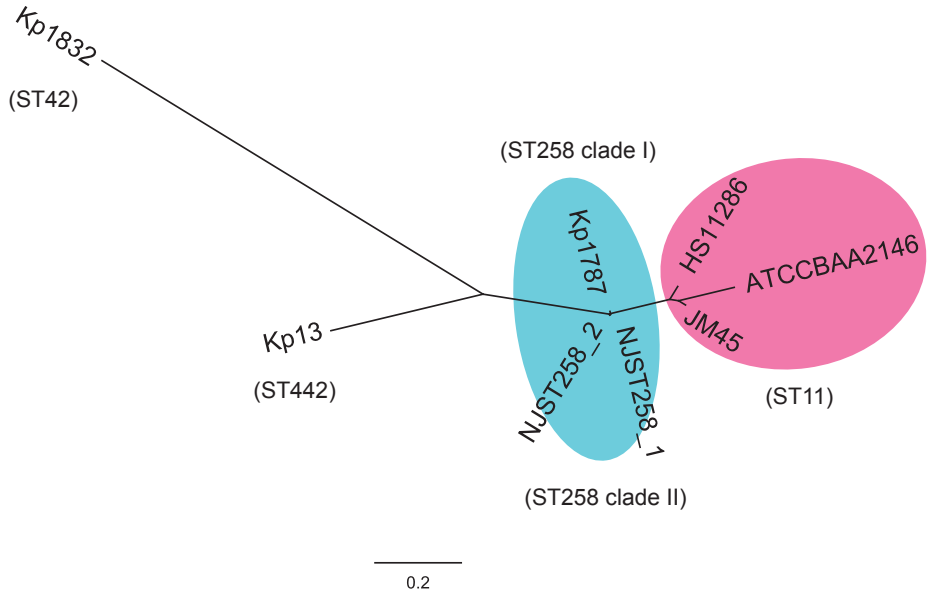

FIG 3 (A) SNP matrix for different *K. pneumonaie* strains. The matrix is illustrated as (total no. of SNPs/no. of SNPs in the ~1.1-Mbp recombination region). Green shading indicates the number of SNPs in ST258 strains compared to that in ST11 strains. Orange shading indicates the number of SNPs in ST258 strains compared to that in ST442 strains. (B) Phylogenetic analysis of the eight isolates based upon 52,135 concatenated SNPs in the core genome.

in the laboratory. In group B *Streptococcus* (GBS), large single chromosomal replacement events and multiple localized recombination events occur naturally and can be reproduced in the laboratory (18). For GBS, conjugation is the molecular pathway for genomic movement (19). It is worth noting that genetic replacement of the GBS *cps* region between unrelated sequence types is the common mechanism by which this species alters its surface antigen composition (18). Similarly, *cps* region replacement-associated capsular switching has also been suggested as being an intrinsic feature throughout the evolutionary history of *Streptococcus pneumoniae* (20).

Here we discovered that ST258 clade II strains are hybrid strains in which 20% of the *K. pneumoniae* ST11 genome was replaced with a homologous ~1.1-Mbp contiguous region from a strain in the ST442 lineage. This region, which includes the previously described region of difference (RD) and capsular polysaccharide biosynthetic genes, has molecular scars of multiple localized recombination events, similar to the phenomenon in *Streptococcus agalactiae* (18, 19). The finding that ST442 and ST258 have a contiguous chromosomal region in common and that the nucleotide boundaries between the ST442 and ST258 clade I and II genomes are indistinguishable (Fig. 2) is evidence that the recombination event creating an ST11 and ST442 hybrid strain likely occurred once, thereby creating the ST258 clade II lineage (Fig. 4).

Based on recent genome-scale studies, there have been numerous putative chromosomal recombination events involving the

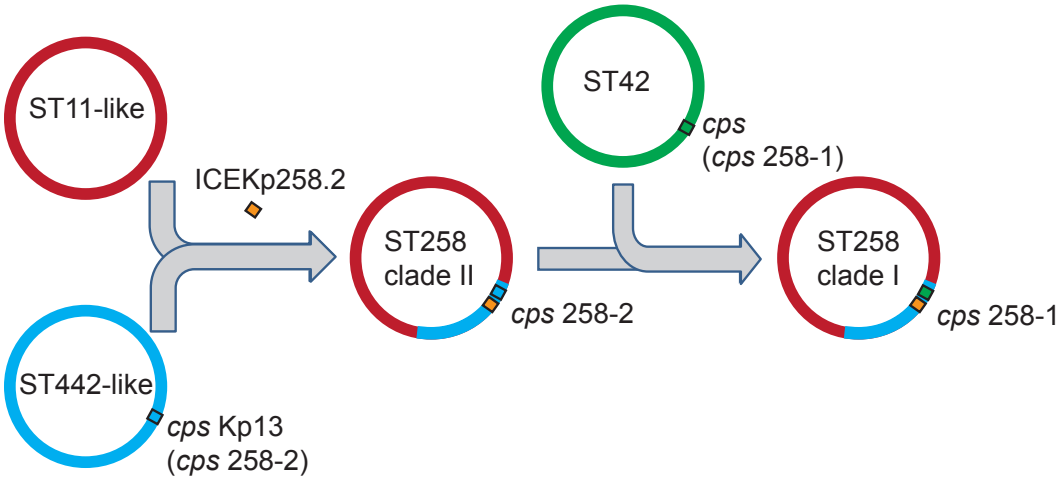

FIG 4 Hypothesized evolutionary history in *K. pneumoniae* ST258 strains.

region encoding the CPS biosynthetic machinery (7). ST258 clades I and II have distinct *cps* regions, and this is also true of the three ST11 strains analyzed in this study (Fig. 1B), further supporting the notion that DNA exchange in and around the *cps* regions may be a general mechanism used by *K. pneumoniae* to rapidly diversify and that novel clades arise through *cps* switching between ST258 (clade I or II) and unrelated *K. pneumoniae* sequence types.

The presence, absence, and diversity of genetic landmarks within the acquired ~1.1-Mbp region provide clues into ST258's recent evolutionary origin and the extent of its genomic plasticity. ICEKp258.2, which is absent in ST442, is present in both ST258 clades, where its chromosomal insertion site is conserved, suggesting that this ICE was acquired after the major genome recombination event that gave rise to ST258 (Fig. 4). In support of the notion that ICEKp258.2 is a relatively recent acquisition, the G+C content of ICEKp258.2 is 37.1%, significantly less than the ~57.5% G+C content of the entire *K. pneumoniae* chromosome. Taken together, these observations provide evidence that ICEKp258.2 is exogenous and was likely acquired once by ST258, before the recombination events involving the *cps* regions in clades I and II (Fig. 4). Moreover, the replacement of the ST258 clade II *cps* region with that from ST42 (thus creating clade I) occurred after the acquisition of ICEKp258.2 (Fig. 4).

Adler and colleagues investigated the association of the ICEKp258.2 with ST258 by testing160 *K. pneumoniae* strains with diverse sequence types for the presence of *pilV*, a gene carried on ICEKp258.2 (21). They found that *pilV* was present only in ST258 and genetically related strains. Based on sequence analysis, ICEKp258.2 harbors a type IV pilus gene cluster and a type III restriction-modification system. A type IV pilus could increase the uptake and exchange of DNA, such as plasmids, as well as facilitate adherence to living and nonliving surfaces—e.g., the human gut or the environment (22)—which may in part explain the high transmissibility of ST258 strains and the movement of KPC genes. Additionally, a type III restriction-modification system could serve in "host specificity" regarding the exchange of certain compatible plasmids and other mobile elements (23). Restriction of plasmids and specific mobile elements may explain the differences observed between ST11 (which lacks ICEKp258.2) and ST258, as the former is associated with a broad range of plasmids and carbapenemases (KPC, VIM, IMP, NDM, and OXA-48) (10–12, 24–28), whereas ST258 strains predominantly harbor KPC. Taken together, the association of ICEKp258.2 with ST258 *K. pneumoniae* strains raises the possibility that this element may contribute to epidemiological success of this sequence type. To investigate whether ICEKp258.2 could potentially be an "epidemic clone-specific" target, we are currently investigating the impact of altering the type IV pilus gene cluster and the type III restriction-modification system in this element.

Taken together, our findings underscore the role of recombination in the rapid evolution of clinical strains of *K. pneumoniae* in both creating hybrid clones and in more localized chromosomal replacements that alter antigenic presentation and ultimately divert the host response.

MATERIALS AND METHODS

Sequence information. Data used in comparative analysis were downloaded from the NCBI database (http://www.ncbi.nlm.nih.gov/genome/genomes/815), including complete genome sequences and annotation of *K. pneumoniae* isolates HS11286 (CP003200) (29), JM45 (CP006656), ATCC BAA-2146 (CP006659) (30), Kp13 (CP003999) (8), NJST258_1(CP006923) (7), and NJST258_2 (CP006918) (7). Additional sequence data were retrieved from our recent study on *K. pneumoniae* ST258 (7).

Genome sequencing and assembly. Strain Kp1832 was selected from one of the seven ST42 *K. pneumoniae* isolates that carry the same *wzy* and *wzi* genes as ST258 *cps-1* strains (7). Genomic DNA isolation and library preparation were performed as described previously (7). The genome was sequenced using an Illumina MiSeq platform, which generated 250-bp paired-end reads. *De novo* assembly for Kp1832 and Kp1787 (a representative ST258 clade I strain, selected from our previous study [7]) was accomplished by using a combination of CLC genomic workbench (v 7.0.3; CLC Bio, Aarhus, Denmark), Mira (31), and Velvet (32). The best assemblies from each method were combined using Geneious Pro software in order to generate the supercontig for the *cps*-harboring element.

Comparative genomics analysis. Visualization of circular genome comparisons was performed using the BLAST ring image generator (BRIG) (33). Prophages were identified by PHAST (34). Insertion sequences were identified using the IS Finder database (http://www-is.biotoul.fr). *De novo* assembled contigs from Kp1832 and Kp1787 were ordered and oriented relative to the NJST258_1 genome and then com-

bined together as a pseudochromosome using the Mauve contig mover (35). Multiple genome sequence alignments and comparison analysis were then performed with Mauve (35). For core genomic analysis, SNPs located on the MGEs, including prophases, ICEs, and insertion elements, as well as those on rRNAs and tRNAs, were excluded. The concatenated SNPs were used to generate a consensus phylogenetic tree by the maximum likelihood method based on the Tamura-Nei model with the MEGA 5 software (36). The SNP distribution among different genome sequences was inferred from genome alignment using Mauve (35), and SNPs were counted on a 1,000-nucleotide (nt) window based on the nucleotide position on NJST258_1.

Nucleotide sequence accession numbers. Illumina short read data for ST258 have been deposited in the Sequence Read Archive (SRA) database under accession no. SRP036874 (7). The Illumina short read data for Kp1832 have been deposited in the SRA database under accession no. SRX512850.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at http://mbio.asm.org/lookup/suppl/doi:10.1128/mBio.01355-14/-/DCSupplemental.

Figure S1, EPS file, 25.9 MB.

Figure S2, EPS file, 1.1 MB.

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B.N.K. discloses that he holds two patents that focus on using DNA sequencing to identify bacterial pathogens.

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