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Plasmid genomic epidemiology of *bla*$_{KPC}$ carbapenemase-producing *Enterobacterales* in Canada, 2010–2021

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ABSTRACT Carbapenems are considered last-resort antibiotics for the treatment of infections caused by multidrug-resistant *Enterobacterales*, but carbapenem resistance due to acquisition of carbapenemase genes is a growing threat that has been reported worldwide. *Klebsiella pneumoniae* carbapenemase (*bla*$_{KPC}$) is the most common type of carbapenemase in Canada and elsewhere; it can hydrolyze penicillins, cephalosporins, aztreonam, and carbapenems and is frequently found on mobile plasmids in the Tn*4401* transposon. This means that alongside clonal expansion, *bla*$_{KPC}$ can disseminate through plasmid- and transposon-mediated horizontal gene transfer. We applied whole genome sequencing to characterize the molecular epidemiology of 829 *bla*$_{KPC}$ carbapenemase-producing isolates collected by the Canadian Nosocomial Infection Surveillance Program from 2010 to 2021. Using a combination of short-read and long-read sequencing, we obtained 202 complete and circular *bla*$_{KPC}$-encoding plasmids. Using MOB-suite, 10 major plasmid clusters were identified from this data set which represented 87% (175/202) of the Canadian *bla*$_{KPC}$-encoding plasmids. We further estimated the genomic location of incomplete *bla*$_{KPC}$-encoding contigs and predicted a plasmid cluster for 95% (603/635) of these. We identified different patterns of carbapenemase mobilization across Canada related to different plasmid clusters, including clonal transmission of IncF-type plasmids (108/829, 13%) in *K. pneumoniae* clonal complex 258 and novel repE(pEh60-7) plasmids (44/829, 5%) in *Enterobacter hormaechei* ST316, and horizontal transmission of IncL/M (142/829, 17%) and IncN-type plasmids (149/829, 18%) across multiple genera. Our findings highlight the diversity of *bla*$_{KPC}$ genomic loci and indicate that multiple, distinct plasmid clusters have contributed to *bla*$_{KPC}$ spread and persistence in Canada.

KEYWORDS plasmid, carbapenemase, antimicrobial resistance, surveillance studies

Carbapenems are considered last-resort antibiotics for the treatment of infections caused by multidrug-resistant Gram-negative bacteria. Following the use of carbapenems in clinical practice, the emergence of carbapenem-resistant pathogens poses a great threat to human health (1). Carbapenem-resistant *Enterobacterales* have been reported worldwide as a consequence largely of the acquisition of carbapenemase genes (2).

Of the different classes of carbapenemases, *Klebsiella pneumoniae* carbapenemase (*bla*$_{KPC}$) is the most commonly identified in many countries including the United States and Canada [reviewed in references (3–6)]. *bla*$_{KPC}$ was the dominant type of carbapenemase isolated from 2010 to 2014 by the Canadian Nosocomial Infection Surveillance Program (CNISP), where they comprised 50%–83% of all carbapenemases analyzed per year (6). From 2017 to 2021, the rates of carbapenemase-producing *Enterobacterales*

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infections in Canada increased by 166% (from n = 20 in 2017 to n = 55 in 2021), and bla_{KPC} remains the most common carbapenemase type detected in Canada (7).

Since the first identification of bla_{KPC} almost 30 years ago (8), bla_{KPC} has been found in over 100 different *K. pneumoniae* sequence types (STs) (9), but the original dissemination was driven primarily by the spread of bla_{KPC}-producing *K. pneumoniae* isolates that are members of clonal complex 258 (9–12). Along with clonal expansion, bla_{KPC} can disseminate through plasmid- and transposon-mediated horizontal gene transfer and has been detected in at least 11 other genera (3, 10, 13–16).

Advances in genome sequencing have enabled detailed characterization of complete bla_{KPC}-encoding plasmids in recent years (13, 15). bla_{KPC} is associated with a variety of plasmid types, including the narrow-host-range IncF-type plasmids which were crucial for the success of *K. pneumoniae* clonal complex 258 (3, 12, 15). bla_{KPC} has also been found on broad-host-range plasmids with IncN, IncR, IncX, ColRNA, IncA/C, or IncP replicons around the world (3, 13, 15, 17–20). Many of these plasmids can have multiple replicons, undergo large rearrangements, and encode other genetic features that ensure their persistence (20).

Here, we applied whole genome sequencing to characterize the molecular epidemiology of bla_{KPC} carbapenemase-producing isolates collected by the Canadian Nosocomial Infection Surveillance Program from 2010 to 2021. Using combined short-read and long-read sequencing of selected representatives to generate complete bla_{KPC}-encoding plasmids, we investigated the diversity of carbapenemase-encoding plasmids among these isolates across Canada and compared them to the global context of bla_{KPC}.

RESULTS

Characteristics of bla_{KPC} carbapenemase-producing isolates

A total of 829 bla_{KPC}-producing isolates were submitted by 34 hospital sites across Canada from 2010 to 2021. We performed short-read and long-read sequencing on a selection of 155 isolates. The bla_{KPC}-producing isolates belonged to 10 genera and 26 different species, with the most common genera being the *Klebsiella pneumoniae* species complex (268/829, 32.3%), *Enterobacter cloacae* complex (203/829, 24.4%), *Citrobacter freundii* complex (132/829, 15.9%), and *Escherichia coli* (73/829, 8.8%) (Fig. 1). The most common sequence types were *K. pneumoniae* ST512 (71/268, 26.4%) and ST258 (35/268, 13.1%) (note that ST512 is a single locus variant of ST258), *E. cloacae* ST316 (53/203, 26.1%), *C. freundii* ST22 (29/132, 32.0%), and *E. coli* ST131 (17/73, 23.2%) (Fig. 1).

Using the StarAMR tool for the detection of resistance genes in the whole genome sequencing data, we observed 93% (773/829) of bla_{KPC}-producing isolates harbored additional β-lactamase genes alongside the bla_{KPC} carbapenemase (Fig. 2). Of the 773 isolates harboring additional β-lactamases, $bla_{TEM\text{-}1B}$ (187/773, 24.2%), $bla_{OXA\text{-}9}$ (146/773, 18.9%), $bla_{TEM\text{-}1A}$ (125/773, 16.2%), $bla_{SHV\text{-}182}$ (91/773, 11.8%), and $bla_{OXA\text{-}1}$ (82/773, 10.6%) were the most common types. Aminoglycoside, sulfonamide, and trimethoprim resistance genes were commonly observed among multiple genera (Fig. 2); the most common genes included *sul1* (482/829, 58.1%), *qacE* (424/829, 51.1%), *aac*(6′)-Ib-cr (357/829, 43.1%), *sul2* (227/829, 27.4%), *aadA2* (225/829, 27.1%), *mphA* (224/829, 27.0%), and OqxA/B (224/829, 27.0%). β-Lactamases were significantly more likely to be found in *Enterobacter* spp., *Citrobacter* spp., or *Klebsiella* spp. than *E. coli* ($P < 0.001$ for all comparisons). Colistin and quinolone resistance genes were significantly more likely to be found in *Enterobacter* spp. than any other genera ($P < 0.001$ for all comparisons). Aminoglycoside, macrolide, sulfonamide, and trimethoprim resistance genes were significantly more likely to be found in *Klebsiella* spp. or *Citrobacter* spp. compared to *Enterobacter* spp. or *E. coli* ($P < 0.035$ for all comparisons). Rifampin resistance genes were significantly more likely to be found in *Citrobacter* spp. than any other genera ($P < 0.001$ for all comparisons).

A total of 844 bla_{KPC} genes were detected among the 829 isolates, indicating an occurrence of 1.7% of isolates harboring >1 copy of bla_{KPC}. The $bla_{KPC\text{-}3}$ variant was the most common (678/844, 80.3%) followed by $bla_{KPC\text{-}2}$ (162/844, 19.2%). Two isolates had

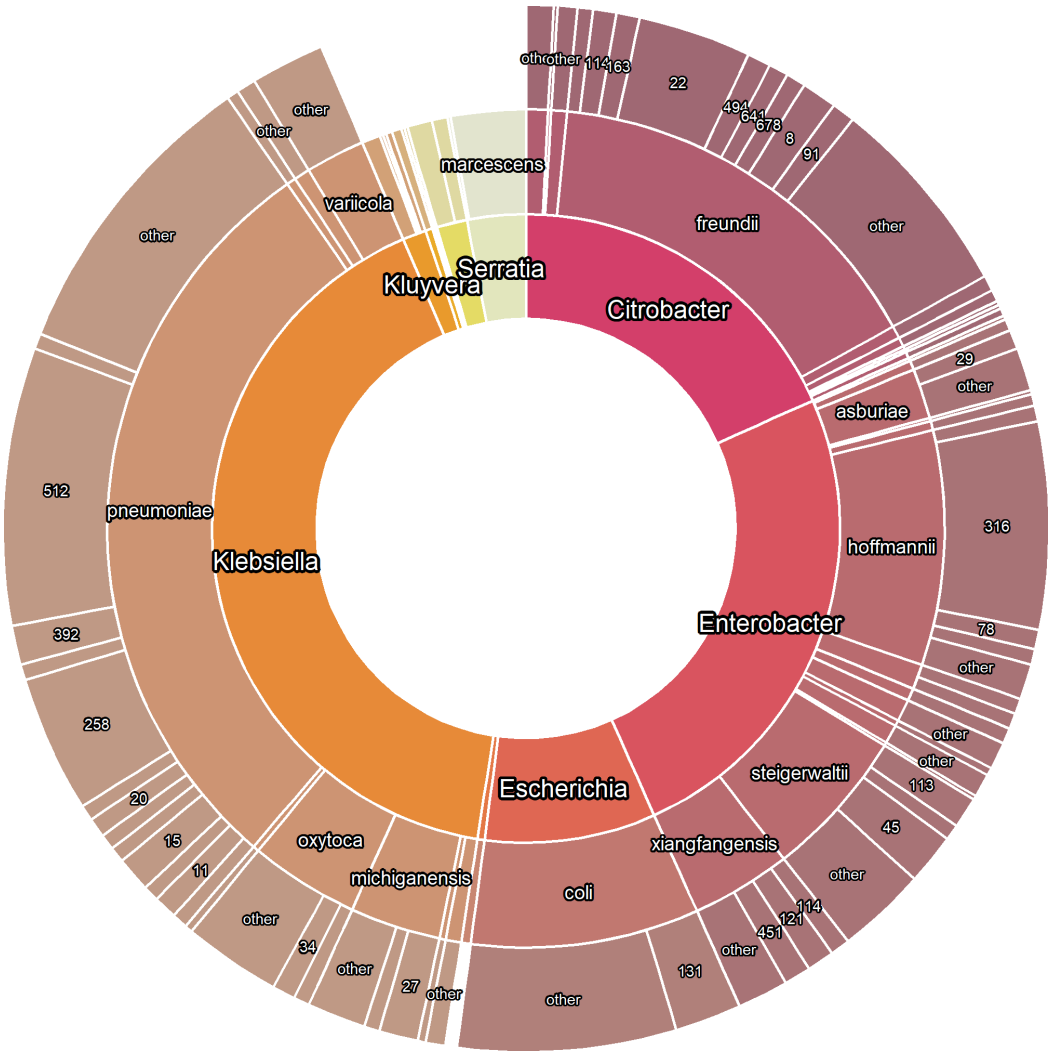

FIG 1 Summary of genera (inner ring), species (middle ring), and multi-locus sequence types (MLST; outer ring) of *bla*$_{KPC}$-encoding isolates included in the study (829 total isolates). MLST profiles found in three or fewer isolates were grouped into "other". Not all labels are displayed.

bla$_{KPC-4}$, one isolate had *bla*$_{KPC-9}$, and one isolate had *bla*$_{KPC-18}$. In addition to *bla*$_{KPC}$, several genomes carried additional carbapenemase genes (7/829, 0.8%; 2 *bla*$_{NDM-1}$, 2 *bla*$_{NDM-5}$, 1 *bla*$_{OXA-232}$, and 2 *bla*$_{VIM-1}$).

Genomic context of *bla*$_{KPC}$ genes

The *bla*$_{KPC}$ genes are often located on the Tn*4401* transposon which facilitates their mobility to other DNA elements and strains (21, 22). In this data set, *bla*$_{KPC}$ was frequently found within the previously described Tn*4401*b isoform (643/829, 77.6%), followed by the Tn*4401*a isoform (132/829, 15.9%), Tn*4401*d (5/829, 0.6%), and Tn*4401*e (1/829, 0.01%). The remainder of *bla*$_{KPC}$ (38/829, 4.6%) were found on a combination of truncated Tn*4401*, a unique isoform of Tn*4401* or complete absence of Tn*4401*.

There were 25 different Tn*4401* isoforms in this data set. The majority of the single nucleotide variants (SNVs) within Tn*4401* observed here differed from those included in TETyper (23), so we generated a custom numbering scheme described in Table S2. The most common SNV profiles were 3 (439/829, 53.0%; A7029G|C8015T), 1 (128/829, 15.4%; none), 2 (115/829, 13.9%; C8015T, differentiates *bla*$_{KPC-2}$ and *bla*$_{KPC-3}$), 7 (56/829, 6.8%; C8015T|G9882T), 8 (18/829, 2.2%; A7029G|C7672T|C8015T), and 4 (10/829, 1.2%; A7029G|C8015T|T9317C) (Fig. 3).

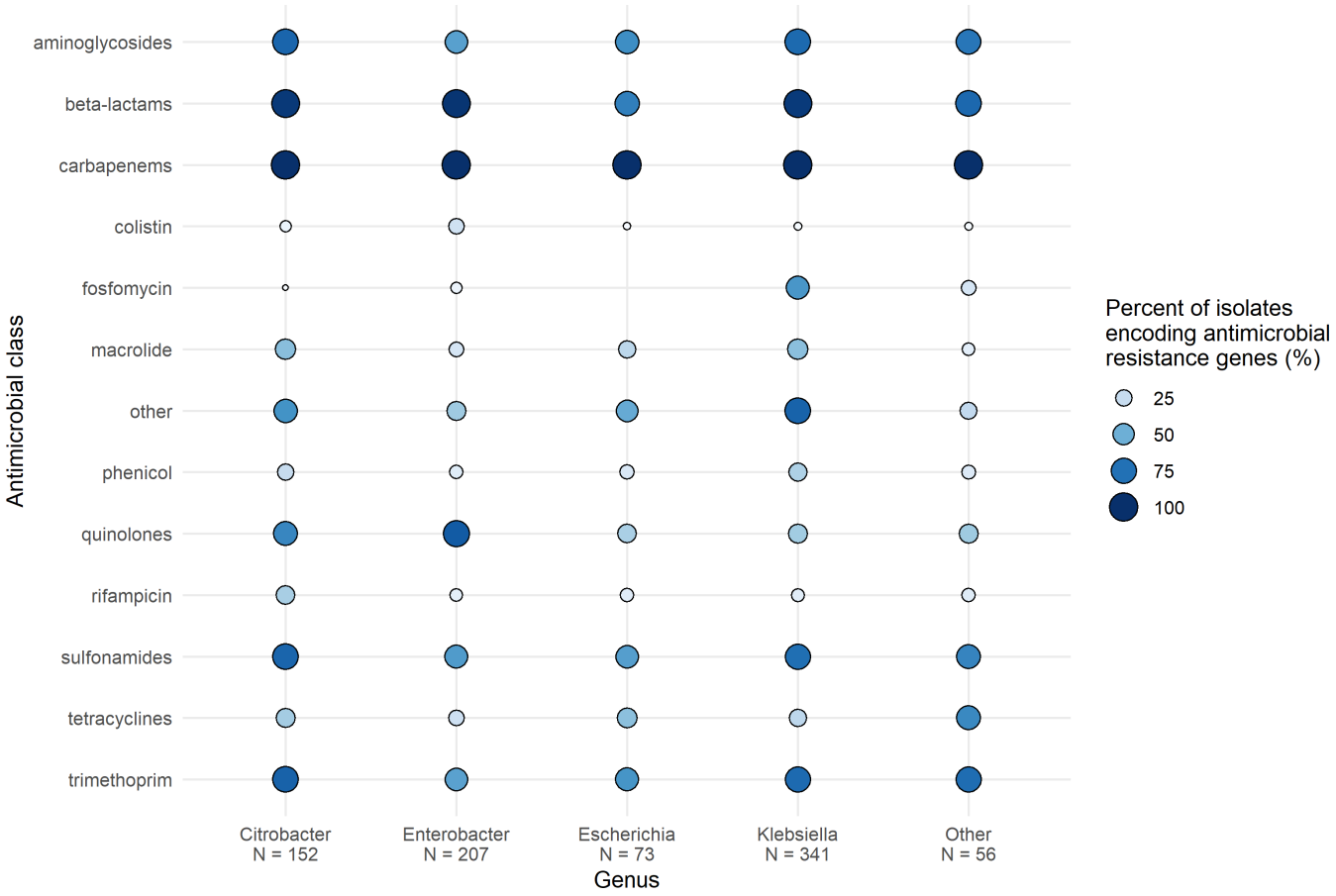

FIG 2 Proportion of isolates encoding antimicrobial resistance genes identified by StarAMR, then categorized by drug class is presented across the top four genera. Values represent proportion of isolates encoding genes belonging to a certain antimicrobial class. "*N*" indicates the number of isolates in that genus. The "Other" genera include *Serratia* spp., *Raoultella* spp., *Pseudescherichia* spp., *Pantoea* spp., *Morganella* spp., *Kluyvera* spp., and *Hafnia* spp.

Complete and circular *bla*$_{KPC}$ plasmids in a global context

Unsurprisingly, we obtained only 59 closed circular plasmids from Illumina-only assemblies. Using a hybrid approach on a subset of 155 isolates, we obtained an additional 143 complete circular plasmids. Complete *bla*$_{KPC}$-encoding plasmids (n = 202) ranged from 7.6 kb to 374.7 kb in size and were distributed among the top species as described above (Fig. 3). Plasmid incompatibility groups included IncL/M (65/202, 32.2%), IncN types (51/202, 25.2%), IncF types (39/202, 19.3%), IncX5 (10/202, 5.0%), and ColRNAI types (21/202, 10.3%), among others. Plasmids were predicted to be conjugative (148/202, 73.2%), mobilizable (36/202, 17.8%), or non-mobilizable (18/202, 8.9%) based on the presence of self-encoded *oriT* sequences, relaxases, and mating pair formation (MPF) proteins detected by MOB-suite (24).

To investigate where these plasmids fit within a global data set, we clustered our 202 complete plasmids alongside 34,513 plasmids present in PLSDB (25) using the MOB-cluster tool from MOB-suite (24, 26). Altogether, the plasmids clustered into 10,996 distinct primary clusters, and our *bla*$_{KPC}$ plasmids grouped into a subset of 28 primary clusters which all contained representative plasmids from PLSDB.

From the above data, we observed 10 primary clusters which represented 87% (175/202) of the Canadian *bla*$_{KPC}$ plasmids from this study. The primary clusters generally reflected incompatibility groups and the major trends of each are summarized in Table 1. Within the global plasmid data set, the presence of *bla*$_{KPC}$ varied between our top primary clusters (Fig. 4); some primary clusters tended to be frequently associated with *bla*$_{KPC}$ (AA014, AA013, AA029, and AA109), whereas others appeared to encode *bla*$_{KPC}$

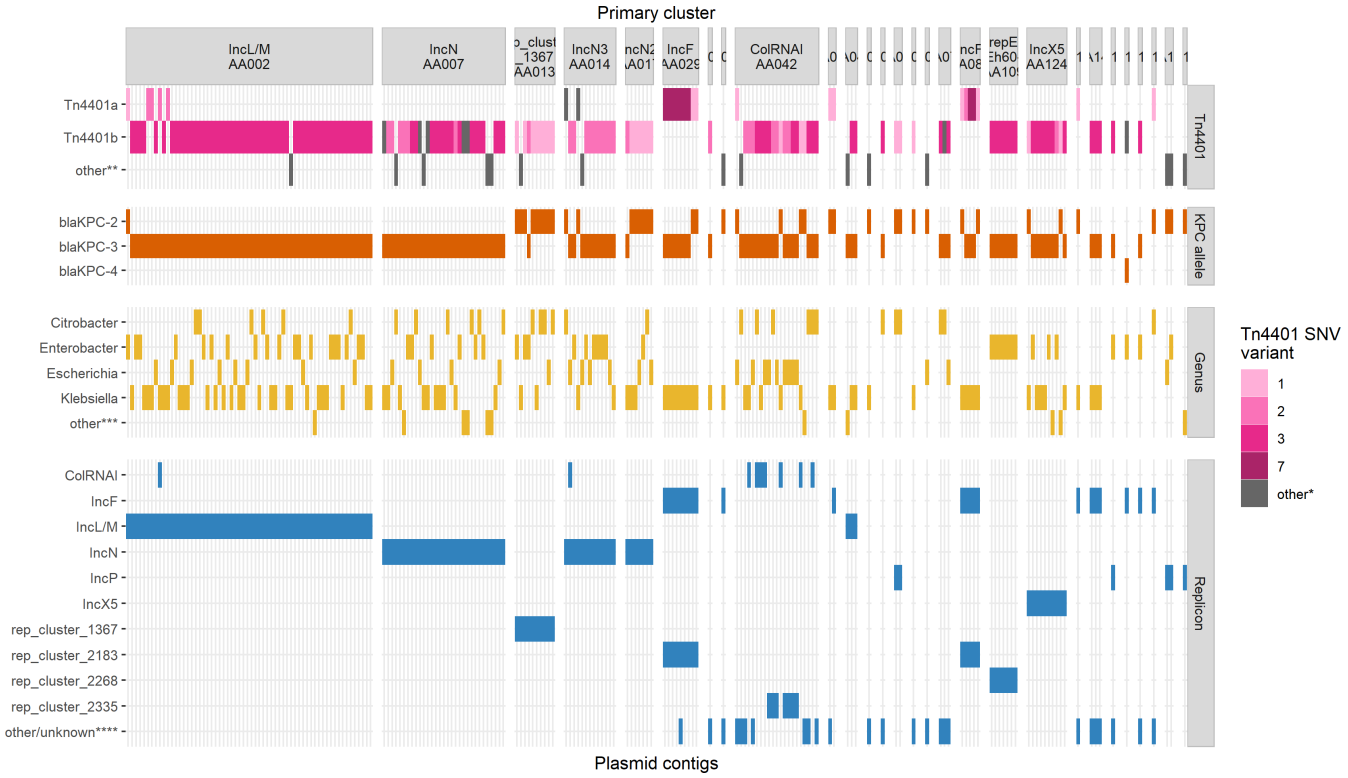

FIG 3 Characteristics of 202 *bla*$_{KPC}$-encoding plasmids sequenced in this study. Groups on the *x*-axis correspond to primary cluster IDs generated among the plasmid database PLSDB and Canadian *bla*$_{KPC}$-encoding plasmids. "Other*" indicates SNVs found in fewer than three plasmids or Tn*4401* absence. "Other**" indicates either truncated, alternative isoforms or absence of Tn*4401*. "Other***" indicates *Hafnia*, *Kluyvera*, *Morganella*, *Raoultella*, or *Serratia* genera. "Other/unknown****" indicates replicons (n = 26) found in fewer than five plasmids or replicons that could not be typed.

more sporadically (AA085 and AA017). Some clusters had a tight distribution of plasmid sizes (AA013 and AA042) whereas others had a broader size distribution (AA085 and AA002). The percentage of core/soft core genes (defined as genes found in >95% plasmids) varied from 3% (AA042) to 28% (AA014) across the primary clusters, indicating some tend to have more diverse or dynamic gene content than others. We also evaluated the core genome size of the Canadian plasmids within each of these primary clusters as we suspected that the Canadian plasmids would be more closely related to each other than to global plasmids. As expected, the percentage of core genes within the Canadian plasmids was higher than the percentage predicted in the global data set with one exception (AA042) (Fig. 4). Several primary clusters had an average of five or more antimicrobial resistance genes (AA002, AA017, and AA085). Certain primary clusters had broad host ranges (AA002, AA007, and AA124), whereas others were only found in a single genus (AA085 and AA109). The dominant type of transposon was Tn*4401*b except for the IncF clusters (AA085 and AA029) where Tn*4401*a was dominant.

Not surprisingly, all primary clusters with the exception of AA042 contain features that support their stability and persistence in the host cell. Of the genes found in >95% of plasmids in each cluster, between one and five genes (representing 2%–33% of genes per cluster) are involved in stability/transfer/defense which includes genes such as partition/stability genes (e.g., *parA*, *parM*, and *stbB*), anti-restriction genes (e.g., *ardA*, *ardB*, and *ardR*), and SOS-inhibition genes (e.g., *psiA* and *psiB*). Different primary clusters had different proportions of stability/transfer/defense gene content; some harbored a single anti-restriction protein (AA124), whereas some encoded two anti-restriction proteins, a plasmid stability protein, an endonuclease, and a stability protein (AA029).

TABLE 1 Summary features of top 10 Canadian *bla*$_{KPC}$-encoding plasmid clusters among PLSDB

Primary cluster ID	No. Canadian plasmids	Median size (kb)	Predicted mobility[a,b]	Replicon type[a,b]	Relaxase type[a,b]	MPF type[a,b]	No. genera	Core genes (%)[d]	*bla*$_{KPC}$ allele[a]	*bla*$_{KPC}$ frequency	Tn*4401* variant[a]	Mean no. ARGs per plasmid[c]	Plasmid taxonomic unit (PTU)[e]
AA002	62	75	Conjugative	IncL/M	MOBP	MPF_I	12	0.09	*bla*$_{KPC}$-3	0.34	Tn*4401*b	5.1	L/M
AA007	31	57.3	Conjugative	IncN	MOBF	MPF_T	15	0.06	*bla*$_{KPC}$-3	0.38	Tn*4401*b	6.1	N1
AA013	10	43.6	Mobilizable	Rep_cluster_1367	MOBC	–[f]	8	0.18	*bla*$_{KPC}$-2	0.86	Tn*4401*b	2.5	E8
AA014	13	55.6	Conjugative	IncN	MOBF	MPF_T	4	0.28	*bla*$_{KPC}$-3	0.71	Tn*4401*b	1.4	N2/3
AA017	7	47	Conjugative	IncN	MOBF	MPF_T	5	0.14	*bla*$_{KPC}$-2	0.18	Tn*4401*b	5	N2/3
AA029	9	113.6	Conjugative	IncFIB, IncFII, rep_cluster_2183	MOBF, MOBF	MPF_F	4	0.04	*bla*$_{KPC}$-2	0.86	Tn*4401*a	3.6	FK
AA042	21	16.5	Mobilizable	ColRNAI_rep_cluster_1857	MOBC	–	7	0.03	*bla*$_{KPC}$-3	1	Tn*4401*b	1.7	Could not be assigned
AA085		185.7	Conjugative	IncFIB, IncFII, rep_cluster_2183	MOBF, MOBF	MPF_F	1	0.06	*bla*$_{KPC}$-2	0.12	Tn*4401*a	5.9	FK
AA109	7	97.9	Non-mobilizable	Rep_cluster_2268 [novel repE(pEh60-7)]	–	MPF_F	1	0.2	*bla*$_{KPC}$-3	0.78	Tn*4401*b	1	Could not be assigned
AA124	10	48.8	Conjugative	IncX5	MOBP	MPF_T	9	0.13	*bla*$_{KPC}$-3	0.4	Tn*4401*b	1.6	New (putative) PTU

[a]Values indicate the most common genotype in the cluster and may not apply to all plasmids in the cluster.
[b]Values obtained from MOB-suite (24, 26). Mobility is assigned based on the presence of relaxase (mobilizable) and/or MPF proteins (conjugative) or absence of both (non-mobilizable).
[c]ARGs = antimicrobial resistance genes.
[d]Core genes represent the number of genes present in >95% of plasmids in the cluster, divided by the total number of non-redundant genes in the cluster.
[e]PTU values obtained from COPLA (27) .
[f]"-" indicates no relaxase or MPF protein detected.

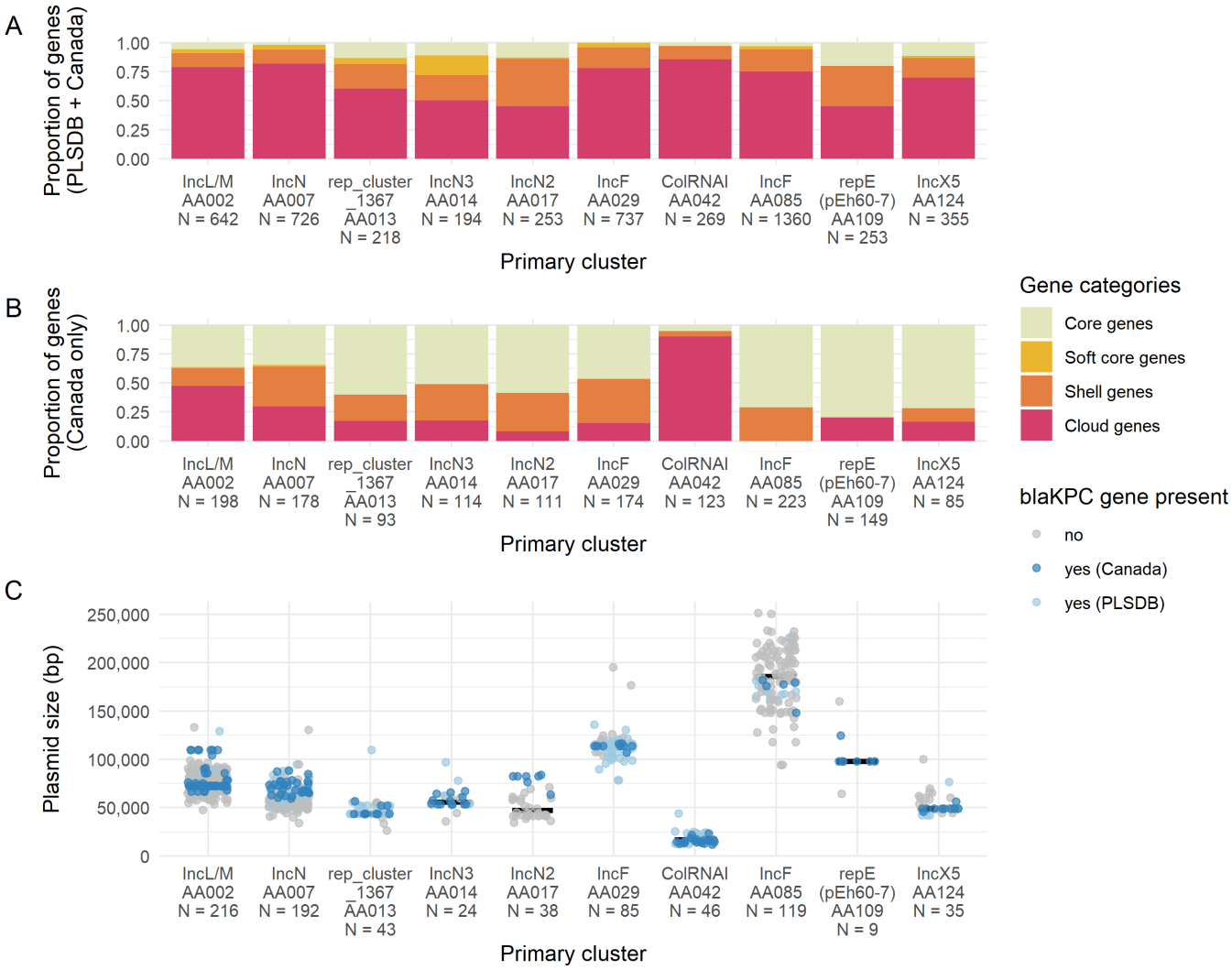

FIG 4 Pangenome size and *bla*$_{KPC}$ prevalence among top 10 MOB-suite primary clusters identified among PLSDB and Canadian *bla*$_{KPC}$-encoding plasmids. Pangenome size was calculated for (A) Canadian and PLSDB plasmids, and (B) Canadian plasmids only. Gene categories represent genes found in 99%–100% of plasmids (core), 95%–99% of plasmids (soft core), 15%–95% of plasmids (shell), and 0%–15% of plasmids (cloud). (C) Plasmid length in base pairs and prevalence of *bla*$_{KPC}$ among plasmids in each primary cluster.

Canadian *bla*$_{KPC}$-encoding plasmids in the top primary clusters

Given the high proportion of conserved genes among Canadian plasmids within each primary cluster (Fig. 4), we focused on the features of these Canadian plasmids separately from the other PLSDB plasmids present in each primary cluster. The primary clusters containing the most *bla*$_{KPC}$-encoding Canadian plasmids were IncL/M replicons (AA002) and IncN replicons (AA007, AA014, and AA017) (Table 1) and are examined in more detail below.

The Canadian plasmids in primary cluster AA002 (IncL/M) were classified into three secondary clusters: AL008 (49/62, 79%), AL013 (8/62, 13%), and AL001 (1/62, 1.6%) (Fig. 5). The first secondary cluster (AL008) had >99% nucleotide identity. They were 72.3 kb isolated from *K. pneumoniae* species complex, *K. aerogenes*, *K. oxytoca*, *C. freundii* complex, *E. cloacae* complex, *E. coli*, and *Raoultella planticola* between 2016 and 2020 from three sites in the same province. These plasmids harbor *bla*$_{KPC-3}$ on Tn*4401*b-3 and do not encode any other resistance genes. The next secondary cluster (AL013) was also shared among multiple genera with >99% nucleotide identity. They were 109.8 kb isolated from *E. coli*, *K. pneumoniae* species complex, and *E. cloacae* complex from one

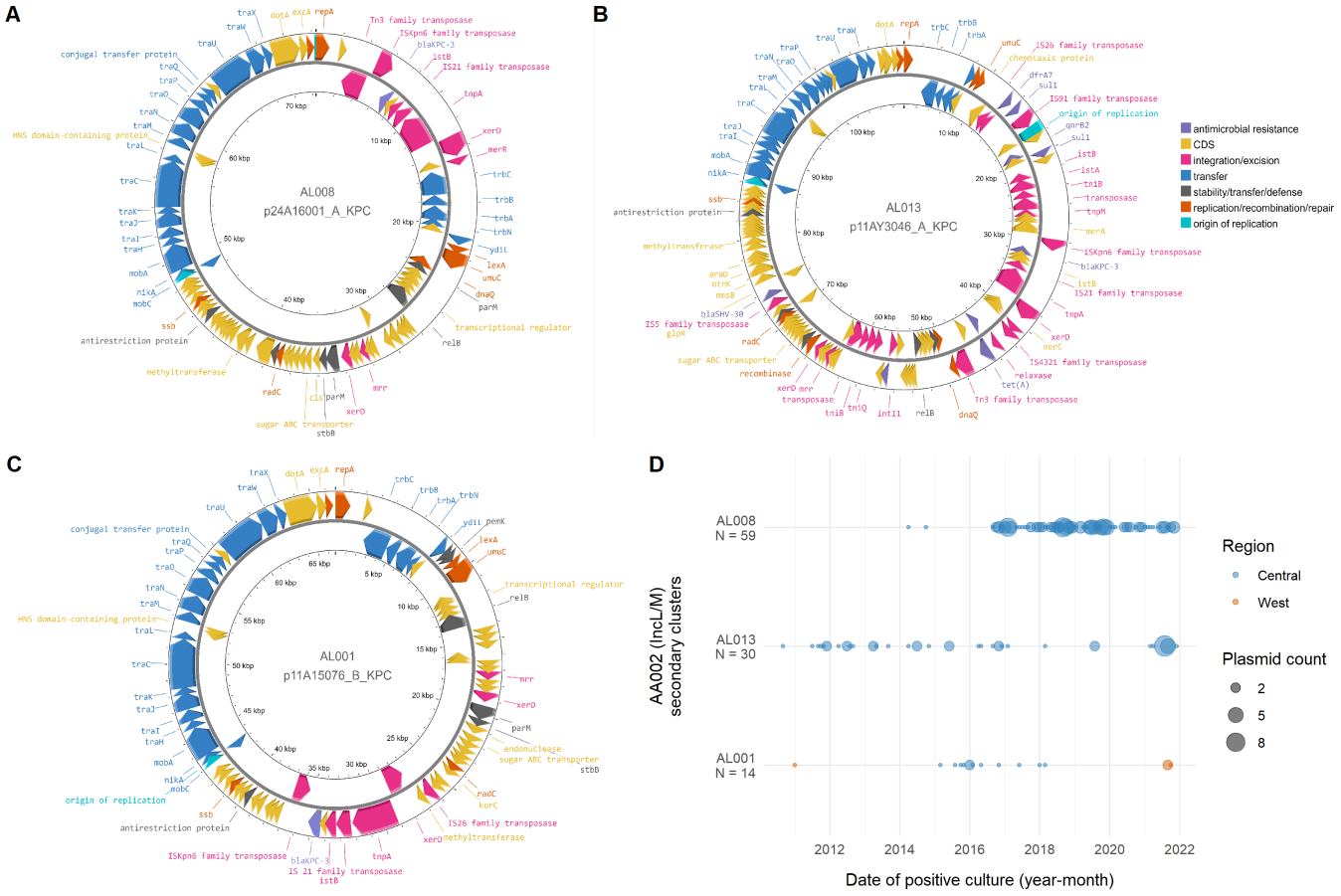

FIG 5 Diversity of secondary clusters within primary cluster AA002 (IncL/M). Plasmid structures of (A) secondary cluster AL008, (B) secondary cluster AL013, and (C) secondary cluster AL001 which grouped within the AA002 (IncL/M) primary cluster. Coding sequences are colored by function, and not all coding sequences are labeled. (D) Date of positive culture and region of plasmids from primary cluster AA002 (IncL/M). Date of positive culture was grouped into year-month bins. The Central region represents the Canadian provinces of Ontario and Québec, and the West region represents British Columbia, Alberta, Saskatchewan, and Manitoba. "N" indicates the number of isolates that contained assembled or predicted plasmids in the respective secondary clusters.

site between 2011 and 2021. In addition to bla_{KPC-3} on Tn*4401*b-3, this plasmid also encodes *ant*(2″)-Ia, bla_{SHV-30}, *dfrA7*, *qacE*, *qnrB2*, *tetA*, and two copies of *sul1*. Structurally, the secondary clusters have similar backbones of transfer and replication genes (Fig. 5). AL008 and AL013 have Tn*4401*b-3 inserted on the same strand, but AL013 contains many additional integration/excision mobile elements, antimicrobial resistance genes, and hypothetical coding sequences. The AL001 plasmid is structurally similar to AL008 plasmids, but has a different locus of insertion of Tn*4401*b-3 on the opposite strand and has a *pemKI* toxin-antitoxin system. The prevalence of these plasmids among different genera and the presence of conjugation genes (MOBP relaxase and MPF_I mating pair formation protein) suggests that regional horizontal transmission has contributed to these secondary clusters' persistence.

Three primary clusters (AA007, AA014, and AA017) were identified as having IncN-type replicons with similar features (Table 1). Each cluster corresponds to a different type of replicon: IncN (AA007), IncN2 (AA017), and IncN3 (AA014). All plasmids in primary cluster AA007 were grouped in a single secondary cluster (31/31, 100%), as did all plasmids in primary cluster AA017 (7/7, 100%); in contrast, plasmids in primary cluster AA014 were split into two secondary clusters: AL059 (11/13, 84%) and AL060 (2/13, 15%). The first secondary cluster (AL059) was isolated from *E. coli*, *K. pneumoniae*, and *E. cloacae* complex from five sites in one province between 2014 and 2020. These plasmids encode bla_{KPC-3} on Tn*4401*b-2 and no other resistance genes with the exception of a

single plasmid with a multi-resistance gene island. The other secondary cluster (AL060) was found in *C. freundii* complex and *K. pneumoniae* complex at two sites in one province. This group has no resistance genes aside from bla_{KPC-2} on Tn*4401*a-2. Primary cluster AA017 plasmids were isolated from *E. coli*, *K. pneumoniae* complex, *E. cloacae* complex, and *Citrobacter farmeri* from two sites in one province between 2016 and 2020. Aside from bla_{KPC-2} on Tn*4401*b-1, other resistance genes found in this cluster include bla_{TEM-1B}, *dfrA25*, *mph(A)*, *qacE*, *qnrB2*, and *sul1*. Plasmids in the largest IncN primary cluster (AA007) were isolated from *E. coli*, *K. pneumoniae* complex, *Klebsiella oxytoca*, *Klebsiella michiganensis*, *Raoultella ornithinolytica*, *E. cloacae* complex, *C. freundii* complex, *Citrobacter amalonaticus*, *Citrobacter koseri*, and *Serratia marcescens*. These plasmids were isolated from 2012 to 2020 from nine sites in four provinces. This group contains bla_{KPC-3} on Tn*4401*b with many of them encoding different combinations of *aac*(6′)-Ib, *aph*(3″)-Ib, *aadA1*, bla_{OXA-9}, bla_{TEM-1A}, *dfrA14*, *qacE*, *qnrS1*, *sul1*, *sul2*, and *tetD*. The prevalence of IncN plasmids in multiple genera from multiple sites in the same province (primary clusters AA017 and AA014) or across multiple provinces (AA007) along with encoded conjugation genes (MOBF relaxase and MPF_T mating pair formation protein) suggests there has been widespread horizontal transmission of these primary clusters in Canada.

Canadian bla_{KPC}-encoding plasmids in the other primary clusters

Plasmids in primary cluster AA042 are small in size (10–20 kb) and grouped into four secondary clusters: AL143 (2/21, 10%), AL144 (17/21, 81%), AL149 (1/21, 5%), and AL150 (1/21, 5%). The largest secondary cluster (AL144) had multiple replicon types identified by MOB-suite, with the most common being ColRNAI_rep_cluster_1987 and rep_cluster_2335. These plasmids were isolated from 14 sites across three provinces from *C. freundii* complex, *E. coli*, *K. pneumoniae* complex, and *S. marcescens* between 2011 and 2021. These plasmids did not contain other resistance genes and most (14/17, 82%) were classified as "mobilizable" by MOB-suite, which indicates they encoded a relaxase or recognizable *oriT* sequence. The core genes in this group include all components of the Tn*4401*b transposon including bla_{KPC-3} which results in integration/excision genes being common in this secondary cluster. The small size of mobilizable plasmids in this primary cluster and their prevalence at various sites indicate that bla_{KPC} spread may be influenced by these smaller vectors.

We detected a novel replicon type in primary cluster AA109, designated as rep_cluster_2268 by MOB-typer, which now has a novel replicon designation of repE(pEh60-7) in PlasmidFinder (personal communication). We obtained complete plasmid sequences for seven isolates which grouped into a single secondary cluster. These plasmids were only found in the *Enterobacter cloacae* species complex, specifically *Enterobacter hormaechei* subsp. *hoffmannii* ST316, at a single site in one province from 2011 to 2020, indicating a very stable plasmid or reservoir of this strain. Occurrences of this plasmid are intermittent from 2011 to 2016 and appear to become more prevalent from 2016 to 2018 with fewer detected in recent years. SNVPhyl (28) was run to analyze SNV differences in the ST316 bla_{KPC}-harboring isolates from this site over the 8-year time span (n = 51), and a maximum of 62 SNVs was identified between isolates using 88.51% of the genome. The novel repE(pEh60-7) plasmids encode bla_{KPC-3} in Tn*4401*b-3 but did not encode any other resistance genes. While they were classified as non-mobilizable by MOB-suite, they do encode a mating pair formation protein and other genes annotated as conjugation machinery which may not be represented in current databases (Fig. 6). Further work will need to be done to determine if these plasmids are conjugative. These plasmids also encode for stability/transfer/defense proteins including a SOS-inhibition protein, stability partition proteins, the *hok-sok* toxin-antitoxin system, and an anti-restriction protein. There were two PLSDB plasmids that grouped in the same primary AA109 cluster but in different secondary clusters, and their gene content is quite diverse compared to the Canadian plasmids (70% identity to NZ_AP022432.1 and 43% identity to NZ_CP080472.1) (Fig. 6). Given these plasmids are only found in one specific sequence type, clonal transmission is likely driving this primary cluster's persistence.

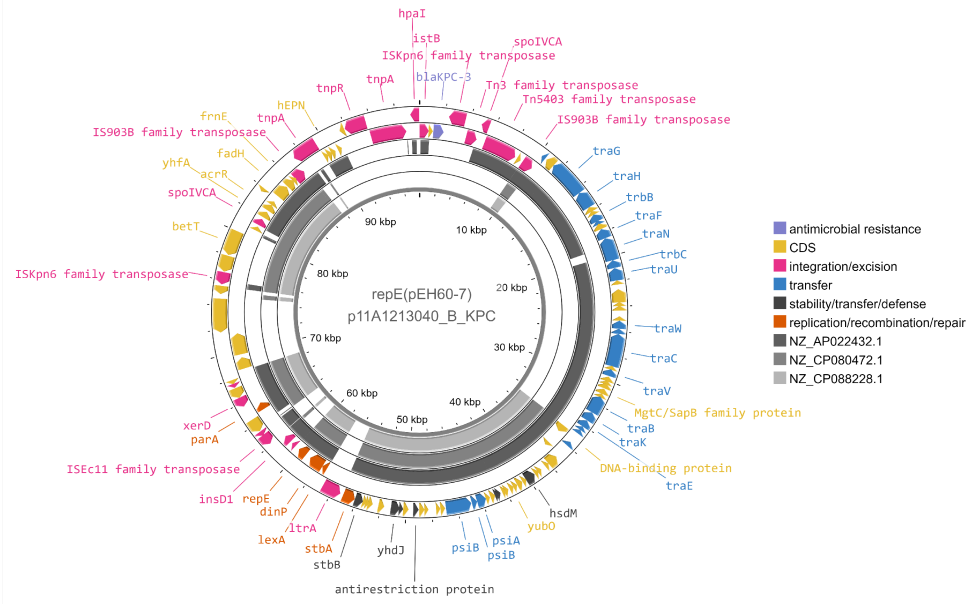

FIG 6 Comparison of p11A1314040_B_KPC, representative of plasmids with novel replicon repE(pEh60-7) and reference NZ_CP088228.1 (pEh60-7), NZ_CP080472.1 (PLSDB), and NZ_AP022432.1 (PLSDB) from cluster AA109. Reference sequences were aligned using blastn+ and an *e*-value cut-off of 0.1. NZ_CP080472.1 and NZ_AP022432.1 were placed in the same primary cluster by MOB-cluster as the reference p11A1314040_B_KPC but in different secondary clusters. Genes are colored by functional classes determined by mobileOG-db, and genes without annotations are hypothetical proteins. Not all gene labels are displayed.

Primary cluster AA124 plasmids were classified as IncX5 replicons grouped into two secondary clusters: AL404 (1/10, 10%) and AL405 (9/10, 10%). The largest secondary cluster (AL405) encoded bla_{KPC-3} on Tn*4401*b-3. These plasmids were isolated from *K. pneumoniae* complex, *Serratia liquefaciens*, *E. cloacae* complex, and *Klebsiella ascorbata* from four sites in two provinces between 2015 and 2021. Eight of these nine plasmids are >99% identical and do not contain any resistance genes aside from bla_{KPC-3} on Tn*4401*b-3. These plasmids are predicted to be conjugative based on the presence of the MOBP relaxase and MPF_T mating pair formation protein. The wide range of genera suggests that these IncX5 plasmids have disseminated horizontally across the country.

Two primary clusters (AA029 and AA085) contained IncF-type replicons that were found exclusively in *K. pneumoniae* isolates. All plasmids encoded two MOBF relaxases and an MPF_F mating pair formation protein which suggests that they are conjugative. Plasmids in primary cluster AA085 were grouped into a single secondary cluster and were isolated from two sites in two provinces from 2011 to 2020. These plasmids were from ST512 (3/5, 60%) and ST258 (2/5, 40%) isolates and varied in size (range: 148–182 kb). All plasmids contained Tn*4401*a, but bla_{KPC-2} was present in ST258 and bla_{KPC-3} was present in ST512, and all but one encoded *aadA2*, *aph*(3′)-Ia, *drfA12*, *mphA*, *qacE*, and *sul1*. Primary cluster AA029 plasmids were grouped into three secondary clusters which reflected the sequence type of the host: AL107 (ST512, 6/9, 67%), AL104 (ST258, 2/9, 22%), or AL106 (ST152, 1/9, 11%). Two of these secondary clusters (AL107, ST512; AL106, ST152) encoded bla_{KPC-3} on Tn*4401*a-7, whereas the other (AL104, ST258) encoded bla_{KPC-2} on Tn*4401*a-1. The secondary clusters AL107 and AL106 (ST512, ST152) were isolated from the same single site from 2010 to 2011, and the ST258 plasmids were isolated from two sites in one province in 2016 and 2019. Both secondary clusters AL107 and AL106 (ST512, ST152) also encoded bla_{OXA-9} and bla_{TEM-1A}. One of the first described pKpQIL plasmids in *K. pneumoniae* ST258 [NC_014016.1 (29)] in PLSDB clustered within AA029 (secondary cluster AL107) and also encoded bla_{OXA-9} and bla_{TEM-1A}. These pKpQIL-type plasmids have been globally disseminated through the

expansion of *K. pneumoniae* clonal complex 258 (9, 29–31), and our results support continued clonal dissemination of these plasmid types in Canada.

Epidemiology of *bla*$_{KPC}$-encoding plasmid clusters across Canada

Long-read data are essential for resolving plasmid structures (32), but this can be costly for large surveillance data sets when performed alongside short-read sequencing, which then makes it difficult to analyze plasmid populations at a broad scale. Consequently, we explored MOB-recon (24) as a tool to predict plasmid cluster presence in isolates with incomplete *bla*$_{KPC}$-encoding contigs (635/829, 76.6%) using a database containing a subset (~20%) of our isolates that were long-read sequenced. We successfully assigned the majority of *bla*$_{KPC}$-encoding contigs to an existing plasmid primary cluster that we defined above, demonstrating that MOB-recon is a feasible approach to predict plasmid cluster membership without long-read sequencing all isolates.

MOB-recon assigned 95% (603/635) of incomplete *bla*$_{KPC}$-encoding contigs to an existing primary cluster, despite approximately half of *bla*$_{KPC}$ contigs (302/635, 48%) not matching any replicon, relaxase, MPF, or *oriT* sequence in the MOB-suite database. The 10 largest primary clusters contained 89% (539/603) of *bla*$_{KPC}$ contigs. Similar to the trends observed above (Table 1), the largest number of *bla*$_{KPC}$ contigs were classified into the IncL/M cluster (AA002, 142/635, 22%) and the IncN clusters (AA007, 149/635, 23%; AA014, 23/635, 3.6%; and AA017, 27/635, 4.3%) (Fig. S1). All contigs assigned to primary cluster repE(pEh60-7) (AA109) (34/635 contigs, 5%) belonged to *E. hormaechei* subsp. *hoffmannii* ST316, and all contigs assigned to the IncF primary cluster (representing both AA029 and AA085) (94/635, 15%) were from the *K. pneumoniae* species complex, which further confirms that these two primary clusters are species-specific. Finally, the remaining contigs were placed in the ColRNAI cluster (AA042, 27/635, 4%) the IncX5 cluster (AA124, 16/635, 3%), and the rep_cluster_1367 (AA109, 27/635, 5%). This reflects the distribution of complete *bla*$_{KPC}$ plasmid clustering alongside PLSDB as described above (Fig. 4; Table 1). Several other replicon types of *bla*$_{KPC}$-encoding contigs were present at low frequencies (21 IncHI2A, 14 IncX3, 12 IncC, and 9 IncP). Those contigs that were unclassified appeared to be chromosomal (located on contigs >1 Mb; 3/32, 9%), coincided with the 10 kb length of Tn*4401* and were filtered out (6/32, 19%), or did not match any plasmids currently in the database, indicating that further sequencing is required to confirm the genomic location of the *bla*$_{KPC}$ in these isolates.

Given the majority of *bla*$_{KPC}$-encoding contigs were predicted to be of plasmid origin, we examined the temporal and geographic patterns of all *bla*$_{KPC}$-encoding plasmids in Canada from 2010 to 2021 (Fig. 7). Geographically, the incidence of *bla*$_{KPC}$-encoding isolates is much higher in the Central region than the West (Central: n = 777/829, 93.7%; West: n = 52/829, 6.3%). The three primary clusters that have persisted over the longest time frame are IncN (AA007), AA002 (IncL/M), and AA0029/AA085 (IncF). Occurrences of AA002 (IncL/M) plasmids were low until 2016, but appear to be found more frequently for the remainder of the study period. Some of the earliest detected plasmids were AA029/AA085 (IncF) plasmids, but these were observed less frequently from 2020 onward, perhaps as AA002 (IncL/M) plasmids increased in prevalence in the Central region. The AA007 (IncN) plasmids appear to be established in the population. Since 2018, there has been an increase in replicons in the "Other" category or those plasmids that do not fall into the top 10 primary clusters, particularly in the West region. This may indicate that other plasmids are becoming dominant in the West region, and further investigation into smaller Western-specific clusters (such as plasmids with IncP6 replicons) is required.

We examined the epidemiology of the three secondary clusters in primary cluster AA002 (IncL/M) in more detail, as this is the only primary cluster wherein we had a substantial number of plasmids in multiple secondary clusters (Fig. 5D). From an epidemiological perspective, AL013 plasmids have been present for a longer time frame at low abundances with an increase in prevalence in 2021, whereas AL008 plasmids have appeared consistently since 2016. It appears that secondary cluster AL001 may be

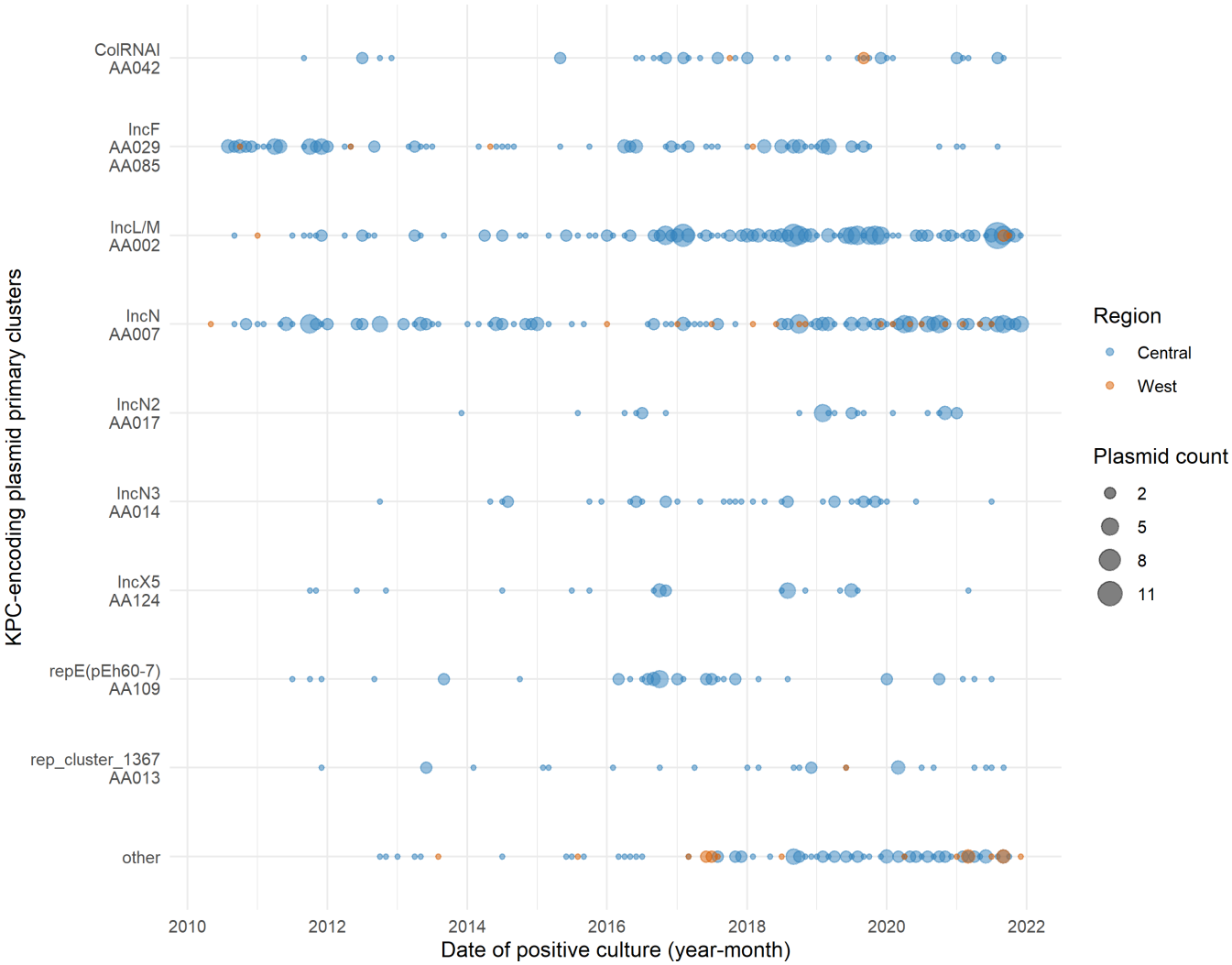

FIG 7 Epidemiology of Canadian *bla*$_{KPC}$-encoding plasmid primary clusters from 2011 to 2021. Date of positive culture was grouped into year-month bins. The Central region represents the Canadian provinces of Ontario and Québec, and the West region represents British Columbia, Alberta, Saskatchewan, and Manitoba. "Other" indicates plasmids which did not group in the top 10 primary clusters.

spreading to the Western region, as this replicon was detected initially in the West in 2010 but not again until 2021 (Fig. 5D). The independent samples collected over long time periods indicate that these plasmids seem to be stably circulating in these regions.

DISCUSSION

We examined the prevalence and distribution of *bla*$_{KPC}$-producing *Enterobacterales* and their plasmids in Canada from 2010 to 2021. Our results suggest that a combination of regional clonal transmission (AA109, AA029, and AA085), regional horizontal transmission (AA017, AA124, AA002, and AA014), widespread horizontal transmission (AA007), and other means of mobilization (AA013 and AA042) has contributed to *bla*$_{KPC}$ prevalence in Canada.

Here, we report on a novel *bla*$_{KPC}$ plasmid, repE(pEh60-7), isolated exclusively from *E. hormaechei* subsp. *hoffmannii* ST316, which has been stably circulating in this population for over 8 years. There have been limited reports on the *E. cloacae* complex ST316 lineage elsewhere; one study identified nine *E. hormaechei* ST316 isolates encoding *bla*$_{KPC-2}$ in the United Kingdom between 2014 and 2016 as part of a larger reference study, however, the plasmid content was not reported (33). Otherwise, eight isolates [one each

from references (34–36), and five from reference (37)] of *E. hormaechei* ST316 have been recently been reported to carry bla_{NDM-1} in Asia. Although conjugation genes were present, the narrow host range of the plasmid suggests that bla_{KPC} persistence is likely due to the ST316 clone and not driven by the plasmid.

We report on some plasmid clusters that are found across a variety of species, supporting evidence of horizontal transfer of bla_{KPC}. One such primary cluster was AA002, which contained the IncL/M plasmids isolated from *Citrobacter* spp., *Escherichia* spp., *Enterobacter* spp., *Klebsiella* spp., and *Raoultella* spp. isolates. The secondary cluster AL008 plasmids had a single resistance gene, but secondary cluster AL013 plasmids encoded fluoroquinolone resistance genes, additional β-lactamase genes, and other antimicrobial resistance genes which may favor their persistence in hospital environments. In addition, all IncL/M plasmids encoded a ParB-like partitioning/stability protein, an anti-restriction protein, a restriction endonuclease (Mrr), single-stranded DNA-binding protein (Ssb), and the RelB antitoxin although no RelE toxin was detected in any plasmid sequence. These features likely contribute to their long-term persistence and stability in Canada. However, bla_{KPC}-encoding IncL/M replicons appear to be rare as they are notably absent from other recent bla_{KPC} surveillance studies (13, 15, 38, 39). One study reported that bla_{KPC-2} being associated with IncL/M replicons a multi-clonal *K. pneumoniae* outbreak in a region in Argentina, but the relatedness between plasmids was not investigated (40). IncL/M plasmids are typically associated with bla_{NDM}, bla_{IMP}, and bla_{OXA}-type carbapenemases (3, 12, 13, 41–43), although a select few cases have been reported in diverse hosts (19, 44–47). The pNE1280 IncL/M plasmid, which was one of the first IncL/M plasmids described carrying bla_{KPC-2} (45), clustered in the AL001 secondary cluster, which was not well represented in the Canadian data set. We demonstrated ongoing bla_{KPC}-encoding IncL/M plasmid transfer across multiple genera which merits further investigation of IncL/M-type replicons as potential vectors for long-term bla_{KPC} persistence.

In contrast to the IncL/M plasmids, the IncN plasmid family had the broadest phylogenetic and geographical distribution across Canada, which is unsurprising given their broad host ranges and their long association with bla_{KPC} genes (3, 48, 49). bla_{KPC}-encoding IncN plasmids have been linked to multi-center outbreaks in Columbia (50) and Germany (51), both facilitated by intra- and inter-species horizontal plasmid transfer. Other bla_{KPC}-encoding IncN plasmids implicated in inter-patient transfer grouped in the same secondary clusters as the Canadian plasmids here [plasmid 12 (49) and pYDC107_70 (52) in AA007/AL033; pKPC-SMH (53) in AA014/AL059], indicating high sequence similarity among Canadian plasmids to those found across the globe. Similar to IncL/M plasmids, all IncN plasmids encoded partitioning/stability proteins (StbA, StbC, and StdB), at least two anti-restriction proteins (ArdA, ArdB, and ArdR), an endonuclease (EcoRII and PemK), and other antimicrobial resistance genes. Given these features are also common in IncL/M plasmids, further investigation is required to explain why the IncN plasmids have a broader geographic and host range distribution in Canada than the IncL/M plasmids.

The IncF-type replicons observed here [IncFIB(pQil)/IncFII(K) and IncFIB(K)/IncFII(K)] are common in bla_{KPC}-producing *K. pneumoniae* species complex isolates detected in other nationwide surveillance programs (12, 13, 15, 17, 18, 38, 39, 54). IncF-type replicons have been implicated in clinical outbreaks of bla_{KPC}-producing *K. pneumoniae* in the Netherlands (55) and the United States (56) as well as cross-species plasmid transmission in the United Kingdom (57). The clonal lineage ST258/512 accounts for 39.6% (106/268) of bla_{KPC}-producing *K. pneumoniae* isolates in our collection (Fig. 1) which were isolated across 3 provinces and 12 sites. This frequency is lower than frequencies observed in Colombia [47% (18)], in the United States [74% CRACKLE-2 (5)], and across Europe [73% EuSCAPE (13), 76% Europe/Israel (39)], but is higher than frequencies reported in the United Kingdom [20% (15)] and Argentina [0% (40)]. Our findings support that IncF-type plasmids in *K. pneumoniae* ST512 and ST258 are important in Canada for bla_{KPC} persistence in the clonal group ST258/512, although there is more diversity in the

sequence type of *bla*$_{KPC}$-encoding *K. pneumoniae* isolates in Canada compared to other parts of the world. Interestingly, IncF-type plasmids were less common in recent years (2020 and 2021), indicating other plasmid types such as IncL/M and IncN-type replicons may be replacing them in the Canadian landscape.

Small cryptic plasmids are often overlooked (58) and underrepresented in plasmid-mediated antimicrobial resistance studies (59). ColRNA-type replicons smaller than 22 kb were the third largest *bla*$_{KPC}$-encoding primary clusters in our data set. ColRNA-type plasmids were predicted to be found in >90% of carbapenemase-producing *Klebsiella pneumoniae* isolates across Europe (13, 60) and around the world (61), although these authors did not investigate if any carbapenemase genes were found on these plasmids. Similarly, ColRNAI replicons were present in >50% of *bla*$_{KPC}$-producing isolates in Colombia (18). Our MOB-recon results agree with these data and predict that 69% (568/829) of Canadian isolates contain ColRNAI-type or rep_cluster_2335 plasmids, the majority (524/568, 92%) of which do not encode *bla*$_{KPC}$ (data not shown). These plasmids can be important vectors for transferring antimicrobial resistance genes between isolates, including the transfer of *bla*$_{KPC}$-type carbapenemases (19, 31, 62). Experimentally, ColRNAI plasmids encoding *bla*$_{KPC}$ and Tn*4401* could mobilize between strains and participate in Tn*4401* transposition events within a patient (63). About half the plasmids we sequenced in this primary cluster do not encode a relaxase but they could be mobilized by the relaxase of a co-resident plasmid if the *oriT* sequence has enough similarity as shown in a previous study (64). Given their high prevalence and ability to mobilize resistance elements, these small plasmids are likely contributing to *bla*$_{KPC}$ dissemination and merit further investigation as potential vectors for *bla*$_{KPC}$ and other antimicrobial resistance spread across the globe.

Plasmid clustering and analyses require a methodology that is tolerant to a small amount of changes given the plasticity of plasmid sequences. Clustering plasmids with MOB-cluster provided a way to analyze subsets of similar plasmids, and MOB-recon allowed us to predict the prevalence of these plasmid clusters in isolates with incomplete assemblies. Canadian plasmids are grouped in two or three secondary clusters within each primary cluster (Mash distance <0.025), with typically a single secondary cluster containing the majority of Canadian plasmids in that primary cluster, indicating many of them have near duplicate sequences (26, 65) and this provided a starting point for investigating clusters of interest in more detail.

Analyzing broad-scale plasmid populations is desirable for surveillance programs, and ideally, every isolate would be sequenced to obtain complete plasmid sequences. Short-read sequencing alone results in many plasmids remaining fragmented (32) but performing long-read sequencing in addition to short-read on large collections of isolates can be cost and time-prohibitive, so we explored using MOB-recon to predict putative plasmids in incomplete assemblies after long-read sequencing a subset of isolates. There is no standardized method for estimating plasmid presence/containment in fragmented assemblies. It is important to recognize that any reference-based method for plasmid reconstruction involving mapping to reference sequences to infer presence assumes that plasmid structures are relatively conserved, and this approach can produce misleading results if plasmid plasticity is high (66, 67). Similar studies have used short-read mappers such as BWA/Bowtie2/SMALT (13, 43, 68, 69) or k-mer-based tools (60, 70) to determine plasmid prevalence in incomplete assemblies, typically using a select few plasmids as references. We use a homology-based clustering approach with MOB-recon (24) to reconstruct plasmids using a relatively large set of complete Canadian plasmids (1,856 plasmids). Previous work has shown that MOB-recon performed well compared to other tools (58, 71, 72), and that *Enterobacterales* plasmid replicons and mobility genes are well represented in the MOB-suite database (58). Using this approach, we were able to predict plasmids for the majority of incomplete *bla*$_{KPC}$-encoding contigs (605/635, 95%). For several primary clusters that were only present in specific sequence types [IncF-type, AA029/AA085 and repE(pEh60-7), AA109], those *bla*$_{KPC}$-encoding contigs that were predicted to be part of plasmids in those primary clusters were from

isolates with matching sequence types, which further supports this methodology for plasmid analysis in large-scale *Enterobacterales* surveillance data and eliminates the requirement to perform long-read sequencing on every single isolate.

In summary, there are multiple distinct clusters of bla_{KPC}-encoding plasmids that circulated in Canada from 2010 to 2021. Certain plasmid clusters spread by horizontal transmission and were found in multiple genera in multiple provinces whereas others persisted through clonal dissemination of the host organism. Our findings highlight the need to integrate targeted long-read sequencing into carbapenemase-producing organism surveillance to generate complete plasmid assemblies and demonstrate that plasmid clustering can facilitate analyses of a large number of plasmids. The characterization of bla_{KPC} plasmids is not only important for outbreak control but also for epidemiological surveillance of antimicrobial resistance, as plasmids encoding bla_{KPC} genes typically carry other antimicrobial resistance genes that can be exchanged between species.

MATERIALS AND METHODS

Surveillance period and PCR confirmation of bla_{KPC} carbapenemase gene

CNISP is a sentinel surveillance system which collects epidemiological and linked microbiology data from 90 Canadian acute-care hospitals across 10 provinces and 2 territories. *Enterobacterales* isolates non-susceptible to a carbapenem (as determined by the submitting site) isolated from patients from 2010 to 2021 were voluntarily submitted to the National Microbiology Laboratory (Winnipeg, Canada) by Canadian hospitals and provincial public health laboratories for carbapenemase gene detection. Multiplex PCR to confirm the carbapenemase gene bla_{KPC} was conducted as previously described (54). A total of 829 isolates encoding bla_{KPC} were collected from 2010 to 2021 from 34 hospitals (Table S1), with one hospital submitting 38% (317/829) of all isolates. Where applicable, the Central region refers to the provinces of Ontario and Québec, and the West region refers to the provinces of British Columbia, Alberta, Saskatchewan, and Manitoba. See the Supplemental Materials for additional information about the surveillance program and isolate eligibility criteria.

Whole genome sequencing

All 829 isolates were sequenced with Illumina MiSeq platforms, and 155 of these were additionally sequenced using Oxford Nanopore Technologies (ONT). Isolates for ONT long-read sequencing represented about 20% of all bla_{KPC} cases stratified across all provinces in Canada. For sites with known outbreaks, we took representative isolates to prevent oversampling of clonal cases. The average Illumina depth of coverage was 123×, and the average ONT depth of coverage was 75×. Genomes were trimmed and filtered before assembly with Unicycler v0.5.0 (73). A total of 111 chromosomes and 1,856 plasmids were completed and circularized in our data set. Details are in the Supplementary Materials, and details on bla_{KPC}-encoding plasmids can be found in Table S2.

Bioinformatic analyses

Organism genus was confirmed using the RefSeq Masher Matches tool (74) and FastANI v1.3 (75) for *Enterobacter* spp. Species complex definitions are in the Supplementary Materials. StarAMR v0.9.1 (76) was used to detect antimicrobial resistance genes using the ResFinder database v2022-05-24 (77) and sequence type using the multi-locus species types database v2.23.0 (78, 79). TETyper v1.1 (23) was used to identify the Tn*4401* isoforms using the reference Tn*4401*b-1 found in the GitHub repository (accession CP017937.1:29609-39614) and variants using an updated SNP profile file (Table S3) and an updated structural profile file (Table S4) based on the literature (22, 23, 45, 80–84). Panaroo v1.3.2 (85) was used to estimate the pangenome and generate core gene alignments for each primary cluster as well as alignments for each of the top four genera

(*Citrobacter*, *Enterobacter*, *Escherichia*, and *Klebsiella*). The MOB-typer tool from MOB-suite v3.1.4 (24, 26) was used to identify plasmid replicons and mobility classes using the default databases.

Plasmid clustering and containment analysis

MOB-suite primary cluster designations are a useful way to broadly cluster plasmids for epidemiological studies, and so plasmids assigned to different primary MOB-clusters are sufficiently unrelated to not be considered as part of an epidemiologically relevant transmission event (26, 65). However, plasmids that share the same primary cluster designation can be examined in more detail through higher resolution subtyping such as secondary cluster designations. If two plasmids are assigned to the same secondary cluster, they have near duplicate sequences and are sufficiently related to be strong candidates for outbreak investigations (26, 65). In addition to secondary cluster designation, epidemiological data are required to best assess direct plasmid transmission.

For plasmid clustering analysis, the PLSDB v2021_06_23v2 (25) database was downloaded and clustered alongside the 202 circular bla_{KPC}-encoding plasmids in this study using MOB-cluster from the MOB-suite v3.1.4 package (24, 26). For plasmid containment analysis, all 1,856 circular plasmids completed in this study (including the 202 bla_{KPC}-encoding plasmids) were clustered using MOB-cluster to create a custom Canadian plasmid database. All 829 isolates were screened for plasmids with MOB-recon using this custom database, and the output was filtered to focus on the reconstructed plasmids containing bla_{KPC}. Further details on plasmid clustering and containment are provided in the Supplementary Materials.

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DATA AVAILABILITY

Raw sequencing reads were deposited to the NCBI SRA archive under BioProject PRJNA855907. See Table S1 for a list of accessions. Complete bla_{KPC}-encoding plasmid sequences were deposited to NCBI GenBank under the accessions listed in Table S2. Updated SNV profiles used for Tn*4401* typing with TETyper are found in Table S3 and updated structural profiles in are found in Table S4.

ADDITIONAL FILES

The following material is available online.

Supplemental Material

Figure S1 (AAC00860-23-s0001.tif). Prediction of blaKPC-encoding plasmids from incomplete assemblies using MOB-recon.

Additional method details (AAC00860-23-s0002.docx). Additional method details.
Table S1 (AAC00860-23-s0003.xlsx). *bla*$_{KPC}$-encoding isolate summary data.
Table S2 (AAC00860-23-s0004.xlsx). Complete *bla*$_{KPC}$-encoding plasmid summary data.
Table S3 (AAC00860-23-s0005.xlsx). Updated Tn*4401* SNV table used for TETyper analysis.
Table S4 (AAC00860-23-s0006.xlsx). Updated Tn*4401* structural table used for TETyper analysis.

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