Uncovering the gut microbiota as a reservoir of ST11 hypervirulent KPC-2-producing Klebsiella pneumoniaeUncovering the gut microbiota as a reservoir of ST11 hypervirulent KPC-2-producing Klebsiella pneumoniae

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Abstract

Background: The emergence and spread of ST11 carbapenem-resistant, hypervirulent K. pneumonia (ST11-CR-HvKP) in China generated great concern from the public health community. The identification of ST11-CR-HvKP strain is expected to become a serious public health issue in China, considering the carbapenem resistance and virulence had converged in an epidemic clone. However, the underlying mechanism that enables its wide dissemination in China remains unclear.

Results: Here, we investigate the prevalence of carbapenemase-producing Enterobacteriaceae (CPE) carriage by inpatients in a teaching hospital over a 1-year period, to identify ST11-CR-HvKP reservoirs, and to understand the transmission of these pathogens across healthcare networks. We identified a high colonization prevalence of CPE (12.4%) among inpatients with diarrhea. Correlations were detected between antibiotic exposure, surgical history, and being CPE positive. A genomic investigation of 65 CRKP isolates indicated a shared bacterial population among various wards. Maximum-likelihood phylogenetic tree demonstrated that these isolates were partitioned into three major clades. An analysis of the wzi locus revealed three different K types (KL105, KL47, and K64) among the ST11 isolates, indicating genetic diversity among these isolates. Our review of the cases showed that these patients had no contact with each other, indicating nosocomial transmission. Genetic and sequence mapping revealed complexity in the existence of virulence plasmids and resistance plasmids in the ST11-CRKP isolates. These data indicate that this process was more complicated than was earlier anticipated, as it may have involved multiple ST11 K. pneumoniae lineages and a variety of virulence plasmids.

Conclusions: Collectively, this work represents the first evidence of gut microbiota may act as the source of ST11-CR-HvKP isolate. Active surveillance approaches, particularly in ICUs based on the results of this study, should be implemented to combat the spread of ST11-CR-HvKP and to improve patient outcomes.

Key words: gut microbiota; hypervirulent; KPC-2; reservoir; genomic characterization

Introduction

Carbapenem-resistant Klebsiella pneumoniae (CRKP), which is associated with high mortality rates of up to 50%, has been identified by the World Health Organization as a critical priority organism [1, 2]. Hypervirulent *K. pneumoniae* (HvKP) causes life-threatening infections, and the *rmpA/rmpA2* genes are associated with its pathogenicity [3]. Recently, a fatal outbreak of ST11 carbapenem-resistant, hypervirulent *K. pneumoniae (*ST11-CR-HvKP) in China generated great concern from the public health community [4]. The identification of this ST11-CR-HvKP strain demonstrated that carbapenem resistance and virulence had converged in an epidemic clone that could become a serious public health issue [5]. Subsequently, cases of ST11-CR-HvKP infection have been documented in diverse regions in China [6–8]. These reports suggest that this clone is widely spread in China; however, the underlying mechanism that enables its wide dissemination remains unclear.

Intestinal tract colonization with carbapenemase-producing Enterobacteriaceae (CPE) may lead to subsequent nosocomial infections in at-risk patients [9–11]. Therefore, we hypothesized that inpatients might have a higher rate of CPE colonization and that intestinal carriage may represent a key factor underlying the epidemiology of ST11-CR-HvKP nosocomial infections in China. The aims of this study were to investigate the prevalence of CPE carriage of inpatients in a teaching hospital in China over a 1-year period, to identify ST11-CR-HvKP reservoirs, and to understand the transmission of these pathogens across healthcare networks.

Materials And Methods

Study design

We conducted a prospective, observational cohort study of inpatients with acute diarrhea between February, 2016 and February, 2017 (Figure S1). Enterobacteriaceae isolates that were cultured from the first clinical samples of respective patients with diarrhea were collected. Duplicate isolates (the same species from the same patient within two weeks of the first positive culture) were excluded in this study. The study was performed at the First Affiliated Hospital of Zhejiang University (FAHZU), China, which is the largest tertiary teaching hospital in Zhejiang Province with 2,500 beds. Clinical data were extracted using centralized queries from clinical and medical record system used for all patients.

Bacterial isolation and identification

Fecal sample (1.0 g) were diluted in 5 ml sterile Luria-Bertani liquid medium and cultured overnight at 37°C. The enrichments were plated on MacConkey agar plates amended with 1 mg/L meropenem for 18–24h at 37°C to isolate potential carbapenem-resistant Enterobacteriaceae (CRE) strains [12]. Colonies with various morphologies were repeatedly streaked on the same medium to obtain pure isolates. Bacterial species were identified by matrix-assisted laser desorption ionization-time of flight massspectrometry (MALDI-TOF MS) and 16S rDNA sequencing using universal prokaryotic primers. CRE isolates were confirmed by susceptibility test with imipenem, ertapenem, and meropenem via broth microdilution method, and CPE isolates carbapenem resistance phenotype [13].

Risk factor analysis

To investigate the risk factors associated with fecal carriage of CPE, carriers were compared with non-carriers in terms of exposure to the different variables studied. Categorical variables were expressed as percentages and were compared using the Chi-squared test or two-tailed Fisher exact test, as appropriate. Independent predictors for CPE were examined by logistic regression analysis. Variables with $P \leq 0.2$ in the univariate analysis were included in a logistic regression model to identify variables with either negative or positive impact on the colonization of CPE in the acute diarrhea inpatients. The strength of associations was determined by calculating the odds ratio (OR) and 95% confidence intervals (CIs).

Variables with a two-tailed P-value of <0.05 were considered statistically significant. All statistical analyses were performed using SPSS version 24.0.

Antimicrobial susceptibility test (AST)

The minimum inhibitory concentrations (MICs) of 14 antimicrobial agents (amikacin, aztreonam, cefotaxime, cefpirome, ceftazidime, ciprofloxacin, colistin, fosfomycin, gentamicin, imipenem, meropenem, piperacillin-tazobactam, tobramycin, and tigecycline) were determined by agar dilution methods, except for colistin and tigecycline, for which broth microdilution was used [14]. The results were interpreted using Clinics and Laboratory Standard Institute guideline (CLSI) breakpoints [13]. Standard reference strains of *E. coli* ATCC 25922and *K. pneumoniae* ATCC 700603 were used as quality control.

Detection of targeted genes

Genomic DNA was extracted from overnight cultures using the Qiagen Blood/Tissue Kit (Qiagen, Hilden, Germany). The presence of carbapenemase encoding genes (NDM, KPC, IMP and VIM) was detected via PCR and sequencing from each colony as described previously [14]. Detection of virulence-associated *rmpA* and *rmpA2* genes in *K. pneumoniae* were conducted as described previously [15].

Molecular typing of KPC–2-producing *K. pneumoniae* isolates

Genetic relatedness of *K. pneumoniae* isolates was assessed by pulsed-field gel electrophoresis (PFGE) [16]. Briefly, DNA fragments were separated in 1% agarose (SeaKem Gold agarose, Lonza) at 14°C and 6 V/cm and with alternating pulses at 120° in a 2–40 s pulse time gradient for 22 h in 0.5 × Tris-boric acid-EDTA (TBE) buffer with CHEF apparatus (Bio-Rad, USA). *Salmonella enterica* serotype Braenderup H9812 was used as a size marker [17]. The dendrogram of PFGE patterns was constructed using BioNumerics v7.6 (Applied Maths, Sint-Martens-Latem, Belgium) with UPGMA clustering. Isolates that exhibited similarity cut-off ≥ 80% were considered a pulsotype.

Illumina sequencing and sequence assembly

To characterize the genetic features and resistome of KPC–2-producing *K. pneumoniae* isolates, whole-genome sequencing (WGS) was performed on all KPC–2-producing *K. pneumoniae.* Whole-cell DNA was extracted from overnight cultures using the Gentra Puregene Yeast/Bact Kit (Qiagen, Hilden, Germany). The harvested DNA was further detected in 1% (w/v) agarose gels, and the concentration and purity of DNA were measured using NanoDrop 2000 UV-Vis Spectrophotometer (Thermo scientific, Waltham, MA, USA) and Qubit®2.0 Fluorometer (Thermo scientific, Waltham, MA, USA). DNA was stored at –20 °C until further processing. The sequencing library was produced by using Illumina Nextera XT Kit and sequenced on Illumina HiSeq X 10-PE150 platform (Illumina, San Diego, CA, USA). A-tailed, ligated to paired-end

adaptors and PCR amplified with a 500 bp insert and a mate-pair library with an insert size of 5 kb were used for the library construction at the Beijing Novogene Bioinformatics Technology Co., Ltd. PCR adapter reads and low-quality reads from the paired-end and mate-pair library were filtered by the step of quality control using Novogene pipeline. Paired reads were assembled into a number of scaffolds using Velvet 1.2.10 [18].

Long-read Pacific Biosciences sequencing and assembly

To further elucidate the genetic environment of *rmpA/rmpA2* carrying *K. pneumoniae* isolates, five randomly selected RmpA and RmpA2 positive isolates were subjected to the PacBio sequencing based on the size of *rmpA/rmpA2* carrying plasmids. The extracted DNA of isolates L39, L201, L388, L482, and L491 were sequenced by long-read single-molecule real-time (SMRT) sequencing technology using the PacBio RS II platform (Pacific Biosciences, Menlo Park, CA, USA). The extracted high-quality, double-stranded DNA (10 μg) was sequenced using P6-C4 chemistry on the PacBio RS II instrumentation by using complexed 20-kb SMRTbell library. Unicycler was used for hybrid assembling the three bacterial genomes from a combination of Illumina short reads and Pacbio long reads [19]. We run Unicycler in three different modes (conservative, normal and bold) that alter its cutoff for minimum acceptable bridge quality to generate the best assemble results.

Bioinformatics analysis and phylogenomic computations

To determine the clonal lineages, the sequence types (STs) of KPC–2-producing *K. pneumoniae* isolates were determined by multilocus sequence typing (MLST) from WGS data in accordance with protocols described on the website (http://bigsdb.pasteur.fr/klebsiella/). ResFinder 3.1 was used to identify antimicrobial resistance genes [20]. Plasmid Finder 1.3 was used to identify the incompatibility type of the plasmids [21]. We further identified the virulence loci with the assembled genome sequences using BIGSdb *Klebsiella* genome database. We generated a heatmap of the virulence loci with Genesis software version 1.7.7. To find the core genes of the KPC–2-producing *K. pneumoniae* genomes, we used Prokka [22] and Roary [23]. Maximum likelihood-based phylogenetic reconstruction was performed with RAxML version 8.2.10 [24]. One hundred bootstrap replicates were evaluated to determine branch support. Maximum-likelihood phylogenetic tree based on core single nucleotide polymorphism (SNP) alignments was visualized with FastTree [25].

Characterization of *rmpA/rmpA2* carrying plasmids

S1 nuclease-pulsed-field gel electrophoresis (S1-PFGE) and Southern blot analysis were performed to estimate the size of the *rmpA/rmpA2* carrying plasmids [26]. The sequence of the plasmid carrying the *rmpA/rmpA2* genes, were assembled using plasmidSPAdes from WGS data [27]. Annotation was performed using the RAST tool [28]. The sequences of represented plasmids was compared against other NCBI accessioned plasmids using BLAST and plotted by BLAST Ring Image Generator (BRIG) [29].

Results

During the study period, we sampled 811 non-duplicate stool samples from 443 inpatients and screened for the presence of CPE strains. Ultimately, 87 CPE isolates from 55 patients were included in the study, and this frequency indicated a high CPE colonization prevalence (i.e., 12.4%, 55/443) among inpatients with diarrhea (Figure S1). Of the 87 isolates, *K. pneumoniae* was the most prevalent species (n = 65), followed by *Proteus mirabilis* (n = 6), and *Escherichia coli (*n = 6) (Table S1). PCR and sequencing revealed that 77 isolates carried bla_{KPC-2}, five carried bla_{NDM-5}, two carried bla_{NDM-1},two carried bla_{IMP-4},andone carried bla_{IMP-26}; furthermore, 49 isolates harbored extended-spectrum β-lactamase (ESBL) genes (Table S2). All of the CPE isolates were multidrug resistant (i.e., phenotypic resistance to three or more drug classes) (Figure S2). Among these CPE isolates, the highest resistance rate (99%) was to imipenem, and most of the isolates were susceptible to colistin (92%) and tigecycline (91%).

Not surprisingly, most of the colonization cases were observed among patients from medical intensive care units (Table S1), since they typically experience prolonged hospital stays that result in exposure to potential risk factors. The patients came from 13 units: 49% (43/87) from intensive care unit (ICU), 9% from hepatobiliary and pancreatic surgery, 8% from emergency intensive care unit (EICU), and the remaining 33% from ten other units. Among the patients, 42 (76%) were male, and the median patient age was 64 years (range, 16–97). Interestingly, correlations were detected between antibiotic exposure (including linezolid, β-lactam-β-lactamase inhibitor, and carbapenems), surgical history, and being CPE positive (Table 1).

To better define the population structure of the 65 CRKPisolates, we further investigated their comprehensive molecular characteristics. Remarkably, an MLST analysis revealed that the *K. pneumoniae* isolates belonged to four STs, indicating low diversity (Figure 1 and Table S1). ST11 was the predominant ST (58/65, 89%), followed by ST37 (n = 5), ST15 (n = 1), and ST107 (n = 1). A notable feature was that patient P1 carried two STs: ST37, which was isolated from three samples, and ST11, which was isolated from eight samples, over the entire study period. Furthermore, ten patients in the study carried multiple isolates. Several major pulsotypes were found via PFGE analysis, which is consistent with the MLST results; however, genetic diversity was observed in the profiles (Figure S3). Roary matrix-based gene sequence analysis generated a large pan-genome of 10,355 gene clusters across 65 full genomes (Figure S4). A maximum-likelihood phylogenetic tree demonstrated that the 65 strains were partitioned into three major clades (Figure 1). Seven non-ST11 isolates were clustered into two separate clades, and the 58 ST11 isolates were grouped into three clusters, despite their high similarity. An analysis of the *wzi* locus revealed three different K types (KL105, KL47, and K64) among the ST11 isolates, indicating genetic diversity among these isolates. Of note, ST11 isolates were detected in patients from 12 wards. Our review of the cases showed that these patients had no contact with each other, making nosocomial transmission less likely.

A broad array of resistance genes associated with various antimicrobials was identified in the *K. pneumoniae* genomes (Figure 2). Isolates encoding carbapenemases (44/65, 68%) also harbored

predicted ESBLs. Among these ESBLs, CTX-M–65 was the most predominant cluster (34/65, 52%), followed by CTX-M–14 (5/65, 8%). A virulence gene analysis revealed that the *K. pneumoniae* isolates carried genes for 45 known virulence factors (Figure 3). As expected, a high prevalence of biofilm-encoding type 3 fimbriae clusters (*mrk)* and yersiniabactin (*ybt)*–associated genes was observed in these isolates.

An *in-silico* analysis identified 14 *rmpA*- and 19 *rmpA2*-positive ST11 CRKP isolates. Interestingly, S1-PFGE and Southern blot analyses clearly showed that at least four types of *rmpA/rmpA2*-positive plasmids were carried by the ST11-CR-HvKP isolates, ranging in size from ~146 kb to ~218 kb (Figure 4). The coexistence of *rmpA* and r*mpA2* on the same plasmid was observed in 14 isolates. PacBio sequencing generated five complete *rmpA/rmpA2*-encodingIncHI1B plasmid sequences that aligned well with the virulence plasmid pLVPK (AY378100), a 219 kb plasmid that harbors a different set of virulence genes, including *iroBCDN, iucABCD, rmpA,* and *rmpA2* (Figure 5).

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Discussion

The first isolation of ST11-CRKP in China occurred at FAHZU in 2004 [30]. Over the last few decades, China has witnessed the emergence and subsequent rapid increase in the number of ST11-CRKP nosocomial infections [31]. However, the role of the gut microbiota in the rapid spread of ST11-CRKP has not been well elucidated. Our experimental evidence, as well as the retrieved clinical data, revealed a disturbing carriage rate of ST11-CRKP (8.6%) among hospitalized patients with acute diarrhea, particularly in ICU wards. Our findings suggest that the gut microbiota could be a major reservoir of ST11-CR-HvKP. Active surveillance approaches, particularly in ICUs based on the results of this study, should be implemented to combat the spread of ST11-CRKP and to improve patient outcomes.

To our knowledge, an association between linezolid exposure CPE colonization in these types of patient populations has not been reported. The lack of such an association might be due to the wide use of linezolid, which eliminates Gram-positive bacteria and disrupts the gut microflora, thus promoting gut colonization by Enterobacteriaceae, especially CPE strains.

A previous investigation speculated that the emergence of ST11-CR-HvKP occurred due to a single genetic event in which a pLVPK-like virulence plasmid was acquired by an ST11-CRKP strain that presumably carried a common plasmid such as pKPC-CR-HvKP4 [4]. The observed complexity in the existence of virulence plasmids and resistance plasmids in the ST11-CRKP isolates indicates that this process was more complicated than was earlier anticipated, as it may have involved multiple ST11 *K. pneumoniae* lineages and a variety of virulence plasmids.

This study has several limitations. First, our study is limited by its single-institution design and 1-year study period; therefore, the findings of this study should be interpreted with caution and might not be generalizable to other hospitals. Second, although we identified 19 fecal ST11-CR-HvKP colonization isolates, none of the colonized patients had ST11-CR-HvKP infections. A previous investigation described the high prevalence and mortality rate of ST11-CR-HvKP meningitis in our hospital [8], and K64 was also the most common serotype detected in this study. Future investigations addressing the transition from ST11-CR-HvKP carriage to infection in high-risk patients will be of great significance.

Conclusions

In conclusion, our genomic epidemiologic investigation demonstrated that the gut microbiota of hospitalized patients served as the reservoir of CPE isolates, particularly ST11-CR-HvKP. Because of the serious clinical outcomes of CRKP infections, more information is needed to understand the potential risk of ST11-CR-HvKP spreading by inpatients and to develop measures for surveillance or control of these risks.

Abbreviations

ST11-CR-HvKP: ST11 carbapenem-resistant, hypervirulent *K. pneumoniae;* CRE: carbapenem-resistant Enterobacteriaceae; CPE: carbapenemase-producing Enterobacteriaceae; CRKP: carbapenem-resistant *K. pneumoniae;* MALDI-TOF MS: matrix-assisted laser desorption ionization-time of flight mass spectrometry; MICs: minimum inhibitory concentrations; CLSI: Clinics and Laboratory Standard Institute guideline; MLST: multilocus sequence typing; SNP: single nucleotide polymorphism; S1-PFGE: S1 nuclease-pulsed field gel electrophoresis.

Declarations

Ethics approval and consent to participate

Informed consent was obtained from all patients according to the ethical protocol approved by the Ethics Committee of FAHZU (no. 2018–1031)

Consent for publication

Not applicable.

Availability of data and material

The whole genome sequences of 65 CRKP have been deposited in GenBank under the BioProject numbers PRJNA390758.

Competing interests

The authors declare that they have no competing interests.

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Authors' contributions

BZ, YX, and LL designed this study. TL and YC collected the fecal samples and screened the isolates. HX performed the microbiological and molecular experiments. LG, XY, CH, SZ, HH, and PS analyzed the data. BZ wrote the manuscript and YX, and LL revised it. All authors read and approved the final manuscript.

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Additional Files

Additional file 1: Table S1. Carbapenemase-producing *Enterobacteriaceae* isolates recovered from stool specimens.

Additional file 2: Table S2. Summary of the carbapenemase-producing Enterobacteriaceae (CPE) isolates and the β-lactamases encoded by the CPE isolates.

Additional file 3: Figure S1. Flowchart of the included cases and the analyses performed. Abbreviations: CPE, carbapenemase-producing Enterobacteriaceae; ARGs, antimicrobial resistance genes.

Additional file 4: Figure S2. Antimicrobial susceptibility profiles of 87 CPE isolates. The MICs were determined via an agar dilution method for all antibiotics except for colistin and tigecycline, for which a broth microdilution method was used. Unless otherwise specified, the susceptibility tests were interpreted according to the 2017 Clinical Laboratory Standards Institute (CLSI) criteria. Pink indicates resistance, blue indicates intermediate, and yellow indicates sensitivity.

Additional file 5: Figure S4. A pan-genome analysis of 65 CRKP strains, performed using Roary. The blue bar indicates the pan-genome of CRKP, including the 10,3554 annotated genes detected among the genomes analyzed in this study.

Additional file 6: Figure S3. A PFGE dendrogram generated using the Bionumerics software showing the genetic relationships between 65 CRKP isolates. The PFGE pattern analysis demonstrated that 61 of the 65 CRKP isolates could be classified into nine pulsotypes: A (n = 2), B (n = 20), C (n = 9), D (n = 8), E (n = 8), F (n = 2), G (n = 2), H (n = 5), and I (n = 5). Four isolates appeared to be singletons. The dashed line corresponds to 80% as the cutoff for a close genetic relationship.

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Tables

Table 1. Characteristics of the study participants and logistic regression analysis of the carbapenemase-producing Enterobacteriaceae (CPE) strains isolated from stool samples from these inpatients.

Variable	CPE-positive group	CPE-negative group	P[a]	P[b]	OR (95% CI)
No. of patients (%)	55 (12)	388 (88)			
Male gender (%)	42 (76)	255 (66)	0.116	0.717	1.143 (0.556-2.350)
Age[c] [median(range)]	64 (16-97)	55 (13-99)	0.047[c]	0.677	1.146 (0.603-2.179)
No. (%) with					
Surgery	16 (29)	54 (14)	0.004	0.047	2.078 (1.008-4.282)
Abdominal pain	13 (24)	130 (34)	0.14	0.578	0.818 (0.402-1.665)
Fever	43 (78)	244 (63)	0.026	0.595	1.257 (0.541-2.919)
No. (%) of hospital-acquired infections	32 (58)	199 (51)	0.124	0.958	0.97 (0.319-2.951)
No. (%) receiving antibiotic therapy					
Cephalosporins	15 (27)	73 (19)	0.141	0.349	1.4 (0.692-2.833)
Carbapenems	42 (76)	221 (57)	0.006	0.156	1.739 (0.81-3.732)
Cephamycin	0 (0)	15 (4)	0.234	-	-
Aminoglycosides	5 (9)	38 (10)	0.869	-	-
Macrolides	3 (6)	18 (5)	0.735	-	-
Glycopeptides	20 (36)	108 (28)	0.192	0.674	1.151 (0.598-2.218)
Oral vancomycin	8 (15)	32 (8)	0.127	0.705	1.196 (0.473-3.021)
Fosfomycin	1 (2)	23 (6)	0.339	-	-
Linezolid	23 (42)	56 (14)	<0.001	0.002	2.871 (1.461-5.642)
Quinolone	16 (29)	160 (41)	0.085	0.174	0.616 (0.306-1.238)
Sulfamethoxazole	4 (7)	68 (18)	0.054	0.457	0.637 (0.194-2.089)
β-lactam/β-lactamase inhibitor	45 (82)	249 (64)	0.01	0.039	2.276 (1.043-4.969)
Tigecycline	15 (27)	63 (16)	0.044	0.67	0.849 (0.401-1.798)

[a] Univariate analysis; categorical variables were compared using x^2 test

. [b] Multivariate analysis of variables (P≤0.20) in univariate analysis was performed using stepwise backward logistic regression. Odds ratios (ORs) and their 95% confidence intervals (CIs) were calculated.

[c] P values were calculated based on a comparison between ages ≥60 and <60 using x^2 test.

Figures

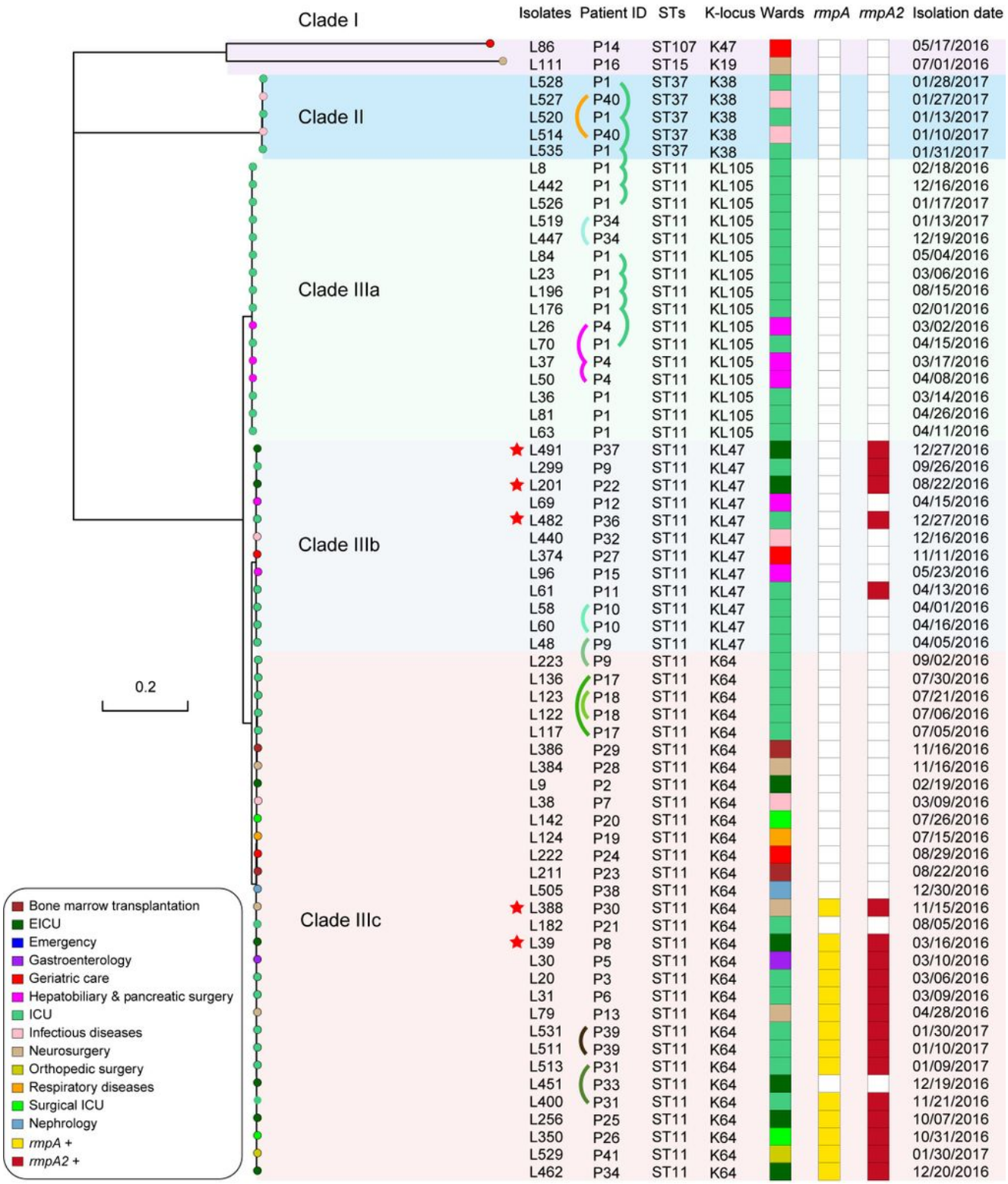

Figure 1

Core genome phylogeny for the CRKP isolates, including the associated multilocus sequence typing result, capsule locus genotypes, wards, virulence genes present, and isolation date. This maximum-likelihood phylogeny tree is based on single-nucleotide polymorphisms (SNPs) in the core genomes of 65

CRKP isolates. The scale bar indicates nucleotide divergence. The origins of the isolates are shown in different colors. Genome clusters are shaded with different colors. The presence of rmpA and rmpA2 are indicated by yellow and red shapes, respectively. The curved lines indicate isolates from the same patient. The asterisks in red indicate isolates were conducted with PacBio sequencing.

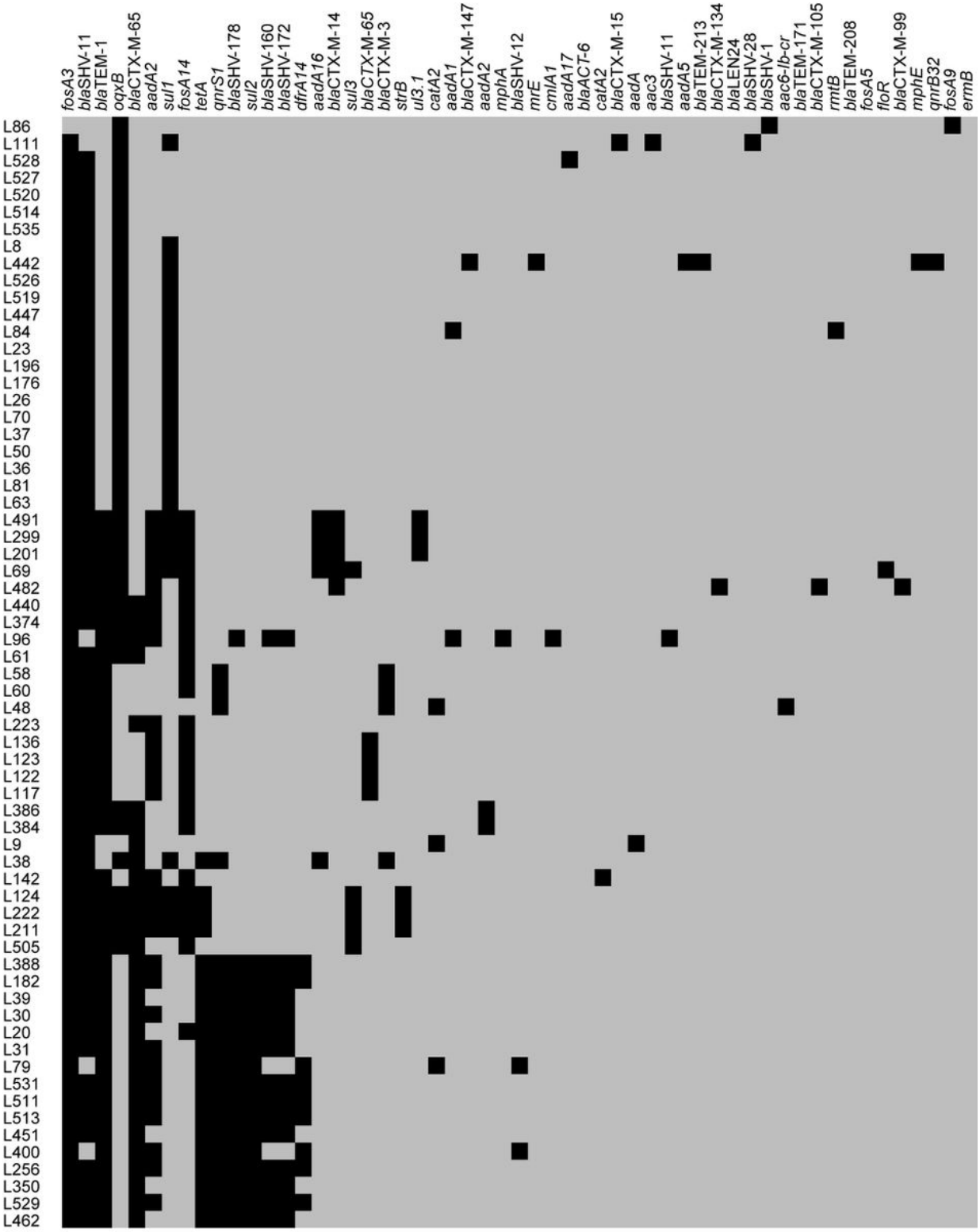

Figure 2

Antimicrobial resistance genes identified in the genomes of CRKP isolates from WGS data. The antimicrobial resistance genes are shown on the right side with their presence indicated by black shapes.

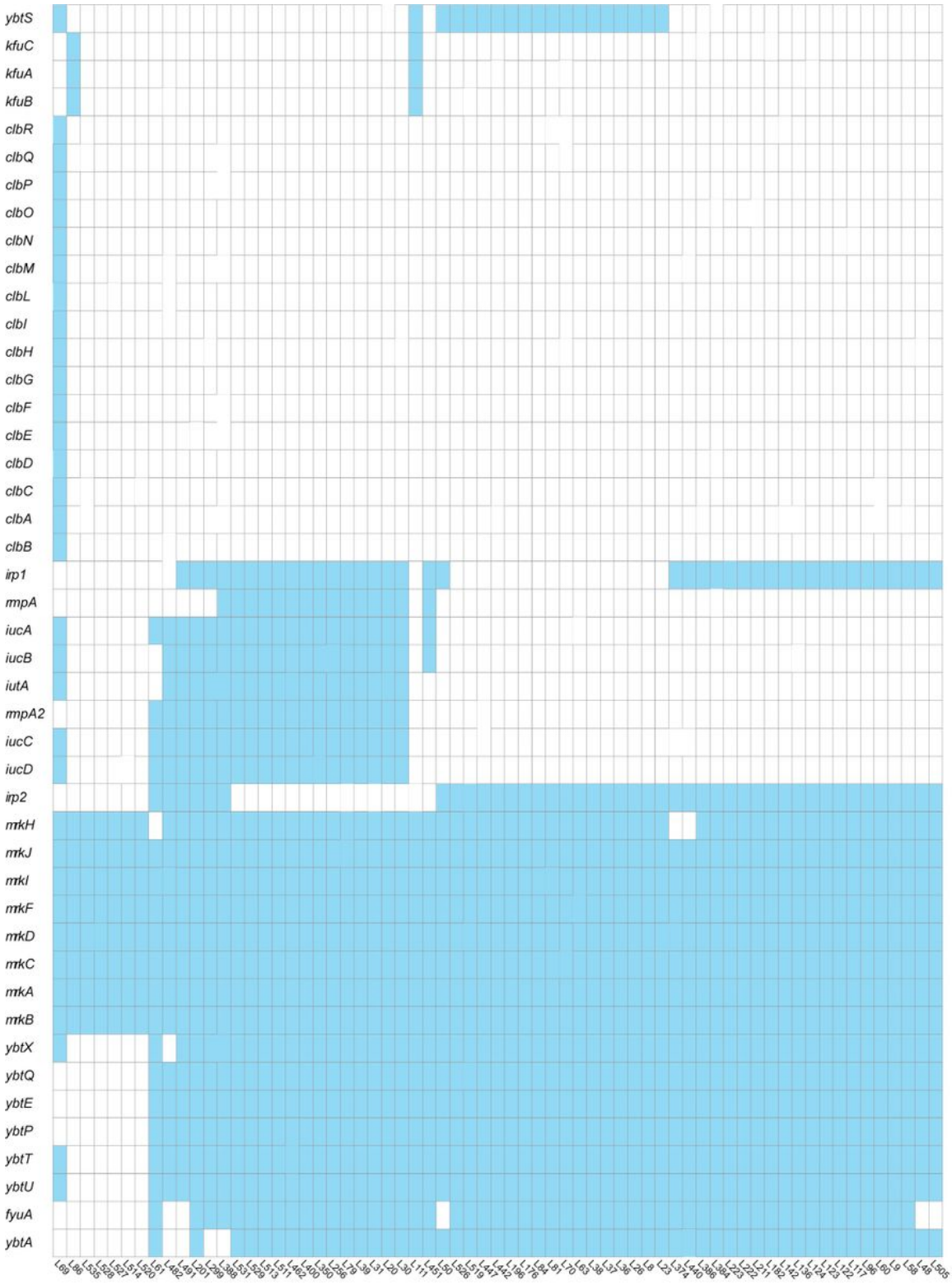

Figure 3

Distribution of virulence-associated genes in Klebsiella pneumoniae strains. Heatmaps were generated by aligning the draft genome sequence of each isolate to the BIGSdb-Kp database. The presence of

virulence genes in a specific genome is represented by a blue box, and the absence of virulence genes is represented by a cream-colored box. Virulence factors are shown on the left side.

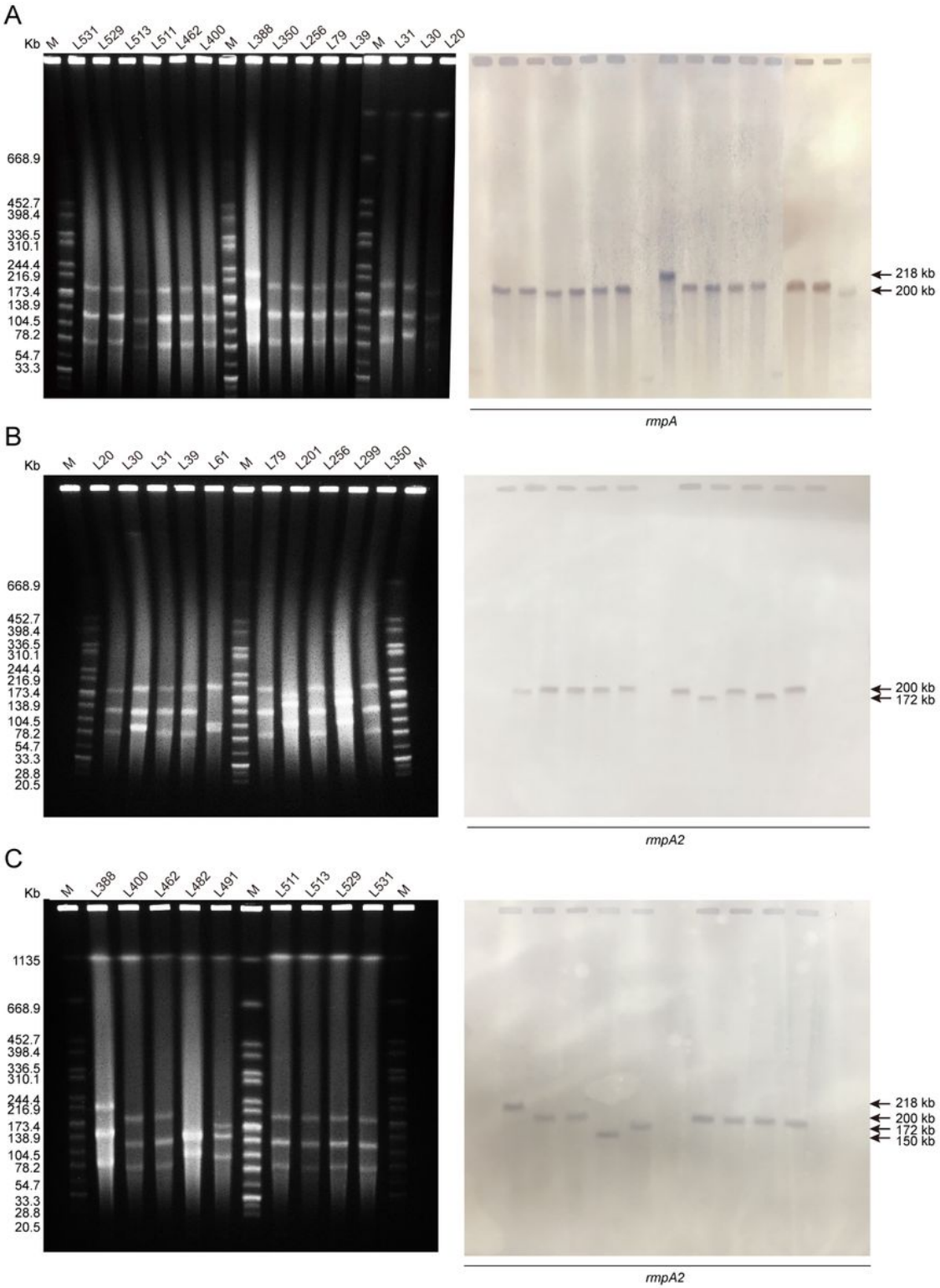

Figure 4

Plasmid analysis of the ST11 CR-HvKP isolates via S1-PFGE and Southern blotting using rmpA and rmpA2 probes. The arrows indicate the locations of the virulence plasmids. S1-PFGE revealed that most of the K. pneumoniae strains harbored three plasmids.

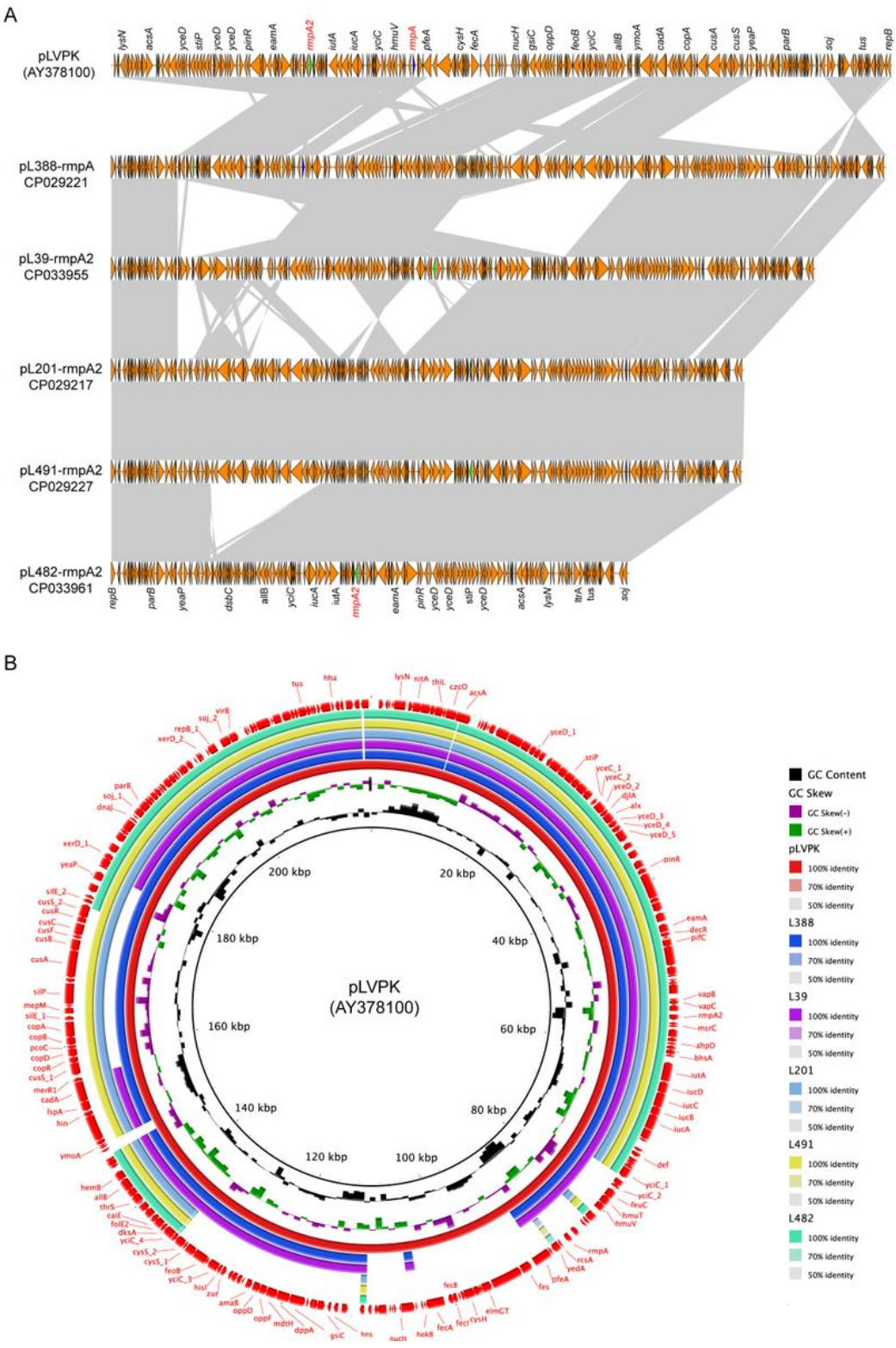

Figure 5

Alignment of rmpA/rmpA2-harbouring plasmids recovered in this work. The circular map was generated with the BLAST Ring Image Generator.

Supplementary Files

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