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SHORT REPORT

ST11 KPC-2-Producing *Klebsiella pneumoniae* Isolated from Patient with Acute Myelocytic Leukemia

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Background: The emergence of the ST11-CRKP (ST11-CRKP) strain is expected to become a serious public health problem in China. As one of the most serious complications in patients with acute myeloid lymphoma, infections can cause systemic infection and life-threatening sepsis, seriously affecting the morbidity, mortality, and quality of life of patients. Thus, ST11-CRKP infections in patients with acute myeloid lymphoma are worthy of our attention.

Aim: To investigate the occurrence and genetic characteristics of the ST11-CRKP from a patient with acute myeloid lymphoma.

Methods: Species identification was determined by MALDI-TOF MS. Antimicrobial susceptibility testing (AST) was conducted by VITEK 2 system with AST-N335 panel. Whole-genome sequencing was performed on the Illumina NovaSeq 6000 platform. Phylogenetic analyses were performed using Snippy based on the core-genome SNPs.

Findings: S1 nuclease pulsed-field gel electrophoresis (S1-PFGE), Southern blot and Whole-genome analysis indicated bla_{KPC-2} genes were located on plasmids with a conserved genetic environment. Moreover, the eight ST11-CRKP strains carry a variety of antimicrobial resistance genes (ARGs) and virulence factors. The ability of biofilm formation of eight strains was verified by a crystal violet assay. Core genome single-nucleotide polymorphism (cgSNP) analysis suggesting a possible bacterial translocation event.

Conclusion: We performed a comprehensive analysis of ST11-CRKP strains from a patient with acute myelocytic leukemia. Our study emphasized the need for continuous surveillance of ST11-CRKP in the clinic especially in the immunocompromised population.

Keywords: KPC-2, acute myelocytic leukemia, whole-genome sequencing, multiple site infections

Introduction

In recent years, antimicrobial resistance (AMR) has become a global concern. It is estimated the morality of 700,000 per year is attributed to infections of antibiotic-resistant bacteria.[1] Carbapenem used to be an important option for the treatment of antibiotic-resistant bacteria. But due to the extensive use of carbapenems in the clinic, the rapid spread of carbapenem-resistant *Klebsiella pneumoniae* (CRKP) is becoming a major threat to public health.[2] Especially, the production of *Klebsiella pneumoniae* carbapenemase (KPC) is the primary mechanism of carbapenem resistance in China.[3] The number of nosocomial infections caused by carbapenem-resistant *K. pneumoniae* ST11(ST11-CRKP) has been increasing rapidly for decades. Given that carbapenem resistance and virulence have converged into an epidemic clone, the emergence of the ST11-CRKP with virulence genes strain is expected to become a serious public health problem in China.[4]

Infection is one of the most serious complications in patients with acute myeloid lymphoma undergoing chemotherapy.[5] They receiving chemotherapy and caused anal mucosa damage, which providing a pathway for pathogens. Therefore, perianal infection is common in patients with hematological malignant tumors.[6] Perianal abscess is the acute manifestation of perianal infection.[7] Perianal infection is often accompanied by severe pain, swelling, and can cause systemic infection

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and life-threatening sepsis, seriously affecting the morbidity, mortality and quality of life of patients.[8] Thus, infections, especially ST11-CRKP infections in patients with acute myeloid lymphoma is worthy of our attention.

In this study, we reported a case of multiple site infections of ST11-CRKP in a patient with acute myeloid lymphoma. A total of eight nonrepeating strains were isolated from this patient during the treatment. The genetic characterizations were determined using whole-genome sequencing data. Furthermore, we investigated the virulence features and genomic differences of the eight strains. To the best of our knowledge, our study first reported the systemic infection caused by ST11-CRKP in a patient with acute myeloid lymphoma.

Materials and Methods

Isolates, Species Identification, and Antimicrobial Susceptibility Testing (AST)

Strains were collected from a patient with acute myeloid lymphoma and was hospitalized in a tertiary hospital in Zhejiang, China. Species identification was performed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS) (Bruker Daltonik GmbH, Bremen, Germany) and Average Nucleotide Identity (Figure S1). The bla_{KPC-2} gene was detected using PCR.

The antimicrobial susceptibility profiles of isolates were determined using VITEK 2 system employing panel AST-N335 with *Escherichia coli* ATCC 25922 as control. The results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI, https://clsi.org) and European Committee on Antimicrobial Susceptibility Testing (EUCAST, https://www.eucast.org/) clinical breakpoints.

Plasmid Analysis and Conjugation Assay

The number and size of the plasmids of strains were characterized using S1-PFGE.[9] The locations of bla_{KPC-2} genes were confirmed by Southern blotting and hybridization with digoxigenin-labelled bla_{KPC-2} specific probes. To verify the transferability of the plasmids carrying bla_{KPC-2} genes, conjugation experiments were conducted with *E. coli* 600 and J53 as the recipient strains.[9,10] The transconjugants were selected on the agar medium supplemented with 200 mg/L rifampicin/sodium azide and 2 mg/L meropenem. If the conjugations were not successful, the final identification of transconjugants was carried out by MALDI-TOF/MS identification, PCR, and AST.

Whole-Genome Sequencing (WGS) and in silico Analyses

Whole-cell DNA was extracted using the OMEGA Bacterial DNA kit (Omega Bio-tek, Norcross, GA, USA). The DNA was then sequenced on the Illumina NovaSeq 6000 (San Diego, CA, USA) platform. The assembly of the genomes were performed using SPAdes v3.9.1. Strain Jinyinhai1 was sequenced on the platform Illumina NovaSeq 6000 (San Diego, CA, USA) and Oxford Nanopore platforms (Oxford Nanopore Technologies, Oxford, United Kingdom). The complete genome of strain Jinyinhai1 was assembled by Unicycler v0.4.2.

The genome sequences were annotated by Prokka.[11] The acquired antimicrobial resistance genes (ARGs) and virulence factors were identified by Resfinder (https://cge.cbs.dtu.dk/services/ResFinder/) and VFDB.[12] Replicon types of plasmids were detected by PlasmidFinder (https://cge.cbs.dtu.dk/services/PlasmidFinder/), and the multilocus sequence typing (MLST) was determined by pubMLST (http://pubmlst.org/ecloacae). Finally, the genetic environments of KPC-2 and circular maps of plasmids were generated with Easyfig 2.3 and BLAST Ring Image Generator (BRIG).[13,14]

String Test and Biofilm Formation

A string test was done using a standard inoculation loop to gently lift a colony of ST11-CRKP grown on a blood agar plate to detect the hypermucoid phenotype, and the string test was rated as positive if a mucoid string of > 5 mm was observed.[15] The abilities of biofilm formation of eight strains were quantitatively determined as a previous study.[16] The interpretation of results as following: non-adherent ($OD \leq ODc$), weakly to moderately adherent ($ODc < OD \leq 2 \times ODc$), and strongly adherent ($2 \times ODc < OD$). Each assay was performed in triplicate.

SNPs Calling and Phylogeny

Core genome single-nucleotide polymorphism (cgSNP) analysis was conducted with Snippy v4.6.0, with the chromosome of *K. pneumoniae* HS11286 (GCA_000240185.2) as the reference. Putative repetitive sections, mobile genetic elements (MGEs), and recombination events were filtered using Gubbins v.2.4.1. Finally, a maximum likelihood tree based was constructed by FastTree v2.1.10 and visualized by iTOL.[17] Pairwise cgSNP differences between strains were determined under SNP-dists v0.4.

Data Availability

All whole-genome sequences were deposited at NCBI with BioProject ID: PRJNA988307.

Results and Discussion

Strain Identification and Case Description

The male patient visited the hospital in October 2021 because of fatigue. After admission, he was diagnosed with acute myeloid leukemia, positive for AML/ETO with ckit D816v mutation, and moderately critical. Chemotherapy began on October 28th, and the regimen was Homoharringtonine combined with aclarubicin and cytarabine (HAA). After one course of chemotherapy, it reached complete remission (CR), but minimal residual leukemia (MRD) was positive. Idarubicin combined with cytarabine (IA) regimen chemotherapy was performed on November 26th, and reached CR with the quantity of AML/ETO was negative after chemotherapy. Moderate dose cytarabine (Ara-c) chemotherapy was performed on December 25th, and reached CR with MRD negative. After IA chemotherapy on February 10th, the quantity of AML/ETO changed to positive. Considering that the MRD of the patients turned positive, lost the best treatment response and had a high risk of recurrence, allogeneic hematopoietic stem cell transplantation (HSCT) was decided. Pre-transplantation pretreatment began on April 1, 2022, and the regimen was FACT (Functional Assessment of Cancer Treatment) (Figure 1A).

The patient was routinely given piperacillin-tazobactam anti-bacterial therapy. Baseline examination before transplantation showed that the patient had no obvious infection lesions and intestinal carbapenem-resistant *Enterobacterales*

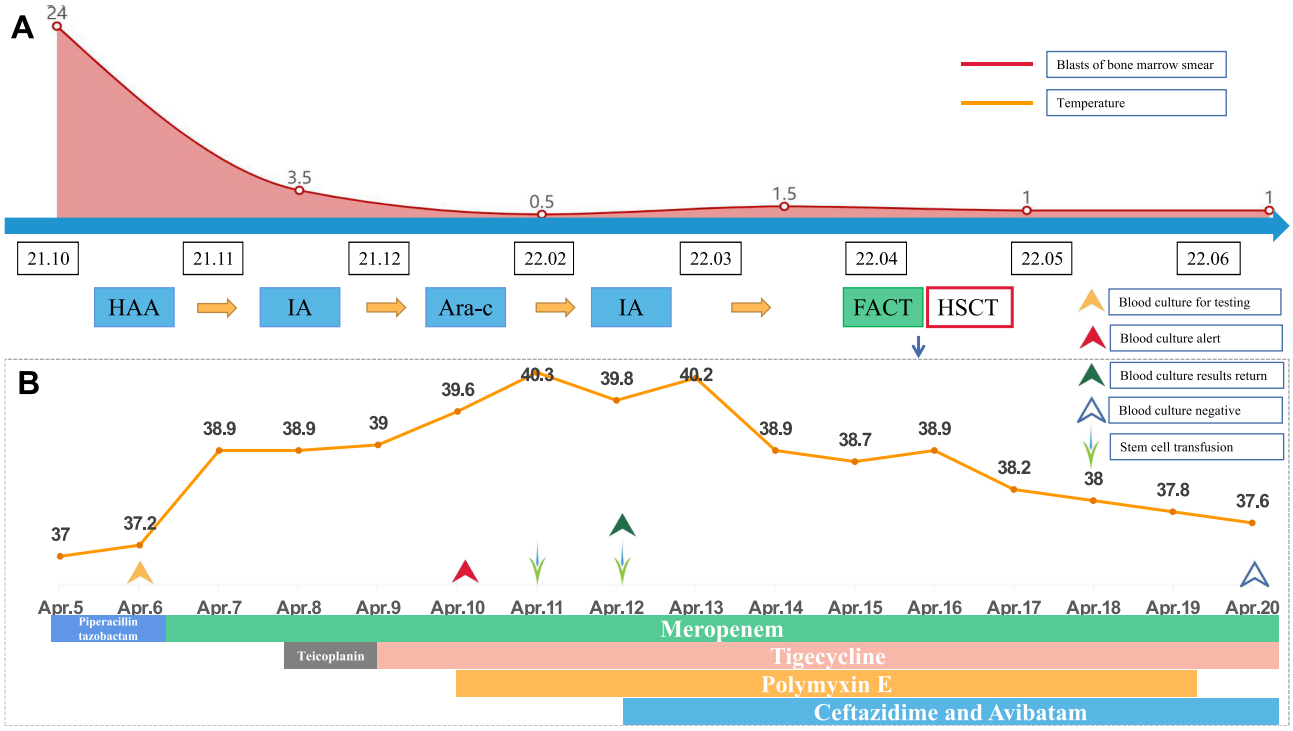

Figure 1 (**A**) Clinical diagnosis and treatment of patients. (**B**) Changes in infection during hospitalization. HAA, homoharringtonine combined aclarubicin and cytarabine; IA, idarubicin combined with cytarabine; Ara-c, cytarabine; FACT, functional assessment of cancer treatment; HSCT, hematopoietic stem cell transplantation.

(CRE) colonization. On April 7, the patient developed chills and fever, with a maximum body temperature of 38.9 °C, accompanied by mild perianal tenderness. The results showed that the white blood cells were 0.1×10^9 /L. Considering the possibility of breakthrough of drug-resistant bacteria, piperacillin tazobactam was discontinued and meropenem was replaced. On April 8, the patient's body temperature was still high, and he was given Teicoplanin to strengthen anti-positive bacteria treatment, but the peak temperature continued to rise the next day, and perianal abscess appeared. Teicoplanin was stopped and replaced with tigecycline for anti-infection. On April 10, the patient's blood culture return indicated multidrug-resistant *K. pneumoniae* bacteremia and the patient was in a state of severe deficiency, and polymyxin E was given to resist infection.

HSCT combined was performed on April 11 and 12. The patient was infected with drug-resistant *K. pneumoniae* in multiple blood and stool cultures and was treated with ceftazidime and avibactam on April 12. On April 14, the patient began to show a peak decrease in body temperature and a progressive increase in creatinine. A total of eight *K. pneumoniae* isolates were isolated from April 9 to 12. The specific isolation time and sources are shown in Table 1. On April 20, polymyxin E was discontinued. After that, the patient's temperature was gradually controlled and the perianal abscess improved (Figure 1B). After infection control, the patient was discharged with no obvious rejection.

Resistome of K. pneumoniae Isolates

Minimum inhibitory concentration (MIC) of eight ST11-CR-HvKP strains indicated its resistance to various types of antibiotics, including ticarcillin/clavulanic acid, piperacillin/tazobactam, ceftazidime, cefoperazone/sulbactam, cefepime, aztreonam, imipenem, meropenem, amikacin, tobramycin, ciprofloxacin, levofloxacin, doxycycline, minocycline, and trimethoprim/sulfamethoxazole (Table 2). All isolates are susceptible to tigecycline except to 22R000695R which is isolated from stool. The usage of tigecycline may have induced its resistance. As shown in Figure 2, various ARGs were found in these strains: aadA2, aadA2b, aadA3, aadA10, aadA16, aac(6')-Ib-cr, aac(6')-Ib, bla_{SHV-11}, bla_{SHV-31}, fosA6, bla_{KPC-2}, aph(6)-Id, sul2, qnrB6, bla_{TEM-1B}, $bla_{CTX-M-65}$ and tet(D).

Toxome and Biofilm Formation Capacity

Screening of virulence factors showed that eight isolates shared the same virulence profiles. These virulence genes encoded multiple functions, including siderophores (including aerobactin-encoded gene *iutAiucAC* and salmochelin-encoded gene *iroE*) and polysaccharide virulence genes (*rmpA* and *rmpA2*). According to the previous study, strains with *rmpA* genes, salmochelin siderophore biosynthesis, aerobactin siderophore biosynthesis were identified to have a hypervirulent phenotype.[9,18] Thus, these eight strains were presumed to have high virulence potential. Additionally, these isolates also contain genes encoded type 3 fimbriae genes (*mrkCDHJ* and *fimCDHK*) which play a significant role in bacterial adherence, the initial step that precedes colonization.[16] According to the results of the string test, all strains were not hypermucoviscous. The biofilm formation capacity was assessed using crystal violet. The mean OD_{600} values of biofilms were shown in Table 3. All strains were classified as weakly to moderately adherent. The biofilm formation abilities were consistent with the results of virulence genes.

Table 1 The Isolation Time and Sources of Eight ST11-CR-HvKP Isolates

Strains	Isolation Time	Source
Jinyinhai1	2022.04.09	Stool
22A008038A	2022.04.10	Blood
22A008014A	2022.04.10	Blood
22A008081B	2022.04.11	Blood
22R000695R	2022.04.12	Stool
Jinyinhai2	2022.04.12	Blood
22A008202A	2022.04.12	Blood
22R000724R	2022.04.14	Stool

Table 2 Antimicrobial Susceptibilities of Eight ST11-CR-HvKP Isolates

	Jinyinhai1	Jinyinhai2	22A008038A	22A008014A	22A008081B	22A008202A	22R000695R	22R000724R
Ticarcillin/ Clavulanic Acid	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R
Piperacillin/ Tazobactam	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R	≥128 / R
Ceftazidime	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R
Cefoperazone/ Sulbactam	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R
Cefepime	≥32 / R	≥32 / R	≥32 / R	≥32 / R	≥32 / R	≥32 / R	≥32 / R	≥32 / R
Aztreonam	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R
Imipenem	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R
Meropenem	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R
Amikacin	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R	≥64 / R
Tobramycin	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R
Ciprofloxacin	≥4 / R	≥4 / R	≥4 / R	≥4 / R	≥4 / R	≥4 / R	≥4 / R	≥4 / R
Levofloxacin	≥8 / R	≥8 / R	≥8 / R	≥8 / R	≥8 / R	≥8 / R	≥8 / R	≥8 / R
Doxycycline	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R
Minocycline	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R	≥16 / R
Tigecycline	2 / S	2 / S	2 / S	2 / S	2 / S	2 / S	≥8 / R	2 / S
Colistin	≤0.5 / S	≤0.5 / S	≤0.5 / S	≤0.5 / S	≤0.5 / S	≤0.5 / S	≤0.5 / S	≤0.5 / S
Trimethoprim/ Sulfamethoxazole	≥320 / R	≥320 / R	≥320 / R	≥320 / R	≥320 / R	≥320 / R	≥320 / R	≥320 / R

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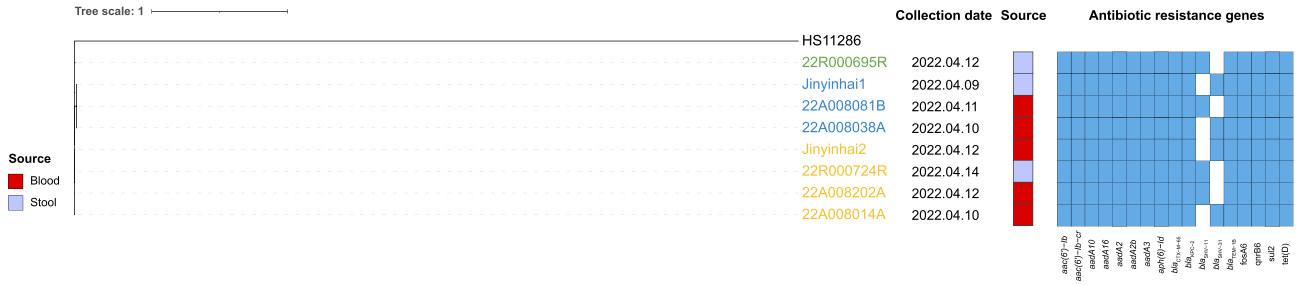

Figure 2 A comparative genome analysis of eight ST11-CR-HvKP isolates based on core genome SNPs. Isolates with zero cgSNP differences were marked by the same color. The collection dates, isolation sources, and antimicrobial resistance genes are shown. Blue indicates that the isolate carries such genes and colorless means that the genes are not present.

Genomic Features of K. pneumoniae Isolates

The result of S1-PFGE and Southern Blotting indicated KPC-2 were located on a ~190 kb plasmid in these strains (Figure 3A). The complete sequence of plasmids of strain Jinyinhai1 were obtained to describe the genomic features better. The WGS data confirmed the KPC-2-carrying plasmid (designated as pJinyinhai1_KPC) was an IncFII/IncR type plasmid with the size of 132,750 bp. To verify the transferability of KPC-2-harbouring plasmids, conjugation assays were performed. However, repeated transformation methods failed, which implied that it was non-conjugative. The OriTFinder results indicated that pJinyinhai1_KPC had incomplete conjugative modules with the absence of relaxase and type IV coupling protein (T4CP) (Table S1).

Bioinformatics analysis indicated that the eight isolates were assigned to sequence type 11 (ST11), a widespread clone in China. Moreover, ST11-CRKP infection cases were recorded in different regions of China.[19] Zheng et al revealed the silent dissemination of ST11-CR-HvKP bacteria in Zhejiang Province, China.[4] In addition, NCBI BLAST analysis revealed that pJinyinhai1_KPC exhibited 100% nucleotide identity with plasmid p3_L39 (accession number: CP033956.1), pDD02391-1 (accession number: CP087640.1), pKP12-KPC (accession number: CP082767.1) and pKPC-2 (accession number: CP130265.1), all of them were from *K. pneumoniae* in China (Figure 3C). The genetic environment analysis showed that these eight strains exhibited 100% nucleotide identity with *K. pneumoniae* C789 (accession number: CP034415.1). All they shared a conserved genetic context: Tn*As1-hin-klcA-bla*$_{KPC-2}$-IS*Kpn27-tnpR* (Figure 3B). Especially, various mobile genetic elements (MGEs) surrounding the *bla*$_{KPC-2}$-harboring region formed a composite transposon-like structure, which promoted its transfer among various plasmids. ST11-CR-HvKP colonizes the patient and may become a reservoir of carbapenemase-encoding and virulence genes, which highlights that an effective prevention strategy should be taken to curb its further dissemination.

Table 3 The Biofilm-Forming Ability of Eight ST11-CR-HvKP Isolates

Strain	OD_{600}	Biofilm Formation Ability
Jinyinhai1	0.18	Weakly to moderately adherent
Jinyinhai2	0.15	
22A008038A	0.17	
22A008014A	0.18	
22A008081B	0.20	
22A008202A	0.17	
22R000695R	0.18	
22R000724R	0.19	
Control	0.09	–

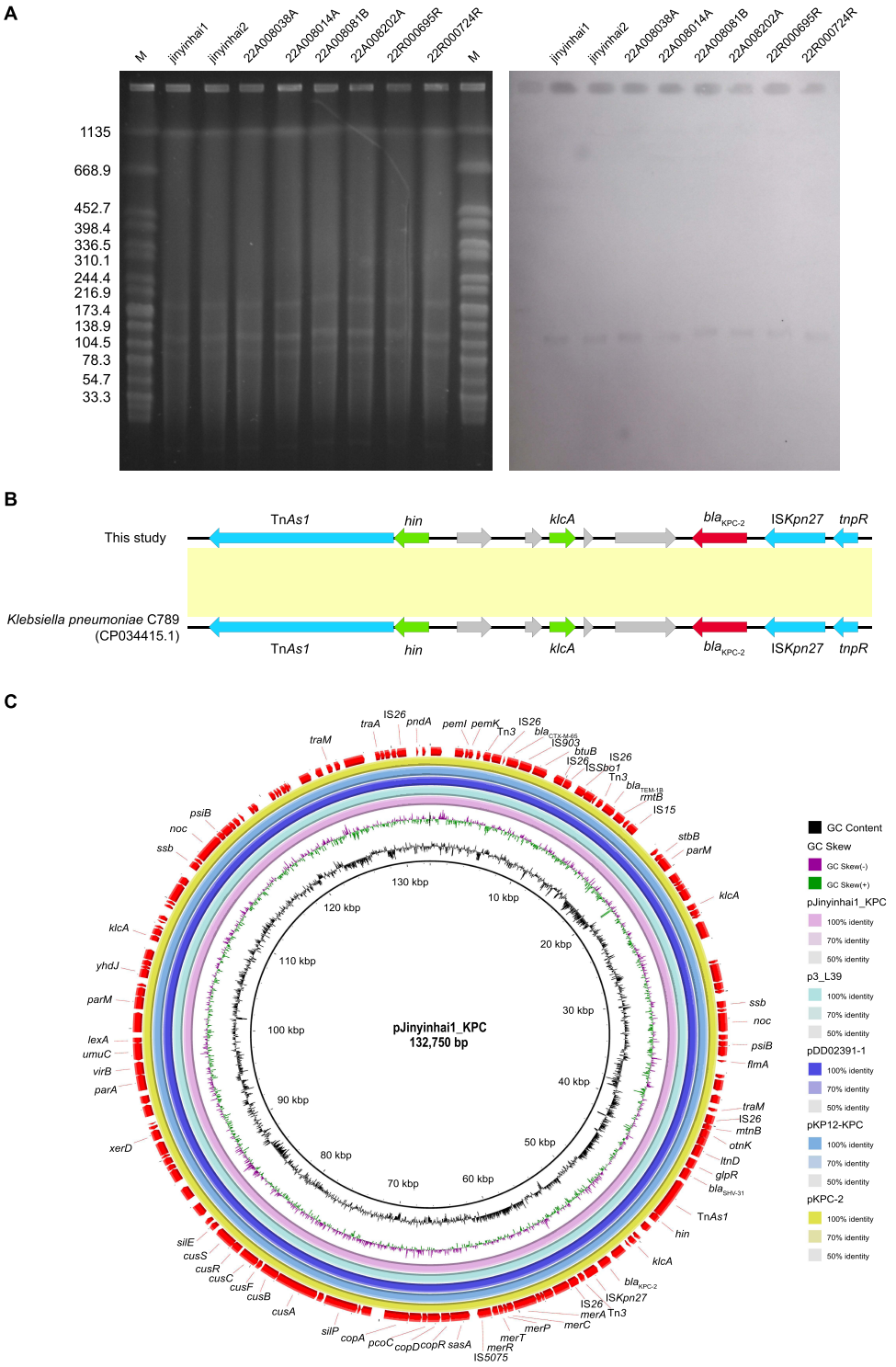

Figure 3 The genetic features of eight ST11-CR-HvKP isolates. **(A)** Plasmid profiles of eight ST11-CR-HvKP. **(B)** Genetic context of bla_{KPC-2} genes in this study. **(C)** Comparative analysis of plasmid pJinyinhai1_KPC detected in *K. pneumoniae* Jinyinhai1.

Analysis of Phylogenetic Relationships

A maximum-like tree was constructed based on cgSNP (Figure 2). The pairwise SNP distances of eight strains were displayed in Table S2. There was no genetic difference between the first ST11-CRKP strain (Jinyinhai1) isolated from stool and the two subsequent strains (22A008081B and 22A008038A) isolated from blood, suggesting a possible

bacterial translocation. Additionally, SNP differences in strains isolated from the same sites within a short time indicated the microevolution of ST11-CRKP during treatment.

Conclusions

In general, we reported ST11-CRKP infections in a patient with acute myelocytic leukemia. The patient's infection was eventually controlled. The WGS data and microbiological analysis elucidated the genetic characterization and antimicrobial resistance mechanisms of the eight strains. The emergence of ST11-CRKP strains is expected to become a threatening public health issue in China, especially in the immunocompromised population. Moreover, multidrug-resistant bacteria make clinical choices extremely limited. Our study emphasized the need for continuous surveillance of ST11-CRKP in the clinic especially in the immunocompromised population.

Ethics Statement

The study was approved by the Clinical Ethics Committee of the Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine [number 2021-070]. Written informed consent has been provided by the patient to have the case details published. All methods were performed in accordance with relevant guidelines and regulations. All investigators adhered to the principles expressed in the Declaration of Helsinki.

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Disclosure

The authors report no conflicts of interest in this work.

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