

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

Can Chen¹, Fan Yang¹, Mantao Chen², Ying Xu¹, Yaping Xie¹ , Ruishan Liu³, Pengfei Shi¹, Shenxian Qian¹

¹Department of Hematology, Affiliated Hangzhou First People's Hospital, Zhejiang University School of Medicine, Hangzhou, Zhejiang, 310006, People's Republic of China; ²Department of Neurosurgery, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, People's Republic of China; ³Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, College of Medicine, Zhejiang University, Hangzhou, People's Republic of China

Correspondence: Shenxian Qian; Pengfei Shi, Email sxqian1028@zju.edu.cn; syfshi@163.com

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 mortality 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

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 ($OD \leq OD_c$), weakly to moderately adherent ($OD_c < OD \leq 2 \times OD_c$), and strongly adherent ($2 \times OD_c < OD$). 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 v2.4.1. 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*

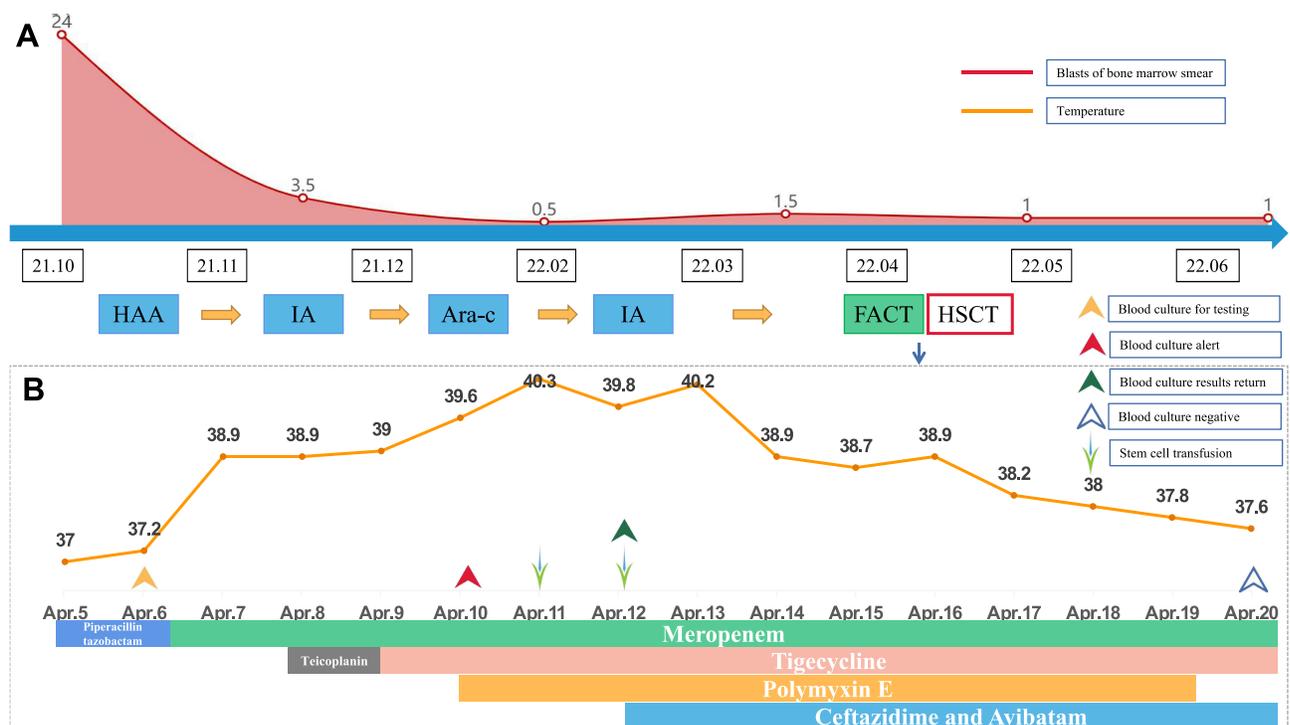


Figure 1 (A) Clinical diagnosis and treatment of patients. (B) Changes in infection during hospitalization. HAA, homoharringtonine combined aclarubicin and cytarabine; IA, idarubicin combined with cytarabine; Ara-c, cytarabine; FACT, functional assessment of cancer treatment; HSCT, hematopoietic stem 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 *iutA*iucAC** 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 OD₆₀₀ 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							
Piperacillin/ Tazobactam	≥128 / R							
Ceftazidime	≥64 / R							
Cefoperazone/ Sulbactam	≥64 / R							
Cefepime	≥32 / R							
Aztreonam	≥64 / R							
Imipenem	≥16 / R							
Meropenem	≥16 / R							
Amikacin	≥64 / R							
Tobramycin	≥16 / R							
Ciprofloxacin	≥4 / R							
Levofloxacin	≥8 / R							
Doxycycline	≥16 / R							
Minocycline	≥16 / R							
Tigecycline	2 / S	2 / S	2 / S	2 / S	2 / S	2 / S	≥8 / R	2 / S
Colistin	≤0.5 / S							
Trimethoprim/ Sulfamethoxazole	≥320 / R							

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.

Funding

This work was supported by the Hangzhou Science and Technology Major Project under [Grant number 202004A15]; the Hangzhou Medical Health Science and Technology Major Project under [Grant number Z20210039] and the Zhejiang Provincial Natural Science Foundation of China [Grant number LHDMZ22H190002].

Disclosure

The authors report no conflicts of interest in this work.

References

1. Inoue H. Strategic approach for combating antimicrobial resistance (AMR). *Glob Health Med.* 2019;1(2):61–64. doi:10.35772/ghm.2019.01026
2. Effah CY, Sun T, Liu S, Wu Y. Klebsiella pneumoniae: an increasing threat to public health. *Ann Clin Microbiol Antimicrob.* 2020;19(1):1. doi:10.1186/s12941-019-0343-8
3. Li D, Li K, Dong H, et al. Ceftazidime-Avibactam Resistance in Klebsiella pneumoniae Sequence Type 11 Due to a Mutation in Plasmid-Borne bla_{kpc-2} to bla_{kpc-33}, in Henan, China. *Infect Drug Resist.* 2021;14:1725–1731. doi:10.2147/IDR.S306095
4. Zheng B, Xu H, Lv T, et al. Stool Samples of Acute Diarrhea Inpatients as a Reservoir of ST11 Hypervirulent KPC-2-Producing Klebsiella pneumoniae. *mSystems.* 2020;5(3):e00498–20. doi:10.1128/mSystems.00498-20
5. Paul M, Gafter-Gvili A, Goldberg E, Yahav D. Infections in hematological cancer patients: the contribution of systematic reviews and meta-analyses. *Acta Haematol.* 2011;125(1–2):80–90. doi:10.1159/000318900
6. Vanhuevozwyn R, Delannoy A, Michaux JL, Dive C. Anal lesions in hematologic diseases. *Dis Colon Rectum.* 1980;23(5):310–312. doi:10.1007/BF02586835
7. Ja R, Al N, Ek J. Anorectal abscess and fistula-in-ano: evidence-based management. *Surg Clin North Am.* 2010;90(1). doi:10.1016/j.suc.2009.10.001
8. Chen CY, Cheng A, Huang SY, et al. Clinical and Microbiological Characteristics of Perianal Infections in Adult Patients with Acute Leukemia. *PLoS One.* 2013;8(4):e60624. doi:10.1371/journal.pone.0060624
9. Liu R, Xu H, Zhao J, et al. Emergence of mcr-8.2-harboring hypervirulent ST412 Klebsiella pneumoniae strain from pediatric sepsis: a comparative genomic survey. *Virulence.* 2023;14(1):233–245. doi:10.1080/21505594.2022.2158980
10. Xu H, Wang X, Yu X, et al. First detection and genomics analysis of KPC-2-producing Citrobacter isolates from river sediments. *Environ Pollut Barking Essex.* 2018;235:931–937. doi:10.1016/j.envpol.2017.12.084
11. Seemann T. Prokka: rapid prokaryotic genome annotation. *Bioinforma Oxf Engl.* 2014;30(14):2068–2069. doi:10.1093/bioinformatics/btu153
12. Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res.* 2022;50(D1):D912–D917. doi:10.1093/nar/gkab1107
13. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. *Bioinforma Oxf Engl.* 2011;27(7):1009–1010. doi:10.1093/bioinformatics/btr039
14. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics.* 2011;12(1):402. doi:10.1186/1471-2164-12-402
15. Zhao J, Zheng B, Xu H, et al. Emergence of a NDM-1-producing ST25 Klebsiella pneumoniae strain causing neonatal sepsis in China. *Front Microbiol.* 2022;13:980191. doi:10.3389/fmicb.2022.980191
16. Hu X, Zhang H, Liu Y, et al. Genetic characterization and virulence determinants of multidrug-resistant NDM-1-producing Aeromonas caviae. *Front Microbiol.* 2022;13:1055654. doi:10.3389/fmicb.2022.1055654

17. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res.* 2021;49(W1):W293–W296. doi:10.1093/nar/gkab301
18. Russo TA, Olson R, Fang CT, et al. Identification of Biomarkers for Differentiation of Hypervirulent *Klebsiella pneumoniae* from Classical *K. pneumoniae*. *J Clin Microbiol.* 2018;56(9):e00776–18. doi:10.1128/JCM.00776-18
19. Wong MHY, Shum HP, Chen JHK, et al. Emergence of carbapenem-resistant hypervirulent *Klebsiella pneumoniae*. *Lancet Infect Dis.* 2018;18(1):24. doi:10.1016/S1473-3099(17)30629-1

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <https://www.dovepress.com/infection-and-drug-resistance-journal>