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ORIGINAL RESEARCH

Genomic Insights into Plasmid Mediated Dissemination of *bla*$_{KPC-2}$ and *bla*$_{CTX-M-14}$ in a Fecal ST595 *Serratia Marcescens* Isolate

Yuyan Wang[1,*], Huanran Zhang[2,*], Yi Liu[3], Yifei Wang[1]

[1]Department of Emergency Medicine, Zhuji Traditional Chinese Medicine Hospital, Zhuji, People's Republic of China; [2]Department of Emergency Medicine, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China; [3]Collaborative Innovation Center for Diagnosis and Treatment of Infectious Diseases, State Key Laboratory for Diagnosis and Treatment of Infectious Diseases, the First Affiliated Hospital, Zhejiang University School of Medicine, Hangzhou, People's Republic of China

*These authors contributed equally to this work

Correspondence: Yifei Wang, Email doctor_wyf1120@163.com

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Objective: *Serratia marcescens* is an opportunistic pathogen with notable antimicrobial resistance features. The emergence of *S. marcescens* harboring the *bla*$_{KPC-2}$ gene, which encodes a carbapenemase conferring resistance to carbapenems, poses a significant threat to public health. This study reports the detailed genomic characteristics of a *bla*$_{KPC-2}$- and *bla*$_{CTX-M-14}$-co-carrying *S. marcescens* strain L4843, isolated from a fecal sample.

Methods: The isolate underwent antimicrobial susceptibility testing (AST) by broth microdilution. Whole-genome sequencing (WGS) was conducted using Illumina NovaSeq and Nanopore platforms. Unicycler was used for assembly and bioinformatics tools identified resistance genes, plasmid types, and mobile elements. Conjugation experiments with *E. coli* J53 as the recipient were carried out on MacConkey agar with sodium azide and imipenem. Transconjugants were confirmed by PCR amplification of resistance genes.

Results: The genome of the *S. marcescens* isolate comprised 5,377,884 bp with a GC content of 59.8%. The *bla*$_{KPC-2}$ gene, responsible for carbapenem resistance, was located on a repB(R1701) plasmid. The plasmid contains several copies of IS*26* and IS*Kpn19* elements, along with Tn*As1* transposons. The isolate also harbored multiple resistance genes, including *bla*$_{CTX-M-14}$, *bla*$_{SRT-1}$, *bla*$_{LAP-2}$, *aac(3)-IId*, and *aac(6')-Ic*. The plasmid sequence was compared with other known sequences (CP047692, CP047686, MT269826), revealing significant homology. The alignment shows a conserved structure around *bla*$_{KPC-2}$, indicating a shared dissemination mechanism for this resistance gene.

Conclusion: The genomic characterization of a *bla*$_{KPC-2}$- and *bla*$_{CTX-M-14}$-co-carrying *S. marcescens* underscores the complexity and mobility of resistance mechanisms within this pathogen. To our knowledge, no published report has documented fecal S. marcescens carrying *bla*$_{KPC-2}$ and *bla*$_{CTX-M-14}$ in China to date, highlighting the surveillance value of our finding.

Keywords: *serratia marcescens*, carbapenem-resistant, KPC-2, repB, R1701, plasmid, mobile genetic elements

Introduction

Serratia marcescens is a Gram-negative bacillus belonging to the Enterobacteriaceae family, recognized for causing nosocomial infections, particularly in immunocompromised patients. Known for causing a variety of infections, including pneumonia, urinary tract infections, and notably, bloodstream infections, *S. marcescens* has become an emerging threat due to its ability to acquire and disseminate resistance genes.[1] The rise of carbapenem-resistant Enterobacteriaceae (CRE), particularly those producing *Klebsiella pneumoniae* carbapenemase (KPC), poses a severe threat to public health because of limited treatment options.[2] The dissemination of the *bla*$_{KPC-2}$ gene is primarily facilitated through plasmids, which can transfer between different bacterial species, exacerbating the spread of resistance. CTX-M-14 is an extended-spectrum β-lactamase (ESBL) enzyme, frequently detected in Enterobacteriaceae and contributing to resistance against third generation cephalosporins.[3] While *S. marcescens* carrying *bla*$_{KPC-2}$ has been described in clinical infections in

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China (eg, ICU isolates), stool carriage in this species remains rare in the Chinese literature to date. These gaps emphasize the importance of stool-based genomic surveillance and motivate the present analysis of a fecal isolate."

This study focuses on the genomic characterization of *S. marcescens* L4843, a bla_{KPC-2}- and $bla_{CTX-M-14}$-co-carrying isolate recovered from a fecal sample in China. Understanding the genetic context of bla_{KPC-2} and other resistance determinants within this isolate is crucial for developing effective strategies to combat CRE.

Materials and Methods

The *S. marcescens* L4843 strain was isolated from a fecal sample of a patient at a tertiary hospital in Hangzhou, China in 2023. The isolate was cultured on MacConkey agar, and species identification was confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry (MALDI-TOF/MS).

The minimum inhibitory concentrations (MICs) for various antibiotics were determined using the broth microdilution method, following the guidelines of the Clinical and Laboratory Standards Institute (CLSI). The antibiotics tested included carbapenems (imipenem, meropenem), β-lactams (ceftazidime, cefepime), aminoglycosides (gentamicin, amikacin), and fluoroquinolones (ciprofloxacin, levofloxacin).

Genomic DNA of *S. marcescens* L4843 was extracted using the QIAamp DNA Mini Kit (Qiagen, Hilden, Germany). The short-read library was prepared using Nextera DNA Flex Library Prep Kit (Illumina Inc., San Diego, CA, USA) for WGS according to the manufacturer's instructions and sequenced on a NovaSeq instrument (Illumina Inc., San Diego, CA, USA). The long-read library was prepared using a Ligation Sequencing Kit 1D (Oxford Nanopore Technologies, Oxford, UK) and sequenced with the MinION sequencer.[4] *De novo* genome assembly was carried by Unicycler v.0.4.8[5] and the genome was subsequently annotated using Prokka 1.14.5.[6] Species identification was corroborated using SpeciesFinder v.2.0 (https://cge.cbs.dtu.dk/services/Species-Finder/). PlasmidFinder and ResFinder were used to identify plasmid replicons and resistance genes, respectively.[7] Integrons were detected using the Integron Finder tool (https://github.com/gem-pasteur/Integron_Finder). Specifically, the SQK-LSK109 kit and R9.4 flow cell (FLO-MIN106) were used for sequencing. MLST analysis was performed using the PubMLST database (https://pubmlst.org/bigsdb?db=pubmlst_serratia_seqdef). Default settings were used for all bioinformatics tools unless otherwise specified. Putative transconjugants were screened by PCR for both bla_{KPC-2} and $bla_{CTX-M-14}$.

Results and Discussion

The isolate *S. marcescens* L4843 demonstrated high-level resistance to carbapenems, with MIC values for imipenem and meropenem exceeding 16 μg/mL. Resistance to other β-lactams, aminoglycosides, and fluoroquinolones was also observed, confirming the multidrug-resistant (MDR) phenotype of this isolate.

The genome of *S. marcescens* L4843 comprises one chromosome and three plasmids. It comprised 5,377,884 bp with a GC content of 59.8%. The genome contained 5,083 coding sequences, 95 tRNA genes, and 6 rRNA operons. MLST analysis identified the isolate as sequence type ST595. The genome of L4843 harbors multiple antimicrobial resistance genes, including bla_{KPC-2}, *aac(3)-IId* and *aac(6')-Ic* conferring resistance to carbapenems and aminoglycosides, respectively. Other resistance genes identified included bla_{SRT-1}, $bla_{CTX-M-14}$, and bla_{LAP-2} (Table S1), which provide resistance to beta-lactams. The presence of these genes correlates with the observed multidrug-resistant (MDR) phenotype, including resistance to imipenem and meropenem. All public repository accessions and direct hyperlinks for the *S. marcescens* L4843 genome and associated datasets (BioProject, BioSample, and WGS assembly) are compiled in (Table S2).

Plasmid analysis of bla_{KPC-2}-carrying repB(R1701) plasmid pL4843-KPC2 provided comprehensive insights into the genetic environment surrounding the bla_{KPC-2} gene (Figure 1A). The bla_{KPC-2} gene is embedded within a complex genetic environment on the plasmid, which includes multiple insertion sequences (IS) and transposons. The bla_{KPC-2} gene is flanked by IS*26* and IS*Kpn27* elements, facilitating its mobility and potential horizontal transfer. The presence of multiple IS elements (IS*26*, IS*Kpn27*, IS*Kpn19*) and transposons (Tn*As1*) indicates a high potential for genetic rearrangements and the acquisition of additional resistance genes. Under the selection used, only bla_{KPC-2} was detected in transconjugants, indicating that $bla_{CTX-M-14}$ did not co-transfer.

Comparative genomics showed that pL4843-KPC2 shares a highly conserved backbone to three previously reported bla_{KPC-2} -bearing plasmids: pC110-KPC (CP047692.1) and p2838-KPC (CP047686.1), both from clinical *S. marcescens* isolates, and

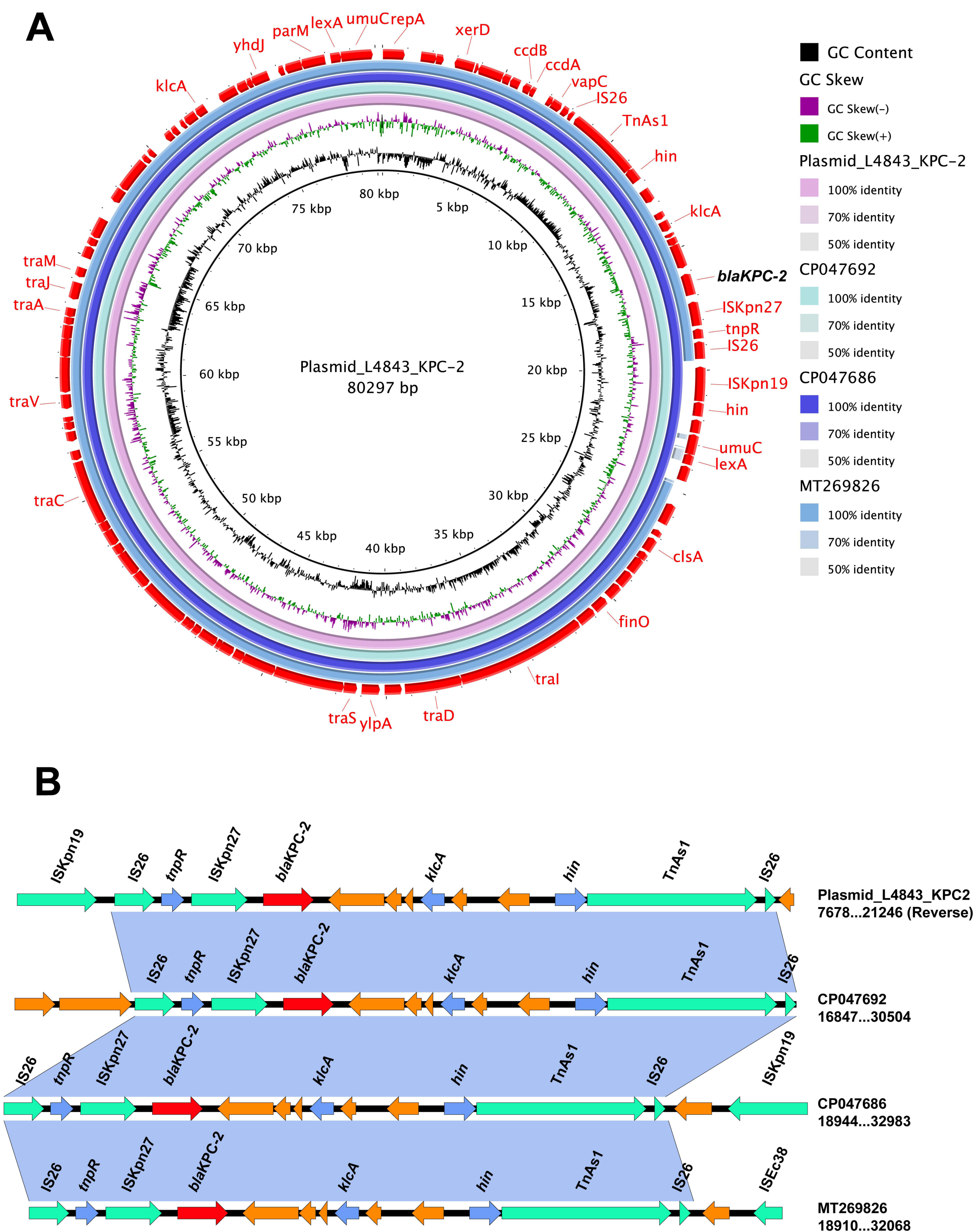

Figure 1 (**A**) Circular BLAST Ring Image Generator map of pL4843-KPC2 (innermost lavender ring) aligned against three reference plasmids: CP047692 (light blue ring), CP047686 (cornflower blue ring) and MT269826 (medium blue ring). Open reading frames (ORFs) are depicted as red arrows on each ring, with the bla_{KPC-2} gene labeled in black. Arrows around the circle correspond sequentially to the various genetic elements. (**B**) Linear comparative alignment generated with Easyfig, showing the pL4843-KPC2 backbone (top track) aligned beneath against CP047692, CP047686 and MT269826. Homologous regions are indicated by shaded arcs: regions with ≥ 99% identity in medium blue. ORFs are shown as colored arrows to reflect its predicted functional category. Mint green: mobile element–associated genes (eg, transposases and insertion sequences). Red: antibiotic resistance genes (eg, *bla*$_{KPC-2}$). Medium blue: conjugation, replication, and stability genes. Orange: hypothetical proteins.

pBSI030-KPC2 (MT269826), from a clinical *Klebsiella pneumoniae* strain. Pairwise alignments demonstrated 100% nucleotide identity across 79% of the pC110-KPC and p2838-KPC backbones, and 99.9% identity over 80% of pBSI030-KPC2 (Figure 1B), yet exhibits sequence divergence outside this conserved core, warranting pL4843-KPC2 as a novel structural variant. These findings underscore both the remarkable stability of the repB(R1701) scaffold and its ability to mediate interspecies dissemination of carbapenemase genes.

Comparative genomics demonstrated that pL4843-KPC2 shares a conserved IncR–IncFII-like backbone with three previously reported bla_{KPC-2}-bearing plasmids: pC110-KPC (CP047692.1) and p2838-KPC (CP047686.1), both originating from clinical *S. marcescens* isolates, and pBSI030-KPC2 (MT269826), derived from a *K. pneumoniae* clinical strain. Pairwise alignments showed 100% nucleotide identity over 79% of the pC110-KPC sequence and p2838-KPC sequence, whereas homology to pBSI030-KPC2 is 99.9% identity across 80% coverage (Figure 1B). This confirming its novel structural variant. These findings underscore both the stability of the repB(R1701) backbone and its capacity for interspecies dissemination of carbapenemase genes.

Beyond stool carriage, *S. marcescens* carrying bla_{KPC-2} and $bla_{CTX-M-14}$ has been reported from non-fecal clinical specimens in China and elsewhere, underscoring clinical relevance. However, as of September 2025, we found no published report of human fecal *S. marcescens* carrying bla_{KPC-2} in China, and existing Chinese CRE carriage studies do not specifically document such stool isolates. Within this context, L4843 adds evidence for a potential intestinal reservoir, which-together with the close backbone homology between pL4843-KPC2 and plasmids reported from *Klebsiella pneumoniae*-supports a risk of interspecies transfer and highlights the value of targeted stool-based surveillance.

Conclusion

In summary, this study provides a comprehensive genomic characterization of a KPC-2- and CTX-M-14-producing *S. marcescens* isolate from a fecal sample. We highlight the complexity and mobility of resistance mechanisms within this pathogen. The presence of bla_{KPC-2} on a transferrable plasmid underscores the role of mobile genetic elements in the dissemination of carbapenem resistance.[8] In conjunction with the apparent scarcity of published fecal *S. marcescens* carrying bla_{KPC-2} in China, our findings highlight the intestinal reservoir as a plausible waypoint for plasmid-mediated and potentially interspecies dissemination of β-lactam resistance, reinforcing the need for integrated stool-based surveillance and infection-control strategies.

Data Sharing Statement

The Whole Genome Shotgun project of *S. marcescens* L4843 has been deposited at DDBJ/ENA/GenBank under the BioProject accession PRJNA1132380 and BioSample accession SAMN42341451, respectively.

Ethical Approval

The isolate was recovered as part of routine clinical microbiology diagnostics. The Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine determined that this work does not constitute human-subject research and waived the requirement for informed consent (Approval No: IIT20230479B).

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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