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Research article

Detection of clinical *Serratia marcescens* isolates carrying bla_{KPC-2} in a hospital in China

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ABSTRACT

Serratia marcescens is an opportunistic and nosocomial pathogen found in the intensive care unit (ICU), but its antimicrobial resistance (AMR) is rarely addressed. Here, we reported two bla_{KPC-2}-positive *S. marcescens* strains, SMBC31 and SMBC50, recovered from the ICU of a hospital in Zhengzhou, China. The minimum inhibitory concentration (MIC) was determined using the broth microdilution method, while S1-PFGE was employed to demonstrate plasmid size approximation. Complete genome sequences were obtained through Illumina NovaSeq 6000 and Oxford Nanopore Technologies. Both strains exhibit resistance to meropenem and harbor the bla_{KPC-2} and bla_{SRT-1} resistance genes. The plasmid pSMBC31-39K in strain SMBC31 and pSMBC50-107K in strain SMBC50 were identified as carrying the bla_{KPC-2} gene. Notably, both of these plasmids were successfully transferred to *Escherichia coli* strain J53. Phylogenetic analysis based on plasmid sequences revealed that pSMBC31-39K exhibited high homology with plasmids found in *Aeromonas caviae*, *Citrobacter* sp., and *Pseudomonas aeruginosa*, while pSMBC50-107K showed significant similarity to those of *E. coli* and *Klebsiella pneumoniae*. Notably, the coexistence of bla_{KPC-2} and bla_{SRT-1} was observed in all 94 KPC-2-producing *S. marcescens* strains by mining all genomes available under the GenBank database, which were mainly isolated from hospitalized patients. The emergence of multidrug-resistant *S. marcescens* poses significant challenges in treating clinical infections, highlighting the need for increased surveillance of this pathogen.

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1. Introduction

Serratia marcescens, a member of the *Enterobacteriaceae*, is an opportunistic pathogen [1]. It can be isolated from various sources including water, soil, animals, insects, plants, and food [2]. *S. marcescens* is commonly found in hospital clinics worldwide and is associated with clinical infections causing a range of diseases, such as meningitis, sepsis, urinary tract infections, skin infections, blood infections and respiratory infections [2]. Moreover, this species exhibits inherent resistance to multiple classes of antibiotics including β-lactams, aminoglycosides, quinolones, macrolides, and polypeptide antimicrobials [1,3]. The treatment of *S. marcescens* infections poses significant challenges due to its intrinsic resistance as well as its ability to acquire additional carbapenem resistance [4,5]. Carbapenem-resistant *S. marcescens* has become increasingly prevalent primarily within hospital settings leading to frequent nosocomial infections [4].

The most prevalent carbapenem resistance genes include bla_{KPC}, bla_{NDM}, bla_{OXA-48}-like, and bla_{VIM} [6]. After the variant KPC-2 was first identified in *Klebsiella pneumoniae* from North Carolina [7,8], bla_{KPC-2}-harboring *Enterobacterales* rapidly spread throughout hospitals [9]. Subsequently, KPC-2 was detected in strains of *Escherichia coli*, *Salmonella enterica*, *Klebsiella oxytoca*, *Acinetobacter mirabilis, Pseudomonas aeruginosa*, *Citrobacter*, and *Enterobacter* [9–11]. In 2007, Zhang et al. initially reported plasmid-mediated KPC-2 in *S. marcescens* isolated from a hospital in Zhejiang, China [12]. Silva et al. also recovered *S. marcescens* producing both KPC-2 and IMP-10 at a Brazilian teaching hospital [13]. Moreover, it has been identified to be harbored on multiple plasmids: IncR, IncP6, IncQ1, IncFII, IncL/M, IncN1, IncA, IncK, IncC, ColRNAI, IncX3 and IncX6 [14–18]. Among these types of plasmids found in *S. marcescens* are specifically the ones belonging to the groups of both IncK and IncX6 [19,20].

In this study, we report bla_{KPC-2}, $bla_{CTX-M-14}$, and bla_{SRT-1}-positive *S. marcescens* based on Oxford Nanopore sequencing platforms. The characteristics of IncP6 and IncR plasmids harboring bla_{KPC-2} were first identified, and the conjugation efficacy of these plasmids was evaluated. Furthermore, additional phylogenetic analysis was performed on similar plasmids as well as all GenBank-recorded bla_{KPC-2}-positive *S. marcescens* strains.

2. Materials and methods

2.1. Bacterial characterization and antimicrobial susceptibility testing

In 2022, two meropenem-resistant strains were isolated from the sputum of patients in Zhengzhou Hospital. Species identification was performed using MALDI-TOF MS (bioMerieux, France) and 16S rDNA sequencing. Antimicrobial resistance (AMR) genes were identified by PCR sequencing and whole genome sequencing. The broth microdilution method recommended by the Clinical and Laboratory Standards Institute (CLSI) was used as a reference to determine the minimum inhibitory concentration (MIC) [21]. Sensitivity testing was performed for fifteen antibiotics: ampicillin (AMP), amoxicillin/clavulanic acid (A/C), gentamicin (GEM), spectinomycin (SPT), tetracycline (TET), florfenicol (FF), sulfisoxazole (SF), trimethoprim/sulfamethoxazole (SXT), ceftiofur (CEF), ceftazidime (CAZ), enrofloxacin (ENR), ofloxacin (OFL), meropenem (MEM), apramycin (AP), mequindox (MEQ).

To determine the bacterial viability of *S. marcescens*, serially diluted cultures were spotted on LB plates supplemented with different concentrations of meropenem (0, 0.25, 0.5, 1, 2, 4, 8,16, 32, 64, 128 μg/mL) and incubated at 37 °C for 12 h. E-tests (Liofilchem®, Italy) were used to verify the meropenem resistance of *S. marcescens* in this experiment. The *E. coli* strain ATCC 25922 was used as a negative control [21].

2.2. Conjugation assay

The bla_{KPC-2}-carrying clinical isolates served as donors, while *E. coli* J53 was utilized as the recipient strain. A 20 μL aliquot of the donor bacteria and a 50 μL aliquot of the overnight cultured recipient bacteria were inoculated onto a 0.22 μm filter membrane placed on an LB plate for overnight incubation at 37 °C. Subsequently, the filter membrane was carefully transferred into a centrifuge tube containing LB broth using sterile forceps. A diluted bacterial solution (10 μL) obtained from aspiration was then inoculated onto screening plates supplemented with NaN3 (100 μg/mL), meropenem (2 μg/mL), and NaN3 (100 μg/mL). Transconjugants were selectively grown in media containing meropenem (2 μg/mL) and sodium azide (100 μg/mL). Conjugation transfer was performed using the method described by Tang et al. [22], and transconjugants were identified by PCR.

2.3. S1-Pulsed-field gel electrophoresis and Southern blot (S1-PFGE)

S1-PFGE was performed to demonstrate the approximate size of plasmids as described in a previous study [23,24]. Genomic DNA from strains was cleaved and embedded in agarose gel blocks, which were subsequently digested with S1 endonuclease (TaKaRa, Dalian, China). The DNA fragments were separated using a CHEF-DR III system (Bio-Rad, Hercules, USA). The plasmid DNA was transferred onto a positively charged nylon membrane and hybridized with a digoxigenin-labeled probe targeting bla_{KPC-2}.

2.4. Whole genome sequencing and data analysis

To further investigate the genetic background of AMR genes in *S. marcescens*, bacterial genomes were extracted using a genomic DNA extraction kit (Generay, Shanghai, China). Short reads were obtained using the NovaSeq 6000 (2 × 150-bp paired-end reads) system (Illumina, USA). In order to remove adaptors and low-quality reads, short reads were optimized using Trimmomatic v0.36. For

long-read sequencing, the libraries were prepared using SQK-LSK109 kit (Oxford Nanopore Technologies [ONT], United Kingdom) and sequenced by a GridION sequencer (ONT). Guppy v3.2.4 was used for base calling of raw fast5 data and removal of adapter sequences. Hybrid de novo assembly of both short and long reads were performed based on the Unicycler v0.4.4 pipeline. ResFinder 4.1 (https://cge.food.dtu.dk/services/ResFinder/) was used to predict acquired AMR genes (90 % identity and 60 % coverage), VirulenceFinder (https://cge.food.dtu.dk/services/VirulenceFinder/) was used to predict virulence genes (90 % identity and 60 % coverage), and PlasmidFinder (https://cge.food.dtu.dk/services/PlasmidFinder/) was used to identify plasmid replicons (95 % identity and 60 % coverage). Similar full-length plasmid sequences with homology greater than 99 % and coverage greater than 85 % were used for analysis by BRIG 0.95.

2.5. Phylogenetic analysis

The plasmids exhibiting more than 85 % query coverage and 99 % identity were selected for kSNP phylogenetic analysis through Blastn. All genomes of *S. marcescens* strains harboring the bla_{KPC-2} gene from the GenBank database were extracted, and phylogenetic analysis was performed using kSNP3.1 with a maximum-likelihood method (k-mer size 19) [22,25]. The data retrieval was performed in October 2022.

3. Results

3.1. Antimicrobial susceptibility test for S. marcescens isolates

The strains SMBC31 and SMBC50, identified as *S. marcescens*, were isolated from the sputum of a patient in Zhengzhou Hospital. The presence of bla_{KPC-2}, $bla_{CTX-M-14}$, and bla_{SRT-1} genes were confirmed through PCR and whole-genome sequencing. These two strains exhibited high resistance to meropenem, ceftiofur, enrofloxacin, ofloxacin, florfenicol, tetracycline, amoxicillin-clavulanate acid, and ampicillin (Table 1). Agar dilution assay (Fig. 1A) revealed that the control strain ATCC 25922 could grow normally on plates without meropenem but failed to grow on plates with meropenem ($\geq$0.25 μg/mL). In contrast, strains SMBC31 and SMBC50 were able to grow under a concentration of 128 μg/mL meropenem. The meropenem resistance of two KPC-2-bearing *S. marcescens* isolates was further verified by E-test strips (0.016–256 μg/mL meropenem), and similar results were obtained (Fig. 1B).

3.2. Characterization of the bla_{KPC-2}-harbor S. marcescens genome

The chromosome of strain SMBC31 is 5,374,300 bp in length. Additionally, it harbors four plasmids pSMBC31-93K (93,837 bp, CP109825), pSMBC31-39K (39,600 bp, CP109826), pSMBC31-5K (5,699 bp, CP109827), pSMBC31-2K (2,883 bp, CP109828). The resistance genes *aac(6')-Ic*, bla_{KPC-2}, bla_{SST-1} and *tet*(41) were found to be associated with aminoglycosides, β-lactams and tetracyclines [10], respectively. Notably, the bla_{KPC-2} gene was identified within the plasmid pSMBC31-39K of IncP6-type. However, none of the other three plasmids harbored any resistance genes. The strain chromosome of strain SMBC50 is 5,450,725 bp in length. In addition, it harbors an IncR-type plasmid pSMBC50-107K (107,807 bp, CP109830), which carries the bla_{KPC-2} gene. Furthermore, the presence of *aac(3)-IId*, *aac(6')-Ic*, $bla_{CTX-M-14}$, bla_{SRT-1}, and *qnrS1* genes was confirmed. Importantly, the above complete plasmid sequences and bla_{KPC-2} genes were verified by S1-PFGE and Southern blot assays (Fig. 2A).

3.3. Characterization of pSMBC31-39K and pSMBC50-107K and conjugation assay

The present study selected 8 plasmids, which exhibited a coverage of over 85 % and a homology of over 99 % to pSMBC31-39K and

Table 1
MICs of strains SMBC31 and SMBC50.

ID	Antibiotics	SMBC31	SMBC50
		MIC (μg/mL)	MIC (μg/mL)
1	Ampicillin, AMP	>512	>512
2	Amoxicillin/clavulanic acid, A/C	>512/256	256/128
3	Gentamicin, GEM	1	32
4	Spectinomycin, SPT	64	64
5	Tetracycline, TET	128	16
6	Florfenicol, FF	128	64
7	Sulfisoxazole, SF	256	64
8	Trimethoprim/sulfamethoxazole, SXT	0.5/9.5	0.25/4.8
9	Ceftiofur, CEF	256	128
10	Ceftazidime, CAZ	64	2
11	Enrofloxacin, ENR	4	32
12	Ofloxacin, OFL	16	32
13	Meropenem, MEM	256	512
14	Apramycin, AP	8	4
15	Mequindox, MEQ	32	32

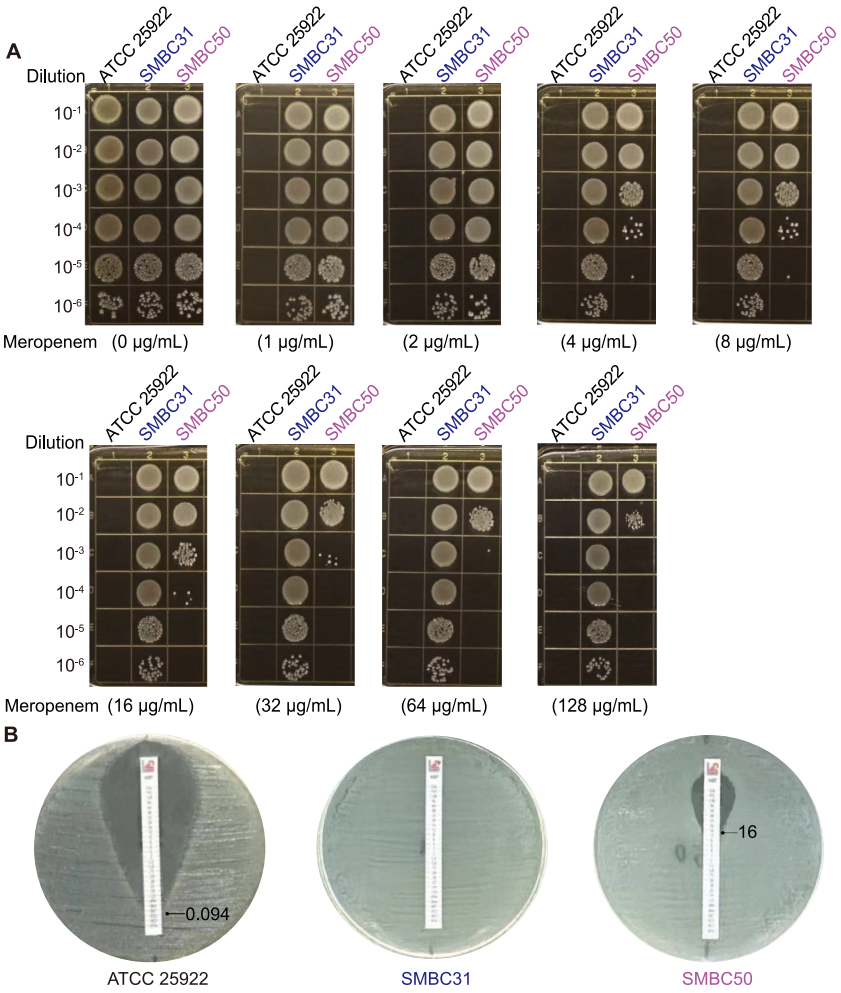

Fig. 1. Antimicrobial susceptibility results for strains SMBC31 and SMBC50. (A) Bacterial viability of *E. coli* strains on LB plates containing different meropenem concentrations. The diluted suspension (10 μL) was inoculated onto the prepared LB plates. (B) E-test assay of strains SMBC31 and SMBC50. *E. coli* ATCC 25922 was used as a negative control.

pSMBC50-107K, respectively, for comparative analysis. As shown in Fig. 3A, pSMBC31-39K and analogous plasmids exclusively carried one acquired AMR gene, namely bla_{KPC-2}. This gene displayed high similarity with *Aeromonas* sp. strain ASNIH3 pKPC-cd17 (NZ_CP026224), *Aeromonas hydrophila* pKPC2_045096 (CP028566), *K. pneumoniae* pA1705-KPC (NZ_MH909348), *K. pneumoniae* strain 11 plasmid P3 (OW970313), *Enterobacter cloacae* p30860-KPC (NZ_MN477223), and *Citrobacter* sp. pCRE12-KPC (MK050973) (Fig. 3A). The bla_{KPC-2} gene is located on pSMBC31-39K between the mobile elements IS*Kpn6* and IS*kpn27*. Additionally, the plasmid harbors BrnT/BrnA toxin-antitoxin system, methionine sulfoxide reductase A (MsrA) and B (MsrB). This plasmid encompasses 10 mobile elements encoding integrase or transposase within pSMBC31-39K.

As shown in Fig. 3B, the *S. marcescens* plasmid pSMBC50-107K exhibited a high degree of similarity to the plasmid pH17-2 (107,793 bp, CP021195) [26] with 95 % coverage and 99.98 % identity. Additionally, eight mobile elements were identified in the plasmid pSMBC50-107K. There are type IV secretion system genes in plasmid pSMBC50-107K, including *oriT* region, Relaxase, T4CP, T4SS gene cluster. This plasmid has eight mobile elements that encode integrase or transposase. A total of 7 genes (*merR-merT--merP-merC-merA-merD-merE*) involved in mercury resistance have been identified in this plasmid. The genetic context surrounding bla_{KPC-2} was conserved and found adjacent to the mobile elements IS*Kpn6* and IS*kpn27* (Fig. 3B). Notably, the genetic context of bla_{KPC-2} gene on the IncR and IncP6 plasmids investigated in this study is conserved, exhibiting a gene arrangement of "*repB*-IS*Kpn6*-bla_{KPC-2}-IS*kpn27*-*pinE*" (Fig. 4). The bla_{KPC-2} plasmids in both strains SMBC31 and SMBC50 were successfully transferred into *E. coli* strain J53 through conjugation, with transfer frequencies of $(1.32 \pm 0.25) \times 10^{-2}$ (Fig. 2B) and $(9.04 \pm 1.01) \times 10^{-4}$ (Fig. 2C), respectively.

3.4. Phylogenetic analysis of plasmids and isolates harboring the bla_{KPC-2} gene

The kSNP phylogenetic analysis of the pSMBC31-39K-like plasmid revealed high homology with pCRE12-KPC and other plasmids,

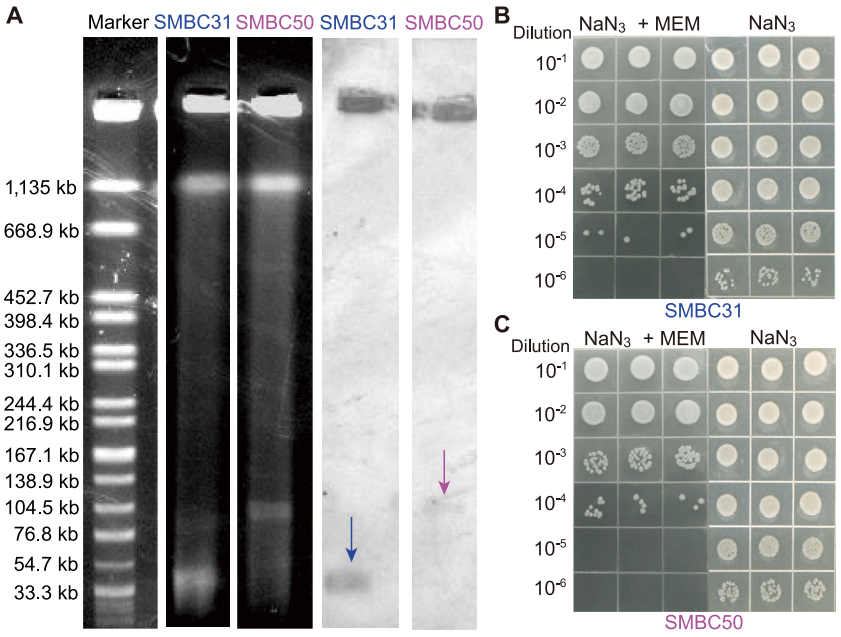

Fig. 2. (A) S1-PFGE and Southern blot for strains SMBC31 and SMBC50. The plasmids harboring blaKPC-2 gene are indicated with the blue and purple arrows on the fingerprints. (B) conjugation assay of SMBC31. (C) conjugation assay of SMBC50. Please refer to Fig. S1 in the supplementary materials for the uncropped version of S1-PFGE and Southern blot. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

indicating a close relationship (Fig. 5A). Notably, the plasmid pCRE12-KPC also originated in China. In contrast to the limited distribution of the pSMBC50-107K-like plasmid, the pSMBC31-39K-like plasmid has been reported in multiple countries including China, Spain, and Japan. Moreover, it was observed that IncP6-type plasmids can be found across various species, such as *K. pneumoniae*, *S. marcescens*, and *Citrobacter*. Plasmid pSMBC50-107K exhibited high similarity to pH17-2 and other related plasmids (Fig. 5B). Importantly, our findings indicate that most IncR plasmids were identified in China, with a predominant isolation from human samples. Additionally, the IncR plasmids carrying the bla_{KPC-2} gene were mainly found in *K. pneumoniae* strains.

At the time of writing, a total of 94 *S. marcescens* strains harboring the bla_{KPC-2} gene were identified in the GenBank database, including strains SMBC31 and SMBC50. The majority of bla_{KPC-2}-positive *S. marcescens* isolates originated from human sources, with China (43.8 %), the United States (25.5 %) and Brazil (21.3 %) being the predominant countries of origin. Notably, strain SMBC31 exhibited high sequence similarity to isolates from China and Italy, while strain SMBC50 showed high homology with multiple Chinese isolates. Interestingly, all bla_{KPC-2} genes co-existed with bla_{SRT-1} in all strains (Fig. 6).

4. Discussion

β-lactam resistance genes are prevalent in clinical isolates [27–29]. The major β-lactamase families include plasmid-mediated extended-spectrum β-lactamases (ESBLs), AmpC cephalosporinases, and carbapenemases that have become a global concern [30]. ESBL-producing and carbapenem-resistant *Enterobacteriaceae* (CRE) pose an urgent threat to public health as they can be transmitted between humans and companion animals [31]. In China, bla_{KPC} and bla_{NDM} are responsible for phenotypic resistance in most of the CRE strains [32]. NDM metallo-β-lactamases are more prevalent in the Indian subcontinent and Eastern Europe, while KPC serine carbapenemases are present more frequently in the Americas, Mediterranean countries, and China [30]. KPC-like carbapenemases have spread worldwide with over 40 variants identified [33], among which KPC-2 or KPC-3 enzymes are the most predominant ones [34,35].

The bla_{KPC-2} gene has been reported in various clinical isolates [9,36], predominantly in *K. pneumoniae* [27]. Furthermore, it has also been found in *Klebsiella michiganensis* [15], *P. aeruginosa* [37], *E. coli* and *Salmonella enterica* [9]. In 2013, a plasmid pCT-KPC334 carrying *fosA3*, bla_{KPC-2}, $bla_{CTX-M-65}$, bla_{SHV-12}, bla_{TEM-1} and *rmtB* genes was identified in *K. pneumoniae* from Guangzhou, China [38]. In 2017, the presence of the bla_{KPC-2} gene on a hybrid plasmid IncHI1B-IncFIB coexisting with the virulence gene *rmpA2* was discovered in *K. pneumoniae* [39]. Notably, an IncHI5 plasmid pK254-KPC_NDM co-carrying bla_{KPC-2} and bla_{NDM-1} in *K. michiganensis* was identified in 2017 [15]. Additionally, another instance of the bla_{KPC-2} gene located on an IncP6-type plasmid pPAEC79 carrying a Tn3-like element was found in *P. aeruginosa* [37]. According to a previous epidemiological report, the carbapenemases in *S. marcescens* species belong to the class A group, including chromosomal location SME type or KPC-2 [40,41]. In this study, we identified two *S. marcescens* isolates with the co-existence of the bla_{KPC-2}, $bla_{CTX-M-14}$ and bla_{SRT-1} genes and the co-existence of the bla_{KPC-2}, and bla_{SRT-1} genes, respectively. We found that all bla_{KPC-2}-positive *S. marcescens* harbored the bla_{SRT} gene in the GenBank database. These

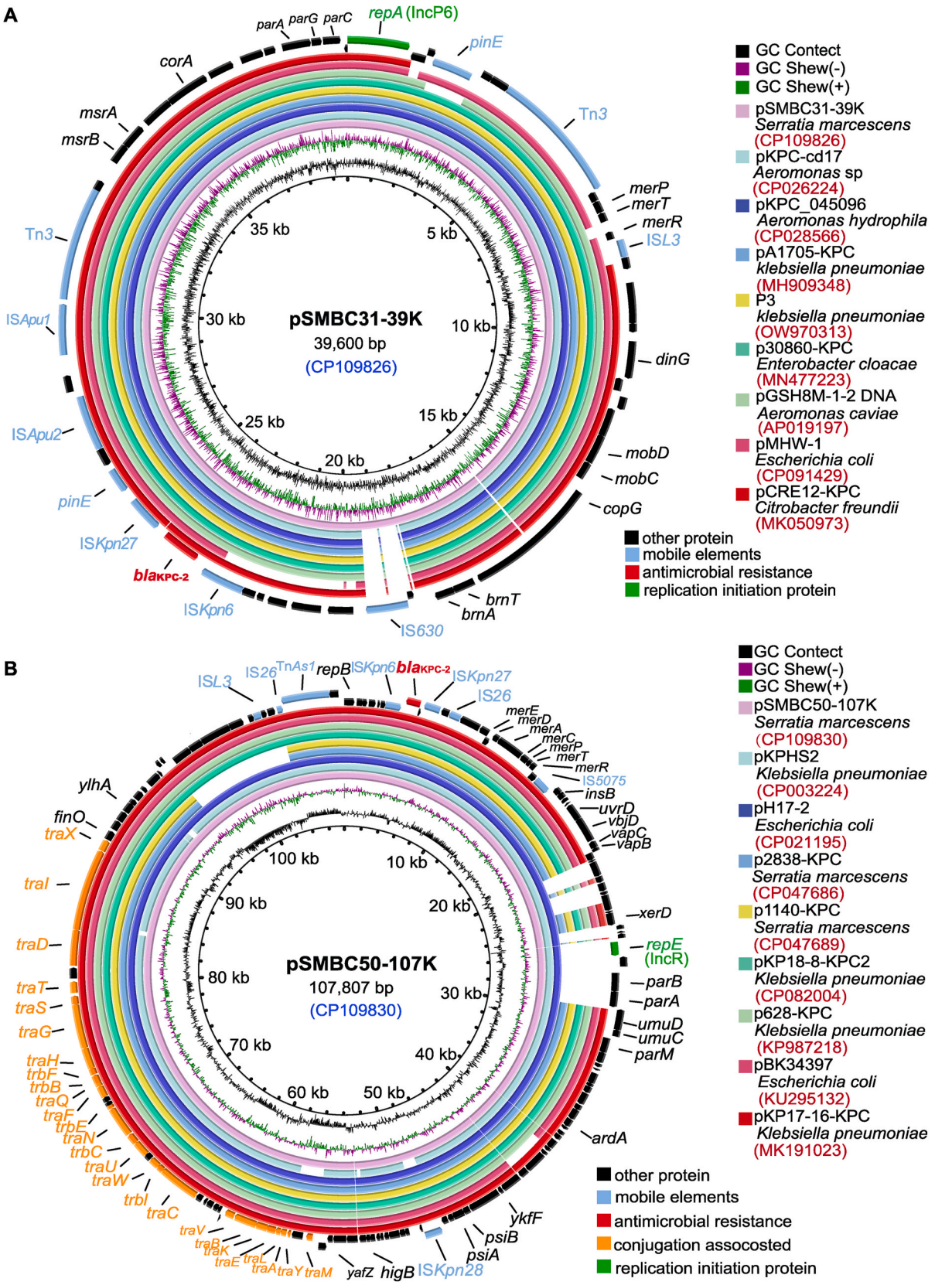

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Fig. 3. Circular diagrams of pSMBC31-39K (A) and pSMBC50-107K (B). From inside to outside, circles indicate the GC content, GC skew, and bla_{KPC-2}-harboring plasmids. Open arrows represent coding sequences in different colors. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

two *S. marcescens* strains exhibited multiple AMR genes similar to previous studies [42].

The bla_{KPC-2} gene in *S. marcescens* was initially reported in a conjugated plasmid in 2007, but no plasmid sequence was identified [12]. In this study, we identified two previously unreported plasmids, IncR and IncP6-type, carrying the bla_{KPC-2} gene. It is noteworthy that most KPC-producing *S. marcescens* strains were isolated from patients in surgical intensive care units, emphasizing the importance of obtaining KPC plasmids within hospital settings. Recently, carbapenem-resistant *S. marcescens* strains isolated in China have been reported to carry bla_{KPC-3} [43] and bla_{KPC-2}, associated with IncX8 [43] and IncX6 [20] plasmids, respectively. We identified bla_{KPC-2}-harboring IncR and IncP6 plasmids in these two *S. marcescens* strains. Importantly, both of these plasmids were capable of successful transfer to the recipient strain *E. coli* strain J53. The observed transfer frequencies of different plasmid types indicate their significant potential for inter-species transfer. Notably, the IncP6-type pSMBC50-107K harbored conjugation elements such as *tra* genes, whereas no conjugation elements were detected in the IncR-type pSMBC31-39K, suggesting alternative mechanisms may facilitate its transfer. Phylogenetic analysis revealed the conservation of pSMBC50-107K-like plasmids (~107 kb) across *K. pneumoniae*, *E. coli*, *S. marcescens*, and *C. koseri*, predominantly transmitted among humans. Further investigation is warranted to ascertain whether this plasmid plays a pivotal role in the dissemination of antimicrobial resistance (AMR) genes. In contrast, pSMBC31-39K-like plasmids displayed wide distribution across various species and hosts.

The study conducted by Matteoli et al. discovered the existence of 12 distinct lineages of *S. marcescens* isolates through a comprehensive genome analysis [44]. Importantly, they found that plasmids carrying bla_{KPC-2} were widely distributed within the Sm7 and Sm9 lineages. It is worth noting that the Sm9 lineage exhibited a unique pattern with an average of 3.26 plasmids per genome, while the Sm7 lineage typically had around one plasmid per genome. In this study, we observed a diverse range of replicon types in plasmids carrying KPC-2 in *S. marcescens*, indicating the absence of any specific preference for particular plasmid types within *S. marcescens* isolates. Strains SMBC31 and SMBC50 exhibited high homology with several *S. marcescens* strains isolated in human hospitals in China, indicating that *S. marcescens* could be disseminated through cloning and scattering across different regions of the country. Phylogenetic analysis revealed that the domestic *S. marcescens* isolates were closely related to the foreign isolates but formed distinct clusters, indicating diverse evolutionary pathways and transmission routes. Since bla_{KPC-2} is frequently transferred by plasmids and insertion elements, identifying the genetic structure surrounding bla_{KPC-2} can facilitate monitoring of horizontal transfer events involving bla_{KPC-2} as well as evolution processes of transplanted plasmids carrying this gene. In this study, the genetic context "*repB*-IS*Kpn6*-bla_{KPC-2}-IS*kpn27*-*pinE*" was present in pSMBC31-39K-like and pSMBC50-107K-like plasmids, implying a high rate of transmission for this region. Additionally, *S. marcescens* has been found to harbor *mcr* gene (*mcr-9*) [44,45], which poses a risk-associated resistance against colistin in *Enterobacteriaceae*. Although *tet*(X) tigecycline resistance gene has not yet been detected in this bacterium, vigilance should be maintained regarding its potential coexistence with multiple important AMR genes mentioned above.

This study reports the first identification of IncR and IncP6 plasmids harboring bla_{KPC-2} in *S. marcescens*, highlighting the transmission pattern and threat of KPC-2 among different species. Urgent action is needed to control its potential rapid spread.

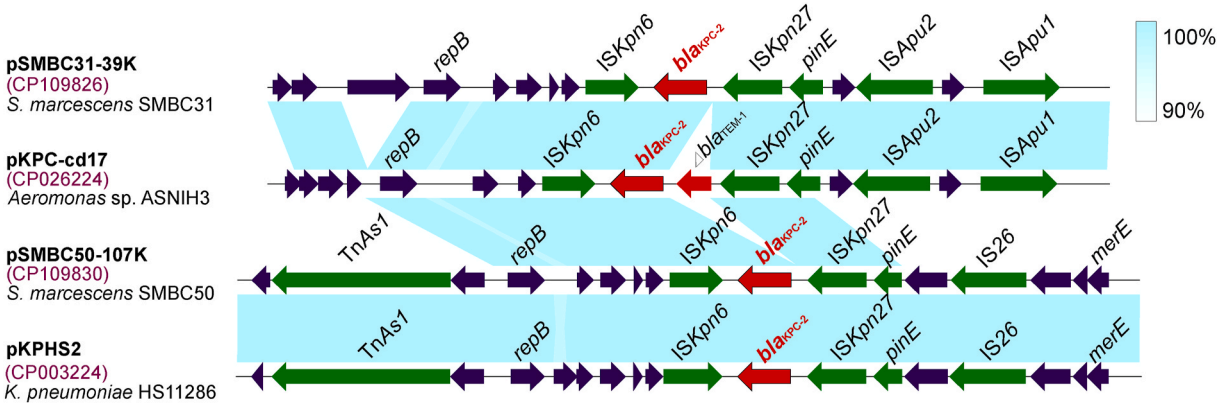

Fig. 4. Linear comparison of the genetic environment of bla_{KPC-2} gene. Open arrows indicate coding sequences (red arrows, AMR genes; green arrows, transfer-related sequences; modena arrows, unclassified) and indicate the direction of transcription. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

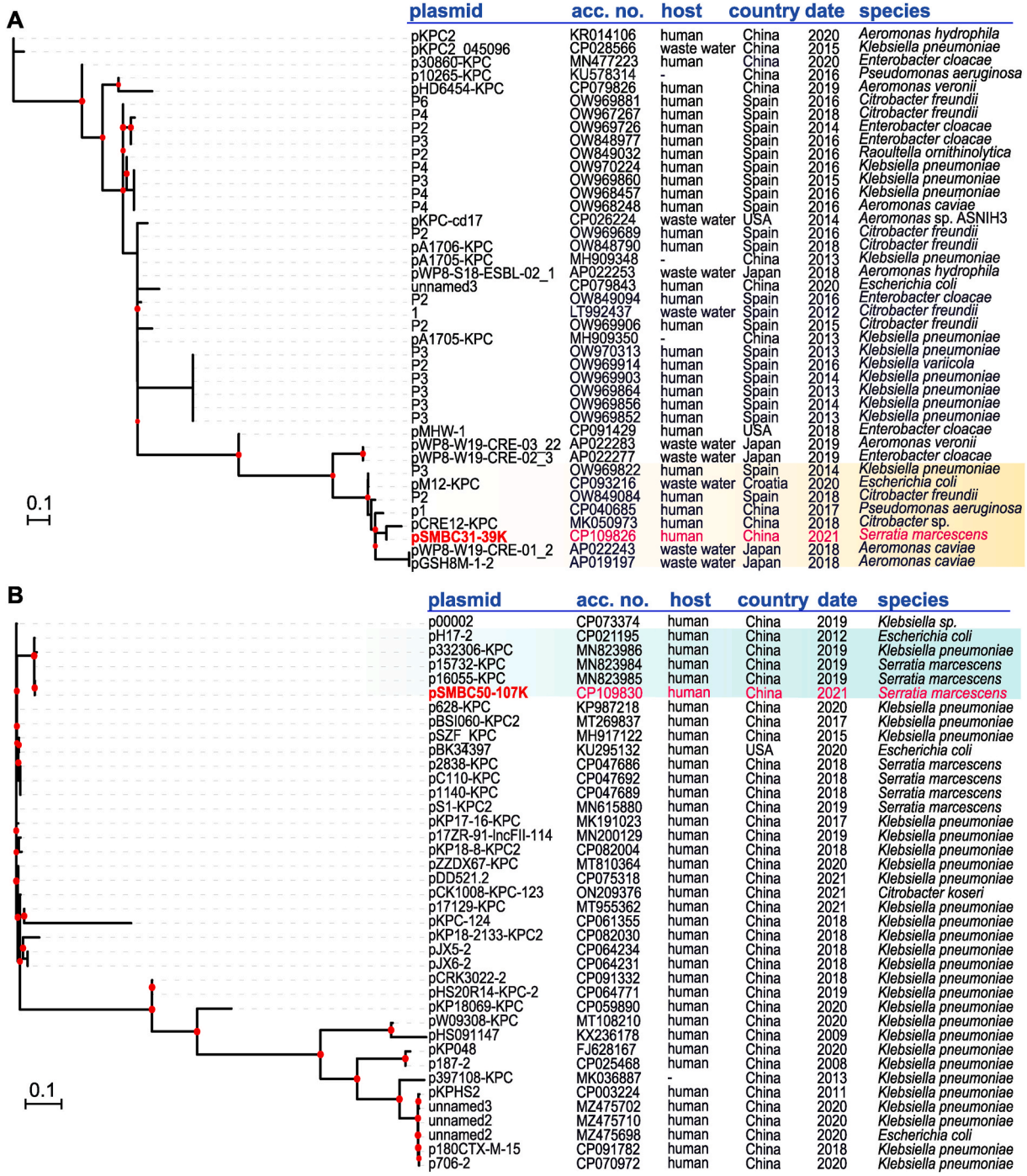

Fig. 5. Phylogenetic tree of *bla*$_{KPC-2}$-harboring plasmid sequences. (A) Phylogenetic tree of pSMBC31-39K (B) Phylogenetic tree of pSMBC50-107K. Similar plasmids (85 % query coverage and 99 % identity) in GenBank were used for SNP phylogenetic tree. Plasmid sequences were aligned for phylogenetic analysis based on the maximum-likelihood method using the bootstrap resampling method with 100 repeats. Bootstrap values above 50 % are shown as red points. Bar, 0.01 nucleotide substitutions per site. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

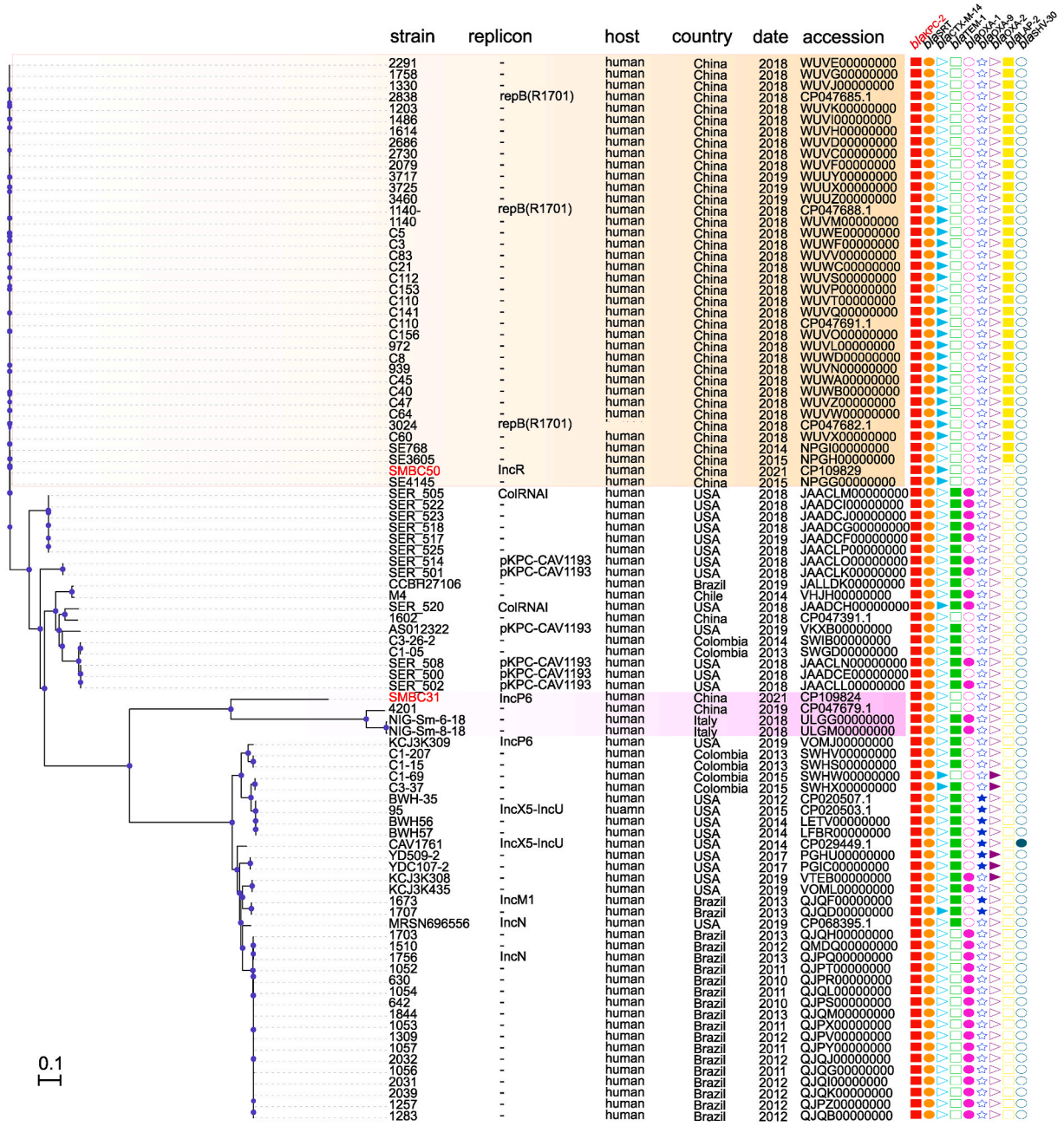

Fig. 6. Phylogenetic analysis of the genome sequences of *bla*$_{KPC-2}$-positive *S. marcescens*. Different colors in the branches indicate clusters corresponding to SMBC31 and SMBC50. Bootstrap values above 50 % are shown as blue points. The Bar scale indicates 0.01 nucleotide substitutions per site. (For interpretation of the references to color in this figure legend, the reader is referred to the Web version of this article.)

5. Conclusion

As an opportunistic pathogen, *S. marcescens* infection poses a significant public safety concern in hospitals due to its wide range of determinants AMR, which limit treatment options and success rates. In this study, we reported two carbapenem-resistant *S. marcescens* strains, SMBC31 and SMBC50, isolated from the ICU of a hospital in Zhengzhou, China. We identified *bla*$_{KPC-2}$-harboring IncR and IncP6 plasmids in these two *S. marcescens* strains. It is noteworthy that both of these plasmids were able to successfully transfer to the recipient strain *E. coli* strain J53. The genetic context IS*481*-*bla*$_{KPC-2}$-IS*1182* is located in pSMBC31-39K and pSMBC50-107K, indicating its potential for transferability. Our findings illustrate the transmission pattern of *bla*$_{KPC-2}$-positive *S. marcescens* and further highlight the threat of KPC-2 among different species, and urgent action should be taken to control the potential rapid spread.

Data availability statement

The whole-genome sequences of *S. marcescens* strains SMBC31 and SMBC50 have been deposited in the GenBank database under accession numbers CP109824-CP109828; CP109829-CP109830.

Ethics approval and consent to participate

Biological samples were collected for bacterial isolation following verbal consent from the patients, ensuring convenient sampling. All activities conducted in this study received approval from the Research Ethics Committee of Zhengzhou People's Hospital (KY2022-013-01).

CRediT authorship contribution statement

Biao Tang: Writing – original draft, Methodology, Funding acquisition. **Haoyu Zhao:** Writing – original draft, Visualization, Methodology, Investigation. **Jie Li:** Writing – original draft, Visualization, Methodology, Investigation. **Na Liu:** Visualization, Resources, Funding acquisition. **Yuting Huang:** Methodology, Investigation. **Juan Wang:** Visualization, Investigation, Conceptualization. **Min Yue:** Writing – review & editing, Supervision, Funding acquisition, Conceptualization.

Declaration of competing interest

The authors declare that they have no competing interests.

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Appendix A. Supplementary data

Supplementary data to this article can be found online at https://doi.org/10.1016/j.heliyon.2024.e29702.

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