Infection and Drug Resistance

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ORIGINAL RESEARCH

Molecular Characterization of a KPC-2- and NDM-1-Producing *Klebsiella michiganensis* Clinical Isolate in Cerebrospinal Fluid

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Objective: *Klebsiella michiganensis* is an emerging pathogen. In this context, we characterised a strain fxq isolated from a cerebrospinal fluid specimen of a patient with tentorial meningioma, and the *K. michiganensis* isolate produced carbapenemases of KPC and NDM types.

Methods: The Phoenix 100 Automated Microbiology System, MALDI-TOF and whole-genome sequencing were used to identify the species. Anti-microbial susceptibility testing was also conducted with the Phoenix 100. The plasmid locations of the bla_{KPC-2} and bla_{NDM-1} genes were determined by S1-nuclease pulsed-field gel electrophoresis and Southern blot. The transfer capacity of plasmids carrying bla_{KPC-2} and bla_{NDM-1} was investigated by conjugation experiments, and the resistance plasmid stability was evaluated by culture and subculture. *K. michiganensis* subtypes were identified by multi-locus sequence typing. We performed whole-genome sequencing to confirm species, characterise plasmids and analyse core genes.

Results: fxq was originally identified as *Klebsiella oxytoca* and showed resistance to imipenem and meropenem, but whole-genome sequencing identified it to be *K. michiganensis*. The strain fxq belonged to the novel sequence type 202 (ST202) and carried the bla_{KPC-2} and bla_{NDM-1} genes located on the pB_KPC InFIA and pE_NDM IncU plasmids, respectively. The bla_{KPC-2}-carrying plasmid was successfully transferred to *Escherichia coli* EC600 by conjugation, whereas the bla_{NDM-1} gene on the pE_NDM plasmid was not. The pB_KPC and pE_NDM plasmids demonstrated high stability.

Conclusion: This work is the first report on a carbapenem-resistant clinical isolate *K. michiganensis* ST202 harbouring the bla_{KPC-2} and bla_{NDM-1} genes encoded by the IncFIA and IncU plasmids, respectively.

Keywords: *Klebsiella michiganensis*, IncU plasmid, carbapenemase gene, NDM, KPC

Introduction

Klebsiella michiganensis was initially discovered in a toothbrush container at a residence in Michigan, USA, and was initially classified as *Klebsiella oxytoca*, with which it shares a close relationship (99% similarity in the nucleotide sequence of the 16S rRNA gene sequence).[1] *K. michiganensis* has also been identified in medical environments, causing diarrhoea in a recipient of a hematopoietic stem cell transplant and producing *K. pneumoniae* carbapenemase (KPC)-2, NDM-1 and NDM-5 carbapenemases in a fluid sample from an abdominal fistula.[2,3] In addition, a patient with cancer in South Africa was found to have OXA-181 and NDM-1-producing *K. michiganensis* in a stool sample.[4] Another case of bloodstream infection in Switzerland was caused by KPC-3-producing *K. michiganensis*.[5] Furthermore, a *K. michiganensis* isolate carrying bla_{VIM-1} was identified in Switzerland from a rectal swab of a Turkish patient.[6] Moreover, a rectal swab sample from Zhejiang province of China revealed a *K. michiganensis* isolate co-producing KPC-2, NDM-1 and IMP.[7] Despite numerous studies focusing on resistance genes, research on the virulence factors of *K. michiganensis* remains scarce. As mentioned above, *K. michiganensis* containing various kinds of carbapenemase genes has been sporadically reported in recent years, which could be a reservoir for the spread of these important resistance genes to other pathogens.

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Here, *K. michiganensis* fxq carrying two carbapenemase genes, bla_{KPC-2} and bla_{NDM-1}, which are located on the IncFIA- and IncU-type plasmids, respectively, is reported. The harbouring plasmids were further characterised. This work is the first report on *K. michiganensis* strains harbouring the bla_{NDM-1} gene on IncU plasmids.

Materials and Methods

Clinical Case and Bacterial Isolates

The strain fxq was acquired from a female patient, aged 58, who was admitted to the hospital because of the recurrence of tentorial meningioma. The patient was admitted to Henan Cancer Hospital in July 2020, which is a 2991-bed special hospital located in Henan, China. The carbapenem-resistant *K. michiganensis* fxq was isolated from a cerebrospinal fluid specimen over the next 3 days after excision of recurrent tumours from the cerebellar tentorium under a high-power microscope. According to the Helsinki Declaration, ethical approval for the study was granted by the Bioethics Committee of Affiliated Cancer Hospital of Zhengzhou University & Henan Cancer Hospital, China. The purpose of the study was explained to the study participants, and informed consent was obtained.

The identification of fxq strain was routinely conducted using a matrix-assisted laser desorption/ionisation time-of-flight (MALDI-TOF) mass spectrometry (Bruker Daltonik, USA) and Phoenix 100 Automated Microbiology System (Becton–Dickinson, USA). The species was confirmed by whole-genome sequencing (WGS) and Type (Strain) Genome Server (https://tygs.dsmz.de/). This server utilised a genome-based phylogeny approach combined with digital DNA:DNA hybridisation and confidence interval support values to accurately infer species identification.

The string test to determine the hypermucoviscosity phenotype was performed by touching a colony grown overnight on a blood agar plate at 37°C with a loop and pulling up. Strains exhibiting a mucoid string with length of 5 mm or longer were defined as hypermucoviscous.

Antibiotic Susceptibility Testing and Resistance Gene

The Phoenix 100 Automated Microbiology System was utilised to measure the fxq strain's minimal inhibitory concentrations, and the results were interpreted based on the criteria set by the Clinical Laboratory Standards Institute (CLSI, 2020), with the exception of polymyxin, which was interpreted according to the criteria established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2020). In this study, susceptibility tests were conducted including 19 antibiotics from 11 different classes of antimicrobials. They were ampicillin, piperacillin/tazobactam, ampicillin/sulbactam and amoxicillin/clavulanate, amikacin and gentamicin, aztreonam, chloramphenicol, cefepime, ceftazidime, cefazolin and cefotaxime, meropenem and imipenem, levofloxacin and ciprofloxacin, trimethoprim–sulfamethoxazole, tetracycline and polymyxin.

The fxq resistance gene was identified using NG-Test® CARBA 5 (Zhongshengzhongjie, Changsha, China).

S1-PFGE and Southern Blot Hybridisation

Southern blot experiments were conducted to determine the plasmid position of the bla_{KPC-2} and bla_{NDM-1} genes. The entire chromosomal DNA underwent digestion using S1-nuclease (TaKaRa, Japan). The fragmented samples were subjected to electrophoresis by using a CHEF-mapper XA pulsed-field gel electrophoresis system (Bio-Rad, United States) for a duration of 18 h at 14°C. The DNA fragments were moved to a nylon membrane with a positive charge (Millipore, USA) and combined with a probe specific to bla_{KPC-2} and bla_{NDM-1}, which was labelled with digoxigenin. An NBT/BCIP colour detection kit (Roche, Germany) was utilised to identify the fragments. *Salmonella enterica* strain Braenderup H9812 served as the reference for size determination.

Bacterial Genotyping

We utilised online MLST tools to conduct multi-locus sequence typing (MLST) analysis. The MLST tool v.2.0 provided by the CGE website (https://cge.cbs.dtu.dk/services/) was utilised for genotypically identified *K. michiganensis* isolates. The MLST scheme included alleles from housekeeping genes. The selected MLST configuration for *K. michiganensis* isolates was identified as "*K. oxytoca*".[8]

Conjugation Experiment and Plasmid Stability

Filter mating experiments were performed to determine the transferability of the bla_{KPC-2} and bla_{NDM-1} plasmids. The fxq strain acted as the donor, whereas rifampin-resistant *E. coli* EC600 was utilised as the recipient. The transconjugants were chosen on Mueller–Hinton (MH) agar with the addition of meropenem (2 μg/mL) and sodium rifampin (200 μg/mL) and identified by PCR to detect resistance genes, whilst antimicrobial susceptibility test of the transconjugants were detected by the Phoenix 100 Automated Microbiology System.

The stability of the resistance plasmids was evaluated. In brief, the fxq strain was individually streaked on MH agar and then incubated at 37°C for 24 h before being transferred to a new MH agar plate. Following 12 consecutive rounds of subculture, a random selection of 10 individual colonies was made for genome extraction. Thereafter, multiplex PCR analysis was conducted to detect the presence of the bla_{KPC-2} and bla_{NDM-1} genes.[9]

WGS and Plasmid Analysis

The genomic DNA of the specimen was obtained by utilising a QIAamp DNA Mini Kit (Qiagen, USA). The WGS procedure utilised the Illumina NovaSeq 6000 system (Illumina Inc., San Diego, CA, USA), along with the long-read MinION sequencer (Nanopore, Oxford, the UK). Unicycler was used to perform the de novo hybrid assembly.

Antimicrobial resistance genes, virulence genes, insertion sequence elements and plasmid replicon types were analyzed by using the Center for Genomic Epidemiology service and several databases, including RseFinder, VFDB, CARD, PlasmidFinder and ISFinder. Putative virulence factors were predicted by VFDB with the BLAST identity value >0.65 and coverage >0.85. We employed oriTfinder in the bla_{KPC-2}- and bla_{NDM-1}-carrying plasmids to rapidly identify the sources of transfer (oriTs) and three additional transfer-related components, namely, type IV secretion system (T4SS), relaxase and type IV coupling proteins (T4CP). The BRIG and Easyfig tools were used to compare the plasmids carrying bla_{KPC-2} and bla_{NDM-1} with other similar plasmids.

Result

Isolate Characteristics and Susceptibility Testing

Firstly, fxq was recognised as *K. oxytoca* and exhibited decreased susceptibility to imipenem and meropenem. Nonetheless, WGS analysis revealed that the strain was indeed *K. michiganensis*. The result of string test is negative, indicating that the strain does not belong to hypermucoviscous. As shown in Table 1, *K. michiganensis* fxq was resistant to ampicillin, ciprofloxacin, levofloxacin, trimethoprim–sulfamethoxazole, gentamicin, cefazolin, cefotaxime, cefepime and ceftazidime, piperacillin–tazobactam, amoxicillin/clavulanate, ampicillin/sulbactam and carbapenems. The isolate was susceptible to aztreonam, polymyxin, amikacin, tetracycline and chloramphenicol. NG-Test® CARBA 5 result indicated that fxq harboured bla_{KPC-2} and bla_{NDM-1} genes.

Bacterial Genotyping and S1-PFGE with Southern Blot Hybridisation

The fxq isolate underwent MLST analysis. The findings showed that the *K. michiganensis* strain was classified as sequence type 202 (ST202), a previously unreported occurrence in carbapenem-resistant *K. michiganensis*. Confirmation of the bla_{KPC-2} and bla_{NDM-1} genes on the plasmid was achieved through S1-PFGE and Southern blot techniques.

Conjugation Experiments and Plasmid Stability

The transferability of the plasmid was further determined by filter mating experiments. The tested fxq isolate was able to effectively transmit its carbapenem resistance to the EC600 strain of *E. coli*. The presence of the bla_{KPC-2} and bla_{NDM-1} genes in *E. coli* EC600 transconjugants was screened using multiplex PCR. Conjugation effectively transferred the bla_{KPC-2} gene to *E. coli* EC600, but the presence of the bla_{NDM-1} gene was not observed in the transconjugants of *E. coli* EC600. Plasmid stability experiments revealed that the pE_NDM and pB_KPC plasmids were stable in the fxq isolate. Given the lack of antibiotics, the strains that were chosen at random all contained the plasmids bla_{KPC-2} and bla_{NDM-1}, which were the same as the original isolate after 12 cycles of subculture on MH agar.

Table 1 Antimicrobial Susceptibility Testing

	MIC (μg/mL)/ antimicrobial susceptibility	MIC breakpoint (μg/mL)[a]		
		S	I	R
Amikacin	<=8/S	≤16	32	≥64
Aztreonam	<=2/S	≤4	8	≥16
Chloramphenicol	<=4/S	≤8	16	≥32
Tetracycline	4/S	≤4	8	≥16
Levofloxacin	8/R	≤0.5	1	≥2
Ciprofloxacin	>2/R	≤0.25	0.5	1
Trimethoprim-sulfamethoxazole	>2/38	≤2/38	-	≥4/76
Polymyxin	<=0.5/S	<=2[b]	-	>2[b]
Gentamicin	>8/R	≤4	8	≥16
Piperacillin-tazobactam	>64/4/R	≤16/4	32/4-64/4	≥128/4
Ampicillin	>16/R	<=8	16	≥32
Amoxicillin-clavulanate	>16/8/R	<=8/4	16/8	≥32/16
Ampicillin-sulbactam	>16/8/R	<=8/4	16/8	≥32/16
Cefazolin	>16/R	<=2	4	≥8
Cefotaxime	>32/R	<=1	2	≥4
Ceftazidime	>16/R	<=4	8	≥16
Cefepime	>16/R	<=2	-	≥16
Imipenem	>8/R	≤1	2	≥4
Meropenem	>8/R	≤1	2	≥4

Notes: [a]CLSI guideline for MIC breakpoints of Enterobacteriaceae except polymyxin. [b]EUCAST guideline for MIC breakpoints of polymyxin.
Abbreviations: S, Susceptible; I, Intermediate; R, Resistant; -, Missing breakpoints.

WGS and Molecular Characterisation

The fxq strain was composed of a chromosome (6,154,540 bp, GC content of 55.89%) and carried five plasmids: fxq plasmid pE_NDM (255,357 bp, GC content of 46.57%), fxq plasmid pA (202,694 bp, GC content of 45.01%), fxq plasmid pB_KPC (140,266 bp, GC content of 54.39%), fxq plasmid pC (117,403 bp, GC content of 53.08%) and fxq plasmid pD (107,094 bp, GC content of 52.64%). The GenBank accession numbers for the nucleotide sequences of the strain were as follows: fxq chromosome (CP094999), pB_KPC (CP095001), pE_NDM (CP095004), pA (CP095000), pC (CP095002) and pD (CP095003). WGS revealed that fxq genome codes for 12 putative virulence factors, and Table 2 for details. However, the presence of *magA, allS, rmpA, mrkD, kfuBC, cf29a, fimH, uge, wabG* and *ureA* genes reported genetic factors implicated in *K. pneumoniae* virulence were not identified in this strain. According to the string test result, the fxq strain exhibited low virulence.

Table 2 Whole Genome Information for Fxq K. Michiganensis Strain

Replicon	Nucleotide Length (bp)	GC%	Antibiotic Resistance Genes	Virulence Genes	GenBank ID
Chromosome	6,154,540	55.89	ND	*RcsAB, AcrAB, ybt, ent, ecp, irp, mgtBC, iuc, luxS, ilpA, sodB, chu*	CP094999
pE_NDM	255,357	46.57	*blaNDM-1, blaDHA-1, qnrB4, qnrS1, ARR-3, dfrA27, sul1, sul2, aac(3)-IId, aadA16, aac(6')-Ib7*	ND	CP095004
pA	202,694	45.01	*qnrS1*	ND	CP095000
pB_KPC	140,266	54.39	bla_{KPC-2}	ND	CP095001
pC	117,403	53.08	ND	ND	CP095002
pD	107,094	52.64	ND	ND	CP095003

Abbreviation: ND, not detected.

The pE_NDM plasmid was a 255,357 bp circular plasmid that was classified as an IncU group structure. Through the oriTfinder server, we found that fxq pE_NDM lacked the oriT region, suggesting that the plasmid was nonconjugative.

Eleven genes were associated with antimicrobial resistance. They were the AmpC β-lactamase gene bla_{DHA-1}; the carbapenemase-encoding gene bla_{NDM-1}; the *qnrB4* and *qnrS1* genes for quinolone resistance; the *ARR-3* gene for rifampicin resistance; the *dfrA27* gene for trimethoprim resistance; the sulphonamide resistance genes *sul1* and *sul*2; and the *aac(3)-IId, aac(6')-Ib7* and *aadA16* genes for aminoglycoside resistance, which was consistent with the phenotype of resistance. Table 2 presents the specifics of drug resistance genes.

A BLAST search of the pE_NDM plasmid sequence against the GenBank database revealed two similar previously published plasmids: plasmid pKOX-R1 (accession no. CP003684) from the *K. oxytoca* strain E718 with 89% coverage and

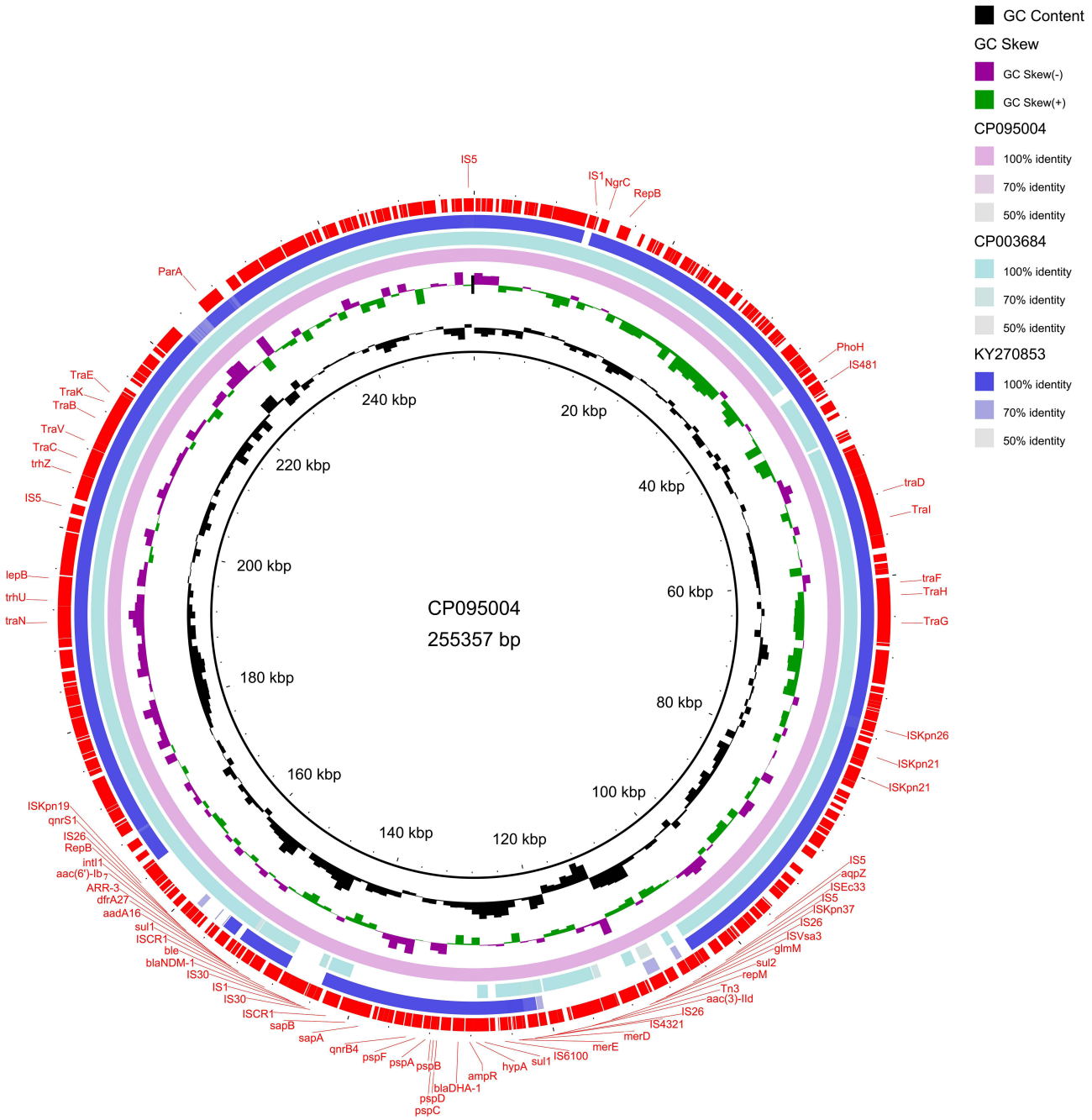

Figure 1 Genomic characterization of pE_NDM in fxq isolate. Sequence alignment of the pE_NDM plasmid in the NCBI GenBank database revealed several identical plasmids from different Gram-negative bacillus strains.

99.42% identity and pYNKP001-dfrA (accession no. KY270853) from the *Raoultella ornithinolytica* strain YNKP001 with 88% coverage and 99.97% identity. In the two plasmids, only pKOX-R1 had the bla_{NDM-1} resistance gene (Figure 1).

The structural characteristics of pE_NDM in comparison with those of pKOX-R1 and pYNKP001-dfrA are presented in Figures 1 and 2. Although pE_NDM had high identity with plasmids pKOX-R1 and pYNKP001-dfrA, the resistance regions differed. The IncU pE_NDM reserves various resistance genes, such as bla_{NDM-1}, *qnrS1, sul1, aadA16, dfrA27, arr-3 and acc(6')-Ib7*, which are located on a complex class 1 integron ISCR1-*sul1-aadA16-dfrA27-arr-3-acc(6')-Ib7-intl1*. Figure 3 shows the regional differences, the sequences ranged from 101,616 bp to 163,283 bp, from 107,547 bp to 195,707 bp and from 182,482 bp to 33,304 bp in lengths of pE_NDM, pKOX-R1 and pYNKP001-dfrA, respectively. For pE_NDM, the maximal conserved region included the downstream sequence, starting from a bleomycin resistance gene (*bleMBL*) and extending to a resistance gene (*qnrS1*), and this sequence comprises several drug resistance genes, including *sul1, aadA16, dfrA17, arr-3, acc(6')-Ib7* and *qnrS1*. On both flanks of the bla_{NDM-1} gene was the mobile genetic element ISCR1. The stability of this region containing bla_{NDM-1} (ISCR1-dsbc-trpF-ble-bla_{NDM-1}) was compromised because of the duplication of *sul1* in its vicinity.

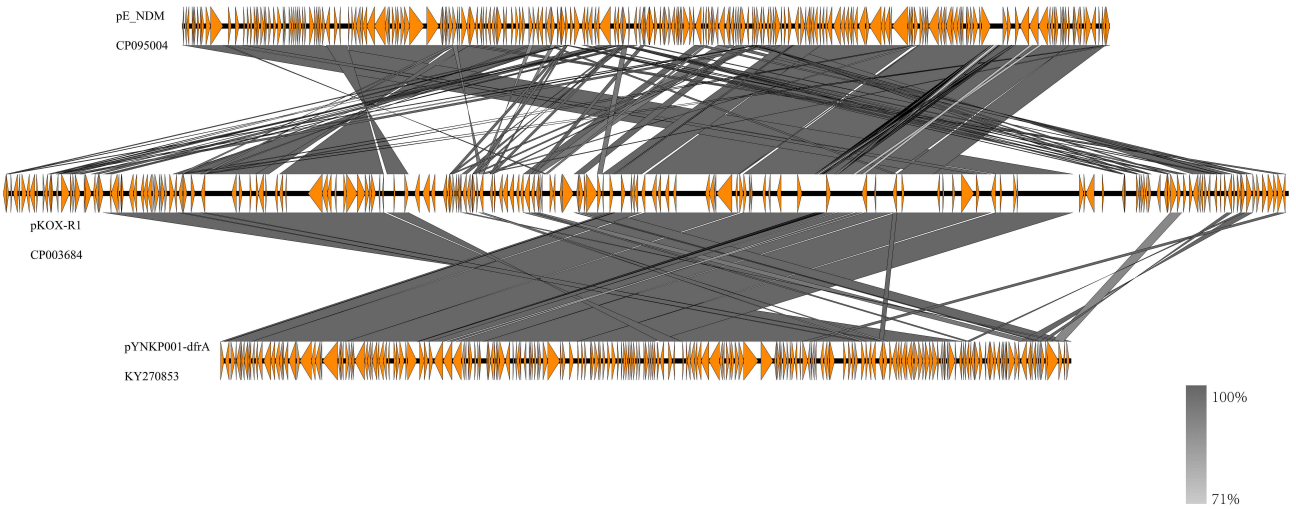

Figure 2 Structural comparison of the related plasmid with pE_NDM. Comparative analysis of the homologous regions shared by three plasmids. pE_NDM, pKOX-R1 and pYNKP001-dfrA isolated from *K. michiganensis* strain fxq, *K. oxytoca* strain E718 and *Raoultella ornithinolytica* strain YNKP001, respectively. The figure was produced using EasyFig v. 2.2.3, and BLASTN was used to compare sequence homology with the following threshold parameters: minimum length of 100 bp accompanied by 90% identity.

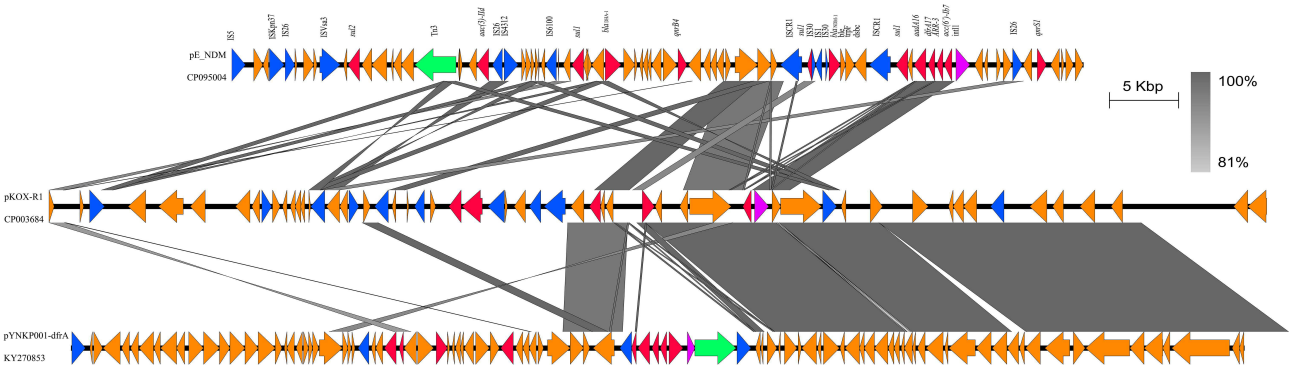

Figure 3 Genetic environments comparison of bla_{NDM-1} gene. Major structural features and comparison of the carbapenemase-producing plasmid with closely related plasmids. Schematic illustration showing the structural features of pE_NDM and the closely related plasmids pKOX-R1 and pYNKP001-dfrA. Grey shading indicates shared regions with a high degree of homology. ORFs are indicated as arrows and coloured according to their putative functions. Antimicrobial resistance genes, IS elements and Tn elements are indicated in red, blue and green respectively, and all other genes are indicated in Orange in the figure.

pB_KPC was a 140,266 bp circular plasmid, and Inc classification showed that the plasmid was grouped into IncFIA replicon types. The results from oriTfinder showed that pB_KPC possessed a full complement of T4SS, relaxase, T4CP and oriTs, suggesting that the plasmid exhibited robust self-transfer capability. This finding was aligned with the results of the conjugation experiments. A BLAST search of the pB_KPC plasmid sequence against the GenBank database revealed three similar previously published plasmids: plasmid pK516_KPC (accession no. CP022349) from the *K. michiganensis* strain K516 with 85% coverage and 99.99% identity, pKP19-3088-159k (accession no. CP063148) from the *K. pneumoniae* strain KP19-3088 with 84% coverage and 100% identity and pBKPC18-1 (accession no. CP022275) from the *Citrobacter freundii* strain C18-1 with 97% coverage and 99.98% identity (Figure 4). All the plasmids were isolated from China. Plasmid comparison indicated a closely related plasmid pBKPC18-1 (accession no. CP022275), which was detected in a *C. freundii* strain from the river sediment of Zhejiang province, China.

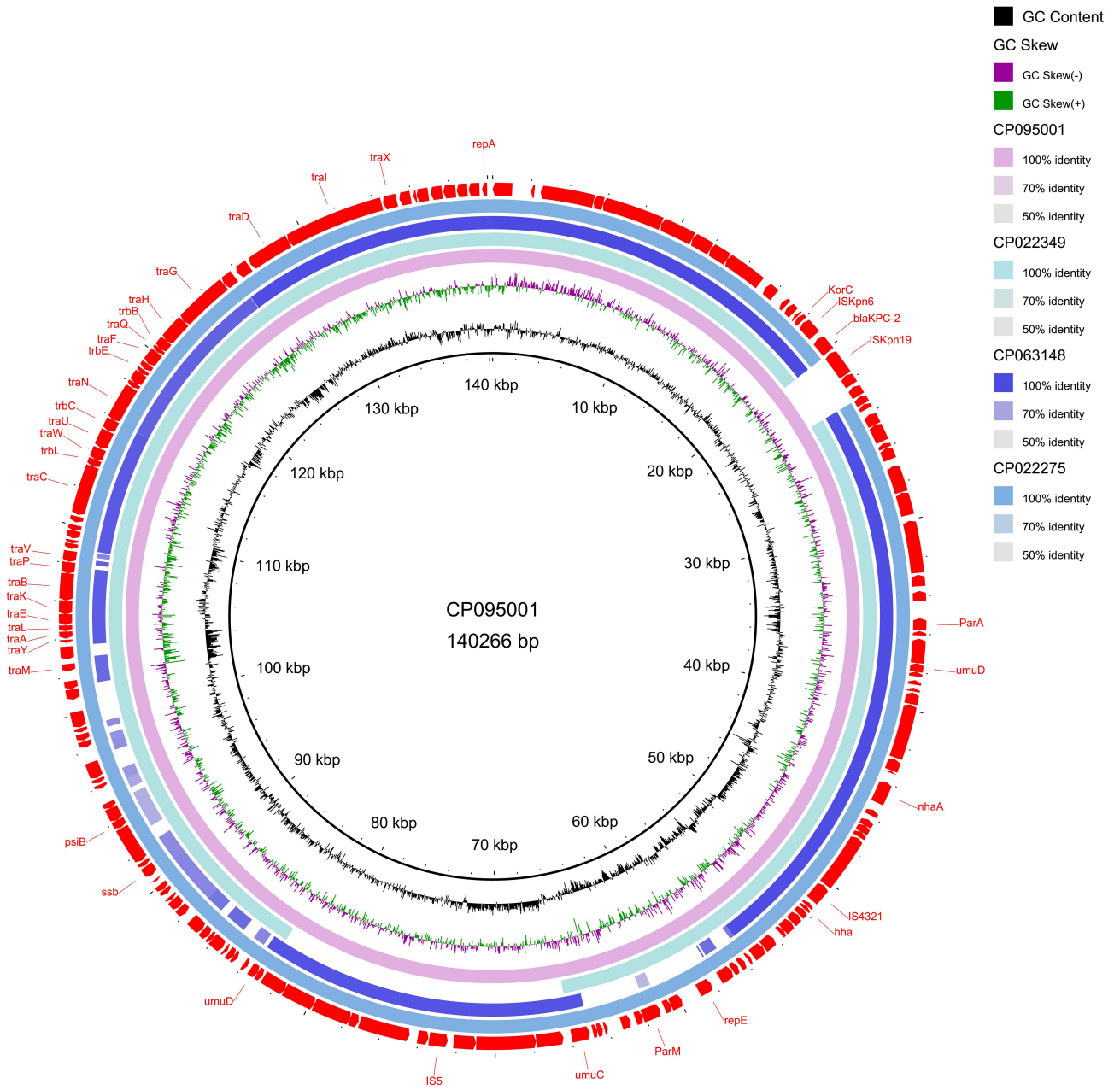

Figure 4 Genomic characterization of pB_KPC in fxq isolate. Sequence alignment of the pB_KPC plasmid in the NCBI GenBank database revealed several highly identical plasmids from different Gram-negative bacillus strains.

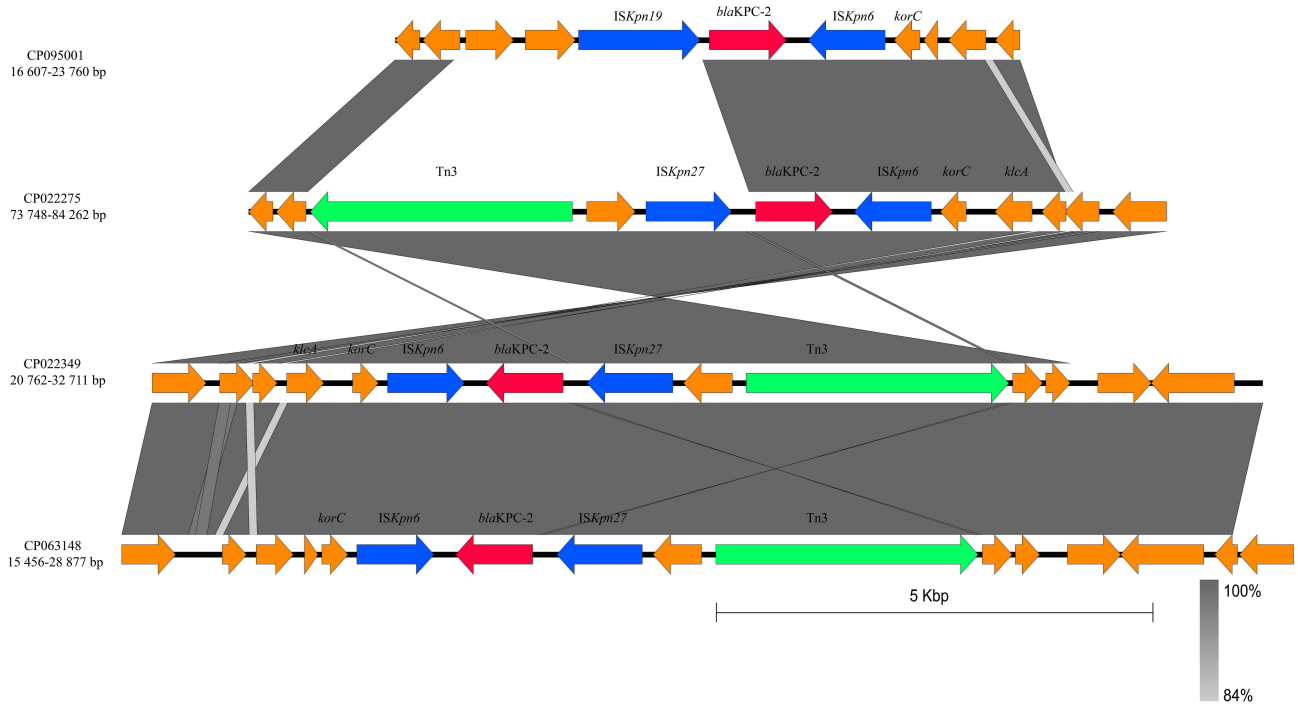

Figure 5 Structural comparison of the related plasmid with pB_KPC. Major structural features and comparison of the carbapenemase-producing plasmid with closely related plasmids. Schematic illustration showing the structural features of pB_KPC and the closely related plasmids pBKPC18-1, pK516_KPC and pKP19-3088-159k. Grey shading indicates shared regions with a high degree of homology. ORFs are indicated as arrows and coloured according to their putative functions. Antimicrobial resistance genes, IS elements and Tn elements are indicated in red, blue and green respectively, and all other genes are indicated in Orange in the figure.

The pB_KPC plasmid contained only the bla_{KPC-2} gene, which was responsible for antimicrobial resistance. This gene was not associated with the Tn4401 transposon, which was a major transposon associated with the bla_{KPC-2} gene in *Enterobacteria*. In the pB_KPC plasmid, the bla_{KPC-2} structure was IS*Kpn6*-bla_{KPC-2}-IS*Kpn19* (Figures 4 and 5), which was nearly identical to pKP19-3088-159k and the backbone structure of bla_{KPC-2} was IS*Kpn6*-bla_{KPC-2}-IS*Kpn27*. By contrast, bla_{KPC-2} was located on a Tn3 transposon element in pK516_KPC and pBKPC18-1 plasmids with the linear structure Tn3-IS*Kpn27*-bla_{KPC-2}-IS*5*-*korC*-*klcA* (Figure 5). These results suggested that the dissemination of bla_{KPC-2} was mediated by diverse mechanisms in these isolates.

Discussion

In clinical settings, *K. michiganensis* is an uncommon Gram-negative bacterium. *K. michiganensis* was first discovered in Michigan, USA, in 2013. Since then, multiple studies have documented a high frequency of clinical instances of infections caused *by K. michiganensis* isolates that are resistant to carbapenem.[2–7,10] *K. michiganensis* has been introduced as a newly recognised human pathogen linked to hospital-acquired infections, this strain was primarily isolated from stool sample, rectal swabs and bloodstream,[2–6] and to our knowledge rarely isolated from cerebrospinal fluid in tentorial meningioma caused by *K. michiganensis* has been documented. This study focused on the *K. michiganensis* strain isolated from cerebrospinal fluid specimen with multiple drug-resistant plasmids containing the bla_{KPC-2} and bla_{NDM-1} carbapenemase genes.

Fxq was classified as carbapenem-resistant *K. michiganensis*, belonging to ST202, which harboured the bla_{KPC-2} and bla_{NDM-1} genes located on the 140,266 and 255,357 bp plasmids, respectively. ST202 carbapenem-resistant *K. michiganensis* has not been reported in the literature. After analysing the genetic information in the GenBank repository (retrieved on 28 July 2023), we determined an absence of ST202 *K. michiganensis* sequences in the database. The emergence of ST202 carbapenem-resistant *K. michiganensis* indicates the diversity of genetic evolution, and the spread of drug-resistant genes between different types of ST types reminds researchers to strengthen the monitoring of drug-resistant strains.

Many of the bla_{NDM-1}-carrying plasmids isolated from *Enterobacterales* that have been published to date belong to the IncA/C group.[11–13] Different countries have identified several isolates belonging to the IncN2, IncL/M, IncFII and IncH groups.[14–17] On

the basis of the replicase sequence, the plasmid pE_NDM is classified as the IncU group. The IncU plasmid incompatibility (Inc) group was designated in 1981, and it is a group of mobile elements with extensively preserved core functions and diverse cassettes containing genes for antibiotic resistance.[18–20] Numerous *Aeromonas* spp. and *E. coli* strains from fish, in addition to clinical settings, contain the IncU Inc group.[18,20–22] The IncU plasmid group members play a significant role in spreading antibiotic resistance in Aeromonas strains that are linked to aquatic environments.[23] IncU plasmids have also been associated with multiple resistance genes, including *qnrS2, aac(6')-Ib-cr, aadA1, aadA2, sulI, sulII, dfrA16 dfrIIc* (*dfrB3*) and *catAII*.[24] Nevertheless, studies regarding IncU plasmids that are resistant to carbapenem are lacking.[25] This report describes the IncU-type $bla_{\text{NDM-1}}$-positive plasmid, which harboured multiple resistance gene. As previously reported for IncU plasmids, various resistance genes, including *qnrS2, dfrA16 dfrIIc* (*dfrB3*) and *catAII, sulI* and *sulII, aac(6')-Ib-cr, aadA1* and *aadA2*, have been described.[24] The backbone structure harboring $bla_{\text{NDM-1}}$ was similar to that reported earlier, and the class 1 integron complex mediated by ISCR1 was unstable.[12,16] Reports have identified ISCR1-$bla_{\text{NDM-1}}$-bearing plasmid heterogeneity in the genomic analysis of a bacterial population.[9,11,12] Although pE_NDM being untransferable, it harboured multiple mobile elements that benefit resistance gene transport among strains. Hence, it is imperative to promptly implement efficacious strategies to mitigate the dissemination of these $bla_{\text{NDM-1}}$-positive plasmids.

pB_KPC belongs to the IncFIA group. The significant spread of KPC is greatly influenced by the transfer of bla_{KPC}-containing plasmids horizontally, both between and within species. Numerous KPC plasmids encompassing various Inc replicon groups, such as IncFIA, IncFII, IncN, IncX, IncR, IncI2, IncA/C, ColE1 and IncL/M, have been discovered. Recently, the significance of these diverse genetic frameworks has been examined.[26] Upon analyzing the similar plasmid of pB_KPC, it was discovered that *Citrobacter freundii* harboring the similar plasmid pBKPC18-1 was present in river water. This finding suggests that there exists cross transmission between environmental and clinical strains, further highlighting the robust transferability of this plasmid type among various strains.

Additionally, we emphasised the gathering of resistance genes in infrequently recognised *K. michiganensis*. Thus, this emerging human pathogen must be monitored to minimise problems associated with it. Nevertheless, the ability to monitor *K. michiganensis* is hindered by the potential misidentification as *K. oxytoca* during routine detection due to the resemblance of protein spectra shared between the two species, coupled with their highly comparable phenotypes, biochemical reactions, and 16S rRNA sequences. In recent years, *K. michiganensis* identification has frequently relied on determining the average nucleotide identity (ANI) of the genome. However, there remains a shortage of straightforward and precise clinical identification methods for routine use, necessitating ongoing research. Hence, additional investigations are required to formulate accurate, uncomplicated and distinct identification techniques.

In conclusion, this study highlighted the genomic features of carbapenem-resistant *K. michiganensis* ST202 harbouring the $bla_{\text{KPC-2}}$ and $bla_{\text{NDM-1}}$ genes encoded by the IncFIA and IncU plasmids, respectively. The $bla_{\text{NDM-1}}$ gene located on the IncU plasmid of *K. michiganensis* has not been described previously in China.

Data Sharing Statement

The authors confirm that the data supporting the findings of this study are available from the corresponding author on reasonable request.

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Disclosure

The authors report there are no competing interests to declare for this work.

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