

Emergence of a novel hybrid plasmid carrying bla NDM-5 in a *Klebsiella pneumoniae* isolate producing KPC-2

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Abstract

Acquisition of plasmid-borne *bla*_{NDM} gene in KPC-2 producing *Klebsiella pneumoniae* (*K. pneumoniae*) largely threatening clinical effectiveness of ceftazidime-avibactam, an effective regiment for treating infections caused by serine carbapenemase producing pathogens. This study reports an extensively drug-resistant (XDR) *K. pneumoniae* clinical strain namely KP234 co-harboring carbapenemase genes *bla*_{NDM-5} and *bla*_{KPC-2}. The phenotype and genotype of *K. pneumoniae* KP234 were investigated by antimicrobial susceptibility testing, phenotypic detection for carbapenemase gene, PCR, conjugation assay, and whole genome sequencing (WGS). Bioinformatics analysis were used to uncover the genetic structures of its plasmid p234-NDM-5 and p234-KPC-2. KP234 displayed an XDR phenotype that only susceptible to colistin. WGS analysis identified five plasmids including plasmid p234-NDM-5 harboring *bla*_{NDM-5} and plasmid p234-KPC-2 harboring *bla*_{KPC-2} in KP234. Comparative genetic analysis revealed p234-NDM-5, a hybrid IncFIA/FIB/FIC plasmid, probably originated from an *bla*_{NDM-5}-bearing plasmid pNDM4 (CP083878) in *Escherichia coli*. Further detailed sequence analysis showed p234-NDM-5 might be generated as a result of an ~ 37 kb p16HN-263 (CP045264) derived fragment integrated into a transposase encoding gene between the gene encoding recombinase family protein and *pemK* gene on plasmid pNDM4. Additionally, this 37 kb insertion segment present in p234-NDM-5 undergone rearrangement events compare with its corresponding region on plasmid p16HN-263, indicating further evolution of such *bla*_{NDM}-bearing plasmids in KPC-2 producing *K. pneumoniae*. Our findings highlight further dissemination of the *bla*_{NDM-5} and *bla*_{KPC-2} into a clinical isolate of KP234 and convergence with multiple resistance genes, which increases the risk of the emergence of XDR strains and threatens the treatment of *Enterobacterales* bacterial infections.

Introduction

Carbapenem-resistant *K. pneumoniae* (CRKP) can lead serious infections to patients in an immunocompromised state, making their hospital stay prolong and increasing their mortality rates (Xiao et al., 2023). The production of carbapenemases is the primary cause of CRKP. The *K. pneumoniae* carbapenemases (KPC) serine carbapenemase and New Delhi β -lactamases (NDM) metallo-beta-lactamase are two common carbapenemases found in CRKP, with KPC enzymes, particularly the KPC-2 variant, being predominant in CRKP strains isolated in China (David et al., 2019). The combination therapy of avibactam, which is able to inhibit serine carbapenemases but ineffective against metallo-beta-lactamases, and ceftazidime has emerged as an effective treatment for infections caused by KPC-producing CRKP. However, recent emergence of KPC enzyme variants such as KPC-74, KPC-113, KPC-90 has led to resistance to ceftazidime-avibactam in CRKP. Additionally, the emergence of NDM enzymes in KPC-producing CRKP has also contributed to resistance to this combination.

Since the first genome of a hypervirulent *K. pneumoniae* strain producing both NDM and KPC enzymes was reported in 2018, the number of reports of CRKP co-producing NDM and KPC has been increasing globally, particularly in China (Gu et al., 2018). The genetic characteristics of plasmids encoding these two enzymes are continuously evolving. In recent years, the detection rate of hybrid multidrug-resistant (MDR) plasmids in clinical samples has increased. MDR mobile genetic elements, such as plasmids, can integrate into other plasmids through insertion sequences like IS26, ISCR2, and IS1 (Li et al., 2023; Ohtsubo et al., 1981). However, studies on the evolutionary trajectories are scarce. We aim to infer the evolutionary characteristics by analyzing the structure of a new hybrid plasmid.

Unlike previously reported CRKP co-producing KPC and NDM strains that carried *bla*_{NDM}-harboring plasmids belonged to IncX3, N2, H11B types (Gao et al., 2020; Hao et al., 2022), the *bla*_{NDM-5} in this study were carried by an IncFIA:IB:IC hybrid plasmid. Herein, we genetically characterized a *bla*_{KPC-2} and *bla*_{NDM-5} positive CRKP named KP234, elucidating the mechanism of *bla*_{NDM}-positive hybrid plasmid formation.

Material and methods

Sample collection and antimicrobial susceptibility testing

A retrospective epidemiologic surveillance study of carbapenem-resistant Enterobacterales (CRE) infection was conducted in The First Affiliated Hospital of Henan University of Chinese medicine. A carbapenem-resistant isolate KP234 was isolated from the sputum sample of a 75-year-old male patient who was diagnosed with severe pulmonary infection in 2022 in our routine detection. The KP234 isolated was identified by using the VITEK 2 system (bioMérieux, Marcy-l'Étoile, France) and 16S-rRNA sequencing. Antimicrobial susceptibility testing was determined by agar dilution (for fosfomycin) and broth microdilution methods following the proposal of the Clinical and Laboratory Standards Institute (CLSI) (CLSI, 2022). And the interpretation of the results of colistin and tigecycline were based on the European Committee on Antimicrobial Susceptibility Testing (EUCAST) guidelines (<http://www.eucast.org>). *E. coli* ATCC25922 was used as the quality-control strain.

Carbapenem resistance genes and phenotypic detection

The presence of carbapenem resistance genes (*bla*_{KPC-2}, *bla*_{NDM}, *bla*_{VIM}, *bla*_{IMP} and *bla*_{OXA}) was investigated using PCR with the primers (Table S1). What's more, phenotypic detection assays were performed using the disc diffusion method with inhibitors (EDTA and aminophenylboronic acid), allowing discriminating between serine- β -lactamases (Class A carbapenemases, e.g. KPC) and metal- β -lactamases (Class B carbapenemases, e.g. NDM) as well as strains producing two carbapenemases (Magiorakos et al., 2012).

Whole-genome sequencing and bioinformatics analysis

The genome of isolate KP234 was extracted using the FastPure bacterial DNA isolation minikit (Vazyme, catalog no. DC103) according to the manufacturer's instructions. The extracted genome was quantified using a Qubit 4 fluorometer and a Nanodrop spectrophotometer. WGS of KP234 was performed utilizing

both Illumina paired-end 2 × 150 bp sequencing and Nanopore MinION long-read sequencing. Genome sequences were completed with a hybrid de novo assembly strategy that combined Illumina short-read and Nanopore long-read data, using Unicycler v.0.4.8 software (Unicycler: Resolving bacterial genome assemblies from short and long sequencing reads). Genome annotation was performed with the RAST tool (<http://rast.nmpdr.org/>) and further refined manually. Multi-locus sequence typing (MLST) of the assembled genome was determined using the mlst software (<https://github.com/tseemann/mlst>). Insertion sequence (IS) elements, antibiotic resistance genes (ARGs), and plasmid replicon types were identified using ISfinder (<https://isfinder.biotoul.fr/>), ResFinder (<https://cge.cbs.dtu.dk/services/ResFinder/>), and PlasmidFinder (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>) with default parameters, respectively. Comparative analysis of plasmid and MDR structures was performed using BRIG and Easyfig tools (Alikhan et al., 2011; Sullivan et al., 2011)..

Conjugation assay

To verify the transferability of the plasmid carrying the carbapenem gene (p234-NDM-5 and p234-KPC-2), conjugation assays were performed using KP234 as the donor strain and rifampicin–tolerant *E. coli* EC600 as the recipient strain. Donor and receptor strains were mixed at a ratio of 1:1 in LB broth and incubated at 37°C for 24 h. Transconjugants were selected using rifampicin (200 µg/mL) and meropenem (1 µg/mL) and identified by PCR and antimicrobial susceptibility testing.

Nucleotide sequence accession numbers

The nucleotide sequences of the KP234 chromosome and four plasmids were submitted in GenBank with the accession numbers CP131916, CP131917, CP131918, CP131919 and CP131920, respectively.

Results

Characteristics of an XDR *K. pneumoniae* isolate KP234

PCR and phenotypic detection for carbapenemase showed that KP234 co-produced NDM-5 and KPC-2 (Supplementary Figure S1). Antimicrobial susceptibility testing showed that KP234 was resistant to multiple categories of antimicrobials, including Penicillins, Antipseudomonal penicillins + β-lactamase inhibitors, Cephalosporins, Carbapenems, Monobactams, Fluoroquinolones, Aminoglycosides, Phosphonic acids, Tetracyclines, and Glycylcyclines (Table 1), exhibiting an XDR phenotype (Magiorakos et al., 2012). However, it remained high susceptibility rates to colistin. The emergence of the XDR phenotype led to the investigation of the resistome in KP234 by using WGS analysis. KP234 also carry multiple resistance genes other than carbapenemase genes, including genes conferring resistance to fosfomycin (*fosA3*), cephalosporin (*bla*_{CTX-M-15}, *bla*_{OXA-1} and *bla*_{CTX-M-65}), sulfonamides (*sul1*), aminoglycosides (*aadA2*), tetracyclines (*tetA*), trimethoprim (*dhfrA12*) and so on.

Table 1
Antimicrobial susceptibility profiles of *K. pneumoniae* KP234.

Antimicrobial category	Antimicrpbial agents	MIC _s ^a (µg/mL)	Susceptibility
Penicillins	Ampicillin	> 256	R
Antipseudomonal penicillins + β-lactamase inhibitors	Piperacillin-tazobactam	> 1024	R
Cephalosporins	Cefazolin	> 256	R
	Ceftazidime	> 64	R
Carbapenems	Imipenem	> 64	R
	Meropenem	> 16	R
Monobactams	Aztreonam	> 64	R
Fluoroquinolones	Ciprofloxacin	> 8	R
Aminoglycosides	Amikacin	> 128	R
Phosphonic acids	Fosfomycin	1024	R
Tetracyclines	Doxycycline	32	R
Glycylcyclines	Tigecycline	4	R
Polymyxins	Colistin	2	I

According to the results of Illumina or Nanopore sequencing data analysis, ST11 strain KP234 has one chromosome (5536,915 bp, CP131916) and four plasmids, including p234-NDM-5 (175,910 bp, CP131917), p234-KPC-2 (84,970, CP131920), p234-4 (10,060 bp, CP131918) and p234-5 (5,596 bp, CP131919) (Supplementary Table S2). Due to *bla*_{NDM-5} and *bla*_{KPC-2} are located on plasmids p234-NDM-5 and p234-KPC-2, respectively, we next do further analysis on plasmids carrying *bla*_{NDM-5} and *bla*_{KPC-2}.

Molecular characterization of plasmid p234-NDM-5 harboring bla_{NDM-5}

The bla_{NDM-5}-bearing MDR plasmid p234-NDM-5 (Fig. 1A), with a length of 175,910 bp and an average GC content of 53% (Supplementary Table S2), was a hybrid plasmid. ResFinder analysis identified 12 resistance genes including carbapenemase resistance gene bla_{NDM-5}, fosfomycin resistance gene fosA3 and cephalosporin resistance gene bla_{CTX-M-15}, bla_{OXA-1} and bla_{CTX-M-65} on plasmid p234-NDM-5.

Additionally, the backbone of p234-NDM-5 included a number of genes involved in replication (IncFIA, IncFIB, and IncFIC), maintenance (*hok-sok*, *ccdAB*, *parDE*, *RelBE*, *pemI/pemK* and *parA*, *parB*) and conjugal transfer (*tra-trb*). Plasmid p234-NDM-5 failed to transfer from the donor strain *K. pneumoniae* to the recipient isolate *E. coli* C600 in the conjugation assay. However, Sequence analysis by oriTfinder6 identified the *oriT* region, relaxase gene and T4SS gene cluster on plasmid p234-NDM-5, but the type IV coupling protein (T4CP) gene was absent. Given that the four components are necessary for conjugation in self-transmissible plasmids, incomplete MOB module might be responsible for the non-conjugative profile of p234-NDM-5.

Antibiotics resistance genes of p234-NDM-5

ARGs were distributed in two distinct MDR regions. A 30,826 bp MDR region was found in p234-NDM-5. A number of ARGs and mobile elements were concentrated in this region (Fig. 1B). The *dfrA12* gene was embedded in a class I integron with the structure of IS26-*intI1*-*dfrA12*-*qacEΔ1*-*sul1*. The bla_{NDM-5} gene was probably disseminated by ΔTn125 and rolling-circle replication with ISCR1. Previous study noted that the Tn125 transposon potentially have played an important role in early plasmid-mediated jumps of bla_{NDM} (Acman et al., 2022). The Tn125 transposon is composed by ISAbal25-bla_{NDM}-ble-trpF-dsbD-cutA1-groES-groEL-ISAbal25 (Yehouenou et al., 2021) while bla_{NDM-5} in this study was located in a structure of qacEΔ1-sul1-ISCR1-qacEΔ1-sul1-ISCR1-dsbD-trpF-ble-bla_{NDM-5}-ΔISAbal25, which is ΔTn125 (Supplementary Figure S2). The mph(A) gene was in a region of IS26-mph(A)-mrx-mphR(A)-IS6100, and there was a region carrying many ARGs (*transposase-bla_{CTX-M-65}-transposase-catB-bla_{OXA-1}-aac(6)-lb-IS26-transposase-tet(A)*). And another 20,345 bp segment organized as IS26-catA2-IS26 and IS26-fosA3-IS26-IS903B-IS26 were located in the variable region.

Proposed formation mechanism of the plasmid p234-NDM-5

It is worth noting that the database does not exist a plasmid identical to plasmid p234-NDM-5, which means that p234-NDM-5 may have been generated through a series of evolution. We then investigated the mechanism of plasmid formation. Comparative analysis shows that p234-NDM-5 displayed 83% query coverage and 100.00% nucleotide identity to plasmid pNDM4 (GenBank accession no. CP083878) (Fig. 1A). The formation of plasmid p234-NDM-5 might have been driven by the recombination of ~ 37 kb segment carrying multiple ARGs on the basis of pNDM4. ~ 37 kb bases originated from p16HN-263 (GenBank accession no. CP045264) from the *K. pneumoniae* strain, were integrated into transposase gene between the gene encoding recombinase family protein and *pemK* gene on plasmid pNDM4 from the *E. coli* strain, making the lack of 2,202 bp of transposase gene and the lack of 33 bp of its downstream. ~37 kb segment is homologous to plasmid p16HN-263. Notably, linear comparison revealed that 37 kb segment including three parts: I, II and III (Fig. 1B). However, the 37 kb segment was rearranged. The sequence of ~ 37 kb segment in plasmid p16HN-263 is II, I and III while in p234-NDM-5 is I, II and III. This shows that I and II swap positions in two plasmids. What's more, III is not adjacent to I and II in p234-NDM-5. Above findings stress the evolutionary potential of the plasmid in this study, because they probably result in the formation of novel multidrug resistance plasmids, which should be monitored.

Molecular characterization of plasmid p234-KPC-2 harboring bla_{KPC-2}

The BLASTN search against the NCBI nucleotide database revealed that p234-KPC-2 displayed 97% query coverage and 99.99% nucleotide identity to plasmid pJNKPN52_kpc_fosA (GenBank accession no. MZ709016.1) from the *K. pneumoniae* strain, 97% query coverage and 99.99% nucleotide identity to plasmid pSW1780-KPC (GenBank accession no. CP073303.1) from the *K. pneumoniae* strain, 97% query coverage and 99.99% to plasmid pEBSI036-2-KPC (GenBank accession no. MT648513.1) from the *K. pneumoniae* strain, 97% query coverage and 99.99% to plasmid pKP19-2029-KPC2 (GenBank accession no. CP047161.1) from the *K. pneumoniae* strain and 97% query coverage and 99.97% to plasmid pKP18-238-KPC2 (GenBank accession no. CP082015.1) from the *K. pneumoniae* strain (Fig. 2A).

The core genetic structure of p234-KPC-2 can be found in different strains (Fig. 2B). The p234-KPC-2 exhibited 100% (7,668 of 7,668 bp) nucleotide sequence identity, 100% query coverage to a corresponding segment of plasmid pQL5-NDM-KPC (GenBank accession no. OR253888) from the *K. pneumoniae* strain QL5, pL2329-KPC-2 (GenBank accession no. CP136845) from the *K. pneumoniae* strain L2329, pJ-JNKPN26-4_HNK (GenBank accession no. OR041629) from the *E. coli* strain J-JNKPN26-4 and pJ-JNKPN26-3_HNK (GenBank accession no. OR041628) from *E. coli* strain J-JNKPN26-3. Recently, a *Citrobacter koseri* strain K1219 (GenBank accession no. CP137007) showed 100% nucleotide sequence identity, 100% query coverage to our p234-KPC-2. Tn6296 transposon potentially has played an important role in early plasmid-mediated jumps of bla_{KPC}. In this study, bla_{KPC-2} was embedded in the structure of *tnpR*-ISKpn27-bla_{KPC-2}-ΔISKpn6-korC-klcA-ΔrepB, which is ΔTn6296.

Discussion

In this study, we molecularly investigated an MDR *K. pneumoniae* ST11 clone harboring five plasmids and 12 resistance genes, including bla_{KPC-2} and bla_{NDM-5}, and characterized the plasmids containing carbapenemases genes. The first report of the co-occurrence of bla_{NDM-1} with bla_{KPC-2} in a clinical isolate was identified in *K. pneumoniae* from India in 2012 (Kumarasamy and Kalyanasundaram, 2012). Currently, Enterobacteriaceae, especially *K. pneumoniae*, is the primary host among strains co-producing NDM and KPC carbapenemases (Table 2). Additionally, we observed that the vast majority of coexistence strains in China, suggesting the need to strengthen the monitoring of clinical *K. pneumoniae* strains harboring both resistance genes (Li et al., 2018).

Table 2
Detailed information of reported strains coproducing *bla*_{NDM} and *bla*_{KPC} isolated from clinical settings.

Isolate name	Species	Year of isolation	Country	MLST	Sample	Carbapenemases resistance genes	Incompatibility and size	Genbank accessic no.
AHSWKP25	<i>K.pneumoniae</i>	2021	China	ST464	Blood	<i>bla</i> _{NDM-1}	IncX3 ,159,349 bp	CP091052
						<i>bla</i> _{KPC-2}	Unknown, 45,886 bp	CP091049
Kp14	<i>K. pneumoniae</i>	2017	Argentina	ST307	Blood	<i>bla</i> _{NDM-1}	IncA/C,145,500 bp	-
Kp2						<i>bla</i> _{KPC-3}	unknown	
C2414	<i>K. pneumoniae</i>	2017	China	ST11	Urine	<i>bla</i> _{NDM-1}	IncN,-	-
						<i>bla</i> _{KPC-2}	IncFII/IncR,-	
C2315	<i>K. pneumoniae</i>	2017	China	ST3493	Intra-abdominal fluid	<i>bla</i> _{NDM-1}	IncHI1B,-	-
						<i>bla</i> _{KPC-2}	IncFIA(HI1)/IncFII,-	
C2343	<i>K. pneumoniae</i>	2017	China	ST3493	Intra-abdominal fluid	<i>bla</i> _{NDM-1}	IncHI1B,-	-
						<i>bla</i> _{KPC-2}	IncFIA(HI1)/IncFII,-	
C2660	<i>K. pneumoniae</i>	2017	China	ST11	Pus	<i>bla</i> _{NDM-1}	IncX3 ,-	-
						<i>bla</i> _{KPC-2}	IncR/IncFII,-	
C2601	<i>K. pneumoniae</i>	2017	China	ST15	Sputum	<i>bla</i> _{NDM-1}	IncN2,-	-
						<i>bla</i> _{KPC-2}	IncFII(Yp),-	
C2972	<i>K. pneumoniae</i>	2016	China	ST15	Sputum	<i>bla</i> _{NDM-1}	IncN2,-	-
						<i>bla</i> _{KPC-2}	IncFII(Yp),-	
C2974	<i>K. pneumoniae</i>	2016	China	ST15	Sputum	<i>bla</i> _{NDM-1}	IncN2,-	-
						<i>bla</i> _{KPC-2}	IncFII(Yp),-	
KP234	<i>K. pneumoniae</i>	2022	China	ST11	Sputum	<i>bla</i> _{NDM-5}	IncFIA/IncFIB/IncFIC,-	-
						<i>bla</i> _{KPC-2}	IncFII/IncR,-	
K210011	<i>Klebsiella michiganensis</i>	2021	China		Rectal swab	<i>bla</i> _{NDM-1}	IncFIB(K)/IncFII(K), 233,442bp	JAHNZR00000000
						<i>bla</i> _{KPC-2}	IncFIB, 101,156bp	
WCHECI-14,653	<i>Enterobacter cloacae</i>	2014	China	ST177	Blood	<i>bla</i> _{NDM-1}	IncFIB/IncFIIY, 109 kb	KP868647
						<i>bla</i> _{KPC-2}	Inc FIIY, 88 kb	KP868646
CCBH14397	<i>Enterobacter hormaechei</i>	2013	Brazil	ST15	Rectal swab	<i>bla</i> _{NDM-1}	IncA/C, 160 kb	-
						<i>bla</i> _{KPC-2}	IncN, 50 kb	
SCLZS47	<i>Citrobacter freundii</i>	2019	China	-	Sewage	<i>bla</i> _{NDM-1}	IncX3 , 56,984bp	CP092495
						<i>bla</i> _{KPC-2}	Unknown, 45,886 bp	CP092494
ZSH6	<i>E. coli</i>	2018	China	ST2	Blood	<i>bla</i> _{NDM-5}	IncX3 , 46,461bp	CM010659
						<i>bla</i> _{KPC-2}	IncP6, ~45.41 kb	CM010658
WLK218	<i>Raoultella ornithinolytica</i>	-	China	-	Sediment	<i>bla</i> _{NDM-1}	IncX3 , 75,415 bp	CP038280
						<i>bla</i> _{KPC-2}	Unknown, 35,262 bp	CP038279
BWH_1_P_GER	<i>Pluralibacter gergoviae</i>	2021	US	-	Respiratory, urine cultures	<i>bla</i> _{NDM-1}	IncHI1B/FIB/IncA/IncM1, -	-

Isolate name	Species	Year of isolation	Country	MLST	Sample	Carbapenemases resistance genes	Incompatibility and size	Genbank accessio no.
BWH_6_P_GER					Respiratory cultures	<i>bla</i> _{KPC-4}	IncHI1B/FIB/IncA/IncX3, -	-
K218	<i>Citrobacter portucalensis</i>	2017	China	-	Blood	<i>bla</i> _{NDM}	IncFII/FIB, 111kb	OL988824
						<i>bla</i> _{KPC}	IncFII, 106 kb	OL988823

ST11-type CRKP is an internationally recognized high-risk clone, prevalent in multiple countries and identified as the predominant clone in China (Liao et al., 2020). The type IV secretion system in ST11 strains facilitates the transfer of genetic elements such as plasmids, exacerbating the spread of resistance genes. Although *K. pneumoniae* strains co-carrying *bla*_{KPC} and *bla*_{NDM} exhibit diverse STs, ST11 remains the most prevalent (Table 2). Furthermore, the diverse STs of *K. pneumoniae* strains (ST11, ST15, ST307, ST464, and ST3493) co-carrying *bla*_{KPC} and *bla*_{NDM} highlight the crucial role of mobile genetic elements, such as plasmids, in mediating the spread of carbapenem resistance genes.

Among these strains co-carrying *bla*_{KPC} and *bla*_{NDM} (Table 2), various distinct plasmid backbones have been identified. Some plasmids exhibit a broad host range (IncA/C, L/M, HI, I1, N, and W), while others are narrow (IncFII, FIA, FIB, and IncX3) (An et al., 2016; Bhattacharjee et al., 2023). The IncX3-type plasmid is a primary vector in mediating the dissemination of *bla*_{NDM} (Wu et al., 2019; Touati et al., 2021) and is the most common subgroup co-harboring *bla*_{KPC} and *bla*_{NDM} (Guo et al., 2022). The structure of the IncX3 plasmid is divided into two parts: the backbone and the accessory module. The backbone is highly conserved, and its related genes play an important role in the stability and spread of the plasmid (Guo et al., 2022). Previously, *bla*_{NDM-5}-positive plasmids carrying multiple plasmid replicons have also been identified other than the single incompatibility type, such as a hybrid IncFIA/IncFIB/IncFIC-type plasmid pCS18F-NDM-Fos (GenBank accession no. CP074577) that is identical to p234-NDM-5 in this study. (Zhao et al., 2022). The type of plasmid replicon is linked to the resistance genes it carries, and the merging of different replicons is expected to increase the strain's resistance capacity (Chen et al., 2024). This finding underscores the need to monitor this novel hybrid plasmid closely.

The transmission of *bla*_{KPC} is commonly linked to mobile genetic elements with a strong ability to disseminate, including Tn4401 and the clonal dissemination of *K. pneumoniae* strains from clonal complex 11 (ST11, ST258) in various countries (Pereira et al., 2015). While in this study, KP234 is disseminated by ΔTn6296. Additionally, NDM is known to be associated with insertion sequences and transposons, such as IS*Aba*125 and Tn125, which is in accordance with the p234-NDM-5. The novel hybrid plasmid in our study is crucial to emphasize the need for antimicrobial resistance surveillance and to actively prevent the spread and evolution of resistance genes in plasmid form. This is a crucial strategy to disrupt the dissemination of resistance.

Conclusion

This study identified a novel hybrid plasmid carrying *bla*_{NDM-5} in *K. pneumoniae* isolate producing KPC-2 from a 75-year-old male patient who was diagnosed with severe pulmonary infection. Plasmid p234-NDM-5 has the potential to evolve into a significant reservoir for the spread of many ARGs. Further research is needed to investigate the prevalence of *bla*_{NDM}-carrying plasmid among different pathogens in clinical settings. What's more, it is important to closely monitor the convergence and dissemination of NDM with KPC in one strain and monitor the evolutionary pathway of this kind of plasmid in the future.

Declarations

Conflict of Interest Statement

The authors declare that the research was conducted in the absence of any commercial or financial relationships that could be construed as a potential conflict of interest.

Author Contribution

Z.Y. and S.Y.J. wrote the main manuscript text, Q.X. and M.Q. prepared figures 1-2. All authors reviewed the manuscript.

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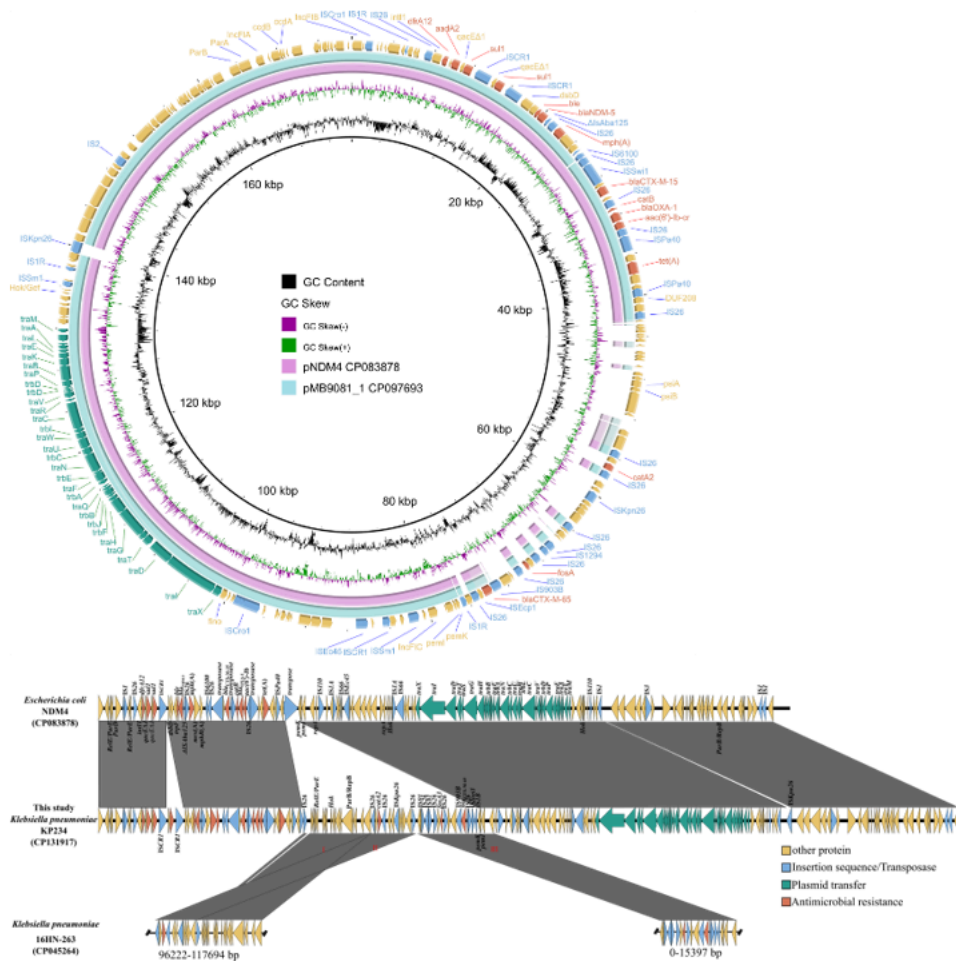
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Figures



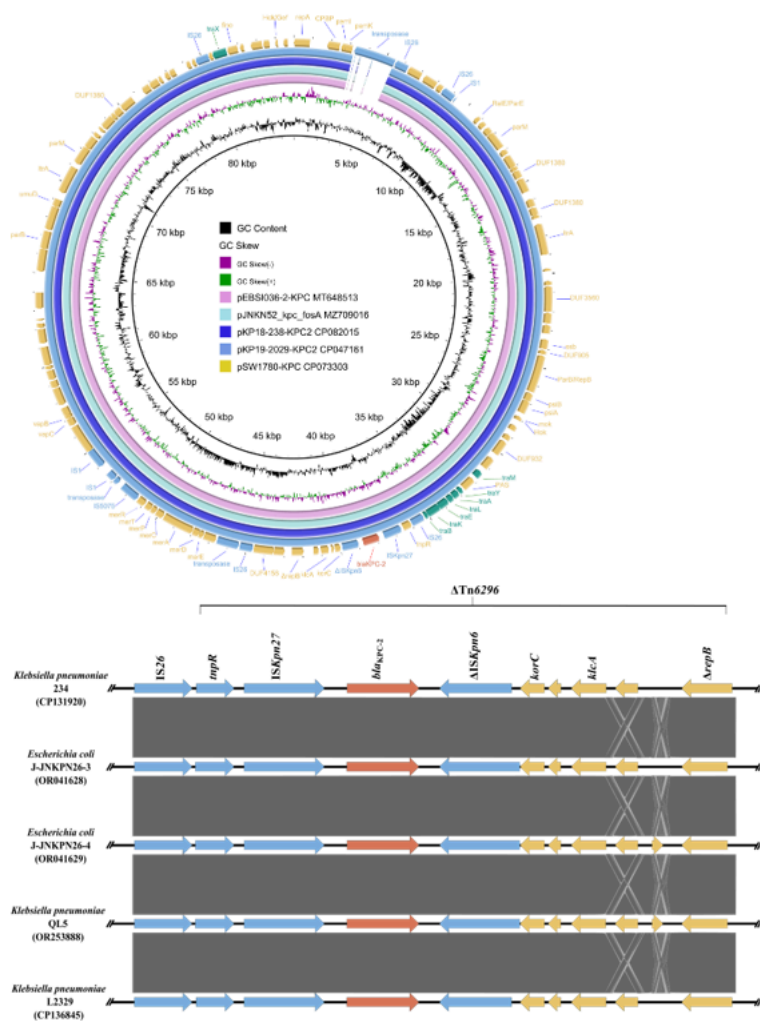


Figure 2

A Plasmid characterization of *bla*_{KPC-2}-bearing plasmid p234-KPC-2 and closely related plasmids. Key features of plasmid were highlighted in different colours. **B** linear depiction of the primary structural characteristics of plasmid p234-KPC-2 compared with others. Key features were highlighted in different colours. Antimicrobial resistance genes were in red.

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