Infection and Drug Resistance

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ORIGINAL RESEARCH

Dissemination of bla_{NDM-5} Driven by Horizontal Transfer of IncFIA Plasmid Between *Escherichia coli* and *Klebsiella pneumoniae* Co-Isolated from a Patient's Ascitic Fluid

Jing Yu[1,2], Yanzi Ding[1,2], Xue Zhang[1,2], Shuhong Tai[1,2], Chengwen Zhang[1,2], Cailin Liu[3], Enwu Yuan[1,2], Yitao Duan[1,2]

[1]Department of Laboratory Medicine, the Third Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, 450052, People's Republic of China; [2]Zhengzhou Key Laboratory for in vitro Diagnosis of Hypertensive Disorders of Pregnancy, Zhengzhou, Henan, 450052, People's Republic of China; [3]Department of Laboratory Medicine, the First Affiliated Hospital of Zhengzhou University, Zhengzhou, Henan, 450052, People's Republic of China

Correspondence: Yitao Duan; Enwu Yuan, Department of Laboratory Medicine, the Third Affiliated Hospital of Zhengzhou University, No. 7 Front Kangfu Street, Zhengzhou, Henan, 450052, People's Republic of China, Email duanyitao@zzu.edu.cn; yuanenwu@126.com

Purpose: Understanding the horizontal transfer of resistance genes, such as bla_{NDM-5}, is pivotal in developing strategies to control the spread of resistance. In this study, we isolated two bacterial strains, *Escherichia coli* (designated GYB01) and *Klebsiella pneumoniae* (designated GYB02), from a single patient. The aim of our research is to explore the biological characteristics of these strains and to investigate the interspecies horizontal transfer of bla_{NDM-5}.

Materials and Methods: Strain identification and antimicrobial susceptibility testing were conducted using the Vitek 2 system. Both GYB01 and GYB02 were sequenced with the Illumina HiSeq platform. Bioinformatics analysis tools, including multilocus sequence typing, PlasmidFinder, ResFinder, and others, were utilized to analyze the strains. Additionally, conjugation assays and *Galleria mellonella* infection assays were employed to assess the strains.

Results: The isolates exhibited similar antimicrobial resistance profiles and both harbored the bla_{NDM-5} gene within the IncFIA plasmids (pGYB01-2, 165.8 kb and pGYB02-2, 211.6 kb, respectively). These plasmids (pGYB01-2 and pGYB02-2) shared over 99% homology, suggesting a common ancestral origin. Conjugation experiments confirmed the transferability of the bla_{NDM-5} carrying IncFIA plasmids among *Enterobacteriaceae*. GYB02 possessed an *iucACD-iutA* gene cluster, exhibited high virulence, and tested positive in the string test.

Conclusion: Our findings provide direct evidence of potential in vivo interspecies transfer of a multidrug-resistant plasmid, thus enriching our understanding of the mechanisms driving multidrug resistance (MDR) and aiding in the formulation of containment and treatment strategies.

Keywords: CRE, NDM-5 carbapenemase, IncFIA plasmid, interspecies horizontal transfer

Introduction

The emergence and spread of antibiotic resistance among bacterial pathogens have become a major global health concern.[1] Of particular concern is the rapid dissemination of carbapenemase-producing *Enterobacteriaceae* (CPE), which are associated with high mortality rates and limited treatment options.[2] The bla_{NDM-5} gene, encoding New Delhi metallo-beta-lactamase-5, is one of the most prevalent carbapenemase genes among *Enterobacteriaceae*.[3,4] This gene confers resistance to carbapenems and other beta-lactam antibiotics, thereby severely compromising the effectiveness of last-resort antibiotics.

The horizontal transfer of antibiotic resistance genes, including bla_{NDM-5}, through mobile genetic elements such as plasmids, integrons, and transposons plays a significant role in the dissemination of antibiotic resistance among

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bacteria.[5,6] The ability of these mobile genetic elements to transfer between different species further contributes to the rapid spread of resistance. Understanding the mechanisms underlying inter-species transfer of antibiotic resistance genes is crucial for developing strategies to contain their spread.

In this study, we simultaneously isolated two strains of interest, *Escherichia coli* (designated as GYB01) and *Klebsiella pneumoniae* (designated as GYB02), from a single patient with an abdominal infection. Both strains demonstrated similar antimicrobial resistance profiles and carried the $bla_{\text{NDM-5}}$ gene. We investigated the possibility of inter-species transfer of $bla_{\text{NDM-5}}$ by conducting whole-genome sequencing (WGS). Our analysis provided direct evidence of the potential in vivo inter-species transfer of a multidrug-resistant plasmid carrying the $bla_{\text{NDM-5}}$ gene. Moreover, this comprehensive genomic analysis of multidrug-resistant *K. pneumoniae* and *E. coli* strains will enhance our understanding of their mechanisms of antimicrobial resistance and shed light on the potential horizontal transmission of antibiotic resistance genes. Ultimately, this research will contribute to the development of strategies for containment and treatment of these bacteria.

Materials and Methods

Isolation and Identification of Bacterial Strains

A 12-year-old patient was admitted to the hospital due to intestinal obstruction. During the course of treatment, carbapenem-resistant *Escherichia coli* (designated as GYB01) and carbapenem-resistant *Klebsiella pneumoniae* (designated as GYB02) were simultaneously isolated from the patient's ascitic fluid. Strain identification was performed using the VITEK-2 system (BioMérieux, Marcy l'Etoile, France).

Antimicrobial Susceptibility Testing

The minimal inhibitory concentration (MIC) was determined using the VITEK 2 system with a Gram-negative antimicrobial susceptibility testing card (AST-GN335). The tested antimicrobials included amikacin, minocycline, aztreonam, cefepime, cefoperazone/sulbactam, ceftazidime, imipenem, meropenem, piperacillin/tazobactam, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, doxycycline, tigecycline, and colistin. *E. coli* ATCC 25922 was used as a quality control strain. The interpretation of the MICs was conducted following the guidelines provided by the Clinical & Laboratory Standards Institute (CLSI, 2023) or the European Committee on Antimicrobial Susceptibility Testing (EUCAST) breakpoints (2023, https://www.eucast.org/).

WGS and Sequence Analysis

The genomic DNA was extracted, analyzed for presence and integrity using agarose gel electrophoresis, and quantified using a Qubit® 2.0 Fluorometer (Life Technologies, ThermoFisher Scientific, USA). The genomic DNA of GYB01 and GYB02 was sequenced using Pacific Biosciences (PacBio) Sequel and Illumina NovaSeq PE150 platform at Beijing Novogene Bioinformatics Technology Co., Ltd. Sequence reads were generated from a 10 kb SMRT Bell library and a 350 bp library. The reads were assembled using Canu (v2.0).[7] The Canu assemblies were subsequently corrected using Racon (v1.4.13) three times.[8] The assemblies were further refined using the Pilon (v1.22) software,[9] which utilized the Illumina clean data for three rounds of correction. This iterative process resulted in the generation of complete circular contigs without any gaps. In addition, the genomic DNA of *E. coli* J53 transconjugants was sequenced using Illumina NovaSeq PE150 platform, generating sequence reads from a 350 bp library. Sequence reads were assembled into scaffolds using SPAdes v 3.13.1.[10] The assembled genome or plasmid sequence was submitted to MLST 2.0 (https://cge.food.dtu.dk/services/MLST), ResFinder 4.6.0 (https://cge.food.dtu.dk/services/ResFinder/), a BLAST search against virulence factor database (VFDB) (http://www.mgc.ac.cn/VFs/search_VFs.htm), and ISfinder (https://isfinder.biotoul.fr/) to identify sequence types (STs), acquired antibiotic resistance genes (ARGs), virulence factors (VFs) and IS elements, respectively. The replicon type and transferability of the plasmids were predicted using PlasmidFinder 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder/) and OriTfinder (https://tool-mml.sjtu.edu.cn/oriTfinder/oriTfinder.html), respectively. Genomics islands were predicted by the SIGI-HMM method implemented in IslandViewer 4 (https://www.pathogenomics.sfu.ca/islandviewer/).

Infection of *Galleria Mellonella* Larvae

The virulence potential of GYB01 and GYB02 strains was assessed using the *G. mellonella* infection model, as previously described with minor modifications.[11] G. *mellonella* larvae were obtained from Henan Keyun Biopesticide Co., Ltd. (Jiyuan, Henan, China). The larvae utilized in the experiments had a weight ranging from 200 to 300 mg. Overnight cultures of GYB01 and GYB02 strains were washed with phosphate-buffered saline (PBS, pH 7.2) and subsequently adjusted to concentrations of 1×10^6 colony-forming units (CFU)/mL using PBS. For each strain, 10 larvae were individually injected with 10 μL of bacterial suspension. Additionally, a group of PBS controls was included. After inoculation, the larvae were placed in 150-mm petri dishes and incubated at 37°C in a dark environment. Survival was monitored and recorded for a duration of 72 hours. The experiments were conducted independently on three separate occasions.

Conjugation Assays

Conjugation assays were conducted to verify the transferability of plasmids carrying $bla_{\text{NDM-5}}$ using the LB broth mating method, as previously described with minor modifications.[12] A NaN_3-resistant *E. coli* J53 was used as a recipient strain. Transconjugants carrying $bla_{\text{NDM-5}}$ were subsequently selected by plating on Mueller-Hinton agar plates (Oxoid Ltd., Hampshire, United Kingdom) containing both 1 mg/L meropenem and 600 mg/L NaN_3. The selected conjugates were then confirmed using the VITEK-2 system. Next-generation sequencing was employed to detect ARGs carried by the conjugates. The MIC values were determined using the VITEK-2 GN335 card. The conjugation frequency (CF) was calculated following previous research: CF = Number transconjugants (CFU/mL)/Numbers of donor and recipient cells (CFU/mL).[13]

Statistical Analysis

Kaplan-Meier survival curves of *G. mellonella* were generated using GraphPad Prism software (Version 9.4.1), and statistical significance was tested with a Mantel-Cox test (Log rank test). P values <0.05 were considered statistically significant.

Results

Clinical Characteristics

The patient, a 12-year-old male, was admitted to our hospital after presenting with a one-day history of fever following intestinal fistula surgery performed six days earlier. On the day of admission, *Enterococcus avium*, carbapenem-resistant *Escherichia coli* (GYB01), and carbapenem-resistant *Klebsiella pneumoniae* (GYB02) were isolated from the ascitic fluid. Subsequently, the patient was treated with meropenem, linezolid, and fosfomycin for the infection. During the treatment process, tracheal intubation, blood transfusion, and nutritional support were also administered. Finally, the patient's condition significantly improved, resulting in his subsequent discharge from our hospital.

Antibiotic Resistance Profiles of GYB01and GYB02

Based on the results of the antibiotic susceptibility test (AST) presented in Table 1, it can be observed that both GYB01 and GYB02 display similar resistance profiles. Both GYB01 and GYB02 exhibited resistance to amikacin, minocycline, aztreonam, cefepime, cefoperazone/sulbactam, ceftazidime, imipenem, meropenem, piperacillin/tazobactam, ciprofloxacin, levofloxacin, trimethoprim/sulfamethoxazole, and doxycycline. However, they showed sensitivity to tigecycline and colistin. AST results revealed that GYB01 and GYB02 strains were multidrug-resistant (MDR) *Enterobacteriaceae*.

Genomic Features of GYB01and GYB02

To investigate the reasons for the identical resistance spectrum of GYB01 and GYB02, we conducted WGS on these two isolated strains. Specific information about the chromosomes and plasmids is presented in Table S1. *E. coli* GYB01 had a 4.9 Mb chromosome and two plasmids: pGYB01-1(107.9 kb) and pGYB01-2 (165.8 kb). The multilocus sequence typing results revealed that GYB01 belonged to ST10. Based on the PlasmidFinder analysis results, the replicon type of pGYB01-1 was determined to be IncI1-I, while pGYB01-2 was classified as IncFIA. The pGYB01-1 plasmid harbored the resistance genes Δ*aac(3)-IId*, Δ*erm*(B), and *mph*(A), whereas the pGYB01-2 plasmid carried the resistance genes *aac(3)-IIa, aac(6')-Ib-cr,*

Table 1 Antimicrobial Susceptibilities and Resistance Genes of GYB01, GYB02, and *E. coli* J53 Transconjugant

Antimicrobial(s)	MIC(s) (μg/mL) / Interpretations[a]				Associated Resistance Gene(s)[c]		
	GYB01	GYB02	*E. coli* J53 transconjugant[b]	*E. coli* J53	GYB01[d]	GYB02[e]	*E. coli* J53 transconjugant[b,f]
Amikacin	≥ 64 / R	≥ 64 / R	≤ 2 / S	≤ 2 / S	*aac(6')-Ib-cr*	*rmtB*	None
Minocycline	≥ 16 / R	≥ 16 / R	≥ 16 / R	2 / S	*tet*(B)	*tet*(B)	*tet*(B)
Aztreonam	≥ 64 / R	≥ 64 / R	≤ 1 / S	≤ 1 / S	$bla_{CTX\text{-}M\text{-}15}$	$bla_{CTX\text{-}M\text{-}55}$	None
Cefepime	≥ 32 / R	≥ 32 / R	2 / R	≤ 1 / S	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, $bla_{NDM\text{-}5}$	$bla_{CTX\text{-}M\text{-}55}$, $bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$
Cefoperazone/sulbactam	≥ 64 / R	≥ 64 / R	≥ 64 / R	≤ 8 / S	$bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$
Ceftazidime	≥ 64 / R	≥ 64 / R	≥ 64 / R	≤ 1 / S	$bla_{CTX\text{-}M\text{-}15}$, $bla_{NDM\text{-}5}$	$bla_{CTX\text{-}M\text{-}55}$, $bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$
Imipenem	8 / R	≥ 16 / R	8 / R	≤ 0.25 / S	$bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$
Meropenem	≥ 16 / R	≥ 16 / R	≥ 16 / R	≤ 0.25 / S	$bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$
Piperacillin/tazobactam	≥ 128 / R	≥ 128 / R	≥ 128 / R	≤ 4 / S	$bla_{CTX\text{-}M\text{-}15}$, $bla_{OXA\text{-}1}$, $bla_{NDM\text{-}5}$	$bla_{SHV\text{-}33}$, $bla_{CTX\text{-}M\text{-}55}$, $bla_{TEM\text{-}1B}$, $bla_{NDM\text{-}5}$	$bla_{NDM\text{-}5}$
Ciprofloxacin	≥ 4 / R	2 / R	≤ 0.25 / S	≤ 0.25 / S	*aac(6')-Ib-cr*	Δ*OqxA*, Δ*OqxB*, *qepA1*	None
Levofloxacin	≥ 8 / R	4 / R	≤ 0.12 / S	≤ 0.12 / S	Unknown	Unknown	None
Trimethoprim/sulfamethoxazole	≥ 16/304 / R	≥ 16/304 / R	≥ 16/304 / R	≤ 1/19 / S	*dfrA17*, *sul1*	Δ*OqxA*, Δ*OqxB*, Δ*dfrA17*, *sul1*	Δ*dfrA17*, *sul1*
Doxycycline	≥ 16 / R	≥ 16 / R	≥ 16 / R	1 / S	*tet*(B)	*tet*(B)	*tet*(B)
Tigecycline	≤ 0.5 / S	≤ 0.5 / S	≤ 0.5 / S	≤ 0.5 / S	None	None	None
Colistin	≤ 0.5 / S	≤ 0.5 / S	≤ 0.5 / S	≤ 0.5 / S	None	None	None

Notes: [a]Interpretations were based on CLSI guidelines, except for cefoperazone/sulbactam interpreted using cefoperazone CLSI breakpoints and tigecycline were interpreted by EUCAST breakpoints. [b]Transconjugant was obtained from a mating experiment using GYB02 as donor and *E. coli* J53 as recipient. [c]Identification of the acquired ARGs and predictions of phenotypes were performed by ResFinder 4.6.0 (https://cge.food.dtu.dk/services/ResFinder/). Mutations (Δ) reached the following conditions: ≥ 98% sequence identity and ≥ 99% alignment coverage. In addition, it has been reported that $bla_{NDM\text{-}5}$ could be responsible for the cefoperazone/sulbactam resistance.[14] [d]Three genes [Δ*aac(3)-IId*, Δ*erm*(B), *mph*(A)] were located on plasmid pGYB01-1, while not included in the table. Another ten genes [*aac(3)-IIa, aac(6')-Ib-cr, aadA5,* $bla_{CTX\text{-}M\text{-}15}$, $bla_{NDM\text{-}5}$, $bla_{OXA\text{-}1}$, *catB3, dfrA17, sul1, tet*(B)] were on plasmid pGYB01-2, with *aac(3)-IIa, aadA5*, and *catB3* also not shown in the table. [e]Four genes ($bla_{SHV\text{-}33}$, Δ*OqxA*, Δ*OqxB*, Δ*fosA6*) were located on the GYB02 chromosome, while Δ*fosA6* not included in the table. Another nine genes [*aadA5,* $bla_{CTX\text{-}M\text{-}55}$, $bla_{NDM\text{-}5}$, $bla_{TEM\text{-}1B}$, Δ*dfrA17, qepA1, rmtB, sul1, tet*(B)] were on plasmid pGYB02-2, with *aadA5* also not shown in the table. [f]Five genes [*aadA5,* $bla_{NDM\text{-}5}$, Δ*dfrA17, sul1, tet*(B)] were detected in the *E. coli* J53 transconjugant, with *aadA5* not being included in the table.

aadA5, bla$_{\text{CTX-M-15}}$, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{OXA-1}}$, *catB3, dfrA17, sul1*, and *tet*(B). Additionally, *K. pneumoniae* GYB02 had a 5.3 Mb chromosome and two plasmids: pGYB02-1 (111.8 kb) and pGYB02-2 (211.6 kb). The multilocus sequence typing results showed that GYB02 belonged to ST35. Based on the PlasmidFinder analysis results, the replicon type of pGYB02-1 was determined to be IncFIB, while pGYB02-2 was classified as IncFIA. The chromosome of GYB02 contained *bla*$_{\text{SHV-33}}$, Δ*OqxA*, Δ*OqxB*, Δ*fosA6*. The plasmid pGYB02-2 carried multiple resistance genes, including *aadA5, bla*$_{\text{CTX-M-55}}$, *bla*$_{\text{NDM-5}}$, *bla*$_{\text{TEM-1B}}$, Δ*dfrA17, qepA1, rmtB, sul1*, and *tet*(B). In contrast, there is no resistance gene present on plasmid pGYB02-1. Interestingly, the blast analysis indicated that the plasmid GYB01-2 exhibited the highest similarity to pGYB02-2 in the NCBI nucleotide collection (nr/nt) database (29 March 2024; Figure 1). The two plasmids, pGYB01-2 and pGYB02-2, exhibited a homology of over 99%. An 8.62-kb resistance island (IS26-*qepA1-rmtB-bla*$_{\text{TEM-1B}}$) partially fills in the disparities observed between the two plasmids, suggesting its potential involvement in their variation. They shared highly similar feature sequences including replicon (both classified as IncFIA), the origin of transfer site (oriT), relaxase gene, gene encoding type IV coupling protein (T4CP), and partial gene clusters for bacterial type IV secretion system (T4SS) (Figure 2). These findings strongly suggest that they likely originated from a common ancestor.

The Transferability of Multidrug-Resistant Plasmids

To evaluate the transferability of multidrug-resistant plasmids, we conducted an analysis using the OriTFinder website. The analysis revealed the presence of complete conjugative modules on both pGYB01-2 and pGYB02-2 plasmids. These modules include oriT, relaxase gene, T4SS, and T4CP. Based on these findings, it can be inferred that these plasmids are capable of horizontal transfer (Figure 2). To further confirm the transferability of the two plasmids harboring *bla*$_{\text{NDM-5}}$, a conjugation assay was conducted using GYB01 or GYB02 strains as the donor strains. GYB01 failed to transfer its *bla*$_{\text{NDM-5}}$ gene into *E. coli* J53 through our study. The *bla*$_{\text{NDM-5}}$-carrying plasmid was successfully transferred from

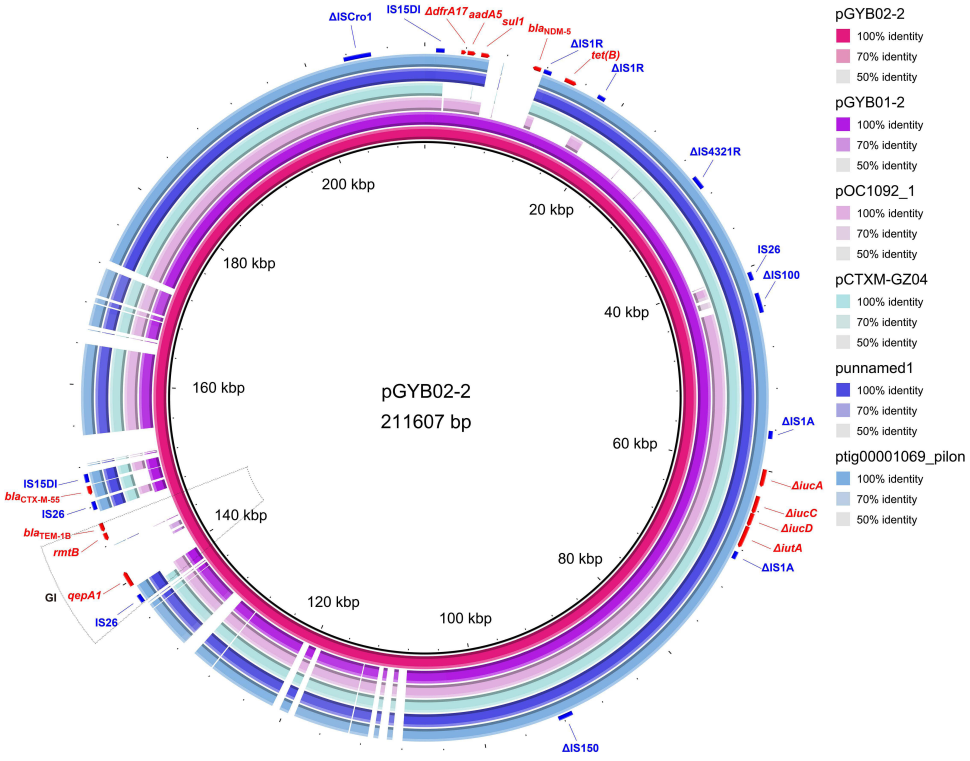

Figure 1 BRIG analysis of the conjugative multidrug resistance plasmid pGYB02-2. Comparative analysis of pGYB02-2 with five closely related plasmids was performed using the BLAST Ring Image Generator (BRIG). The concentric rings display similarity between the pGYB02-2 sequence in the inner ring and the other sequences in the outer rings. The various color levels indicate a BLAST result with a matched degree of shared regions, as shown to the right of the ring. The predicted ARGs and VFs are shown in red, and the IS elements are shown in blue. Mutations (Δ) reached the following conditions: ≥ 95% sequence identity and ≥ 98% alignment coverage. A GI is indicated within the dotted box.

Abbreviation: ARGs, antibiotic resistance genes; VFs, virulence factors; IS, insertion sequence; GI, gene island.

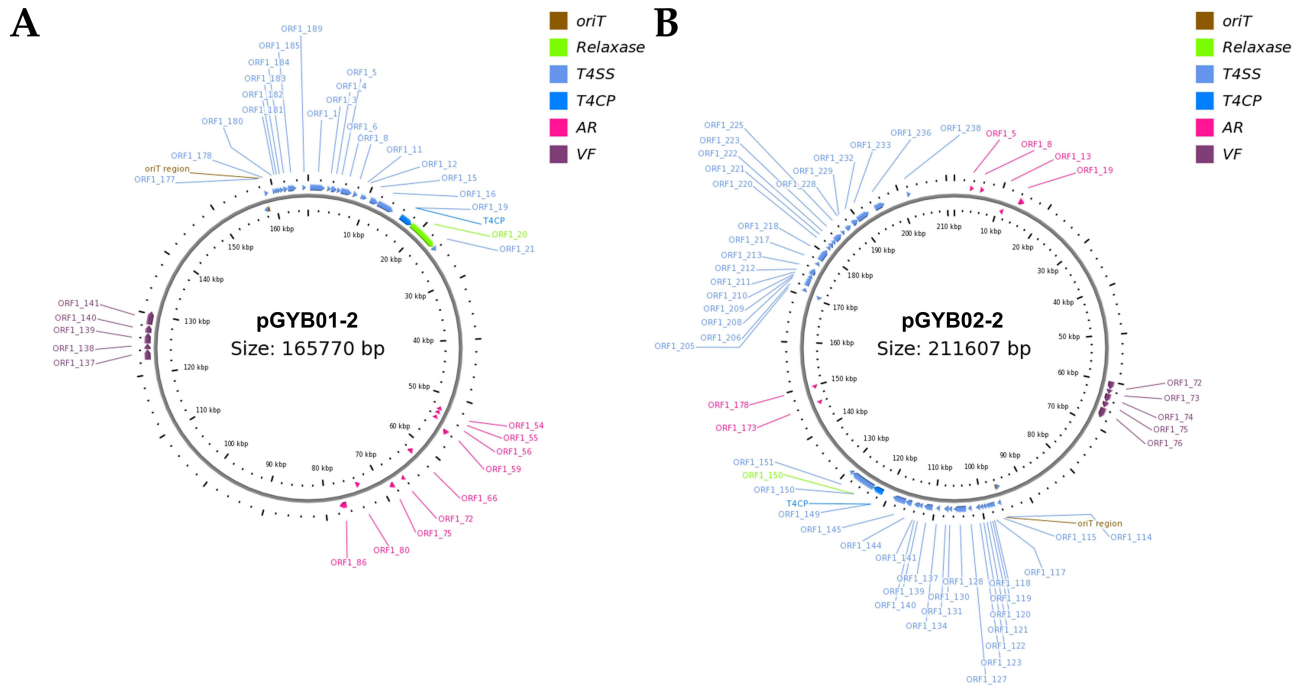

Figure 2 Two conjugative plasmids pGYB01-2 (**A**) and pGYB02-2 (**B**).
Abbreviation: AR (ARGs), antibiotic resistance genes; VF, virulence factors.

GYB02 to *E. coli* J53 with a frequency of 2×10^{-4}-2×10^{-3} per recipient cell. The *E. coli* J53 transconjugant exhibited resistance to minocycline, cefepime, cefoperazone/sulbactam, ceftazidime, imipenem, meropenem, piperacillin/tazobactam, trimethoprim/sulfamethoxazole, and doxycycline (Table 1). Simultaneously, the WGS data confirmed the presence of *aadA5, bla*$_{\text{NDM-5}}$, Δ*dfrA17, sul1, tet*(B) in the *E. coli* J53 transconjugant (Table 1).

Virulence Assessment of GYB01 and GYB02 in a *G. Mellonella* Infection Model

The virulence potential of GYB01 and GYB02 was assessed using a *G. mellonella* infection model. As depicted in Figure 3, the survival rates of *G. mellonella* larvae injected with GYB02 were notably lower compared to those injected

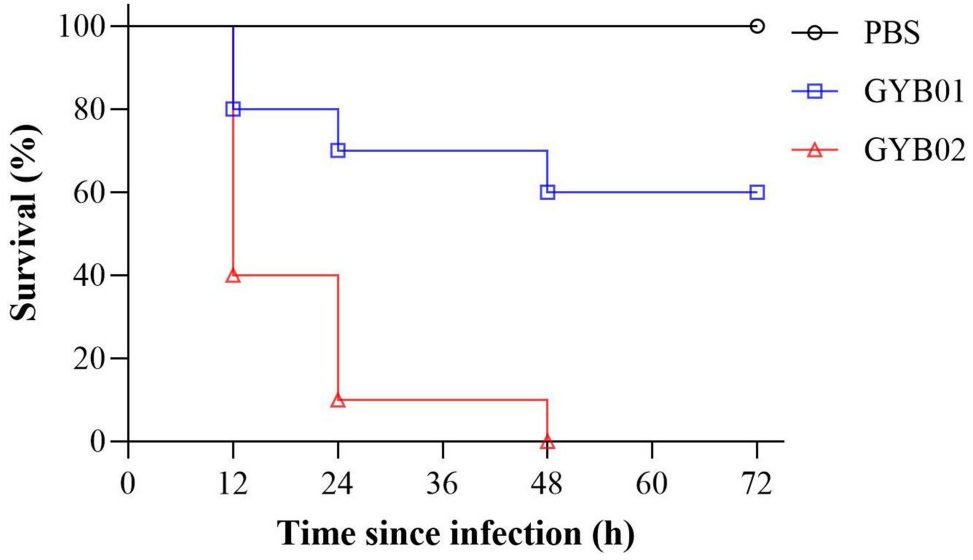

Figure 3 Kaplan-Meier survival curves of *Galleria mellonella* infected with GYB01 and GYB02. *G. mellonella* larvae (n = 10) were inoculated with 1×10^{6} colony-forming units (CFU)/10 μL per larva of GYB01, GYB02 or PBS (phosphate-buffered saline). Survival was recorded at 12-hour intervals for a total duration of 72 hours. Curves represent average counts for triplicate experiments.

with GYB01 ($P = 0.0023$; Survival analysis, Log-rank Mantel-Cox test). At 12 hours, the survival rate for GYB02 was 40%, while for GYB01 it was 80%. At 24 hours, the survival rate for GYB02 decreased to 10%, whereas for GYB01 it was 70%. Finally, at 48 hours, the survival rate for GYB02 dropped to 0%, while for GYB01 it remained at a relatively higher rate of 60%. There was no lethality observed among the PBS-inoculated control group. The results indicate that GYB02 strain exhibited higher virulence compared to GYB01 strain. Considering the similarity of virulence genes (*ΔiucA, ΔiucC, ΔiucD*, and *ΔiutA*) found on the plasmid it is probable that dispersion in pathogenicity between the two strains is attributed to virulence genes originating from the chromosomes (Table S1). Meanwhile, GYB02 tested positive for the string test and also harbored various virulence factors, including *ΔiucA, ΔiucC, ΔiucD*, and *ΔiutA* (Figure 1). Taken together, GYB02 was identified as a hypervirulent *Klebsiella pneumoniae* (hvKp) strain.

Discussion

Both GYB01 and GYB02 exhibited similar antibiotic resistance profiles, demonstrating resistance to a broad range of antibiotics commonly used to treat *Enterobacteriaceae* infections. WGS revealed that both strains harbored an IncFIA plasmid carrying the $bla_{\text{NDM-5}}$ gene, which confers resistance to carbapenems. When we used the BLAST server to search for similarities between the pGYB02-2 sequence and those deposited in the NCBI nucleotide collection (nr/nt) database (29 March 2024), we were surprised to find that the pGYB01-2 is most similar to pGYB02-2 (99.96% identity and 89% coverage), followed by pOC1092_1 (GenBank accession no. CP128670.1), pCTXM-GZ04 (CP042337.1), punnamed1 (CP027130.1), and ptig00001069_pilon (CP021880.1) (≥ 99.8% identity and ≥ 70% coverage) (Figure 1). Both strains were concurrently isolated from the patient's ascitic fluid, and the conjugation experiment demonstrated the ability of the $bla_{\text{NDM-5}}$ carrying plasmid pGYB02-2 to transfer from *Klebsiella pneumoniae* to *Escherichia coli*. These findings suggested a possible transmission event between these two strains. Multiple studies have shown that similar plasmids carrying the carbapenemase gene have been identified in different bacteria from the same patient. These include the $bla_{\text{NDM-1}}$ gene on IncFIA plasmids in *K. pneumoniae* and *Proteus mirabilis*,[15] the $bla_{\text{KPC-2}}$ gene on ColRNAI plasmids in *K. pneumoniae, Citrobacter europaeus*, and *Morganella morganii*,[16] as well as the $bla_{\text{KPC-3}}$ gene on IncFIB plasmids in *K. pneumoniae* and *Serratia marcescens*.[17] This suggests the presence of interspecies transmission of carbapenemase genes in patients with multiple bacterial infections. In addition, plasmid pGYB02-2 is larger than pGYB02-1, possibly due to breakage and recombination events that occurred during the conjugation process.[18] The $bla_{\text{NDM-5}}$ gene in the plasmid pGYB02-2 was found within a genetic context that includes IS15DI-*dfrA17-aadA5-sul1-*$bla_{\text{NDM-5}}$-ΔIS1R. Within the NCBI nucleotide collection (nr/nt) database there were a total of 47 sequences exhibiting a high similarity to the genetic context (≥ 90% identity and coverage), with 42 of them originating specifically from plasmids of *Escherichia coli* (17 August 2024; data not shown). Interestingly, in comparison to the plasmid pCTXM-GZ04 (Figure 1), it appears that pGYB01-2 and pGYB02-2 have acquired the complete gene structure (IS15DI-*dfrA17-aadA5-sul1-*$bla_{\text{NDM-5}}$-ΔIS1R). This finding suggests that the identified gene structure may serve as a transferable unit that contributes to the dissemination of multidrug-resistant genes, such as $bla_{\text{NDM-5}}$.

The clinical characteristics of the patient revealed that he presented with fever following intestinal fistula surgery and was subsequently diagnosed with an infection caused by *Enterococcus avium*, carbapenem-resistant *Escherichia coli* (GYB01), and carbapenem-resistant *Klebsiella pneumoniae* (GYB02). Based on the patient's history of intestinal surgery, the uncommon occurrence of a simultaneous infection involving three bacteria, and the presence of these bacteria in the human gut microbiota, there was speculation that these bacteria, which were known to be linked to abdominal infections, might have originated from the intestine. Unfortunately, a fecal culture was not performed in this case.

Our results demonstrated that GYB02 exhibited significantly higher virulence compared to GYB01, as evidenced by lower survival rates of *G. mellonella* larvae injected with GYB02. Additionally, GYB02 tested positive for the string test and harbored multiple resistant genes and virulence factors, indicating that it is the carbapenem-resistant hypervirulent *Klebsiella pneumoniae* (CR-HvKP) strain. Infections caused by CR-HvKP have been documented in various regions,[19,20] underscoring the critical importance of strengthening surveillance efforts, implementing effective infection control measures, and advancing the development of novel therapeutic interventions.

Conclusions

Our study provides direct evidence of potential in vivo inter-species transfer of a multidrug-resistant plasmid carrying the bla_{NDM-5} gene. This comprehensive genomic analysis of MDR *K. pneumoniae* and *E. coli* provides a deep understanding of their MDR mechanism and elucidates the potential horizontal transmission of antibiotic resistance genes, thus providing a basis for the containment and treatment of these bacteria. Furthermore, the potential transmission of the carbapenemase gene facilitates the conversion of HvKP into CR-HvKP, which may cause severe infections.

Nucleotide Sequence Accession Numbers

The sequences mentioned in the present study were submitted to the GenBank nucleotide database with the following accession numbers: CP125731 (chromosome of GYB01), CP125732 (pGYB01-1), CP125733 (pGYB01-2), CP125734 (chromosome of GYB02), CP125735 (pGYB02-1), and CP125736 (pGYB02-2).

Ethics Approval

Institutional approval was required to publish the case details. And this study has been approved by the Ethics Committee of the Third Affiliated Hospital of Zhengzhou University to publish the case details (Ethical Approval Number: 2023-256-01). The study was conducted in accordance with the Declaration Helsinki, with data anonymized and patient confidentiality ensured.

Consent for Publication

Written informed consent was obtained from the patient's parents for publication of this case report. A copy of the written consent is available by request.

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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