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SHORT REPORT

Genomic Characterization of a Carbapenem-Resistant *Raoultella planticola* Strain Co-Harboring bla_{IMP-4} and bla_{SHV-12} Genes

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Abstract: *Raoultella planticola* is an emerging bacterial pathogen responsible for causing infections in both humans and animals. Unfortunately, sporadic reports of carbapenem-resistant *R. planticola* (CRRP) have been documented worldwide. Here we first reported the complete genome sequence of a CRRP isolate RP_3045 co-carrying bla_{IMP-4} and bla_{SHV-12}, recovered from a patient in China, and its genetic relatedness to 82 *R. planticola* strains deposited in the NCBI GenBank database, sourced from humans, animals, and the environment. Whole-genome sequencing was performed using the Illumina NovaSeq 6000 and Oxford Nanopore MinION platforms. Phylogenetic analysis was also performed and visualized using a single nucleotide polymorphism (SNP)-based strategy. The complete genome of *R. planticola* strain RP_3045 was determined to be 6,312,961 bp in length, comprising five contigs that included one chromosome and four plasmids. RP_3045 was found to be multidrug-resistant and harbored several antimicrobial resistance genes, including both bla_{IMP-4} and bla_{SHV-12} genes located on a single plasmid. The most closely related strain was hkcpe63, recovered from humans in Hong Kong, China, in 2014, with 506 SNP differences. *R. planticola* strains were distributed globally and exhibited strong associations among isolates obtained from different sectors. This study provides evidence for the potential of *R. planticola* to disseminate carbapenem resistance across different sectors, highlighting the critical need for active and continuous surveillance of CRRP.

Keywords: *Raoultella planticola*, bla_{IMP-4}, bla_{SHV-12}, antimicrobial resistance

Introduction

Carbapenems were considered the preferred treatment for severe gram-negative bacterial infections in clinical settings due to their broad-spectrum antibacterial activity and low toxicity. However, their extensive use has led to the development and global spread of carbapenem resistance, causing major public health concerns.[1,2] Carbapenem resistance in gram-negative bacteria is mainly attributed to the production of carbapenemases, which include class A (KPC and GES), class B (IMP, NDM, and VIM), and class D (OXA-48 and its variants).[3] Notably, carbapenemase genes are primarily located on conjugative plasmids, facilitating the horizontal transfer of carbapenem resistance between different species and severely limiting treatment options.[4,5] Although carbapenemases have been frequently identified in other genera, their emergence in the *Raoultella* genus has been uncommon.

Raoultella are non-motile, gram-negative, aerobic bacilli that include several species, i.e., *R. planticola, R. terrigena, R. ornithinolytica*, and *R. electrica*. Originally classified under the genus *Klebsiella*, they were reclassified as *Raoultella* in 2001 based on the 16S rRNA and *rpoB* genes.[6] *Raoultella* were previously regarded as environmental organisms that were mostly found in water and soil.[7,8] However, in recent years, they have emerged as virulent pathogens causing human and animal infections worldwide, with *R. planticola* being the most common species.[9,10] Carbapenems are highly effective against

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R. planticola, but reduced susceptibility has been observed due to the acquisition of carbapenemase genes, such as bla_{KPC-2}, bla_{OXA-48}, and bla_{NDM-1}.[7,8,10,11] Carbapenem-resistant *R. planticola* (CRRP) has been frequently recovered from humans, animals, and the associated environment globally, including in China, Japan, the USA, Germany, and Canada.[7,8,10–12] This highlights the potentially underappreciated importance of this bacterial species in the transmission of antimicrobial resistance, making it difficult to effectively treat severe infections and posing a significant threat to human health.

Whole-genome sequencing (WGS) has completely revolutionized the field of bioscience and is now recognized as a crucial tool in understanding the spread of pathogenic bacteria. This technology plays an important role in identifying transmission routes, discerning evolutionary patterns, and understanding antimicrobial resistance mechanisms.[13] Continuous advancements and breakthroughs in WGS technology have enabled the generation of extensive sequence data with reduced costs. Whole-genome comparisons can also identify similarities and differences between genomes, providing insights into evolutionary relationships and genetic variation in antimicrobial resistance determinants.[14,15] However, genomic studies on *R. planticola* regarding evolution and genetic variation are extremely limited.

In this study, to our knowledge for the first time, we characterized the genomic environment of a CRRP isolate obtained from one patient in China, which co-carried bla_{IMP-4} and bla_{SHV-12} and conferred elevated levels of carbapenem resistance. Additionally, we performed a thorough comparative genomic analysis to understand the phylogenetic relationship and dissemination of antimicrobial resistance among the global *R. planticola* strains.

Materials and Methods

Antimicrobial Susceptibility Testing

In April 2021, *R. planticola* strain RP_3045 was isolated from a patient's bronchoalveolar lavage fluid (BALF) in Hangzhou, Zhejiang Province, China. The strain was identified using VITEK 2 (bioMérieux, Marcy-l'Étoile, France), matrix-assisted laser desorption/ionization-time-of-flight mass spectrometry (MALDI-TOF-MS, Bruker, Billerica, MA, United States), and 16S rRNA gene sequencing.

R. planticola strain RP_3045 was subjected to antimicrobial susceptibility testing using the broth microdilution method, evaluating clinically relevant antimicrobial agents commonly used in infection treatment. The tested agents comprised cefuroxime, cefoxitin, ceftazidime, ceftriaxone, aztreonam, cefepime, ertapenem, imipenem, meropenem, amoxicillin-clavulanic acid, cefoperazone-sulbactam, amikacin, levofloxacin, fosfomycin, trimethoprim-sulfamethoxazole, colistin, and tigecycline. The minimum inhibitory concentrations (MICs) were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) 2020 guidelines, and the results of tigecycline and colistin were interpreted according to the European Committee on Antimicrobial Susceptibility Testing (EUCAST) 10.0 guidelines. *Escherichia coli* ATCC 25922 was used as a quality control strain for MIC determination.

Whole-Genome Sequencing and Bioinformatics Analysis

The genomic DNA of *R. planticola* strain RP_3045 was extracted and purified using the QIAamp DNA Mini Kit (Qiagen, Valencia, CA, United States). WGS was performed on both the Illumina NovaSeq 6000 (Illumina, San Diego, CA, USA) and Oxford Nanopore MinION (Oxford Nanopore Technologies, Oxford, UK) platforms.[16] The resulting hybrid genome sequences were assembled using Unicycler v0.4.8, incorporating both short, accurate Illumina reads and long, less accurate Nanopore reads in conservative mode.[17] The highly accurate Illumina reads were aligned against Nanopore reads for error correction, resulting in a high-quality genome assembly. Further refinement involved multiple rounds of polishing with the BWA-MEM algorithm using Illumina paired-end reads and Pilon v1.2.3.[18] The quality assessment of the assembled genome was conducted using CheckM v1.0.18.[19]

The complete genome sequence of RP_3045 was automatically annotated using the NCBI Prokaryotic Genomes Annotation Pipeline (PGAP).[20] acquired antimicrobial resistance genes, virulence genes, and plasmid incompatibility types were identified using ABRicate 1.0.1 in tandem with ResFinder 4.1, VFDB 2022, and PlasmidFinder 2.1 databases.[21–23] Genomic islands, insertion sequence (IS) elements, prophage sequences, clustered regularly interspaced short palindromic repeat (CRISPR) sequences, secondary metabolite gene clusters, and integrative and conjugative elements (ICEs) were predicted with Island Viewer 4, ISfinder 1.0, Prophage Hunter, CRISPRCasFinder 1.0, antiSMASH 6.1.1, and ICEberg 3.0

tools, respectively.[24–29] Circular comparisons among multiple plasmids were generated using the BLAST Ring Image Generator (BRIG).[30] The genetic surroundings of antimicrobial resistance genes and the homologous regions between plasmids were generated using Easyfig 2.2.5.[31]

Conjugation Assay

Conjugation experiments were conducted using the filter mating method. *R. planticola* strain RP_3045 was used as the donor, and the sodium azide-resistant *E. coli* strain J53 served as the recipient. Overnight cultures of *R. planticola* RP_3045 and *E. coli* J53 in Luria-Bertani (LB) broth were adjusted to a 0.5 MacFarland standard. After incubating a 5 μL aliquot of each culture in 1 mL of fresh LB broth for 4 h, RP 3045 (40 μL) and J53 (40 μL) were conjugated on sterilized GF/A glass microfiber filters (Whatman International Ltd., Maidstone, England), and the filter was incubated on an LB agar plate overnight. Transconjugants were selected on Muller-Hinton agar plates containing meropenem (4 mg/L) and sodium azide (100 mg/L). The presence of *the blaIMP-4* gene in transconjugants was confirmed both using PCR and Sanger sequencing.

Phylogenetic Analysis

As of 27 September 2023, genomic sequences and corresponding metadata for 82 *R. planticola* strains deposited in the NCBI GenBank database, excluding five strains with inconclusive or failed taxonomy, were obtained. Whole-genome sequences of all strains were uploaded and analyzed on the CSI Phylogeny 1.4 pipeline (https://cge.food.dtu.dk/services/CSIPhylogeny/), which recognizes, filters, and validates the location of single nucleotide polymorphisms (SNPs). Based on a concatenated alignment of the high-quality SNPs, a phylogenetic tree was constructed between *R. planticola* strain RP_3045 and the 82 *R. planticola* strains with the default parameters. Visualization and annotation of the phylogenetic tree were performed using the Interactive Tree of Life (iTOL) V6.[32]

Results and Discussion

Genomic Characteristics

The complete genome sequence of *R. planticola* strain RP_3045 consisted of five contigs comprising 6,312,961 bp. Among them, contig 1 (5,722,527 bp) belonged to the chromosome, and the remaining contigs all belonged to different plasmids (contig 2: 260,053 bp; contig 3: 212,569 bp; contig 4: 113,157 bp; and contig 5: 4655 bp). A total of 5,941 coding sequences (CDSs) and 125 RNAs (25 rRNA, 87 tRNA, and 13 ncRNA) genes were identified in RP_3045, with an overall G+C content of 55.1%. The genome included at least 58 genomic islands and numerous IS elements, with the majority belonging to the IS*3*, IS*5*, and IS*1202* families. Three CRISPR sequences were predicted, but no prophages. Additionally, four putative secondary metabolite gene clusters were identified in the genome, including those for aryl polyenes, O-antigen, and enterobactin biosynthesis, which play crucial roles in antimicrobial activity, outer membrane structure, and iron acquisition, respectively.

Phenotypic and Genotypic Antimicrobial Resistance

R. planticola strain RP_3045 was found to be multidrug resistant (Table 1). According to the antimicrobial susceptibility testing data, RP_3045 was resistant to β-lactams (cefuroxime, cefoxitin, ceftazidime, ceftriaxone, aztreonam, cefepime, ertapenem, imipenem, meropenem, amoxicillin-clavulanic acid, and cefoperazone-sulbactam), fluoroquinolones (levofloxacin), fosfomycins (fosfomycin), and glycylcyclines (tigecycline). However, it remained susceptible to aminoglycosides (amikacin), sulphonamides (trimethoprim-sulfamethoxazole), and polypeptides (colistin). In accordance with its multidrug-resistant profile, RP_3045 harbored several antimicrobial resistance genes that mediated resistance to β-lactams ($bla_{\text{IMP-4}}$, $bla_{\text{PLA-5A}}$, and $bla_{\text{SHV-12}}$), aminoglycosides [*aph(3')-Ib, aph(6)-Id*, and *aac(3)-IId*], fluoroquinolones (*qnrS1*), fosfomycins (*fosA* and *fosA3*), sulphonamides (*sul1* and *sul2*), phenicols (*catA2*), and antiseptics (*qacE*) (Table 1). The resistance to third-generation cephalosporins and carbapenems was attributed to the presence of two extended-spectrum β-lactamase (ESBL) genes, namely $bla_{\text{SHV-12}}$ and $bla_{\text{PLA-5A}}$, as well as one carbapenemase gene, $bla_{\text{IMP-4}}$. To our knowledge, we are the first to report a CRRP isolate co-carrying $bla_{\text{IMP-4}}$, $bla_{\text{SHV-12}}$, and $bla_{\text{PLA-5A}}$ in China. Nevertheless, the global detection of carbapenemase and ESBL-co-producing *R. planticola* strains highlights the

Table 1 Antimicrobial Susceptibility and Antimicrobial Resistance Genes of the *R. Planticola* Strain RP_3045

Antimicrobial Agents	MIC (mg/L)	Susceptibility	Antimicrobial Resistance Genes
β-lactams			
Cefuroxime	>64	R	bla_{PLA-5A}, bla_{SHV-12}, bla_{IMP-4}
Cefoxitin	>64	R	
Ceftazidime	>64	R	
Ceftriaxone	>64	R	
Aztreonam	>128	R	
Cefepime	32	R	
Ertapenem	16	R	
Imipenem	16	R	
Meropenem	8	R	
Amoxicillin-clavulanic acid	>32	R	
Cefoperazone-sulbactam	>64	R	
Aminoglycosides			
Amikacin	≤2	S	*aph(3')-Ib, aph(6)-Id, aac(3)-IId*
Fluoroquinolones			
Levofloxacin	2	R	*qnrS1*
Fosfomycins			
Fosfomycin	>512	R	*fosA, fosA3*
Sulphonamides			
Trimethoprim-sulfamethoxazole	≤20	S	*sul1, sul2*
Lipoglycopeptides			
Colistin	0.5	S	
Glycylcyclines			
Tigecycline	2	R	–

need for heightened attention.[7,8,12] Besides the ESBLs, the presence of additional resistance genes in *R. planticola* strain RP_3045 enabled it to endure the onslaught of antimicrobials used to treat infections. Fortunately, colistin was still efficacious in vitro and could serve as an effective anti-infective option.

Virulence Characteristics

In silico analysis revealed the presence of 21 virulence genes in *R. planticola* strain RP_3045. Gene clusters encoding type 1 fimbriae (*fimD*) and type 3 fimbriae (*mrkABC*) were identified, indicating their involvement in facilitating bacterial adherence to surfaces and contributing to biofilm formation. Furthermore, the presence of genes encoding capsule formation (*galF, gnd, ugd*, and *wzi*) and lipopolysaccharide (LPS) synthesis (*manB* and *manC*) suggested a role in immune evasion. The AcrAB efflux pump genes (*acrA* and *acrB*) confer antimicrobial resistance and are associated with evading innate immune defense, while the enterobactin genes (*entBFS* and *fepADG*) facilitate iron uptake, enhancing bacterial survival in host environments. The RcsAB regulatory system (*rcsB*) is involved in the control of capsule synthesis, and the type VI secretion system genes (*hcp*/*tssD* and *tssF*) suggest a function in delivering effectors for host interactions.

Genetic Features and Transferability of *BlaIMP-4*-Bearing Plasmid p3045-1

Plasmid p3045-1 is a 260,053 bp circular plasmid with no replicon typing predicted. BLAST search of the plasmid sequences against NCBI GenBank database showed that p3045-1 is highly similar to several bla_{IMP}-harboring IncU-type plasmids isolated in China, eg, pRo24724 (GenBank accession no. CP021328) from *Raoultella ornithinolytica*, as well as pRes_C1672 (GenBank accession no. CP073918) and p13450-IMP (GenBank accession no. MF344564) from *Klebsiella pneumoniae* (Figure 1A). However, p3045-1 also possessed several unique genes encoding ATPase, MbcA/ParS/Xre family antitoxin, and two site-specific integrases.

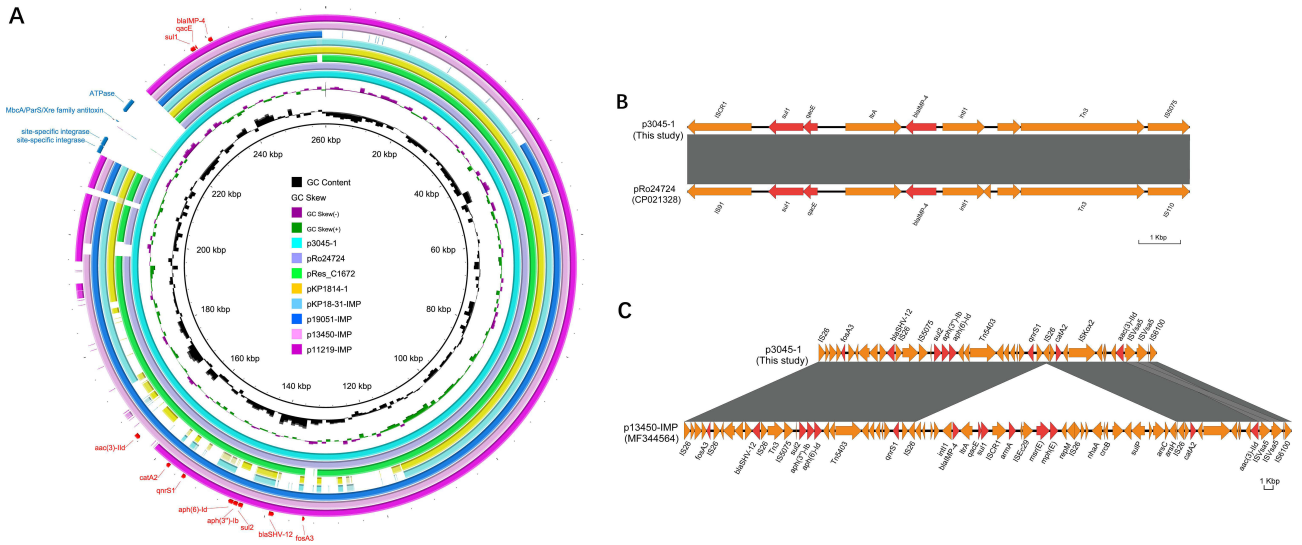

Figure 1 Genetic organization of plasmids carrying the bla_{IMP} gene. (**A**) Circular comparison of plasmid p3045-1 with homologous plasmids harboring the bla_{IMP} gene in NCBI GenBank database. In the outer circle, red arrows represent antimicrobial resistance genes, while blue arrows denote unique genes specific to plasmid p3045-1. (**B**) Structural alignment of the genetic environments surrounding the bla_{IMP-4} gene. (**C**) Structural alignment of the genetic environments surrounding the bla_{SHV-12} gene. Antimicrobial resistance genes are depicted by red arrows, while additional coding sequences are indicated by Orange arrows. Regions of >90% nucleotide sequence identity are shaded in gray.

Besides bla_{PLA-5A} and *fosA*, all other antimicrobial resistance genes, including carbapenemase gene bla_{IMP-4} and β-lactamase gene bla_{SHV-12}, were located on plasmid p3045-1. The genetic context analysis of the bla_{IMP-4} gene revealed its placement within a class 1 integron. Furthermore, it was observed to be preceded by the highly conserved *intI1*-ORF-Tn *3*-IS*5075* segment, while the *ltrA-qacE-sul1*-IS*CR1* segment was located in the downstream region (Figure 1B). Another multidrug-resistant region, carrying bla_{SHV-12}, *fosA3, sul2, aph(3')-Ib, aph(6)-Id, qnrS1, catA2*, and *aac(3)-IId*, was also found on plasmid p3045-1. The bla_{SHV-12} gene was flanked by two copies of IS*26* transposases, with IS*5075* lying upstream; however, no integrase was identified in the adjacent position (Figure 1C).

To evaluate the transferability of bla_{IMP-4} and bla_{SHV-12} carrying plasmid, conjugation experiments were conducted using RP_3045 as the donor and *E. coli* strain J53 as the recipient. Despite repeated attempts, no transconjugants were obtained, indicating the non-transferability of the bla_{IMP-4}-bearing plasmid p3045-1. This observation is in line with the absence of detectable conjugative modules on the aforementioned plasmid, including integrase, relaxase, T4CP (Type IV coupling protein), VirB4/TraU or full T4SS (Type IV secretion system), Rep (Replication initiator protein), Tra (Translocation protein), OriT (Origin site of DNA transfer).

Global Prevalence and Phylogenetic Analysis of *R. planticola* Strains

Phylogenetic comparison of *R. planticola* strain RP_3045 with a total of 82 *R. planticola* strains from NCBI GenBank database was performed with categories of phylogroups, antimicrobial resistance genes, continent, isolation sources, and collection years (Figure 2). The majority of the 83 *R. planticola* strains were isolated from humans (n=45, 54.2%) and the environment (n=27, 32.5%), followed by animals (n=9, 10.8%) and unknown sources (n=2, 2.4%). Collections of strains span from 1954 to 2022, with most being collected from 2011–2020 (n=52, 62.7%). These *R. planticola* strains were mainly found in North America (n=28, 33.7%), followed by Asia (n=21, 25.3%), Europe (n=19, 22.9%), Oceania (n=4, 4.8%), Africa (n=2, 2.4%), and South America (n=2, 2.4%).

According to the phylogenetic analysis, the closest relatives of RP_3045 were hkcpe63 (GenBank accession no. DACSEQ010000000) recovered from a human rectal specimen in Hong Kong, China in 2014 (differing by 506 SNPs), as well as 2022HL-01644 (GenBank accession no. ABHTPZ010000000) and 2022HL-01626 (GenBank accession no. ABHTOY010000000) from human rectal specimens in the USA in 2022 (both differing by 518 SNPs). Strain hkcpe63 also harbored bla_{IMP-4} and bla_{PLA-5A}, but not bla_{SHV-12}. All 83 *R. planticola* strains were examined for their antimicrobial resistance gene profiles, revealing random acquisition of carbapenemase genes in these isolates. Among

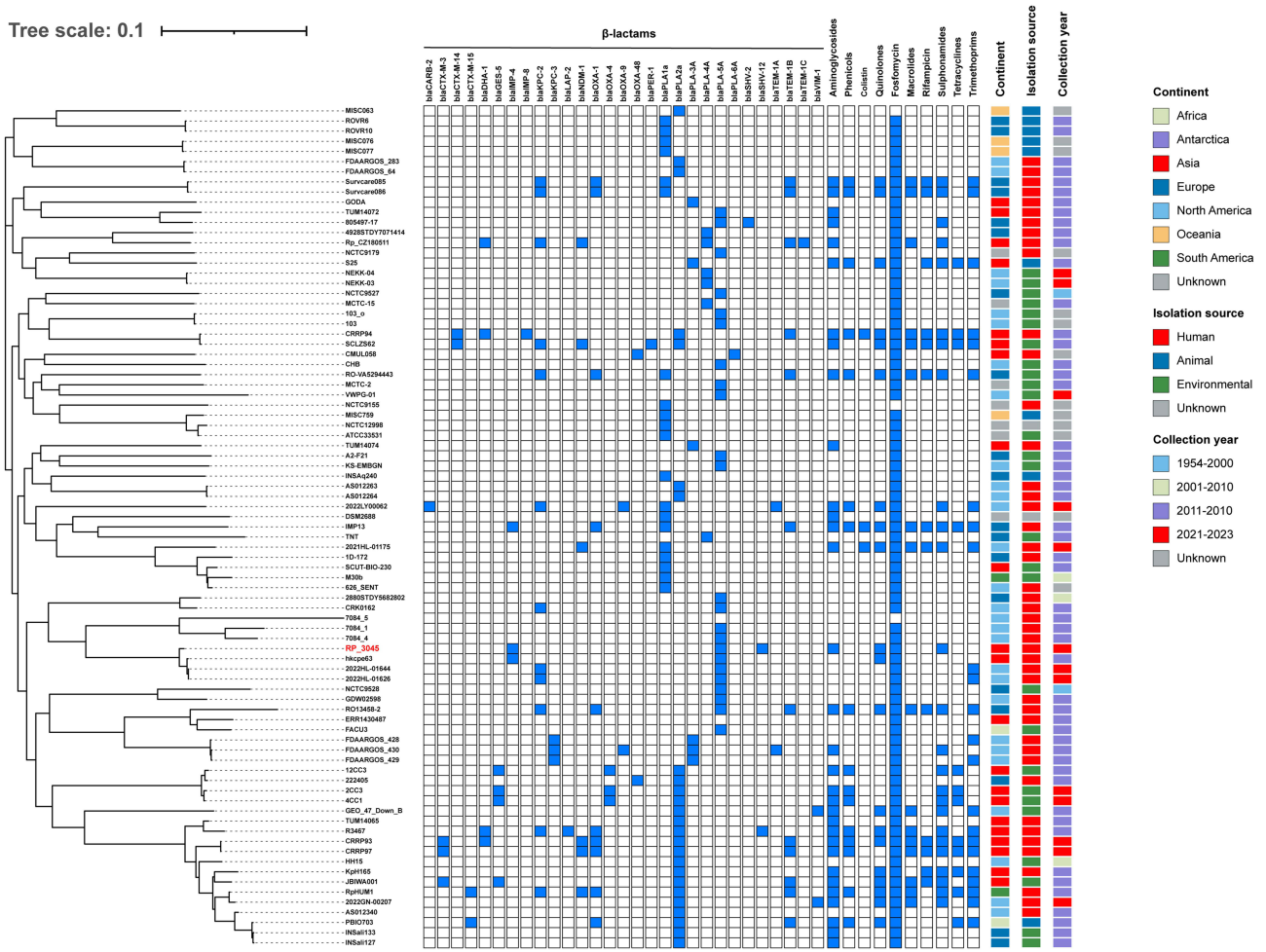

Figure 2 Phylogenetic relationship and antimicrobial resistance genes distribution between *R. planticola* RP_3045 and a total of 82 *R. planticola* strains currently deposited in NCBI GenBank database. Strain IDs are labeled in compliance with the tree, with RP_3045 highlighted in red. The distance of SNPs is represented by the branch length. Antimicrobial classes are labeled at the top of the figure. Blue cells indicate the presence of the antimicrobial resistance genes, and white cells indicate their absence. The continent, isolation source, and collection year are indicated for each strain.

these genes, $bla_{\text{KPC-2}}$ appeared most frequently (n=10, 13.0%), followed by $bla_{\text{TEM-1B}}$ (n=8, 10.4%) and $bla_{\text{OXA-1}}$ (n=7, 9.1%). Besides RP_3045, only two strains, namely IMP13 (GenBank accession no. CAIZTF010000000) isolated from humans in the United Kingdom in 2017 and hkcpe63, contained the $bla_{\text{IMP-4}}$ gene.

Notably, the transmission of *R. planticola* strains with resistance genes is easier within or among humans, animals, and the associated environment, spanning across the globe. Strain 626_SENT (GenBank accession no. JUZO01000000), which was isolated from a clinical patient in the USA in 2015, was present in a cluster with SCUT-BIO-230 (GenBank accession no. JANHGU010000000), obtained from organic-polluted wastewater in China in 2020 (differing by 580 SNPs), and M30b (GenBank accession no. VIIV01000000), recovered from explosives-contaminated soils in Colombia in 2009 (differing by 1062 SNPs). Another noteworthy instance was strain PBIO703 (GenBank accession no. CADCYG010000000), isolated from Musca domestica in Rwanda in 2014. It displayed a close relationship with INSali127 and INSali133 (GenBank accession no. LSUV01000000 and LSUW01000000), obtained from vegetables in Portugal in 2013 (differing by 92 and 89 SNPs, respectively), as well as AS012340 (GenBank accession no. VKWN01000000), recovered from a patient with lung disease in the USA in 2014 (differing by 838 SNPs). These findings indicated the global distribution of this bacterium and even its resistance determinants across diverse sectors.

Conclusion

In summary, this study firstly represented the complete genome sequence of a CRRP isolate RP_3045 co-carrying bla_{IMP-4} and bla_{SHV-12}, recovered from a patient in China. Furthermore, comparative genomic analysis showed a close genetic connection between *R. planticola* strains recovered from humans, animals, and the environment. The study provides valuable insights into the genetic relationships and resistance patterns of *R. planticola* strains, shedding light on their adaptability and dissemination in diverse ecological niches.

Data Sharing Statement

The genome sequences for both the chromosome and plasmids of *R. planticola* strain RP_3045 have been deposited in NCBI GenBank under accession no. CP114772-CP114776.

Ethics Approval

This study was reviewed and approved by the Ethics Committee of Sir Run Run Shaw Hospital, Zhejiang University School of Medicine, China. The study was considered exempt from the requirement of informed consent as no identifiable patient information was collected, and only residual samples (after routine clinical testing) were collected in the clinical laboratory.

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Disclosure

The authors report no conflicts of interest in this work.

References

1. Salamzade R, Manson AL, Walker BJ, et al. Inter-species geographic signatures for tracing horizontal gene transfer and long-term persistence of carbapenem resistance. *Genome Med*. 2022;14(1):37. doi:10.1186/s13073-022-01040-y
2. Theuretzbacher U, Bush K, Harbarth S, et al. Critical analysis of antibacterial agents in clinical development. *Nat Rev Microbiol*. 2020;18(5):286–298. doi:10.1038/s41579-020-0340-0
3. Nordmann P, Poirel L. Epidemiology and Diagnostics of Carbapenem Resistance in Gram-negative Bacteria. *Clinl Infect Dis*. 2019;69(Suppl 7):S521–S528. doi:10.1093/cid/ciz824
4. Gao H, Liu Y, Wang R, Wang Q, Jin L, Wang H. The transferability and evolution of NDM-1 and KPC-2 co-producing Klebsiella pneumoniae from clinical settings. *EBioMedicine*. 2020;51:102599. doi:10.1016/j.ebiom.2019.102599
5. Kopotsa K, Osei Sekyere J, Mbelle NM. Plasmid evolution in carbapenemase-producing Enterobacteriaceae: a review. *Ann N Y Acad Sci*. 2019;1457(1):61–91. doi:10.1111/nyas.14223
6. Drancourt M, Bollet C, Carta A, Rousselier P. Phylogenetic analyses of Klebsiella species delineate Klebsiella and Raoultella gen. nov., with description of Raoultella ornithinolytica comb. nov., Raoultella terrigena comb. nov. and Raoultella planticola comb. nov. *Int J Syst Evol Microbiol*. 2001;51(Pt 3):925–932. doi:10.1099/00207713-51-3-925
7. Li Y, Qiu Y, Gao Y, et al. Genetic and virulence characteristics of a Raoultella planticola isolate resistant to carbapenem and tigecycline. *Sci Rep*. 2022;12(1):3858. doi:10.1038/s41598-022-07778-0
8. Zou H, Berglund B, Wang S, et al. Emergence of bla(NDM-1), bla(NDM-5), bla(KPC-2) and bla(IMP-4) carrying plasmids in Raoultella spp. in the environment. *Environ Pollut*. 2022;306:119437. doi:10.1016/j.envpol.2022.119437
9. Fukuda Y, Adachi S, Saito M, Shoji R, Togashi A. A rare pathogen Raoultella planticola caused urinary tract infection in child with congenital anomalies of kidney and urinary tract: case report. *Transl Pediatr*. 2021;10(9):2387–2391. doi:10.21037/tp-21-170
10. Tseng SP, Wang JT, Liang CY, Lee PS, Chen YC, Lu PL. First Report of bla(IMP-8) in Raoultella planticola. *Antimicrob Agents Chemother*. 2014;58(1):593–595. doi:10.1128/AAC.00231-13
11. Zhao Y, Tang N, Jia R, et al. Co-existence of the carbapenem resistance genes bla(KPC-2) and bla(NDM-1) in a Raoultella planticola isolate in China. *J Global Antimicrob Resist*. 2020;23:327–328. doi:10.1016/j.jgar.2020.09.015
12. Tufa TB, Fuchs A, Feldt T, et al. CTX-M-9 group ESBL-producing Raoultella planticola nosocomial infection: first report from sub-Saharan Africa. *Ann Clin Microbiol Antimicrob*. 2020;19(1):36. doi:10.1186/s12941-020-00380-0
13. Schurch AC, Arredondo-Alonso S, Willems RJL, Goering RV. Whole genome sequencing options for bacterial strain typing and epidemiologic analysis based on single nucleotide polymorphism versus gene-by-gene-based approaches. *Clin Microbiol Infect*. 2018;24(4):350–354. doi:10.1016/j.cmi.2017.12.016
14. Ruan Z, Feng Y. BacWGSTdb, a database for genotyping and source tracking bacterial pathogens. *Nucleic Acids Res*. 2016;44(D1):D682–D687. doi:10.1093/nar/gkv1004

15. Feng Y, Zou S, Chen H, Yu Y, Ruan Z. BacWGSTdb 2.0: a one-stop repository for bacterial whole-genome sequence typing and source tracking. *Nucleic Acids Res*. 2021;49(D1):D644–D650. doi:10.1093/nar/gkaa821
16. Wu Y, Jiang T, Bao D, et al. Global population structure and genomic surveillance framework of carbapenem-resistant Salmonella enterica. *Drug Resist Updates*. 2023;68:100953. doi:10.1016/j.drup.2023.100953
17. Wick RR, Judd LM, Gorrie CL, Holt KE. Unicycler: resolving bacterial genome assemblies from short and long sequencing reads. *PLoS Comput Biol*. 2017;13(6):e1005595. doi:10.1371/journal.pcbi.1005595
18. Walker BJ, Abeel T, Shea T, et al. Pilon: an integrated tool for comprehensive microbial variant detection and genome assembly improvement. *PLoS One*. 2014;9(11):e112963. doi:10.1371/journal.pone.0112963
19. Parks DH, Imelfort M, Skennerton CT, Hugenholtz P, Tyson GW. CheckM: assessing the quality of microbial genomes recovered from isolates, single cells, and metagenomes. *Genome Res*. 2015;25(7):1043–1055. doi:10.1101/gr.186072.114
20. Tatusova T, DiCuccio M, Badretdin A, et al. NCBI prokaryotic genome annotation pipeline. *Nucleic Acids Res*. 2016;44(14):6614–6624. doi:10.1093/nar/gkw569
21. Bortolaia V, Kaas RS, Ruppe E, et al. ResFinder 4.0 for predictions of phenotypes from genotypes. *J Antimicrob Chemother*. 2020;75(12):3491–3500. doi:10.1093/jac/dkaa345
22. Carattoli A, Zankari E, García-Fernández A, et al. In silico detection and typing of plasmids using PlasmidFinder and plasmid multilocus sequence typing. *Antimicrob Agents Chemother*. 2014;58(7):3895–3903. doi:10.1128/AAC.02412-14
23. Liu B, Zheng D, Zhou S, Chen L, Yang J. VFDB 2022: a general classification scheme for bacterial virulence factors. *Nucleic Acids Res*. 2022;50(D1):D912–d917. doi:10.1093/nar/gkab1107
24. Bertelli C, Laird MR, Williams KP, et al. IslandViewer 4: expanded prediction of genomic islands for larger-scale datasets. *Nucleic Acids Res*. 2017;45(W1):W30–w35. doi:10.1093/nar/gkx343
25. Blin K, Shaw S, Kloosterman AM, et al. antiSMASH 6.0: improving cluster detection and comparison capabilities. *Nucleic Acids Res*. 2021;49(W1):W29–w35. doi:10.1093/nar/gkab335
26. Couvin D, Bernheim A, Toffano-Nioche C, et al. CRISPRCasFinder, an update of CRISRFinder, includes a portable version, enhanced performance and integrates search for Cas proteins. *Nucleic Acids Res*. 2018;46(W1):W246–w251. doi:10.1093/nar/gky425
27. Siguier P, Perochon J, Lestrade L, Mahillon J, Chandler M. ISfinder: the reference centre for bacterial insertion sequences. *Nucleic Acids Res*. 2006;34(Database issue):D32–36. doi:10.1093/nar/gkj014
28. Song W, Sun HX, Zhang C, et al. Prophage Hunter: an integrative hunting tool for active prophages. *Nucleic Acids Res*. 2019;47(W1):W74–w80. doi:10.1093/nar/gkz380
29. Wang M, Liu G, Liu M, et al. ICEberg 3.0: functional categorization and analysis of the integrative and conjugative elements in bacteria. *Nucleic Acids Res*. 2024;52(D1):D732–D737. doi:10.1093/nar/gkad935
30. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics*. 2011;12:402. doi:10.1186/1471-2164-12-402
31. Sullivan MJ, Petty NK, Beatson SA. Easyfig: a genome comparison visualizer. *Bioinformatics*. 2011;27(7):1009–1010. doi:10.1093/bioinformatics/btr039
32. Letunic I, Bork P. Interactive Tree Of Life (iTOL) v5: an online tool for phylogenetic tree display and annotation. *Nucleic Acids Res*. 2021;49(W1):W293–W296. doi:10.1093/nar/gkab301