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SHORT REPORT

Genomic Characterization of Intestinal Colonizing *Pseudomonas juntendi* Strains Harboring bla_{VIM-2}

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Introduction: To characterize the genomic architecture of carbapenemase-producing *Pseudomonas juntendi* harboring bla_{VIM-2}, elucidate genetic mechanisms underlying carbapenem resistance, and evaluate mobile genetic element (MGE)-mediated dissemination pathways using Oxford Nanopore and Illumina sequencing were combined for hybrid genome assembly approaches.

Methods: Hybrid Nanopore-Illumina whole-genome sequencing was applied on two *P. juntendi* isolates (L2353hy/L2891hy) recovered from distinct human fecal samples. L2353hy and L2891hy were identified as *P. juntendi* by ANI analysis. Comparative pangenomics identified resistance determinants and phylogenetic relationships, and SNP distances were calculated using SNP-dists. Plasmid profiles were verified using S1 nuclease pulsed-field gel electrophoresis (S1-PFGE).

Results: Both strains exhibited a multidrug resistance profile, comprising 13 antimicrobial resistance genes (ARGs), including bla_{VIM-2}, $bla_{OXA-246}$, and *tet(A)*. Core genome phylogeny demonstrated clonal propagation of two VIM-producing *P. juntendi* strains. Notably, these two isolates were closely linked to *P. juntendi* yb_3 (a fish intestinal isolate; Wenzhou, China).

Conclusion: This study reports two clonally related *P. juntendi* strains harboring bla_{VIM-2} isolated from human fecal microbiota, expanding the genomic understanding of carbapenem-resistant *P. juntendi*. The close phylogenetic relationship between these human isolates and an animal-derived strain (*P. juntendi* yb_3) underscores bidirectional resistance gene flow at the human-animal interface. Our findings support a One Health-oriented surveillance approach to mitigate the dissemination of carbapenemase-producing pathogens.

Keywords: *Pseudomonas juntendi*, bla_{VIM-2}, whole-genome sequencing, antimicrobial resistance

Introduction

Pseudomonas juntendi, first described in 2019 from sputum samples of patients in Japan and Myanmar,[1] is an emerging member of the Pseudomonas putida group with increasing clinical relevance. Once considered an under-studied environmental species, it is now increasingly recognized as a potential nosocomial pathogen. Emerging clinical evidence highlights its role in opportunistic infections, particularly among immunocompromised individuals.[2] This Gram-negative bacterium is associated with multidrug-resistant (MDR) healthcare-associated infections, including ventilator-associated pneumonia and catheter-related bacteremia, with mortality rates exceeding 28% in ICU settings.[3]

The clinical significance of this species is compounded by its genomic plasticity, which facilitates the acquisition of horizontally transferred resistance determinants. Of particular concern is bla_{VIM-2}, a metallo-β-lactamase gene conferring carbapenem resistance, which has been detected in *P. juntendi* isolates from clinical and environmental sources,[4,5] However, the genetic context and transmission dynamics of this resistance gene remain poorly characterized.

A critical knowledge gap persists regarding the clonal spread and genomic stability of bla_{VIM-2} harboring *P. juntendi* in human populations, particularly within the intestinal microbiota—a potential reservoir for horizontal gene transfer. This study investigates two MDR *P. juntendi* strains isolated from human fecal specimens, aiming to characterize their resistomes, plasmid profiles, and evolutionary links to aquatic reservoirs using hybrid whole-genome sequencing.

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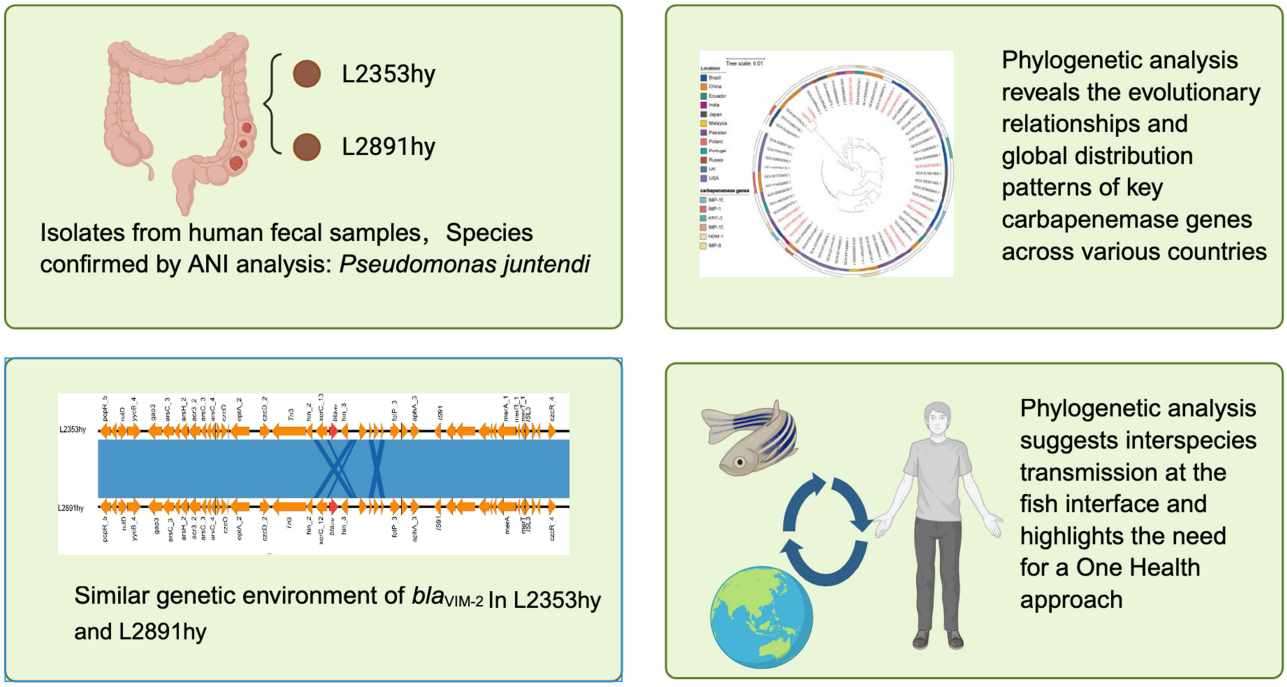
Graphical Abstract



Materials and Methods

Bacterial Isolation and Phenotyping

P. juntendi strain L2353hy was isolated from a 56-year-old male with diarrhea in July 2020, whereas strain L2891hy was isolated from a 71-year-old male with diarrhea who was treated at the First Affiliated Hospital, College of Medicine, Zhejiang University, October 2020. Species identification was confirmed by ANI analysis using JSpeciesWS (http://jspecies.ribohost.com/jspeciesws/) against 58 global *P. juntendi* genomes (NCBI Pathogen Detection Database, Table S1). Antimicrobial susceptibility profiles were determined using CLSI M100-Ed34 (2024)-recommended broth microdilution (MIC) and disk diffusion for carbapenems (meropenem and imipenem), cephalosporins (ceftazidime and cefepime), aminoglycosides (amikacin), and fluoroquinolones (ciprofloxacin).

Genomic Characterization

Hybrid Nanopore Minion (Oxford Nanopore Technologies, Oxford, UK) and Illumina NovaSeq (San Diego, CA, USA, 150bp paired-end) sequencing were performed on the extracted genomic DNA (QIAamp DNA Mini Kit). The hybrid assembly used Unicycler v0.5.0[6] with SPAdes-polished[7] Illumina reads, achieving >100× coverage (Q30 ≥95%). Genome annotation incorporated Prokka[8] v1.14.6, CheckM v1.2.0[9] and RASTtk (https://www.bv-brc.org/app/Annotation) v3.47.11. Resistance determinants were identified using ResFinder v4.1, and plasmid types were determined using PlasmidFinder 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder). CARD v3.2.4 (https://card.mcmaster.ca/), with ≥90% identity threshold. Virulence-associated genes were identified using the Virulence Factor Database, (VFDB) (https://www.vfdb.org/). Plasmid replicon typing combined PlasmidFinder 2.1 (https://cge.food.dtu.dk/services/PlasmidFinder-2.0/) and further verified using S1 nuclease pulsed-field gel electrophoresis (S1-PFGE, CHEF Mapper XA System).

Phylogenetic Analysis

Core genome SNP phylogeny was reconstructed using Parsnp v1.7.4 (Harvest Suite, https://github.com/marbl/parsnp). The pairwise SNP distances were calculated via SNP-dists v0.7.0 (https://github.com/tseemann/snp-dists). Phylogenetic trees were

visualized using iTOL v6.7 with bootstrap support (1000 replicates). Comparative analysis included 58 global isolates (Table S1) from 10 countries.

Results

The genomes of strains L2353hy (5,820,718 bp) and L2891hy (5,699,712 bp) were assembled with a GC content of 62% and contained 5287 and 5116 coding DNA sequences (CDSs), respectively. Completeness was assessed using CheckM, which confirmed 100% completeness and no contamination by either strain. These high-quality assemblies provided a robust basis for further functional and comparative genomic studies. Average nucleotide identity analysis of *P. juntendi* strains L2353hy and L2891hy, and 58 global isolates revealed high nucleotide conservation (Figure 1A). A heat map was constructed using ANI values, with a color gradient representing identity percentages ranging from 75% (blue) to 100% (red). The established species boundary threshold (95% ANI) confirmed that both the clinical strains were *P. juntendi*. Notably, L2353hy and

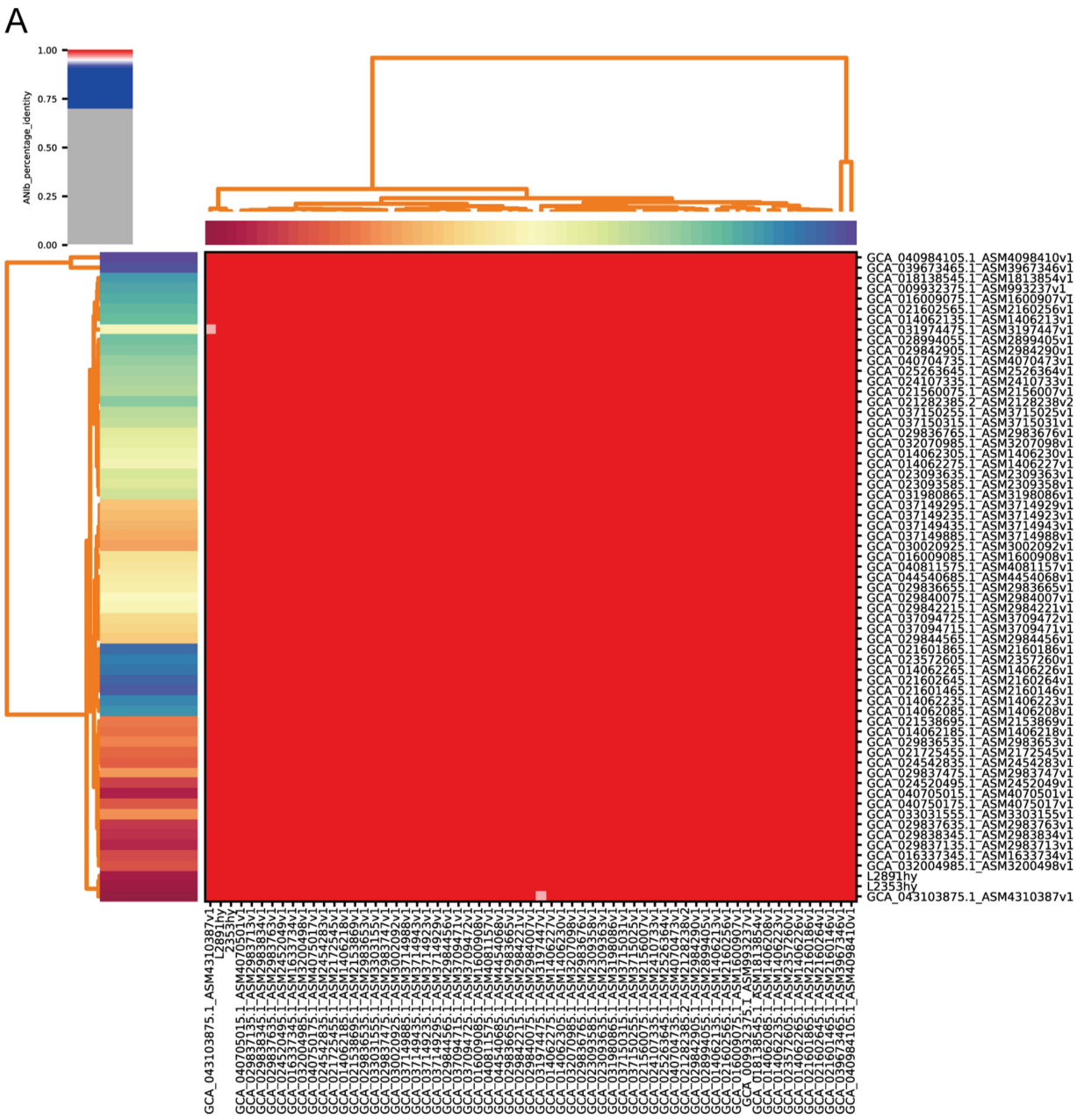


Figure 1 Continued.

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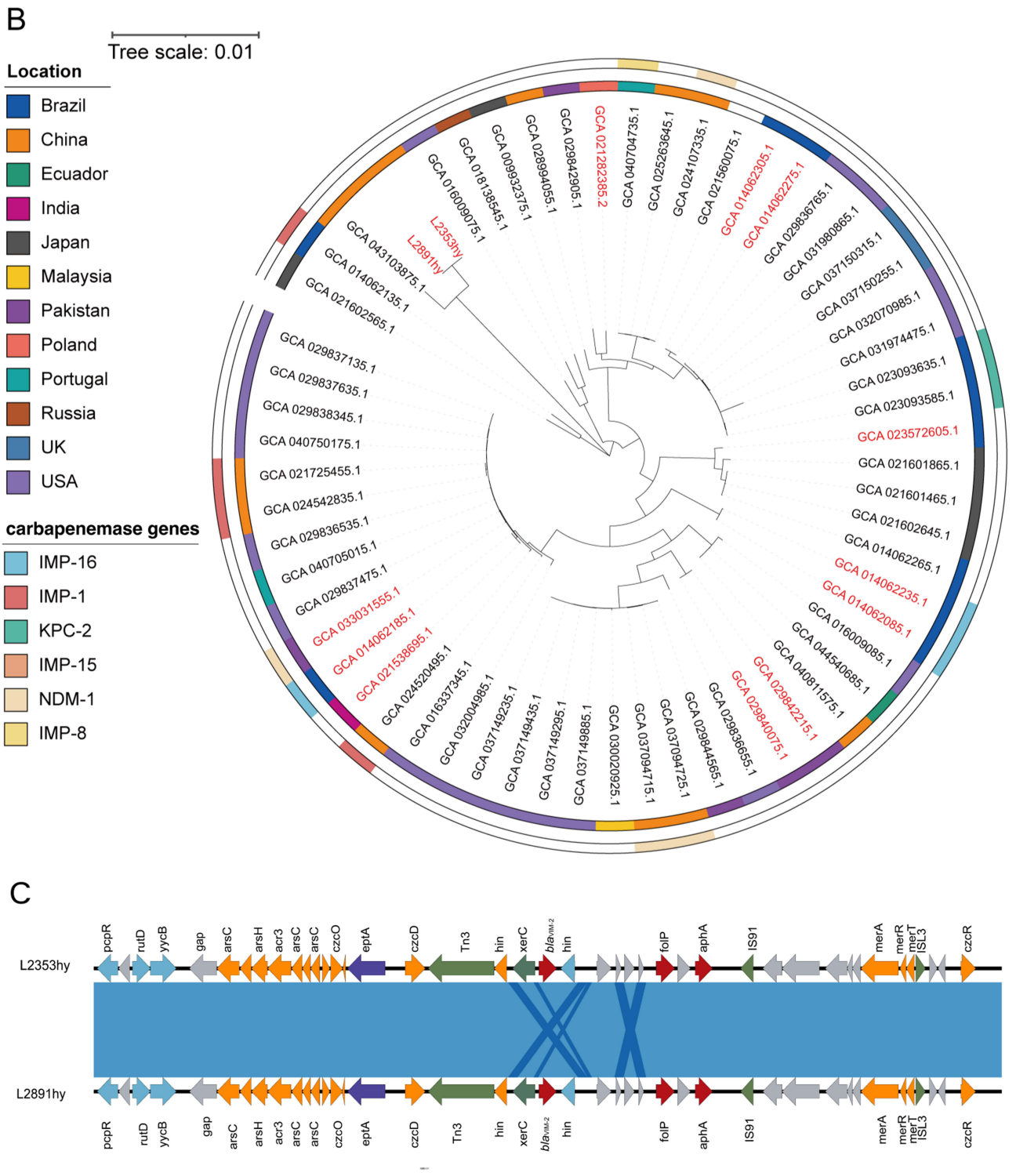

Figure 1 Comparative genomic analysis and plasmid distribution. (**A**) The heatmap was constructed using ANI values, with a color gradient from 75% (blue) to 100% (red), showing genetic similarity among strains. (**B**) Prevalence of key carbapenemase genes (IMP-16, IMP-1, KPC-2, etc) across different countries. Strains harboring bla_{VIM-2} are highlighted in red. The tree scale indicates evolutionary relationships at 0.01 substitutions per site. (**C**) Genetic environment of bla_{VIM-2} created by Easyfig. Arrows indicate: antibiotic resistance (red), heavy metal resistance (Orange), membrane modification (purple), mobile genetic elements (green), regulatory proteins (blue), and metabolic or hypothetical proteins (gray). Blue shading indicates homologous regions.

L2891hy exhibited 99.9% ANI values, which indicated clonal relatedness. Furthermore, both strains shared 99.2% ANI with *P. juntendi* yb_3(GCA_043103875.1; fish intestinal isolate; Wenzhou, China) (Figure 1B).

Identification of virulence factors showed that both strains harbored chromosomally encoded virulence determinants, including lipopolysaccharide biosynthesis genes (*waaF*), transcriptional regulators (*vfr* and *rpoNS*), flagellar assembly and motility genes (*flgCGHI, fleQ, flhA, fleN, cheY, pa1464, fliAGIMNPQ, motC*), iron acquisition systems (*pvdS* and *mbtH-like*), alginate biosynthesis machinery (*algU, alg8, algIA*), twitching motility components (*pilH*), and catabolite-repression regulators (*crc*). These findings suggest that *P. juntendi* possesses a robust genetic arsenal for host colonization, biofilm formation, and immune evasion, potentially contributing to its persistence in clinical settings.

ResFinder analysis identified 13 resistance genes in L2353hy and L2891hy, including aminoglycoside resistance genes (*aadA13, aph(3')-VIb, aac(6')-IIa*), β-lactam resistance genes ($bla_{\text{VIM-2}}$ and $bla_{\text{OXA-246}}$), quinolone resistance gene *qnrVC6, p*henicol resistance gene *cmlA1, r*ifampin resistance gene *arr-3, s*ulfonamide resistance gene *sul1, t*etracycline resistance gene *tet(A), and t*rimethoprim resistance gene *dfrB4*. The presence of $bla_{\text{VIM-2}}$ within the conserved integron structure highlighted the role of this species as a reservoir for carbapenem resistance.

PlasmidFinder analysis confirmed the absence of plasmid replicons in both L2353hy and L2891hy isolates. S1 nuclease pulsed-field gel electrophoresis (S1-PFGE) showed no plasmid bands for either strain (Figure S1). These results indicate that all identified resistance genes, including $bla_{\text{VIM-2}}$, are chromosomally encoded, highlighting the genomic integration of resistance determinants in these clinical isolates.

Gene environment analysis revealed a diverse array of sequences surrounding $bla_{\text{VIM-2}}$, with the conserved structure sequence eptA-czcD-Tn3-*hin-xerC-* $bla_{\text{VIM-2}}$-*hin*-hypothetical protein-*folP*-hypothetical protein--*aphA*-IS91 present in two strains. The predominant MGEs surrounding $bla_{\text{VIM-2}}$ were Tn3 and IS 91 (Figure 1C).

Discussion

Core genome phylogeny revealed that L2353hy and L2891hy clustered within a distinct clade with three closely related *P. juntendi* strains: *P. juntendi* yb_3, *P. juntendi* 12815 (GCA_014062135.1; human urine isolate; Brazil), *P. juntendi* BML-PP047 (GCA_021602565.1; human urine isolate; Japan). This close phylogenetic relationship suggests a potential zoonotic transmission pathway, with aquatic reservoirs serving as sources for clinically relevant *P. juntendi* strains. The clustering of human isolates from geographically distinct regions (China, Brazil, and Japan) further underscores the global dissemination of this lineage.

Although $bla_{\text{VIM-2}}$ is typically embedded within integrons that are not autonomously transferable, their association with mobile genetic elements (MGEs) facilitates horizontal dissemination[10] The increasing global prevalence of $bla_{\text{VIM-2}}$-mediated carbapenem resistance poses a significant threat to public health, particularly in healthcare settings where MDR pathogens thrive.[11] This study identified *P. juntendi* as a potential reservoir for carbapenem-resistance genes. The close relationship between human and animal strains suggests bidirectional resistance gene flow at the human-animal interface. By analyzing the genetic environment surrounding bla_{VIM} in two strains (L2353hy and L2891hy), we identified a highly conserved gene cluster, including lipopolysaccharide biosynthesis genes (waaF), transcriptional regulators (vfr, rpoNS), flagellar assembly and motility genes (flgCGHI, fleQ, flhA, fleN, cheY, pa1464, fliAGIMNPQ, motC), iron acquisition systems (pvdS, mbtH-like), alginate biosynthesis machinery (algU, alg8, algIA), twitching motility components (pilH), and catabolite repression regulators (crc). These findings suggest that *P. juntendi* possesses a robust genetic arsenal for host colonization, biofilm formation, and immune evasion, potentially contributing to its persistence in clinical settings. This region not only contains multiple antibiotic resistance genes ($bla_{\text{VIM-2}}$, *aphA, merA, merR and merT*) but also includes several recombinases (Tn3 and IS91) and other accessory proteins. This structure suggests that this area may constitute a multidrug resistance island (MDRI) with the potential for rapid dissemination through horizontal gene transfer. The high-quality genomes generated here enhance genomic resources for *P. juntendi* and inform future surveillance of carbapenemase-carrying pathogens across ecological settings.

Conclusion

The detection of $bla_{\text{VIM-2}}$ harboring *P. juntendi* in human fecal microbiota adds to the growing evidence of its role as a reservoir for carbapenem resistance. The close genomic relationship between human and animal isolates highlights

potential bidirectional transmission at the human-animal interface. These findings support the adoption of a One Health approach to surveillance for carbapenemase producing *Pseudomonas* species.

Data Sharing Statement

The whole-genome sequencing data for the samples analyzed in this study were deposited in the NCBI BioSample database under accession numbers SAMN49107627/SAMN49107628 and BioProject accession number PRJNA1277365.

Ethical Approval

This study utilized residual clinical samples collected during routine diagnostic procedures. As the study involved retrospective analysis of anonymized samples and data, the requirement for informed consent was waived by the Clinical Research Ethics Committee of the First Affiliated Hospital, Zhejiang University School of Medicine. The study was approved by the same committee (Approval No. 2021-IIT-631). All methods were carried out in accordance with the relevant guidelines and regulations.

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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