Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Open Access Full Text Article

ORIGINAL RESEARCH

Comprehensive Genome Analysis of a Human-Derived β-Lactam-Resistant *Klebsiella variicola* Isolate from China

Taif Shah[1], Jie Li[2,3], Zahir Shah[4], Yunlan Lu[2], Weihong Qin[2]

[1]State Key Laboratory of Conservation and Bio-Resources in Yunnan, Yunnan University, Kunming, Yunnan, People's Republic of China; [2]Kunming Customs Port Outpatient Department, Yunnan International Travel Health Care Center, Kunming, Yunnan, People's Republic of China; [3]Faculty of Life Science and Technology, Kunming University of Science and Technology, Kunming, Yunnan, People's Republic of China; [4]College of Veterinary Sciences, The University of Agriculture, Peshawar, KP, Pakistan

Correspondence: Taif Shah; Weihong Qin, Email taifshah@yahoo.com; qinwh19@sina.com

Infection and Drug Resistance downloaded from https://www.dovepress.com/
For personal use only.

Introduction: *Klebsiella variicola* is an emerging bacterial pathogen commonly associated with opportunistic human infections.
Methods: In this study, we characterized and analyzed the core genome of a β-lactam-resistant *K. variicola* subsp. *variicola* isolate from a human in Kunming, Yunnan, China.
Results: The human-derived β-lactam-resistant *K. variicola* genome was assembled into 37 contigs, with a total genome size of 5,667,700 bp. *K. variicola* comprises multiple antimicrobial resistance (AMR) genes, including blaLEN-22, *fosA, OqxA*, and *OqxB*, as well as the virulence-associated factors *fimH, nlpI*, and *iutA*. The β-lactam-resistant *K. variicola* genome comprised K and O (KL30 and O3/O3a) serotypes. We also identified 29 SNPs from three different snpEff categories: 18 low, five moderate, and six modifiers. Subsequently, we identified five assembled replicons, including three plasmids. Plasmid pKp5-1, plasmid p15WZ-82_res, and plasmid pKP91, and two phage regions: phage region-1, which resembled Entero-phage-HK446, and phage region-2, which was questionable with phage-Erwini-vB-EhrS-59. Furthermore, secondary metabolites, such as redox-cofactor, butyrolactone, azole-containing-RiPP, terpene-precursors (two distinct clusters), NRP-metallophores, and RiPP-like gene clusters were also identified in the *K. variicola* genome.
Discussion: Identifying key antibiotic resistance determinants, virulence factors, capsule serotypes, secondary metabolites, plasmids, and phage replicons emphasizes the zoonotic potential of this pathogen. Given the potential zoonotic implications, a multidisciplinary approach should be used to prevent the spread of β-lactam-resistant *K. variicola*.
Keywords: *Klebsiella variicola*, antimicrobial resistance, virulence, secondary metabolite genes, plasmids, phages

Introduction

The *Klebsiella pneumoniae* complex belongs to the Enterobacteriaceae family and comprises seven related species, including *Klebsiella variicola*.[1] *K. variicola* is an emerging bacterial pathogen that can colonize humans, animals, and birds.[1,2] *K. variicola* was first identified as a new *Klebsiella* species in plants in 2004,[3] followed by *Klebsiella quasipneumoniae* and *Klebsiella similipneumoniae* in 2014.[4] The clinical significance of *K. variicola* is obscured by imprecise diagnoses that understate its prevalence, resulting in knowledge gaps and clinical implications for the healthcare system. According to Martínez-Romero et al (2018), some *K. variicola* and *K. quasipneumoniae* isolates previously thought to be *K. pneumoniae* were correctly identified using rpoB phylogenies and average nucleotide identity (ANI) methods.[5] Advanced diagnostic tools, such as MALDI coupled to time-of-flight mass spectrometry can correctly identify bacterial pathogens.[6] In addition, whole genome sequencing (WGS) and multilocus sequence typing (MLST) based on seven gene alleles (gapA, infB, mdh, pgi, phoE, rpoB, and tonB) analysis can identify emerging bacterial pathogens.[6]

The current global antibiotic burden may increase gene mutations, recombination, lateral gene transfer, and phage populations in specific environments.[7] Improper waste discharge from human communities, hospitals, and poultry farms into the open environment contributes to the spread of AMR (antimicrobial resistance) pathogens.[7,8] A study tested 55

Received: 13 June 2025
Accepted: 29 August 2025
Published: 2 October 2025

© 2025 Shah et al. This work is published and licensed by Dove Medical Press Limited. The full terms of this license are available at https://www.dovepress.com/terms.php and incorporate the Creative Commons Attribution – Non Commercial (unported, v4.0) License (http://creativecommons.org/licenses/by-nc/4.0/). By accessing the work you hereby accept the Terms. Non-commercial uses of the work are permitted without any further permission from Dove Medical Press Limited, provided the work is properly attributed. For permission for commercial use of this work, please see paragraphs 4.2 and 5 of our Terms (https://www.dovepress.com/terms.php).

positive *K. pneumoniae* isolates from 120 hospitalized patient samples, and most were resistant to cefazolin, ceftriaxone, ampicillin, ceftazidime, and cefepime. Some isolates tested positive for extended-spectrum β-lactamases, with the overall rates of multidrug-resistant (MDR) and extensively drug-resistant isolates being 57.2% and 10.9%, respectively.[9] Consequently, the presence of resistant pathogens and antibiotic residues in surface and underground water promotes the growth of resistomes.[7,10] *K. variicola*, similar to other *Klebsiella*, causes hospital- and community-acquired bloodstream, respiratory tract, and urinary tract infections, as well as surgical wounds in humans and animals.[8]

Several human-derived *K. variicola* isolates are known to carry high-risk AMRs and virulence factors (VFs). A study in the United States found five *K. variicola* clones associated with infections in trauma patients. Compared to *K. pneumoniae, K. variicola* is more likely to be involved in bloodstream infections and less likely to be MDR phenotypes. None of the *K. variicola* isolates were found to have hyper-microviscosity.[11] De-Campos et al (2021) characterized five *K. variicol*a strains previously identified as *K. pneumoniae*. A closer genomic look revealed that three of the five strains shared a close genomic cluster with *K. variicola*, with the remaining two being *K. pneumoniae* and *K. quasipneumoniae*. All five strains were MDR, with three exhibiting virulence phenotypes, ie, the ability to invade epithelial cells and survive in human blood and serum.[12] These findings revealed the emergence of new *K. variicola* clones with pathogenic potential for various hosts, raising serious concerns about human health. *K. variicola*, isolated from the blood and cerebrospinal fluid of patients with post-surgical meningitis in Japan,[13] may aid physicians in identifying pathogens and managing *Klebsiella* phylogroup infections effectively. MDR *K. variicola* str. UFMG-H9 and UFMG-H10, isolated from the urine of healthy zebuine cattle (*Gyr* heifers) at the Agricultural Institute of Minas Gerais in Brazil, had a higher genome similarity to isolates from the human urinary tract than those from bovine feces/milk samples.[14] Given *K. variicola*'s presence in human feces, the observed MDR phenomenon raises concerns about its potential as a human pathogen. Some *Klebsiella* species develop drug-resistance phenomena, making treating infections more difficult.[8] In this context, antibiotic-resistant *Klebsiella* infections have responded to bacteriophages (simply phages) that target specific bacteria;[15] however, this is not always the case, as phage-mediated transduction can spread antibiotic-resistance determinants in various niches.[16]

Phages are abundant and diverse predators that influence the structure of the microbial community in the ecosystem and have tremendous therapeutic potential for bacterial infections. Phages can have two lifestyles: virulent, which means that they always kill their host through direct replication, and temperate, which means that they can integrate into the host genome as prophages to form lysogens.[17] WGS analysis has helped phage therapy against emerging bacterial pathogens by identifying species-specific phage receptors, AMRs, and VF determinants, which are critical for developing effective phage-based treatments.[18,19] Recent genomic analyses of *K. variicola* have revealed distinct outer membrane structures and capsule serotypes that influence phage susceptibility, which can guide the selection and engineering of lytic phages for this species.[19] In addition, phage detection within the *K. variicola* genome sheds light on phage-host interactions and the potential resistome. Our findings may be essential for optimizing phage combinations, preventing the evolution of resistance, and ensuring the efficacy of phage therapy as an alternative to antibiotics in the treatment of *Klebsiella* infections. Similar to phages, plasmids are well-known mobile genetic elements (MGEs) that allow for the horizontal transfer of resistance genes that encode β-lactams and VFs.[15] Molecular techniques, such as PCR-based replicon typing and conjugation assays, have long been used to identify plasmids; however, WGS has significantly improved plasmid detection by enabling the precise characterization of plasmid gene content and evolutionary relationships. *K. variicola* harbors a variety of plasmids, some of which are shared with *K. pneumoniae*, indicating frequent intraspecies genetic exchange.[19] Plasmid-associated genes for siderophore production, capsule biosynthesis, and other VFs have been shown to improve bacterial fitness in ecosystems.[15,19]

In this study, we characterized and comprehensively analyzed the core genome of a β-lactam-resistant *K. variicola* isolate from a diarrhea patient in China. We performed taxonomic annotation, phylogenetic analysis, MLST, and capsule serotyping of the β-lactam-resistant *K. variicola* isolate. We also revealed several AMR genes, VFs, and MGEs, including plasmids and phage types, in the β-lactam-resistant *K. variicola*. Our findings emphasize the importance of human-derived β-lactam-resistant *K. variicola* genomic surveillance in tracking the emerging drug resistance threats.

Materials and Methods

Isolation and Identification of Klebsiella Species

Clinical human fecal samples (suffering from diarrhea) were collected aseptically in 5 mL sterile tubes in Kunming, Yunnan, China, on April 22, 2025. Bacteria were grown and isolated on EMB agar using the serial dilution method following overnight incubation in a 37°C shaker. Three suspected *Klebsiella* isolates from the MacConkey agar (Hope Bio-Technology Co., Qingdao, China) plates were transferred to 50 mL sterile tubes containing BHI broth and incubated at 37°C for 24 h. After incubation, the culture tubes were centrifuged at 10,000 *g* for 10 min. The collected bacterial pellet was resuspended in sterile PBS (1.5 mL) and centrifuged for purification. A QIAamp DNA mini kit (Qiagen, Germany) was used to extract bacterial DNA, according to the manufacturer's instructions. High-quality extracted DNA was tested for purity and concentration. The Nanodrop-2000 detected DNA purity, and a Qubit-4 fluorometer (Thermo Fisher Scientific, USA) was used. Finally, the extracted DNA was confirmed using a 1% agarose gel and stored at –80°C until further analysis.

This study complies with the Declaration of Helsinki and was approved by the ethics committee of Yunnan International Travel Healthcare Center, Kunming, Yunnan, China (protocol no. 2023001). Informed consent was obtained from the study participants before study commencement.

The extracted DNA was used to amplify the 16S rRNA gene using specific primers 27F and 1492R for species identification. The PCR assay was performed in a 25 µL reaction mixture containing 12.5 µL of 2× Taq Green PCR master mix (Thermo Fisher Scientific, USA), 0.5 µL of each forward and reverse primer, two µL of the DNA template, and 9.5 µL of nuclease-free water. The PCR conditions were set as an initial denaturation step at 95°C for 3 m, followed by 32 cycles of denaturation at 94°C for 20s, annealing at 50°C for 20s, and extension at 72°C for 1.2 m, and a final extension at 72°C for 10 m. The amplified PCR product was cleaned with ExoSAP-IT before sequencing using the BigDye terminator (v. 3.1 kit: Thermo Fisher Scientific, USA) and the SeqStudio Genetic Analyzer. Chromas (v. 2.6.6) was used to inspect raw chromatograms and assess sequence quality. Low-quality and primer sequences were trimmed to produce high-confidence reads. BioEdit (v. 7.2.6) was used to assemble forward and reverse sequences into consensus sequences, which were then compared to the NCBI nucleotide (BLASTn) database for closest taxonomy matches based on sequence identity and E-values.

Whole Genome Sequencing and Analysis

DNA Library Preparation, Draft Genome Sequencing, and Assembly

Following high-quality bacterial DNA isolation using the QIAamp DNA mini kit (Qiagen, Germany), a DNA library was prepared using the Rapid Barcoding kit (RBK004), following the manufacturer's instructions. Libraries containing a *Klebsiella* isolate barcode were sequenced using GridION (flow cells, active pore number ≥800). The fast5 format was converted to fastq files using Guppy's high-accuracy mode and the MinKNOW software. The *Klebsiella* isolate was de-barcoded using the same software used for base calling. The quality of the trimmed data was checked using MultiQC (v. 1.25). FiltLong (v. 0.2.0) was used for quality filtering. The filtered reads were assembled using the Unicycler assembler (v. 0.4.8). We then used Racon (v. 1.4.12) in conjunction with Pilon software with a minimum contig length of 300 bp and contig coverage of 5 bp to improve the draft genome assembly and call sequence variants of various sizes, such as large insertions and deletions, and to fill gaps. The CheckM (v. 2) software was used to assess the quality of the assembled genome. The QUAST quality assessment tool (v. 2.3) was used to evaluate the draft genome assembly. The assembled genome was annotated with the NCBI prokaryotic genome pipeline and the rapid annotation subsystem technology (RAST) tool, while VIGOR4 annotated phage sequences in the bacterial genome.

Taxonomic Annotation and Phylogenetic Analysis

The human-derived β-lactam-resistant *K. variicola* genome was annotated and compared with other *Klebsiella* to understand potential genes for functional categorization (systems and subsystems), phylogenetic analysis, and MLST typing, which was determined using a PubMLST database that uses seven housekeeping gene alleles (gapA, infB, mdh, pgi, phoE, rpoB, and tonB). The assembled *K. variicola* genome was annotated using Pharokka (v. 1.2.1). The MLST profile was used for in silico taxonomic classification. Pairwise BLASTp and Markov cluster algorithms were used to find similarities between the coding

sequences (CDSs). The Krona and Sankey tools generate Krona and Sankey plots, space-filling displays with coloring, and interactive polar coordinates, allowing complex taxonomic hierarchies. Single-nucleotide polymorphisms (SNPs) with distinct snpEff impact categories, such as low, moderate, and modifiers, were revealed in the *K. variicola* genome using the PATRIC database. The PATRIC database contains reference and representative *Klebsiella* genomes that were used to build phylogenetic trees using fast bootstrapping to support the tree values. The MinHash tool identified the genome most closely, and a phylogenetic tree was constructed using PATRIC global protein families from related *Klebsiella* genomes. The MUSCLE program aligned multiple-protein sequences, and the nucleotides of each sequence were mapped to the protein alignment. Amino acid and nucleotide alignments were combined to create a data matrix, which was then analyzed as a phylogenetic tree using the RA×ML program.

Prediction of AMR Genes, VFs, Capsule Serotypes, and Secondary Metabolites

The ABRicate and AMRFinderPlus tools evaluated AMR determinants and VFs in the human-derived β-lactam-resistant *K. variicola*. In addition, ResFinder (with strict parameters) and the Comprehensive Antibiotic Resistance Database (CARD; v. 4.0.0: with strict/perfect criteria, ie, cut_off: strict/perfect) were used to identify the AMRs. Efflux pump-coding genes and VFs were annotated using the ABRicate pipeline with minimum percentage identity (70) and coverage thresholds (50). Kaptive (v. 2.0) was used to determine capsule serotypes in the *K. variicola* genome. Minimum inhibition concentration (MIC) assay was performed to determine the resistance potential of the *K. variicola* isolate. In addition, the *K. variicola* genome sequence was examined for the presence of secondary metabolite biosynthetic gene clusters using the antiSMASH server (v. 7.0).

Prediction of Plasmid Types and Phage Replicons

PlaScope (v. 0.9) was used to identify and locate the plasmid replicons. Circular plasmid maps were created using the Proksee server. The quality and completeness of the phage genomes were evaluated using QUAST and CheckV tools (with default settings). Pharokka was used to complete the draft genome annotation, which was screened for the presence of tRNAs using the tRNAscan-SE (v. 2.0.11). Circular plots were created using the Pharokka software. Proteome-based phylogeny helped us to understand the relationships between the human-derived β-lactam-resistant *K. variicola* phages and previously characterized phages using NCBI BLASTn. The VICTOR tool was used to study the evolutionary relationships among the Enterobacteriaceae phages. We used a viral intergenomic distance calculator to compare the ANI of our phages with that of other phage genomes. Genomes with >95% ANI were identified to belong to the same phage species. The Clinker pipeline was used to compare gene content and synteny between phage genomes based on a 50% similarity threshold. The phage TB server determined phage interactions with the host bacteria.

Results

Klebsiella Species Identification

Two suspected *Klebsiella* colonies were isolated from EMB agar plates. A 16S rRNA gene PCR assay confirmed the presence of the *K. variicola* isolates. The 16S rRNA gene query for *K. variicola* showed the highest similarity (100%) with *K. variicola* str. F2R9 (NR_025635.1), 99.71% with *K. pneumoniae* str. JCM1662 (NR_112009.1), 99.63% with *K. pneumoniae* str. DSM 30104 (NR_117686.1), and *K. pneumoniae* str. ATCC 13883 (NR_119278.1) in the GenBank database. After WGS quality filtering, the total genomic length of the human-derived β-lactam-resistant *K. variicola* was 5,667,700 bp, with an average depth of 261.6.

In silico Taxonomic Classification and Phylogenetic Analysis

Krona and Sankey plots visualized bacterial taxonomy at the species level. Kraken's taxonomic classification system, which employs exact k-mers, was matched to its lowest ancestor. The Kraken's classified human-derived β-lactam-resistant *K. variicola* to species *K. variicola, K. pneumoniae, K. michiganensis*, and other closely related species (Supplementary Figure S1). In addition, the human-derived β-lactam-resistant *K. variicola* subspecies variicola sequence reads were assembled into 37 contigs with an average GC content of 57.23%, an N50 of 516,484 bp, 5529 CDSs, 80 tRNAs, three rRNA genes, three plasmids, and two phage regions (Table 1).

 https://doi.org/10.2147/IDR.S544005

Table 1 Genome Assembly and Annotations of a Human-Derived β-Lactam-Resistant *K. Variicola*

Genome Annotation	Number
Contigs	37
GC content	57.23%
Contig L50	4
Genome length	5,667,700 bp
Contig N50	516,484
CDS	5519
tRNA	80
rRNA	3
Hypothetical protein	752
Proteins with functional assignments	4767
Proteins with EC number assignments	1482
Proteins with GO assignments	1227
Proteins with pathway assignments	1095
Proteins genus-specific family assignments	5343
Proteins with cross-genus family assignments	5373

PATRIC genome annotation revealed that a human-derived β-lactam-resistant *K. variicola* isolate exhibited high similarity to reference genomes. Comparative genomic profiling revealed the closest matches to *K. variicola* str. LEMB11 (GenBank: CP045783), *K. pneumoniae* str. HS11286 (CP003223), *K. quasivariicola* str. 08A119 (CP084768), *K. africana* str. 200023 (CP084874), and *K. quasipneumoniae* str. KqPF26 (CP065838). In addition, our findings suggest a close phylogenetic relationship between the human-derived β-lactam-resistant *K. variicola* isolate and other members of the *K. pneumoniae* complex. Notably, our human-derived β-lactam-resistant *K. variicola* isolate clustered in the *K. variicola* str. LEMB11 clade, which was previously isolated from Garam Masala in the United Kingdom. Phylogenetic analysis of similar *Klebsiella* genomes suggested shared evolutionary features and potential functional overlap between specific taxa (Figure 1). Furthermore, ribosomal MLST analysis identified the assembled genome "rST 200763" with 100% similarity to *K. variicola*. According to the core genome MLST based on seven gene alleles (gapA, infB, mdh, pgi, phoE, rpoB, and tonB), the identified *K. variicola* subspecies variicola was grouped into sequence type 146 (ST146).

K. variicola Genomic Features and SNP Annotations

Comprehensive genome analysis was performed using the RAST tool, which assigned a unique genome identifier (244366.1524) to the human-derived β-lactam-resistant *K. variicola*. This pathogen contains 752 hypothetical proteins and 4767 functionally assigned proteins. Functional assignments included 1482 proteins with Enzyme Commission (EC) numbers, 1227 proteins with Gene Ontology (GO), and 1095 proteins assigned to pathways. PATRIC identified two types of protein families, ie, 5345 proteins belonging to the genus-specific families and 5376 belonging to the cross-genus families (Table 1). A circular *K. variicola* genome structure was used to assess the genetic diversity of core structure and functional genes (Figure 2a). These include, from the outer to the inner rings, contigs, CDS on both strands, RNA genes, CDS with homology to known AMR genes, VFs, and GC content. The CDS colors on the forward and reverse strands represent systems and subsystems (a collection of proteins) collaborating to perform specific biological, molecular, and cellular processes in the human-derived β-lactam-resistant *K. variicola* (Figure 2b).

The human-derived β-lactam-resistant *K. variicola* genome analysis revealed 29 SNPs with three distinct snpEff impact categories (18 low, five moderates, and six modifiers). Moderate missense mutations are known to alter protein sequences. Low-category snpEff impact synonymous mutations do not affect protein function, whereas modifier-category mutations occur in non-coding intron regions. The impact is determined by the type of mutation (whether the SNP causes a non-synonymous mutation or is located in the coding regions, introns, or promoter regions). Such classification allows

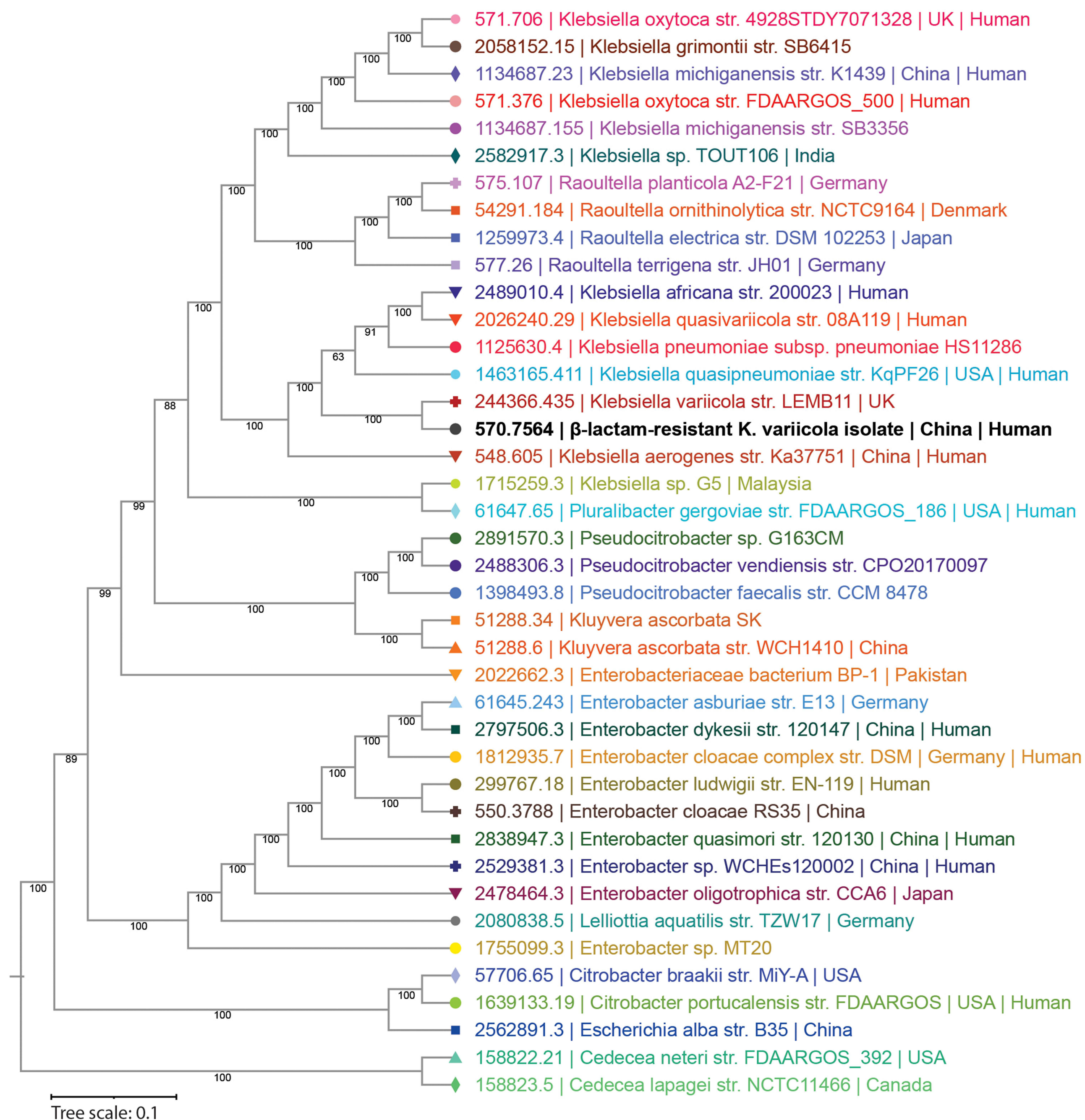

Figure 1 Phylogenetic analysis based on WGS of *K. variicola* genomes and its closely related species. Our human-derived β-lactam-resistant *K. variicola* isolate from China (as shown with black bold texts) was clustered into the *K. variicola* LEMB11 clade, previously isolated in the United Kingdom. Phylogenetic construction determined the position of our *K. variicola* isolate among other reference genomes obtained from the PATRIC global protein families. Protein sequences of PGFams were aligned with the MUSCLE tool, and nucleotide sequences were overlaid onto the protein alignments for codon structure maintenance. Concatenated amino and nucleotide alignments were utilized to create a data matrix that was subsequently used to input phylogenetic inference into RaxML.

us to understand how damaging each SNP may be associated with gene function. We identified 16 synonymous mutations in the low-snpEff impact category. A non-synonymous SNP occurs in the coding region (exon), altering the amino acid sequence and, as a result, gene function. All five non-synonymous mutations found in this study were in the moderate category, with only one insertion mutation in the modifier category (Supplementary Table S1).

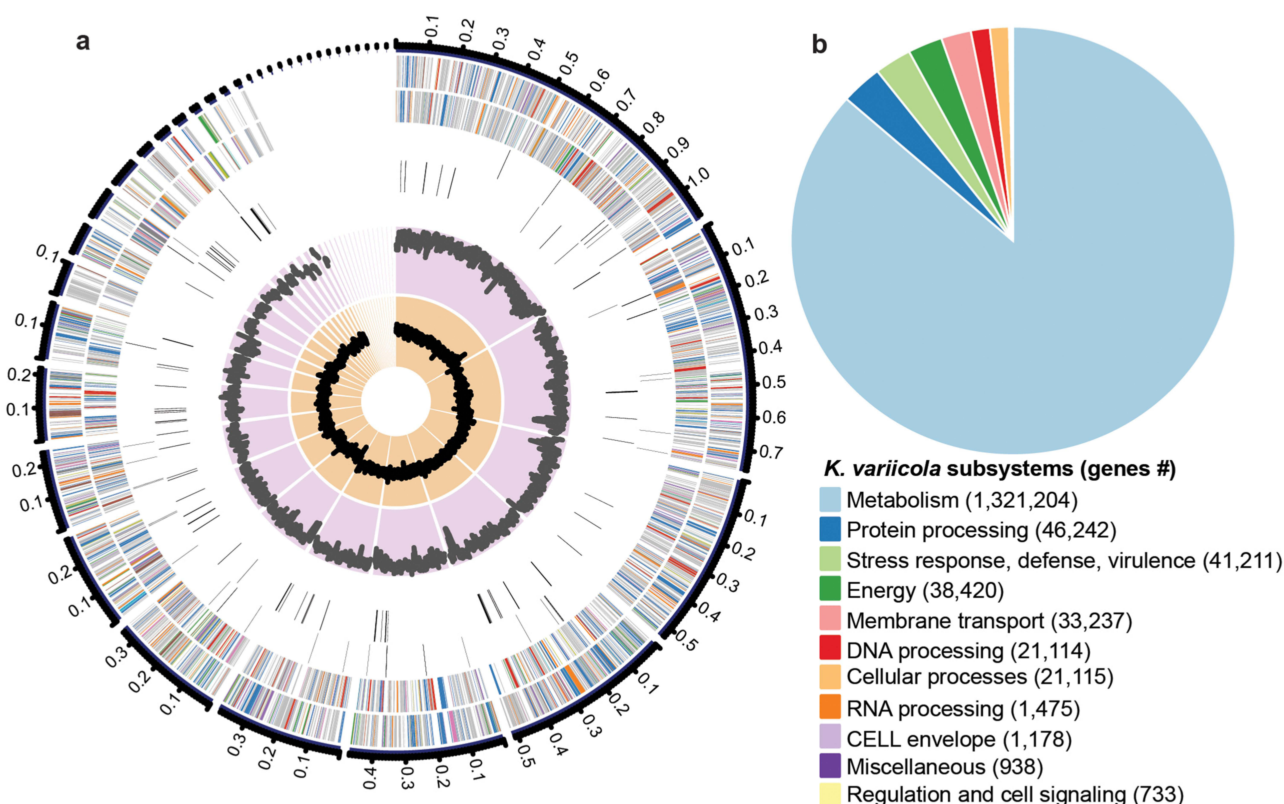

Figure 2 Beta-lactam-resistant *K. variicola* circular genomic graph. (**a**) The *K. variicola* circular graph was created to evaluate the diversity of core and accessory genes. (**b**) *K. variicola* genome structure and functional annotation to various subsystems.

GO Enrichment Analysis

GO enrichment analysis classified the human-derived β-lactam-resistant *K. variicola* genome into biological, cellular, and molecular processes (Figure 3). According to the biological process, the majority of the genes were annotated to cellular process (n = 1066), metabolic process (n = 906), biosynthesis process, gene expression, and translation (Figure 3a). In addition, the GO molecular function category was enriched with catalytic activity (n = 805), binding (n = 775), small molecule binding (n = 294), transferase activity (n = 270), and enzyme regulator activity (n = 21) (Figure 3b). Moreover, the GO cellular process was enriched with anatomical entity (n = 1301), cytoplasm (n = 746), intracellular (n = 825) activities, intracellular non-membrane-bounded organelle (n = 68), ribonucleoprotein complex (n = 15), and proton-transporting ATP synthase complex (n = 8) (Figure 3c). The detailed values of genes involved in GO enrichment processes are shown in the Supplementary Table S2.

Phenotypes, Acquired AMRs, Mutations Mediating AMR Genes, and VFs

The human-derived *K. variicola* phenotype comprised multiple AMR genes, including *OqxB* resistance to fluoroquinolone, ciprofloxacin, nalidixic acid, rimethoprim, and chloramphenicol. *fosA* was resistant to fosfomycin, whereas β-lactam-*LEN22* (*blaLEN22*) was resistant to amoxicillin, ampicillin, iperacillin, ticarcillin, and cephalothin (Table 2). In addition, several acquired AMR genes were also revealed in the human-derived β-lactam-resistant *K. variicola* isolate. A chromosomal gene *blaLEN22* (accession no. AM850912) was resistant to amoxicillin, ampicillin, piperacillin, ticarcillin, and cephalothin. *FosA* (ACZD01000244) was resistant to fosfomycin, whereas *OqxB* and *OqxA* (EU370913) were resistant to ciprofloxacin, nalidixic acid, trimethoprim, and chloramphenicol. We also revealed several chromosomal mutations mediating AMR genes, such as *acrR* (accession no. AJ318073.1), *ompK35* (accession no. AJ011501.1), *ompK36* (Z33506.1), *ompK37* (AJ011502.1), *ramR* (KY465996.1), gyrB, and rpsL (Supplementary Table S3). In addition, CARD annotation (with strict cutoff parameters) identified multiple MDR efflux pump genes

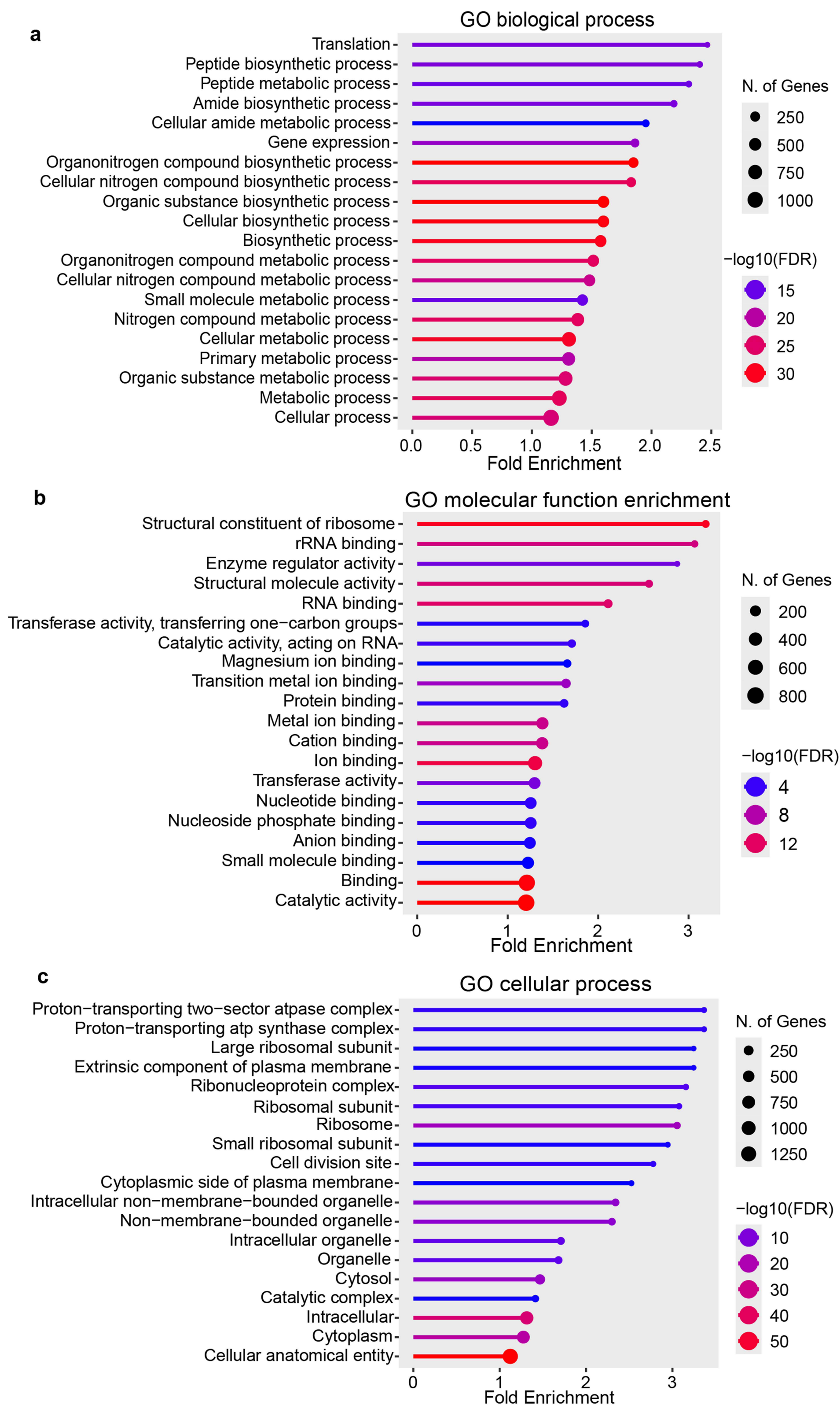

Figure 3 GO enrichment analysis classified all the genes into biological process, molecular function, and cellular process. (**a**) In the biological process, the majority of the genes were enriched in cellular, metabolic, biosynthetic, and gene expression. (**b**) The GO cellular category was enriched with anatomical entities, cytoplasm, and intracellular activities. (**c**). The GO molecular category was enriched with catalytic activity, binding, and small molecule binding.

Table 2 Core Genomic Characteristics of a Human-Derived β-Lactam-Resistant *K. Variicola*

MGE_resistance	**Phenotype**	**Accession no.**	**Position in Contig**	**Coverage (%)**	**Identity (%)**
blaLEN22	Piperacillin, amoxicillin, cephalothin, ampicillin, ticarcillin	AM850912	22,246-23,106	100.0	99.9
fosA	Fosfomycin	ACZD01000244	24,673-25,092	100.0	94.0
OqxB	Trimethoprim, chloramphenicol, cetylpyridinium chloride, benzylkonium chloride, ciprofloxacin, nalidixic acid	EU370913	422,497-425,649	100.0	97.7
OqxA	Trimethoprim, chloramphenicol, cetylpyridinium chloride, benzylkonium chloride, ciprofloxacin, nalidixic acid	EU370913	425,673-426,848	100.0	96.3
MGE_virulence	**Protein function**	**Accession no.**	**Position in contig**	**Coverage (%)**	**Identity (%)**
fimH	Type 1 fimbriae	NA	66540-67,028	100.0	95.1
mrkA	Type 3 fimbrial major subunit	ABW83989	84,741-85,355	100.0	92.4
iutA	Ferric aerobactin receptor	FLWH01000001	270,692-272,881	100.0	94.6
iutA	Lipoprotein NlpI precursor	CP000243	63,206-64,090	99.9	90.2
Plasmid/replicon types	**Accession no.**	**Position in contig**	**Coverage (%)**	**Identity (%)**	**Mobility**
K. variicola plasmid pKp5-1/FIA	KF954760	21,700-21,994	100.0	97.3	Non-mobilizable
K. variicola plasmid p15WZ-82_res/IncFII	CP000966	39,407-39,636	100.0	100.0	Non-mobilizable
K. variicola plasmid pKP91/IncFIB	JN233704	4006-4561	99.3	96.1	Non-mobilizable
Surface polysaccharide typing					
K locus	Predicted capsule type	Confidence	O locus	Capsule type	Confidence
KL30	K30	Typeable	O3/O3a	O3/O3a	Typeable

https://doi.org/10.2147/IDR.S544005

(*KpnF, KpnE*, and *qacG*) and major facilitator superfamily (MFS) efflux pump genes (*leuO, emrR, KpnG*, and *KpnH*). We also identified a general bacterial porin gene, *OmpK37*, with reduced permeability to β-lactam in human-derived *K. variicola* (Supplementary Table S4). Moreover, the presence of mobile-virulence-associated genes *fimH* (type-1 fimbriae), *nlpI* (NlpI lipoprotein precursor), and *iutA* (ferric aerobactin receptor) could play key roles in bacterial pathogenesis (Table 2). Moreover, the MIC susceptibility profile of the *K. variicola* isolate revealed an MDR phenotype, which was resistant to a variety of antibiotics, including β-lactam antibiotics, aminoglycosides, ciprofloxacin, enrofloxacin, doxycycline, tetracycline, chloramphenicol, fosfomycin, and linezolid. The isolate was resistant to imipenem but susceptible to meropenem. Amikacin, colistin, azithromycin, florfenicol, and sulfadiazine sensitivity remained unchanged. Overall, the findings suggest that the isolate has broad-spectrum resistance (Supplementary Table S5). Further, the β-lactam-resistant *K. variicola* genome was predicted to have human pathogenic capacity due to the presence of virulence-associated proteins, bacteriocin, invasin, type-VI secretion protein VasK, hydrolase, fimbrial adhesin, quinoprotein glucose dehydrogenase, ferric hydroxamate uptake, HTH-type transcriptional regulator MalT, fimbrial outer membrane usher protein, phosphoglycerol transferase, LPS-assembly protein LptD, translocation and assembly subunit TamB, ferrienterobactin receptor, ß-mannosidase B, glucose-1-phosphatase, outer membrane protein-W, and periplasmic alpha-amylase (Supplementary Table S6).

K. variicola Serotypes and Metabolic Traits

The human-derived β-lactam-resistant *K. variicola* also contained capsule serotypes, the K locus (KL30) based on the *wzi* gene, and the O locus (O3/O3a). Seven biosynthetic gene clusters (BGCs) involved in the biosynthesis of secondary metabolites were also identified in the *K. variicola* genome (Figure 4). BGCs associated with the production of redox cofactors (Figure 4a), butyrolactone (Figure 4b), azole-containing RiPP (ribosomally synthesized and post-translationally modified peptides) (Figure 4c), two distinct terpene precursors (Figure 4d and e), NRP-metallophores (non-ribosomal peptide siderophores) (Figure 4f), and RiPP-like compounds (Figure 4g) were identified in the bacterial genome. The presence of these BGCs may suggest *K. variicola's* potential metabolic capacity for producing bioactive compounds, which may help bacterial adaptation and survival in host tissues and extreme environments.

Annotation of Plasmid and Phage Replicons

The human-derived *K. variicola* genome comprised multiple AMR genes, VFs, and other crucial MGEs, some of which are shown in the circular graph (Figure 5a). We identified three non-mobilizable plasmids in the β-lactam-resistant *K. variicola* that were compatible with other *K. variicola* strains. Among these plasmids, *K. variicola* plasmid p15WZ-82_res/FIA, which was a non-mobilizable plasmid, showed 97.3% nucleotide identity with the previously sequenced plasmid (accession no. KF954760). In addition, *K. variicola* plasmid p15WZ-82_res/IncFII showed 100% nucleotide identity and coverage with the previously known plasmid (CP000966). *K. variicola* plasmid pKP91/IncFIB showed 96.1% nucleotide identity with the known plasmid (JN233704). Notably, several AMR, acquired, and other genetic elements with potential transfer capability were revealed on the plasmids. MGEs involved in integration/excision, replication/recombination, stability, transfer, and defense were shown on the *K. variicola* plasmid pKp5-1 (Figure 5b). *K. variicola* plasmid p15WZ-82_res carried AMR genes *traI, traU*, and *traN*, along with other MGEs (Figure 5c). *K. variicola* plasmid pKP91 carried a few crucial MGEs, such as *relE* and *tnpA*, compared to the other plasmids (Figure 5d). Moreover, the human-derived β-lactam-resistant *K. variicola* genome was intact, with two phage regions, phage region-1 and phage region-2. Phage region-1, comprising 62 phage genes, 42,050 bp in length, and a GC content of 48.78%, resembles Entero-phage-HK446 (accession no. NC_019714.1). Phage region-2 of 20,961 bp with a GC content of 51.79% was questionable with phage-Erwini-vB-EhrS-59 (NC_048198). The number and position of phage capsids, integrases, tails, hypothetical proteins, transposases, portals, terminases, and proteases are shown in Figure 6.

Discussion

The global emergence of a β-lactam-resistant *K. variicola* in humans has raised concerns about its potential role in zoonotic transmission. In this study, we characterized and comprehensively analyzed the genome of a ß-lactam-resistant *K. variicola* str. carrying *blaLEN*-22 isolated from human feces in Kunming. blaLEN-22 has shown resistance to several

Figure 4 Prediction of BGCs in the *K. variicola* genome. (**a**) BGCs associated with the redox cofactor, (**b**) butyrolactone, (**c**) azole-containing RiPP, (**d** and **e**) two terpene precursors, (**f**) NRP-metallophores, and (**g**) RiPP-like compounds were annotated in the human-derived β-lactam-resistant *K. variicola* assembled genome.

drugs, including ampicillin, amoxicillin, cephalothin, piperacillin, and ticarcillin, demonstrating *K. variicola*'s adaptability to antibiotic pressure. Our findings focused on AMRs, VFs, capsule serotypes, and plasmid and phage replicons in *K. variicola*, thereby emphasizing their epidemiological relevance. In addition, this study contributes to the importance of surveillance and genomic features in preventing drug-resistant microbes in humans and animals. *K. variicola* genomic surveillance revealed *blaLEN*-22 is a primary β-lactam determinant that degrades β-lactam antibiotics and makes them ineffective.[20] Our findings are consistent with a previous study in which several ß-lactamase genes, including *blaLEN*-2, *blaLEN*-16, *blaLEN*-17, *blaLEN*-19, *blaLEN*-21, *blaLEN*-22, and *blaLEN*-24, were found in *K. variicola* isolated from humans and animals,[15] demonstrating their widespread presence in various niches.

In addition to *blaLEN*-22, the human-derived *K. variicola* str. contained other AMR genes *acrA, acrAB, emrR, kpnG, kpnH, rsmA*, and *oqxA*, which confer resistance to ciprofloxacin, tetracycline, gentamicin, fosfomycin, amoxicillin, chloramphenicol, and fluoroquinolone, indicating an MDR phenotype. Furthermore, efflux pump genes and the porin

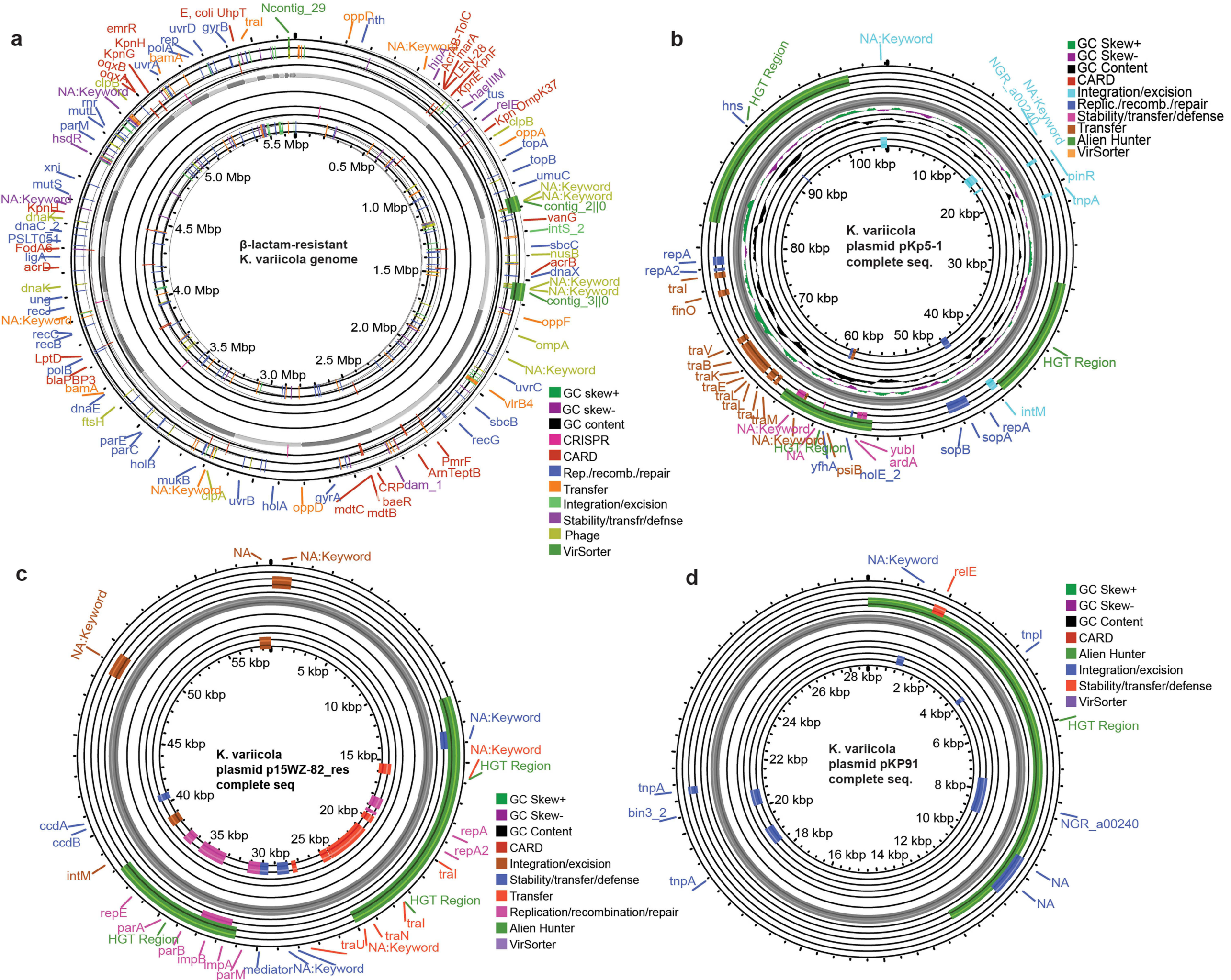

Figure 5 Beta-lactam-resistant *K. variicola* genomic graph and plasmids with various genes. (**a**) Several important genes are shown on the bacterial circular genome. Three plasmids were identified in the β-lactam-resistant *K. variicola*. (**b**) *K. variicola* plasmid pKp5-1 with IncFIA replicon, (**c**) *K. variicola* plasmid p15WZ-82_res with IncFII replicon, and (**d**) *K. variicola* plasmid pKP91 with IncFIB(K) replicon were compatible with the *K. variicola* species.

modification system suggest a multifactorial resistance mechanism that enhances the ability of the bacterium to resist broad-spectrum antimicrobial therapies.[21] Our findings are consistent with previous reports that *K. variicola* exhibits high levels of drug resistance in humans.[22] *Klebsiella* species, particularly *K. variicola*, harbor VFs, including *iroE* (siderophore esterase-iroE), *clpV* (type VI secretion system tssH), *icmF* (type VI secretion protein), *iutA* (ferric aerobactin receptor), *acrB* (acriflavine resistance protein-B), *acrB* (acriflavine resistance protein-B), KPK_RS07970 (capsule assembly protein Wzi), *wbdA* (LPS immune modulation), *hlyA* (hemolysin-A exotoxin), and *gspD* (T2SS secretin variant PulD). These VFs allow bacteria to colonize and persist in various hosts, resulting in chronic infections. *K. variicola* biofilm formation capability is particularly concerning because biofilms act as AMR reservoirs and facilitate horizontal gene transfer.[23] The interaction between resistance and VFs demonstrates that β-lactam-resistant *K. variicola* can adapt to different niches, posing infection prevention and treatment challenges.

In this study, the β-lactam-resistant *K. variicola* was closely related to isolates from human and animal infections, indicating potential human-animal transmission dynamics.[23] Our findings are consistent with those of previous studies showing zoonotic potential of *K. variicola* as a reservoir for various AMRs and VFs.[8,15] In addition, detection of capsular K and O loci in *K. variicola* sheds light on its pathogenic potential. The K KL30 and O O3/O3a loci predicted the conserved capsular serotypes for this species. KL30 has been previously identified in clinical ST29 isolates and may be associated with increased virulence and immune evasion.[24] Similarly, the presence of the O3b serotype in *K. variicola*

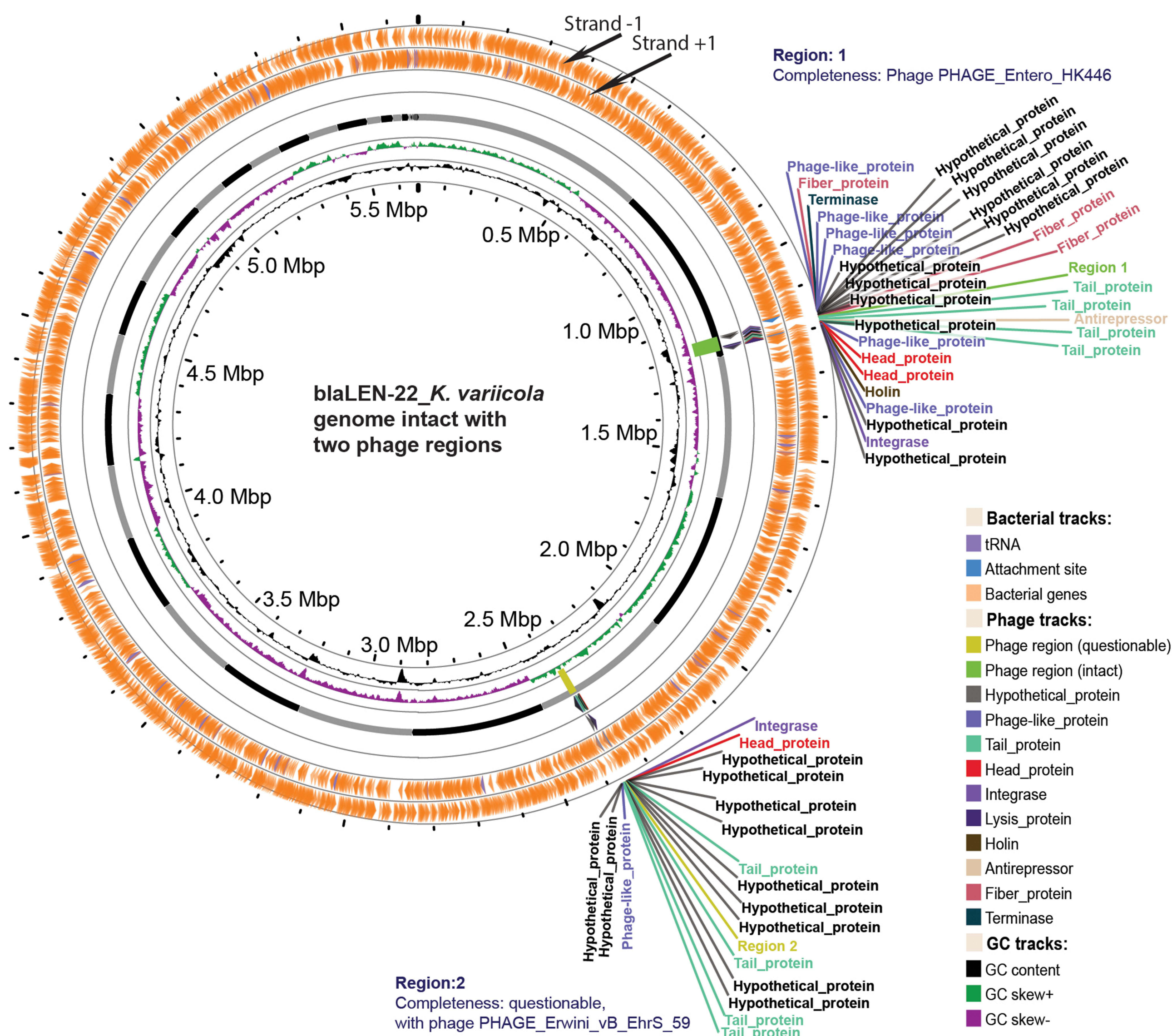

Figure 6 Identification of two intact phage regions with the *K. variicola* genome. The *K. variicola* genome was intact with two phage regions: phage region-1, which resembled Entero-phage-HK446, and phage region-2, which was completely questionable with phage-Erwini-vB-EhrS-59.

may influence serum resistance and immune system interactions, allowing the pathogen to survive and cause infections.[24] The presence of these serotypes in the *K. variicola* genome demonstrates their importance in evolutionary conservation and potential pathogenicity.

Identifying biosynthetic gene clusters in the β-lactam-resistant *K. Variicola* emphasizes its ability to produce a diverse range of secondary metabolites.[25] Interestingly, we discovered gene clusters for the biosynthesis of redox cofactors, butyrolactones, azole-containing RiPPs, two terpene precursor clusters, NRP metallophores, and RiPP-like molecules. The presence of two distinct terpene precursor clusters demonstrates the metabolic versatility of the pathogen. Redox cofactors play central roles in regulating intracellular redox balance and can influence reactive oxygen species production, further enhancing microbial survival in extreme environments.[26] Butyrolactones are quorum-sensing signaling molecules that regulate gene expression according to the cell density. Azole-containing molecules, such as RiPPs and RiPP-like compounds, have shown antibacterial activity and an intricate architecture, suggesting that they may be involved in habitat colonization and species competition. Terpenes produced from terpene precursor clusters exhibit a wide range of biological activities, including antimicrobial and immunomodulatory functions. NRP metallophores are

selective siderophores that aid metal ion acquisition under limiting conditions. The presence of these secondary metabolites suggests that *K. variicola* may not only exist in different environmental niches but also actively engage in microbial interactions with hosts.[27] The genomic potential of this organism may be the key to its involvement in beneficial and harmful interactions with hosts. Additional metabolomic profiling is required to determine the precise function of these biosynthetic gene clusters and assess their biomedical relevance.[26,27]

The presence of MGEs, including plasmids and phage replicons, increases the likelihood of horizontal gene transfer, which can accelerate the spread of resistance in bacterial populations.[15] These findings emphasize the importance of a One-Health approach to monitor and control the spread of MDR pathogens across different ecological niches. Three plasmids (IncFIA (pBK30683), pBK30683, and IncFII) in a human-derived β-lactam-resistant *K. variicola* have raised serious concerns because of their essential roles in AMRs and VF transmission. IncF plasmids, particularly those from the FIA and FII replicon families, are among the most frequently associated with MDR in Enterobacterales, particularly in *Klebsiella* species.[28] These plasmids contain β-lactamase determinants with a high transfer efficiency through conjugation, making them important contributors to global antibiotic resistance. Plasmid pBK30683, which belongs to the IncFIA and IncFII types, is likely a hybrid replicon capable of hosting AMR.[29] IncFIA and IncFII within a single plasmid structure can increase stability and adaptability, allowing AMRs to persist in *Klebsiella* hosts even without selective pressure.

IncFII plasmids contain *blaCTX*-M, *blaNDM*, and *mcr1*, which confer resistance to broad-spectrum β-lactamases, carbapenemases, and colistin, respectively.[29] Their ability to incorporate resistance islands from other MGEs accelerates the spread of MDR phenotypes.[30] Given the increasing prevalence of IncF-type plasmids in clinical and environmental niches, healthcare centers should investigate their stability, transmission, and recombination potentials. Molecular epidemiology and WGS studies can be used to monitor the spread of these plasmids and to detect emerging resistance patterns. Strategies, such as plasmid curing, phage therapy, and novel antimicrobial agents, should be investigated to address the threat of resistance-encoding determinants in emerging pathogens.[29,30] In addition to plasmids, the discovery of two phage replicons, Entero-phage-HK446 and phage-Erwini-vB-EhrS-59, which contain 62 phage genes, sheds light on the bacterial ecosystem diversity and functional potential. Entero-phage-HK446 and phage-Erwini-vB-EhrS-59 belong to the enterobacteria and Erwinia phage groups, respectively, and are known to infect Enterobacterales.[31] Genetic analysis of these phages revealed a wide range of genes involved in phage functions such as replication, virion assembly, and host cell lysis. Phage lysin, holins, and tail fiber protein genes are especially important for host recognition and infection.[31,32] The detection of such phages in emerging pathogens may highlight their therapeutic potential, particularly in phage therapy against AMR bacterial infections. Furthermore, phage genes may underlie the complexities of phage-bacteria interactions, implying that these phages could play essential roles in the microbial ecosystem, promoting horizontal gene transfer and bacterial evolution.[16] Future research should focus on functional validation of these genes and the application of phages in biocontrol strategies, particularly in human and animal settings.

Beta-lactam-resistant *K. variicola* isolated from human feces emphasizes the importance of enhanced antimicrobial stewardship and surveillance programs. Local clinics and hospitals are essential to monitor emerging pathogens in the human population. The high levels of AMR genes in *K. variicola* may emphasize routine antibiotic use, which may contribute to the evolution of resistance phenotypes. Stricter preventive measures (such as prudent antibiotic use) are required to slow the spread of resistant pathogens.[33] Furthermore, genomic surveillance should track emerging bacterial pathogens, their resistance, and their virulence determinants.

Conclusion

In conclusion, our findings offer genomic insights into the human-derived β-lactam-resistant *K. variicola* isolated from humans. Identifying key AMR genes, virulence-associated factors, capsule typing, secondary metabolite genes, plasmids, and phage determinants highlights the importance of the ongoing surveillance of this emerging bacterial pathogen. Given the potential zoonotic consequences, a One-Health approach should be used to prevent β-lactam-resistant *K. variicola* infections in humans.

Data Sharing Statement

The whole-genome nucleotide sequence data of this isolate was deposited in the GenBank database under bioproject number PRJNA1255725.

Funding

This study was supported by the National Key R&D Program of China (2022YFC2302700 & 2023YFC2307400).

Disclosure

The authors declare no competing interests in this work.

References

1. Rodríguez-Medina N, Rodríguez-Santiago J, Alvarado-Delgado A, et al. Comprehensive study reveals phenotypic heterogeneity in Klebsiella pneumoniae species complex isolates. *Sci Rep*. 2024;14(1):5876. doi:10.1038/s41598-024-55546-z
2. Sun Y, Zheng C, Zhou J, et al. Pathogen profile of Klebsiella variicola, the causative agent of banana sheath rot. *Plant Dis*. 2023;107(8):2325–2334. doi:10.1094/pdis-09-22-2018-re
3. Rosenblueth M, Martínez L, Silva J, Martínez-Romero E. Klebsiella variicola, a novel species with clinical and plant-associated isolates. *Syst Appl Microbiol*. 2004;27(1):27–35. doi:10.1078/0723-2020-00261
4. Brisse S, Passet V, Grimont PAD. Description of Klebsiella quasipneumoniae sp. nov. isolated from human infections, with two subspecies, Klebsiella quasipneumoniae subsp. quasipneumoniae subsp. nov. and Klebsiella quasipneumoniae subsp. similipneumoniae subsp. nov. and demonstration that Klebsiella singaporensis is a junior heterotypic synonym of Klebsiella variicola. *Int J Syst Evol Microbiol*. 2014;64(Pt 9):3146–3152. doi:10.1099/ijs.0.062737-0
5. Martínez-Romero E, Rodríguez-Medina N, Beltrán-Rojel M, et al. Genome misclassification of Klebsiella variicola and Klebsiella quasipneumoniae isolated from plants, animals and humans. *Salud Publica Mex*. 2018;60(1):56–62. doi:10.21149/8149
6. Ohama Y, Nomura Y, Mizoguchi M, Higurashi Y, Okamoto K, Harada S. Accurate identification of Klebsiella variicola by MALDI-TOF mass spectrometry in clinical microbiology laboratories. *Microbiol Spectr*. 2022;10(5):e0284422. doi:10.1128/spectrum.02844-22
7. Larsson DGJ, Flach C-F. Antibiotic resistance in the environment. *Nat Rev Microbiol*. 2022;20(5):257–269. doi:10.1038/s41579-021-00649-x
8. Li L, Zhang Y, Wang W, Chen Y, He F, Yu Y. Global emergence and genomic epidemiology of bla(NDM)-carrying Klebsiella variicola. *Infect Drug Resist*. 2024;17:1893–1901. doi:10.2147/idr.S460569
9. Hussein RA, Al-Kubaisy SH, Al-Ouqaili MTS. The influence of efflux pump, outer membrane permeability and β-lactamase production on the resistance profile of multi, extensively and pandrug resistant Klebsiella pneumoniae. *J Infect Public Health*. 2024;17(11):102544. doi:10.1016/j.jiph.2024.102544
10. Akhter S, Bhat MA, Ahmed S, Siddiqui WA. Antibiotic residue contamination in the aquatic environment, sources and associated potential health risks. *Environ Geochem Health*. 2024;46(10):387. doi:10.1007/s10653-024-02146-5
11. Kiley JL, Mende K, Beckius ML, et al. Resistance patterns and clinical outcomes of Klebsiella pneumoniae and invasive Klebsiella variicola in trauma patients. *PLoS One*. 2021;16(8):e0255636. doi:10.1371/journal.pone.0255636
12. de Campos TA, de Almeida FM, de Almeida APC, et al. Multidrug-resistant (MDR) Klebsiella variicola strains isolated in a Brazilian hospital belong to new clones. *Front Microbiol*. 2021;12:604031. doi:10.3389/fmicb.2021.604031
13. Akine D, Sasahara T, Watanabe S, et al. Post-surgical meningitis caused by Klebsiella variicola. *IDCases*. 2019;18:e00622. doi:10.1016/j.idcr.2019.e00622
14. Giannattasio-Ferraz S, Ene A, Johnson G, et al. Multidrug-resistant Klebsiella variicola isolated in the urine of healthy bovine heifers, a potential risk as an emerging human pathogen. *Appl Environ Microbiol*. 2022;88(9):e0004422. doi:10.1128/aem.00044-22
15. Lekota KE, Mabeo RO, Ramatla T, et al. Genomic insight on Klebsiella variicola isolated from wastewater treatment plant has uncovered a novel bacteriophage. *BMC Genomics*. 2024;25(1):986. doi:10.1186/s12864-024-10906-x
16. Wang Y, Zhang Z, Kang J, et al. Phages in different habitats and their ability to carry antibiotic resistance genes. *J Hazard Mater*. 2024;469:133941. doi:10.1016/j.jhazmat.2024.133941
17. Shaidullina A, Harms A. Toothpicks, logic, and next-generation sequencing: systematic investigation of bacteriophage-host interactions. *Curr Opin Microbiol*. 2022;70:102225. doi:10.1016/j.mib.2022.102225
18. Dicks LMT, Vermeulen W. Bacteriophage-host interactions and the therapeutic potential of bacteriophages. *Viruses*. 2024;16(3):478. doi:10.3390/v16030478
19. Nie W, Qiu T, Wei Y, Ding H, Guo Z, Qiu J. Advances in phage–host interaction prediction: in silico method enhances the development of phage therapies. *Briefings Bioinf*. 2024;25(3). doi:10.1093/bib/bbae117
20. Smith HZ, Hollingshead CM, Kendall B. Carbapenem-Resistant Enterobacterales. In: *StatPearls*. StatPearls Publishing Copyright © 2025, StatPearls Publishing LLC.; 2025.
21. Silva KPT, Sundar G, Khare A. Efflux pump gene amplifications bypass necessity of multiple target mutations for resistance against dual-targeting antibiotic. *Nat Commun*. 2023;14(1):3402. doi:10.1038/s41467-023-38507-4
22. Dong N, Yang X, Chan EW, Zhang R, Chen S. Klebsiella species: taxonomy, hypervirulence and multidrug resistance. *EBioMedicine*. 2022;79:103998. doi:10.1016/j.ebiom.2022.103998
23. Castro J, Araújo D, Oliveira H, et al. Multidrug-resistant Klebsiella pneumoniae and Klebsiella variicola isolated from patients in Portuguese hospitals: genomic and phenotypic characterization. *The Microbe*. 2024;5:100172. doi:10.1016/j.microb.2024.100172
24. Klaper K, Pfeifer Y, Heinrich L, et al. Enhanced invasion and survival of antibiotic- resistant Klebsiella pneumoniae pathotypes in host cells and strain-specific replication in blood. *Front Cell Infect Microbiol*. 2025;15:1522573. doi:10.3389/fcimb.2025.1522573
25. Dat TTH, Steinert G, Cuc NTK, Cuong PV, Smidt H, Sipkema D. Diversity of bacterial secondary metabolite biosynthetic gene clusters in three Vietnamese sponges. *Mar Drugs*. 2022;21(1):29. doi:10.3390/md21010029
26. Wei X, Ning W, McCadden CA, et al. Exploring and expanding the natural chemical space of bacterial diterpenes. *Nat Commun*. 2025;16(1):3721. doi:10.1038/s41467-025-57145-6
27. Ayikpoe RS, Shi C, Battiste AJ, et al. A scalable platform to discover antimicrobials of ribosomal origin. *Nat Commun*. 2022;13(1):6135. doi:10.1038/s41467-022-33890-w

28. Mahajan P, Kumar M, Bhalla GS, Tandel K. Plasmid-based replicon typing: useful tool in demonstrating the silent pandemic of plasmid-mediated multidrug resistance in Enterobacterales. *Med J Armed Forces India*. 2024;81(3):301–306. doi:10.1016/j.mjafi.2024.02.004
29. Castañeda-Barba S, Top EM, Stalder T. Plasmids, a molecular cornerstone of antimicrobial resistance in the one health era. *Nat Rev Microbiol*. 2024;22(1):18–32. doi:10.1038/s41579-023-00926-x
30. Wang Y, Dagan T. The evolution of antibiotic resistance islands occurs within the framework of plasmid lineages. *Nat Commun*. 2024;15(1):4555. doi:10.1038/s41467-024-48352-8
31. Dion MB, Oechslin F, Moineau S. Phage diversity, genomics and phylogeny. *Nat Rev Microbiol*. 2020;18(3):125–138. doi:10.1038/s41579-019-0311-5
32. Chevallereau A, Pons BJ, van Houte S, Westra ER. Interactions between bacterial and phage communities in natural environments. *Nat Rev Microbiol*. 2022;20(1):49–62. doi:10.1038/s41579-021-00602-y
33. Dhaka P, Chantziaras I, Vijay D, et al. Can improved farm biosecurity reduce the need for antimicrobials in food animals? A scoping review. *Antibiotics*. 2023;12(5):893. doi:10.3390/antibiotics12050893

Infection and Drug Resistance

Dovepress
Taylor & Francis Group

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit http://www.dovepress.com/testimonials.php to read real quotes from published authors.

Submit your manuscript here: https://www.dovepress.com/infection-and-drug-resistance-journal