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ORIGINAL RESEARCH

In-silico Analysis of a Novel MCR-1.1 Variant on an IncX4 Plasmid Attenuating Colistin Resistance in Multidrug-Resistant *Escherichia coli* ST131

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Introduction: The emergence of mcr-1.1-mediated colistin resistance in *Escherichia coli* poses a significant threat to last-resort antibiotic therapy. This study investigates a novel variant of mcr-1.1 found in a highly virulent E. coli ST131 strain isolated from a pediatric patient with severe aplastic anemia and recurrent infections.

Methods: Blood samples were collected from a 4-year-old patient, and the *E. coli* isolate underwent antimicrobial susceptibility testing, multi-locus sequence typing, serotyping, and whole-genome sequencing. In-silico analyses included molecular docking and molecular dynamics simulations to assess the structural and functional impact of the mcr-1.1 variant. Horizontal gene transfer experiments evaluated plasmid mobility.

Results: The *E. coli* ST131 isolate harboured a mcr-1.1 gene located on a stable IncX4 plasmid and exhibited a multidrug-resistant phenotype. A missense mutation (T797C) led to an F265L substitution in the MCR-1.1 enzyme, reducing its phosphoethanolamine transferase activity. This mutation likely impairs lipid A modification, decreasing colistin resistance. Molecular modeling supported the reduced binding affinity of the mutated MCR-1.1 for lipid A. The plasmid demonstrated a horizontal transfer frequency of 1.3×10^{-2}. Phylogenetic analysis showed close relatedness to global ST131 clones.

Conclusion: This novel mcr-1.1 variant potentially restores colistin susceptibility in a globally prevalent *E. coli* lineage. The findings highlight a unique resistance attenuation mechanism and offer a promising avenue for restoring colistin efficacy. Further *in-vivo* validation is warranted to explore therapeutic strategies exploiting such mutations.

Keywords: colistin susceptible, MCR1.1. variant, *Escherichia coli* ST131, IncX4 plasmid, multidrug resistance

Introduction

Colistin, a polymyxin class antibiotic, has regained prominence in clinical settings due to the global rise of multidrug-resistant (MDR) Gram-negative infections.[1] Despite its initial withdrawal from widespread use in the mid-20th century owing to nephrotoxicity and neurotoxicity,[2] colistin is now a critical last-resort therapy against carbapenem-resistant pathogens such as *Enterobacteriaceae, Acinetobacter baumannii*, and *Pseudomonas* spp.[3,4] Its bactericidal mechanism involves interaction with the lipid A component of lipopolysaccharide (LPS) in the outer membrane, disrupting membrane integrity and leading to bacterial cell death.[5] This makes it one of the few effective treatments against infections that do not respond to 3rd-generation cephalosporin antibiotics. Resistance to colistin has traditionally been chromosomal, involving modifications to LPS structure that reduce antibiotic binding.[6] These chromosomal mutations are only vertically transmissible, and since the discovery of the *mcr* gene, polymyxins were considered one of the last classes of antibiotics for which resistance was not horizontally transferable between cells and remained effective against Gram-negative bacteria.[7] However, the discovery of plasmid-

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mediated colistin resistance genes, notably the mobile colistin resistance gene *mcr-1*, has introduced a new dimension to antimicrobial resistance by facilitating horizontal gene transfer. Since its initial detection in *E. coli* from food animals in China in 2015,[8] multiple mcr gene variants (*mcr-1* to *mcr-10*) have been identified across diverse ecological niches and bacterial hosts. The *mcr-1* gene encodes a phosphoethanolamine (pEtN) transferase that modifies lipid A, thereby reducing colistin's affinity and efficacy.[9,10] Among these, *mcr-1* remains the most widespread, with several alleles including *mcr-1.1* to *mcr-1.36*.[11] These alleles have been found on various plasmid backbones, including IncI2, IncX4, IncHI1, and IncHI2, often in combination with other antimicrobial resistance determinants such as extended-spectrum beta-lactamases (ESBLs) and carbapenemases.[12–14] Particularly concerning is the pandemic *E. coli* ST131 lineage, which frequently harbours such resistance elements, posing significant clinical challenges due to its virulence and resistance profile.[15] In this study, we focus on characterizing a novel mcr-1.1 variant carried on an IncX4 plasmid isolated from an MDR *E. coli* ST131 clinical strain. We aim to elucidate the genetic and proteomic basis of reduced colistin resistance associated with this variant through comprehensive genomic sequencing, molecular docking, molecular dynamics simulations, and horizontal gene transfer assays. A present study focuses on the novel *mcr-1.1* variant, carried by the IncX4 plasmid, which has recently emerged. This variant reduces resistance, a change linked to a specific mutation site within the gene. The IncX4 plasmid is noted for its stability and efficient dissemination across bacterial populations, raising concerns about the spread of *mcr-1.1* in clinical settings.[16] Rapid and accurate detection of *mcr* genes in clinical and environmental samples is critical to managing and controlling the spread of resistance. Additionally, the fitness cost associated with carrying this gene and the stability of *mcr*-carrying plasmids in bacterial populations need to be thoroughly understood. Furthermore, novel therapeutic strategies, including the development of new antibiotics or adjuvants that can restore colistin efficacy, are urgently needed as antibiotic classes targeting the lipopolysaccharide transporter.[17] Through our integrative genomic and proteomic analyses, we investigate how the novel *mcr-1.1* variant impacts bacterial physiology, antibiotic resistance, and virulence. Our findings seek to provide critical insights into the molecular mechanisms underlying this mutation and to identify potential targets for therapeutic intervention to combat colistin-resistant infections.

Materials and Methods

This study was performed in accordance with the Declaration of Helsinki and the institutional biosafety and ethical guidelines of Shenzhen Children's Hospital. Approval was obtained from the hospital's ethics committee. Written informed consent was secured from the legal guardian of the pediatric participant.

Isolation and Identification of Bacterial Isolate

Upon admission on October 24, 2022, to Shenzhen Children's Hospital, the 4-year-old girl was diagnosed with severe aplastic anaemia-chemotherapy received chemotherapy in the past, but recurrent fever, along with other medical conditions. We do not have a record for before-admission antibiotic therapy at home. Our immediate focus was on isolating and identifying the causative agents responsible for her recurring infections and symptoms. To achieve this, we collected blood cultures and perianal swab specimens under sterile conditions to ensure accurate microbiological assessment. These samples were cultured on blood agar plates and then incubated at 37°C, 18–24 hrs. We closely monitored microbial growth and identified colonies using colony characteristics, followed by biochemical tests using the API-20 strip, as well as microscopy and 16_S RNA PCR assay and sequencing. Universal primers 27F (5'-AGAGTTTGATCCTGGCTCAG-3') and 1492R (5'-GGTTACCTTGTTACGACTT-3') were used, and laboratory protocol was well established in our laboratory. PCR products were purified and subjected to Sanger sequencing for species verification. Genomic DNA was extracted using the boiling and cold method as previously described from the EC-22-087 strain.[18](pp2014–2018) Identifying any pathogenic organisms was critical for customizing targeted antimicrobial therapy.

Screening for mcr-1 Gene and Mutation Analysis

We performed a PCR assay to screen for the *mcr-1* gene as part of our project and discovered a mutation at the 797 bp position when compared to the *mcr-1* gene reported in China in 2015,[8] which we designated as the wild type in this study and present denoted as novel *mcr-1.1*. The primers for *mcr-1* (forward primer 5'-CGGTCAGTCCGTTTGTTC-3' and reverse primer 5'-CTTGGTCGGTCTGTAGGG-3') and the protocol were adopted from our laboratory.[18] PCR conditions

were as follows: initial denaturation at 95°C for 5 minutes; 30 cycles of 95°C for 30 seconds, 58°C for 30 seconds, and 72°C for 45 seconds; final extension at 72°C for 7 minutes. Amplicons were purified and sequenced by Sanger sequencing to identify nucleotide changes. The genomic DNA of $DH_{10}B$ was used as a control. Based on the PCR assays and further antimicrobial susceptibility for colistin, pursue this isolate for further genomic study. Simultaneously, we closely monitored the patient's blood counts, liver and kidney function tests, and inflammatory markers to evaluate her response to treatments and to promptly identify any complications arising from severe aplastic anaemia and ongoing infections.

Antimicrobial Susceptibility

The minimum inhibitory concentrations (MICs) for 18 antimicrobial agents, including beta-lactams, aminoglycosides, fluoroquinolones, polymyxins, and trimethoprim-based combinations, were determined using the VITEK® 2 automated system (bioMérieux, AST-N246 card) (Table 1). MICs for colistin and polymyxin B were further verified by broth microdilution according to EUCAST 2024 guidelines. Quality control was ensured using *E. coli* ATCC 25922.

Genome Sequencing and Assembly

Genomic DNA (gDNA) from the EC-22-087 isolate was purified using the QIAamp DNA Mini Kit (Qiagen, Germany) following the manufacturer's protocol. Whole-genome sequencing was performed on the Illumina MiSeq platform (Sangon Biotechnology, China), utilizing a paired-end library with 300 bp insert sizes prepared using the MiSeq Reagent Kit v2. The raw reads were assembled de novo using CLC Genomics Workbench version 9.0.1 (Qiagen Bioinformatics). The assembled genome was subsequently visualized and scaffolded using the Contiguity tool (available at https://github.com/mjsull/Contiguity).

Multi-Locus Sequencing Typing, Serotyping and Phylogenetic Group

Sequence typing of the EC-22-087 strain was conducted using the *E. coli*-specific MLST scheme provided by PubMLST (https://pubmlst.org/organisms/escherichia-spp; accessed December 22, 2023). O- and H-antigen profiles were identified using SerotypeFinder version 2.0.1, K database version 1.0.0 (https://cge.food.dtu.dk/services/SerotypeFinder/; accessed

Table 1 MICs for Conventional Antibiotics Studies for E. coli Strain EC-22-087

Antibiotics	Mics (mg/L)	Conclusion	Resistance Genes
Amikacin	4	S	NA
Amoxicillin-clavulanic acid	8	S	NA
Ampicillin	64	R	bla_{TEM-1}
Cefazolin	64	R	$bla_{CTX-M-15}$
Cefepime	2	R	$bla_{CTX-M-15}$
Cefoxitin	4	S	NA
Ceftazidime	16	R	$bla_{CTX-M-15}$
Ceftriaxone	64	R	$bla_{CTX-M-15}$
Ciprofloxacin	16	R	*gyr*A
Colistin	1	S	*mcr*-1.1
Gentamicin	16	R	aac(3)-IIa
Meropenem	0.5	S	NA
Nitrofurantoin	16	S	NA
Norfloxacin	8	R	*gyr*A
Piperacillin-tazobactam	4	S	NA
Polymyxin B	1	S	*mcr*-1.1
Ticarcillin-clavulanic acid	4	S	NA
Tobramycin	4	R	*aac*(3)-IIa
Trimethoprim	16	R	*drf*A
Trimethoprim-sulfamethoxazole	320	R	*drf*A

Abbreviations: NA, Not Detected.

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January 4, 2024). Phylogenetic grouping was assessed using a triplex PCR method that targets the *chu*A, *yja*A, and *Tsp*E4-C2 genes, following established protocols. As part of the validation, EC-19-322 (a previously characterized laboratory strain) was used as a positive control, and *P. aeruginosa* ATCC 25923 served as the negative control. The isolate's phylogenetic relatedness to other ST131 *E. coli* strains was subsequently evaluated.

Genome Annotation and Comparative Genomics

Genome annotation of the EC-22-087 isolate was conducted using Prokka, a prokaryotic genome annotation pipeline (http://vicbioinformatics.com/; accessed January 5, 2024). To identify acquired antimicrobial resistance genes, ResFinder version 4.4.3 (http://genepi.food.dtu.dk/resfinder; last accessed January 5, 2024) was employed. Additionally, the raw Illumina sequence reads were screened against the ARG-ANNOT database (https://www.mediterranee-infection.com/acces-ressources/base-de-donnees/arg-annot-2/) for a more comprehensive resistome profile. Specific chromosomal loci such as *str*A, *Amp*C, *tmr*B, and *tet*A were manually examined for mutations known to be associated with resistance phenotypes. Comparative sequence analysis of the chromosome and plasmid was carried out using BLASTn to identify homologous elements and structural variations.[19]

Measurement of Conjugation Transfer Rate

To evaluate the horizontal transferability of the novel *mcr-1.1* allele, a conjugation assay was performed using colistin-susceptible *E. coli* J53 (sodium azide resistant) as the recipient and EC-22-087 as the donor. Equal volumes (2 mL) of overnight donor and recipient cultures were combined and resuspended in 20 mL of Luria-Bertani broth at a 1:2 donor-to-recipient ratio. The mixed suspension was incubated for 6 hours at 37°C. Following mating, 100 µL aliquots were plated on Mueller–Hinton agar containing 100 mg/L sodium azide and 2 mg/L polymyxin B to select for transconjugants. Colonies were screened by PCR for the presence of *mcr-1.1* and confirmed by sequencing. The conjugation efficiency (R) was calculated using the formula:

$$R = \frac{\text{Number of Transconjugants}}{\text{Number of Recipients}}$$

In-Silico Structural Analysis of Novel MCR-1.1

We prepared both the wild-type MCR-1 protein and the novel MCR-1.1 model. The MCR-1 protein sequence was obtained from the UniProt database https://www.uniprot.org/ and its model was prepared using homology modelling via SWISS-MODEL software, selecting templates with high sequence identity to MCR-1. The quality of the resulting models was evaluated using ProSA-web to ensure structural integrity. The amino acid sequences of both proteins were compared to identify any variations. Molecular docking was conducted for both the wild-type MCR-1 and novel MCR-1.1 using AutoDock Vina and Schrödinger's Glide software. Both rigid and flexible docking protocols were employed to explore various binding conformations in lipid-A present in the bacterial cell wall. The active site of the MCR proteins was defined, and a grid box was generated to confine the docking process. Initial screening was done under default settings, followed by refined docking with optimized parameters for promising candidates. The binding affinities of the ligands were assessed using scoring functions from the docking software, and the top-ranked poses were analysed for interactions with key residues in the binding site, particularly focusing on their mode of action on the lipid-A in the cell wall. To further assess the stability of the ligand-protein interactions, molecular dynamics (MD) simulations were performed using GROMACS or AMBER. The systems were solvated, ionized, and equilibrated, and the resulting trajectories were analysed to evaluate the stability of the complexes. Although this study primarily focuses on in-silico methods, future work will involve experimental validation of the top predicted ligands through in vitro and in vivo assays to confirm their efficacy and safety.

Results

Identification and Characterization of EC-22-087

We successfully isolated and identified an *E. coli* strain from the blood and perianal swab specimens collected from the patient. The strain exhibited characteristic features, including lactose fermentation and beta-haemolysis on blood agar plates (data not

shown). Further biochemical identification using the API-20E system confirmed the *E. coli* identity, consistent with the known biochemical profile for this species (Table S1). Further confirmed by using 16S rRNA gene sequencing (Figure S1). Due to limited access to the patient's full medical history, certain contributing factors such as cumulative antibiotic exposure or microbiological cultures from prior infections could not be comprehensively analyzed, which represents a limitation of this study. PCR amplification and subsequent Sanger sequencing revealed a positive result for the *mcr-1* gene, indicating the presence of a single-point mutation at position T797C (Figure 1). This mutated gene, referred to as the novel *mcr1.1* deposited to the gene bank and obtained an accession number of PQ037029. The identification of this *E. coli* strain, particularly with the *mcr-1.1* gene, underscores the importance of ongoing surveillance and tailored antimicrobial therapy, especially in immuno-compromised patients like the one described in this case. The laboratory investigation supports the infection as WBC

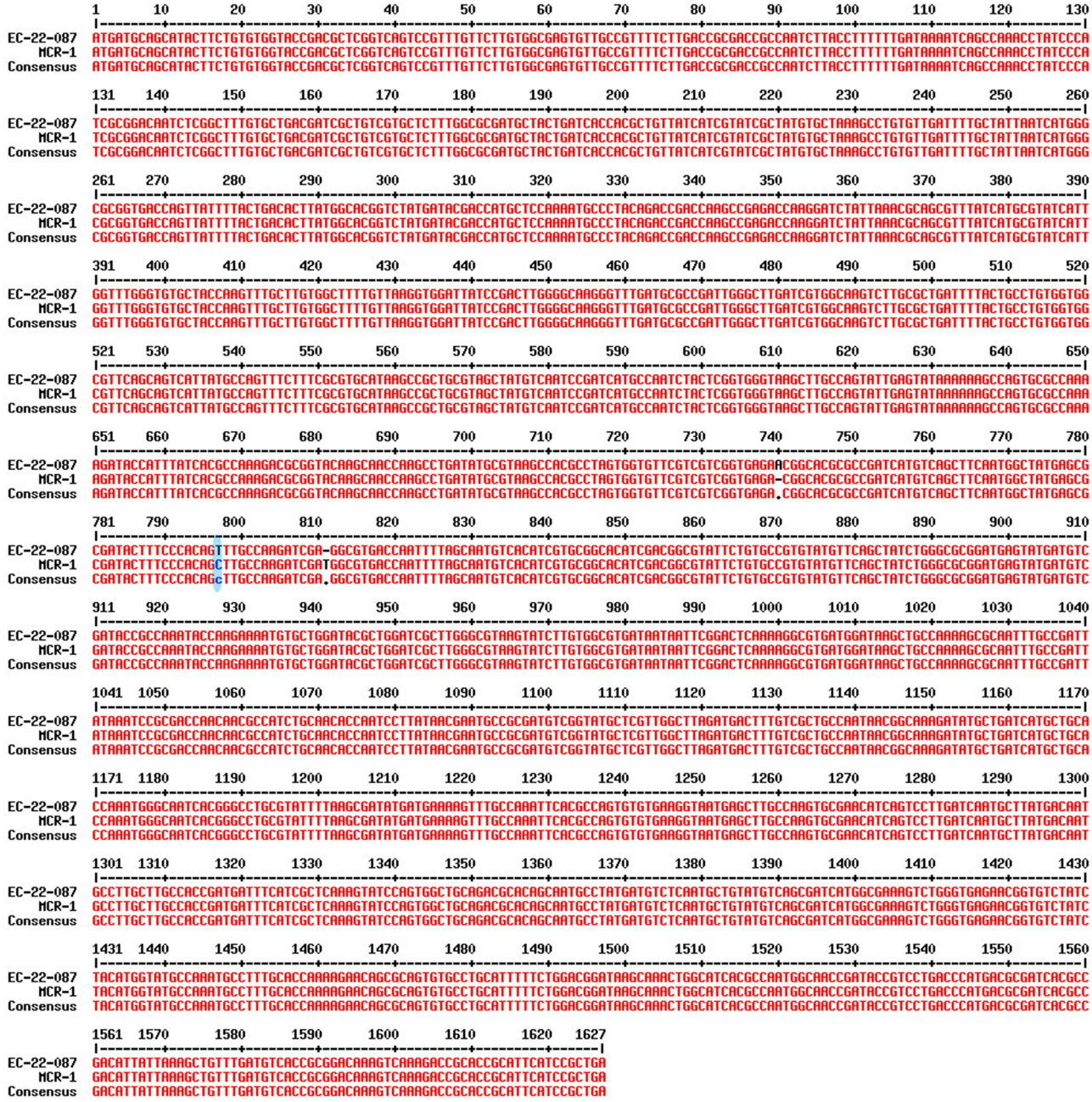

Figure 1 Identification of the *mcr-1.1* (this Study) Gene Mutation in *E. coli* EC-22-087 Sanger Sequencing Results with *mcr-1* gene.
Notes: The mutation at position T797C is highlighted in blue, indicating a single-point mutation.

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0.12×10^9/L, Neutrophils 0.01×10^9/L, haemoglobin 47g/L, and platelets 2×10^9/L/C-reactive protein was 243mg/L, procalcitonin 428.99ng/mL, Additional Kidney dysfunction, electrolyte imbalance, and signs of pneumonia on chest X-ray.

Susceptibility Testing

The strain EC-22-087 was resistant to multiple beta-lactams (ampicillin, cefazolin, ceftriaxone, ceftazidime, cefepime), and for non-beta-lactams (gentamicin, tobramycin, fluoroquinolones, trimethoprim, and trimethoprim-sulfamethoxazole). But was susceptible to colistin, polymyxin-B, meropenem, nitrofurantoin, cefoxitin, amoxicillin-clavulanic acid and piperacillin-tazobactam (Table 1). Antimicrobial susceptibility revealed the MDR phenotype.

Genomic Analysis of EC-22-087

Whole-genome sequencing revealed that the EC-22-087 isolate possesses a circular chromosome measuring 5,120,866 base pairs, with an average GC content of 50.4%, and a single plasmid. This plasmid, designated p22087A, spans 241,164 bp and harbours the novel *mcr-1.1* gene conferring reduced colistin resistance. Multilocus sequence typing (MLST) analysis classified the strain as belonging to sequence type ST131. Serotyping identified the isolate as O45:H17, and it carries the *fim*H27 allele. Phylogenetic grouping categorized EC-22-087 within subgroup B2. Comparative genome analysis indicated a high level of genetic similarity to the human bloodstream *E. coli* isolate O25b: H4-ST131 (GenBank accession number SAMN40477384), as illustrated in Figure 2. Many ST131 strains, including this one, are associated with multidrug resistance plasmids and frequently harbour virulence-associated genes.

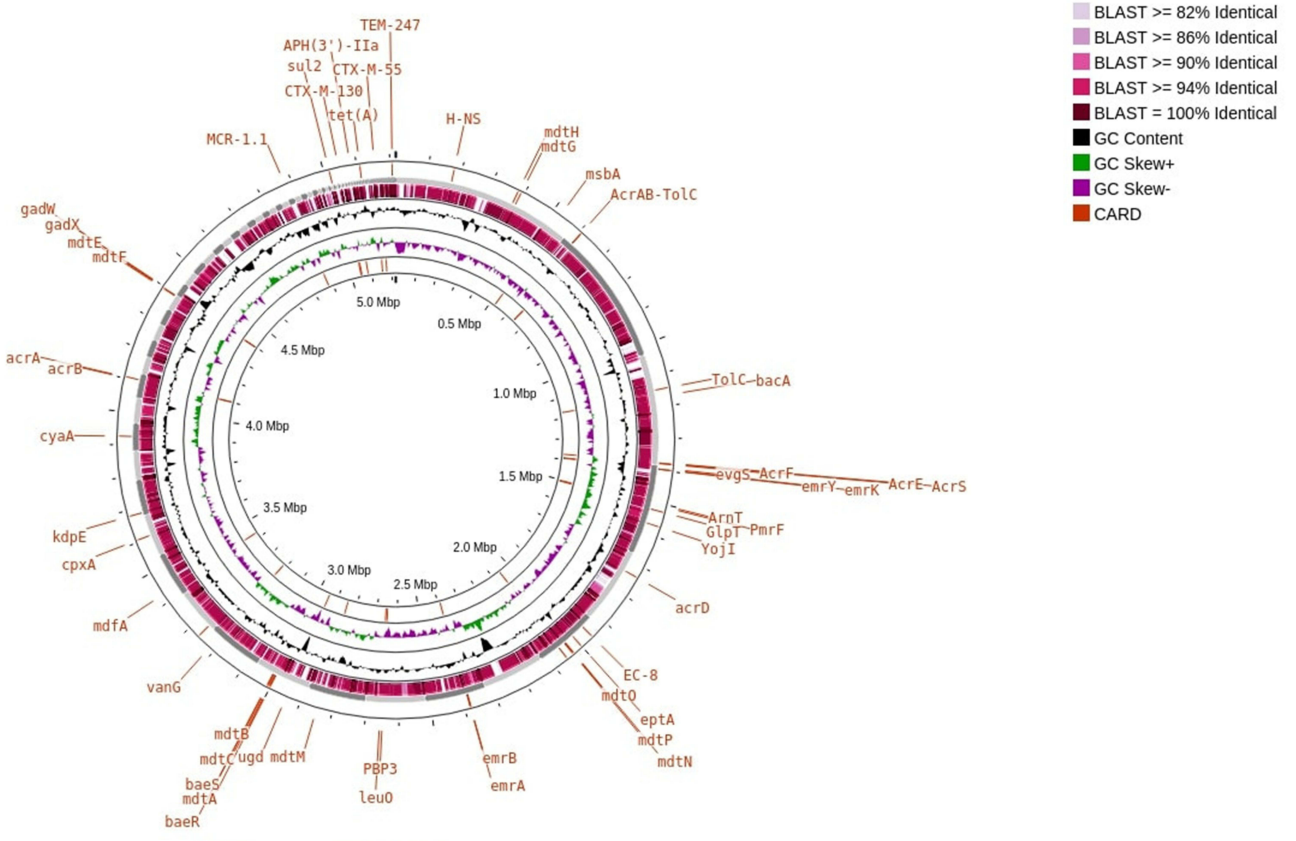

Figure 2 Whole Genome Analysis and Comparison of *E. coli* EC-22-087 with Human Bacteraemia Isolate O25b: H4-ST131 (GenBank accession number SAMN40477384).

Analysis of MDR Plasmid p22087A

The *mcr-1.1* variant associated with colistin susceptibility was found on a multidrug resistance (MDR) plasmid of the IncX4 type, designated p22087A, which spans 241 kilobases. This plasmid exhibited a horizontal transfer frequency of 1.3×10^{-2}. Comparative sequence analysis revealed 99% nucleotide identity and 89% query coverage with the previously characterized *mcr-1* carrying plasmid pSA26-MCR1 (GenBank: KU743384). Notably, p22087A harbours a distinct MDR region comprising three resistance genes, including $bla_{\text{CTX-M-15}}$, which is absent in pSA26-MCR1. In both plasmids, the *mcr* gene is embedded within an ISApal1-associated mobile element and is positioned outside the MDR gene cluster. Although the ISApal1-*mcr* cassette is structurally conserved between the two plasmids, in pSA26-MCR1 the downstream *hp*1 gene is disrupted by a secondary ISApl1 insertion bearing a predicted pap2-like phosphatase. This structural divergence may have functional implications in plasmid evolution or resistance expression.

In-silico Analysis

The in-silico proteomic analysis identified a significant point mutation at position 797 in the gene sequence, specifically the transition from thymine (T) to cytosine (C) (T797C). This mutation results in a substitution of the amino acid phenylalanine (F) with alanine (A) at position 265 within the protein structure. The replacement of phenylalanine with alanine, a smaller and less hydrophobic amino acid, may disrupt these interactions (Figure 3). The protein–protein docking of both MCR 1 and the novel MCR 1.1 with 2 phosphatases revealed multiple favourable interaction clusters based on weighted scores and energy values. The structural validation of the models was confirmed using Ramachandran plots and ProSA, as shown in Figure 4. These analyses demonstrated that the majority of residues in both MCR 1 and MCR 1.1 lie within the favoured regions of the Ramachandran plot, indicating good stereochemical quality of the models. The ProSA results further validated the models, showing Z-scores well within the range of native proteins, suggesting that the predicted structures are reliable and of high quality (Figure 4). The MCR 1 and 2 phosphatase complex, the most favourable cluster (Cluster 0), had a representative weighted score of −594.7 and a lowest energy value of −718.0, indicating strong binding affinity. Other clusters, such as Cluster 1 and Cluster 2, showed similar results with weighted scores of −596.1 and −660.4, and lowest energy values of −764.0 and −858.4, respectively. This suggests that the complex has multiple stable conformations, supported by strong binding energies. In the case of the novel MCR 1.1 and 2 phosphatase complex, the docking results also indicated strong interactions. The top cluster (Cluster 0) had a representative weighted score of −592.0 and a lowest energy value of −764.9. Other clusters, such as Cluster 1 and Cluster 2, displayed weighted scores of −691.1 and −602.2, with the lowest energy values of −691.1 and −795.2, respectively. These results highlight the structural similarities and potential variations in the binding interfaces of MCR 1 and MCR 1.1 with 2 phosphatases, with both variants showing strong binding affinities across multiple conformations (Figure 5). The pharmacophore analysis for both MCR 1 and MCR 1.1 revealed key interactions, including hydrogen bonding, hydrophobic, and electrostatic interactions between critical residues of the proteins, emphasizing their functional binding potential. In particular, several conventional hydrogen bonds and hydrophobic contacts were observed between key active site residues, supporting their potential biological relevance. The full docking data, along with detailed information on the Ramachandran plots and ProSA analysis are provided in the Table S2.

Additional Resistance Genes

The multidrug resistance (MDR) region of plasmid p22087A carries 15 additional resistance genes, along with three predicted efflux pump systems, which likely contribute to resistance against a broad spectrum of antibiotic classes, including aminoglycosides, beta-lactams, macrolides, sulfonamides, tetracyclines, and trimethoprim. Beyond the plasmid, 14 resistance determinants were identified on the EC-22-087 chromosome. These genes were localized to four distinct chromosomal regions, two of which included nearly identical genomic islands harboring *sul*1, *aad*A1, and *erm*(B). Point mutations in the *gyr*A gene specifically S83L and D87N substitutions were detected, suggesting fluoroquinolone resistance. The in-silico resistance profile derived from genome data was consistent with the phenotypic resistance pattern observed using the VITEK system (Table 1). A comprehensive inventory of resistance genes identified in EC-22-087 is summarized in Table 2.

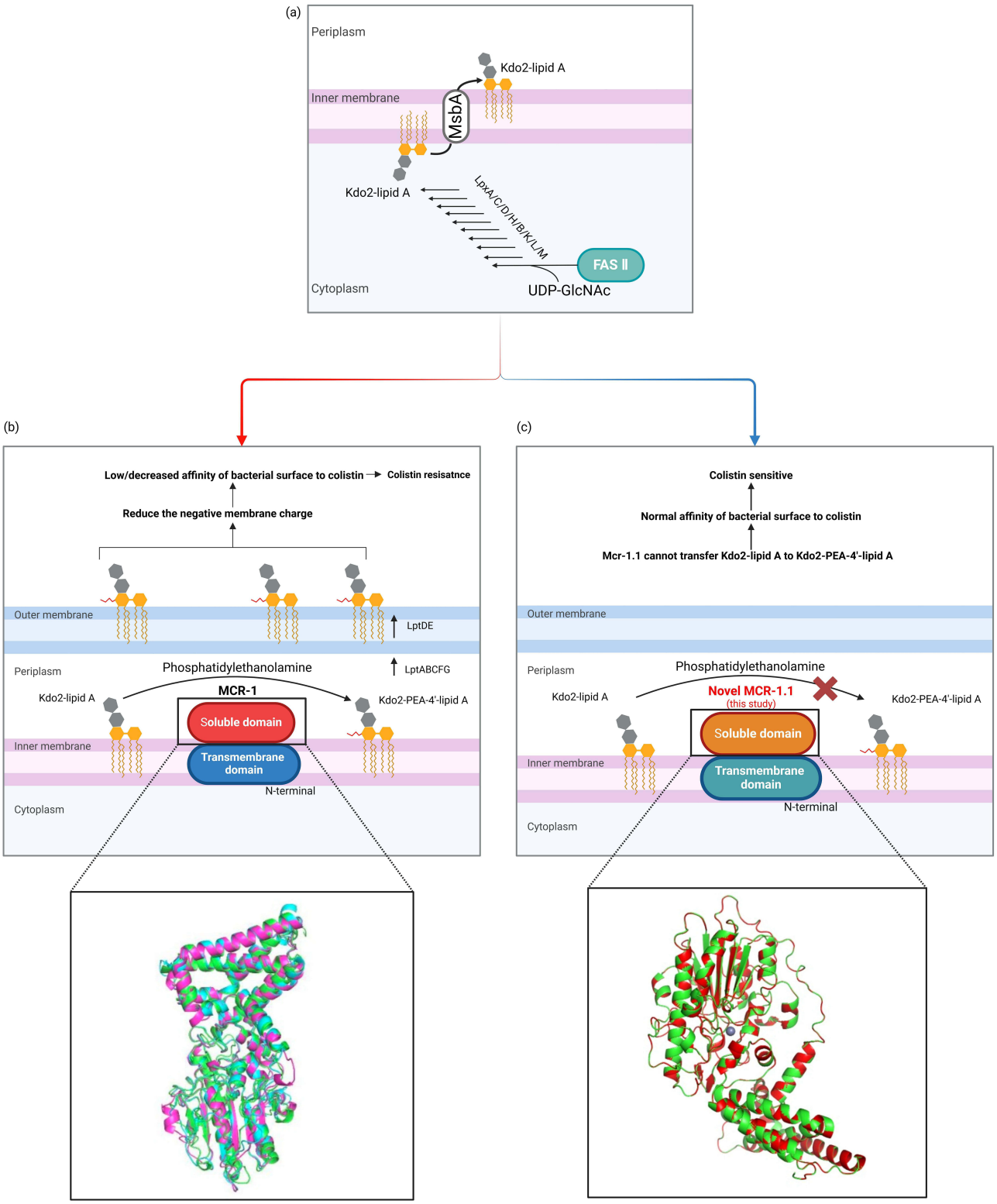

Figure 3 Impact of the T797C Nucleotide Mutation Leading to F265L Amino Acid Substitution: Comparative Analysis Between MCR-1 and MCR-1.1 (This Study).
Notes: (**a**) explains the role of lipid-A as the target for colistin; (**b**) the mechanism by which MCR-1 confers resistance to colistin by modifying lipid-A; (**c**) MCR-1.1 mutation affects this mechanism, leading to increased susceptibility to colistin. The protein model was generated using the SWISS-MODEL server based on a homologous MCR-1 structure, and visualized using PyMOL.
Abbreviations: PE, phosphatidylethanolamine; PEA, phosphoethanolamine.

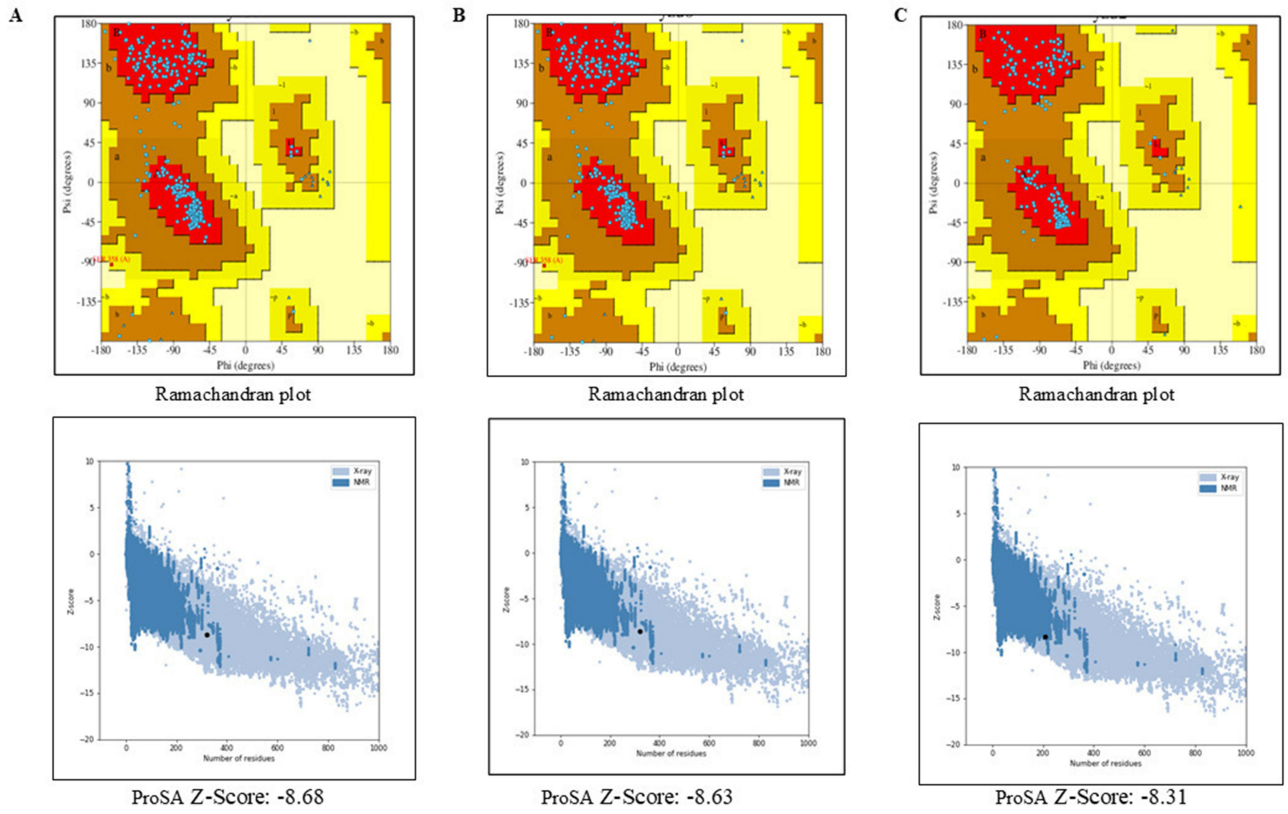

Figure 4 Structural Validation of MCR-1 and MCR-1.1 Models in Complex with 2 Phosphatase: Ramachandran Plots and ProSA Analysis.
Notes: (**A**) Ramachandran Plots of MCR-1 and ProSA Analysis; (**B**) Ramachandran Plots of MCR-1.1 (this study) and ProSA Analysis; (**C**) Ramachandandran Plots of 2 phosphatase and ProSA Analysis.

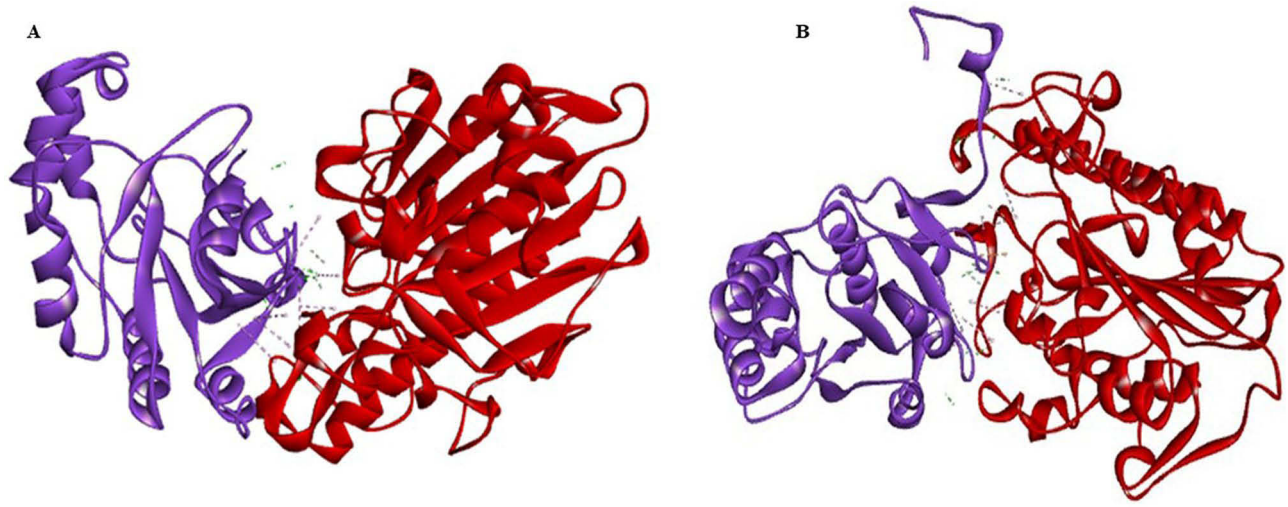

Figure 5 Binding Affinities and Interaction Clusters of MCR-1 and MCR-1.1 with 2 Phosphatase.
Notes: (**A**) Docking Clusters of MCR-1 with 2 Phosphatase; (**B**) Docking Clusters of MCR-1.1 (this study) with 2 Phosphatase. The dotted lines represent predicted hydrogen bonds between the protein and ligand. The purple domain corresponds to the putative active site region, while the red domain indicates the mutation site (Phe265). These clarifications have been added to the figure legend for better understanding.

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Table 2 Comprehensive List of Resistance Genes Identified in E. coli Strain EC-22-087

Antibiotic(s)	Genetic Environment of Resistance Gene	Resistance Genes
Aminoglycosides	Chromosome	*str*A
Aminoglycosides		*str*B
Cephalosporins		bla_{SHV-11}
Cephalosporins		*amp*C
Erythromycin		*erm*(B)
Fluoroquinolones		*gyr*A
Fluoroquinolones		*par*C
Penicillin		bla_{TEM}
Spectinomycin, streptomycin		*aad*A1
Sulfonamide		*sul*1
Sulfonamide		*sul*2
Tunicamycin		*tmr*B
Tetracycline		*tet*R
Tetracycline		*tet*A
Trimethoprim		*dfr*A1
Aminoglycosides	p22087	*aac*(3)-lia
Aminoglycosides		*str*A
Aminoglycosides		*str*B
Erythromycin		*ere*(A)
Macrolides		*mph*(A)
Penicillin		bla_{TEM}
Rifampin		*arr*-2
Spectinomycin, streptomycin		*aad*A1
Sulfonamide		*sul*1
Sulfonamide		*sul*1
Tetracycline		*tet*B
Trimethoprim		dfra7
Tunicamycin		*tmr*B
β-Lactams		$bla_{CTX-M-15}$

Discussion

The emergence of plasmid-borne colistin resistance genes such as *mcr-1* has raised substantial concerns within the global health community, particularly because colistin remains a critical therapeutic option for treating infections caused by multidrug-resistant Gram-negative bacteria.[20] Since its initial identification, *mcr* and its various alleles have been detected in over 25 countries across Asia, Europe, North Africa, the Middle East, and North America.[21] The *mcr-1.1* variant was initially observed in *E. coli* strains isolated from animals in Serbia, most notably associated with ST410, ST58, and ST641 lineages.[22] To date, more than 35 allelic variants of the *mcr-1* gene have been identified, including mcr-1.36. These variants arise due to point mutations within the mcr-1 coding sequence, each potentially altering the structure or function of the encoded phosphoethanolamine transferase. Such mutations can modulate the level of colistin resistance, plasmid stability, or fitness cost associated with resistance gene carriage. Monitoring these allelic variants is essential for understanding the molecular evolution, epidemiological spread, and potential clinical impact of colistin resistance mechanisms.[11] In this study, we describe a novel *mcr-1.1* variant identified in an ST131 *E. coli* isolate (EC-22-087) recovered from a clinical case in Shenzhen, China, which exhibited reduced colistin resistance.

The EC-22-087 strain, isolated from both bloodstream and perianal swab samples, demonstrated broad-spectrum resistance to multiple antibiotic classes, including β-lactams, aminoglycosides, and fluoroquinolones. However, the strain remained susceptible to colistin (MIC=1 mg/L), polymyxin B, meropenem, and certain β-lactam/β-lactamase inhibitor combinations.

This susceptibility profile is noteworthy, as it mirrors reports on other attenuated *mcr* variants such as *mcr*-3.4 and *mcr*-1.35, which have shown compromised resistance functionality.[23] Previous studies have demonstrated that agents such as silver nitrate ($AgNO_3$) can inhibit the enzymatic activity of MCR proteins, thereby restoring the antibacterial efficacy of colistin against mcr-positive strains.[23] This pharmacological suppression of MCR activity, along with natural attenuating mutations such as the A267F substitution described in our study, highlights promising strategies to restore colistin susceptibility. Further exploration of such adjuvant therapies, including metal-based inhibitors, efflux pump blockers, or lipid A-modifying enzyme antagonists, may offer novel therapeutic options for managing multidrug-resistant infections.

Phylogenomic comparisons revealed that EC-22-087 shares significant genomic similarity with the *E. coli* ST131 O25b:H4 strain previously linked to human bacteremia cases in Spain.[24] Although the strain from our study is serotyped as O45:H17, its placement within the same ST131 lineage suggests comparable virulence potential. In contrast, Forde et al 2018 reported an ST95 isolate (O2:K1:H4) from a patient in Qatar, typically characterized by lower resistance, illustrating the spectrum of resistance within pathogenic *E. coli* lineages.[25] ST131, particularly the H30-Rx clade, has emerged globally as a high-risk clone known for its multidrug-resistant phenotype and its association with both urinary and bloodstream infections.[26,27] In the United States, ST131 has been implicated in a substantial proportion of drug-resistant *E. coli* infections. The co-occurrence of *mcr-1* with other resistance determinants in this lineage amplifies its threat to public health.[28] Although recent surveillance indicates a decline in *mcr-1.1* prevalence in China, and European studies report only 0.2% positivity in clinical *E. coli* isolates,[29] the gene's plasmid-borne nature continues to pose a risk of horizontal dissemination. In our case, mcr-1.1 was located on a stable IncX4-type plasmid (p22087A) with a conjugation frequency of 1.3×10^{-2}. This plasmid also carries $bla_{\text{CTX-M-15}}$ and demonstrates high transferability. A similar plasmid, pMS8345A (IncHI2), has been described with a larger resistance gene repertoire, although differences in backbone structure and insertion sequence arrangements between pMS8345A, pSA26-MCR1, and p22087A suggest independent evolutionary origins.[25]

IncX4 plasmids are known facilitators of *mcr-1* spread among *Enterobacteriaceae*. Mobilisation of the *mcr*-1-hp1 cassette is thought to involve ISApl1-mediated transposition, possibly triggered by variant inverted repeat regions (IRRs).[30] MCR-1 belongs to the phosphoethanolamine transferase family, which modifies lipid A by substituting phosphate groups with ethanolamine residues, reducing the net negative charge on the bacterial outer membrane.[12] This charge alteration diminishes the binding affinity of cationic antimicrobial peptides, such as colistin, thus conferring resistance.[31] The in-silico analysis of EC-22-087 revealed a resistome comprising 29 genes, encoded both chromosomally and on plasmids. This extensive resistance gene repertoire aligns with prior reports of ST131 strains from other regions of China and globally.[32–34] Interestingly, although the novel MCR-1.1 shares approximately 85% amino acid identity with putative PAP2-family proteins (with 50% query coverage), it lacks the characteristic conserved motifs associated with enzymatic function. This suggests the presence of a nonfunctional or truncated phosphatase-like element. Molecular docking simulations predicted a lower binding affinity between the MCR-1.1 variant and lipid A compared to the wild-type enzyme, supporting the hypothesis of reduced functional activity. However, these results are hypothesis-generating and require experimental confirmation. Phenylalanine, a bulky and hydrophobic amino acid, plays a crucial role in maintaining the protein's interaction with other molecules, particularly with the lipid-A component of the LPS. While this interaction is proposed based on docking data, prior literature does not identify phenylalanine 265 among key lipid A binding residues.[35] The specific F265L mutation observed in MCR-1.1 lies within a conserved domain and may play a role in altering membrane interaction. The alteration in the amino acid sequence at this critical position is hypothesized to reduce the binding affinity of the protein for colistin, an antibiotic that targets lipid-A. This reduced binding could lead to a decrease in the protein's ability to confer resistance to colistin, making the bacterium more susceptible to this antibiotic. This finding is significant as it suggests a potential vulnerability in the pathogen that could be targeted to overcome drug resistance. Phenylalanine, a bulky hydrophobic residue, likely stabilizes lipid A binding; its substitution with a smaller alanine could impair this function.[36] This proposed mechanism helps explain why EC-22-087 remains susceptible to colistin, unlike typical *mcr*-1-positive strains. Prior studies describing *mcr-1*-mediated resistance often lack structural or mutation-based functional validation,[25] underscoring the novelty and importance of our findings. Nonetheless, this study has limitations. Our analysis is based on a single clinical isolate, which restricts the generalizability of the results. Additionally, while the isolate was collected in Shenzhen, local epidemiology may not reflect

national or international patterns. Finally, the proposed functional impairment of MCR-1.1 is based solely on in-silico modelling and requires confirmation through in-vitro or in-vivo experiments.

Conclusion

This study reports a novel mcr-1.1 variant in a clinical *E. coli* ST131 isolate (EC-22-087) exhibiting reduced colistin resistance despite harbouring extensive chromosomal and plasmid-borne resistance genes. The gene was located on a highly transferable IncX4 plasmid alongside $bla_{\text{CTX-M-15}}$ underscoring its potential for horizontal spread. Structural modeling suggests that the F265L substitution in MCR-1.1 may impair lipid A binding, correlating with the observed colistin susceptibility. These findings highlight the complexity of resistance evolution and suggest that naturally attenuated mcr variants may offer a therapeutic window. Functional validation and broader surveillance are warranted to assess the clinical significance of such variants.

Data Sharing Statement

The datasets generated and/or analyzed during the current study are available from the corresponding author, Sandip Patil (sandippatil1309@yahoo.com), upon reasonable request.

Ethics Approval

This study was conducted according to the guidelines of the Declaration of Helsinki and approved by the Institutional Ethics Committee, reference number: 2018 (013) dated 2018/09/03, which complies with international ethical standards.

Consent for Publication

The patient concerned was obtained from the legal guardian of the patient, as data used for research and publication purposes

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 declare that the research was conducted without any commercial or financial relationships that could be construed as a potential conflict of interest.

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