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ORIGINAL RESEARCH

Genomic Characteristics of a Carbapenem-Resistant *Escherichia coli* Co-Carrying bla_{NDM-5} and *mcr-1.1* from a Urinary Tract Infection in China

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Objective: The global emergence of multidrug-resistant *Escherichia coli* strains, particularly those co-carrying plasmid-mediated colistin and carbapenemase genes, represents a significant public health concern. The coexistence of these resistance determinants severely limits the therapeutic options available for treating infections caused by MDR pathogens.

Methods: Antimicrobial susceptibility testing was performed by broth microdilution assay. Clermont Typing platform was employed to classify the phylogenetic group. The BacWGSTdb 2.0 online platform was utilized to determine sequence types and perform the core genome cgMLST analysis by comparing with publicly available *E. coli* isolates. Serotype, FimH type, Inc groups and antimicrobial resistance genes were carried out using SerotypeFinder 2.0, CHTyper 1.0, PlasmidFinder 2.1 and ResFinder 3.2 databases, respectively.

Results: *E. coli* SM107 was resistant to nearly all the tested antibiotics excluding nitrofurantoin. The complete genome sequence comprises a single chromosome along with six plasmids and was assigned to sequence type (ST)1011. The bla_{NDM-5} gene was discovered in a 119,850 bp IncI1-1-type plasmid with IS*26*, IS*3000*, IS*30* and IS*5* exist downstream of the bla_{NDM-5} gene with IS*26* situated upstream as a truncated fragment. The *mcr-1.1* gene in a 111,450 bp IncI2-type plasmid. cgMLST analysis highlighting the emergence of multidrug-resistant *E. coli* ST1011 strains that harbor both colistin and carbapenem resistance genes.

Conclusion: We report the emergence of a ST1011 *E. coli* strain in China co-carrying the *mcr-1.1* and bla_{NDM-5} genes. This highlights the urgent need for alternative therapeutic strategies, strengthened antimicrobial stewardship, and enhanced genomic surveillance to curb the dissemination of high-risk resistance plasmids and safeguard the effectiveness of last-resort antibiotics.

Keywords: *Escherichia coli*, bla_{NDM-5}, *mcr-1.1*, whole genome sequencing, antimicrobial resistance, nitrofurantoin

Introduction

Escherichia coli is one of the most important and prevalent pathogens associated with clinical infectious diseases, particularly in urinary tract infections (UTIs).[1] The emergence of MDR *E. coli*, particularly those producing extended-spectrum β-lactamases (ESBLs) or exhibiting carbapenem resistance, poses a growing threat to global public health. These strains frequently harbor resistance genes on mobile genetic elements, such as plasmids, which facilitate horizontal gene transfer and dissemination among bacterial populations.

The World Health Organization (WHO) released an updated Bacterial Priority Pathogens List (BPPL) in 2024, reaffirming Carbapenem-resistant Enterobacteriaceae (CRE) as a critical global public health threat. It remains a major concern due to their high level of antimicrobial resistance, limited treatment options, and rapid dissemination in both

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healthcare and community settings. Among CRE, the most commonly identified carbapenemases are KPC, NDM, and OXA-48.[2–4] The $bla_{\text{NDM-1}}$ gene, conferring resistance to most β-lactams, was first discovered in 2009,[5] which has rapidly spread across the globe,[4,6,7] leading to the emergence of 96 distinct NDM variants. Among these, $bla_{\text{NDM-1}}$ and $bla_{\text{NDM-5}}$ have become the most prevalent variants worldwide.[8] The majority of $bla_{\text{NDM-5}}$-harboring plasmids have been classified as IncX3-type.[9,10]

Colistin is regarded as a last-resort antibiotic against CRE infections.[11,12] Nevertheless, its extensive and inappropriate application in livestock production and clinical settings has accelerated the rise and international spread of colistin-resistant strains. The discovery of the plasmid-borne *mcr-1* gene highlights a growing threat to the efficacy of last-resort antibiotics.[13] Since its initial identification, at least ten *mcr* variants (designated *mcr*-1 through *mcr*-10) have documented globally over the past ten years. Among them, *mcr-1* is frequently found on three primary plasmid types: IncI2, IncX4, and IncHI2.[9,10]

The simultaneous presence of *mcr-1* and $bla_{\text{NDM-5}}$ genes in gram-negative bacteria presents a significant threat to the treatment of MDR and extensively drug-resistant (XDR) infections.[10] This dual resistance, compounded by the limited availability of effective treatment options, exacerbates the challenge in managing such infections. Indeed, recent reports have shown a growing frequency of *mcr-1* detection in CRE isolates, emphasizing its continued emergence and global dissemination. Among carbapenemases, NDMs are the primary enzymes coexisting with *mcr-1* in carbapenem-resistant *E. coli*, with $bla_{\text{NDM-5}}$ being the most prevalent variant.[14]

In our study, we identified and analyzed a multidrug-resistant *E. coli* strain from China that harbored a $bla_{\text{NDM-5}}$-harbouring IncI1-I plasmid and an *mcr-1.1*-carrying IncHI2 plasmid. These findings offer important insights into the genetic characteristics, evolutionary trends, and epidemiology of the $bla_{\text{NDM-5}}$ and *mcr-1* resistance genes.

Materials and Methods

E. coli SM107, a carbapenem-resistant strain, was recovered from a urine sample of a 40-year-old female patient in Taizhou, Zhejiang, China, in October 2022. The patient had been hospitalized following surgery for a malignant tumor. Identification of the isolate was carried out using MALDI-TOF MS (matrix-assisted laser desorption/ionization time-of-flight mass spectrometry).

Antimicrobial susceptibility testing was conducted via the broth microdilution method in accordance with Clinical and Laboratory Standards Institute (CLSI) guidelines. The antibiotics included amikacin, gentamicin, tobramycin, aztreonam, ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefotetan, ceftazidime, cefotaxime, ceftriaxone, cefepime, ertapenem, imipenem, ciprofloxacin, levofloxacin, tetracycline, minocycline, tigecycline, trimethoprim-sulfamethoxazole, colistin and nitrofurantoin. Antimicrobial susceptibility results were interpreted according to the CLSI M100 (31th Edition),[15] except for tigecycline and colistin, which were evaluated using Enterobacterales breakpoints established by the European Committee on Antimicrobial Susceptibility Testing (EUCAST).[16] *E. coli* ATCC 25922 was used as the quality control strain.

Chromosome and plasmid sequences were obtained using a combination of Oxford Nanopore (MinION system, Nanopore, Oxford, UK) and Illumina (NovaSeq system, Illumina Inc., San Diego, USA) sequencing platforms. Genome annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline (PGAP).

Phylogenetic grouping was assigned using the Clermont Scheme in the Clermont Typing platform. Sequence typing and core genome multilocus sequence typing (cgMLST) analyses were conducted on the BacWGSTdb 2.0 online server by comparing the isolate with genetically related *E. coli* strains available in public databases.[17,18] Serotype, FimH type, plasmid incompatibility (Inc) groups and antimicrobial resistance genes were identified using SerotypeFinder 2.0,[19] CHTyper 1.0,[20] PlasmidFinder 2.1,[21] and ResFinder 4.0[22] databases, respectively, all accessible via the Center for Genomic Epidemiology platform. The genetic contexts of plasmid carrying the *mcr-1.1* gene and $bla_{\text{NDM-5}}$ gene were analyzed using BLAST Ring Image Generator (BRIG) and EasyFig (v2.3).[23] Core genome single nucleotide polymorphisms (cgSNPs) were identified, and a maximum-likelihood phylogenetic tree was constructed using CSI Phylogeny v1.4.[24] Phylogenetic trees were visualized and annotated with the Interactive Tree of Life (iTOL) version 5 web server.[25] The complete genome sequences of the chromosome and plasmids from *E. coli* SM107 have been deposited in the NCBI GenBank database under accession numbers CP130667-CP130673.

Results and Discussion

E. coli SM107 exhibited resistance to almost every antibiotic that was tested, with the exception of nitrofurantoin. These antibiotics included ampicillin, ampicillin/sulbactam, piperacillin/tazobactam, cefazolin, cefotetan, ceftazidime, cefotaxime, ceftriaxone, cefepime, ertapenem, imipenem, ciprofloxacin, levofloxacin, tetracycline, minocycline, tigecycline, trimethoprim-sulfamethoxazole, fosfomycin, and colistin.

The complete genome sequence of *E. coli* SM107 consists of seven fully assembled and circularized contigs, totaling 5,511,563 bp. The chromosome is represented by Contig 1, comprising 5,166,015 bp, while the remaining contigs represent distinct plasmids: contig 2 (119,850 bp), contig 3 (111.450 bp), contig 4 (105678 bp), contig 5 (5772 bp), contig 6 (1597 bp) and contig 7 (1201 bp) (Table 1). The genome has a GC content of 50.5% and encodes a total of 5301 coding sequences (CDSs) along with 123 RNA genes, including 93 tRNAs, 22 rRNAs, and 8 non-coding RNAs (ncRNAs). *E. coli* SM107 was classified as ST1011. *In silico* serotyping and phylogenetic analysis identified *E. coli* SM107 as nontypeable O-:H16 and placed it in phylogroup E.

E. coli SM107 consists of genes that confer resistance to aminoglycosides [*aph(6)-Id, aac(3)-IId, aadA22, aph(3')-Ia*], β-lactams ($bla_{\text{OXA-1}}$, $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-64}}$, and $bla_{\text{NDM-5}}$), tetracyclines [two copies of *tet*(A) and *tet*(M)], macrolides [*mph*(A), *mph*(E), *msr*(E), *mdf*(A) and *erm*(B)], polymyxins (*mcr-1.1*), sulphonamides (*sul*2 and *sul*1) and efflux pumps (Table 2).

Table 1 Genome Sequence of *E. coli* Isolate SM107

Contig	Genetic Material	Plasmid Type	Size (bp)	GC Content (%)	Antimicrobial Resistance Gene(s)
1	Chromosome	/	5,166,015	50.5	*mph*(E), *msr*(E), *sul*1, $bla_{\text{OXA-1}}$, *sul*2, *aph(6)-Id, tet*(A), *flo*R, *mph*(A), *aac(3)-IId, sul*1, $bla_{\text{TEM-1B}}$, *mdf*(A)
2	pSM107_NDM	IncI1-I	119,850	50	$bla_{\text{NDM-5}}$, *aadA22, tet*(M), *erm*(B)
3	pSM107_MCR	IncI2	111,450	45	*mcr*-1.1
4	pSM107_1	IncFIB	105,678	50	*aph(3')-Ia, tet*(A), $bla_{\text{CTX-M-64}}$
5	pSM107_2	ColRNAI	5772	49	
6	pSM107_3	Col (MG828)	1597	46	
7	pSM107_4	Unknown	1201	53	

Table 2 Genotyping and Phenotypic Resistance Profile of the *E. coli* SM107

Antimicrobial Agents	MIC (mg/L)	Susceptibility	Antimicrobial Resistance Genes
Aminoglycosides			*aph(6)-Id, aac(3)-IId,*
Amikacin	≥32	R	*aadA22, aph(3')-Ia*
Gentamicin	≥16	R	
Tobramycin	≥16	R	
β-lactams			$bla_{\text{OXA-1}}$, $bla_{\text{TEM-1}}$, $bla_{\text{CTX-M-64}}$, $bla_{\text{NDM-5}}$
Ampicillin	≥32	R	
Ampicillin/Sulbactam	≥32	R	
Piperacillin/Tazobactam	≥128	R	
Cefazolin	≥64	R	
Cefotetan	≥64	R	
Cefotaxime	≥32	R	
Ceftazidime	≥64	R	
Ceftriaxone	≥64	R	
Cefotaxime	≥64	R	
Cefepime	≥64	R	
Aztreonam	≥128	R	
Ertapenem	≥16	R	
Imipenem	≥8	R	

(*Continued*)

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Table 2 (Continued).

Antimicrobial Agents	MIC (mg/L)	Susceptibility	Antimicrobial Resistance Genes
Fluoroquinolones			
Ciprofloxacin	≥4	R	
Levofloxacin	≥8	R	
Tetracyclines			*tet*(A), *tet*(M)
Tigecycline	≥128	R	
Minocycline	≥16	R	
Tetracycline	≥1	R	
Sulfonamides			*sul*1, *sul*2
Trimethoprim/sulfamethoxazole	≥512/16	R	
Macrolide			
Clarithromycin	≥2	R	*mph*(A), *mph*(E), *msr*(E), *mdf*(A), *erm*(B)
Polymyxins			
Colistin	≥32	R	*mcr-1.1*
Nitrofuran			
Nitrofurantoin	≤16	S	
Other			
Fosfomycin	≥128	R	

Abbreviations: MIC, minimum inhibitory concentration; S, susceptible; R, resistant.

The $bla_{\text{NDM-5}}$ gene was found in the 119,850 bp IncI1-1 type plasmid pSM107_NDM. According to a BLAST analysis, pSM107_NDM shared 87% query coverage and over 99% sequence identity with the IncI-type plasmid p2_025970 from *E. coli* strain WCHE025970. Similarly, pSM107_NDM had 83% query coverage and over 99% identity with the IncI-type plasmid pEC6563-NDM5 form *E. coli* strain EC6563 isolated from a urine sample in Hangzhou, Zhejiang, China, in November 2020 (Figure 1A). Both plasmids pSM107_NDM and pEC6563-NDM5 contain a single copy of $bla_{\text{NDM-5}}$, with the IS*26* insertion sequence located upstream and the IS*26*, IS*30*, IS*5* insertion sequence located downstream (Figure 1B). Notably, *E. coli* isolate EC6563 was a novel IncI1-I plasmid carrying $bla_{\text{NDM-5}}$ coexisted with an IncHI2 plasmid harboring *mcr-1.1*.[26] The $bla_{\text{NDM-5}}$-carrying IncI1-I plasmid pEC6563-NDM5 exhibited high conjugation efficiency, which may provide a favourable platform for the dissemination of carbapenem resistance genes in both clinical and environmental settings.[26] These findings from pSM107_NDM further validate the regional dissemination of the $bla_{\text{NDM-5}}$-carrying IncI1-I plasmid in Zhejiang Province, as previously observed in EC6563.[26]

The *mcr-1.1* gene was discovered in pSM107_MCR, a 111,450 bp IncI2 plasmid. A BLAST analysis revealed that pSM107_MCR only 56% query coverage with IncHI2-type plasmid pGZ49266 and pHNSD133-MCR (Figure 2). Significantly, this limited homology suggests that *mcr-1.1* is not limited to high-homology IncHI2 plasmids and may also disseminate via genetically diverse plasmids, highlighting a potential challenge for surveillance and control efforts.

According to the genetic environment analysis, IS*26*, IS*3000*, IS*30* and IS*5* located upstream of the $bla_{\text{NDM-5}}$ gene, whereas IS*26* was found downstream to be a truncated fragment inside the pSM107_NDM plasmid (Figure 3A). The genetic environment of the *mcr*-1.1 revealed that DNA topoisomerase III situated downstream within the pSM107_MCR plasmid (Figure 3B). Analysis of the genetic environment of the $bla_{\text{CTX-M-64}}$ revealed that IS*1B* was present upstream of the $bla_{\text{CTX-M-64}}$, whereas IS*Ecp1*, Tn3 and IS*1A* were located downstream in the form of a truncated fragment inside the pSM107_1 (Figure 3C). The genetic contexts of the $bla_{\text{NDM-5}}$, *mcr-1.1* and $bla_{\text{CTX-M-64}}$ genes identified in this study suggest complex mobilization mechanisms that facilitate horizontal gene transfer and the dissemination of multidrug resistance.

The phylogenetic relationship between *E. coli* SM107 and 31 ST1011 *E. coli* strains that are currently deposited in the NCBI GenBank database and have cgMLST distances less than 1000 were examined in order to assess the genomic epidemiological traits of *E. coli* strains globally (Figure 4). The strains included isolates from humans, chickens, pigs, ducks, fish, reptiles, wastewater, and shrimp, spanning a range of years from 1985 to 2022, and suggests the ability of this

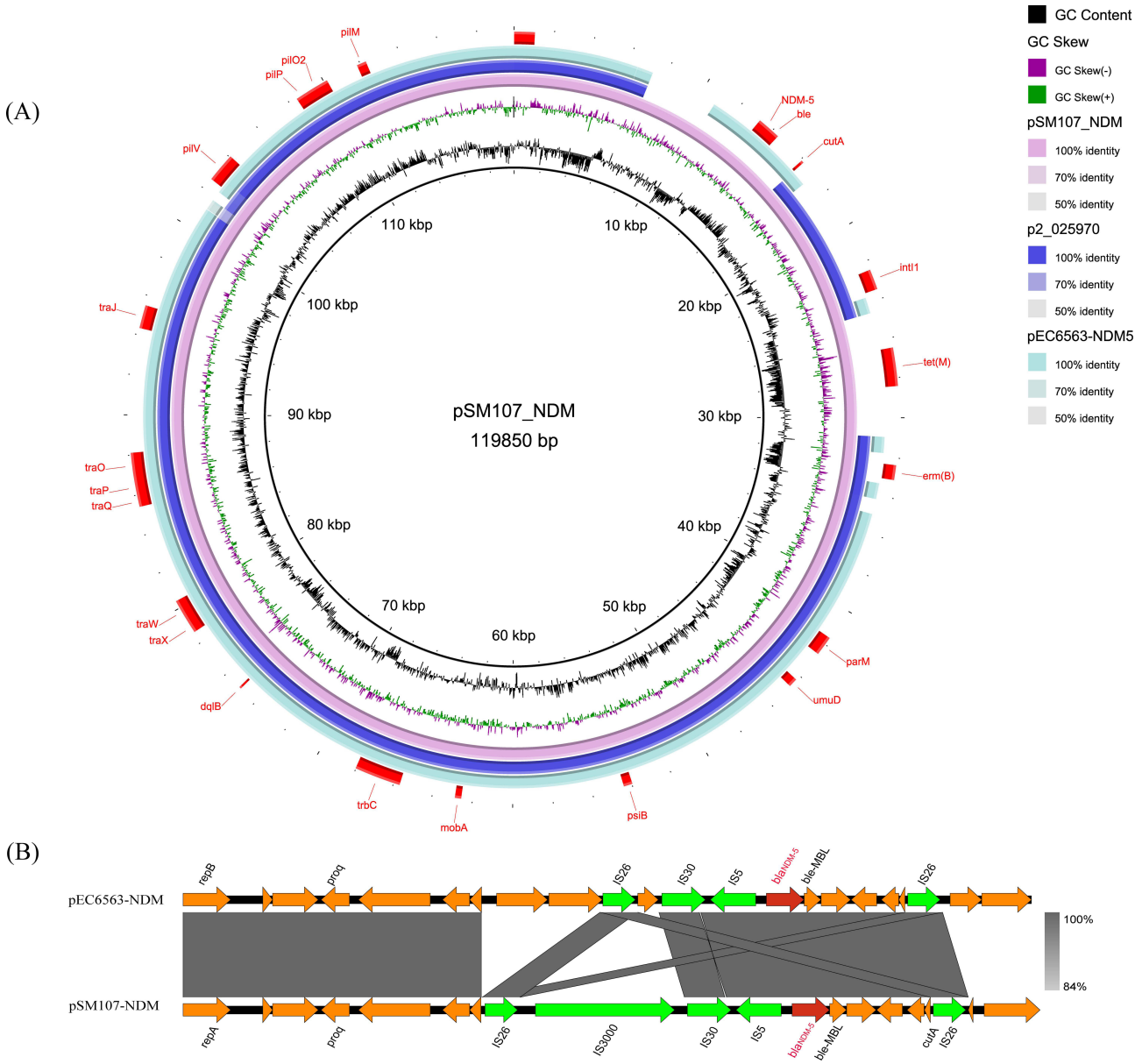

Figure 1 Comparative analysis between the pSM107_NDM and other related plasmids. (**A**) Plasmid structure of pSM107_NDM. pSM107_NDM was used as the reference plasmid to perform genome alignment with p2_025970 and pEC6563-NDM5. (**B**) Schematic illustration comparing the structural features of plasmid pSM107_NDM with sequences of plasmid pEC6563-NDM5. Grey shading and squares indicate homologies between the corresponding genetic loci on each plasmid. Arrows indicate open reading frames, with arrowheads indicating the direction of transcription: red, antibiotic resistance-encoding genes; green, IS truncated fragment genes; other genes are shown by Orange arrows.

lineage to transmit across diverse host species may play a key role in its successful adaptation to various environments. While most of the ST1011 strains are isolated from China (21 out of 32), the data also include strains from other countries, including the United States, Pakistan, the Netherlands, Germany, and the United Kingdom. The distribution of *E. coli* ST1011 across multiple countries suggests that this lineage is globally disseminated, with the ability to spread across different regions and countries.

Common resistance determinants included those conferring resistance to β-lactams ($bla_{\text{TEM-1B}}$, $bla_{\text{OXA-1}}$, $bla_{\text{CTX-M-64}}$) , aminoglycosides [*aac(3)-IId, aad*A2], and tetracyclines [*tet*(A), *tet*(M)]. In addition, genes related to phenicol resistance [*mph*(A), *mph*(E)], sulfonamide resistance (*sul*1, *sul*2), and quinolone resistance (*oqx*A, *oqx*B) were frequently detected. Interestingly, *mcr-1.1*, which confers resistance to colistin, was present in the majority of the strains (18 out of 32), highlighting the worldwide dissemination of this lineage carrying colistin-resistance gene. A nationwide surveillance

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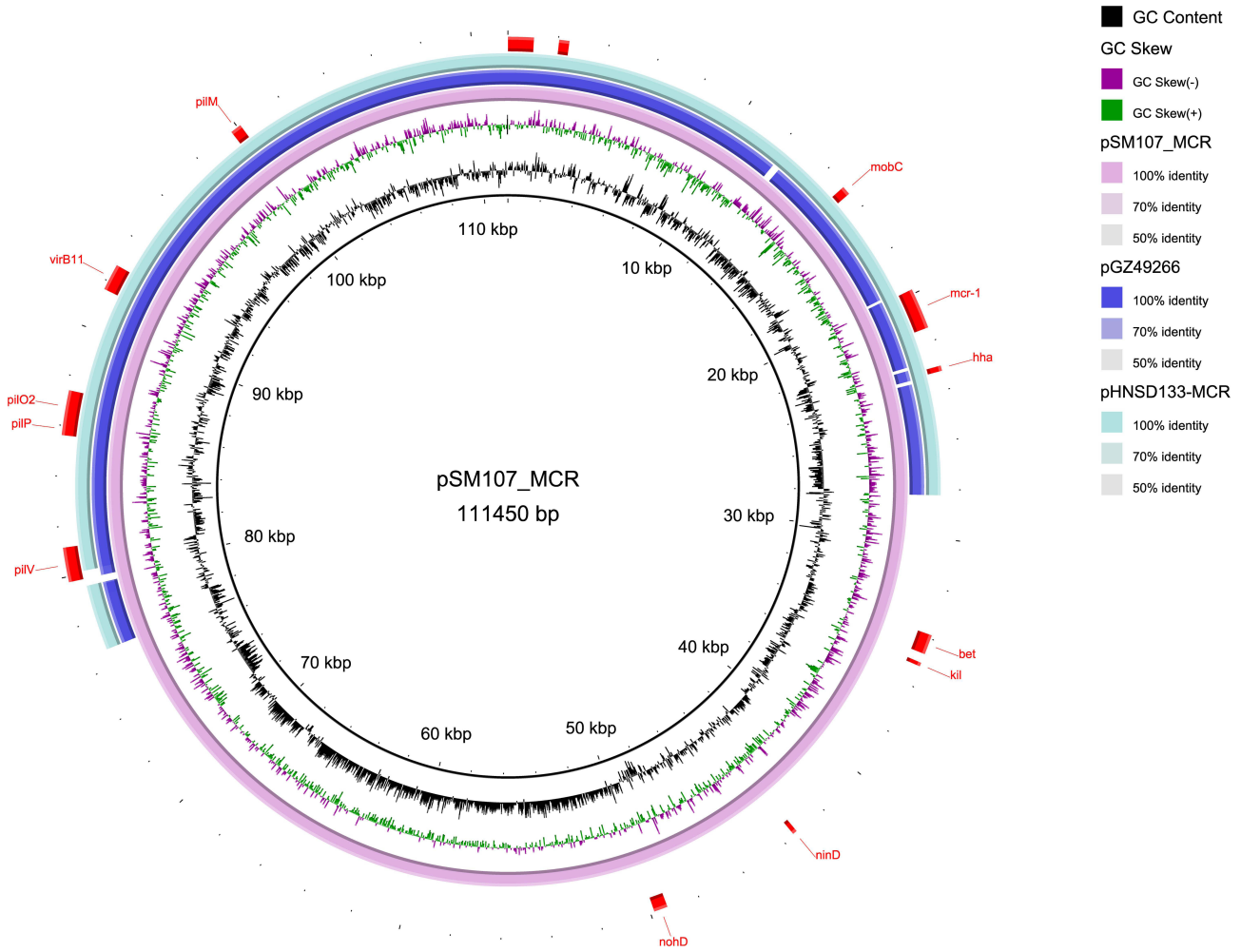

Figure 2 Comparative analysis between the pSM107_MCR plasmid and other similar plasmids. Plasmid structure of pSM107_MCR. pSM107_MCR was used as the reference plasmid to perform genome alignment with pGZ49266 and pHNSD133-MCR.

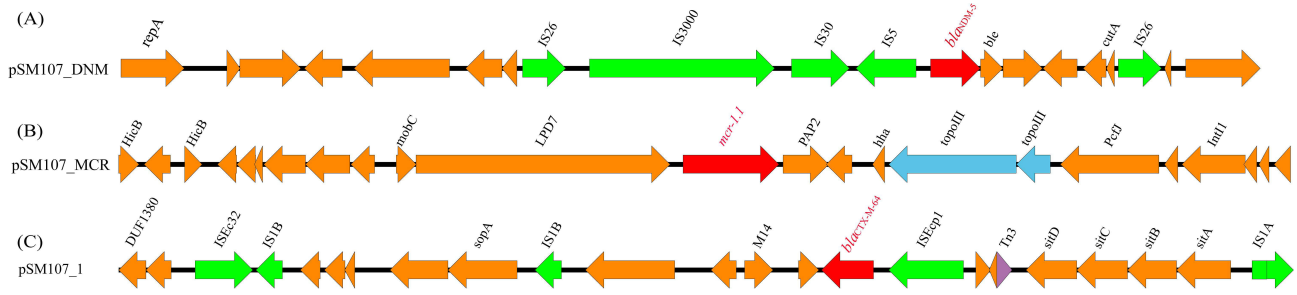

Figure 3 Genetic environment of carbapenem resistance genes in *E. coli* SM107. (**A**) Genetic structure of plasmid pSM107_DNM carrying bla_{NDM-5}; (**B**) Genetic structure of plasmid pSM107_MCR harboring *mcr-1.1*; (**C**) Genetic context of $bla_{CTX-M-64}$ in pSM107_1. The red arrows represent the resistance genes, the green arrow represents the IS truncated fragment genes, the purple arrow represents the Tn truncated fragment, the blue arrow represents the DNA topoisomerase III, whereas the Orange arrows represent additional coding sequences (CDSs).

study in the Czech Republic identified several prevalent *mcr*-associated lineages, with ST1011 being the most common one (15.6%).[27] Similarly, a study in Lebanon on the clonal and epidemiological characteristics of colistin resistance linked to *mcr* genes in the poultry sector also emphasized that ST1011 is among the most widely identified clones carrying the *mcr* gene.[28] The bla_{NDM} gene, associated with carbapenem resistance, was found in 4 strains. The closest phylogenetic relative of *E. coli* SM107 identified in this study is the ST1011 strain *E. coli* strain NC3_1, isolated from

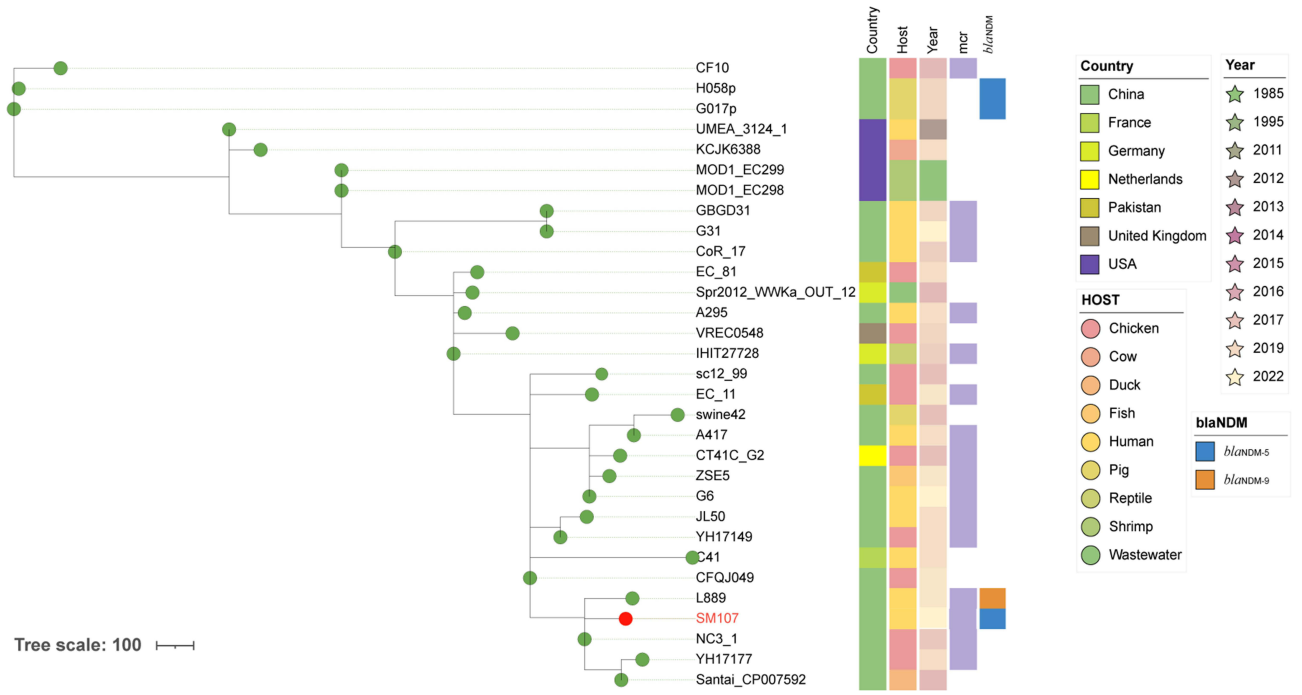

Figure 4 The phylogenetic relationship between *E. coli* SM107 and a total of 31 *E. coli* ST1011 strains retrieved from the NCBI GenBank database (Data as of April 1, 2025). The distance of alleles is represented by the branch length. The positions represent the country, host, the isolation date, *mcr-1.1* and bla_{NDM}. The strain *E. coli* SM107 from this study is highlighted in red.

chicken in Shandong, China, in 2013. Both strains carry the *mcr-1.1* gene but not the bla_{NDM-5} gene and differ by 111 cgMLST alleles, indicating that the plasmid harboring bla_{NDM-5} might be acquired horizontally later on.

Conclusions

Our study described an ST1011 *E. coli* strain recovered from China harboring both *mcr-1.1* and bla_{NDM-5} and characterized the plasmids carrying these resistance determinants. The coexistence of carbapenem and colistin resistance genes in a single isolate is of significant clinical concern. In addition to genomic characterization, the phenotypic profile of the isolate was largely consistent with the resistance determinants identified in the genome. Notably, the observed susceptibility of certain CRE strains to nitrofurantoin suggests its potential utility in treating urinary tract infections caused by these multidrug-resistant pathogens. These findings highlight the need for strengthened antimicrobial stewardship and continued genomic surveillance to limit the spread of high-risk resistance plasmids and preserve the effectiveness of last-resort antibiotics.

Ethics

The study was conducted in accordance with the Declaration of Helsinki and approved by the Ethics Committee of the Sanmen People's Hospital (Approval No. 2025018). This study was exempt from the requirement of informed consent as no identifiable patient information was collected and it exclusively focused on the genomic characteristics of bacteria.

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Disclosure

The authors report no conflicts of interest in this work.

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