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SHORT REPORT

Emergence of a Multidrug-Resistant *Escherichia coli* Co-Carrying a New *mcr-1.33* Variant and bla_{NDM-5} Genes Recovered from a Urinary Tract Infection

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Background: Carbapenem-resistant Enterobacterales (CRE) are a significant threat to worldwide public health, resulting in increased morbidity, death, hospitalization time and healthcare expenses. Here, the genomic and phylogenetic characteristics of a multidrug-resistant *Escherichia coli* isolate carrying both the new *mcr-1.33* variant and bla_{NDM-5} gene obtained from a urinary tract infection in China are investigated.

Methods: Antimicrobial susceptibility of *E. coli* 779 was evaluated by using the broth microdilution method. Short-read Illumina NovaSeq 6000 and long-read Oxford Nanopore MinION platforms were applied to sequence the bacterial whole genomic DNA and then de novo assembled. The genome sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline and further subjected to identify the sequence type (ST), capsular type, and antibiotic resistance genes. BacWGSTdb 2.0 was used to perform the core genome multilocus sequence typing (cgMLST) analysis with other closely related *E. coli* isolates deposited in the public database.

Results: *E. coli* 779 was resistant to aztreonam, levofloxacin, fosfomycin, cefoxitin, cefepime, cefotaxime, imipenem, meropenem, polymyxin, and tigecycline. The complete genome sequence of *E. coli* 779 is made up of nine contigs totaling 5,667,876 bp, including one chromosome and eight plasmids. The isolate was assigned to ST101, serotype O-:H31, and phylogroup B1. The colistin resistance gene *mcr-1.33* (located in a 242,460 bp IncHI2/IncHI2A plasmid) and the β-lactam resistance gene bla_{NDM-5} (located in a 46,161 bp IncX3 plasmid) were among the 27 antimicrobial resistance genes discovered. The closest relative of *E. coli* 779, another ST101 strain (*E. coli* 443) obtained from a sewage sample in Shandong, China in 2015, differs by only 24 cgMLST alleles.

Conclusion: We discovered the first multidrug-resistant ST101 *E. coli* strain with plasmid-mediated *mcr-1.33* variant and bla_{NDM-5} gene in China. These findings would help us to better understanding the genomic traits, antimicrobial resistance mechanisms and epidemiological aspects of this bacterial pathogen.

Keywords: whole genome sequencing, *Escherichia coli*, multidrug-resistance, *mcr-1.33*, bla_{NDM-5}, urinary tract infection

Introduction

Carbapenems are the last-line β-lactams for the treatment of severe infections caused by multidrug-resistant Gram-negative bacteria. However, their effectiveness is being hampered by the increasing incidence of carbapenem-resistant Enterobacterales (CRE), which poses a significant threat to worldwide public health.[1] Class A carbapenemase (KPC), class B metallo-β-lactamases (NDM), and class D carbapenemases (OXA-48), are three of the most predominant carbapenemases that have been widely reported in CRE. CRE that produce New Delhi metallo-β-lactamase (NDM) in particular exhibit a high degree of resistance to nearly all presently available β-lactam antibiotics. Colistin and tigecycline are presently regarded as antibiotics of last choice in the treatment of severe CRE infections.[2] However, the inappropriate use of colistin in the livestock industry and hospitals have led to the global emergence of colistin-resistant pathogens. The recent identification of plasmid-mediated mobile colistin resistance gene *mcr-1* in CRE is a key signal that the antibiotic golden age may be in jeopardy.[3] Since the emergence of *mcr-1* in Enterobacterales, ten major *mcr* variants (*mcr-1* to *mcr-10*) have been reported worldwide in the past five years. To make matters worse, further colistin and carbapenem-resistant clinical isolates have been reported, including the

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advent of MCR-1 and NDM-5-producing *Escherichia coli* isolates in the United States, leaving patients with extremely limited treatment options.[4] Here, we firstly reported the co-existence of a novel plasmid-borne *mcr-1.33* variant and the bla_{NDM-5} genes in a multidrug-resistant *Escherichia coli* isolate in China.

Materials and Methods

In November 2016, a carbapenem-resistant strain of *E. coli* 779 was discovered from a urine sample of a 29-year-old woman hospitalized with fever. The bacterial species was identified using a MALDI Biotyper (Bruker Daltonics, Billerica, MA, USA) and verified using 16S rRNA gene sequencing. VITEK 2 Compact (bioMérieux, France) antimicrobial susceptibility testing revealed that *E. coli* 779 was resistant to a variety of antimicrobial agents. *E. coli* 779 was tested for antimicrobial resistance against the following antimicrobial agents: amikacin, aztreonam, levofloxacin, fosfomycin, cefoxitin, cefepime, cefotaxime, imipenem, meropenem, colistin, and tigecycline using the broth microdilution method. Except for the breakpoints of colistin and tigecycline for Enterobacterales, which were interpreted using the European Committee on Antimicrobial Susceptibility Testing (EUCAST) criteria (v.9.0, 2019), minimum inhibitory concentrations (MICs) of other antimicrobial agents were interpreted using Clinical and Laboratory Standards Institute (CLSI) 2020.

Short-read Illumina NovaSeq 6000 and long-read Oxford Nanopore MinION platforms were applied to sequence the bacterial whole genomic DNA and then de novo assembled. The genome sequence was annotated using the NCBI Prokaryotic Genome Annotation Pipeline and further subjected to identify the sequence type (ST), capsular type, and antibiotic resistance genes. BacWGSTdb 2.0 was used to perform the core genome multilocus sequence typing (cgMLST) analysis with other closely related *E. coli* isolates deposited in the public database.[5,6] ABRicate 1.0.1 was used in conjunction with ResFinder 4.1, CARD 2020, SerotypeFinder 2.0, and PlasmidFinder 2.1 to identify acquired antimicrobial resistance genes (ARGs), serotype and plasmid incompatibility (Inc) groups.[7–9] IslandViewer 4, ISfinder 1.0, PHASTER 2016, CRISPRCasFinder 1.0, and antiSMASH 5.2.0 with default parameters predicted genomic islands, insertion sequence (IS) elements, prophage sequences, clustered regularly interspaced short palindromic repeat (CRISPR) sequences, and secondary metabolite gene clusters, respectively.[10–13] The genome sequences of the chromosome and plasmids of *E. coli* 779 have been deposited in NCBI GenBank under accession numbers CP086220-CP086228.

Results and Discussion

The complete genome sequence of *E. coli* 779 is made up of nine contigs that have been closed and circularized, totaling 5,667,876 bp. Contig 1 (5,091,593bp) belonged to the chromosome, whereas the others belonged to different plasmids (contig 2, 242,460 bp; contig 3, 114,310 bp; contig4, 97,374 bp; contig 5, 62,813 bp; contig 6, 46,161 bp; contig 7, 5773 bp; contig 8, 4018 bp; and contig 9, 3374 bp). The strain's overall G+C content was 50.37%, and a total of 5675 coding sequences (CDSs), 127 RNAs (96 tRNA, 22 rRNA, and 9 ncRNA) genes were found. *In silico* serotyping and phylotyping of *E. coli* 779 revealed that it belonged to the nontypeable O-:H31 and phylogroup B1.

The resistome of *E. coli* 779 is made up of genes that are responsible for resistance to aminoglycosides [*aadA1, aadA2, aadA5, aac(3)-IV, aph(3'')-Ib, aph(3')-Ia, aph(4)-Ia, aph(6)-Id*], β-lactams ($bla_{CTX-M-14}$ and bla_{NDM-5}), tetracyclines [*tet*(A)], macrolides [*mdf*(A) and *mph*(A)], phenicols (*cmlA1, catA1* and *floR*), trimethoprim (*dfrA14* and *dfrA17*), sulphonamide (*sul1, sul2* and *sul3*), fosfomycin (*fosA3*) and colistin (*mcr-1*). The multidrug resistance characteristic to aminoglycosides, third-generation cephalosporins, carbapenems, quinolones, tetracyclines, and colistin was adequately explained by these findings (Table 1). Interestingly, the *mcr-1.33* gene is a novel variant allele of the mobile colistin resistance gene *mcr-1*, firstly reported in this study and submitted to the GenBank database (GenBank accession no. OL624718). The allele of *mcr-1.33* gene is a new variant of the *mcr-1* gene with one nonsynonymous mutation E209G. The bla_{NDM-5} gene was discovered in p779-5-NDM, a 46,161 bp IncX3 plasmid (Figure 1). Analysis of the genetic environment revealed that an insertion sequence IS*Aba125* exists upstream of bla_{NDM-5} as a truncated fragment harboring IS*5* and IS*3000*. The bla_{NDM-5} gene is downstream of the ble_{MBL}, *trpF, tat* and IS*26* elements (Figure 2). A BLAST analysis revealed that p779-5-NDM had 100% query coverage and 100% identity with several bla_{NDM-5}-bearing plasmids, ie, p2D-NDM-5 in *E. coli* strain 2D isolated from a bloodstream infection in China (Figure 1).[14] The plasmid carrying *mcr-1.33* was 242,460 bp long and contained two replicons of IncHI2 and IncHI2A type. The genetic environment of the *mcr* gene revealed that an insertion sequence IS*Apl1* lies downstream of *mcr-1.33*, and

Table 1 Minimal Inhibitory Concentrations (MICs) of *Escherichia coli* 779

Antimicrobials	MIC (μg/mL)
Aztreonam	64
Levofloxacin	64
Fosfomycin	64
Cefoxitin	128
Cefepime	>128
Cefotaxime	>128
Imipenem	32
Meropenem	>128
Colistin	8
Tigecycline	4
Amikacin	4

the *terB, terC, terD, terF*, and *pap2* genes located upstream of the *mcr* gene (Figure 1). According to the result of BLAST analysis, p779-1-mcr had 100% query coverage and 99.98% identity with plasmid pSH15G1428 (NCBI GenBank accession number MK477605.1) in a *Salmonella* strain SH15G1428 obtained from a diarrheal outpatient in Shanghai, China (Figure 1).

The genome of *E. coli* 779 also contains at least 104 genomic islands and several IS elements, the bulk of which belong to the IS*6*, IS*3* and IS*66* families. In the genome, a total of 13 prophage and 5 CRISPR sequences association with type I-E, the specificity of PAM selection resides with Cas1-2, can be predicted. The presence of two putative secondary metabolite gene clusters, including the turnerbactin and O-antigen biosynthetic gene clusters, can also be predicted. According to Achtman's MLST scheme, *E. coli* 779 belongs to ST101, a widely dispersed clonal lineage globally. The phylogenetic connections between *E. coli* 779 and a total of 86 ST101 *E. coli* strains currently deposited in the NCBI GenBank database were examined to evaluate the genomic epidemiological features of *E. coli* strains in a global context. According to the findings of the current investigation, the closest relative of *E. coli* 779, another ST101 strain (*E. coli* strain 443) discovered from sewage in Shandong, China in 2015, differs by only 24 cgMLST alleles. Interestingly, the Shandong strain also had the $bla_{\text{NDM-5}}$ gene but not the *mcr* gene, suggesting that the *mcr*-carrying plasmid was acquired horizontally later on. Furthermore, we discovered that 19 of the 86 ST101 strains were from Shandong, China, based on the results of cgMLST analysis.

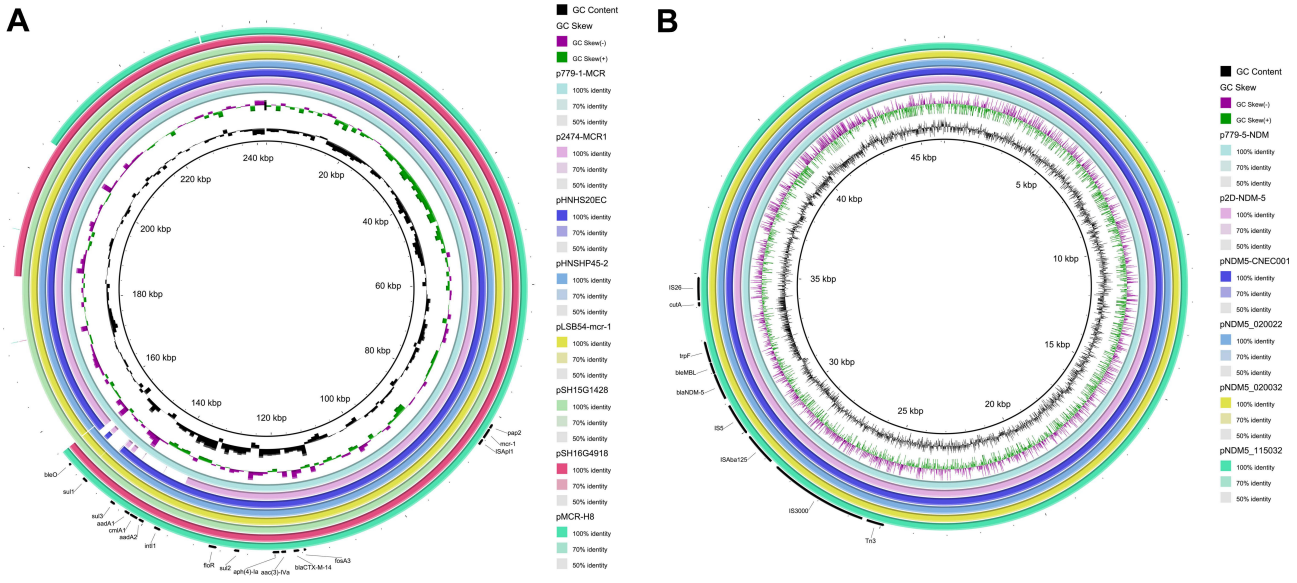

Figure 1 Circular comparative analysis of the *mcr-1* (**A**) and $bla_{\text{NDM-5}}$ (**B**) bearing plasmids characterized in this study and deposited in GenBank database. Antimicrobial resistance genes and insertion sequence elements were labeled at the outmost ring.

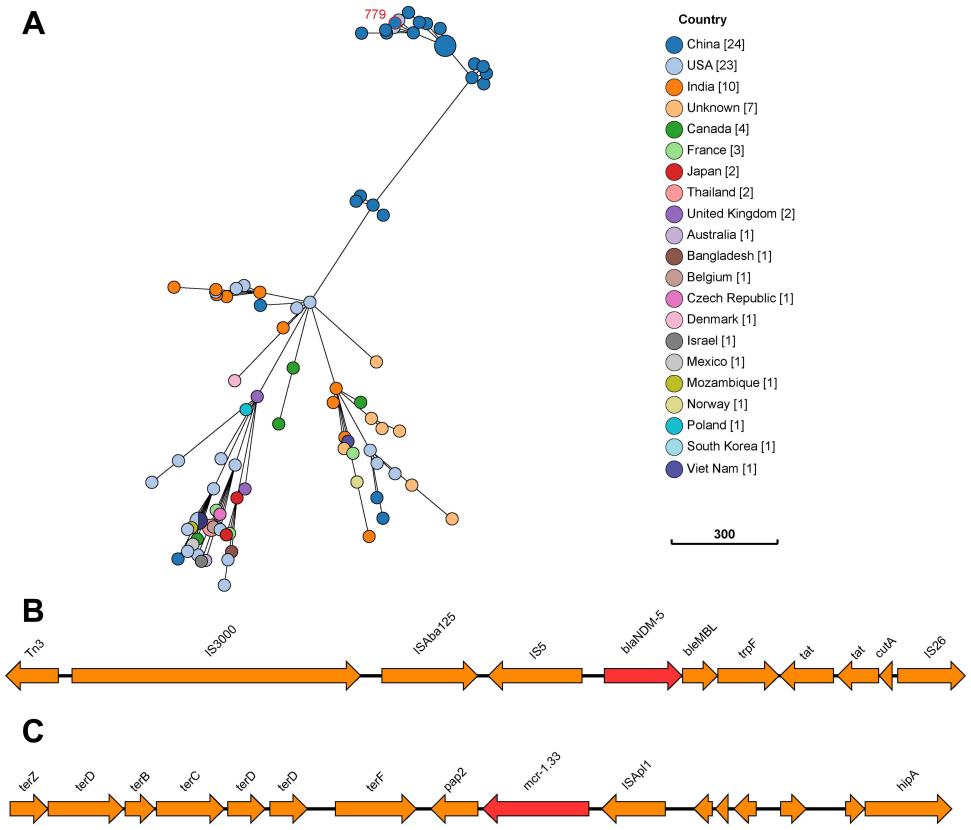

Figure 2 (**A**) Phylogenetic relationship between *Escherichia coli* 779 and 86 ST101 *E. coli* strains currently deposited in the NCBI GenBank database. The lines that link the circles represent the clonal connection between the various strains. The distance between core genome multilocus sequence typing (cgMLST) loci is represented by the branch length. In square brackets is the number of isolates retrieved from each country. (**B**) The genetic environment of the bla_{NDM-5} gene on the plasmid p779-5-NDM. The red arrow represents the colistin resistance gene bla_{NDM-5}, whereas the Orange arrows represent additional coding sequences (CDSs). (**C**) The genetic context of the *mcr-1.33* gene on plasmid p779-1-mcr. The red arrow represents the colistin resistance gene *mcr-1.33*, while the Orange arrows represent additional coding sequences (CDSs).

Conclusion

In summary, we reported a ST101 *E. coli* strain in China that has both the plasmid-borne bla_{NDM-5} gene and a new *mcr-1* allelic variant *mcr-1.33*. The prevalence of *E. coli* isolates carrying both carbapenem and colistin resistance determinants raises major concerns, emphasizing the critical need for closely monitoring the evolution of this novel *mcr* variant in clinical settings. These findings will also aid in the future understanding of the genetic traits, antibiotic resistance mechanisms, and epidemiological characteristics of this bacterial pathogen.

Ethics Approval and Consent to Participate

This study was conducted in accordance with the Declaration of Helsinki and obtained approval from Medical Ethics Committee at the Sanmen People's Hospital. Written informed consent was provided by the patient to allow the case details to be published.

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Disclosure

The authors declare that they have no competing interests.

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