

Escherichia coli ST542 Co-Harbours *bla*_{NDM-1} and *mcr-1* in a Pig Farm, China

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Carbapenems are potent antibiotics utilized to treat serious infections caused by multidrug-resistant (MDR) Gram-negative bacteria.¹ However, the clinical use of carbapenems has led to the emergence and global dissemination of carbapenem-resistant Enterobacteriaceae (CRE), which pose a significant threat to effective clinical therapy.¹ The genes encoding carbapenemases, particularly the New Delhi metallo- β -lactamase gene *bla*_{NDM}, have been widely distributed among humans and detected in animals, food, and the environment.^{1,2} Colistin is considered as the last therapeutic resort for treating MDR Gram-negative bacteria, especially CRE bacteria.^{1,3} However, its efficacy has been undermined by the emergence of various antimicrobial resistance mechanisms, such as mutations in the two-component regulatory systems PmrA-PmrB and PhoP-PhoQ.³ Particularly, the global spread of plasmid-mediated colistin resistance gene *mcr*, first identified in 2015, has further exacerbated this issue.³ Here, we report the recent identification of *Escherichia coli* ST542 strains co-producing MCR-1 and NDM-1 isolated from a pig farm in Yangzhou, China.

On 17 May 2022, a total of 126 samples were collected from a pig farm in Yangzhou, China, including pig feces ($n = 93$), feed ($n = 3$), pig drinking water ($n = 5$), and floor swabs from pens ($n = 25$). A single sample was collected per pig, with no more than five samples taken from pigs in the same house. The samples were incubated in buffered peptone water (BPW) broth for 18–24 hours and subsequently inoculated onto MacConkey agar. One suspected *E. coli* isolate was selected from each sample and confirmed using matrix-assisted laser desorption/ionization time-of-flight mass spectrometry. In total, 107 *E. coli* isolates were obtained, among which three (2.80%) isolates, namely YZ22PE67, YZ22PE68 and YZ22PE106 from pig feces co-carried the *bla*_{NDM-1} and *mcr-1* genes, confirmed by PCR and sequencing as previously described.^{3,4} Minimum inhibitory concentrations (MICs) of 16 antimicrobial agents were determined using the microdilution method (cation-adjusted Mueller–Hinton broth) or the agar dilution method (Mueller–Hinton agar; limited to fosfomycin). *E. coli* ATCC 25922 served as the quality control strain. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100, 32nd edition, or the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org). As shown in Table 1, three NDM- and MCR-producing *E. coli* isolates were resistant to meropenem and colistin, as well as to ampicillin, cefazolin, cefotaxime, streptomycin, tetracycline, chloramphenicol, florfenicol, sulfamethoxazole/trimethoprim. However, they remained susceptible to gentamicin, amikacin, tigecycline, nalidixic acid, ciprofloxacin, and fosfomycin (Table S1).

To further characterize these *E. coli* isolates, whole-genome sequencing was performed using Illumina HiSeq technology (Illumina, San Diego, CA, USA). The library was prepared with the NEBNext Ultra II DNA Library Prep Kit for Illumina (New England Biolabs, USA), generating 150 bp paired-end reads. Subsequently, genomic DNA was

Table 1 Characteristics of *Mcr-I* and *bla*_{NDM-1}-Carrying *Escherichia coli* Isolates in This Study

| Strain | ST | Resistance Genes | Resistance Patterns ^a | Location (Plasmid) | |
|-----------|-----|--|--|---------------------------------|---|
| | | | | <i>bla</i> _{NDM-1} | <i>mcr-I</i> |
| YZ22PE67 | 542 | <i>bla</i> _{TEM-176} / <i>bla</i> _{NDM-1} / <i>mcr-I</i> / <i>aph</i> (3')-Ia/ <i>aadA1</i> / <i>aadA2</i> / <i>tet</i> (A)/ <i>cmiA1</i> / <i>floR</i> / <i>qnrS1</i> / <i>sul3</i> / <i>dfrA12</i> / <i>dfrA14</i> | AMP/CFZ/CTX/MEM/CL/STR/TET/CHL/FFC/SXT | IncFII pYUYZPE67-1 (80,201 bp) | IncI2 pYUYZPE67-2 (63,656 bp) IncX4 pYUYZPE67-5 (33,309bp) |
| YZ22PE68 | 542 | <i>bla</i> _{TEM-176} / <i>bla</i> _{NDM-1} / <i>mcr-I</i> / <i>aph</i> (3')-Ia/ <i>aadA1</i> / <i>aadA2</i> / <i>tet</i> (A)/ <i>cmiA1</i> / <i>floR</i> / <i>qnrS1</i> / <i>sul3</i> / <i>dfrA12</i> / <i>dfrA14</i> | AMP/CFZ/CTX/MEM/CL/STR/TET/CHL/FFC/SXT | IncFII pYUYZPE68-1 (82,203 bp) | IncI2 pYUYZPE68-2 (63,079 bp) |
| YZ22PE106 | 542 | <i>bla</i> _{TEM-176} / <i>bla</i> _{NDM-1} / <i>mcr-I</i> / <i>aph</i> (3')-Ia/ <i>aadA1</i> / <i>tet</i> (A)/ <i>floR</i> / <i>qnrS1</i> / <i>dfrA14</i> | AMP/CFZ/CTX/MEM/CL/STR/TET/CHL/FFC/SXT | IncFII pYUYZPE106-2 (80,201 bp) | IncI2 pYUYZPE106-3 (63,656 bp) |

Notes: ^aAMP, ampicillin. *E. coli* ATCC 25922 served as the quality control strain. Results were interpreted according to the Clinical and Laboratory Standards Institute (CLSI) M100, 32nd edition, or the European Committee on Antimicrobial Susceptibility Testing (EUCAST; www.eucast.org).
Abbreviations: CFZ, cefazolin; CTX, cefotaxime; MEM, meropenem; CL, colistin; STR, streptomycin; TET, tetracycline; CHL, chloramphenicol; FFC, florfenicol; SXT, sulfamethoxazole/trimethoprim.

sequenced on the Oxford Nanopore MinION platform (Oxford Nanopore Technologies, Oxford, UK). The library was constructed using the Ligation Sequencing Kit (SQK-LSK109) and Native Barcoding Expansion (EXP-NBD104/114). Following library preparation, the DNA library was loaded onto the MinION R9.4 flow cells to read the DNA sequence in real-time, generating long-read data. The Illumina short reads and MinION long reads were then subjected to hybrid assembly using Unicycler v0.5.0 to obtain complete genome sequences. The sequences were analyzed using the Center for Genomic Epidemiology (CGE) pipeline (<https://www.genomic epidemiology.org/>) and BLAST (<http://blast.ncbi.nlm.nih.gov/Blast.cgi>). All whole-genome sequences have been deposited in GenBank under accession number PRJNA1175595. Three NDM- and MCR-producing *E. coli* strains belonged to sequence type 542 (ST542). Previously, *E. coli* ST542 strains carrying carbapenemase gene *bla*_{NDM-5} or *mcr-I* were reported in patients or animals in China.^{5,6} In addition to *bla*_{NDM-1} and *mcr-I*, all three isolates harbored the β-lactam resistance genes *bla*_{TEM-176}, the aminoglycoside resistance genes *aph*(3')-Ia and *aadA1*, the tetracycline resistance gene *tet*(A), the phenicol exporter gene *floR*, the quinolone resistance gene *qnrS1*, and the trimethoprim resistance gene *dfrA14*. Notably, YZ22PE67 and YZ22PE68 also contained the aminoglycoside resistance genes *aadA2*, the chloramphenicol-resistant gene *cmiA1*, the sulfonamide resistance genes *sul3* and the trimethoprim resistance gene *dfrA12* (Table 1). These findings are consistent with the results of antimicrobial susceptibility testing.

Three NDM- and MCR-producing isolates contained multiple plasmids (Table S2). Specifically, three similar *bla*_{NDM-1}-carrying plasmids (80,201 or 82,203 bp), designated as pYUYZPE67-1, pYUYZPE68-1, and pYUYZPE106-2 were obtained, respectively (Table S2). These plasmids belonged to IncFII plasmids and were highly similar (>99.9%) in organization to some *bla*_{NDM-1}-bearing IncFII plasmids from China, such as plasmids pDXLP15_1 (*E. coli*, OR965474.1, pork), plasmid unnamed1 from *Salmonella enterica* strain sg1722-2 (CP081190.2, child feces), and plasmid unnamed4 from *E. coli* strain EC1722-1 (CP081195.1, child feces) (Figure S1). Each of them contained a 9067-bp core *bla*_{NDM-1} structure (IS26-*tat*-*trpF*-*ble*_{MBL}-*bla*_{NDM-1}-IS*Aba125*-ΔIS3000-IS26). Furthermore, three *bla*_{NDM-1}-carrying plasmids pYUYZPE67-1, pYUYZPE68-1, and pYUYZPE106-2 were successfully transferred to *E. coli* C600 with frequencies of 2.42×10^{-3} , 3.11×10^{-2} , and 1.32×10^{-4} transconjugants per recipient, respectively.

The *mcr-I* gene was located on IncI2 plasmids in three NDM- and MCR-producing *E. coli* isolates, with an additional *mcr-I*-carrying IncX4 plasmid identified in isolate YZ22PE67 (Tables 1 and S2). Three IncI2 plasmids pYUYZPE67-2 (63,656 bp), pYUYZPE68-2 (63,079 bp), and pYUYZPE106-3 (63,656 bp) were similar to other *mcr-I*-carrying IncI2 plasmids from China, such as pYULNMC7-MCR (*E. coli*, chicken meat, CP126506) and the first reported *mcr-I*-carrying plasmid pHNSHP45 (*E. coli*, pig, KP347127) (Figure S2). Notably, the mobile element IS*Apl1*, which is associated with *mcr-I* mobilization, was absent in our *mcr-I*-bearing IncI2 plasmids (Figure S2A). The *mcr-I*-bearing IncX4 plasmid pYUYZPE67-5 (33,309 bp) was organized similarly to other *mcr-I*-carrying IncX4 plasmids from animals or food products, including regions for replication, conjugal transfer, maintenance and stability (Figure S2B).

Additionally, one copy of IS26 was inserted into the backbone of pYUYZPE67-5 at the same site as observed in plasmids like pHNSHP10 (*E. coli*, pig, MF774182) and pFS170G (*E. coli*, pig, KX711707), generating 8-bp direct repeats (5'-CTGTGTGA-3') (Figure S2). Both *mcr-I*-carrying plasmids in YZ22PE67 (pYUYZPE67-2 and pYUYZPE67-5) could be successfully transferred to *E. coli* C600 at frequencies of 2.68×10^{-3} and 3.05×10^{-3} transconjugants per recipient, respectively. The remaining *mcr-I*-carrying IncI2 plasmids, pYUYZPE68-2 and pYUYZPE106-3, were transferable at a frequency of 2.71×10^{-3} and 3.34×10^{-6} transconjugants per recipient, respectively.

The coexistence of carbapenemase genes (*bla*_{NDM}/*bla*_{KPC}) and *mcr* has been reported in some Enterobacteriaceae species, such as *E. coli* and *Klebsiella pneumoniae* from humans, animals, and food.^{7–9} The concurrent presence of carbapenemase genes and *mcr* in Enterobacteriaceae significantly limits treatment options and complicates infection management. Furthermore, the dissemination of such organisms can occur through multiple transmission routes, including the food chain, environmental contamination, and direct contact with animals, thereby posing a serious threat to public health. Consequently, it is essential to implement control measures to reduce and prevent the transmission of such organisms in animal husbandry. Furthermore, exploring alternative strategies, such as bacteriophages and antimicrobial peptides, is critical for preventing and treating infections in both animals and humans, without relying solely on antibiotics.^{10,11}

Author Contributions

All authors made a significant contribution to the work reported, whether that is in the conception, study design, execution, acquisition of data, analysis and interpretation, or in all these areas; took part in drafting, revising or critically reviewing the article; gave final approval of the version to be published; have agreed on the journal to which the article has been submitted; and agree to be accountable for all aspects of the work.

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Disclosure

The authors report no conflicts of interest in this work.

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