Co-production of MCR-1 and NDM-5 in Escherichia coli isolated from a colonization case of inpatient

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Abstract: Colistin is increasingly used as an antibiotic of last resort for treating carbapenem-resistant Enterobacteriaceae. Mobile colistin resistance gene mcr-1 has been increasingly reported in Enterobacteriaceae around the world. Of particular concern is the spread of mcr-1 into carbapenemase-producing Enterobacteriaceae, which results in highly drug-resistant strains that are potentially untreatable. Notably, such mcr-1-carrying isolates harboring carbapenemase genes have been reported in animals and patients with infection. Here, we report an Escherichia coli strain carrying co-transferable mcr-1-harboring IncX4 and blaNDM-5-harboring IncX3 plasmids, which was recovered in the context of fecal colonization.

Keywords: Escherichia coli, MCR-1, NDM-5, co-production

Introduction
Colistin is increasingly used as an antibiotic of last resort for treating multidrug-resistant Gram-negative bacteria, especially carbapenem-resistant Enterobacteriaceae. In December 2015, transmissible colistin resistance gene mcr-1, encoding phospho-ethanolamine transferase, was reported from China. The gene mcr-1 has since been increasingly reported in Enterobacteriaceae around the world.1,2 Of particular concern is spread of mcr-1 into carbapenemase-producing Enterobacteriaceae, which results in highly drug-resistant strains that are potentially untreatable.

Methods and results
A total of 349 non-duplicate Escherichia coli clinical isolates were collected from serial fecal specimens in a hospital with capacity of 3,000 beds in Guangzhou, China in 2016 for the purpose of routine drug resistance monitoring. All isolates were characterized by API 20E system (BioMerieux, Marcy l’Etoile, France) and 16S rDNA sequencing. The mcr-1 gene was detected using polymerase chain reaction (PCR) and sequencing.2 As a result, 88 (25.2%) isolates were positive for mcr-1. This mcr-1 positivity was higher than found in our previous study (11.3%),2 indicating that the prevalence of mcr-1 in E. coli is still increasing in this area. Susceptibility testing was performed for routine resistance observation; the results showed that one of the strains, E. coli GB788, was resistant to both colistin and carbapenem (Table 1). PCR and sequencing confirmed that E. coli GB788 carried both mcr-1 and blaNDM-5 genes. E. coli GB788 was then subjected to phylogenetic typing and multilocus sequence typing for molecular epidemiology.2 As a result, E. coli GB788 was classified as phylogenetic group A and sequence type 46 which has been sporadically identified among drug-resistant E. coli strains.3
E. coli GB788 was recovered from a 52-year-old female patient, who was admitted to the hospital after suffering from periarthritis shoulder. The patient did not have any signs of infection, was not receiving any antibiotics, and did not have any significant travel history.

Streptomycin-resistant E. coli C600 was used as the recipient for the conjugation experiment to identify the mobility of drug resistance genes. Three different types of transconjugants (E. coli C600 GB788-colistin (CL), E. coli C600 GB788-imipenem (IMP), and E. coli C600 GB788-CL/IMP) were obtained, including those harboring mcr-1, blaNDM-5, or both (Table 1), with 6–7 of 15 colonies positive for both genes. For detecting the stability of these plasmids, plasmid stability experiment was performed. Three independent lineages of each plasmid-containing strain were grown overnight at 37°C in 1 mL of Luria-Bertani (LB) broth. Bacterial cells were washed by spinning down overnight cultures and resuspending the pellet in 1 mL of LB without antibiotics. An aliquot of 1 µL of washed cells was transferred into 1 mL of LB and incubated overnight at 37°C. Serial passaging of 1 µL of overnight culture to 1 mL of LB was performed daily, approximating ten generations of growth per passage. Every 20 generations, samples were diluted and plated on LB plates. Then, 50 colonies from each lineage were screened on LB plates supplemented without colistin to determine the fraction of plasmid-containing cells. The results showed that no plasmid was lost even after 100 generations for all three types of transconjugants, which indicated that mcr-1 and blaNDM-5 can transfer alone or co-transfer at a certain frequency and subsequent stability. PCR-based replicon typing was performed to detect the plasmid type, which was determined by a PCR-based replicon typing method. Plasmid DNA was amplified by five multiplex and three simplex PCRs using 18 pairs of primers as reported previously that are recognized as Inc (incompatibility) replicon types: FIA, FIB, FIC, HI1, HI2, I1-Ic, L/M, N, P, W, T, A/C, K, B/O, X, Y, F, and FIIA. Pulsed field gel electrophoresis analysis of S1 nuclease-digested DNA (S1-PFGE), followed by southern blotting were conducted for identifying the location of mcr-1 and blaNDM-5 carrying plasmid. Whole-cell DNA of all mcr-1-producing isolates was extracted and embedded in gold agarose gel plugs (SeaKem Gold Agarose; Lonza, Rockland, ME, USA). The plugs were digested with S1 nuclease (TaKaRa, Dalian, China), and the DNA fragments were separated by PFGE. Southern blotting hybridizations of plasmid DNA were performed with a digoxin-labeled mcr-1 probe according to the manufacturer’s instructions (Roche Diagnostics, Mannheim, Germany). The results showed that mcr-1 was located on ~33 kb IncX4 plasmid, while blaNDM-5 was located on ~45 kb IncX3 plasmid (Figure S1). IncX4 and IncX3 are the common host-range plasmids for mcr-1 and blaNDM-5, respectively.

Table 1: Characterization of the Escherichia coli GB788 and its transconjugants

| Characteristics | E. coli GB788 | E. coli C600 Transconjugants | E. coli C600 Transconjugants | E. coli C600 Transconjugants |
|----------------|-------------|------------------------------|------------------------------|------------------------------|
| Rescore Inpatient | E. coli C600 GB788-CL/IMP | E. coli C600 GB788-CL | E. coli C600 GB788-IMP |
| Isolation site | Feces | GB788-CL | GB788-IMP |
| MLST | ST46 | ST46 | |
| Phylogenetic group | A | A | |
| Plasmid replicon type | IncX3, IncX4 | IncX3, IncX4 | IncX4 | IncX3 |
| Minimum inhibitory concentration (µg/mL) | | | | |
| Colistin | 16 | 16 | 16 | 0.25 |
| Polymyxin B | 16 | 16 | 16 | 0.5 |
| Tigecycline | 0.5 | 0.25 | 0.25 | 0.5 |
| Ampicillin | >256 | >256 | 64 | >256 |
| Amoxicillin–clavulanic acid | >256 | 256 | 8 | 16 |
| Cefotaxime | 128 | 64 | ≤1 | 64 |
| Ceftazidime | >256 | >256 | ≤1 | >256 |
| Cefepime | 32 | 8 | ≤0.5 | 8 |
| Gentamicin | 31 | 8 | ≤0.25 | 31 |
| Amikacin | 4 | 2 | 2 | 2 |
| Ertapenem | 16 | 8 | ≤0.25 | 8 |
| Imipenem | 16 | 4 | 0.25 | 4 |
| Meropenem | 16 | 8 | ≤0.125 | 8 |
| Fosfomycin | 64 | 16 | 16 | 16 |
| Nitrofurantoin | 32 | ≤8 | ≤8 | ≤8 |
| Ciprofloxacin | 1 | ≤0.03 | ≤0.03 | ≤0.03 |

Abbreviations: MLST, multilocus sequence typing; CL, colistin; IMP, imipenem.
For detailed genome characteristics of *E. coli* GB788, whole genome sequencing was performed. The genome DNA of *E. coli* GB788 was extracted and DNA libraries were constructed with 350 and 6,000 bp DNA fragments. With Illumina Hiseq4000 platform, 13,531,019 50-bp paired-end reads were produced from 6,000 bp DNA fragment library, while 5,333,992 150-bp paired-end reads were produced from 350 bp library. Reads were assembled with SPAdes. The predicted open reading frames were annotated using the gene ontology, Kyoto Encyclopedia of Genes and Genomes, Swiss-Prot, non-redundant, and cluster of orthologous groups of proteins databases. Assembled contigs were aligned with the reference genome of *E. coli* strain K-12 substrain MG1655 (NC_000913) by using BLASTp and BLASTn. As a result, several genomic insertion and deletion segments were identified in *E. coli* GB788 (Figure S2A). Forty-five resistance genes including *mcr-1* and *bla*NDM-5 were identified according to the Comprehensive Antibiotic Resistance Database using identity matches of >60% and E values of <1e−5 (Figure S2B). This Whole Genome Shotgun project has been deposited at DDBJ/ENA/GenBank under the accession NEFV00000000. The version described in this paper is version NEFV01000000.

To obtain the complete sequence of *mcr-1* and *bla*NDM-5-harboring plasmids, pGB788-CL and pGB788-IMP were extracted from transconjugants. The DNA libraries were constructed with 350 bp DNA fragments. Thus, 1,201,548 and 1,236,894 150-bp paired-end reads were produced with Illumina Hiseq4000 platform for the two plasmids, respectively. The scaffolds were assembled with SPAdes, and the scaffold with targeted genes of *mcr-1* and *bla*NDM-5 was selected as the candidates. Assembly results showed that the size of *mcr-1*-harboring plasmid pGB788-CL and *bla*NDM-5-harboring plasmid GB788-IMP were 30,988 and 45,793 bp, respectively, which were consistent with the results of plasmid size determination by S1-PFGE and southern blotting (Figure S3).

**Conclusion**

Although the coexistence of *bla*NDM-5 and *mcr-1* has been reported in infection cases from specimens such as bloodstream, urinary tract, kidney, bile, and liver infections, this study is the first to report such an occurrence from a fecal specimen of a colonized individual. Emergence of coexistence of *mcr-1* and carbapenemase genes in Enterobacteriaceae may seriously compromise the effectiveness of antimicrobial therapy. Therefore, close monitoring and routine investigations are required to establish measures to control dissemination of such strains in both infected and colonized individuals. The clinical isolate samples used in this research were part of the routine hospital laboratory procedure. Oral consent of the patients was obtained.

**Disclosure**

The authors report no conflicts of interest in this work.

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Supplementary material

Figure S1 Location of mcr-1 and blaNDM-5 in Escherichia coli GB788.

Notes: (A) S1-PFGE analysis. (B) Southern hybridization with the mcr-1 and blaNDM-5 probe, respectively. Lane M, DNA ladder (New England Bio-Labs, Beverly, MA, USA); lane 1, E. coli GB788; lane 2, E. coli C600 GB788-CL; lane 3, E. coli C600 GB788-IMP; lane 4, E. coli C600 GB788-CL/IMP.

Abbreviations: S1-PFGE, pulsed field gel electrophoresis analysis of S1 nuclease-digested DNA; CL, colistin; IMP, imipenem.

Figure S2 Genomic maps and resistance genes of Escherichia coli GB788.

Notes: (A) Comparison of GB788 and reference sequence NC_000913. Orange bands represent assembly result of GB788 that has similar sequence with reference sequence. Outer bands with different colors represent different COG function annotations. (B) Resistance genes distribution in GB788 genome. Different color bands represent different COG function annotations.

Abbreviation: COG, cluster of orthologous groups of proteins.
Figure S3 Structure map of (A) pGB788-IMP and (B) pGB788-CL.

Note: Plasmid analysis of pGB788-IMP and pGB788-CL using GCview plasmid sequencing.

Abbreviations: CDS, coding sequence; GC, guanine-cytosine; CL, colistin; IMP, imipenem.