Co-Existence of mcr-1 and blaNDM-5 in an *Escherichia coli* Strain Isolated from the Pharmaceutical Industry, WWTP

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Abstract: The emergence of the plasmid-borne colistin-resistant gene (mcr-1) poses a great threat to human health. What is worse, the recent observations of the co-existence of mcr-1 with other antimicrobial resistance genes in some bacteria cause further concern. Here, we present the first report of a wild *Escherichia coli* strain that co-carries an mcr-1 encoding plage-like IncY plasmid (pR15_MCR-1) and a blaNDM-5 encoding IncX3 plasmid (pR15_NDM-5) from a pharmaceutical industry, wastewater treatment plant, in China. This study highlights the spreading of *E. coli* carrying both mcr-1 and blaNDM-5 genes in the pharmaceutical industry.

Importance: *Escherichia coli* strains that carry both mcr-1 and blaNDM-5 genes are of great health concern and are already found in humans and animals worldwide, yet there is a paucity of observations of this resistant strain in the environment. Here we present the first isolation of an *E. coli* strain (R15) that co-carries mcr-1 and blaNDM-5 genes from a wastewater treatment plant in China. Whole-genome sequencing indicated that R15 harbored two plasmids, pR15_MCR-1 and pR15_NDM-5, that carry mcr-1 and blaNDM-5, respectively. The observation of this wild-derived *E. coli* strain that carries mcr-1 and blaNDM-5 genes simultaneously calls for the urgency to improve monitoring and reducing its further spreading.

Keywords: mcr-1, blaNDM-5, wastewater treatment plant, *Escherichia coli*

The transferable colistin resistance gene, mcr-1, has been a growing concern worldwide because colistin is considered as the last resort in the treatment of multidrug-resistant pathogens.1 Recently, reports of bacteria that carry the mcr-1 gene along with other types of antimicrobial resistance genes (ARGs), especially beta-lactam resistance genes, have highlighted the threat of these pan-drug resistant pathogens to public health. Several cases of infection caused by a pathogen which harbored mcr-1 and beta-lactam resistance genes were reported worldwide.2–4 In addition, bacteria that carried mcr-1 and beta-lactam resistance genes were also found in the natural environment. The occurrence of the mcr-1 gene in extended-spectrum beta-lactamase (ESBL)-producing *Escherichia coli* from well water was reported in rural China.5 In our previous study, we isolated six mcr-1-positive ESBL-producing *E. coli* strains from farm soils.6 Wastewater treatment plants (WWTPs), which have been taken as deep reservoirs of ARGs and antimicrobial-resistant bacteria (ARB), are believed to offer the opportunity for ARGs to flow into susceptible disease-causing bacteria.7,8 Thus, the surveillance of these environment-derived bacteria that carry the mcr-1
gene with other types of ARGs is of great importance and the transmission patterns of these bacteria between different habitats should be elucidated.

In our previous study, we obtained several ESBL-producing E. coli strains from a WWTP located in Taizhou, Zhejiang province, China. This WWTP treats the wastewater from a pharmaceutical company which produces antimicrobial agents including beta-lactam antibiotics such as cefaclor, cefixime, cefditoren pivoxil, cefprozil, cefdinir, and cefixime. Here, we reported the isolation of another strain, E. coli strain R15, from this WWTP. This strain is unique for the co-carrying of both mcr-1 and blaNDM-5 genes.

The isolation and identification of R15 was performed as described previously. Antimicrobial susceptibility testing by VITEK 2 Compact (bioMérieux, France) showed that R15 was resistant to various types of antimicrobial agents, including ampicillin (MIC ≥ 32 μg/mL), amoxicillin (MIC ≥ 32 μg/mL), piperacillin (MIC ≥ 128 μg/mL), cefazolin (MIC ≥ 64 μg/mL), cefoxitin (MIC ≥ 64 μg/mL), ceftriaxone (MIC ≥ 64 μg/mL), cefepime (MIC ≥ 64 μg/mL), ertapenem (MIC ≥ 8 μg/mL), imipenem (MIC ≥ 16 μg/mL), amikacin (MIC ≥ 64 μg/mL), gentamicin (MIC ≥ 16 μg/mL), tobramycin (MIC ≥ 16 μg/mL), ciprofloxacin (MIC ≥ 4 μg/mL), levofloxacin (MIC ≥ 8 μg/mL), nitrofurantoin (MIC ≥ 128 μg/mL) and trimethoprim (MIC ≥ 320 μg/mL). Resistance to colistin (MIC = 8 μg/mL) was examined using broth microdilution test as described before.

To investigate the genotype in correspondence to its antimicrobial phenotype, whole-genome sequencing of R15 was performed using Illumina HiSeq 2000 sequencer (Illumina, USA) as described previously and reads were assembled into 140 contigs (PSSH0000000.1) using SPAdes software. Analyzing with the draft genome sequence of R15 by MLST 2.0 showed that it belonged to ST-744. Further, we used ResFinder to predict the resistance genes encoded by strain R15 and 20 resistance genes were identified, including one copy of mcr-1, blaCTX-M-14, blaNDM-5, blaTEM-1B, fosA3, floR, aac(3)-IV, aph(3’)-Ib, aph(3’)-Ila, aph(4’)-Ia, aph(6’)-Ia, rmtB, mdf(A), mph(A), qacA, qacB, tet(B), and dfrA17, and two copies of sul2 genes. Some of these resistance genes might be responsible for the resistant phenotype of strain R15. For example, three beta-lactam resistance genes, blaCTX-M-14, blaNDM-5, blaTEM-1B, which were harbored by strain R15, might be related to its resistance to several beta-lactams antimicrobial agents such as ampicillin, amoxicillin, piperacillin, and cefazolin, etc. In addition, one mcr-1 gene was also identified which might be responsible for the resistance of colistin of strain R15. However, here we only represented required resistance genes, so there might be some other mechanisms that exist in strain R15 which may also be related to its resistance phenotype. To a further extent, the co-existence of mcr-1 and blaCTX-M in E. coli strains has been observed in different sources of samples. Meanwhile, E. coli strains with both mcr-1 and blaNDM-5 were also found in humans and animals. However, R15 was isolated from a WWTP which treats wastewater generated by a pharmaceutical factory. To the best of our knowledge, R15 is the first reported wild-derived E. coli strain that harbored both mcr-1 and blaNDM-5 genes in China.

To gain insight into the locations and the gene environments of the mcr-1 and blaNDM-5 genes in R15, whole-genome sequencing using Pacific Biosciences (PacBio) RSII sequencing system with single-molecule real-time (SMRT) analysis was performed. Two complete plasmids that carry mcr-1 (pR15_MCR-1) and blaNDM-5 (pR15_NDM-5) were assembled from the sequencing reads. The complete sequences of these two plasmids were deposited in GenBank with the accession numbers, MK256965 (pR15_MCR-1) and MK256964 (pR15_NDM-5).

Plasmid pR15_MCR-1 is 109,908 bp in length with a G+C content of 47.0%. Plasmid typing revealed that it belongs to the IncY group. Nucleotide sequence alignment indicated that pR15_MCR-1 possesses 98% homology and 74% coverage with plasmid pMCR-1-P3 (KX880944). Further comparison of the gene arrangement of these two plasmids revealed the absence of the second ISApI element downstream of mcr-1 gene in pR15_MCR-1 (Figure 1A). The absence of this second ISApI element was also reported in other studies and was considered as the indication of the dynamic changes of TnApI during the transposition process. Furthermore, an insertion of approximately 11 kb in pR15_MCR-1 was found downstream of the mcr-1 gene. This insertion showed 99% nucleotide homology to the chromosome of a porcine extraintestinal pathogenic E. coli strain PCN033 (CP006632) isolated in China. An insertion element IS609 was found adjacent to this insertion that may be responsible for its transfer (Figure 1A). Since its first discovery, the mcr-1 gene has been found in various incompatibility groups of plasmids, including IncX4, IncI2, IncI, IncFII, IncHI1, IncHI2, IncX1-X2, IncX3-X4, and IncI2-IncFIB. Plasmid pR15_MCR-1, identified in the present study, belongs to the IncY incompatibility group. This group of plasmids has commonly been reported to be carrying the blaCTX-M-15 gene. Moreover, IncY plasmids contain a portion of phage-related sequences, which imply that phages may play an important role in disseminating of the mcr-1 gene. To date, there have only been two studies...
Figure 1 Lineal comparison of antimicrobial-resistant regions. (A) Comparison of the mcr-1 coding region of plasmid pR15_MCR-1 (MK256965) with plasmid pMCR-1-P3 (KX880944) and the chromosome of E. coli PCN033 (CP006632). (B) Comparison of the blaNDM-5 coding region of plasmids pR15_NDM-5 (MK256964) and pNDM_MGR194 (KF220637). Genes are portrayed by arrows and colored according to their functions.

reporting the carrying of the mcr-1 gene in IncY group plasmids. One was pMCR-1-P3, identified in E. coli strain P3 isolated from pig anal swabs in Shandong province in China.20 The other was pHYEC7-mcr1, which was harbored by E. coli strain HYEC7 isolated from a fecal sample collected from a pig farm in Guangdong province in China.21 Thus, both plasmids were harbored by E. coli strains of animal origin. However, pR15_MCR-1 in the present study was identified from an E. coli isolated from a WWTP in Zhejiang province in China, and this WWTP has no contact with animal waste. Thus, our report is the first discovery of an IncY plasmid that carries the mcr-1 gene in an E. coli strain of environmental origin. Our work highlights the dissemination of this kind of plasmid in the environment.

Plasmid pR15_NDM-5, which carries the blaNDM-5 gene, is 46,161 bp in length. This plasmid belongs to the IncX3 group which was reported to be the most common Inc type plasmid that harbored blaNDM gene in China.22 Nucleotide BLAST revealed that pR15_NDM-5 was highly similar to pNDM-MGR194 (99% identity and 100% coverage).23 Furthermore, genetic environment characterization revealed a structure of IS26-dsbC-trpF-bleMBL-bleNDM-5-IS5 in both plasmids (Figure 1B). Thus, our discovery of this plasmid supplements previous studies and further highlights the dissemination of blaNDM-5 gene-carrying IncX3 group plasmids in WWTP.

In conclusion, we report the complete sequences of an IncY type mcr-1 carrying plasmid and an IncX3 type blaNDM-5 carrying plasmid in an E. coli strain isolated from WWTP. There is a growing body of literature that reports the co-existence of mcr-1 and blaNDM-5 genes in E. coli strains of clinic-origin and animal-origin. However, our study represents the first report of a wild-derived E. coli strain that harbors mcr-1 and blaNDM-5 genes simultaneously. To lower the risk of the dissemination of this multidrug-resistant strain in the environment, more surveillance is needed in future.

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Disclosure
The authors report no conflicts of interest in this work.

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