Co-Existence of *mcr-1* and *bla*_{NDM-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 phage-like IncY plasmid (pR15_MCR-1) and a *bla*_{NDM-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 *bla*_{NDM-5} genes in the pharmaceutical industry.

Importance: *Escherichia coli* strains that carry both *mcr-1* and *bla*_{NDM-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 *bla*_{NDM-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 *bla*_{NDM-5}, respectively. The observation of this wild-derived *E. coli* strain that carries *mcr-1* and *bla*_{NDM-5} genes simultaneously calls for the urgency to improve monitoring and reducing its further spreading.

Keywords: *mcr-1*, *bla*_{NDM-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*

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Figure 1 Lineal comparison of antimicrobial-resistant regions. **(A)** Comparison of the *mcr-1* coding region of plasmid pR15_MCR-I (MK256965) with plasmid pMCR-I-P3 (KX880944) and the chromosome of *E. coli* PCN033 (CP006632). **(B)** Comparison of the *bla*_{NDM-5} coding region of plasmids pR15_NDM-5 (MK256964) and pNDM_MGR194 (KF220657). 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-I-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 *bla*_{NDM-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 *bla*_{NDM} 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*-*ble*_{MBL}-*bla*_{NDM-5}-IS5 in both plasmids (Figure 1B). Thus, our discovery of this plasmid supplements previous studies and further highlights the dissemination of *bla*_{NDM-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 *bla*_{NDM-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 *bla*_{NDM-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 *bla*_{NDM-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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