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SHORT REPORT

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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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.[9] This WWTP treats the wastewater from a pharmaceutical company which produces antimicrobial agents including beta-lactam antibiotics such as cefaclor, ceftizoxime, ceftibuten, cefuroxime sodium, 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 $bla_{\text{NDM-5}}$ genes.

The isolation and identification of R15 was performed as described previously.[9] 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 (MI C≥ 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.[4]

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[10] and reads were assembled into 140 contigs (PSSH00000000.1) using SPAdes software.[11] Analyzing with the draft genome sequence of R15 by MLST 2.0[12] showed that it belonged to ST-744. Further, we used ResFinder 3.2[13] to predict the resistance genes encoded by strain R15 and 20 resistance genes were identified, including one copy of *mcr-1*, $bla_{\text{CTX-M-14}}$, $bla_{\text{NDM-5}}$, $bla_{\text{TEM-1B}}$, *fosA3, floR, aac(3)-IV, aph(3")-lb, aph(3')-lla, aph (4)-la, aph(6)-ld, rmtB, mdf(A), mph(A), oqxA, oqxB, 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, $bla_{\text{CTX-M-14}}$, $bla_{\text{NDM-5}}$, $bla_{\text{TEM-1B}}$, 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 $bla_{\text{CTX-M}}$ in *E. coli* strains has been observed in different sources of samples.[14] Meanwhile, *E. coli* strains with both *mcr*-1 and $bla_{\text{NDM-5}}$ were also found in humans and animals.[2–4,15,16] 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 $bla_{\text{NDM-5}}$ genes in China.

To gain insight into the locations and the gene environments of the *mcr-1* and $bla_{\text{NDM-5}}$ 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 $bla_{\text{NDM-5}}$ (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 IS*Apl*1 element downstream of *mcr-1* gene in pR15_MCR-1 (Figure 1A). The absence of this second IS*Apl*1 element was also reported in other studies and was considered as the indication of the dynamic changes of Tn*Apl* during the transposition process.[3,17] 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.[18] An insertion element *IS*609 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, Incl2, IncP, IncFI, IncFII, IncHI1, IncHI2, IncX1-X2, IncX3-X4, and Incl2-IncFIB.[17] 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 $bla_{\text{CTX-M-15}}$ gene.[19] 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.[20] To date, there have only been two studies

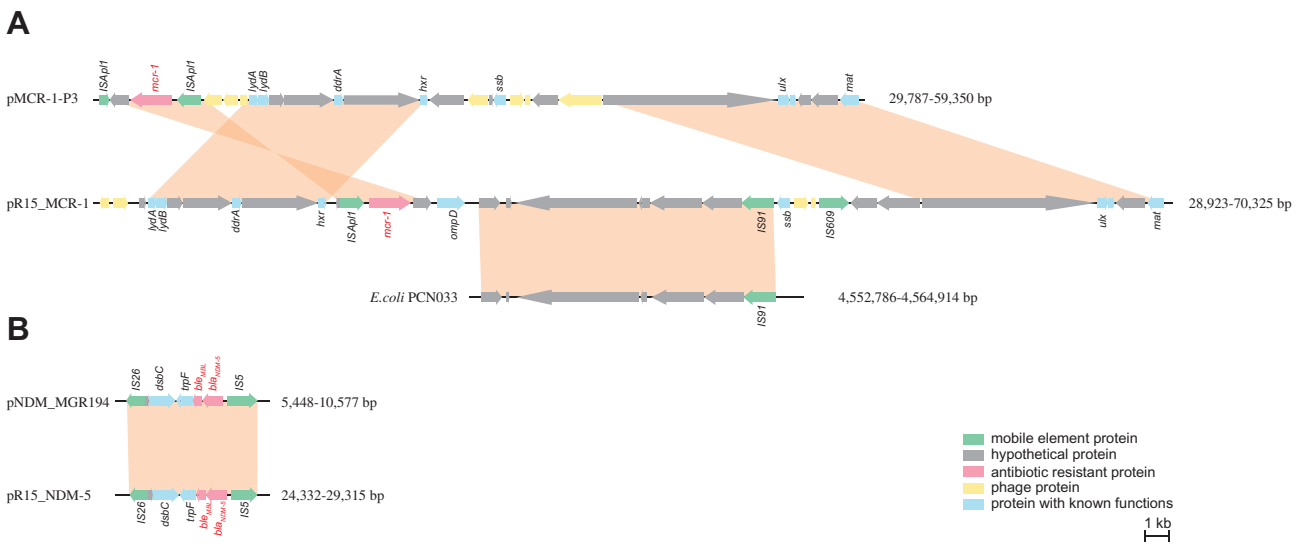

Figure 1 Lineal comparison of antimicrobial-resistant regions. (**A**) Comparison of the *mcr-1* coding region of plasmid pR15_MCR-1 (MK256965) with plasmid pMCR-1-P3 (KX880944) and the chromosome of *E. coli* PCN033 (CP006632). (**B**) Comparison of the bla_{NDM-5} coding region of plasmids pR15_NDM-5 (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-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 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 IS*26-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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