AMERICAN SOCIETY FOR MICROBIOLOGY | Antimicrobial Agents and Chemotherapy®

New Variant of *mcr-3* in an Extensively Drug-Resistant *Escherichia coli* Clinical Isolate Carrying *mcr-1* and bla_{NDM-5}

Lu Liu,[a,b] Yu Feng,[a,b] Xiaoxia Zhang,[a,b] Alan McNally,[e] Zhiyong Zong[a,b,c,d]

Center of Infectious Diseases, West China Hospital, Sichuan University, Chengdu, China[a]; Division of Infectious Diseases, State Key Laboratory of Biotherapy, Chengdu, China[b]; Department of Infection Control, West China Hospital, Sichuan University, Chengdu, China[c]; Center for Pathogen Research, West China Hospital, Sichuan University, Chengdu, China[d]; Institute of Microbiology and Infection, College of Medical and Dental Sciences, University of Birmingham, Birmingham, United Kingdom[e]

ABSTRACT A colistin- and carbapenem-resistant *Escherichia coli* clinical isolate was found to carry two plasmid-borne colistin-resistant genes, *mcr-1* and the newly identified *mcr-3*, and a carbapenemase gene, bla_{NDM-5}. *mcr-3* is a new variant (*mcr-3.5*) in the isolate and encodes three amino acid substitutions compared with the original MCR-3. *mcr-3* was carried by a Tn*As3*-like transposon on a self-transmissible IncP plasmid in the isolate, highlighting that *mcr-3* may have widely spread.

KEYWORDS colistin, resistance, plasmids, *Escherichia coli*, *Enterobacteriaceae*

The emergence of multidrug resistance (MDR) in Gram-negative pathogenic bacteria represents one of the greatest global public health threats of the 21st century. In particular, the rise in incidence of MDR *Escherichia coli* has coincided with an increase in cases of bacteremia infections caused by *E. coli* (1). The evolution of MDR *E. coli* has been progressive in nature, with initial emergence due to dissemination of plasmids containing extended-spectrum-β-lactamase (ESBL)-encoding genes such as $bla_{CTX-M-15}$ (2). Infection with ESBL-producing *E. coli* is usually treated with carbapenem antibiotics, but *E. coli* could acquire carbapenemase genes such as bla_{NDM-5} (3) to become carbapenem resistant. The remaining possible treatment for infections caused by carbapenem-resistant isolates is primarily colistin. However, increasing use of this antimicrobial in clinical and veterinary practice has led to the emergence of mobile colistin resistance genes, *mcr*, which have been reported in various species of the *Enterobacteriaceae* family (4).

Five plasmid-borne colistin resistance genes, *mcr-1* (5), *mcr-2* (6), *mcr-3* (7), *mcr-4* (8), and *mcr-5* (9), have now been reported, with *mcr-3* having been identified in China in the past few months. The MCR-3 protein has only 32.5%, 31.7%, 49.0%, and 34.7% amino acid identity to MCR-1, MCR-2, MCR-4, and MCR-5, respectively (7–9), suggesting that it is not a recently evolved variant of MCR-1 but rather a distinct class of enzyme. Here we report the first ever clinical isolation of an *E. coli* strain carrying plasmid-borne bla_{NDM-5}, *mcr-1*, and *mcr-3* genes.

E. coli strain WCHEC-LL123 was recovered from an abdominal abscess of a 43-year-old male patient in January 2017 in China. The strain was resistant to aztreonam (MIC, 16 μg/ml), ceftazidime (MIC, >512 μg/ml), ceftazidime-avibactam (MIC, >512/4 μg/ml), ciprofloxacin (MIC, 128 μg/ml), colistin (MIC, 8 μg/ml), imipenem (MIC, 32 μg/ml), meropenem (MIC, 64 μg/ml), piperacillin-tazobactam (MIC, >512/4 μg/ml), and trimethoprim-sulfamethoxazole (MIC, 64/1216 μg/ml) but was susceptible to amikacin (MIC, 8 μg/ml), aztreonam-avibactam (MIC, <0.125/4 μg/ml), and tigecycline (MIC, 0.25 μg/ml), as determined using the broth dilution method of the Clinical and Laboratory

Received 23 August 2017 **Returned for modification** 25 September 2017 **Accepted** 27 September 2017

Accepted manuscript posted online 2 October 2017

Citation Liu L, Feng Y, Zhang X, McNally A, Zong Z. 2017. New variant of *mcr-3* in an extensively drug-resistant *Escherichia coli* clinical isolate carrying *mcr-1* and bla_{NDM-5}. Antimicrob Agents Chemother 61:e01757-17. https://doi.org/10.1128/AAC.01757-17.

Address correspondence to Zhiyong Zong, zongzhiy@scu.edu.cn.

Standards Institute (10). The patient recovered after repeated surgeries, drainage of the abscess, and antimicrobial treatment with tigecycline.

A draft genome sequence of the strain was generated on the Illumina HiSeq X10 platform, which generated 5,751,013 clean reads assembled into 275 contigs (176 >1,000 bp; *N50* 88,902 bp) with a 50.4% GC content using SPAdes (11). Strain WCHEC-LL123 belonged to phylogenetic group A, as determined using PCR as described previously (12), and sequence type 206 (ST206), as determined using the genomic sequence to query the *E. coli* multilocus sequence typing database (http://enterobase.warwick.ac.uk/species/index/ecoli). Antimicrobial resistance genes were identified from genome sequences using the ABRicate program (https://github.com/tseemann/abricate). Strain WCHEC-LL123 had both *mcr-1* and *mcr-3* plasmid-borne colistin resistance genes and the bla_{NDM-5} carbapenemase gene. In addition, the strain had 19 antimicrobial resistance genes mediating resistance to aminoglycosides [*aac(3)-IVa*, *aadA1*, *aadA2*, *aadA5*, *aph(3′)-Ia*, *aph(4)-Ia*], β-lactams ($bla_{CTX-M-14}$, $bla_{TEM-106}$), fosfomycin (*fosA*), phenicol (*catA2*, *cmlA1*, *floR*), quinolones (*oqxA*, *oqxB*), tetracycline [*tet(A)*], sulfonamides (*sul1*, *sul2*, and *sul3*), and trimethoprim (*dfrA17*).

At least 8 variants of *mcr-1* have been found up to now, and the *mcr-1* gene in our strain is identical to the original *mcr-1* variant. However, the *mcr-3* in our strain is a novel variant; compared with the original *mcr-3* variant on plasmid pWJ1 (GenBank accession no. KY924928) it has 3 nucleotide differences (99.82% identity), which lead to 3 amino acid substitutions (M23V, A456E, and T488I). In GenBank, there are three additional *mcr-3* variants in species other than *Aeromonas* spp., which are believed to be the origin of *mcr-3*. Compared to the original MCR-3, the three additional *mcr-3* variants encode MCR-3 proteins with a single amino acid substitution, D295E in *Klebsiella pneumoniae* strain PB395 (GenBank accession no. NZ_FLWO01000034) from humans in Thailand, G373V in *K. pneumoniae* strain PB517 (GenBank accession no. NZ_FLXA01000011) from humans in Thailand, and M23V in *Citrobacter freundii* strain D36-1 (GenBank accession no. NZ_NEFW01000046) from swine in China. The *mcr-3* variant in our strain was therefore assigned the designation *mcr-3.5* here. The MIC of colistin for the *E. coli* transconjugant containing *mcr-3.5* was 4 μg/ml (see Table 1), which was identical to that for *E. coli* containing the original *mcr-3* variant (7). This suggests that the amino acid substitutions of MCR-3.5 have not altered its activity against colistin.

Plasmids carrying *mcr-1*, *mcr-3.5*, and bla_{NDM-5} were circularized using PCR and Sanger sequencing to fill in gaps between contigs. Plasmid replicon types were determined by using the PlasmidFinder tool at http://genomicepidemiology.org/. Conjugation experiments were carried out in broth and on filters with the azide-resistant *E. coli* strain J53 as the recipient. The *mcr-3.5* gene was carried by a self-transmissible 52.2-kb IncP plasmid, designated pMCR3_LL123. pMCR3_LL123 is almost identical to two *mcr-1*-carrying IncP plasmids, both of which were recovered from sewage of the same hospital in 2015, pMCR_1511 (GenBank accession no. KX377410) found in a *K. pneumoniae* isolate (13) and pMCR_1622 (GenBank accession no. KY463452) found in an *E. coli* isolate (ST7068), but there are two differences. First, there is no *mcr-3* on pMCR_1622, while a truncated *mcr-3* (a remnant with the last 645 bp of the 1,626-bp *mcr-3*) is present on pMCR_1511 (Fig. 1), which is identical to the corresponding region of the original *mcr-3* variant and does not have the mutations seen in *mcr-3.5*. The truncated *mcr-3* on pMCR_1511 had a genetic context similar to that of *mcr-3.5* on pMCR3_LL123 (Fig. 1). The mechanism for the truncation of *mcr-3* on pMCR_1511 was not clear. Second, the composite transposon formed by IS*Apl1* carrying *mcr-1* on pMCR_1511 (13) and the IS*Apl1-mcr-1*-orf element on pMCR_1622 with the open reading frame (ORF) referring to a gene encoding a putative phosphoesterase were absent from pMCR3_LL123 (Fig. 1). Nonetheless, the identical backbone suggests that pMCR3_LL123, pMCR_1511, and pMCR_1622 were derived from a common ancestor. IncP is a broad-host-range type, and the association of *mcr-3* with an IncP plasmid is worrisome because it may have the potential to mediate the dissemination of *mcr-3* beyond the *Enterobacteriaceae* family and *Aeromonas* spp.

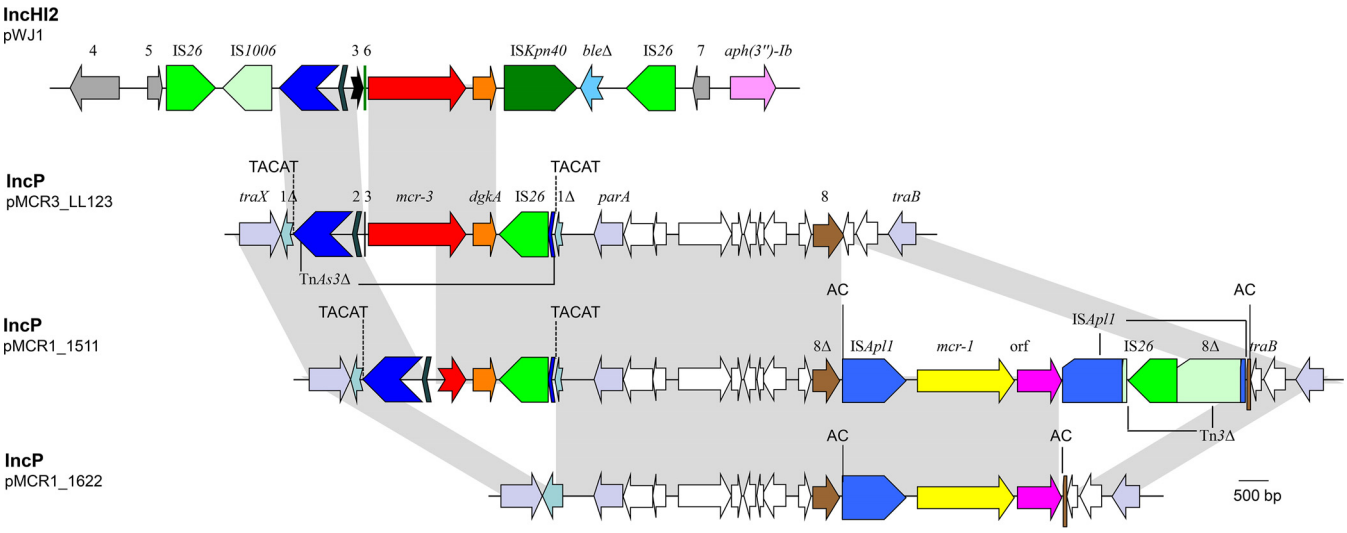

FIG 1 The genetic context of *mcr-3* on pMCR3_LL123 (second from top). The genetic context of *mcr-3* on pWJ1 (top), that of the truncated *mcr-3* on pMCR_1511 (third from top), and the corresponding region on non-*mcr-3*-carrying plasmid pMCR_1622 (bottom) are shown for comparison. The 5-bp direct repeats (DRs) characteristic of the transposition of Tn*3* family transposons, including Tn*As3* in this case, are indicated. *dgkA* refers to a gene encoding diacylglycerol kinase. The numbers represent genes or mobile genetic elements, including: 1, an open reading frame (ORF) of unknown function, which is broken into two parts (two 1Δ); 2, IS*Enca1* truncated; 3, a gene encoding an NimA/NimC family protein, which is truncated with a 219-bp remnant on pWJ1 and a 24-bp remnant on pMCR3_LL123; 4, a gene encoding a DUF4942 domain-containing protein; 5, an ORF of unknown function; 6, a truncated (41-bp remnant) IS*Kpn40*; 7, an ORF of unknown function; 8, an ORF of unknown function, which is interrupted by the composite transposon formed by IS*Apl1* carrying *mcr-1* on pMCR_1511 and the IS*Apl1-mcr-1*-orf element on pMCR_1622 with the 2-bp direct repeats (AC) characteristic of the insertion of IS*Apl1*.

Analysis of the complete sequence of pMCR3_LL123 identified that *mcr-3* was located on the transposon Tn*As3*, which belongs to the Tn*3* family and was originally identified in *Aeromonas salmonicida*. Both boundaries (inverted repeats [IRs]) of Tn*As3* were present on pMCR3_LL123 and were flanked by a 5-bp repeat (TACAT) (Fig. 1), which is characteristic of the transposition of Tn*As3*. Tn*As3* is inserted into a gene which has no known function and is located between the *parA* gene encoding plasmid partition and the *traX* gene encoding plasmid conjugation (Fig. 1). However, the transposase gene *tnpA* is interrupted with only 861 bp of 2,967 bp remaining. It is therefore unlikely that the transposition of Tn*As3* is due to its own transposase. It is known that two IRs of the Tn*3* family of transposons can be transposed together with their intervening genetic components in the presence of transposases encoded by other Tn*3* family transposons (14). However, the mechanism mediating the introduction of *mcr-3* into Tn*As3* warrants further investigation.

The *mcr-1* gene was carried by a separate 223-kb IncHI2 plasmid in strain WCHEC-LL123, which was also self-transmissible and was highly similar (99% identity) to the corresponding region of pMCR_1613 (GenBank accession no. CP019214), an IncHI2 plasmid with an additional IncN replicon in *E. coli* strain WCHEC1613 (ST48) isolated from the sewage of the same hospital in 2015. In strain WCHEC-LL123, *mcr-1* was located in a composite transposon formed by two copies of IS*Apl1*, which was inserted into a gene encoding a putative signal peptidase I/pili assembly chaperon protein belonging to the IncHI2 backbone with the characteristic 2-bp direct target repeats.

The coexistence of two colistin resistance plasmids in a single bacterial isolate is intriguing. The MICs of colistin against *E. coli* transconjugants containing *mcr-3* or *mcr-1* were 4 μg/ml, while that against WCHEC-LL123 (containing both *mcr-1* and *mcr-3*) was 8 μg/ml (Table 1). This suggests that the coexistence of *mcr-1* and *mcr-3* may not provide a significant additive effect. Astonishingly, the carbapenemase-encoding bla_{NDM-5} gene was found on a third resistance plasmid, a self-transmissible 47-kb IncX3 plasmid. This is identical to pNDM5_WCHEC0215 (GenBank accession no. KY435936), a bla_{NDM-5}-carrying IncX3 plasmid in an ST167 *E. coli* clinical isolate from the same hospital in 2013 (15), except for several single nucleotide variations.

In conclusion we report the clinical isolation from a patient in China of an *E. coli*

TABLE 1 MICs of colistin against WCHEC-LL123 and its transconjugants

Strain	Description	*mcr* gene	MIC of colistin (μg/ml)
WCHEC-LL123	Wild isolate	*mcr-1*, *mcr-3.5*	8
TxLL123-1	Transconjugant, J53 acquired the *mcr-1*-carrying plasmid from WCHEC-LL123	*mcr-1*	4
TxLL123-3	Transconjugant, J53 acquired the *mcr-3*-carrying plasmid from WCHEC-LL123	*mcr-3.5*	4
J53	Recipient strain	—[a]	1

[a]No *mcr* gene.

strain which carries three distinct resistance plasmids, one carrying *mcr-1*, one carrying *mcr-3*, and one carrying bla_{NDM-5}. The isolation of this strain is highly significant not only from a clinical treatment perspective but also from the perspective of the evolution of antimicrobial resistance. It also suggests that clinical strains of *E. coli* are capable of acquiring and maintaining multiple large MDR plasmids with no apparent loss of fitness with respect to ability to successfully colonize and cause clinical infections in patients.

Accession number(s). A draft whole-genome sequence of strain WCHEC-LL123 has been deposited into GenBank under the accession no. NMQY00000000. The complete sequence of pMCR3_WCHEC-LL123 has been deposited into GenBank under the accession no. MF489760.

ACKNOWLEDGMENTS

This work was supported by grants from the National Natural Science Foundation of China (projects 81222025 and 81572030) and a joint grant from the National Natural Science Foundation of China (project 8151101182) and the Newton Advanced Fellowship, Royal Society, United Kingdom (NA150363).

REFERENCES

1. de Kraker ME, Davey PG, Grundmann H, BURDEN study group. 2011. Mortality and hospital stay associated with resistant *Staphylococcus aureus* and *Escherichia coli* bacteremia: estimating the burden of antibiotic resistance in Europe. PLoS Med 8:e1001104. https://doi.org/10.1371/journal.pmed.1001104.
2. Coque TM, Novais A, Carattoli A, Poirel L, Pitout J, Peixe L, Baquero F, Canton R, Nordmann P. 2008. Dissemination of clonally related *Escherichia coli* strains expressing extended-spectrum β-lactamase CTX-M-15. Emerg Infect Dis 14:195–200. https://doi.org/10.3201/eid1402.070350.
3. Hornsey M, Phee L, Wareham DW. 2011. A novel variant, NDM-5, of the New Delhi metallo-β-lactamase in a multidrug-resistant *Escherichia coli* ST648 isolate recovered from a patient in the United Kingdom. Antimicrob Agents Chemother 55:5952–5954. https://doi.org/10.1128/AAC.05108-11.
4. Poirel L, Jayol A, Nordmann P. 2017. Polymyxins: antibacterial activity, susceptibility testing, and resistance mechanisms encoded by plasmids or chromosomes. Clin Microbiol Rev 30:557–596. https://doi.org/10.1128/CMR.00064-16.
5. Liu Y-Y, Wang Y, Walsh TR, Yi L-X, Zhang R, Spencer J, Doi Y, Tian G, Dong B, Huang X, Yu L-F, Gu D, Ren H, Chen X, Lv L, He D, Zhou H, Liang Z, Liu J-H, Shen J. 2016. Emergence of plasmid-mediated colistin resistance mechanism MCR-1 in animals and human beings in China: a microbiological and molecular biological study. Lancet Infect Dis 16:161-168. https://doi.org/10.1016/S1473-3099(15)00424-7.
6. Xavier BB, Lammens C, Ruhal R, Kumar-Singh S, Butaye P, Goossens H, Malhotra-Kumar S. 2016. Identification of a novel plasmid-mediated colistin resistance gene, mcr-2, in *Escherichia coli*, Belgium, June 2016. Euro Surveill 21 (27):pii=30280. https://doi.org/10.2807/1560-7917.ES.2016.21.27.30280.
7. Yin W, Li H, Shen Y, Liu Z, Wang S, Shen Z, Zhang R, Walsh TR, Shen J, Wang Y. 2017. Novel plasmid-mediated colistin resistance gene *mcr-3* in *Escherichia coli*. mBio 8:e00543-17. https://doi.org/10.1128/mBio.00543-17.
8. Carattoli A, Villa L, Feudi C, Curcio L, Orsini S, Luppi A, Pezzotti G, Magistrali CF. 2017. Novel plasmid-mediated colistin resistance *mcr-4* gene in *Salmonella* and *Escherichia coli*, Italy 2013, Spain and Belgium, 2015 to 2016. Euro Surveill 22(31):pii=30589. https://doi.org/10.2807/1560-7917.ES.2017.22.31.30589.
9. Borowiak M, Fischer J, Hammerl JA, Hendriksen RS, Szabo I, Malorny B. 18 September 2017. Identification of a novel transposon-associated phosphoethanolamine transferase gene, *mcr-5*, conferring colistin resistance in d-tartrate fermenting *Salmonella enterica* subsp. *enterica* serovar Paratyphi B. J Antimicrob Chemother. https://doi.org/10.1093/jac/dkx327.
10. Clinical and Laboratory Standards Institute. 2017. Performance standards for antimicrobial susceptibility testing; 23rd informational supplement. CLSI M100-S27. Clinical and Laboratory Standards Institute, Wayne, PA.
11. Bankevich A, Nurk S, Antipov D, Gurevich AA, Dvorkin M, Kulikov AS, Lesin VM, Nikolenko SI, Pham S, Prjibelski AD, Pyshkin AV, Sirotkin AV, Vyahhi N, Tesler G, Alekseyev MA, Pevzner PA. 2012. SPAdes: a new genome assembly algorithm and its applications to single-cell sequencing. J Comput Biol 19:455–477. https://doi.org/10.1089/cmb.2012.0021.
12. Clermont O, Bonacorsi S, Bingen E. 2000. Rapid and simple determination of the *Escherichia coli* phylogenetic group. Appl Environ Microbiol 66:4555–4558. https://doi.org/10.1128/AEM.66.10.4555-4558.2000.
13. Zhao F, Feng Y, Lu X, McNally A, Zong Z. 2017. IncP plasmid carrying colistin resistance gene *mcr-1* in *Klebsiella pneumoniae* from hospital sewage. Antimicrob Agents Chemother 61:e02229-16. https://doi.org/10.1128/AAC.02229-16.
14. Szuplewska M, Czarnecki J, Bartosik D. 2014. Autonomous and non-autonomous Tn*3*-family transposons and their role in the evolution of mobile genetic elements. Mob Genet Elements 4:1–4. https://doi.org/10.1080/2159256X.2014.998537.
15. Yang P, Xie Y, Feng P, Zong Z. 2014. bla_{NDM-5} carried by an IncX3 plasmid in *Escherichia coli* of the sequence type 167. Antimicrob Agents Chemother 58:7548–7552. https://doi.org/10.1128/AAC.03911-14.