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Various Sequence Types of *Escherichia coli* Isolates Coharboring bla_{NDM-5} and *mcr-1* Genes from a Commercial Swine Farm in China

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ABSTRACT Sixteen different sequence types (STs) of *Escherichia coli* isolates from a commercial swine farm in China were confirmed to coharbor the carbapenem resistance gene bla_{NDM-5} and the colistin resistance gene *mcr-1*. Whole-genome sequencing revealed that bla_{NDM-5} and *mcr-1* were located on a 46-kb IncX3 plasmid and a 32-kb IncX4 plasmid, respectively. The two plasmids can transfer together with a low fitness cost, which might explain the presence of various STs of *E. coli* coharboring bla_{NDM-5} and *mcr-1*.

KEYWORDS *Escherichia coli*, carbapenems, bla_{NDM-5}, colistin, *mcr-1*, fitness cost

Carbapenemase-producing *Enterobacteriaceae* has become a major public health threat around the world (1). The recently identified carbapenemase New Delhi metallo-β-lactamase confers resistance to all β-lactam antimicrobials except monobactam (2). The NDM-5-encoding gene bla_{NDM-5} was first identified in an *Escherichia coli* strain recovered from a patient in the United Kingdom in 2011 (3). Since then, bla_{NDM-5} was identified in many countries, such as Algeria (4–6), the United States (7), Australia (8), China (9–12), Denmark (13), Japan (14), India (15), and the United Kingdom (3). The widespread occurrence of NDM-5 in recent years should arouse our attention. Colistin is a critically important medication for humans in the treatment of carbapenemase-producing *Enterobacteriaceae*, and it has been widely used in veterinary medicine in China (16, 17). The first plasmid-mediated colistin resistance gene, *mcr-1*, was reported in *E. coli* in 2015 (18). In a short period, colistin-resistant *E. coli* carrying the *mcr-1* gene were reported worldwide (19, 20). Recently, *mcr-1* was reported to coexist with bla_{NDM} (21–23) and bla_{CTX-M} (24), which brought great challenges for the treatment of bacterial infection. In the present study, we are the first to report the presence of isolates of various sequence types of *E. coli* coharboring bla_{NDM-5} and *mcr-1* genes from a commercial pig farm in China.

A total of 105 anal swabs samples from swine were collected from a commercial pig farm on 1 October 2015 in Sichuan province. *E. coli* strains were selected by eosin-methylene blue agar, and only 1 isolate was picked up from each sample. All 105 isolates were identified by BD Phoenix 100 diagnostic systems (Sparks, MD). Sixty-four strains were nonsusceptible to imipenem and polymyxin B, identified by the agar dilution method according to Clinical and Laboratory Standards Institute guidelines (25). Isolates were divided into 16 different clones by pulsed-field gel electrophoresis after XbaI digestion according to the standard PulseNet conditions (26) (Fig. 1). Phy-

Received 14 October 2016 **Returned for modification** 11 November 2016 **Accepted** 29 November 2016

Accepted manuscript posted online 19 December 2016

Citation Kong L-H, Lei C-W, Ma S-Z, Jiang W, Liu B-H, Wang Y-X, Guan R, Men S, Yuan Q-W, Cheng G-Y, Zhou W-C, Wang H-N. 2017. Various sequence types of *Escherichia coli* isolates coharboring bla_{NDM-5} and *mcr-1* genes from a commercial swine farm in China. Antimicrob Agents Chemother 61:e02167-16. https://doi.org/10.1128/AAC.02167-16.

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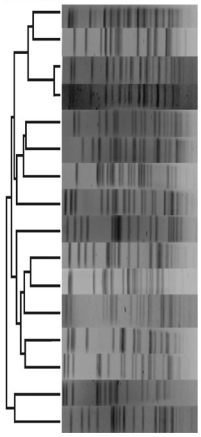

PFGE profiles	Pattern	No. of isolates	ST	Phylogenetic group	Resistant phenotype
	ECMY2	4	1114	A	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC-GEN-SXT-PB-CIP
	ECMY12	2	167	A	IPM-MEM-AMC-FOX-CTX -DO-FFC-GEN-SXT-PB-CIP
	ECMY4a	6	410	A	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC-GEN-SXT-PB-CIP
	ECMY4b	3	90	A	IPM-MEM-AMC-FOX-CTX -DO-FFC- SXT-PB-CIP
	ECMY16	1	4429	A	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC-GEN-SXT-PB-CIP
	ECMY5	1	4656	A	IPM-MEM-AMC-FOX-CTX -DO-FFC-GEN-SXT-PB-CIP
	ECMY1	2	156	B1	IPM-MEM-AMC-FOX-CTX -DO-FFC -SXT-PB-CIP
	ECMY14	7	54	A	IPM-MEM-AMC-FOX-CTX-FFC-GEN-SXT-PB-CIP
	ECMY13	8	4463	B1	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC- SXT-PB-CIP
	ECMY17	1	3331	A	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC-GEN-SXT-PB-CIP
	ECMY6	6	165	A	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC-GEN-SXT-PB-CIP
	ECMY9	4	1178	A	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC-GEN-SXT-PB
	ECMY18	4	1437	A	IPM-MEM-AMC-FOX-CTX -DO-FFC -SXT-PB-FOS
	ECMY10	2	None[1]	B1	IPM-MEM-AMC-FOX-CTX-ATM -FFC-SXT-PB
	ECMY11	1	2439	A	IPM-MEM-AMC-FOX-CTX -DO-FFC-GEN-SXT-PB-CIP
	ECMY3	12	48	A	IPM-MEM-AMC-FOX-CTX-ATM-DO-FFC-GEN-SXT-PB-CIP

FIG 1 PFGE profiles, antimicrobial resistance phenotypes, phylogenetic groups, and sequence types (STs) of 16 different *E. coli* strains. [1]Failure to find any corresponding ST type with MLST database via BLAST. Seven housekeeping gene allele types, i.e., *adk*, *fumC*, *gyrB*, *icd*, *mdh*, *purA*, *recA*, were identified as follows: 10, 11, 4, 8, 274, 8, 42. IPM, imipenem; MEM, meropenem; AMC amoxicillin-clavulanate; FOX, cefoxitin; CTX, cefotaxime; ATM, aztreonam; CIP, ciprofloxacin; DO, doxycycline; FFC, florfenicol; FOS, fosfomycin; GEN, gentamicin; SXT, trimethoprim-sulfamethoxazole; PB, polymyxin B.

logenetic group (A, B1, B2, and D) typing showed that all 16 different clones belonged to group A ($n = 13$) and B1 ($n = 3$) (27). Multilocus sequence typing (MLST) was performed as previously described (http://mlst.warwick.ac.uk/mlst/dbs/Ecoli). All 16 clones belonged to different sequence types. The antimicrobial resistance profile, phylogenetic group, and sequence type of each different clone are shown in Figure 1.

The carbapenemase-encoding genes bla_{GES}, bla_{KPC}, bla_{IMP}, bla_{NDM}, bla_{OXA-48}, and bla_{VIM} and the colistin resistance gene *mcr-1* were screened in all 16 different clones by PCR as described previously (18, 28, 29). The results showed that they all carried bla_{NDM-5} and *mcr-1*. Very recently, *mcr-1* and bla_{NDM-5} coharbored by *E. coli* ST156 and ST648 were found in a Chinese hospital (23). To the best of our knowledge, this is the first report of the presence of diverse *E. coli* strains coharboring bla_{NDM-5} and *mcr-1* on a commercial swine farm.

Whole-genome sequencing for 16 different clones was performed on the Illumina MiSeq (Majorbio, Shanghai, China) using a 400-bp paired-end TruSeq library with a 2 × 300 run. The paired-end reads were assembled *de novo* using the SOAP v2.04 and GapCloser v1.12. The gaps between different contigs were closed by PCR and sequencing. The genetic environments of bla_{NDM-5} and *mcr-1* were analyzed by using the BLAST program (http://blast.ncbi.nlm.nih.gov/Blast.cgi). Sequence analysis revealed that bla_{NDM-5} and *mcr-1* were located on two different plasmids, which showed 100% nucleotide identity in all 16 strains. The plasmid carrying bla_{NDM-5} belonged to the IncX3 group and was designated pECNDM101. It had a length of 46,165 bp with 46.66% G+C content, which had a 9-bp nucleotide substitution, 1-bp insertion, and 3-bp deletion compared with pNDM_MGR194 (GenBank accession number KF220657). An IS*5* was inserted with IS*Aba125* upstream of bla_{NDM-5}, which was also found in isolates in India (15), Japan (14), and China (9, 10).The plasmid carrying *mcr-1* belonged to the IncX4 group and was designated pECMCR-1101. It had a length of 32,751 bp with 41.96% G+C content, which had a 559-bp deletion between 1,170 bp and 1,171 bp, an 8-bp nucleotide substitution, and a 6-bp insertion compared with pICBEC72Hmcr (GenBank accession number CP015977), which was recovered from a clinical *E. coli* strain in Brazil.

Conjugation experiments were carried out among the 16 different clones with rifampin-resistant *E. coli* EC600 as the recipient. Transconjugants were selected on three different Mueller-Hinton agar (Oxoid) plates that contained 400 μg/ml rifampin with 10 μg/ml imipenem or 2 μg/ml polymyxin B. Positive transconjugants were identified by

TABLE 1 Transconjugative frequencies of pECNDM101 and pECmcr-1101 plasmids in 16 different *E. coli* strains

E. coli strain (transconjugant)	Transconjugative frequencies[a]		
	pECNDM101[b]	pECmcr-1101[c]	pECNDM101 and pECmcr-1101[d]
ECMY1	1.1×10^{-3}	8.7×10^{-6}	4.0×10^{-6}
ECMY2	1.1×10^{-2}	9.2×10^{-7}	5.5×10^{-7}
ECMY3	7.7×10^{-2}	4.6×10^{-6}	2.8×10^{-6}
ECMY4a	5.3×10^{-2}	1.6×10^{-5}	5.7×10^{-6}
ECMY4b	1.3×10^{-2}	9.4×10^{-7}	4.3×10^{-7}
ECMY5	1.7×10^{-2}	5.3×10^{-6}	1.2×10^{-6}
ECMY6	8.0×10^{-3}	6.3×10^{-6}	8.9×10^{-7}
ECMY9	5.7×10^{-4}	5.8×10^{-5}	2.5×10^{-5}
ECMY10	1.1×10^{-3}	7.1×10^{-7}	4.3×10^{-7}
ECMY11	5.3×10^{-3}	5.6×10^{-5}	2.3×10^{-5}
ECMY12	1.8×10^{-3}	3.0×10^{-5}	1.7×10^{-5}
ECMY13	5.6×10^{-3}	5.3×10^{-7}	2.9×10^{-7}
ECMY14	1.4×10^{-3}	3.0×10^{-6}	1.3×10^{-6}
ECMY16	9.1×10^{-4}	6.6×10^{-6}	3.9×10^{-6}
ECMY17	2.2×10^{-3}	1.7×10^{-5}	1.2×10^{-5}
ECMY18	1.8×10^{-3}	5.0×10^{-5}	1.6×10^{-5}

[a]The results shown are presented as the average value of 3 parallel experiments.
[b]Imipenem was used as selection pressure in conjugation experiments.
[c]Polymyxin B was used as selection pressure in conjugation experiments.
[d]Imipenem and polymyxin B were used as selection pressure in conjugation experiments.

detection of antimicrobial resistance profiles and screening for the presence of bla_{NDM-5} and *mcr-1*. Conjugation frequencies were calculated as the number of transconjugants per recipient cell and are shown in Table 1. When imipenem was used as the selection pressure, transfer frequencies of bla_{NDM-5} varied from 7.7×10^{-2} to 5.7×10^{-4}. When polymyxin B was used as the selection pressure, transfer frequencies of *mcr-1* varied from 5.8×10^{-5} to 5.3×10^{-7}, and similar transfer frequencies of 2.9×10^{-7} to 2.5×10^{-5} were detected when imipenem and polymyxin B were used as the selection pressure. It was very interesting that the cotransfer of bla_{NDM-5} and *mcr-1* was detected in a few transconjugants when using polymyxin B or imipenem alone. Three different transconjugants were obtained, MYNDM-5 carrying pECNDM101, MYmcr-1 carrying pECMCR-1101, and MYNDM-5+mcr-1 carrying pECNDM101 and pECMCR-1101.

The fitness costs of pECNDM101 and pECMCR-1101 were determined by growth curves and competition experiments as previously described (30, 31). Growth curves of transconjugant MYNDM-5 and recipient *E. coli* EC600 were similar, and they arrived at the same concentration at the stationary phase (optical density at 600 nm [OD_{600}] = 1.166). However, transconjugants MYmcr-1 and MYNDM-5+mcr-1 had a slight growth disadvantage and a lower concentration at the stationary phase (OD_{600} = 1.132 and 1.130, respectively) (Fig. 2A). For the competition experiment, a constant increase in the proportion of transconjugant MYNDM-5 was observed from day 3 on. In contrast, constant decreases in the proportion of transconjugants MYmcr-1 and MYNDM-5+mcr-1 were observed from day 2 on and day 1 on, respectively (Fig. 2B). Transconjugants MYmcr-1 and MYNDM-5+mcr-1 presented competitive disadvantages of ~11.45% and ~9.67% per 10 generations relative to those of *E. coli* EC600. Interestingly, transconjugant MYNDM-5 showed a competitive advantage (~3% per 10 generations) (Fig. 2C), which might contribute to the propagation of bla_{NDM-5}.

In conclusion, to our knowledge, our study is the first to report that a bla_{NDM-5}-carrying IncX3 plasmid (pECNDM101) and an *mcr-1*-carrying IncX4 plasmid (pECmcr-1101) coexist in various sequence types of *E. coli* on a commercial pig farm, and they can transfer together at a low fitness cost. Note that the cotransfer of bla_{NDM-5} and *mcr-1* by an IncX3-X4 hybrid plasmid was detected in a clinical *E. coli* isolate in China very recently (32). The results highlight that a swine farm is an important reservoir of *E. coli* carrying bla_{NDM-5} and *mcr-1*, which presents a serious challenge for public health via food-chain transmission.

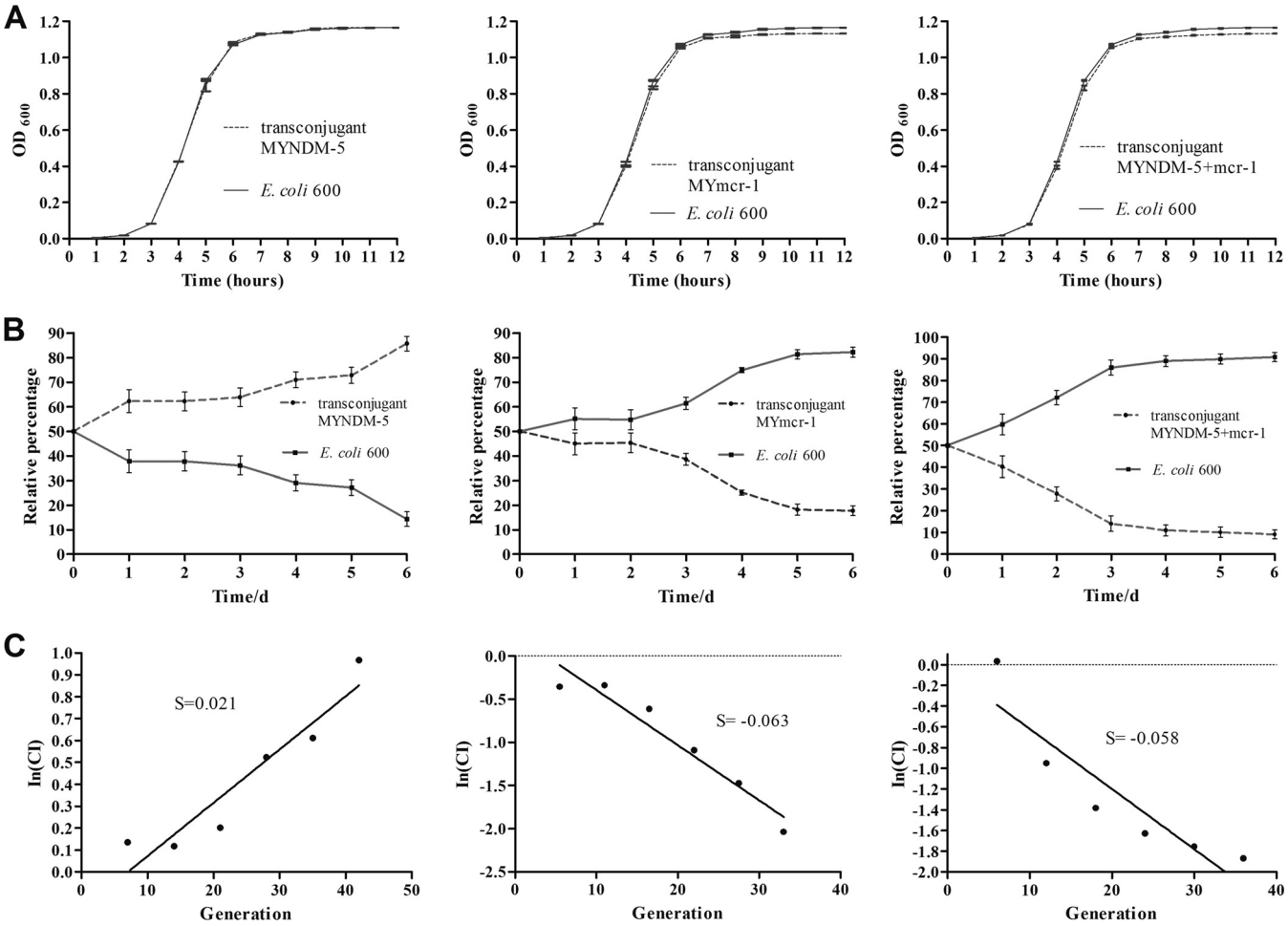

FIG 2 Fitness costs of pECNDM101 and pECmcr-1101 in *E. coli* EC600. (A) Comparison of the growth kinetics of recipient *E. coli* EC600 and three transconjugants (MYNDM-5, MYmcr-1, and MYNDM-5+mcr-1) without any antibiotics. Growth curves represent the average results of three independent experiments. (B) Growth competition between recipient *E. coli* 600 and three transconjugants (MYNDM-5, MYmcr-1, and MYNDM-5+mcr-1). The initial ratio was 1:1. Competition experiments were repeated three times. (C) The selection coefficient (S) used to estimate the difference between the relative fitness of two competitors over the entire competition experiment, and S was calculated as the slope of the linear regression model ln(CI)/ln(d), where CI is the CFU ratio of the resistant and susceptible strains and d is the dilution factor.

Accession number(s). The complete nucleotide sequences of the bla_{NDM-5}-carrying IncX3 plasmid (pECNDM101) and the *mcr-1*-carrying IncX4 plasmid (pECmcr-1101) characterized in this study were submitted to GenBank and assigned accession numbers KX507346 and KX570748, respectively.

ACKNOWLEDGMENTS

This work was supported by the 973 National Basic Research Program of China (project number 2013CB127200), the General Program of the National Natural Science Foundation of China (grant number 31100102), and the Science and Technology Pillar Program in Sichuan Province (grant numbers 2013NZ0025, 13ZC2578, and 2012GZ0001-1).

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