

Emergence of two *Escherichia coli* strains co-harboring *mcr-1* and *bla*_{NDM} in fresh vegetables from China

This article was published in the following Dove Press journal:
Infection and Drug Resistance

Bao-Tao Liu¹
Feng-Jing Song²

¹Department of Basic Veterinary Medicine, College of Veterinary Medicine, Qingdao Agricultural University, Qingdao, People's Republic of China;

²Institute of Plant Protection, Qingdao Academy of Agricultural Sciences, Qingdao, People's Republic of China

Background: The concurrence of *mcr* and carbapenemase genes among *Enterobacteriaceae* has been a great clinical concern. In our study, we aimed to investigate the prevalence of *mcr*-positive carbapenem-resistant *Enterobacteriaceae* (CRE) in fresh vegetables and shed light on the possibility of transmission of *mcr*-positive CRE via fresh vegetables.

Methods: In this study, 712 fresh vegetable samples from 10 provinces in China were collected between May 2017 and Dec 2018 and were screened for *mcr* and carbapenemase genes. Antibiotic susceptibilities for isolates co-harboring carbapenemase genes and *mcr* were determined by an agar dilution or a broth microdilution method. Pulsed-field gel electrophoresis (PFGE) and multilocus sequence typing (MLST) analysis were also performed. Transferability of the carbapenemase/*mcr*-bearing plasmids was determined by conjugation, replicon typing and S1-PFGE-Southern blotting. The sequences of these plasmids were analyzed by using whole-genome sequencing with Illumina HiSeq platform.

Results: Two *E. coli* isolates concomitantly carrying *mcr-1* and *bla*_{NDM-5/9} from leaf rape and spinach, respectively, were found and both isolates showed multidrug resistance. Notably, *mcr-1*-positive 690 harboring *bla*_{NDM-5} and 701 carrying *bla*_{NDM-9} belonged to ST156 and ST2847, respectively, similar to the prevalent MLST types of *E. coli* co-carrying *mcr-1* and *bla*_{NDM} from avian in our previous study. *mcr-1* was on ~33-kb IncX4 plasmid or ~60-kb IncI2 plasmid, while *bla*_{NDM-5/9} was on ~46-kb IncX3 plasmid or ~120-kb untypable plasmid. The plasmids were highly similar to those from animals and clinical patients reported in various countries.

Conclusion: *E. coli* isolates concomitantly carrying *mcr-1* and *bla*_{NDM-5/9} in fresh vegetables may serve as a direct source of pathogens in humans, and such discovery in fresh vegetables emphasizes the importance of prompt surveillance and intervention in limiting the spread of *E. coli* co-carrying *bla*_{NDM} and *mcr-1*. To our knowledge, this is the first report of *Enterobacteriaceae* co-carrying *bla*_{NDM} and *mcr-1* in fresh vegetables.

Keywords: carbapenem resistance, colistin resistance, coexistence, plasmids, *Enterobacteriaceae*

Introduction

With the increasing carbapenem consumption in the past two decades, unprecedented global increase has been observed in the populations of carbapenem-resistant *Enterobacteriaceae* (CRE), posing colistin as the last therapeutic resort for the treatment of such organism. However, the efficacy of the drug has been challenged by the emergence of mobilized colistin resistance (*mcr*) genes.¹ Of great clinical concern is the concurrence of *mcr* and carbapenemase genes among *Enterobacteriaceae*. In fact, *mcr* has been found in CRE isolates from food animals² and humans,³⁻⁶ around the world, especially in China. High prevalence of *mcr*-positive CRE isolates has been found among

Correspondence: Bao-Tao Liu
Department of Basic Veterinary Medicine,
College of Veterinary Medicine, Qingdao
Agricultural University, Qingdao 266109,
People's Republic of China
Tel +86 1 346 587 7602
Email liubaotao-1986@163.com

Feng-Jing Song
Institute of Plant Protection, Qingdao
Academy of Agricultural Sciences,
Qingdao, People's Republic of China
Email lcsfj1130@163.com

various origins in China, including humans,^{3,7-9} retail meat,¹⁰ food animals,^{11,12} dogs,¹¹ birds and flies.¹¹

Consumption of fresh vegetables has increased over the recent years, because vegetables can provide essential components for humans.¹³ However, fresh vegetables eaten raw have been linked with outbreak of foodborne diseases¹⁴ and have often served as resistance gene “reservoirs”. For example, cephalosporin-resistant *Enterobacteriaceae* were found on 5.2% of the 1216 vegetables obtained from Dutch stores during 2012 and 2013.¹⁵ About 25.4% of the 169 vegetables imported from the Dominican Republic, India, Thailand and Vietnam in 2014 harbored one or more extended-spectrum-beta-lactamase-producing *Enterobacteriaceae*.¹⁶

Recently, *mcr*-positive isolates have been also found in one lettuce sample in Portugal¹⁷ and in two imported vegetable samples in Switzerland.¹⁸ In China, 9 of the 916 vegetables (0.98%) sampled in Guangzhou carried *mcr-1*,¹⁹ while 19 of the 528 fresh vegetables (3.60%) were found to harbor *mcr-1*-positive isolates in our previous report.²⁰ In addition, CRE isolates were also sporadically found in fresh vegetables, including one *bla*_{OXA-181}-positive *Klebsiella variicola* in Switzerland,²¹ three *bla*_{OXA-181}-positive *K. pneumoniae* in Algeria,²² one *E. coli* co-harboring *bla*_{NDM-1} and *bla*_{KPC-2} in China²³ and twelve isolates in our previous study in China.²⁴ All these findings suggest that *Enterobacteriaceae*-producing carbapenemases and *mcr*-positive isolates might have emerged and distributed in fresh vegetables around the world, especially in China. However, isolates co-harboring *mcr* and carbapenemases-encoding genes have not been isolated in those previous studies. Considering the widespread of *mcr* and carbapenemases-encoding genes in China, it is crucial to identify *mcr*-positive CRE in fresh vegetables. In this study, we identified two *mcr*-positive *E. coli* strains producing NDM-5/9, recovered from fresh vegetables in China and the characteristics of resistance plasmids were also analyzed.

Materials and methods

Samples and identification of *Enterobacteriaceae* co-harboring *mcr* and carbapenemases genes

Seventeen different types of fresh vegetables from 72 supermarkets and farmer's markets in 29 cities or districts of 10 provinces (Shandong, Shanghai, Beijing, Hubei, Henan, Heilongjiang, Yunnan, Tianjin, Shanxi and Jiangsu) in China were purchased between May 2017 and December 2018. In total, 712 fresh vegetable samples were collected, including

cucumber (n=125), tomato (n=114), romaine lettuce (n=76), curly endive (n=53), green pepper (n=50), coriander (n=50), leaf rape (n=49), spinach (n=49), mungbean sprouts (n=35), chili pepper (n=28), leaf lettuce (n=20), soybean sprouts (n=18), pakchoi (n=17), garland chrysanthemum (n=10), carrot (n=9), green shallots (n=5) and eggplant (n=4). These samples were processed with Mueller-Hinton (MH) broth co-harboring vancomycin (8 mg/L), colistin (2 mg/L) and meropenem (1.0 mg/L) to select *Enterobacteriaceae* carrying both carbapenemase and *mcr* genes using the similar protocol as previously reported.²⁰ The MH broth with survived bacteria was diluted in series of 1:10 and 100 μ L appropriate dilution was spread onto MH agar plates supplemented with both colistin (2 mg/L) and meropenem (1.0 mg/L). Presumptive *Enterobacteriaceae* colonies on the MH plate were selected for screening the carbapenemase-encoding genes (*bla*_{NDM}, *bla*_{KPC}, *bla*_{IMP}, *bla*_{VIM}, *bla*_{SPM}, *bla*_{AIM}, *bla*_{DIM}, *bla*_{GIM}, *bla*_{SIM}, *bla*_{BIC} and *bla*_{OXA-48}) using primers previously described,²⁵ and the presence of *mcr* (*mcr-1*, *mcr-2*, *mcr-3*, *mcr-4*, *mcr-5*, *mcr-6*, *mcr-7* and *mcr-8*) was also determined.¹ *mcr*-positive isolates carrying carbapenemase-encoding genes were identified by *rpoB* sequence analysis.²⁶

Antimicrobial susceptibility testing

Susceptibility testing to 17 antimicrobial agents was determined by the agar dilution method according to the Clinical and Laboratory Standards Institute (CLSI) criteria.²⁷ The 17 antimicrobial agents were the following ones: cefotaxime, ceftiofur, meropenem, ampicillin, enrofloxacin, ciprofloxacin, levofloxacin, nalidixic acid, streptomycin, amikacin, gentamicin, kanamycin, doxycycline, tetracycline, tigecycline, florfenicol and fosfomycin. The results except tigecycline were interpreted according to the CLSI breakpoints.²⁷ The MIC method for colistin and breakpoints for colistin and tigecycline were recommended by the 2017 EUCAST (http://www.eucast.org/clinical_breakpoints/).

MLST and PFGE typing

MLST²⁸ of the *E. coli* isolates carrying both carbapenemase and *mcr* genes in this study was performed to compare with such isolates of other origins. Clonal relationships of isolates in this study and those with the same MLST types from other sources were also investigated by PFGE using *Xba*I enzyme as previously described.²⁹ The *Xba*I-digested DNA of *Salmonella Braenderup* strain H9812 was used as a reference.

Plasmid conjugation and incompatibility typing

Plasmid conjugation experiment was performed between the *mcr*-positive isolates carrying carbapenemase and streptomycin-resistant recipient *E. coli* C600 using the broth-mating method.³⁰ *mcr*-positive transconjugants were selected on eosin methylene blue agar plates containing both streptomycin (2000 mg/L) and colistin (2 mg/L), while MacConkey agar plates supplemented with both streptomycin (2000 mg/L) and meropenem (0.8 mg/L) were used to select transconjugants with carbapenemase genes. Antimicrobial susceptibility testing and PCRs mentioned above were subsequently performed to confirm the transconjugants, followed by Enterobacterial repetitive intergenic consensus PCR as previously described.³¹ Incompatibility (Inc) groups of plasmids within the transconjugants were assigned by the PCR-based replicon typing method,³² and the IncX and IncI2 replicons were also detected as previously described.^{33,34}

Plasmid analysis

To analyze the location of *mcr* and carbapenemase genes, S1 nuclease-PFGE and Southern Hybridization blot were performed twice on the transconjugants and their donors. *E. coli* C600 lacking plasmid was used to confirm the specific for *mcr* or carbapenemase gene probe. To determine whether the *bla*_{NDM}/*mcr*-1-bearing plasmids in this study were similar to the reported plasmids of other origins, total genomic DNA (including the chromosome and corresponding plasmid) from the two *mcr*-1-positive transconjugants and two *bla*_{NDM}-positive transconjugants were extracted and sequenced using Illumina HiSeq PE150, respectively. After assembling the sequence reads and cleaning out *E. coli* C600 chromosomal DNA sequences, plasmid contigs were obtained. All plasmids in this study were then subjected to PlasmidFinder 2.0 (<https://cge.cbs.dtu.dk/services/PlasmidFinder/>) and ResFinder 3.1 (<https://cge.cbs.dtu.dk/services/ResFinder/>) to analyze the plasmid replicons and antimicrobial resistance genes, respectively. Functional annotation was performed using the NCBI Prokaryotic Genome Annotation Pipeline server, and the BLASTn implemented in software BRIG was used for sequence comparison.³⁵

Results and discussion

Among the 712 fresh vegetable samples collected in 10 provinces from China in this study, two isolates from leaf

rape and spinach in two supermarkets of Shandong province, respectively, carried both *bla*_{NDM-5/9} and *mcr*-1. To our knowledge, this is the first report of isolates co-carrying *bla*_{NDM-5/9} and *mcr*-1 in fresh vegetables.

The two isolates were designated 690 and 701 here. *rpoB* sequence analysis showed that both isolates were *E. coli*. Isolate 690 concomitantly harbored *bla*_{NDM-5} and *mcr*-1, while 701 carried both *bla*_{NDM-9} and *mcr*-1 (Table 1). Both isolates showed resistance to all beta-lactams, tetracyclines, fluoroquinolones, fosfomycin and colistin tested, which were therapeutic agents in clinics in many countries.³⁶ Notably, both isolates remained susceptible to amikacin and tigecycline, similar to the *E. coli* isolates producing both NDM and MCR-1 from humans in China.⁹

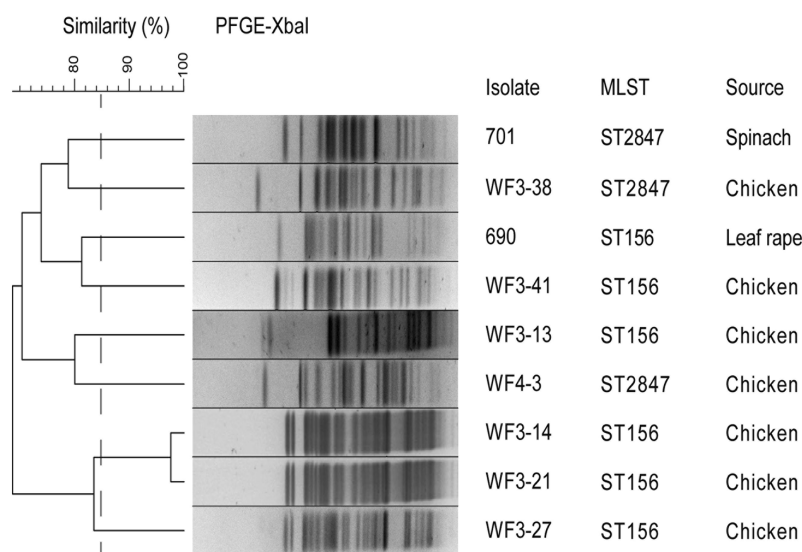
MLST analysis showed that isolates 690 and 701 belonged to ST156 and ST2847, respectively. ST156 and ST2847 were also found in *E. coli* isolates co-carrying *mcr*-1 and *bla*_{NDM} from avian in Shandong of China in 2015, in our previous study,¹² suggesting that the MCR-1-NDM producers in vegetables might have originated from animals because vegetables might be fertilized with manure and wastewater from livestock. In fact, the final effluent applied to farmland in the piggery wastewater treatment system in China has been proved to contain considerable amounts of *bla*_{NDM} and *mcr*-1,³⁷ which will further support our hypothesis. We then performed PFGE using *Xba*I enzyme to compare the clonal relationships of isolates in this study and isolates of ST156 and ST2847 types from avian we previously reported.¹² The results showed that the ST156 isolate 690 was different from the five ST156 isolates from avian, and isolate 701 of ST2847 was also different from the two ST2847 isolates of avian origin (Figure 1), suggesting that such isolates in vegetables in this study were not directly derived from the avian feces we studied previously and more *E. coli* isolates co-carrying *mcr*-1 and *bla*_{NDM} from other animal farms should be investigated in the future. Notably, ST156 type of *E. coli* isolates producing NDM-5 and isolate carrying *mcr*-1 were also found in human from China in 2016⁹ and human from Brazil in 2016,³⁸ respectively, while ST2847 *E. coli* isolated from a patient in Hong Kong in 2004 was found to carry both *bla*_{CTX-M-65} and *fosA3*.³⁹ Thus, the presence of ST156 and ST2847 *E. coli* isolates producing both NDM-5/9 and MCR-1 in vegetables still represents a threat to human health.

For both isolates, we obtained two different transconjugants harboring *mcr*-1 or *bla*_{NDM-5/9}, respectively (Table 1). *fosA3*, conferring resistance to fosfomycin, was found in

Table I Characteristics of the two *E. coli* strains 690 and 701 from fresh vegetables and their transconjugants with plasmids either harboring *mcr-1* or *bla_{NDM-5/9}*

Strain	Resistance genes	MICs (μg/mL)				Resistance profiles	Plasmids carrying <i>mcr-1</i> or <i>bla_{NDM}</i> in transconjugants	
		COL	MEM	CTX	FOS		Replicon type	Size (kb)
690	<i>mcr-1</i> , <i>bla_{NDM-5}</i> , <i>fosA3</i>	16	>16	>64	>256	MEM, CTF, CTX, AMP, COL, DOX, TET, GEN, KAN, NAL, ENR, LEV, CIP, FOS, FFL		
COL690	<i>mcr-1</i>	16	0.125	0.031	4	COL, STR	X4	~33
MER690	<i>bla_{NDM-5}</i>	0.25	>16	>64	8	MEM, CTF, CTX, AMP, STR	X3	~46
701	<i>mcr-1</i> , <i>bla_{NDM-9}</i> , <i>fosA3</i>	16	>16	>64	>256	MEM, CTF, CTX, AMP, COL, DOX, TET, GEN, STR, NAL, ENR, LEV, CIP, FOS, FFL		
COL701	<i>mcr-1</i>	16	0.125	0.031	4	COL, STR	I2	~60
MER701	<i>bla_{NDM-9}</i> , <i>fosA3</i>	0.25	>16	>64	>256	MEM, CTF, CTX, AMP, FOS, STR,	UT	~110
C600		0.25	0.125	0.031	4	STR		

Abbreviations: AMP, ampicillin; CTX, cefotaxime; CTF, ceftiofur; MEM, meropenem; COL, colistin; GEN, gentamicin; KAN, kanamycin; STR, streptomycin; CIP, ciprofloxacin; LEV, levofloxacin; ENR, enrofloxacin; NAL, nalidixic acid; DOX, doxycycline; TET, tetracycline; FFL, florfenicol; FOS, fosfomycin; UT, untypable; C600, recipient strain in the conjugation experiment.

**Figure 1** XbaI-PFGE patterns of isolates in this study and isolates of ST156 and ST2847 types co-carrying *mcr-1* and *bla_{NDM}* from avian.

bla_{NDM-9}-positive transconjugants MER701 using primers described previously,⁴⁰ while no other resistances were co-transferred with colistin/meropenem resistance in the other 3 transconjugants. Interestingly, there were additional bigger bands marked with arrows in all 4 transconjugants and some donors in the S1-PFGE (Figure 2A and C) and these bands could be also hybridized with the corresponding *mcr-1*/

bla_{NDM} probe (Figure 2B and D), although these experiments were performed several times. These bigger bands were the portion of the *mcr-1/bla_{NDM-5/9}*-carrying plasmids not exposed to S1 nuclease in the S1-PFGE experiment, according to the findings of the previous study in which the S1-PFGE method was established.⁴¹ Thus, all the 4 transconjugants carried only one plasmid and *mcr-1* was located on

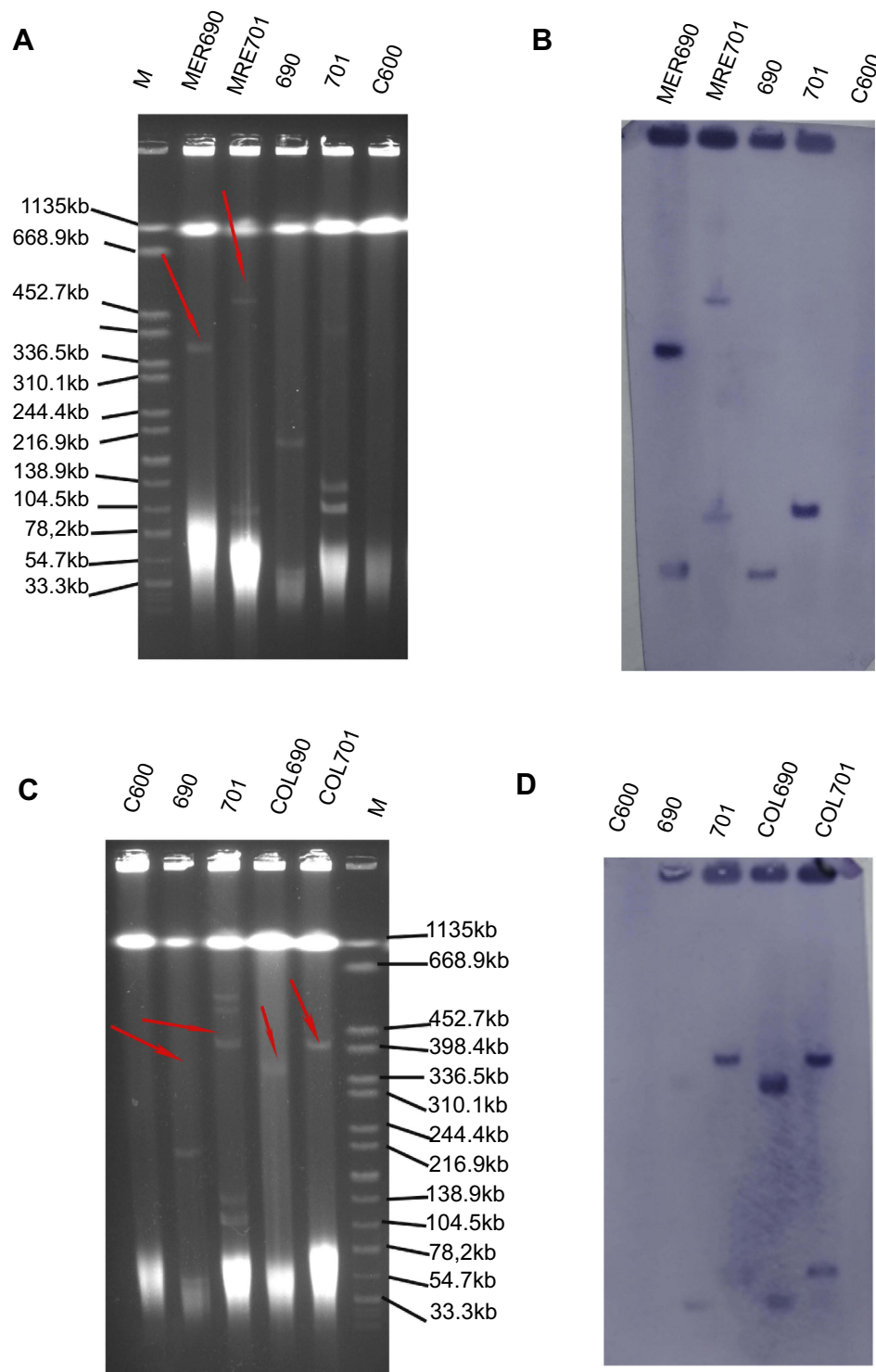


Figure 2 Analysis of the location of *mcr-I/bla*_{NDM} among transconjugants and their donors. **(A)** S1 nuclease-PFGE of transconjugants and their donors carrying *bla*_{NDM}. **(B)** Southern blot hybridization with the *bla*_{NDM} probe. **(C)** S1 nuclease-PFGE of transconjugants and their donors carrying *mcr-I*. **(D)** Southern blot hybridization with the *mcr-I* probe. Lane M: chromosomal DNA of *Salmonella enterica* serotype Braenderup H9812 digested with *Xba*I serving as size markers. The red arrows indicate bigger bands, which are portion of the *mcr-I/bla*_{NDM-5/9}-carrying plasmids not exposed to S1 nuclease in the S1-PFGE experiment.

IncX4 type plasmid of ~33 kb in COL690, while COL701 carried IncI2 type ~60 kb plasmid harboring *mcr-I* (Figure 2C and D). *bla*_{NDM-5} was on IncX3 plasmid of ~40 kb in transconjugant MER690, however, *bla*_{NDM-9} and *fosA3*

were on the same ~110 kb plasmid which was untappable in MER701 (Figure 2A and B and Table 1).

From the results of plasmid sequences, both *mcr-I*-bearing plasmids did not carry any other antibiotic resistance

gene besides *mcr-1* and this could account for the resistance phenotypes of transconjugants COL690 and COL701 (Table 1). Comparison of the pCOL690T (accession no. VMKQ000000000) to several previously reported ~33-kb IncX4 plasmids showed that it aligned very well to pCSZ4

(KX711706) (100% in coverage and 99% in identity) from *E. coli* of pork origin in China (Figure 3A). Notably, pCOL690T (VMKQ000000000) from leaf rape in this study was also highly similar to plasmids pKP15450-MCR-1 (MH715959) from clinical *Klebsiella pneumoniae*

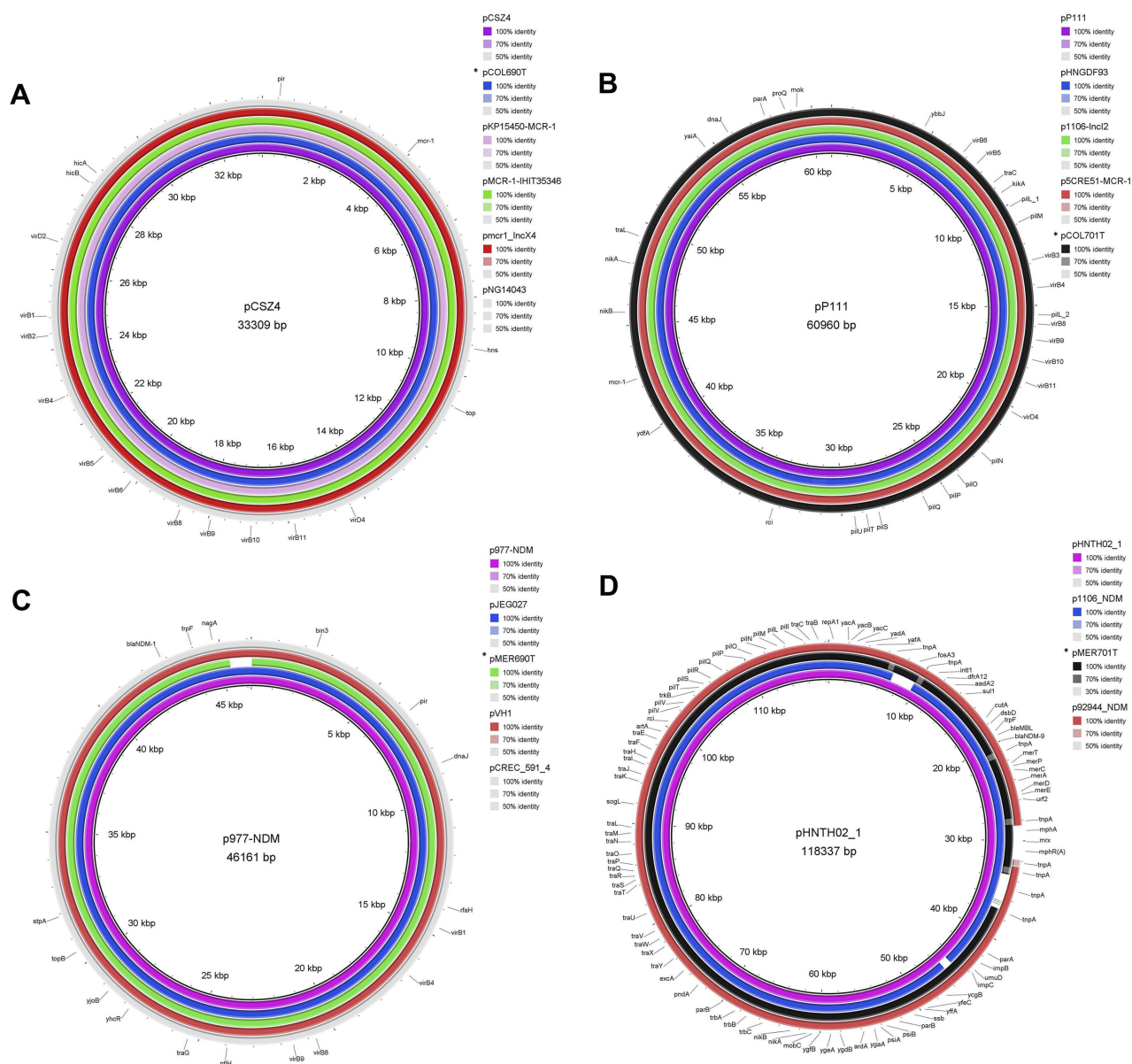


Figure 3 Sequence alignment of *bla*_{NDM}-bearing or *mcr-1*-bearing plasmids. (A) The plasmid pCSZ4 (KX711706) (purple ring) from *E. coli* of pork origin in China was used as a reference to compare with the IncX4 plasmids. The ring light purple, green, red, gray and blue rings represent pKP15450-MCR-1 (MH715959) from clinical *K. pneumoniae* in Taiwan, pMCR-1-IHIT35346 (KX894453) from *E. coli* of pig origin in Germany, pmcr1_IncX4 (KU761327) from clinical *K. pneumoniae* in China, pNG14043 (KY120364) from clinical *S. typhimurium* in Taiwan and pCOL60T (VMKQ000000000) in this study, respectively. The outer circle with black arrows signifies annotation of the reference sequence. (B) The plasmid pP111 (KY120365) from *S. typhimurium* of pig in Taiwan was used as a reference to compare with the IncI2 plasmids. The purple, blue, green, red and black rings represents the reference plasmid, pHNGDF93 (MF978388) from fish *E. coli* in China, p1106-Incl2 (MG825374) from *E. coli* of chicken in China, pSCRE51-MCR-1 (CP021176) from clinical *E. coli* in Taiwan, and pCOL701T (VMKR000000000) in this study, respectively. The outer circle with black arrows signifies annotation of the reference sequence. (C) The plasmid p977-NDM (MG825382) from *E. coli* of pork origin in China was used as a reference to compare with the IncX3 plasmids. The purple, blue, green, red and gray rings represent the reference plasmid, pJEG027 (KM400601) from clinical *K. pneumoniae* in Australia, pMER690T (VMKS000000000) from leaf rape in this study, pVH1 (CP028705) from *E. coli* of cucumber in China, and pCREC-591_4 (CP024825) from clinical *E. coli* in South Korea, respectively. The outer circle with black arrows signifies annotation of the reference sequence. (D) The plasmid pHNT02-1 (MG196294) from *E. coli* of retail meat in China was used as a reference (purple ring). The blue, dark, and red rings represent plasmid p1106-NDM (MG825375) from *E. coli* of chicken, pMER701T (VMKT000000000) in this study, and p2944-NDM (MG838206) from clinical *E. coli*, respectively, in China. The outer circle with black arrows signifies annotation of the reference sequence. *represents plasmids in this study.

in Taiwan, pmer1_IncX4 (KU761327) from clinical *K. pneumoniae* in China and pNG14043 (KY120364) from clinical *Salmonella Typhimurium* in Taiwan (Figure 3A). Furthermore, the pCOL690T in the *mcr-1*-positive NDM-5-producing *E. coli* in this study also showed high similarity to plasmid pMCR-1-IHIT35346 (KX894453) from *E. coli* co-producing OXA-181-carbapenemase and *mcr-1* of pig origin in Germany. Plasmid pCOL701T (VMKR00000000) from *E. coli* of spinach in this study aligned well to IncI2 plasmid pP111 (KY120365) (99% in coverage and 100% in identity) from *S. Typhimurium* of pig in Taiwan, pHNGDF93 (MF978388) from fish *E. coli* and p1106-IncI2 (MG825374) from *E. coli* of chicken in China (Figure 3B). Notably, the pCOL701T (VMKR00000000) in NDM-9-producing *E. coli* in this study was also highly similar to p5CRE51-MCR-1 (CP021176) from clinical *E. coli* co-producing NDM-9 and MCR-1 in Taiwan. All these findings suggested that highly similar IncX4 or IncI2 plasmids have disseminated *mcr-1* among different *Enterobacteriaceae* species in food and animals around the world, and these plasmids can spread to carbapenemase-producing clinical isolates to threaten human health.

The pMER690T plasmid (VMKS00000000) did not carry any other resistance gene besides *bla*_{NDM-5} (Figure 3C), and it aligned very well to IncX3 plasmid p977-NDM (MG825382) (100% in coverage and 99% in identity) from *E. coli* of pork origin in China (Figure 3C). Notably, the pMER690T from leaf rape in this study was also highly similar to plasmids pCREC-591_4 (CP024825) from clinical *E. coli* in South Korea, and pJEG027 (KM400601) from clinical *K. pneumoniae* in Australia. Interestingly, the pMER690T (VMKS00000000) in the *mcr-1*-positive *E. coli* producing NDM-5 in this study also showed high similarity to plasmid pVH1 (CP028705) from *E. coli* of cucumber in China in our previous report,²⁴ in which only CRE isolates were isolated. Replicon untypable plasmid pMER701T (VMKT00000000) carried resistance genes *fosA3*, *dfiA12*, *aadA2*, *sul1*, *ble*_{MBL}, *bla*_{NDM-9}, and *mph(A)*, which were all centralized in the multidrug resistance region (Figure 3D). pMER701T (VMKT00000000) in this study aligned well to plasmids pHNTH02-1 (MG196294) (100% in coverage and 99% in identity), p1106-NDM (MG825375) and p92944-NDM (MG838206), and these three plasmids were from *E. coli* of retail meat, chicken and human, respectively, in China (Figure 3D). These findings suggested that pMER690T- and pMER701T-like plasmids have been disseminated among different *Enterobacteriaceae* species of various origins around the world, especially China.

Conclusion

In summary, we reported for the first time two clonally unrelated *E. coli* harboring both *bla*_{NDM-5/9} and *mcr-1* in fresh vegetables in China. The dissemination of *mcr-1* was mediated by IncX4 or IncI2 plasmid, while *bla*_{NDM-5/9} was on IncX3 or untypable plasmid. All the plasmids in this study were highly similar to the plasmids from animals and clinical isolates in various countries. The emergence of *mcr-1*-positive bacteria producing NDM in fresh vegetables is alarming and constitutes a food safety issue. Further investigations are required for monitoring such organisms in fresh vegetables to ensure food safety in China and other countries.

Acknowledgments

This study was supported by the National Natural Science Foundation of China (number 31502122), the Scientific and Technological Projects of Qingdao (19-6-1-94-nsh) and the Advanced Talents Foundation of Qingdao Agricultural University (number 663/1115014).

Disclosure

The authors report no conflicts of interest in this work.

References

- Wang X, Wang Y, Zhou Y, et al. Emergence of a novel mobile colistin resistance gene, *mcr-8*, in NDM-producing *Klebsiella pneumoniae*. *Emerg Microbes Infect.* 2018;7(1):122. doi:10.1038/s41426-018-0124-z
- Roschanski N, Fischer J, Falgenhauer L, et al. Retrospective analysis of bacterial cultures sampled in german chicken-fattening farms during the years 2011-2012 revealed additional VIM-1 carbapenemase-producing *Escherichia coli* and a serologically rough *Salmonella enterica* serovar infantis. *Front Microbiol.* 2018;9:538. doi:10.3389/fmicb.2018.00538
- Li Y, Sun QL, Shen Y, et al. Rapid increase in prevalence of carbapenem-resistant *Enterobacteriaceae* (CRE) and emergence of colistin resistance gene *mcr-1* in CRE in a hospital in Henan, China. *J Clin Microbiol.* 2018;56(4). doi:10.1128/JCM.01932-17
- Huang TD, Bogaerts P, Berhin C, et al. Increasing proportion of carbapenemase-producing *Enterobacteriaceae* and emergence of a MCR-1 producer through a multicentric study among hospital-based and private laboratories in Belgium from September to November 2015. *Euro Surveill.* 2017;22(19):30530. doi:10.2807/1560-7917.ES.2017.22.19.30530
- Mediavilla JR, Patrawalla A, Chen L, et al. Colistin- and carbapenem-resistant *Escherichia coli* harboring *mcr-1* and *bla*_{NDM-5}, causing a complicated urinary tract infection in a patient from the United States. *MBio.* 2016;7(4). doi:10.1128/mBio.01191-16
- Delgado-Blas JF, Ovejero CM, Abadia-Patino L, Gonzalez-Zorn B. Coexistence of *mcr-1* and *bla*_{NDM-1} in *Escherichia coli* from Venezuela. *Antimicrob Agents Chemother.* 2016;60(10):6356-6358. doi:10.1128/AAC.01319-16
- Feng S, Shen C, Chen H, et al. Co-production of MCR-1 and NDM-5 in *Escherichia coli* isolated from a colonization case of inpatient. *Infect Drug Resist.* 2018;11:1157-1161. doi:10.2147/IDR.S171164

8. Quan J, Li X, Chen Y, et al. Prevalence of *mcr-1* in *Escherichia coli* and *Klebsiella pneumoniae* recovered from bloodstream infections in China: a multicentre longitudinal study. *Lancet Infect Dis*. 2017;17(4):400–410. doi:10.1016/S1473-3099(16)30528-X
9. Shen Z, Hu Y, Sun Q, et al. Emerging carriage of NDM-5 and MCR-1 in *Escherichia coli* from healthy people in multiple regions in China: a cross sectional observational study. *EClinicalMedicine*. 2018;6:11–20. doi:10.1016/j.eclinm.2018.11.003
10. Yao X, Doi Y, Zeng L, Lv L, Liu JH. Carbapenem-resistant and colistin-resistant *Escherichia coli* co-producing NDM-9 and MCR-1. *Lancet Infect Dis*. 2016;16:288–289. doi:10.1016/S1473-3099(16)00057-8
11. Wang Y, Zhang R, Li J, et al. Comprehensive resistome analysis reveals the prevalence of NDM and MCR-1 in Chinese poultry production. *Nat Microbiol*. 2017;2:16260. doi:10.1038/nmicrobiol.2016.251
12. Liu BT, Song FJ, Zou M, Zhang QD, Shan H. High incidence of *Escherichia coli* strains coharboring *mcr-1* and *bla_{NDM}* from chickens. *Antimicrob Agents Chemother*. 2017;61(3). doi:10.1128/AAC.02347-16
13. Callejon RM, Rodriguez-Naranjo MI, Ubeda C, Hornedo-Ortega R, Garcia-Parrilla MC, Troncoso AM. Reported foodborne outbreaks due to fresh produce in the United States and European Union: trends and causes. *Foodborne Pathog Dis*. 2015;12(1):32–38. doi:10.1089/fpd.2014.1821
14. Jung Y, Jang H, Matthews KR. Effect of the food production chain from farm practices to vegetable processing on outbreak incidence. *Microb Biotechnol*. 2014;7(6):517–527. doi:10.1111/1751-7915.12178
15. van Hoek AH, Veenman C, van Overbeek WM, Lynch G, de Roda Husman AM, Blaak H. Prevalence and characterization of ESBL- and AmpC-producing Enterobacteriaceae on retail vegetables. *Int J Food Microbiol*. 2015;204:1–8. doi:10.1016/j.ijfoodmicro.2015.03.014
16. Zurfluh K, Nuesch-Inderbinen M, Morach M, Zihler Berner A, Hachler H, Stephan R. Extended-spectrum-beta-lactamase-producing Enterobacteriaceae isolated from vegetables imported from the Dominican Republic, India, Thailand, and Vietnam. *Appl Environ Microbiol*. 2015;81(9):3115–3120. doi:10.1128/AEM.00258-15
17. Jones-Dias D, Manageiro V, Ferreira E, et al. Architecture of class 1, 2, and 3 integrons from gram negative bacteria recovered among fruits and vegetables. *Front Microbiol*. 2016;7:1400. doi:10.3389/fmicb.2016.01400
18. Zurfluh K, Poirel L, Nordmann P, Nuesch-Inderbinen M, Hachler H, Stephan R. Occurrence of the plasmid-borne *mcr-1* colistin resistance gene in extended-spectrum-beta-lactamase-producing enterobacteriaceae in river water and imported vegetable samples in Switzerland. *Antimicrob Agents Chemother*. 2016;60(4):2594–2595. doi:10.1128/AAC.00066-16
19. Luo J, Yao X, Lv L, et al. Emergence of *mcr-1* in *Raoultella ornithinolytica* and *Escherichia coli* isolates from retail vegetables in China. *Antimicrob Agents Chemother*. 2017;61(10). doi:10.1128/AAC.01139-17
20. Liu BT, Li X, Zhang Q, Shan H, Zou M, Song FJ. Colistin-resistant *mcr*-positive enterobacteriaceae in fresh vegetables, an increasing infectious threat in China. *Int J Antimicrob Agents*. 2019;54(1):89–94. doi:10.1016/j.ijantimicag.2019.04.013
21. Zurfluh K, Poirel L, Nordmann P, Klumpp J, Stephan R. First detection of *Klebsiella variicola* producing OXA-181 carbapenemase in fresh vegetable imported from Asia to Switzerland. *Antimicrob Resist Infect Control*. 2015;4:38. doi:10.1186/s13756-015-0080-5
22. Touati A, Mairi A, Baloul Y, et al. First detection of *Klebsiella pneumoniae* producing OXA-48 in fresh vegetables from Bejaia city, Algeria. *J Glob Antimicrob Resist*. 2017;9:17–18. doi:10.1016/j.jgar.2017.02.006
23. Wang J, Yao X, Luo J, Lv L, Zeng Z, Liu JH. Emergence of *Escherichia coli* co-producing NDM-1 and KPC-2 carbapenemases from a retail vegetable, China. *J Antimicrob Chemother*. 2018;73(1):252–254. doi:10.1093/jac/dkx335
24. Liu BT, Zhang XY, Wan SW, Hao JJ, Jiang RD, Song FJ. Characteristics of carbapenem-resistant enterobacteriaceae in ready-to-eat vegetables in China. *Front Microbiol*. 2018;9:1147. doi:10.3389/fmicb.2018.01147
25. Poirel L, Walsh TR, Cuvillier V, Nordmann P. Multiplex PCR for detection of acquired carbapenemase genes. *Diagn Microbiol Infect Dis*. 2011;70(1):119–123. doi:10.1016/j.diagmicrobio.2010.12.002
26. Mollet C, Drancourt M, Raoult D. *rpoB* sequence analysis as a novel basis for bacterial identification. *Mol Microbiol*. 1997;26(5):1005–1011. doi:10.1046/j.1365-2958.1997.6382009.x
27. CLSI. Performance standards for antimicrobial susceptibility testing; twenty-fifth informational supplement. CLSI document M100-S25. Wayne (PA): Clinical and Laboratory Standards Institute; 2015.
28. Wirth T, Falush D, Lan R, et al. Sex and virulence in *Escherichia coli*: an evolutionary perspective. *Mol Microbiol*. 2006;60(5):1136–1151. doi:10.1111/j.1365-2958.2006.05172.x
29. Saenz Y, Brinas L, Dominguez E, et al. Mechanisms of resistance in multiple-antibiotic-resistant *Escherichia coli* strains of human, animal, and food origins. *Antimicrob Agents Chemother*. 2004;48(10):3996–4001. doi:10.1128/AAC.48.10.3996-4001.2004
30. Chen L, Chen ZL, Liu JH, Zeng ZL, Ma JY, Jiang HX. Emergence of RmtB methylase-producing *Escherichia coli* and *Enterobacter cloacae* isolates from pigs in China. *J Antimicrob Chemother*. 2007;59(5):880–885. doi:10.1093/jac/dkm065
31. Versalovic J, Koeuth T, Lupski JR. Distribution of repetitive DNA-sequences in eubacteria and application to fingerprinting of bacterial genomes. *Nucleic Acids Res*. 1991;19(24):6823–6831. doi:10.1093/nar/19.24.6823
32. Carattoli A, Bertini A, Villa L, Falbo V, Hopkins KL, Threlfall EJ. Identification of plasmids by PCR-based replicon typing. *J Microbiol Methods*. 2005;63(3):219–228. doi:10.1016/j.mimet.2005.03.018
33. Johnson TJ, Bielak EM, Fortini D, et al. Expansion of the IncX plasmid family for improved identification and typing of novel plasmids in drug-resistant Enterobacteriaceae. *Plasmid*. 2012;68(1):43–50. doi:10.1016/j.plasmid.2012.03.001
34. Chen L, Chavda KD, Al Laham N, et al. Complete nucleotide sequence of a *bla_{KPC}*-harboring IncI2 plasmid and its dissemination in New Jersey and New York hospitals. *Antimicrob Agents Chemother*. 2013;57(10):5019–5025. doi:10.1128/AAC.01397-13
35. Alikhan NF, Petty NK, Ben Zakour NL, Beatson SA. BLAST Ring Image Generator (BRIG): simple prokaryote genome comparisons. *BMC Genomics*. 2011;12:402. doi:10.1186/1471-2164-12-402
36. Falagas ME, Kastoris AC, Kapaskelis AM, Karageorgopoulos DE. Fosfomycin for the treatment of multidrug-resistant, including extended-spectrum beta-lactamase producing, Enterobacteriaceae infections: a systematic review. *Lancet Infect Dis*. 2010;10(1):43–50. doi:10.1016/S1473-3099(09)70325-1
37. Yang F, Gu Y, Zhou J, Zhang K. Swine waste: a reservoir of high-risk *bla_{NDM}* and *mcr-1*. *Sci Total Environ*. 2019;683:308–316. doi:10.1016/j.scitotenv.2019.05.251
38. Rossi F, Girardello R, Morais C, et al. Plasmid-mediated *mcr-1* in carbapenem-susceptible *Escherichia coli* ST156 causing a blood infection: an unnoticeable spread of colistin resistance in Brazil? *Clinics (Sao Paulo)*. 2017;72(10):642–644. doi:10.6061/clinics/2017(10)09
39. Ho PL, Chan J, Lo WU, et al. Prevalence and molecular epidemiology of plasmid-mediated fosfomycin resistance genes among blood and urinary *Escherichia coli* isolates. *J Med Microbiol*. 2013;62(Pt 11):1707–1713. doi:10.1099/jmm.0.062653-0
40. Hou J, Huang X, Deng Y, et al. Dissemination of the fosfomycin resistance gene *fosA3* with CTX-M beta-lactamase genes and *rmtB* carried on IncFII plasmids among *Escherichia coli* isolates from pets in China. *Antimicrob Agents Chemother*. 2012;56(4):2135–2138. doi:10.1128/AAC.05104-11
41. Barton BM, Harding GP, Zuccarelli AJ. A general method for detecting and sizing large plasmids. *Anal Biochem*. 1995;226(2):235–240. doi:10.1006/abio.1995.1220

Infection and Drug Resistance

Dovepress

Publish your work in this journal

Infection and Drug Resistance is an international, peer-reviewed open-access journal that focuses on the optimal treatment of infection (bacterial, fungal and viral) and the development and institution of preventive strategies to minimize the development and spread of resistance. The journal is specifically concerned with the epidemiology of

antibiotic resistance and the mechanisms of resistance development and diffusion in both hospitals and the community. The manuscript management system is completely online and includes a very quick and fair peer-review system, which is all easy to use. Visit <http://www.dovepress.com/testimonials.php> to read real quotes from published authors.

Submit your manuscript here: <http://www.dovepress.com/infection-and-drug-resistance-journal>