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Detection of the *mcr-1* Colistin Resistance Gene in Carbapenem-Resistant *Enterobacteriaceae* from Different Hospitals in China

Hua Yu,[a] Fen Qu,[b] Bin Shan,[c] Bin Huang,[d] Wei Jia,[e] Cha Chen,[f] Aiqing Li,[g] Minhui Miao,[g] Xin Zhang,[a] Chunmei Bao,[b] Yunmin Xu,[c] Kalyan D. Chavda,[h] Yi-Wei Tang,[i] Barry N. Kreiswirth,[h] Hong Du,[g] Liang Chen[h]

Sichuan Academy of Medical Science & Sichuan Provincial People's Hospital, Chengdu, Sichuan, China[a]; The Center of Clinical Diagnosis Laboratory, 302 Hospital of PLA, Beijing, China[b]; Department of Laboratory Medicine, The First Affiliated Hospital of Kunming Medical University, Kunming, Yunnan, China[c]; Department of Laboratory Medicine, The First Affiliated Hospital of Sun Yat-sen University, Guangzhou, Guangdong, China[d]; Center of Medical Laboratory, the General Hospital of Ningxia Medical University, Yinchuan, China[e]; Department of Laboratory Medicine, The Traditional Chinese Medical Hospital of Guangdong, Guangzhou, Guangdong, China[f]; Department of Clinical Laboratory, The Second Affiliated Hospital of Soochow University, Suzhou, Jiangsu, China[g]; Public Health Research Institute Tuberculosis Center, New Jersey Medical School, Rutgers University, Newark, New Jersey, USA[h]; Department of Laboratory Medicine, Memorial Sloan Kettering Cancer Center, New York, New York, USA[i]

The spread of the plasmid-mediated colistin resistance gene, *mcr-1*, into carbapenem-resistant *Enterobacteriaceae* (CRE) clinical isolates poses a significant threat to global health. Here we report the identification of three *mcr-1*-harboring carbapenem-resistant *Escherichia coli* strains, collected from three patients in two provinces in China. Our results show that *mcr-1*-harboring CRE strains have started to spread in different hospitals in China. In addition, this report presents the first description of chromosomal integration of *mcr-1* into a carbapenem-resistant *E. coli* strain.

Polymyxins B and E (also known as colistin) are among some antibiotics of the last resort used to treat serious infections caused by carbapenem-resistant *Enterobacteriaceae* (CRE). However, the recent discovery of a plasmid-mediated colistin resistance gene, *mcr-1*, sounded the alarm that last-resort antibiotics may be in jeopardy (1). Of particular concern is the spread of *mcr-1* into CRE, creating extensively drug-resistant isolates causing untreatable disease. We have recently reported the cooccurrence of NDM-5 carbapenemase and MCR-1 within the same clinical isolate from a tertiary hospital in eastern China (2). The isolates coproducing MCR-1 and NDM-5 were nonsusceptible to nearly all antimicrobial agents tested (2). It is worrisome that these MCR-1-producing CRE isolates may spread further into hospital settings and within high-risk patients, thereby causing untreatable infections. Here we conducted a molecular screening study for clinical CRE isolates collected from six tertiary hospitals in six provinces in order to explore the dissemination of MCR-1-producing CRE in China.

A total of 264 clinical CRE isolates were collected from six large regional hospitals in northern (Beijing), eastern (Suzhou), southern (Guangzhou), northwestern (Yinchuan), and southwestern (Chengdu and Kunming) China between January 2014 and December 2015. They were isolated from respiratory tract ($n = 119$), urine ($n = 50$), blood ($n = 38$), intra-abdominal ($n = 22$), skin and soft tissue ($n = 17$), rectal swab ($n = 9$), wound ($n = 5$), and other sites ($n = 4$) of 251 unique patients and included 160 *Klebsiella pneumoniae*, 36 *Escherichia coli*, 19 *Enterobacter cloacae*, 17 *E. aerogenes*, 11 *K. oxytoca*, 5 *Citrobacter freundii*, 4 *Serratia*, 3 *Morganella morganii*, 3 *Providencia rettgeri*, and 3 *K. ozaenae* spp. and 3 other species. Species identification was performed using matrix-assisted laser desorption ionization–time of flight mass spectrometry (Bruker Microflex LT). PCR detection of carbapenemase genes (bla_{KPC}, bla_{NDM}, bla_{VIM}, $bla_{OXA48\text{-}like}$, and bla_{IMP}) showed that 134 isolates were positive for bla_{KPC}, 69 for bla_{NDM}, 18 for bla_{IMP}, and 7 for bla_{VIM}, while 59 were negative for any carbapenemase gene. Thirty-one isolates were found to carry more than one carbapenemase. We then tested for the presence of *mcr-1* using a previously published PCR method (1) and identified a total of five *mcr-1*-harboring CRE isolates from four different patients in three different hospitals, including three carbapenem-resistant *E. coli* isolates and two previously reported sequence type 25 (ST25) *K. pneumoniae* isolates coproducing MCR-1 and NDM-5 (2, 3). In this report, the clinical and molecular characteristics of the three carbapenem-resistant *E. coli* isolates are described.

Patient 1 was a male in his late 40s with a medical history of high-level paraplegia and nephropyelitis over 20 years. The patient had had long-term urinary catheterization and had a history of recurring urinary tract infections, for which he was admitted to hospital A in Chengdu, Sichuan (southwestern China), in February 2015. On the first day of admission, an *E. coli* strain (CDA6) was isolated from a catheter-associated urine specimen. CDA6 was resistant to all β-lactams tested, except for aztreonam, and was resistant to ciprofloxacin, levofloxacin, moxifloxacin, piperacillin-tazobactam, co-trimoxazole, and colistin (Table 1).

Patient 2 was a female who was almost 50 years old who had rectal cancer with liver metastases. The patient underwent rectal

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Address correspondence to Hong Du, hong_du@126.com, or Liang Chen, Chen11@njms.rutgers.edu.

H.Y. and F.Q. contributed equally to this article.

TABLE 1 Characteristics of MCR-1-producing *E. coli* strains and their *E. coli* J53 transconjugants[a]

E. coli strain	ST	β-Lactamase(s)	Plasmid	MIC (μg/ml)[b]															
				SAM	AMC	CFZ	CAZ	CTX	FEP	ATM	IMP	MEM	AMK	GEN	CIP	LVX	MXF	TZP	SXT	TET	CHL	COL
CDA6	167	NDM-5, CMY-2, TEM-1	ND	>16/8	>16/8	>16	>16	>32	>16	≤2	>8	>8	≤8	<1	>2	>8	>4	>64/4	>2/38	≤2	8	>2
BJ10	156	NDM-5, TEM-1	ND	>16/8	>16/8	>16	>16	>32	>16	>16	>8	>8	≤8	>8	>2	>8	>4	>64/4	>2/38	>8	>16	>2
BJ13	457	CTX-M-14, CMY-2, TEM-1	ND	>16/8	>16/8	>16	>16	>32	>16	16	>8	4	≤8	>8	>2	>8	>4	>64/4	≤0.5/9	>8	8	>2
CDA6-mcr-T	ND	—	IncX4	≤4/2	≤8/4	≤4	≤1	≤1	≤2	≤2	≤1	≤1	≤8	≤2	≤0.5	≤1	≤1	≤4/2	≤0.5/9	≤2	≤4	>2
BJ13-mcr-T	ND	—	IncI2	≤4/2	≤8/4	≤4	≤1	≤1	≤2	≤2	≤1	≤1	≤8	≤2	≤0.5	≤1	≤1	≤4/2	≤0.5/9	≤2	≤4	>2
CDA6-NDM-T	ND	NDM-5	IncX3	>16/8	>16/8	>16	>16	>32	>16	≤2	>8	>8	≤8	≤2	≤0.5	≤1	≤1	>64/4	≤0.5/9	≤2	≤4	≤0.5
BJ10-NDM-T	ND	NDM-5	IncX3	>16/8	>16/8	>16	>16	>32	>16	≤2	>8	>8	≤8	≤2	≤0.5	≤1	≤1	64/4	≤0.5/9	≤2	≤4	≤0.5

[a] CDA6-mcr-T and BJ13-mcr-T, *mcr-1 E. coli* J53 transconjugants; CDA6-NDM-T and BJ10-NDM-T, bla_{NDM-5} *E. coli* J53 transconjugants. No *mcr-1* transconjugant was obtained from BJ10. ND, not determined; —, negative by PCR.

[b] MICs were determined using a MicroScan WalkAway Plus system. SAM, ampicillin-sulbactam; AMC, amoxicillin-clavulanic acid; CFZ, cefazolin; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; ATM, aztreonam; IMP, imipenem; MEM, meropenem; AMK, amikacin, GEN, gentamicin; CIP, ciprofloxacin; LVX, levofloxacin; MXF, moxifloxacin; TZP, piperacillin-tazobactam; SXT, trimethoprim-sulfamethoxazole; TET, tetracycline; CHL, Chloramphenicol; COL, colistin.

cancer surgery and liver tumor resection in early 2015 at a cancer hospital in Beijing. She was admitted to hospital B in Beijing (northern China) a few weeks later due to intra-abdominal infection and liver abscess. On the second day of admission, a multidrug-resistant (defined as resistant to three or more antimicrobial classes) (4) *E. coli* strain (BJ10) was isolated from the ascites. Susceptibility testing showed that this isolate was resistant to colistin and all β-lactam antimicrobial agents, including imipenem, meropenem, and aztreonam, but remained susceptible to amikacin (Table 1).

Patient 3 was a nearly 50-year-old female with a medical history of cirrhosis lasting more than a decade. In September 2015, the patient was admitted to a local hospital due to symptoms of fatigue, abdominal pain, jaundice, and ascites. One month later, the patient was transferred to hospital B in Beijing. The patient underwent therapeutic plasma exchange 2 weeks later in order to improve liver function. In early November, a multidrug-resistant *E. coli* isolate (BJ13) was collected from a bile culture. The isolate was resistant or intermediately resistant to all β-lactams, as well as to gentamicin, ciprofloxacin, levofloxacin, moxifloxacin, piperacillin-tazobactam, tetracycline, and colistin (Table 1).

Multilocus sequence typing showed that the three *E. coli* isolates belonged to three unrelated STs: ST167, ST156, and ST457, respectively (5) (Table 1). PCR and sequencing of the carbapenemase genes revealed that CDA6 and BJ10 carried bla_{NDM-5}, the same bla_{NDM} variant we previously reported having found in the two MCR-1-producing ST25 *K. pneumoniae* isolates (2, 3). BJ13 was negative for all carbapenemase genes tested, but it harbored $bla_{CTX-M-14}$ and bla_{CMY-2} and had a premature stop codon at amino acid 303 in porin gene *ompC*, which likely explains its carbapenem resistance.

The *mcr-1* gene from isolates CDA6 and BJ13 were successfully transferred to recipient *E. coli* $J53^{AZ-R}$ strains by conjugation, whereas conjugation transfer for BJ10 was unsuccessful. Plasmid DNA from BJ10 was therefore extracted and used in electroporation experiments with *E. coli* DH10B as the recipient, but this transfer was also not successful. To our surprise, results of pulsed-field gel electrophoresis (PFGE) with S1 nuclease treatment (S1-PFGE), followed by Southern hybridization probing with an *mcr-1*-specific fragment, indicated that the *mcr-1* gene in BJ10 is located on the chromosome (data not shown). Whole-genome sequencing of BJ10 was then conducted using an Illumina NextSeq platform to confirm the chromosomal integration of *mcr-1*. *De novo* assembly (6) and BLASTn analysis (http://blast.ncbi.nlm.nih.gov/Blast.cgi) revealed that *mcr-1* in BJ10 is located on a Tn*9*-like composite transposon (tentatively named Tn*Apl*), with two directly repeated IS*Apl1* elements flanking the *mcr-1–pap2* gene cassette (Fig. 1). Tn*Apl* was integrated in the BJ10 chromosome within the intergenic region between two hypothetical protein genes (corresponding to locus_tag ECSE_1545 and ECSE_1546 in ST156 *E. coli* strain SE11), with 2-bp (TG) putative target site duplications (Fig. 1). To our best knowledge, this report presents the first description of chromosomal integration of *mcr-1* in a composite transposon.

The bla_{NDM-5} genes from CDA6 and BJ10 were successfully transferred to *E. coli* $J53^{AZ-R}$ through conjugation. PCR-based plasmid replicon typing (PBRT) showed that bla_{NDM-5} was carried by IncX3 plasmids in both NDM-5-producing isolates (7, 8). The *mcr-1*-harboring plasmid from CDA6 belonged to the

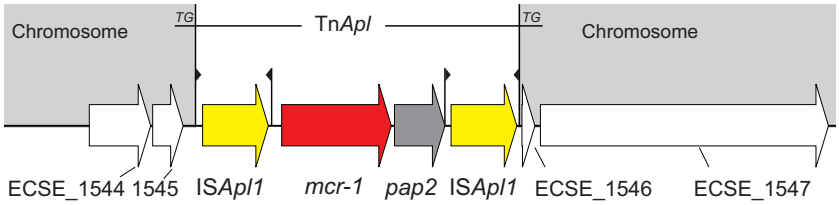

FIG 1 Chromosomal integration of *mcr-1* gene. Arrows denote open reading frames (ORF), with red, gray, yellow, and white arrows denoting *mcr-1*, *pap2*, ISA*pl1*, and neighboring chromosomal genes (illustrated by locus_tag in ST156 strain SE11 [GenBank accession no. AP009240]), respectively. The small black arrows adjacent to ISA*pl1* denote the inverted reverse repeats (IRR). The 2-bp putative target duplicated sequences for TnApl1 are underlined.

IncX4 group, while the *mcr-1* plasmid from BJ13 belonged to IncI2 group (Table 1). S1-PFGE of the transconjugants showed that the *mcr-1*-harboring plasmids from CDA6 (IncX4) and BJ13 (IncI2) were ~35 kb and ~65 kb in size, respectively, while the bla_{NDM-5}-harboring plasmids from CDA6 and BJ10 were ~45 kb in size. No ISA*pl1* gene was found upstream of *mcr-1* in CDA6 and BJ13 by PCR, but the downstream *pap2* gene was identified in all three isolates. Antimicrobial susceptibility testing showed that the *mcr-1* transconjugants were resistant to colistin but susceptible to all other agents tested, suggesting that no additional resistance genes coexist on the *mcr-1* plasmids. The bla_{NDM-5} transconjugants were resistant to all β-lactams and inhibitors, except for aztreonam (Table 1).

Taking the results together, our current and previous studies have demonstrated the emergence of MCR-1-producing CRE from multiple hospitals in China (2, 3). This study extended our knowledge of emerging *mcr-1*, and the results show that this resistance gene has spread into different geographic regions in China, has been found in multiple bacterial species, and has moved to the chromosome. Our findings underline the importance of continuous microbiological and molecular surveillance with regard to further dissemination of *mcr-1*.

Nucleotide sequence accession number. The draft genome sequence of *E. coli* strain BJ10 has been deposited within the GenBank whole-genome shotgun (WGS) database under accession no. LWQZ00000000.

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B.N.K. discloses that he holds two patents that focus on using DNA sequencing to identify bacterial pathogens.

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