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MCR-1 and OXA-48 *In Vivo* Acquisition in KPC-Producing *Escherichia coli* after Colistin Treatment

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ABSTRACT The spread of *mcr-1*-encoding plasmids into carbapenem-resistant *Enterobacteriaceae* raises concerns about the emergence of untreatable bacteria. We report the acquisition of *mcr-1* in a carbapenem-resistant *Escherichia coli* strain after a 3-week course of colistin in a patient repatriated to France from Portugal. Whole-genome sequencing revealed that the *Klebsiella pneumoniae* carbapenemase-producing *E. coli* strain acquired two plasmids, an IncL OXA-48-encoding plasmid and an IncX4 *mcr-1*-encoding plasmid. This is the first report of *mcr-1* in carbapenemase-encoding bacteria in France.

KEYWORDS colistin, *Escherichia coli*, KPC-28, KPC-3, OXA-48, antibiotic resistance, β-lactamases, carbapenemase, *mcr-1*

Colistin is a last-resort antibiotic reserved for treating multidrug-resistant Gram-negative bacilli. However, the increased use of colistin in clinical treatment and agricultural and animal production has led to the emergence of bacterial resistance to the drug. In November 2015, the first transferable plasmid-mediated colistin resistance gene, *mcr-1*, was detected in China in retail meat and human samples (1). The gene was observed worldwide a few months later. The spread of *mcr-1*-encoding plasmids into carbapenem-resistant *Enterobacteriaceae* (2–5) is causing concern about the rise of untreatable bacteria (6).

In this work, we report an *in vivo* acquisition of *mcr-1* in carbapenemase-producing *Escherichia coli* after a 3-week course of colistin in a patient repatriated to France from Portugal in 2016. The 44-year-old man was hospitalized in Portugal for 2 months after a traffic accident. He had multiple traumas, including a thoracic injury associated with a respiratory *Enterobacter cloacae* infection that was treated with a combination of piperacillin and tazobactam. Carbapenemase-producing *Enterobacteriaceae* (CPE) belonging to the species *Klebsiella pneumoniae* and *E. coli* were isolated from a stool sample after treatment. A second episode of lower respiratory tract infection involving *Acinetobacter baumannii*, *Pseudomonas aeruginosa*, and the carbapenemase-producing *K. pneumoniae* was successfully treated by a 20-day course of colistin. The patient was then repatriated to an intensive care unit in France, where two *E. coli* isolates designated WI1 and WI2 were recovered from a feces CPE screening sample taken on the patient's admission with CarbaSmart medium (bioMérieux, La Balme, France).

The *E. coli* WI1 and WI2 isolates were resistant to penicillins, oxy-imino-

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TABLE 1 Susceptibilities of the studied strains to β-lactams, by microdilution method

Strain	Carbapenemase-encoding genes	MIC (μg/ml) of[a]:							
		AMX	FOX	CAZ	CTX	FEP	IPM	ETP	MEM
WI1	bla_{KPC-3}	>256	16	>256	>32	12	4	6	2
WI2	bla_{KPC-28}, bla_{OXA-48}	>256	8	>256	8	6	1	3	0.38
E. coli DH5α-KPC-3	bla_{KPC-3}	>256	8	4	1	1	1	0.125	0.25
E. coli DH5α KPC-28	bla_{KPC-28}	64	8	64	0,5	1	0.125	0.032	0.032
E. coli DH5α		1	4	0.06	0.06	0.032	0.05	0.006	0.006

[a]AMX, amoxicillin; FOX, cefoxitin; CAZ, ceftazidime; CTX, cefotaxime; FEP, cefepime; IPM, imipenem; ETP, ertapenem; MEM, meropenem.

cephalosporins, and carbapenems. The colistin MIC (broth microdilution method) was in the sensitive range (0.25 μg/ml) for the WI1 isolate. In contrast, the MIC was in the resistance range for the *E. coli* WI2 isolate, with a value of 4 μg/ml, as usually observed for *mcr-1*-harboring *E. coli*. The whole-genome sequence (WGS) of strain WI2 was determined by hybrid *de novo* assembly of 2× 150-bp paired-end reads generated with Illumina sequencing technology (San Diego, CA, USA) and long reads generated with Pacific Biosciences RS II SMRT technology (Menlo Park, CA, USA). WGS of strain WI1 was determined by *de novo* assembly of 2× 150-bp paired-end reads (Illumina) and mapping to the genome of strain WI2. *De novo* assemblies were performed with SPAdes (7), the mappings with Burrows-Wheeler aligner (8), and the final polishing of the assembly with Pilon (9). The average depth sequencing (ADS) was 125× and 145× for WI1 and WI2 chromosomes (4.8 Mb), respectively. Three plasmids were detected in strain WI1 (ADS, 282× to 320×; sizes, 54,502 to 83,831 bp) and five in strain WI2 (ADS, 81× to 213×; sizes, 33,304 to 83,832 bp). The plasmid content of the strains and the size of the plasmids were confirmed with plasmid DNA extracted by alkaline lysis, as previously described (10).

The antibiotic resistance genes were detected with the Comprehensive Antibiotic Resistance Database (CARD) (11). *E. coli* WI1 did not harbor *mcr-1* but did harbor the carbapenemase-encoding gene bla_{KPC-3}. In contrast, *E. coli* WI2 harbored the *mcr-1* gene and two carbapenemase-encoding genes, bla_{OXA-48} and a bla_{KPC-3} variant gene designated bla_{KPC-28} (accession number KY282958). The sequence of bla_{KPC-28} was confirmed by PCR and Sanger sequencing. The deduced amino acid sequence of KPC-28 was derived from KPC-3 by two amino acid deletions in the catalytic pocket at positions 241 and 242. The KPC-28- and KPC-3-encoding open reading frames were cloned in *E. coli* DH5α with pBK-CMV vector (Stratagene, San Diego, CA, USA). MIC values suggested that the deletions at positions 241 and 242 decrease the activity against amoxicillin and carbapenems but improve the activity against ceftazidime (Table 1). Isolates WI1 and WI2 shared the other antimicrobial resistance gene contents (*strA*, *strB*, *folP*, and *tetBDR*), and no mutation was detected in chromosomal genes involved in quinolone (*gyrA*, *gyrB*, *parC*, and *parE*) and colistin (*mgrB*, *pmrAB*, and *phoPQ*) resistance.

The isolates were typed from WGSs by assigning sequence types according to the MLST Warwick University website. WI1 and WI2 belonged to sequence type ST1288 and *E. coli* phylogroup C (12). Single nucleotide polymorphism calling was performed from alignments generated by parsnp in deeply sequenced regions (>60×) (13), which were filtered for repeat elements, phages, and putative recombination events. Among 4,219,421 bp, WI1 and WI2 diverged by only four single nucleotide variants (SNVs) and were therefore determined to be two isolates of the same strain.

The assembled genomes were analyzed by PlasmidFinder (http://www.genomicepidemiology.org/) using the *Enterobacteriaceae* database with the detection thresholds set at 95% sequence identity. Three replicons (IncN, IncFII, and IncI1) were shared by both isolates. However, WI2 contained two additional replicons (IncX4 and IncL). The 33,304-bp-long IncX4 plasmid, designated pWI2-mcr, harbored *mcr-1* and encoded no other antimicrobial resistance gene. The most closely related plasmid is the unpublished *mcr-1*-harboring plasmid pICBEC72Hmcr characterized in Brazil

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(CP015977). The pWI2-mcr sequence covered 99.98% of the pICBEC72Hmcr plasmid sequence and differed from the latter by only four SNVs.

A 62,645-bp-long IncL plasmid, designated pWI2-OXA48, carried bla_{OXA-48}. pWI2-OXA48 differed from the pOXA-48a reference plasmid by a 2,762-bp deletion (14). The deletion occurred within *orf25* at base 22,738, leading to the suppression of *ccgA1* and *orf26* genes. The deleted region was replaced by insertion sequence IS*1R*, which is probably involved in this novel arrangement within the backbone of a pOXA-48-like plasmid (15).

In the WI1 and WI2 isolates, bla_{KPC-3} and bla_{KPC-28} were carried by ST15-IncN 54,518-bp and 54,533-bp plasmids, designated pWI1-KPC3 and pWI2-KPC28, respectively. The plasmid pWI2-KPC28 differed from pWI1-KPC3 by the deletion of 6 bp, which generated the new bla_{KPC} variant. A 21-bp deletion occurred within hypothetical protein (49.871 to 50,728 bp) in pWI1-KPC3. No additional resistant gene was detected in these plasmids. The two strains also contained two identical plasmids devoid of antibiotic resistance genes and belonging to incompatibility groups IncI1 (83,831 bp) and IncFII (60,622 bp).

In conclusion, our data support the *in vivo* acquisition of *mcr-1*- and bla_{OXA-48}-bearing plasmids by a KPC-producing *E. coli* probably following treatment with colistin. The emergence of multidrug-resistant isolates, such as *E. coli* WI2, that need to be carefully monitored is becoming a major burden on health care systems worldwide.

Accession number(s). The complete genome sequences of WI1 and WI2 strains were deposited in EMBL/GenBank under assembly accession numbers LT838196, LT838197, LT838198, and LT838199 (WI1) and LT838200, LT838201, LT838202, LT838203, and LT838204 (WI2).

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