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Coproduction of MCR-1 and NDM-1 by Colistin-Resistant *Escherichia coli* Isolated from a Healthy Individual

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The first transferable plasmid-mediated colistin resistance gene, *mcr-1* was reported in *Escherichia coli* isolates from food animals, food, and patients in China and now has been reported worldwide (1). Furthermore, cocarriage of *mcr-1* and bla_{NDM} has been reported in *E. coli* and other members of the family *Enterobacteriaceae* from a chicken meat sample; patients with peritonitis, urinary tract infections, and rectal cancer; and a Muscovy duck, all in China. Most recently, *E. coli* carrying *mcr-1* and bla_{NDM-5} was isolated from a patient with a urinary tract infection in the United States (2–7). Here, we report asymptomatic carriage of *E. coli* harboring both *mcr-1* and bla_{NDM-1} in an otherwise healthy individual.

A total of 151 nonduplicate, serial fecal specimens were collected from 98 inpatients and 53 healthy individuals at Guangdong General Hospital in Guangzhou, China, during the first week of January 2016 for the purpose of detecting extended-spectrum β-lactamase-producing *Enterobacteriaceae*. Each sample was screened on a Columbia blood agar plate without any antibiotics and then subcultured on MacConkey agar with 2 μg/ml cefotaxime. Colonies selected from the MacConkey agar were identified to the species level by the API 20E system (bioMérieux, Marcy l'Etoile, France) and 16S rRNA gene sequencing (8). As a result, 73 nonduplicate *E. coli* isolates were collected from 58 inpatients and 15 healthy individuals. Of these isolates, 17 were found to harbor *mcr-1* by PCR assay and sequencing performed as previously described (1, 8). Of the 17 *mcr-1*-carrying *E. coli* isolates, 2 were from clinical cultures of inpatients, 12 were from rectal surveillance cultures of inpatients, and 3 (*E. coli* GB049, GB090, and GB135) were from healthy individuals who provided rectal cultures with stool specimens after consent during outpatient visits for their annual physical examinations. Since carriage of *mcr-1* by healthy individuals is of particular epidemiologic interest, we analyzed these three strains further.

E. coli GB049 was recovered from a 23-year-old male, *E. coli* GB090 was from a 68-year-old female, and *E. coli* GB135 was from a 56-year-old female. These healthy individuals were all nonvegetarian, living in the city >10 km from commercial animal farms, drinking the municipal water, had received a secondary or tertiary education, had a mid to high socioeconomic status, and had traveled overseas. The individual with *E. coli* GB049 traveled in India for 5 days in September 2015; and the other two individuals with *E. coli* GB090 and GB135 traveled to the United States for 9 and 15 days, respectively, in December 2015. In addition, the individual with *E. coli* GB090 had taken oral amoxicillin for several days for her respiratory symptoms 3 months earlier.

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TABLE 1 Characterization of three *mcr-1*-positive *E. coli* isolates from healthy individuals and their transconjugants

Parameter	*E. coli* GB049	Transconjugant of *E. coli* GB049	*E. coli* GB135	*E. coli* GB090	Transconjugant of *E. coli* GB090	*E. coli* EC600
Isolation date	1 January 2016		4 January 2016	2 January 2016		
Source	Healthy individual		Healthy individual	Healthy individual		
Isolation site	Feces		Feces	Feces		
MLST result	ST1193		ST38	ST744		
Phylogenetic group	D		D	B2		
Resistance gene(s)[a]	*mcr-1*, bla_{TEM-1}	*mcr-1*, bla_{TEM-1}	*mcr-1*, bla_{TEM-1}	*mcr-1*, bla_{NDM-1}, bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-15}$ $bla_{CTX-M-14}$, *fosA3*, *qnrB*, *qnrS*, *aac(6′)-Ib-cr*	*mcr-1*, $bla_{CTX-M-15}$, bla_{TEM-1}	
Plasmid replicon type(s)	IncI2	IncI2	IncFIB, IncN	IncFI, IncN, IncP, IncK	IncFI	
MIC (μg/ml) of:						
Colistin	8	16	16	16	16	0.25
Polymyxin B	16	16	16	16	16	0.5
Tigecycline	0.5	0.25	0.5	1	0.5	0.25
Ampicillin	>256	>256	>256	>256	>256	8
Amoxicillin-clavulanic acid	16	4	16	128	8	2
Cefotaxime	≤1	≤1	≤1	128	256	≤1
Ceftazidime	≤1	≤1	≤1	>256	64	≤1
Cefepime	≤0.5	≤0.5	≤0.5	16	32	≤0.5
Gentamicin	2	≤1	≤1	128	4	≤1
Amikacin	4	2	2	8	4	2
Ertapenem	0.125	≤0.063	0.125	4	0.125	≤0.063
Imipenem	0.125	≤0.063	≤0.063	1	0.25	0.125
Meropenem	≤0.063	≤0.063	≤0.063	1	0.125	≤0.063
Fosfomycin	16	≤8	≤8	>512	16	≤8
Nitrofurantoin	≤8	≤8	16	32	16	≤8
Ciprofloxacin	0.125	0.064	0.064	16	0.064	≤0.032

[a]Determined by PCR and sequencing.

Antimicrobial susceptibility testing was performed by the agar dilution method and interpreted according to the breakpoints of the European Committee on Antimicrobial Susceptibility Testing (EUCAST, 2016) for colistin, polymyxin B, and tigecycline and those of the Clinical and Laboratory Standards Institute (CLSI, 2015) for other antimicrobials. All three isolates were resistant to colistin and polymyxin B but susceptible to tigecycline and amikacin. In addition, *E. coli* GB090 was also resistant to cephalosporins, ertapenem, fosfomycin, and ciprofloxacin.

PCR was performed to identify β-lactamase, plasmid-mediated quinolone resistance, and 16S rRNA methyltransferase genes as described previously (9–11). Sanger sequencing of both strands of all PCR products was performed. As a result, in addition to *mcr-1*, *E. coli* GB090 was positive for bla_{NDM-1}, bla_{TEM-1}, bla_{SHV-12}, $bla_{CTX-M-15}$, $bla_{CTX-M-14}$, *fosA3*, *qnrB*, *qnrS*, and *aac*(*6′*)*-Ib-cr*.

All three *mcr-1*-positive *E. coli* isolates from healthy individuals were subjected to pulsed-field gel electrophoresis (PFGE) as previously described (12, 13). The three isolates were clonally unrelated by PFGE (data not shown). *E. coli* GB090 was then subjected to phylogenetic typing and multilocus sequence typing (MLST) according to the protocol at http://mlst.warwick.ac.uk/mlst/dbs/Ecoli (9) and classified into phylogenetic group B2 and sequence type 744 (ST744), which belongs to the ST10 complex. *E. coli* B2 ST744 was also reported in *mcr-1*-carrying *E. coli* from a human bloodstream infection and imported chicken meat in Denmark (14). Phylogenetic group B2 ST10 complex strains, along with phylogenetic group B2 ST131 and ST405 strains, have been implicated as vehicles driving the international spread of $bla_{CTX-M-15}$ (15).

Rifampin-resistant *E. coli* EC600 was used as the recipient for the conjugation and transformation experiments. The transconjugants or transformations were selected on lysogeny broth (LB) agar plates containing rifampin (500 μg/ml) plus colistin (4 μg/ml), cefotaxime (16 μg/ml), ertapenem (2 and 4 μg/ml), or fosfomycin (256 μg/ml). As a result, colistin-resistant transconjugants containing *mcr-1*, $bla_{CTX-M-15}$, and bla_{TEM-1} were successfully obtained from plates containing either colistin or cefotaxime for *E. coli* GB090, which was positive for an IncFI replicon by PCR-based replicon typing (16) (Table 1). We could not obtain transconjugants or transformants containing bla_{NDM-1}. In order to determine the locations of the *mcr-1* and bla_{NDM} genes, DNA linearization of

E. coli GB090 with S1 nuclease, followed by PFGE and Southern hybridization using *mcr-1* and bla_{NDM} gene probes, was conducted as previously described (17).The results indicated that *mcr-1* was located on an ~33-kDa plasmid together with the β-lactamase genes $bla_{CTX\text{-}M\text{-}15}$ and $bla_{TEM\text{-}1}$, whereas bla_{NDM} was not located on this plasmid (see Fig. S1 in the supplemental material). Taking the results together, we speculate that $bla_{NDM\text{-}1}$ may be located on the chromosome of *E. coli* GB090. In addition, colistin-resistant transconjugants containing *mcr-1* were successfully obtained for *E. coli* GB049 but not *E. coli* GB135.

The genomic DNA of *E. coli* GB090 was extracted and digested with EcoRI and XbaI and then ligated to cloning vector pBC-SK(–). The ligation products were transferred to *E. coli* DH10B by electroporation and cultured on LB agar supplemented with chloramphenicol (30 μg/ml) and colistin (4 μg/ml) or ertapenem (2 and 4 μg/ml), which yielded colistin- and ertapenem-resistant transformants, respectively. Sequencing of the recombinant plasmids carried by these *E. coli* transformants revealed ~6.1- and ~8.1-kb inserts containing the *mcr-1* and $bla_{NDM\text{-}1}$ genes, respectively.

Sequence analysis of the ~6.1-kb genetic environment surrounding *mcr-1* revealed 100% identity with that of pHNSHP45 in our previous study (1), which was also similar to the sequence found in various *mcr-1*-carrying plasmids, with up to 99% identity with pA31-12 from *E. coli* strain A31-12 (accession no. KX034083), pAF23 from *E. coli* strain Af23 (KX032519), pSCS23 from *Salmonella enterica* strain SC23 (KU934209), and pABC149-MCR-1 from *E. coli* strain ABC149 (KX013538). The genetic environment surrounding $bla_{NDM\text{-}1}$ was identical to that found in various $bla_{NDM\text{-}1}$-carrying plasmids in *Enterobacteriaceae* in China, including pKP04NDM (KU314941), pNDM-HN380 (JX104760), pKPN5047 (KC311431), and pNDM-SX04 (KC876051) in *K. pneumoniae*; pYE315203 (JX254913) and p112298-NDM (KP987216) in *Citrobacter freundii*; pNDM-HF727 in *Enterobacter cloacae* (KF976405); and a plasmid in *Raoultella planticola* (KF877335). These results indicate that the genetic structures of both *mcr-1* and $bla_{NDM\text{-}1}$ have likely already spread to different species of *Enterobacteriaceae*.

Recovery of *mcr-1*- and $bla_{NDM\text{-}1}$-carrying *E. coli* from a healthy individual suggests that *Enterobacteriaceae* with extreme antimicrobial resistance may already circulate in the community in China. Our findings underline the importance of continuous microbiological and molecular surveillance with regard to further dissemination of *mcr-1* in the community. The high rate of *mcr-1*-carrying *E. coli* isolation from both hospitalized and nonhospitalized patients also suggests intestinal colonization as a key source in its dissemination.

Accession number(s). The nucleotide sequences obtained in this study were submitted to the GenBank database and assigned accession no. KX886345 and KX886346.

SUPPLEMENTAL MATERIAL

Supplemental material for this article may be found at https://doi.org/10.1128/AAC.01962-16.

TEXT S1, PDF file, 0.1 MB.

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