Colistin- and Carbapenem-Resistant *Escherichia coli* Harboring *mcr-1* and *blaNDM-5*, Causing a Complicated Urinary Tract Infection in a Patient from the United States

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ABSTRACT  Colistin is increasingly used as an antibiotic of last resort for the treatment of carbapenem-resistant Gram-negative infections. The plasmid-borne colistin resistance gene *mcr-1*, was initially identified in animal and clinical samples from China and subsequently reported worldwide, including in the United States. Of particular concern is the spread of *mcr-1* into carbapenem-resistant bacteria, thereby creating strains that approach pan-resistance. While several reports of *mcr-1* have involved carbapenem-resistant strains, no such isolates have been described in the United States. Here, we report the isolation and identification of an *Escherichia coli* strain harboring both *mcr-1* and carbapenemase gene *blaNDM-5* from a urine sample in a patient without recent travel outside the United States. The isolate exhibited resistance to both colistin and carbapenems, but was susceptible to amikacin, aztreonam, gentamicin, nitrofurantoin, tigecycline, and trimethoprim-sulfamethoxazole. The *mcr-1* and *blaNDM-5*-harboring plasmids were completely sequenced and shown to be highly similar to plasmids previously reported from China. The strain in this report was first isolated in August 2014, highlighting an earlier presence of *mcr-1* within the United States than previously recognized.

IMPORTANCE  Colistin has become the last line of defense for the treatment of infections caused by Gram-negative bacteria resistant to multiple classes of antibiotics, in particular carbapenem-resistant *Enterobacteriaceae* (CRE). Resistance to colistin, encoded by the plasmid-borne gene *mcr-1*, was first identified in animal and clinical samples from China in November 2015 and has subsequently been reported from numerous other countries. In April 2016, *mcr-1* was identified in a carbapenem-susceptible *Escherichia coli* strain from a clinical sample in the United States, followed by a second report from a carbapenem-susceptible *E. coli* strain originally isolated in May 2015. We report the isolation and identification of an *E. coli* strain harboring both colistin (*mcr-1*) and carbapenem (*blaNDM-5*) resistance genes, originally isolated in August 2014 from urine of a patient with recurrent urinary tract infections. To our knowledge, this is the first report in the United States of a clinical bacterial isolate with both colistin and carbapenem resistance, highlighting the importance of active surveillance efforts for colistin- and carbapenem-resistant organisms.
nephrostomy tubes were clamped 5 days prior to presentation, and he then developed subjective fever, chills, and generalized weakness. Laboratory testing on presentation indicated a leukocyte count of 14.1 × 10^9 cells/μl, associated with pyuria. The initial antimicrobial regimen included intravenous piperacillin-tazobactam and vancomycin. No fistula was seen on imaging studies, and the nephrostomy tubes were unclamped.

The susceptibility results of urine cultures obtained prior to the initiation of antimicrobial therapy are shown in Table 1. A clean-catch urine culture grew greater than 100,000 CFU per ml of *Pseudomonas aeruginosa*. A urine culture obtained from the nephrostomy tube grew greater than 100,000 CFU per ml of *P. aeruginosa*.

| Antimicrobial agenta | MIC (μg/ml) | E. coli | MIC (μg/ml) | Interpretation | K. pneumoniaeb | MIC (μg/ml) | Interpretation | P. aeruginosac | MIC (μg/ml) | Interpretation |
|---------------------|------------|---------|-------------|---------------|----------------|-------------|---------------|---------------|-------------|---------------|
| Amikacin            | ≤16        | S       | ≤16         | S             | ≤16            | S           | >32           | R             |             |               |
| Ampicillin          | >16        | R       | >16         | R             | >16            | R           | N/R           |               |             |               |
| Ampicillin-sulbactam| >16/8      | R       | ≤8/4        | S             | ≤8/4           | S           | N/R           |               |             |               |
| Aztreonam           | ≤8         | S       | ≤8          | S             | ≤8             | S           | 16            | I             |             |               |
| Cefazolin           | >16        | R       | ≤8          | S             | ≤8             | S           | N/R           |               |             |               |
| Ceftazime           | >16        | R       | ≤8          | S             | ≤8             | S           | >16           | R             |             |               |
| Ciprofloxacin       | >16        | R       | ≤1          | S             | ≤1             | S           | >2            | R             |             |               |
| Colistin            | 3          | Rd      | N/R         |               | 2              | Sb           |               |               |             |               |
| Ertapenem           | >4         | R       | ≤1          | S             | ≤1             | S           | >4            | R             |             |               |
| Gentamicin          | ≤4         | S       | ≤4          | S             | ≤4             | S           | >8            | R             |             |               |
| Imipenem            | >8         | R       | ≤4          | S             | ≤4             | S           | >8            | R             |             |               |
| Levofloxacin        | >4         | R       | ≤2          | S             | ≤2             | S           | >4            | R             |             |               |
| Meropenem           | >8         | R       | ≤4          | S             | ≤4             | S           | >8            | R             |             |               |
| Nitrofurantoin      | ≥32        | S       | ≤32         | S             | 64             | I           | >64           | R             |             |               |
| Piperacillin-tazobactam | ≥64  | R       | ≤16         | S             | ≤16            | S           | 64            | S             |             |               |
| Ticarcillin         | ≥2         | S       | ≥2          | S             | ≤2             | S           | N/R           |               |             |               |
| Trimethoprim-sulfamethoxazole | ≥2/38 | S | ≥2/38 | S | ≥2/38 | S | N/R |               |             |               |

a Antimicrobial susceptibilities for all agents (except colistin) were obtained using the MicroScan Walkaway Plus System (Beckman Coulter, Brea, CA).
b Isolated from nephrostomy tube drainage culture.
c Isolated from clean-catch urine culture.
d Colistin MIC values were determined using Etest strips (bioMérieux, Marcy-l’Etoile, France). The MICs for *E. coli* and *P. aeruginosa* were 3 μg/ml and 2 μg/ml, respectively.

e Abbreviations: MIC, minimum inhibitory concentration; Interpretation; R, resistant; I, intermediate; S, susceptible; N/R, not reported.

Since 2014, our laboratory has used molecular methods to analyze clinical isolates of Gram-negative bacteria obtained from this affiliated tertiary-care hospital, including 16S sequencing; multilocus sequence typing (MLST); and PCR detection of carbapenemases, AmpC β-lactamases, extended-spectrum β-lactamase genes, and, more recently, the mcr-1 gene. Using these methods, the *E. coli* isolate from the study case (named MCR1_NJ) was shown to carry both mcr-1 and blaNDM-5 genes. Whole-genome sequencing of *E. coli* strain MCR1_NJ was performed using an Illumina NextSeq platform (San Diego, CA), and the resistome was investigated using ResFinder 2.1 (14). In addition to mcr-1 and blaNDM-5, strain MCR1_NJ was found to harbor resistance genes for aminoglycosides [strA, strB, and (aac(6')-Ib-cr)], β-lactams (blaOXA-1, blaVIM-2), chloramphenicol (catB3 and floR), fluoroquinolones [tet(A)], rifampin (arr-3), sulfonamides (sul1 and sul2), and tetracycline (tet(A)). The mcr-1- and blaNDM-5-harboring plasmids from *E. coli* strain MCR1_NJ were transferred to *E. coli* DH10B by electroporation, thereby confirming the mutually exclusive presence of mcr-1 and blaNDM-5 in the resulting transformants. The conjugability of the mcr-1 plasmid was further confirmed by experiments using *E. coli* J53 Azt as the recipient strain. Plasmid DNA was isolated using a Qiagen Plasmid Midi kit (Hilden, Germany) and subjected to complete plasmid sequencing using Illumina NextSeq as described previously (6). Sequencing reads were assembled de novo using SPAdes software (15), and gaps were closed by Sanger sequencing as described previously (6, 16). The mcr-1-harboring plasmid from *E. coli* MCR1_NJ (subsequently named pMCRI-NJ-IncX4) was 33,395 bp in length and had 100% BLAST query coverage and 99.6% nucleotide identity to conjugative plasmid pMCRI-IncX4 (GenBank accession no. KU761327), which we previously described in CTX-M-55-producing *E. coli* and NDM-5-producing *K. pneumoniae* strains from Chinese hospitals (5, 6). The blaNDM-5-harboring plasmid (named pNDM5-NJ-IncX3) was 39,520 bp in length and closely related (100% nucleotide identity and 79% query coverage) to pNDM5-IncX3.
Strain MCR1_NJ was shown by MLST to be a single-locus variant of ST405, associated with E. coli phylogroup D (17). ST405 is classified as one of the main extraintestinal pathogenic E. coli (ExPEC) lineages (18), and is associated with the global spread of extended-spectrum β-lactamases, most notably CTX-M-15 (18–20). AmpC cephemases and NDM metallo-β-lactamases have also been reported in ST405 (20), including NDM-1 (21) and NDM-4 (22). Consequently, ST405 may also be involved in the global dissemination of polymyxin-resistant E. coli strains (22). Moreover, whereas strain MCR1_NJ was susceptible to various antibiotics, including gentamicin and trimethoprim-sulfa-methoxazole, studies of ST405 strains from multiple countries suggest that they are typically resistant to these as well (20). Worrisomely, ST405 has been frequently associated with community onset urinary tract infections (23–25). Dissemination of mcr-1 within this global lineage may therefore contribute to further spread of polymyxin resistance within ESBL- and carbapenemase-producing E. coli (and other Enterobacteriaceae) strains.

In summary, we report the isolation and identification of an E. coli strain harboring both mcr-1 and blaNDM-5 from a urine of a patient without recent travel outside the United States. This strain was isolated in August 2014, highlighting an earlier presence of mcr-1 within the region than previously known and raising the likelihood of ongoing undetected transmission. Active surveillance efforts involving all polymyxin- and carbapenem-resistant organisms are imperative in order to determine mcr-1 prevalence and prevent further dissemination.

**Accession number(s).** The complete nucleotide sequences of plasmids pMCR1-NJ-IncX4 and pNDM5-NJ-IncX3 have been deposited as GenBank accession no. KX447768 and KX447767, respectively. The draft genome sequence of E. coli strain MCR1_NJ was deposited as GenBank accession no. MAJK00000000.

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