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.

Polyoxyns are increasingly used as antibiotics of last resort for clinical infections caused by carbapenem-resistant Gram-negative bacteria, especially carbapenem-resistant Enterobacteriaceae (CRE) (1). The recent discovery of transmissible colistin resistance in China (2) has led to numerous reports of the plasmid-borne gene mcr-1 in animal, food, and clinical samples worldwide (3). Of particular concern is the spread of mcr-1 into CRE, thereby creating highly drug-resistant strains that are potentially untreatable. Several reports outside the United States have already described mcr-1 in bacterial strains harboring carbapenemases, including NDM-1 (4), NDM-5 (5–7), NDM-9 (8), KPC-2 (9), OXA-48 (10), and VIM-1 (11). Most recently, mcr-1 was detected in two clinical carbapenem-susceptible Escherichia coli strains from the United States, isolated from urine cultures obtained in April 2016 (12) and May 2015 (13), respectively. Here, we report the identification from urine of an E. coli strain harboring both mcr-1 and carbapenemase gene blaNDM-5, originally isolated in August 2014 from a U.S. patient with recurrent urinary tract infections and no recent travel history.

A 76-year-old man presented to a tertiary-care hospital in New Jersey with subjective fever and flank pain in August 2014. The patient had emigrated from India and resided continuously in the United States for 1 year prior to this presentation. He had a history of prostate cancer treated with radiation therapy and subsequently developed recurrent urinary tract infections. He had recently undergone cystoscopy to evaluate the possibility of colovesicular fistula formation. The procedure was complicated by bladder perforation requiring bilateral nephrostomy tube placement. The
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 × 10³ cells/µl, associated with pyuria. The initial antimicrobial regimen included intravenous piperacillin resistant). Susceptibility testing results showed that the isolate was resistant to colistin and all β-lactams (including carbapenem) and remained susceptible to ampicillin, gentamicin, nitrofurantoin, tigecycline, and trimethoprim-sulfamethoxazole (Table 1). The susceptibility results of urine cultures obtained prior to the initiation of antimicrobial therapy are shown in Table 1. A clean-catch urine culture was obtained after the patient had remained afebrile and the leukocyte count had normalized. A sterile clean-catch urine culture was obtained after antimicrobial therapy was initiated, thereby confirming the mutually exclusive presence of mcr-1 and blaNDM-5 in the resulting transformants. The conjugal transfer of the mcr-1 plasmid was further confirmed by experiments using E. coli J53 Az1 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 MCRI_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

### Table 1 Antimicrobial susceptibilities of Gram-negative bacterial species isolated from urine cultures and nephrostomy tube drainage obtained from the case patient

<table>
<thead>
<tr>
<th>Antimicrobial agent</th>
<th>E. coli</th>
<th>C. koseri</th>
<th>K. pneumoniae</th>
<th>P. aeruginosa</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>MIC (µg/ml)</td>
<td>Interp</td>
<td>MIC (µg/ml)</td>
<td>Interp</td>
</tr>
<tr>
<td>Amikacin</td>
<td>≤16</td>
<td>S</td>
<td>≤16</td>
<td>S</td>
</tr>
<tr>
<td>Ampicillin</td>
<td>&gt;16</td>
<td>R</td>
<td>&gt;16</td>
<td>R</td>
</tr>
<tr>
<td>Ampicillin-sulbactam</td>
<td>&gt;16/8</td>
<td>R</td>
<td>≤8/4</td>
<td>S</td>
</tr>
<tr>
<td>Aztreonam</td>
<td>≤8</td>
<td>S</td>
<td>≤8</td>
<td>S</td>
</tr>
<tr>
<td>Cefazolin</td>
<td>&gt;16</td>
<td>R</td>
<td>≤8</td>
<td>S</td>
</tr>
<tr>
<td>Ceftazidime</td>
<td>&gt;16</td>
<td>R</td>
<td>≤8</td>
<td>S</td>
</tr>
<tr>
<td>Ciprofloxacin</td>
<td>&gt;2</td>
<td>R</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Colistin</td>
<td>3</td>
<td>R</td>
<td>N/R</td>
<td></td>
</tr>
<tr>
<td>Ertapenem</td>
<td>&gt;4</td>
<td>R</td>
<td>≤1</td>
<td>S</td>
</tr>
<tr>
<td>Gentamicin</td>
<td>≤4</td>
<td>S</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Imipenem</td>
<td>&gt;8</td>
<td>R</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Levofoxacin</td>
<td>&gt;4</td>
<td>R</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Meropenem</td>
<td>&gt;8</td>
<td>R</td>
<td>≤4</td>
<td>S</td>
</tr>
<tr>
<td>Nitrofurantoin</td>
<td>≥32</td>
<td>R</td>
<td>≤32</td>
<td>64</td>
</tr>
<tr>
<td>Piperacillin-tazobactam</td>
<td>&gt;64</td>
<td>R</td>
<td>≤16</td>
<td>S</td>
</tr>
<tr>
<td>Tigecycline</td>
<td>≤2</td>
<td>S</td>
<td>≤2</td>
<td>S</td>
</tr>
<tr>
<td>Trimethoprim-sulfamethoxazole</td>
<td>≥2/38</td>
<td>S</td>
<td>≤2/38</td>
<td>S</td>
</tr>
</tbody>
</table>

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.

### Abbreviations
- MIC, minimum inhibitory concentration
- Interp, interpretation
- R, resistant
- I, intermediate
- S, susceptible
- N/R, not reported.

### Notes
- Isolated from clean-catch urine culture.
- Isolated from nephrostomy tube drainage culture.
- Colistin MIC values were determined using Etest strips (bioMérieux, Marcy-l’Étoile, France). The MICs for E. coli and P. aeruginosa were 3 µg/ml and 2 µg/ml, respectively.

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 MCRI_NJ) was shown to carry both mcr-1 and blaNDM-5 genes. Whole-genome sequencing of E. coli strain MCRI_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 MCRI_NJ was found to harbor resistance genes for aminoglycosides [strA, strB, and aac(6')-Ib-cr], β-lactams (blaOXA-1), chloramphenicol (catB3 and floR), fluoroquinolones [aac(6')-Ib-cr], rifampin (arr-3), sulfonamides (sul1 and sul2), and tetracycline (tetA).
and blaNDM-5 were observed in plasmid pMCR1-NJ-IncX4 or pNDM5-NJ-IncX3, respectively.

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 cephalosporinases 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 producing E. coli strains (22). Moreover, whereas strain MCR1_NJ was susceptible to various antibiotics, including gentamicin and trimethoprim-sulfamethoxazole, studies of ST405 strains from multiple countries has classified as one of the main extraintestinal pathogenic E. coli.

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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References


