

Detection of *mcr-1*-Carrying *Escherichia coli* Causing Bloodstream Infection in a New York City Hospital: Avian Origins, Human Concerns?

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The spread of *mcr-1* in the United States remains poorly defined. *mcr-1*-producing *Escherichia coli* that also carried *bla*_{SHV-12} was detected in a hospitalized patient. No additional cases were identified during screening of 801 Gram-negative isolates. Genomic sequencing identified an IncX4 *mcr-1*-harboring plasmid and ST117 clonal background associated with avian pathogenic *E coli*.

Keywords. avian pathogenic *E coli*; colistin; IncX4 plasmid; *mcr-1*; polymyxin B.

Polymyxins (including polymyxin B and colistin) are considered antibiotics of “last resort” for treating infections with multidrug-resistant Gram-negative bacteria (MDR-GNB). *mcr-1*, a plasmid-associated gene for colistin resistance, was described in China in 2015 [1]. The possibility of horizontal transfer of colistin resistance has raised significant concern regarding spread of *mcr-1*. After initially being described in isolates from food animals, human carriage of *mcr-1* was noted in *Escherichia coli* and *Klebsiella pneumoniae* in urine and blood samples from hospitalized patients. By January 2016, cases had been reported from 19 countries worldwide [2]. In the United States, 12 human *mcr-1* cases have been reported in 9 states to date [3–7]. Most *mcr-1* infections have occurred in patients that had not received polymyxins, leading to speculation that colonization with *mcr-1* may have occurred after ingestion of contaminated meat products [2]. Underdetection of colistin resistance is possible

because many microbiology laboratories do not perform routine colistin susceptibility testing on carbapenem-susceptible isolates [8]. An *mcr-1*-producing *E coli* isolate was detected during our center’s participation in the SENTRY Antimicrobial Surveillance Program [3]. In this study, we describe the clinical characteristics, molecular survey, and bacterial genomics of this *mcr-1* infection from May 2015.

METHODS

After detection of the *mcr-1*-positive isolate, we conducted a molecular survey for *mcr-1*-positive isolates with retrospective and prospective components. Our institution is a 2200-bed urban campus in Northern Manhattan. The microbiology laboratory processes samples from both inpatients and outpatients from our institution and a smaller affiliated community hospital also situated in Northern Manhattan. For the retrospective survey, we screened routinely collected MDR-GNB clinical isolates from 2011 to 2016 for the presence of *mcr-1* by polymerase chain reaction (PCR) [1]. These included (1) all carbapenem-resistant isolates that had undergone polymyxin B susceptibility testing and were found to be resistant to polymyxin B (polymyxin B mean inhibitory concentration [MIC], >2 mcg/mL [9]) as well as (2) all *E coli* isolates resistant to third-generation cephalosporins (whether polymyxin B susceptibility was performed or not). For the prospective survey, we conducted polymyxin B susceptibility testing on all Gram-negative isolates resistant to third-generation cephalosporins for 1 month. Gram-negative bacteria known to be intrinsically polymyxin resistant were not tested. Polymyxin B susceptibility testing was performed with E-test (bioMérieux, Durham, NC). Approval was granted by the Columbia University Institutional Review Board (IRB-AAAQ9741).

Whole-genome sequencing (WGS) of the *mcr-1*-positive isolate was performed using the HiSeq 2500 sequencer (Illumina, San Diego, CA). Sequences obtained were de novo assembled in contigs using Velvet after optimization of settings with VelvetOptimiser [10, 11]. Automatic annotation of the genome was performed using RAST [12]. The multilocus sequence type (MLST), resistome, and plasmid type were characterized using ResFinder 2.1, PlasmidFinder 1.3, and MLST 1.8, respectively [13–15]. Additional characterization of chromosomal resistance determinants was performed using CARD Resistance Gene Identifier [16]. Findings were confirmed by performing pairwise alignment of the relevant genes to wild-type *E coli* K-12 MG1655 (GenBank accession no. U00096) in Geneious 9.0.2 [17]. The GenBank Genome database was searched for complete *E coli* genomes, and these were characterized using MLST 1.8 [15]. Multiple sequence alignment of the study isolate and reference *E coli* ST117 genomes was performed using

Received 2 March 2017; editorial decision 24 May 2017; accepted 24 May 2017.

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Mauve in the progressiveMauve setting [18, 19]. Genomes were visualized with BRIG [20]. Plasmid sequences were aligned using Geneious 9.0.2 and visualized using EasyFig [17, 21].

The complete nucleotide sequences of plasmid pKP1749_MCR1 has been deposited as GenBank accession no. CP0000000. The draft genome of *E coli* strain KP1749 was deposited as GenBank accession no. MAJK0000000.

RESULTS

The patient was a man in his 50s with a past history of sickle cell anemia, recurrent cholangitis, and cholecystectomy who was admitted for elective biliary surgery in May 2015. The patient had no prior documented history of receiving polymyxins but had received a course of ciprofloxacin for cholangitis during an admission in another institution 4 months prior. He had a history of travel to the Dominican Republic in 2013. The patient was noted to be febrile at 38.2°C on postoperative day 1. Vancomycin 1 gram q12h and meropenem 500 mg q8h were commenced empirically according to the patient's renal function. The patient's modified Pitt bacteremia score was 3 [22].

Blood cultures taken during the febrile episode and intraoperative biliary fluid cultures were positive for *E coli* with the following MICs on the Vitek-2 instrument (bioMerieux): ceftriaxone 2 mcg/mL, ceftazidime 16 mcg/mL, piperacillin-tazobactam <4 mcg/mL, cefepime <1 mcg/mL, meropenem <0.25 mcg/mL, amikacin <2 mcg/mL, tobramycin <1 mcg/mL, gentamicin <1 mcg/mL, levofloxacin >8 mcg/mL, trimethoprim/sulfamethoxazole >320 mcg/mL, and minocycline 4 mcg/mL. The isolate was resistant to fosfomycin on disk diffusion susceptibility testing with a zone of 18 mm noted [23]. Ertapenem E-test indicated an MIC of 0.006 mcg/mL (bioMerieux). Polymyxin B susceptibility testing was not initially performed due to susceptibility of the isolate to many β -lactam antibiotics. Polymyxin B susceptibility

testing and *mcr-1* PCR were performed retrospectively after detection of *mcr-1* in the SENTRY program. Both isolates had a polymyxin B MIC of 4 mcg/mL and were *mcr-1* positive by PCR [24]. On postoperative day 5, the patient's antibiotic regimen was switched to cefepime 2 grams q8h, metronidazole 500 mg q8h, and fluconazole 400 mg q24h, and he completed 2 weeks of therapy with that regimen. The patient was discharged home on postoperative day 14 having had no further fever, abdominal symptoms, or cultures positive for *E coli*.

For the retrospective survey, *mcr-1* PCR was performed on 633 *E coli* isolates (selected as described above) and 171 polymyxin B-resistant isolates (163 *K pneumoniae*, 1 *E coli*, and 7 *Acinetobacter baumannii* isolates, respectively). Median polymyxin B MIC for polymyxin B-resistant isolates was 16 mcg/mL (interquartile range, 12–32 mcg/mL). All isolates tested were negative for *mcr-1*. For the prospective survey, 94 Gram-negative isolates were identified over the 1-month period, and all were tested for polymyxin B resistance according to criteria described above. No isolate had a polymyxin MIC >2 mcg/mL, hence none underwent further *mcr-1* PCR.

We then carried out WGS on the blood culture isolate (named KP1749). In addition to *mcr-1*, the isolate harbored resistance genes for β -lactams (*bla*_{SHV-12}, *bla*_{TEM-1B}), aminoglycosides [*aph*(3')-IIa, *aph*(6)-Ic, *aadA2*, *aph*(3')-Ia, *strA*, *strB*], sulphoamides (*sul1*, *sul2*), trimethoprim (*dfrA12*), chloramphenicol (*catA1*), macrolides (*mphA*), fosfomycin (*fosA*), and tetracycline [*tet*(A)]. Of note, *bla*_{SHV-12} codes for an extended-spectrum β -lactamase [25]. Chromosomal fluoroquinolone resistance mutations were detected in *gyrA* (S83L and D87N) and *parC* (S80I) [26]. KP1749 was compared with the 2 *E coli* ST117 isolates with complete genomes available on GenBank (GenBank accession nos. CP010157.1 from China and NZ_CP013025.1 from New York) (see Figure 1, Panel A). KP1749 shared 99.7%

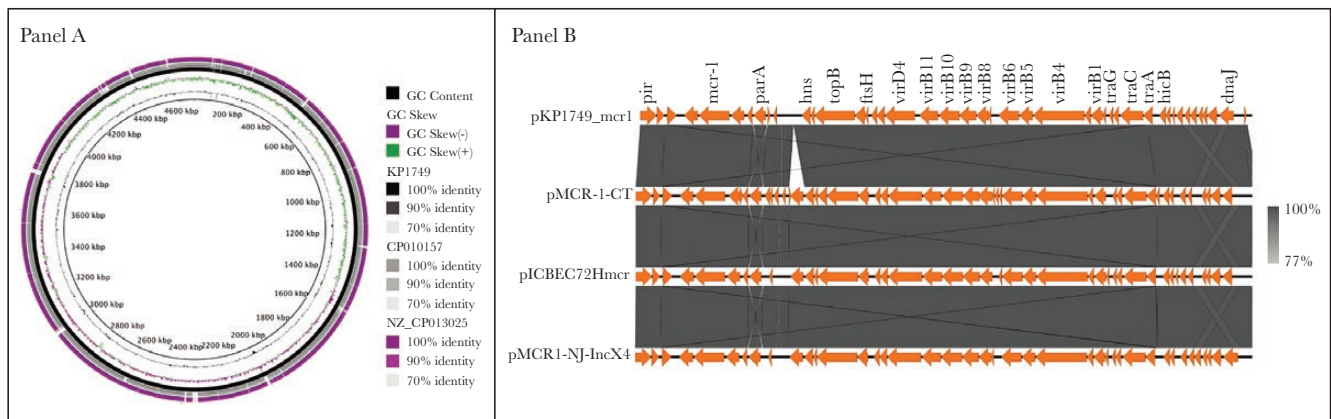


Figure 1. (Panel A) Comparative genomic analysis of KP1749 compared with 2 reference *Escherichia coli* ST117 genomes identified in GenBank. (Panel B) Backbone of pKP1749_MCR1 plasmid (GenBank accession no. CP0000000) of size 33.3 kb carrying *mcr-1* from a human *E coli* strain isolated in New York. pKP1749_MCR1 was compared with plasmid pMCR-1-CT (GenBank accession no. CP018773) from an *E coli* strain isolated in Connecticut, pICBEC72Hmcr IncX4 plasmid (GenBank accession no. CP015977) carrying the *mcr-1* gene in a human *E coli* strain isolated in Brazil, and plasmid pMCR1-NJ-IncX4 (GenBank accession no. KX447768) from a human *E coli* strain isolated in New Jersey.

and 99.65% nucleotide identity, respectively, with the isolates across the core genome. Multilocus sequence type analysis indicated that the isolate was ST117, an important avian pathogenic *E coli* lineage also noted to cause disease in humans [27].

The *mcr-1* gene was carried on an IncX4 plasmid (named pKP1749_MCR1) 33.3 kbp in length. There was a high level of architectural conservation with *mcr-1*-carrying IncX4 plasmids from Brazil, Connecticut, and New Jersey (GenBank accession nos. CP015977, CP018773, and KX447768, respectively) (Figure 1, Panel B) [5, 28]. pKP1749_MCR1 lacked an IS26 insertion sequence present in the other plasmids but otherwise differed 8 single-nucleotide polymorphisms (SNPs) to pMCR-1-CT (Connecticut), 10 SNPs to pICBEC72Hmcr (Brazil), and 95 SNPs to pMCR1-NJ-IncX4 (New Jersey). An IncI1 plasmid ~113.2 kbp in length was found to harbor *bla*_{SHV-12} and resistance genes for multiple other antibiotic classes: aminoglycosides, *aph(3')-IIa*, *aph(6)-Ic*, *aadA2*, *aph(3')-Ia*, *strA*, and *strB*; trimethoprim, *dfpA12*; sulphonamides, *sul1*; macrolides, *mphA*.

DISCUSSION

We describe a human infection with an *mcr-1*-producing *E coli* belonging to the ST117 lineage. This is one of the first cases of *mcr-1* noted in a US hospital setting and preceded the initial description of *mcr-1* in 2015 [1]. We were unable to identify further cases during a molecular survey for *mcr-1*, suggesting lack of widespread local circulation of the plasmid. In addition, other *mcr-1* isolates from the United States are of different clonal background (ST405 and ST457) [4, 5].

The ST117 clonal background of this isolate raises the possibility that it was acquired in the community from an avian source. *Escherichia coli* ST117 is a major pathogen in the poultry industry and may form a reservoir of human extraintestinal pathogenic *E coli* and antimicrobial resistance [27]. *mcr-1* and multiple *mcr-1*-carrying IncX4 plasmids have also been identified in poultry isolates from Asia, Europe, Latin America, and Africa [28, 29]. *mcr-1* was detected in 12 of 980 isolates in a collection of avian pathogenic *E coli* [30]. There are now several reports that suggest a possible association between *E coli* ST117 and *mcr-1* carriage. Initially, 2 of 3 isolates carrying *mcr-1* during a 2016 survey in the Netherlands were *E coli* ST117 from retail chicken meat [31]. Two recent Chinese studies reported 3 and 2 *mcr-1* carrying *E coli* ST117 in human samples, respectively [32, 33].

Therefore, poultry may be a reservoir of *E coli* carrying *mcr-1*, particularly in regions where colistin is routinely used in animal husbandry such as China and Latin America [34]. This patient had traveled to the Dominican Republic, and much of our center's patient population is of Dominican background, suggesting a possible epidemiological link [35]; however, no cases of *mcr-1* have yet been reported from that country. A human *mcr-1* case from Connecticut reported recent travel to the Caribbean and consumption of chicken and goat meat purchased from a live animal market [6]. The high degree of

similarity between pKP1749_MCR1 and IncX4 *mcr-1* carrying plasmids from Connecticut, New Jersey, and Brazil suggests the contribution of this plasmid in disseminating *mcr-1* through the Americas [28].

CONCLUSIONS

The spread of *mcr-1* to carbapenem-resistant *Enterobacteriaceae*-endemic regions such as the north-eastern United States is an active concern. An *E coli* isolate harboring both *mcr-1* and *bla*_{NDM-5} has already been noted in New Jersey [5]. Fortunately, isolate KP1749 retained susceptibility to multiple β -lactam agents, thus allowing adequate treatment and a satisfactory outcome. Since the description of *mcr-1*, several other transferable mechanisms of colistin resistance have been identified [2]. Therefore, we need to develop more active surveillance approaches for detecting resistance to colistin. Although phenotypic susceptibility testing forms the cornerstone, there are limitations in accuracy depending on the method used [8]. This problem is further compounded by the finding that most *mcr-1*-producing isolates have lower colistin MICs in the range of 2–4 mcg/mL, and some isolates that are phenotypically susceptible may produce *mcr-1* [2]. Therefore, our study may have underdetected *mcr-1*, both due to issues with polymyxin susceptibility testing and because our screening strategy required isolates to initially have resistance to third-generation cephalosporins. Integrating molecular techniques such as WGS may have an increasingly important role in stopping the spread of transferable colistin resistance by providing valuable epidemiological data and allowing detection of multiple resistance mechanisms.

Acknowledgments

Disclaimer. The funders had no role in study design, data collection and interpretation, or the decision to submit the work for publication.

Financial support. This work was funded by grants from the National Institutes of Health/National Institute of Allergy and Infectious Diseases (R01 AI116939; to A-C. U.). Merck provided funding for research.

Potential conflicts of interest. All authors: No reported conflicts of interest.

All authors have submitted the ICMJE Form for Disclosure of Potential Conflicts of Interest. Conflicts that the editors consider relevant to the content of the manuscript have been disclosed

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